MANCHESTER 1824
The University of Manchester

Translating Science: Translation of Japanese Scientific Research Articles into English and a Critical Analysis of Cross-Cultural Differences in Organisational Schemata and the Metadiscourse of Making Claims

A dissertation submitted to The University of Manchester for the degree of Master of Arts in the Faculty of Humanities

2016

UID: 7555155

School of Arts, Languages and Cultures

Table of Contents

List of Abbreviations

Term	Abbreviation
Context Frame	CF
Create a Research Space	CARS
Introduction Methods Results Discussion	IMRD
Move one	M1
Move three	M3
Move two	M2
Research article	RA
Source text	ST
Source text *n* Page *n* Line *n* e.g. Source Text 1, Page 1, Line 1	ST*n*P*n*L*n* e.g. STP1L1
Target audience	TA
Target text	TT
Target Text *n* Page *n* Line(s) *n* e.g. Target Text 1 Page 1 Line 1	T*n*P*n*l*n* e.g. T1P1L1
Text one	T1
Text three	T3
Text two	T2

Abstract

The influence of English as a *lingua franca* of science continues to spread within the international disciplinary community and the role of translation is therefore becoming increasingly significant in the dissemination of scientific knowledge. It is undeniable that Japan is a major player within the scientific community, particularly in terms of diabetes research. However, Japanese to English translation of scientific research articles still poses various difficulties due to conflicting norms and styles. In this thesis, the translation of three scientific articles in the field of diabetes research are critically analysed in order to explore common translational difficulties and provide translation strategies for localising a Japanese scientific research article. In translating these articles I identified two major translational issues. The first issue relates to differences in textual organisation. Swale's IMRD structure was applied in order to reorganise the target texts to Anglophone norms, Swale's CARS model was applied to improve the delineation of the research objectives and an Abstract was created. The second issue identified was the differences in terms of adherence to adequacy and acceptability conditions which could have influence on the research claims of the target texts. In order to address adequacy conditions, thematic connections were analysed, then unclear referents were specified and context frames and explicit anaphoric reference were added. In order to address acceptability conditions, strategic textual omissions were explored and hedging strategies between the source text and target text were compared and adapted. The aforementioned findings recorded within this thesis provide insight into possible issues and strategies for the translation of scientific research articles from Japanese into English.

Declaration

I declare that this dissertation is my own original work unless referenced to the contrary and no portion of the work referred to in the dissertation has been submitted in support of an application for another degree or qualification of this or any other university or other institute of learning.

Intellectual Property Statement

i. The author of this dissertation (including any appendices and/or schedules to this dissertation) owns certain copyright or related rights in it (the "Copyright") and she has given The University of Manchester certain rights to use such Copyright, including for administrative purposes.

ii. Copies of this dissertation, either in full or in extracts and whether in hard or electronic copy, may be made **only** in accordance with the Copyright, Designs and Patents Act 1988 (as amended) and regulations issued under it or, where appropriate, in accordance with licensing agreements which the University has entered into. This page must form part of any such copies made.

iii. The ownership of certain Copyright, patents, designs, trademarks and other intellectual property (the "Intellectual Property") and any reproductions of copyright works in the dissertation, for example graphs and tables ("Reproductions"), which may be described in this dissertation, may not be owned by the author and may be owned by third parties. Such Intellectual Property and Reproductions cannot and must not be made available for use without the prior written permission of the owner(s) of the relevant Intellectual Property and/or Reproductions.

iv. Further information on the conditions under which disclosure, publication and commercialisation of this dissertation, the Copyright and any Intellectual Property and/or Reproductions described in it may take place is available in the University IP Policy, in any relevant Dissertation restriction declarations deposited in the University Library, and The University Library's regulations.

Dedications

I would like to take advantage of this space to express my sincere thanks to my family for their continued support (and tolerance!) throughout my academic career. I would like to thank my Mum and Dad for never pressuring me and believing in me when I lacked that belief myself. I would like to thank my brother, Mark, for all the counsel he has provided when I needed it. I would also like to thank my lovely Nanny for all of her assurances and positivity. Finally, I'd like to thank my cats Mao and Sherlock for unwittingly being the best stress relievers possible.

I would also like to express my gratitude to all of the staff in both the Department of Japanese Studies and Department of Chinese Studies for their continued support from undergraduate until now. In particular I would like to highlight Jonathan Bunt for his guidance as my academic advisor over the course of my undergraduate degree and beyond. I would also like to express particular thanks to Dr Aya Homei who inspired me and encouraged me to pursue translation studies and consistently went above and beyond to volunteer her time and help as a teacher, language specific tutor and dissertation supervisor. I would also like to thank Dr Erica Baffelli who directed me and guided me in my application for the Sasakawa scholarship and thus made reading a masters a possible path for me.

Finally, I'd like to express my deep gratitude to the Sasakawa Foundation for their financial support that allowed me to read this master's programme and to continue pursuing my passions.

Translation Section

Source Texts

Text 1

Yamamoto, Hiroshi (2014) '糖尿病合併症の成因・病態・克服に関する基礎的研究' [Fundamental Research on the Causes, Pathology and Subjugation of Diabetes Complications], *糖尿病 [Diabetes]* 57(10): 765-711.

Text 2

Ishihara, Hisamitsu (2009) '2 型糖尿病発症における膵 b 細胞障害の分子機構' [The molecular mechanism of pancreatic beta cell damage in the pathogenesis of Type 2 diabetes], *糖尿病 [Diabetes]* 52(11): 884-866.

Text 3

Watada, Hirotaka (2009) '膵 b 細胞容積調節機構に関する研究' [The Clinical Application of the Mechanism for Regulating Beta Cell Mass in the Treatment of Type 2 Diabetes], *糖尿病 [Diabetes]* 52(11): 881-883.

Author's Note

The translation is presented in a facing page format, as stipulated by the Guidelines for MATIS/MACINT Dissertations. However, as restructuring forms a major component of the analysis undertaken there are a number of Appendices for reference. There is a bilingual table of source text and target text before restructuring in Appendix A, target texts with numbered sentences in the structure of the source text available in Appendix B and target texts which have been restructured but still have original sentence numbers included in Appendix C.

Source Text 1, Page 1

ハーゲドーン賞受賞講演

糖尿病合併症の成因・病態・克服に関する基礎的研究

山本博

はじめに

本賞に冠せられた Hagedorn の名にはじめて接したのは，須藤憲三金澤醫科大學醫化學初代教授の著書「醫化學的微量測定法」1）を紐解いたときであった．須藤先生は，尿糖測定法を確立するなど，わが国糖尿病研究の草分け的存在で「，栄養」の命名者としても知られる．

「醫化學的微量測定法」では「一二滴の血液を用いて容易に且つ正確に」血糖を定量できる測定として Hagedorn-Jensen 法が紹介されている．

私は恩師岡本宏先生の下で糖尿病研究に着手し，富山医科薬科大学および前任の東北大学時代には，主として膵ランゲルハンス島の分子生物学的研究に取り組んだ．アロキサンとストレプトゾトシンが膵ランゲルハンス島の DNA 鎖を切断することの発見 2）や“，Molecularbiologyoftheisletsof Langerhans”3）の編纂に関わった．

血管生物学研究から糖尿病合併症研究へ

1990 年金沢大学赴任後，血管生物学に取り組み，血管構成細胞種の共存培養系を確立して，周皮細胞が内皮細胞の増殖を抑制し，プロスタサイクリン産生能を保持するとともに，過酸化脂質による内皮細胞障害を防止することを明らかにした 4）．これにより，糖尿病網膜症ではなぜ周皮細胞喪失に伴って血管新生が起こるかの一端が説明されるようになった．

Target Text 1, Page 1

Winner of the Hagedorn Prize: Hiroshi Yamamoto

Fundamental Research on the Causes, Pathology and Subjugation of Diabetes Complications

Abstract

The decline in β cell mass and vascular complications associated with Type 2 diabetes is well established. Although no viable therapies are currently available, a primary factor associated with these changes, the AGE-RAGE pathway, has become a promising therapeutic target. In this paper we use transgenic mice that overexpress or lack the RAGE gene to investigate the relationship between the AGE-RAGE interaction and diabetic complications. We explore two potential therapies through the creation of a decoy RAGE protein and a RAGE antagonist. Our results show a definite relationship between the AGE-RAGE interaction and diabetic complications with RAGE contributing to the apoptotic-induced reduction in β cell mass and impaired glucose tolerance associated with Type 2 diabetes. In identifying a potential therapy, we successfully promoted the solubilisation of membrane-bound RAGE by increasing cAMP concentration. We also successfully identified potential RAGE antagonists in the form of low-molecular weight RAGE, using this information to assess the agonist/antagonist activity of AGE-derived food products. The results presented in this paper highlights the viability of using the AGE-RAGE interaction as a therapeutic target to develop a potential therapy to treat Type 2 diabetes and its complications.

1. Introduction

In the 1990s, the co-cultivation of constituent vascular cell types was established within vascular biology. Using this technique, it was found that pericytes inhibit the proliferation of endothelial cells, while preserving the production of prostacyclin and preventing endothelial cell damage by lipid peroxides[4]. This discovery gave an insight into why neovascularization and pericyte loss are observed simultaneously in cases of diabetic retinopathy. However,

Source Text 1, Page 2

では，糖尿病状態で各種血管細胞に特徴的な変化を来す primary の要因は何か？この問いに答えるため，周皮細胞や内皮細胞の純培養系を用い，種々の環境要因を探索した結果，同定された因子が advancedglyca-tionendproducts（以下，AGE）であった５，６（）Fig.1）．AGE は，糖のカルボ二ル基と蛋白のアミノ基とが非酵素的に反応する結果，不可逆的に形成される産物の総称である．AGE の形成，蓄積は慢性的な高血糖状態で加速的に進行する．ブドウ糖は有効な燃料分子であるが，糖化という負の側面をもつ．大阪大学の垂井清一郎先生は「，脊椎動物への進化の段階で遊離のブドウ糖を血糖として採用し閉鎖循環系のなかを循環させるに至ったのが，そもそも糖尿病の個体が出現するきっかけであり，糖尿病は宿命的に合併症発現のリスクを担った疾患なのではなかろうか」と記されている７）．

AGE-RAGE は糖尿病合併症の一成因–遺伝子改変動物を用いた証明

AGE の血管細胞作用の少なくとも一部は，特異受容体 receptorforAGE（以下，RAGE）を介する．RAGE は，パターン認識受容体に分類され，さまざまな病原体関連分子パターン pathogen-associatedmolecularpattern（PAMP）や傷害関連分子パターン damage-associatedmolecularpattern（DAMP）をリガンドとして認識する（Fig.2A）．

教室の山本靖彦准教授ら８，９）は，血管細胞で RAGE を過剰に発現するトランスジェニックマウス，内在性 RAGE 遺伝子を欠損したマウスを作製し，糖尿病を誘発して，合併症を解析した．すると，RAGE 過剰発現マウスは糖尿病腎症８）および網膜症１０）指標の増悪を示し（Fig.2BandC），他方，RAGE 欠損マウスは糖尿病腎症を発症しなかった９（）Fig.2D）．以上の結果は，糖尿病合併症の発症に AGE と RAGE の相互作用が機能的に関わっていることを示す.

Target Text 1, Page 2

the primary factor that induces the characteristic changes observed in every type of vascular cell in those with diabetes had not been identified. In order to determine this instigator, an axenic culture of endothelial cells and pericytes was used to test the effect of a variety of environmental factors. This investigation identified the primary factor as advanced glycation end products (AGE)[5,6]. (Figure 1)

AGE is the term used to describe the product formed following an irreversible non-enzymatic reaction between the carbonyl group on a sugar and the amino group on a protein impairing its functionality. Glucose, despite being an efficacious source of respiratory derived energy, still has the issue of glycation. Accordingly, the process of AGE formation and accumulation is accelerated by a chronic hyperglycaemic state. As diabetes is characterised by a hyperglycaemic state, it has been noted that it would not surprising to find that AGE formation is associated with diabetes and is an evitable complication of the disease[7].

It is thought that a degree of AGE derived complications associated with vascular cells is caused by interaction with the specific receptor for AGE (RAGE). RAGE is classified as a pattern recognition receptor which is recognised as a ligand by various pathogen-associated molecular patterns (PAMP) and damage- associated molecular patterns (DAMP) (Figure 2a). The AGE-RAGE interaction is considered to be a potential contributor to the established cell dysfunction in pancreatic β cells within the islets of Langerhans and the decline in β cell mass which accompanies the progression of Type 2 diabetes. Thus firstly, this paper seeks to further clarify the relationship between AGE-RAGE, decline in beta cell mass and consequent diabetes complications.

Despite the hypothesis that AGE-RAGE interaction is a potential therapeutic target in the treatment of Type 2 diabetes, there have been no viable therapies developed as of yet. It is proposed that the first theoretically conceivable strategy is to inhibit the formation of AGE. Interestingly, it has been reported that angiotensin receptor blocker (ARB) has AGE inhibiting activity[16]. However, the mechanism of action for the majority of currently developed AGE inhibitors is to target the covalent bonds of intermediate compounds generated during AGE formation. These intermediate compounds require therapeutic concentrations close to an equimolar which results in stoichiometric issues. Another option that has been explored is

Source Text 1, Page 3

ヒト RAGE 遺伝子の転写調節機構

教室の Tanaka ら[11]は，ヒト RAGE 遺伝子の転写調 節機構を調べた．その結果，AGE 自身が転写因子 nu- clear factor-κB（NF-κB）を活性化して RAGE 遺伝子 の転写を活性化する，という positive なフィードバッ クの仕組みが見い出された（Fig. 3）．これが，糖尿病状 態で認められる見かけ上 constitutive な RAGE 発現 や AGE-RAGE 共局在の分子的基礎と考えられた． RAGE 後の多様なシグナル経路の中でも糖尿病では とくに NF-κB を介する経路が重要と考えられる．

AGE-RAGE は膵 β 細胞不全にも関わる

2 型糖尿病の進行に伴い膵ランゲルハンス島 β 細胞 の機能不全や β 細胞塊の減少が起こることが知られ ている．この現象に AGE-RAGE が関係していないか どうかが教室の Han ら[12]によって調べられた．する と，意外なことに，正常な β 細胞では細胞表面に RAGE 蛋白は検出されなかった．ところが，2 型糖尿病 モデル動物である *ob*/*ob* マウスや *db*/*db* マウスでは加 齢に伴って膵 β 細胞の RAGE 蛋白陽性率が増大し た．そこで，*db*/*db* マウスと RAGE 欠損マウスを交配 させると，糖尿病の進行に伴う耐糖能異常とアポトー シスによる β 細胞塊の減少が改善されることが見い 出された（Fig. 4）．さらに，MIN-6 細胞を用いた解析で，遊離脂肪酸とレプチン受容体アンタゴニストの投 与により細胞表面 RAGE 蛋白発現が誘導され AGE 曝露による細胞死がもっとも顕著になることが観察された．以上の結果から，2 型糖尿病に伴う膵 β 細胞不全 のメカニズムとして従来想定されてきた lipotoxicity と glucotoxicity の実体の少なくとも一部は遊離脂肪 酸と AGE によって担われているものと考えられた（Fig. 4）．

Target Text 1, Page 3

the development of a drug to break down already formed AGE. This type of drug belongs to a category of drugs called AGE breakers. However, an AGE breaker which is sufficiently efficacious at breaking down AGE is yet to be developed.

Two promising forms of targeting the AGE-RAGE interaction are firstly, the addition of an extracellular decoy receptor for AGE that protects the vascular cells and secondly the invention of a RAGE antagonist.

The current paper will firstly, further analyse and investigate the role of the AGE-RAGE interaction and its associated pathways in the decline of beta cell mass and the appearance of diabetes complications. Secondly, this paper will investigate and critically analyse the potential of proposed therapeutic targets for disrupting the AGE-RAGE interaction including decoy RAGE targets and RAGE antagonists.

2. Method

2.1

Transgenic mice that over-expressed RAGE within their vascular cells and mice that endogenously lacked the RAGE gene were created in order to investigate the relationship between AGE-RAGE and diabetic complications. Diabetes was then induced within the mice and the resulting complications analysed. Next, the transcriptional regulatory mechanism for the human RAGE gene was investigated[11].

Following the investigation of the transcriptional regulatory mechanism for the human RAGE gene, an investigation was carried out to find out whether there is a connection between the AGE-RAGE interaction and Type 2 diabetes pathogenesis. The cell surface of normal pancreatic β cells were analysed in animal models of type 2 diabetic mice including ob/ob mice and db/db mice for RAGE proteins. An age variable was also introduced. The db/db RAGE deficient mice were then crossbred. Non-esterified fatty acids and leptin receptor antagonists were then administered to MIN-6 cells, causing them to induce the expression of RAGE proteins on the cells' surface. This was followed by a study into the relation of RAGE to other pathologies using a subacute inflammation mechanism.

Source Text 1, Page 4

RAGE が関係するその他の病態

東北大学久保裕司博士ら１３，１４）との共同研究で，RAGE が，亜急性炎症モデルで上皮間葉移行に関わることや，phosphatidylserine を特異的に認識してアポトーシス細胞の貪食に関わることが見い出された．また，金沢大学における学際的研究により，アミロイドβ1-42 ペプチドの脳内への移行が RAGE 欠損や後述する可溶型 RAGE 蛋白の過剰発現で有意に抑制されることが明らかにされた１５）．

AGE-RAGE ターゲッティング

以上述べてきた知見から，AGE-RAGE は糖尿病とその合併症および各種ヒト疾患の治療標的候補と考えられる．理論上考えうる主な AGE-RAGE 標的療法の方針と手段を Table 1 に示す．

第一は，AGE 形成の阻害である．が，これまで開発されてきた AGE 形成阻害剤の多くは AGE 形成中間体への共有結合を作用機構としており，中間体に対して等モル近い薬剤を必要とするという化学量論的な問題がある．興味深いことに，angiotensin receptor blocker（ARB）が AGE 形成阻害活性をもつことが報告されている１６）．

第二は，すでに形成された AGE を分解する薬物である．このカテゴリーに属する薬物は AGE breaker とよばれる．が，未だ効率よく AGE を分解できる breaker の開発には至っていない．

可溶型 RAGE

第三は，AGE を細胞外で補足し血管細胞を保護するデコイ受容体である（Fig. 5）．教室の Yonekura（現金沢医科大学教授）ら[17]は，ヒト血管細胞ポリソームのスクリーニングでオルタナティブ RNA スプライシングにより生成されるデコイ RAGE 蛋白を同定し，esRAGE（endogenous secretory RAGE）と命名した．Motoyoshi ら[18]は，細

2.2

Next ways of targeting the AGE-RAGE interaction were investigated. The first therapeutic target investigated was the invention of a decoy RAGE protein. A decoy RAGE protein was identified and named endogenous secretory RAGE (esRAGE) by screening human vascular cell polysomes. It was then formed through alternative RNA splicing[17]. Next, the feasibility of creating soluble RAGE was investigated. This was achieved through MM9 induced ectodomain shedding by increasing the concentration of intracellular cyclic AMP[18]. This process induced membrane-bound RAGE proteins to convert into soluble RAGE proteins

The next option explored was the invention of a RAGE antagonist. Analysis of fluorescence resonance energy transfer (FRET) was carried out using RAGE antibodies, whilst undergoing ligand stimulation. Then a pharmacological assessment was carried out in which low-molecular weight AGE of a maximum molecular weight of 300 was prepared and investigated for signs of antagonist activity[19]. Following this analysis, using the three-dimensional structure of the human RAGE protein established in earlier research[20], low-molecular weight compounds were screened *in silico* by their structural information. A pharmacological assessment ensued in order to identify potential agents. Finally, an assessment of RAGE as an agonist/antagonist was carried out using the aforementioned screening method. Soy sauce, coffee, red wine and cola were used within this assessment.

3. Results

3.1

Analysis of the transgenic mice for diabetic complications revealed that both the indexes for diabetic nephropathy[8] and diabetic retinopathy[10] increased in species that overexpress RAGE (Figure 2B and 2C), whilst RAGE deficient mice did not develop any symptoms (Figure 2D). These results indicate that there is a functional interaction between the pathogenesis of diabetes complications and the AGE- RAGE interaction.

Investigations into the transcriptional regulatory mechanism of the human RAGE gene showed that AGE activates the transcription factor nuclear factor-κB（NF-κB）as well as

細胞内サイクリック AMP 濃度が 上昇すると，MMP9 によるエクトドメインシェディン グにより，膜結合型 RAGE 蛋白から可溶型 RAGE 蛋 白への転換が誘導されることを示した．

RAGE アンタゴニストの開発と食品 AGE の評価

第四は，RAGE アンタゴニストの開発である．RAGE 抗体を用いた fluorescence resonance energy transfer （FRET）解析を行うと，リガンド刺激前後で RAGE monomer に由来すると考えられる蛍光の強度は変化 せず，オリゴマーに由来すると考えられる蛍光の強度が増大した．細胞内に情報を送るアゴニスティックなリガンドは RAGE 受容体をオリゴマー化するものと考えられる．そこで，分子量 300 ほどの低分子 AGE を調製し，薬理学的な評価を行ったところ，低分子 AGE は RAGE に対してアンタゴニスト活性を示すこ とが見い出された 19）．

大阪大学小林祐次名誉教授のグループとの共同研究 で，ヒト RAGE 蛋白の三次元構造を決定した２０）．構造 情報に立脚した低分子化合物の in silico スクリーニン グと，その後の薬理学的な評価により，RAGE 拮抗活 性を示す数種の候補物質も得られた．

ある種の食品は AGE に富み，色，香り，味などの風 味の一部は AGE に由来する．摂取した食品に含まれ る AGE の約 10 %が循環血中に回収され，48 時間後に は 70 %が体内に留まる２１）．欧米では従来，食品中の AGE を有害視する考え方が支配的であったが，食品 AGE のもつ生物学的作用についてはなお検証の必要 があると考えられる．Munesue ら１９）は RAGE アゴニズ ムƒアンタゴニズムの観点から醤油，コーヒー，赤ワイ ン，コーラをモデルとした評価を行った．その結果， 醤油，コーヒー，赤ワインは高分子 AGE の RAGE アゴニスト活性を中和することが見い出され，このア ンタゴニスト活性は低分子画分に回収された．

Target Text 1, Page 5

promoting the transcription of the RAGE gene, establishing a positive feedback loop. This research forms the molecular basis for the apparent constitutive RAGE expression and AGE-RAGE colocalization in the observed diabetic state. The NF-κ B intermediary pathway is considered to be particularly important in regard to diabetes among the numerous RAGE-flanking signalling pathways.

In analysing the connection between AGE-RAGE and the aforementioned aspects of Type 2 diabetes pathogenesis, the surprising discovery was that the RAGE protein was not detected on the cell surface of normal β cells [12]. However, in animal models of type 2 diabetic mice, the ratio of RAGE protein positive pancreatic β cells within ob/ob mice and db/db mice was observed and increased with age. It was discovered that when the db/db RAGE deficient mice were crossbred, the apoptosis induced reduction in β cell mass and impaired glucose tolerance associated with diabetes improved (Figure 4).

However, the most remarkable observation was cell death due to AGE exposure. In this analysis, free fatty acids and leptin receptor antagonists were administered to MIN-6 cells which caused the MIN-6 cells to induce the expression of RAGE proteins on the cells' surface, and promoted cell death when exposed to AGE.

There were various discoveries concerning RAGE and its relationship to the epithelial-mesenchymal transition using a subacute inflammation model[13,14], in addition to discoveries concerning RAGE specifically recognising phosphatidylserine and its involvement in the phagocytosis of apoptotic cells. Moreover, interdisciplinary research demonstrated that both RAGE deficiency and soluble RAGE overexpression significantly suppresses the uptake of amyloid β 1-42 peptide into the brain[15].

This supports the hypothesis that AGE-RAGE can be considered as a possible target for treatment of all kinds of human diseases including diabetes and the complications associated with diabetes.

3.2

Initial efforts to identify potential AGE-RAGE therapeutic treatments focused on establishing a decoy RAGE protein. An endogenous secretory RAGE (esRAGE) was created by screening

Source Text 1, Page 6

まとめ

AGE を含むリガンドと RAGE との相互作用は，糖 尿病における血管障害および β 細胞不全の成因の一 つと考えられ，糖尿病の一次・二次・三次予防上の標 的となると考えられる．RAGE アンタゴニスト薬やデ コイバリアント産生誘導法の開発により糖尿病合併症を克服できる日が来ることを期待したい．

著者の COI（conflicts of interest）開示：特になし

謝　辞

厳しく，また，温かく，私の糖尿病研究をお見守りいただきました恩師岡本宏東北大学名誉教授に深甚の謝意を表 します．折にふれ encourage いただきました金沢大学名誉 教授竹田亮祐先生に心より感謝申し上げます．今回の受賞 は真に共同研究者各位との共同研究の賜物であります．以 下にお名前を掲げ，心からの謝意を表します．

the polysomes for human vascular cells and splicing it with alternative RNA. It demonstrated that it is possible to cause membrane-bound RAGE proteins to become soluble through ectodomain shedding by MMP9 when the concentration of intracellular cyclic AMP is increased.

The second method investigated was the invention of a RAGE antagonist. Analysis of fluorescence resonance energy transfer (FRET) was carried out using RAGE antibodies. There was no change in fluorescence intensity originating from what is believed to be a RAGE monomer. However, at the same approximate time of ligand stimulation, the fluorescence intensity, which is believed to originate from an oligomer, increased. It is thought that the intracellular signals sent from agonist ligands make the RAGE receptors oligimerize. So, when a subsequent pharmacological assessment was carried out in which low-molecular weight AGE of a maximum molecular weight of 300 was prepared, it was discovered that low-molecular weight AGE showed signs of RAGE antagonist activity[19]. Next, the three-dimensional structure of the human RAGE protein, established in earlier collaborative research, was used in order to screen low-molecular weight compounds *in silico* based on their structural information[20]. Through subsequent pharmacological assessment, several potential agents which showed RAGE antagonist activity were obtained.

Finally, using the previous research as a basis, an assessment of RAGE as an agonist/antagonist was carried out. It was found that soy sauce, coffee and red wine neutralised the RAGE agonist activity of the high molecular weight AGE and that it was low-molecular weight fractions that reversed the activity into becoming that of a RAGE antagonist.

4. Discussion

4.1

Our investigations found that the ratio of RAGE protein positive pancreatic β cells within ob/ob mice and db/db mice increased with age. Moreover we found that when the db/db RAGE deficient mice were crossbred, the apoptosis induced reduction in β cell mass and

Target Text 1, Page 7

impaired glucose tolerance that is associated with advancement of diabetes showed signs of improvement (Figure 4).

The above results are in line with prevailing theories on the mechanism behind pancreatic β cell deficiency which is associated with Type 2 diabetes. These theories postulate that free fatty acids and AGE at least play a part in the lipotoxicity and glucotoxicity contributing to the mechanism of β cell deficiency. Thus our results also support the targeting of AGE-RAGE in order to treat type 2 diabetes.

4.2

The interaction between RAGE and its ligands, including AGE, is now considered to be one of the causes of diabetic angiopathy and β cell failure in diabetes. Accordingly they are considered to be the primary, secondary and tertiary targets for prevention of these disorders.

In our study, several potential agents which showed RAGE antagonist activity were obtained. This could represent an important step in the development of an AGE-RAGE therapeutic target.

Conventionally, the dominant view has been that food derived AGE is regarded as harmful to health. The taste of varieties of food products which are rich in AGE partially originates from AGE itself. This includes the colour, aroma and flavour of AGE rich food. Of the intake of food derived AGE, approximately 10% is absorbed into the blood stream. Yet after 48 hours, 70% of the AGE consumed remains within the body[21]. Thus, it is also thought that further investigation into the biological effects of AGE in food products is required before the development of a RAGE antagonist.

However, to conclude, there is great reason to hope that the development of a RAGE antagonist drug or a method of inducing the production of a decoy variant of the RAGE protein will soon become a reality. These developments could mean the elimination of diabetes complications in the near future.

Target Text 1, Page 8

Conflicts of interest (COI): none to declare.

Acknowledgements

I would like to express my profound gratitude to my respected emeritus professor Hiroshi Okamoto of Tohoku University for his stern but warm guidance concerning my diabetes research. I would also like to express heartfelt thanks to emeritus Professor Ryousuke Takeda of Kanazawa University for his frequent encouragement. This prize truly is a result of the collaborative research efforts of every member involved.

Words: 2,247

Source Text 2, Page 1

リリー賞受賞講演

2型糖尿病発症における膵b細胞障害の分子機構

石原寿光

はじめに

2 型糖尿病は，膵 b 細胞からのインスリン分泌障害と骨格筋・脂肪組織や肝臓でのインスリン抵抗性が複雑に絡み合って，発症・進展する疾患である．私が糖尿病を専門とすることを決意し，医局の研究室で研究を始めた当初，私のまわりではインスリン抵抗性に関する研究がより盛んに行われていた．膵島あるいは膵細胞は単離することが簡単ではなく，研究材料を豊富に得られないことが，研究を進めるうえで足かせとなっていた．

b 細胞の機能の研究–engineeringofnutrient-stimulatedinsulinsecretion

幸運なことに，1990 年に，今日最も代表的なインスリン分泌細胞株として世界中で広く使用されている MIN6 細胞が樹立された．これによって，研究材料が得にくいという困難さはかなり解消された．2 型糖尿病の発症初期あるいは耐糖能異常の段階において，インスリン分泌応答はしばしば遅延過大反応と記述されるように，絶対量の低下は認められない．このため，1990 年代前半，b 細胞の異常は量の低下ではなく，むしろグルコース濃度認識機構の異常であると考えられていた．私も，b 細胞のグルコース濃度認識機構の詳細を解明することが，2 型糖尿病におけるインスリン分泌異常の治療，ひいては 2 型糖尿病の治療に役立つと考えた．そこで，MIN6 細胞を用い，遺伝子工学の技術を取り入れて，どのような遺伝子を導入した場合にグルコースによるインスリン分泌応答が変化するかを解析していった．グルコースなどの栄養源(nutrient)によるインスリン分泌を engineering して検討するという方法であった．

Target Text 2, Page 1

Winner of the Lilly Prize: Hisamitsu Ishihara

The molecular mechanism of pancreatic beta cell damage in the pathogenesis of Type 2 diabetes

Abstract

Type 2 diabetes is a complex disease caused by impaired insulin secretion by the pancreatic beta cells and complicated by insulin resistance in the skeletal muscle, adipose tissue and liver. There has been mounting academic interest into insulin resistance. However, there is still not a comprehensive view of the mechanism of insulin secretion in response to glucose, or the mechanism of beta cell apoptosis and its association with endoplasmic stress. We analysed the insulin secretion response upon the introduction of different genes using genetically engineered MIN6 cells. *Wfs1* disrupted mice were analysed and crossbred to investigate the beta cell stress response. We found that the forced expression of necessary transporter proteins resulted in insulin secretion in response to nutrients other than glucose. Pyruvic acid was also found to be intertwined within the explanation of the mechanism of insulin secretion and its glucose specificity. We found that expression of eIF4E-binding protein 1: 4e-bp1 increased under endoplasmic reticulum stress-induced apoptosis and a lack of 4e-bp1 caused advancing beta cell damage and worsened glucose intolerance. This indicated that 4e-bp1 is beneficial to long-term survival of beta cells, as it regulates protein synthesis in beta cells under endoplasmic reticulum stress. Our research provides new perspectives on the mechanisms of insulin secretion and cell death in beta cells and how these factors contribute to diabetes pathogenesis, thereby offering potential new therapeutic targets.

Source Text 2, Page 2

1.グルコース応答性インスリン分泌機構

まず，解糖系の酵素に注目して取り組み，グルコース取り込みを行うグルコース輸送担体 GLUT1 とグルコースリン酸化を行うヘキソキナーゼIの過剰発現の効果を解析した 1)．また，解糖系とミトコンドリア代謝との連携のうえで重要なグリセロール 3 リン酸脱水素酵素 2)や乳酸脱水素酵素 3)，ミトコンドリアでのアデノシン 5'-三リン酸(ATP)産生を抑制する脱共役タンパク UCP14)の強制発現の効果を検討した．それらの結果をまとめると，①グルコースリン酸化過程が b 細胞における解糖系の律速過程であり，グルコース代謝流量を規定しグルコースセンサーとしての役割を担っていること，②b 細胞においては解糖系とミトコンドリア代謝の連関効率が高いこと，そして，③ミトコンドリア代謝が ATP をはじめインスリン分泌のシグナル形成に重要な役割を果たしていること，が明らかとなった．

2.インスリン分泌のグルコース特異性

Nutrient によるインスリン分泌機構を engineering する過程で，b 細胞がインスリン分泌をグルコースに限定して起こすために，他の細胞と異なった特徴を有していることに気がついた．すなわち，b 細胞はグルコース以外の nutrient によってインスリン分泌を起こさないために，それらに対する細胞膜上の輸送担体をもっていないことが明らかとなった．実際，TCA サイクルの中間体であるジカルボン酸を細胞に取り込むジカルボン酸輸送担体を発現させたところ，これらの分子に対してインスリンを分泌するようになった 5)．また，留学中の成果であるが，ピルビン酸輸送担体を発現させることにより，膵島がピルビン酸に対してインスリン分泌を起こすようになることも観察された 3)．これは，ピルビン酸逆説の解明に繋がる興味深い結果であった．

Target Text 2, Page 2

1. **Introduction**

1.1

Type 2 diabetes is a complex disease in which insulin secretion from the pancreatic beta cells is impaired. It is complicated by insulin resistance in the skeletal muscle, adipose tissue and liver which results in the pathogenesis and progression of the disease. Since the 1990s there has been mounting academic interest into insulin resistance. In order to research insulin resistance islets of Langerhans need to be isolated. However the process of isolating the islets of Langerhans or pancreatic beta cells is extremely complicated. A lack of these resources in large quantities can become a major stumbling block for the progression of diabetic research. Fortunately, in 1990 the MIN6 cell, which is still currently used globally and considered the most typical insulin-secreting cell line, was created. This solved the issue of obtaining sources of research materials for investigation into beta cell function and insulin resistance.

It has been noted that at the stage of initial pathogenesis of Type 2 diabetes, or at the stage of impaired glucose tolerance, there is frequent latent excessive response to insulin secretion. This has meant that it is not possible to observe the absolute amount of beta cell decline. Thus, since the first half of the 1990s, there has been a shift in research interest. The prevailing view became to make the research target the abnormalities in the mechanism for recognising glucose concentration, rather than researching the decline in amount of beta cells which results in abnormalities.

Despite this shift in research interest, there is still not a comprehensive view of the mechanism of insulin secretion in response to glucose. The present study seeks not only to assist in elucidating the details of the mechanism for glucose concentration recognition in beta cells but also assist in the treatment of type 2 diabetes through the treatment of insulin secretion disorders.

Source Text 2, Page 3

b 細胞量の維持機構の研究

1.b 細胞の生存と小胞体ストレスジュネーブ大学での留学を終え，岡芳知教授のもとで，同教授が山口大学の谷澤幸生教授らとともに発見したウオルフラム症候群原因遺伝子 Wfs1 の解析に携わった．Wfs1 遺伝子破壊マウスの膵島では，グルコースによるインスリン分泌応答の異常が生じ，その過程に b 細胞内でのカルシウム動態の異常が関与していることを明らかにした 6)．また，同時に，WFS1 タンパクが欠損する状態では，b 細胞での小胞体ストレス応答の亢進が認められ，b 細胞がアポトーシスに陥りやすくなっていることが明らかとなった 7,8)．小胞体ストレス応答亢進の一因は，b 細胞内でのカルシウム動態の異常であると考えられる．すなわち，Wfs1 遺伝子破壊マウスの膵島では，インスリン分泌機能の低下とともに，アポトーシスが亢進する結果，膵 b 細胞量の低下も起こり，個体としてのインスリン分泌不全に陥って糖尿病が発症すると考えられた．

2.b 細胞のストレス応答と翻訳制御

さらに，Wfs1 遺伝子破壊マウス膵島における小胞体ストレス誘導アポトーシスの分子機構を検討する過程で，翻訳開始因子(eIF)4E 結合蛋白 1(eIF4E-bindingprotein1:4E-BP1)の発現が増加していることを見出した 9)．4E-BP1 の増加は，Wfs1 遺伝子破壊マウス膵島に限ったものではなく，インスリン分子の異常による小胞体ストレス亢進によって糖尿病を発症する Akita マウスの膵島でも認められた．この小胞体ストレス応答における 4E-BP1 の発現増加は，ストレス応答のマスター転写因子 ATF4 による 4E-BP1 の転写活性化によることを明らかにし，4E-BP1 遺伝子のイントロン 1 の内部に ATF4 の結合領域を見出した．Wfs1 遺伝子破壊マウスや Akita マウスにおける 4EBP1 誘導の意義を解析するため，Akita マウスあるいは Wfs1 遺伝子破壊マウスと 4E-BP1 遺伝子破壊マウスを交配して 2 重変異マウスを作製し，解析した．Akita マウスおよび Wfs1 遺伝子破壊マウスのいずれにおいても，4E-BP1 の欠損が b 細胞障害を進行させ，耐糖能障害を悪化させることが観察された．小胞体スト

Target Text 2, Page 3

1.2

Wolframin's Syndrome, a disease which Tanizawa et al. discovered, is caused by a mutation in the *wfs1* gene. It is linked with defects in the endoplasmic reticulum stress response and a reduction in beta cell mass. A study which analysed the islets of Langerhans of *wfs1* disrupted mice found that glucose stimulated insulin secretion became dysfunctional and that the process of insulin secretion is connected to a dysfunction in calcium movement within beta cells[6]. Moreover, it was also observed that there is acceleration of the endoplasmic reticulum stress response in conditions in which there is a lack of the wfs1 protein. From this, it has become evident that under wfs1 deficient conditions, it is likely that beta cell apoptosis will be induced[7,8]. It is now hypothesised that one of the causes of this acceleration in the endoplasmic reticulum stress response is the dysfunction of calcium movement within the beta cells of *wfs1* disrupted mice. In other words, as well as a decline in the functionality of insulin secretion in the islets of Langerhans of *wfs1* disrupted mice, there is also a decrease in pancreatic beta cell mass due to accelerated apoptosis. It is thought that these two factors lead to hyposecretion of insulin, which in turn results in the pathogenesis of diabetes. The current study seeks to further elucidate the details of the mechanism of beta cell endoplasmic reticulum stress induced apoptosis in order to attempt to prevent hyposecretion of insulin.

2. Method

2.1

In order to investigate the mechanisms of insulin secretion in response to a variety of nutrients, including glucose, techniques of genetic engineering were applied. Analysis using MIN6 cells which incorporated genetic engineering technology was carried out. This analysis tested how the insulin secretion response changed upon introduction of different kinds of genes. First, research efforts were focused on the enzymes in the glycolytic pathway before the efficacy of over-expressing the glucose transporter GLUT1 which uptakes glucose was analysed. Subsequently the overexpression of hexokinase I, which carries out glucose

Source Text 2, Page 4

レス下のb細胞では，タンパク合成を抑制しておくことが長期的な生存にとっては有利であり，その役割を4E-BP1が担っているものと考えられた．

おわりに

b細胞障害が2型糖尿病発症において不可欠であることは疑いがなく，そこには，b細胞量の低下と機能不全の両者が存在するものと思われる．Wfs1遺伝子の変異による細胞内カルシウム動態の異常が，インスリン分泌を低下させるとともにアポトーシスを亢進させるように，ある1つの細胞機能の異常は，細胞の生存と高度に分化した機能であるインスリン分泌の両者に多かれ少なかれ影響を与えると考えられる．b細胞のインスリン分泌と生死のメカニズムの全貌を解明し，2型糖尿病におけるその障害を明らかにして，糖尿病の治療に役立てられるよう取り組んでいきたい．

謝辞

このたびのリリー賞受賞にあたり，糖尿病の臨床，教育ならびに基礎研究のすべてにわたって，これまでご指導いただきました東北大学大学院医学系研究科糖尿病代謝科岡芳知教授に深謝いたします．また，長い間，b細胞研究の全般にわたってご教示いただきました朝日生命新人病研究所菊池方利所長，分子生物学の初歩からご指導いただきましたMIN6細胞の樹立者である大阪大学医学部幹細胞制御分野宮崎純一教授，また，留学当時から今日まで貴重なご助言を下さったGeneve大学ClaesB.Wollheim教授に心から感謝いたします．さらに，深夜まで実験・研究をともに行って下さった多くの先生方に厚くお礼申し上げます．

Target Text 2, Page 4

phosphorylation, was tested[1]. Finally, taking into account the link between the glycolytic pathway and mitochondrial metabolism, the effect of forcing uncoupling protein 1[4] (UCP1) expression, which has a suppressive role in the production of glycerol-3-phosphate dehydrogenase[2], lactic dehydrogenase[3] and adenosine 5'-triphosphate (ATP), was analysed.

2.2

In order to examine the beta cell stress response and the regulation of translation, the molecular mechanism of endoplasmic reticulum stress induced apoptosis in the islets of Langerhans of *wfs1* disrupted mice was analysed. Upon increased expression of translation initiation factor eIF4E-binding protein1: 4e-bp1), a second generation cross was created by cross-breeding either the Akita mice or *wfs1* disrupted mice with the *4e-bp1* deleted mice, the F2 mice were then analysed in order to ascertain the significance of 4e-bp1 induction.

3. Results

3.1

Investigations into the mechanisms of insulin secretions using the forced expression of UCP1 discovered that the rate-determining step of the glycolytic pathway in pancreatic beta cells was determined as the glucose phosphorylation process. It also determined the role of the glucose sensor as regulating glycolytic flux. Furthermore, it was found that there is a highly functional association between the glycolytic pathway and mitochondrial metabolism. It was clarified that mitochondrial metabolism not only fulfils an important role for ATP, but it also plays a pivotal position in the formation of insulin secretion signalling. In the process of engineering a nutrient-stimulated insulin secretion mechanism, it became clear that in order to ensure that beta cells only responded to glucose, beta cells possess different characteristics to other cells types within the islets of Langerhans. Namely, it became apparent that there were no transporters on the cell membranes of beta cells for types of nutrients other than glucose in order to ensure that beta cells do not secrete insulin in response to nutrients other than glucose. In fact, dicarboxylic acid, an intermediary product of the TCA cycle, forced the expression of dicarboxylic acid transporters. Upon expression of the transporters, insulin secretion was observed in response to dicarboxylic acid[5]. Pyruvic acid-stimulated insulin

Target Text 2, Page 5

secretion was also observed in the islets of Langerhans due to the forced expression of a transporter for pyruvic acid[3]. This was an interesting result which has tied pyruvic acid to the mechanism of insulin secretion.

3.2

Upon analysing the molecular mechanism of endoplasmic reticulum stress induced apoptosis in the islets of Langerhans of *wfs1* disrupted mice, it was discovered that the expression of translation initiation factor eIF4E-binding protein 1: 4e-bp1 increased[9]. The increase in 4e-bp1 was not only observed in the islets of Langerhans of *wfs1* disrupted mice but was also observed in the islets of Langerhans of Akita mice. Pathogenesis of diabetes was generated in the Akita mice through endoplasmic reticulum stress caused by insulin secretion dysfunction.

Evidence was found that the increased expression of 4e-bp1, which occurs in the endoplasmic reticulum stress response, is caused by the transcription activation of *4e-bp1* by ATF4, the master transcription factor for the stress response. Moreover, (43) the binding site for ATF4 was discovered within intron 1 of *4e-bp1*.

After creating two second generation mice crosses by cross-breeding either the Akita mice or *wfs1* disrupted mice with the 4E-BP1 gene deleted mice in order to analyse the significance of 4e-bp1 induction, it was observed that the lack of 4e-bp1 caused advancing beta cell damage and worsened glucose intolerance in the F2 mice of both crosses. This is indicative that *4e-bp1* fulfils a role in the regulation of protein synthesis in beta cells under endoplasmic reticulum stress. This regulation of protein synthesis is significant as it is beneficial to the long-term survival of beta cells and therefore beneficial in the prevention of beta cell apoptosis.

Target Text 2, Page 6

4. Discussion

4.1

We sought to elucidate the details of the mechanism for recognising glucose concentration in beta cells. From our results it is clear that beta cells possess different characteristics to other cells within the islets of Langerhans, due to their lack of transporters in the cell membrane. The resulting insulin secretion from forced expression of membrane transporters offers further insight into mechanisms of inducing insulin secretion and suggests that cells which are normally unresponsive to certain nutrients could be activated by expressing the protein needed for the metabolism of that nutrient. This result is further compounded by the unexpected observation that the forced expression of a carrier for pyruvic acid caused the islets of Langerhans to secrete insulin in response to pyruvic acid. This is a result which has paradoxically tied pyruvic acid to an explanation of the mechanism of insulin secretion and its glucose specificity.

4.2

There is no longer any doubt that beta cell damage is essential to Type 2 diabetes pathogenesis. However, it is now thought that there are in fact two factors contributing to this: a decrease in beta cell mass as well as beta cell dysfunction. As discussed, dysfunction in calcium movement within the cell due to *wsf1* gene mutation causes a decrease in insulin secretion, in addition to an acceleration of apoptosis caused by a lack of 4e-bp1. From these results, it is now thought that just one type of cell dysfunction will have an effect to a greater or lesser extent on not only cell survival but also on the highly specialised function of insulin secretion. Our results thus provide a potential therapeutic target for treating type 2 diabetes in *4e-bp1*.

The aforementioned research has contributed to advancing the understanding of mechanism of insulin secretion, loss of beta cell mass and the pathogenesis of type 2

Target Text 2, Page 7

diabetes. Moving forward, I would like to endeavour to elucidate a comprehensive view of the mechanisms of insulin secretion and cell death in beta cells, to further the understanding of type 2 diabetes and then apply this knowledge in the treatment of type 2 diabetes.

Acknowledgments

On this occasion of being awarded the Lilly Prize, I would like to express my sincere gratitude to Professor Yoshitomo Oka of the Diabetes Metabolism department of Tohoku University School of Medicine for all his guidance across all of the basic research, clinical diabetes research and education undertaken. Additionally, I'd like to express my appreciation for the mentoring I received throughout all of my research into beta cells in general from the director of the Asahi Life institute for research into new and emerging diseases Mr. Masatoshi Kikuchi. I'd also like to express my sincere gratitude to Professor Junichi Miyazaki who specialises in the field of stem cell management at Osaka University's Faculty of Medicine and established the MIN6 cell, for his guidance during my initial stages of molecular biological research. Equally, I'd like to express my sincere thanks to Professor Claes B. Wolheim of Genevia University for all his valuable advice given since my time studying abroad up until today. Lastly, I would like to express my sincere gratitude to all of my numerous mentors who all went above and beyond, even going to the extent of helping me perform experiments and research late into the night.

Words: 2,043

Source Text 3, Page 1

リリー賞受賞講演

膵β細胞容積調節機構に関する研究

綿田裕孝

IPF-1

研究開始時の私共の疑問は，なぜ，インスリンは，ほぼ膵β細胞に限局して発現するのかということであった．この疑問の解決のための第一歩として，インスリン遺伝子の転写調節機構の解明に携わりたいと考えた．そのためにインスリン遺伝子エンハンサー領域に結合する転写因子 IPF-1 に焦点を当て実験することとした．その結果，IPF-1 が膵β細胞のブドウ糖センサーである膵β細胞型グルコキナーゼ遺伝子および IAPP 遺伝子プロモーターに結合し，それぞれの遺伝子の転写活性化を行うことを見出した 1)．ただし，当時の遺伝子発現調節メカニズムの検討は，主にレポーター遺伝子アッセイやゲルシフトアッセイなどを用いて行っており，IPF-1 が本当にゲノムに存在するそれぞれの遺伝子のプロモーターに結合し，遺伝子発現を活性化させるのかということに関しては，さらなるデータが必要と考えた．

そこで，膵 a 細胞株 aTC1 細胞に外来性に IPF-1 遺伝子を発現させた．すると，Betacellulin 存在下で極めて低レベルではあるものの，インスリン，グルコキナーゼ，IAPP という膵β細胞特異的遺伝子の発現が誘導されることを見出した 2)．この結果は，当初の

Target Text 3, Page 1

Winner of the Lilly Research Award: Hirotaka Watada

The Clinical Application of the Mechanism for Regulating Beta Cell Mass in the Treatment of Type 2 Diabetes

Abstract

Diabetes, a disease caused by a lack of insulin secretion, is among the top-ten causes of death globally. However, a viable method of increasing insulin secretion is yet to be established. In order to establish this method, the gene expression patterns of pancreatic β cells must be established by elucidating their developmental process, allowing this process to be imitated to induce pancreatic β cell differentiation and increased beta cell mass. We analysed AR42J-B13 cells and various mouse models in order to identify crucial transcription factors which control pancreatic β cell differentiation and influence cell mass. We found and classified the mechanism of insulin gene regulation and used it to transform non-pancreatic β cells into pancreatic β cells. We also established the roles of the vasculature of the pancreatic β cells and autophagy in increasing β cell mass. The results discussed in this paper provide a method of inducing pancreatic β cell differentiation which is likely to have clinical applications in the development of a therapy to treat diabetes and also provide possible therapeutic targets for increasing β cell mass which could offer a radical cure for type 2 diabetes.

1. Introduction

Diabetes is a disease defined by the hypo- or complete impairment of insulin secretion, which results in hyperglycaemia. This lack of insulin secretion causes various complications, making diabetes one of the top ten leading causes of death globally. This in turn has resulted in an influx of research interest into the establishment of a method for increasing insulin secretion. In order to establish a method of promoting insulin secretion, previous research has addressed whether insulin expression is confined to pancreatic β cells by investigating the regulatory mechanism of insulin gene transcription. Accordingly, the focus of investigation was on Insulin Promoter Factor-1 (IPF-1), a transcription factor which binds to the insulin gene promoter region. The term IPF-1 has since been replaced by the term Pancreatic and Duodenal Homeobox 1 (Pdx1). It was found that IPF-1/Pdx1 binds to the promoter regions of

Source Text 3, Page 2

研究目的のとおり，IPF-1 が各膵β細胞特異的遺伝子の発現を直接活性化するという強い証拠となったが，同時に，筆者らは，この実験結果を受け，内因性インスリン遺伝子が発現している細胞を類膵β細胞と仮に呼ぶとすれば，本実験の結果は，IPF1 遺伝子発現が非膵β細胞を類β細胞化したと解釈できるかもしれないと考えた．もし，そうだとすると，転写因子を用いて内因性遺伝子発現を変化させる分化誘導法は，将来的には，糖尿病患者に不足している膵β細胞を補充する新規治療法の開拓につながるのではないかと考えた．そのためには，細胞内での遺伝子発現パターンを膵β細胞にできるだけ近似させなければならないわけであり，その目的のためには，生理的な膵β細胞の発生過程を解明し，その過程を模倣することで膵β細胞分化誘導法を考案することが重要ではないかと考えた．なお，これらの研究結果を報告する前後で，IPF-1 の統一呼称名が Pdx1 となり，MODY の原因遺伝子であることも報告された．

膵β細胞発生分化過程と転写因子カスケード

そこで，筆者は，膵β細胞発生過程を調節している転写因子カスケードの解明に携わった．膵臓は，発生学的には一層の内胚葉上皮細胞に由来する．この一部の細胞が膵内分泌前駆細胞となり，膵内分泌前駆細胞から数々の分化ステップを経て，成熟した膵β細胞ができる．膵前駆細胞には Pdx1 が発現しており，内胚葉上皮細胞から，膵前駆細胞への分化に大きな役割を果たすと考えられている．膵前駆細胞から膵内分泌前駆細胞への分化に関わる転写因子が Neurogenin3(Ngn3)である．筆者らは，Ngn3 遺伝子の発現調節機構を解明し，HNF3β や HNF6 などの内胚葉に発現する転写因子，Notch シグナル，Activin や HGF シグナルなどが極めて複雑に Ngn3 の遺伝子発現に関与していることを見出した 3)．一方，膵β

Target Text 3, Page 2

glucokinase (a glucose sensor in the pancreatic β cells) or the promoter region of islet amyloid polypeptide (IAPP) genes stimulating their transcription. [1]. However, at the time of this study investigation into the mechanisms of regulating gene expression was mainly carried out using reporter gene and gel shift assays. However, in order to investigate whether IPF-1/Pdx1 actually does bind to the respective gene promoters present on the genome and cause this activation of the gene expression, further data was necessary. Therefore, the exogenous expression of IPF-1/Pdx1 was forced in a pancreatic **α** cell line, **α**TC1, and examined. It was discovered that once IPF-1/Pdx1 was expressed, albeit in the presence of extremely low levels of Betacellulin, this induced the expression of genes specific to pancreatic β cells: insulin, glucokinase and $IAPP$ [2]. This research, in addition to the discovery that IPF-1/Pdx1 is a causative gene of Maturity Onset Diabetes of the Young (MODY) provided strong evidence that IPF-1/Pdx1 directly activates the expression of each type of pancreatic β cell specific genes. For example, if endogenous cells which express the insulin gene can be induced into a type of pancreatic β cell, then in consideration of the results of the aforementioned research, it could be interpreted that the expression of IPF-1/Pdx1 can induce non-pancreatic β cells to become a type of pancreatic β cell. By building upon this research, it is conceivable that in the near future, this method of inducing differentiation using transcription factors to change the endogenous gene expression could be used in a trailblazing new method to treat diabetes sufferers with insufficient levels of β cells by replenishing the β cells. In order to achieve this, it is necessary to approximate the intracellular gene expression patterns of pancreatic β cells as closely as possible. However, in order to approximate pancreatic cell gene expression patterns, it is imperative to elucidate the physiological developmental process of pancreatic β cells. Then, this developmental process would need to be imitated in order to devise a method of inducing pancreatic β cell differentiation. This paper seeks to first establish the pancreatic β cell development and differentiation process, identify the associated transcription factor cascade and then establish the factors which increase the cell mass of existing pancreatic β cells. These discoveries could then be applied in a novel treatment for type 2 diabetes where there is a decline in β cell mass.

Source Text 3, Page 3

細胞分化因子，Pax4，Nkx2.2 遺伝子の発現調節機構を検討すると，Ngn3 が発現すると自動的にこれらの転写因子が発現するかのように，Ngn3 と HNF 転写因子群との協調作用により遺伝子発現が調節されていることが明らかになった 4)．一方，Nkx2.2 遺伝子の下流に存在する転写因子 Nkx6.1 の発現調節機構は，転写後発現調節機構も含めて，極めて複雑に調節されていることを見出した 5,6)．なお，Nkx6.1 の下流に MafA という強力なインスリン遺伝子転写活性化因子が存在することは，明らかにされていた．

そこで，発現調節機構が複雑で，かつ膵β細胞分化に重要な転写因子群として，Pdx1，Ngn3，Nkx6.1 を選別し，非膵β細胞から膵β細胞への分化誘導を試みた．膵前駆細胞のモデル細胞株である AR42J-B13 細胞は Pdx1 をもともと発現している．この細胞に Ngn3 を強制発現させると，Nkx2.2 や Pax4 の発現が認められた．そこに，Nkx6.1 を発現させてもインスリンの発現は認められなかったが，代わりに MafA を強制発現させるとインスリンの発現が著明に認められた 7)．ちょうどこの論文を報告したとき，Melton らのグループは膵外分泌細胞に Pdx1，Ngn3，MafA を強制発現することで，膵β細胞への分化誘導に成功したことを Nature 誌に報告した．これらの結果から，膵β細胞の発生分化機構を解明し，それらの知識を集積させると，将来的な新規膵β細胞分化誘導法の確立に役立つ可能性が強く示唆された．

既存の膵β細胞の容積を増加させるために

–膵β細胞容積に影響を与える因子の解明–(Fig.)

以上のような膵β細胞分化誘導法は，膵β細胞容積が低下している糖尿病の将来の治療として有用である．膵β細胞容積増加のためのその他の戦略としては，膵β細胞容積に影響を与える因子を解明し，その因子が 2 型糖尿病状態下で作用低下しているのであれば，

Target Text 3, Page 3

2. Methods

Initial research focused on elucidating the transcription factor cascade which regulates that pancreatic β cell developmental process. Upon classifying the crucial transcription factors in pancreatic β cell differentiation Pdx1, Neurogenin-3 (Ngn3) and NK6 homeobox 1 (Nkx6.1), differentiation of non-pancreatic β cells into pancreatic β cells was then attempted using the mechanism for regulating gene expression in AR42J-B13 cells, the cell line model for pancreatic precursor cells.

Next, research into the factors which influence pancreatic β cell mass was carried out in order to increase pancreatic β cell mass. This involved research into the vasculature of the pancreatic islets of Langerhans. A model of vascular insufficient islets of Langerhans was used in order to investigate pancreatic β cell function. This model used pancreatic β cell specific vascular endothelial growth factor (VEGF)-A knockout mice.

Next, the mechanism of autophagy was investigated. The state of autophagy within pancreatic β cells of diabetic mouse models, including db/db mice, was analysed. Then, in order to investigate the significance of autophagy in pancreatic β cells, pancreatic β cell specific autophagy-specific gene 7 (*atg7*) knockout mice were created. These mice were created by knocking out the pancreatic β cell specific form of *atg7* which is essential in the mechanism of autophagy.

3. Results

The mechanism for regulating the expression of the Ngn3 gene was elucidated. It was discovered that transcription factors such as HNF3β and HNF6, which are expressed in the endoderm, as well numerous signalling pathways such as Notch signalling, Activin signalling and Hepatocyte growth factor (HGF) signalling have an involvement in the gene expression of Ngn3[3] which is extremely complex. Upon examination of the mechanism for regulating the gene expression of the pancreatic β cell differentiation factors Paired box gene 4 (Pax4) and NK2 Homeobox 2 (Nkx2.2), it was found that Pax4 and Nkx2.2 were automatically expressed when Ngn3 was expressed. Thus it was shown that the Ngn3 and Hepatocyte nuclear factors (HNF) groups of transcription factors regulate gene expression through synergistic action[4].

Source Text 3, Page 4

それを補うようにすれば，2 型糖尿病の新規治療法の確立が可能と考えられる．そこで，まず着目したのが，膵ランゲルハンス島の血管構築である．健常者では膵 β 細胞容積増加時に膵ラ氏島の血管密度が増加し，逆に 2 型糖尿病では，膵ラ氏島の血管密度が減少することとが知られている．すなわち，膵ラ氏島血管と膵 β 細胞機能とには明確な相関があるが，これが原因か，ただの相関であるのかは明らかでなかった．そこで，膵ラ氏島の血管不全モデルとして，膵 β 細胞特異的血管内皮細胞増殖因子(VEGF)-A ノックアウトマウスを用いて，膵 β 細胞機能を検討した．その結果，膵 β 細胞の血管構築は正常な膵 β 細胞機能に必須であるが，定常状態の膵 β 細胞容積には無関係であること，骨髄移植の膵 β 細胞容積増加機能には必須であるが，インスリン抵抗性による膵 β 細胞容積増加機構には正常の血管構築は必須ではないことが明らかになった 8,9)．

次に，着目したのはオートファジー機構である．オートファジーは不要な蛋白を除去する細胞内浄化という点で重要である．膵 β 細胞におけるオートファジーの状態を検討すると，インスリン抵抗性がオートファジーを誘導するものの，dβ/dβ マウスなどの糖尿病モデルマウスの膵 β 細胞ではオートファジー不全を示唆する結果が得られた．

次に，膵 β 細胞におけるオートファジーの意義を検討する目的でオートファジー機構に必須な ATG7(autophagy-specificgene7)を膵 β 細胞特異的にノックアウトした膵 β 細胞特異的 ATG7 ノックアウトマウスを作成した．その結果，膵ラ氏島における恒常的オー'トファジー不全は，ミトコンドリアでのアデノシン 5-三リン酸(ATP)産生能の低下を介して，ブドウ糖応答性インスリン分泌低下をもたらすことが示唆された．また，インスリン抵抗性による誘導性オートファジーは，膵 β 細胞増殖促進とアポトーシスの抑制を介して，膵 β 細胞容積を増加させるのに必須な機構であることが明らかになった 10)．以上をあわせると，2 型糖尿病モデルマウスで認められる膵 β 細胞におけるオートファジー不全は，ブドウ糖応答性インスリン分泌低下の原因となり，また，インスリン抵抗性による膵 β 細胞容積増加不

Target Text 3, Page 4

It was also discovered that the mechanism for gene regulation, including the mechanism of post transcriptional regulation of the transcription factor Nkx6.1 which lies downstream from transcription factor Nkx2.2, is extremely intricately regulated[5,6]. Furthermore, it was proven that the potent insulin gene transcription activator MafA lies downstream of Nkx6.1.

After classifying the crucial transcription factors Pdx1, Ngn3 and Nkx6.1 in terms of pancreatic β cell differentiation, differentiation of non-pancreatic β cells into pancreatic β cells was attempted in the cell line model for pancreatic precursor cells AR42J-B13. It was found that the AR42J-B13 cells expressed Pdx1 from the offset. The expression of Nkx2.2 and Pax4 was observed when the AR42J-B13 cells were forced to express Ngn3. Insulin expression was not observed when there was forced expression of Nkx6.1. Instead, insulin expression was clearly observed when there was forced expression of MafA[7].

Through an investigation using (VEGF)-A knockout mice it was shown that although the pancreatic β cells vasculature is crucial in normal pancreatic β cell function, it is unrelated to pancreatic β cell mass in a steady state. Moreover, it was found that although normal vasculature is essential for an increase in the function of pancreatic β cells derived from a bone marrow transplant, normal vasculature is not required for the mechanism of increasing pancreatic β cell mass when due to insulin resistance[8,9].

In analysing the state of autophagy within pancreatic β cells, results were obtained that suggested that there was an autophagy deficiency in the pancreatic β cells of diabetic mouse models including db/db mice. In order to investigate the significance of autophagy in pancreatic β cells, pancreatic β cell specific *atg7* knockout mice were analysed. This analysis suggested that a deficiency of constitutive autophagy causes a reduction in glucose stimulate insulin secretion through a reduction in mitochondrial adenosine-5-triphosphate (ATP) productivity. Moreover, it was also found that induction of autophagy triggered by insulin resistance is a necessary mechanism for increasing pancreatic β cell mass through the promotion of the proliferation of pancreatic β cells and the suppression of apoptosis[10]. It was also proven that an autophagy deficiency could also cause the insulin resistance-induced impairment of the process of generating pancreatic β cell mass.

Source Text 3, Page 5

全の原因となりうることが明らかになった．今後，この膵β細胞オートファジー不全を改善させることが出来れば，2型糖尿病の根本治療に役立つかもしれない．

さいごに

今後も，リリー賞受賞を励みに，糖尿病における膵β細胞の病態解明，糖尿病治療につながる研究を行ってゆきたい．

謝辞

今回の受賞は，関係の諸先生方，同僚の皆さま方のご指導，ご鞭撻なしにはなし得なかったことで，この場を借りて深謝いたします．特に終始お世話になった恩師の河盛隆造先生に厚く御礼申し上げたいと存じます．

さらに，大阪大学第一内科で直接ご指導頂いた梶本佳孝先生，留学中，UCSF にてご指導頂いた MichaelS.German 先生をはじめとする諸先生方，さらにこれまで共同研究などをしてくださった大変多くの先生方にも厚く御礼申し上げたいと思います．

Target Text 3, Page 5

4. Discussion

In order to establish a method of promoting insulin secretion, it is necessary to understand the pancreatic β cell development and differentiation process and identify the associated transcription factor cascade. The pancreas is derived from one embryological germ layer, namely the epithelial cells of the endoderm. This section of cells become pancreatic endocrine precursor cells. These pancreatic endocrine precursor cells then go through numerous steps of differentiation involving various transcription factors to become a mature pancreatic β cell. It has long been thought that the expression of Pdx1 fulfils a considerable role in the development of pancreatic precursor cells through the differentiation of the pancreatic endoderm epithelial cells into pancreatic precursor cells. Our research elucidated both the mechanisms regulating the expression of the Ngn3 gene and its relationship with Pax4 and Nkx2.2. It also proved that the potent insulin gene transcription activator MafA lies downstream of Nkx6.1. This classification of the crucial transcription factors in the pancreatic β cell process meant that differentiation of non-pancreatic β cells into pancreatic β cells could be attempted. The results of this attempt strongly suggest that the elucidation of the mechanism for the development and differentiation of pancreatic β cells and the gathering of extensive information on this mechanism could be practically applied in the establishment of a new future technique to induce differentiation into pancreatic β cells.

Moreover, at precisely the same time as the publication of this paper, Melton et al. published a paper in *Nature* that they also succeeded in inducing differentiation into pancreatic β cells by forcing the expression of Pdx1, Ngn3 and MafA but in exocrine cells. Therefore methods of inducing pancreatic β cell differentiation, such as those attempted in this paper, are likely to be valuable in the development of a future therapy to treat cases of diabetes where pancreatic β cell mass is in decline.

Another strategy for increasing pancreatic β cell mass involves elucidating the factors which influence pancreatic β cell mass. The first area that attracted research attention was the vasculature of the pancreatic islets of Langerhans. It is already established that when pancreatic β cell mass increases in healthy individuals then the vascular density of the islets of Langerhans also increases. However under the same conditions, the vascular density of

Target Text 3, Page 6

the islets of Langerhans decreases in individuals with type 2 diabetes. In other words, although there is an evident correlation between the islets of Langerhans vasculature and pancreatic β cell function, it is not clear whether this pertains to a causative relationship or whether it is just mere correlation. In this paper it was found that although normal vasculature is essential for an increase in the function of pancreatic β cells from a bone marrow transplant it is not required for the mechanism of increasing pancreatic β cell mass when due to insulin resistance.

Next therefore the focus of research was the mechanism of autophagy. Autophagy has an important function in the process of intracellular cleaning by removing unnecessary proteins. Although, insulin resistance induces autophagy, in analysing the state of autophagy within pancreatic β cells, results were obtained that showed a deficiency of constitutive autophagy causes a reduction in glucose stimulated insulin secretion through a reduction in mitochondrial adenosine-5-triphosphate (ATP) productivity. It was therefore suggested that induction of autophagy triggered by insulin resistance is a necessary mechanism for increasing pancreatic β cell mass through the promotion of the proliferation of pancreatic β cells and the suppression of apoptosis. This means that the autophagy deficiency observed in the pancreatic β cells of type 2 diabetic mouse models causes a reduction in glucose stimulated insulin secretion and a reduction in the generation of beta cell mass.

It is thought that it would be possible to establish a new therapeutic technique for type 2 diabetes through supplementing the factors which influence pancreatic β cell mass. Therefore our research suggests that if we are able to improve pancreatic β cell autophagy deficiencies then this could offer a radical new cure for type 2 diabetes in the future.

In consideration of the encouragement offered to me by the award of the Lilly prize and the promising research outlined above, I will continue to engage in research into finding a cure for diabetes and into the elucidation of the pathology of pancreatic β cells in individuals with diabetes.

Target Text 3, Page 7

Acknowledgments

On this occasion of being awarded the Lilly Prize, I would like to take the opportunity to express my sincere thanks to all the professors involved in my research and to all of my colleagues. I could not have accomplished this without all your guidance and encouragement. I would also particularly like to express my deep gratitude to Dr Ryuzo Kawamori who oversaw my research from conception to completion.

Next, I'd like to express deep gratitude to all of the various mentors who guided me throughout my research, including Dr Yoshitaka Kajimoto who directly supervised me in Osaka University's Internal Medicine department and Dr Michael S. German who supervised me during my research abroad at UCSF. Finally, I'd like to thank the numerous colleagues who assisted me in collaborative research.

Words: 2,296

Total translation word count: 6,586

Critical Analysis

1. Introduction

Research articles (RAs) written in English are the most likely to be cited in other scientific articles (Englander 2014: 9), as a result 'English now acts as the international language of science' (Montgomery 2009: 7). However, the second largest contributor of funds to scientific research globally is Japan (Englander 2014: 11). The goal of scientific research is to disseminate findings to the wider global scientific community and a *lingua franca* of science, such as English, makes the concept of global dissemination not only more efficient but entirely more plausible (Montgomery 2009: 11). Therefore, the role of Japanese to English translation of scientific research is increasingly important in order to give Japan a voice as the wider scientific community continues to internationalise.

This dissertation focuses on the translation of three Japanese RAs into English. Through subsequent analysis the texts are adapted according to conventions of Anglophone scientific RAs and the theory supporting the applied translation strategies is explained.

1.1 The Source Material

The source material is three RAs which were originally published in the Japanese journal *Tounyoubyou [Diabetes]* and were written by three different Japanese researchers who were awarded prizes for their findings in the field of diabetes research. Text 1 is 3,300 characters, Text 2 is 2,767 characters and Text 3 is 3,141 characters, all excluding references and diagrams which have not been commissioned for localisation. The function of the source material, as with any RA, was to disseminate the results of the research as widely as possible, but it also particularly emphasises the achievements of the researchers of the respective articles. A significant feature of the source material is that the layout does not conform to the Introduction, Methods, Results and Discussion structure but rather gives a chronological, narrative account of the research, focusing on the actions of the researchers and their achievements.

1.2 The Translation Brief

The client

The end client is the Japan Diabetes Society (JDS). The client's aim is to promote research into the study of diabetes in order to find a cure. The client has invested in the development of academic research programs and due to the anticipation that the incidence of diabetes is set to increase in Asia, JDS is hoping to further its global leadership role.

Distribution

In accordance with the manuscript requirements of *Diabetes*, the translated manuscript will be submitted as a word document (Diabetes 2013). If approved for submission, the articles will be published as a collection of essays which highlight the contributions of Japanese researchers to the field. The deadline is 15th September in order to enter into the lengthy review process and publication is anticipated in 2017. The journal will be available internationally both in print and online.

Target audience

There are two target audiences to consider. The first is the editors at *Diabetes* and the second is the international scientific community. In order to meet the requirements of the editors, the manuscript will be translated in accordance with the format requirements of *Diabetes*, namely by conforming to the Introduction, Methods, Results, Discussion (IMRD) structure, with the addition of Abstracts. In order to meet the expectations of the international scientific community, the RAs will be translated in accordance with Anglophone disciplinary-specific norms. These norms include considerations such as tenor, hedging strategies, layout and argumentation structure.

Text function

The function of these articles, as with any scientific RA, is to disseminate the research findings as widely as possible and for the author to receive recognition for their contributions to the field.

There are three text functions the articles must fulfil. The first text function is to be referential-informative due to the necessity of clearly outlining the research contributions, relevant theory and pertinent past research. The second function is to be expressive-evaluative; this function is required as the RAs need to critically discuss how the research has contributed something novel to the field. The final text function is to be persuasive-informative. The RAs need to be persuasive-informative as, firstly, they need to convince the editors that the research is worthy of publication and secondly, convince the scientific community that the research is contributing something significant to the field.

To summarise, in order to fulfil these three textual functions, the one key element that must be communicated is that the RAs have addressed a gap within the field and that the knowledge obtained from the research findings is significant.

1.4 Background to the Critical Analysis

Skopos theory stipulates that the purpose or 'skopos' (Vermeer 1989/2000: 230) of the target text (TT) is the factor that determines the translation strategy (Nord 2006: 30). Namely, the translation brief determines whether a text needs to be 'translated' or 'paraphrased' (Vermeer 1989/2000: 203-231). It is the translator's responsibility to reduce 'communicative suffering' resulting from misunderstanding (Nord 2006: 36). In order to reduce communicative suffering, the text must conform to the generic conventions of the target culture (TC) as non-conformance draws the reader's attention away from the ideational content and impedes effective information processing (Nord 2006: 39). Consequently, by conforming to generic conventions, the text appears to be professionally written and this increases the likelihood of the success of the TT. Thus, the aim of this translation is to produce a text that is well-written and conforms to the generic conventions of the Anglophone scientific community.

There are three points of agreement concerning scientific RAs within the Anglophone scientific community. Firstly, the RA is the most important manner of disseminating scientific knowledge, secondly, the RA is written for scientists in the field and finally, the RA represents the community's notion of valid argumentation (Englander 2014: 23). This means that publishing research in the form of an RA and using valid argumentation schemata will result

in the broadest potential dissemination of the RA. Thus, key considerations whilst writing the RA is Anglophone disciplinary-specific conventions and negotiating the reader-writer relationship in terms of the writer's role within the scientific community.

2. Critical Analysis: Chapter 1

Non-Anglophone scientific publishing often has its own writing conventions (Englander 2014: 57) which can lead to differences in argumentation structure and metatext (Uysal 2014: 180). In translation, differences in conventions must be understood so that a text isn't misinterpreted (Englander 2014: 57). When localising an RA, it is imperative to consider whether the source text's (ST) conventions, namely the textual organisation and argumentation structure, will impede or facilitate the comprehension of the reader within the target culture (TC) (Fukuoka and Spyridakis 2002: 99). Thus, when localising these documents, I took into consideration the conventional differences between the ST and TT in terms of the macrostructure and organisational schemata of the RA.

Readers use their knowledge of conventional organisational schemata in order to comprehend a text (Fukuoka and Spyridakis 2002: 99). Accordingly, communicative suffering will occur when a reader encounters an unfamiliar organisation for a given genre (Fukuoka and Spyridakis 2002: 100). A sense of organisational schemata is developed through texts in the same genre (Fukuoka and Spyridakis 2002: 100) and the scientific genre has a highly conventionalised rhetoric style to which all scientists must conform (Englander 2014: 90). These genre-specific conventions constrain and conventionalise the RA (Swales 1990: 125). This conventionalised organisation of the RA has resulted in the Introduction-Methods-Results-Discussion (IMRD) macrostructure (Bennett 2007: 161; Englander 2014: 39; Kawase 2015: 116; Swales 1990: 133; Teufel 1998: 44). Moreover, the structure within these individual sections is also organised and hierarchal (Bennett 2007: 161). This manner of standardisation is important within the sciences as it facilitates community shared knowledge (Bazerman 1988: 303). In terms of the translations undertaken, this expectation of the scientific community is compounded by the requirements of the journal (Okamura 2006: 72), which stipulate an IMRD structure (Diabetes 2013). This adaptation to the conventional IMRD

structure will help ensure the text's suitability for international rather than national dissemination (Englander 2014: 63).

2.1 Introduction

In order to adapt the ST to the IMRD structure, I applied Swales' IMRD structural model and Create a Research Space (CARS) model. Firstly, this involved revising the Introduction. The Introduction fulfils a crucial role in the RA as it is the section most read by reviewers and it establishes three key points: that the area of study is important, that the researcher is familiar with previous work and that there is still something unknown in the field (Englander 2014: 41). Based on this established textual function, Swales' established the CARS model which is composed of three 'moves'. Move one (M1) is the establishment of a territory; this involves 'claiming centrality and/or making topic generalisations and/or reviewing previous research' (Swales 1990: 140-141). I translated Text 1 (T1) to establish M1 by examining previous research. Introductions involve necessary choices in regard to the amount of background information (Swales 1990: 137) and in T1 there was information present about the author's research career. Thus, sentences Source Text 1 Page 1 Lines 5-13 (ST1P1L5-13) were omitted as it would have distracted focus from the object of research. M1 in Target Text 1 Page 1 Lines 22- Target Text 1 Page 2 Line 12 (T1P1L22-T1P2L12) contextualises the research by presenting what has been discovered in the field thus far.

There can be more than one move cycle within a RA and these can be cycled as different gaps and research interests are addressed (Swales 1990: 162). In Text 2 (T2) there are two CARS cycles, one for each area of research. In order to make these distinct research niches clear in T2, I divided the introduction into two sections (1.1 and 1.2) and went through each of the three moves within each section. T2, like T1, contextualises the research with previous research (T2P2L5-19) but adopts a firmer stance to the direction of research to justify the direction of their own research efforts, established through the use of the adjective 'prevailing' (T2P2L16-19). In T2, the author's opinion (ST2P1L6-8) is repurposed into a centrality claim, which is of a more appropriate tenor, concerning research interest (T2P2L5-7) which maintains focus on the research area's importance. M1 is cycled again in T2 in order to contextualise the second research objective; this involved restructuring the RA so that it addresses previous research in the Introduction (T2P3L2-10), rather than later on in the article (ST2P3L2-11). This ensured that all research objectives were clear from the beginning. It also

takes a stance in agreement with previous research (T2P3L8-10), setting the text up to build upon the 'evident' research as correct. Text 3 (T3) was unique in that the ST starts with their research question and due to the narrative and chronological style of the paper does not seem to have a M1. In order to create a M1, I firstly contextualised the research by inserting a paragraph making centrality claims about research interest in diabetes and the morbidity rates of diabetes (T3P1L20-23). I then re-contextualised the initial research about IPF-1 (ST3P1L9-ST3P2L11) to be the M1 discussion of previous research, as this is what supports the research later on and thus serves the same purpose (T3P1L24-T3P2L10). These re-workings of T1, T2 and T3 served to establish a territory and therefore a direction for the research.

Move two (M2) of the CARS model is the establishment of a niche. This involves 'counter-claiming, indicating a gap, raising a question or continuing a tradition' (Swales 1990: 140-141) and is therefore where the central argument for the paper is developed. It is often found that 'Japanese scientists tend to avoid an overt M2 where they would state an obvious gap in the literature' (Englander 2014: 61) and this trend was also found in the three STs. The establishment of a M2 either involved making a gap more emphatically stated or stating a gap in research that was only contextually implied. Most M2s are signalled by adversatives or negation in order to make the gap explicit (Swales 1990: 154-155) and adversatives were used for this purpose in the three TTs. Examples of M2s in the TTs included the insertion of a completely new sentence highlighting the gap (T1P2L22-24;T2P2L20-21), the rearrangement of sentences (T1P2L26-29), the use of emphatics to make the gap more explicit such as the adversative 'however' (T3P2L21-23) (ST3P2L6-9) or the addition of adverbs such as 'sufficiently' in order to highlight an inadequacy and therefore a gap (T1P3L2) (ST1P4L15-17). Finally, the deliberate lexical selection of reporting verbs are a powerful rhetorical tool in establishing a research space as they can indicate whether a claim is substantiated (Swales 1990: 151). I employed a modal reporting verb 'it could be interpreted that' (T3P2L15) in order to indicate that further research is needed as the claim is not proven and thus indicates a gap. All these moves serve to justify the need for the next move, Move 3 (M3).

Move three (M3) is occupying the niche, this involves 'outlining the research purpose, announcing present research or research findings' (Swales 1990: 140-141). M3 serves to substantiate the gap identified in M2 and identify the main purpose of the research (Swales

1990: 159). However, it is often found that Japanese writers tend to delay the introduction of the text's purpose (Gosden 1995: 50). In the three TTs, this involved moving information mentioned later regarding the research purpose into the Introduction (T1P3L4-6) (ST1P4L19) or inserting new sentences so that the RA's purpose was clear (T1P2L19-21; T1P3L4-11; T2P2L21-24; T2P3L15-17; T3P2L24-28).

The main rhetorical function of the Introduction is to justify the research by placing it within the context of previous research and arguing the novelty (Kawase 2015: 116). The CARS model helps to achieve this by contextualising the basis of the research in M1, introducing something unknown and novel in M2 and substantiating that in M3. The use of the CARS model is also conducive to the established structure of the 'IMRD hourglass' (Englander 2014: 40) in which the Introduction includes general statements about the area of research, establishes what isn't known, narrows the focus of the research and then returns to broader statements about the potential implications of the research. The use of the CARS model in reformulating the Introduction helps the three TTs address the expressive-evaluative textual function, as the CARS structure explicitly evaluates previous research to find a gap in M2 and evaluates the potential contribution to the field of the research to be undertaken in M3. In addition, it also contributes to the 'persuasive-informative' requirement of the translation brief. The conversion from a chronologically ordered research document to one that outlines why the research is significant and necessary not only assists in satisfying the requirement of the journal editors that the research should represent 'a significant advance in diabetes research' (Diabetes 2013), but also clarifies the objectives and role of the text within the field for the second TA, the scientific community, meaning that the research is more likely to be understood and accepted.

2.2 Methods

As the STs closely fit a narrative discourse style by explaining findings in a chronological order (Hinds 1976: 45), there was no discrete Methods section. Thus, it was necessary to create Methods sections for the TTs in order to conform to an IMRD structure. Life Sciences' Methods are 'swift, presumptive of background information, not designed for easy replication and with little statement of rationale or discussion of choices made' (Swales 1990: 170). Consequently, the purpose of the TTs' Methods was not reproducibility but procedural (Swales 1990: 121), mainly functioning to show that the researcher is familiar with credible

practices in the field (Englander 2014: 45). This meant when constructing a Methods section, there was little requirement for explanation as procedures are standardised (Englander 2014: 45) or elaboration as conventionally Methods 'read like checklists' (Swales 1990: 168). A common tool for narrative continuity within Anglophone Methods is the marked theme of the 'to+ verb' construct (Martínez 2003: 113). I employed this construct to assist in establishing both narrative continuity and coherence in the Methods (T1P3L15; T1P3L20; T1P4L16; T2P3L21; T2P4L6; T2P4L11; T3P3L10; T3P3L12). Another key feature utilised to adhere to Anglophone generic conventions of the Methods was the use of external temporal textual themes (Martínez 2003: 111), namely being written chronologically. This was underlined by temporal markers such as 'then' (T1P3L16; T1P3L24; T1P3L25; T1P4L5; T1P4L11; T2P4L11; T3P3L5' T3P3L16), 'next' (T1P3L17; T3P3L9; T3P3L15), 'following' (T1P3L19; T1P3L26; T1P4L13), 'first' (T1P3L2; T2P3L25) and 'finally' (T1P4L16; T2P4L1). The last consideration in the creation of the Methods was the length. Although limited by the information available in the STs, I aimed to make the Methods brief in accordance with the trend of Methods being reduced, sometimes to only a paragraph (Englander 2014: 47). However, I would also highlight to the commissioner that more information concerning the methodology may be required by the journal editors. The production of a Methods section again serves to fulfil the 'persuasive-informative' function of the translation brief by convincing the reader that the researcher is credible as they conform to generic conventions, thereby assisting in persuading the reader to accept the research findings.

2.3 Results

The Results section functions to highlight interesting and important findings, guiding the reader as to how the results should be interpreted (Englander 2014: 48-50). The main changes made from ST to TT were insertion of phrases to guide the reader (discussed further in Chapter 2) and strategic lexical selection to describe the results. For example, this included the insertion of the phrase 'these results indicate that' (T1P5L25), 'is significant as' (T2P5L21) and the adjectives 'remarkable' (T1P5L13) and 'particularly important' (T1P5L4). These additions help to fulfil the persuasive-informative function as they guide the reader toward the researcher's desired interpretation of the results and their significance.

2.4 Discussion

Merely reporting results without employing language strategies is insufficient to fulfil the desired persuasive-evaluative function (Sionis 1995: 105). Accordingly, dialogic intervention is increased in the Discussion as results are explained (Martínez 2003: 107). Although there is more variety in both the Results and Discussion (Swales 1990: 170) some general trends have been noted. Typically, in the Discussion, specific results are summarised, the results are broadly discussed (Englander 2014: 51) connected to the wider field (Englander 2014: 40) and their contribution to the field is explained (Englander 2014: 50). In accordance with the consensus of research objectives within the sciences (Swales 1990: 175), Swales devised an 8 move scheme that outlines the most frequently observed moves within an Anglophone Discussion (Swales 1990: 172-173). I applied this structure in the translation of the Discussions in order to conform to expected structural schemata and thus facilitate comprehension. The first optional move is the restatement of background information (Swales 1990: 172-173). This mainly involved reminding the reader of the research objectives, particularly if there were multiple objectives, such as in T2 (T2P6L3-4; T2P6L16-17) and T3 (T3P5L2-10; T3P6L9-10). The second move is a statement of results, which is restated for later contextualisation and discussion (T1P6L25-T1P7L2; T2L6L4-5; T2L6L20-23; T3P5L10-13; T3P6L4-7; T3P6L11-14). Next are four optional moves to help the reader understand the significance of the specific results. Move three is a statement of an (un)expected outcome, move four is reference to previous research for comparison or support in order to strengthen the later claims, move five is an explanation of the results to help ensure that the referential-informative textual function is achieved, move five can then be built upon by move six, exemplification (Swales 1990: 172-173). T1 uses move three (T1P7L3-4) to validate the results further by saying that the outcome is in line with what was expected, move four (T1P7L4-6) in order to further support their result with previous research and move five (T1P7L6-7) to explain the link between what was hypothesised and what was found. T2 uses a move three in order to highlight the novelty of the outcome, in that it was unexpected (T2P6L9-11) and a move five for each research objective in order to further explain the significance of their results (T2P6L6-9; T2P6L20-23). In T3, move threes used unexpected outcomes in order to highlight the author's contribution to changing the knowledge in the field, both highlighted by the adversative 'although' (T3P6L4-7; T3P6L11-14). T3 also employs a move four, discussing

supporting research in order to further validate their results through corroboration (T3P5L20-22). T3 had two move fives which were employed to highlight the significance of their findings (T3P5L13-15; T3P6L14-19).

Move seven is arguably the most significant move within a Discussion. It moves the discussion from the specific results to the general contribution to the field. It is also the move in which general claims can be made. However, this was a move that was often either not stated or not explicitly stated in the STs. This may be due to a difference in traditional Japanese argumentation schematas, specifically the ki-shou-ten-ketsu [起承転結] (Hinds 1983: 80). 'Ki' marks the beginning of the argument, 'shou' is argument development and 'ten' is the introduction of a sub-theme. One of the distinct differences between this schemata and Anglophone schemata lies in 'ketsu'. 'Ketsu' although translated as 'conclusion' differs to the Anglophone concept in that it 'need not be decisive. All it needs to do is to indicate a doubt or ask a question' (Hinds 1983: 80) and thus 'ketsu' can leave the Anglophone reader unsure of what to take away from the RA. This meant that when translating the TTs it was necessary to insert sentences which linked the results to wider implications (T1P7L6-7; T1P7L13-14; T2P6L12-13'T2P6L23-T2P7L1) or made the contribution to the field more explicit with the addition of phrases such as 'now' or 'therefore our research suggests' (T1P7L9-14;T3P6L22-23) (ST1P6L2-4; ST3P4L23-ST3P5L1). Move seven is important for outlining how the gap identified in the Introduction has been successfully filled and thus argues for the importance of the research. Therefore, the explicit use of move seven helps to fulfil the persuasive-informative textual function.

There is a final optional move, move eight, which is a space where the researcher can make recommendations about future research thus strengthening the claim (T2P7L1-3). It can also offer a way to broaden the scope of the potential contribution of the research discussed within the paper (T3P6L22-29). Alternatively, it can discuss how the research could fit in with other research and progress forward, such as in T1, where there is a second move 4 (T1P7L15-19) which serves as the basis for move eight (T1P7L19-21).

Reformulating the Discussion to meet the conventional structure, with a particular emphasis on move seven, assists the Anglophone reader to understand what has been achieved, thus fulfilling the referential-informative function and illuminating how the findings fit in with the

field, consequently satisfying the expressive-evaluative function. It also offers a space to generalise the research findings and speculate on their potential contribution, thereby helping to fulfil the persuasive-informative function that the research is novel and significant.

2.5 Abstract

An abstract is an important aspect of the RA as it functions to sell the RA to the reader and to provide the reader with sufficient information about the research to make an informed decision about reading the RA (Englander 2014: 53). Due to the important role of the abstract and its requirement for submission to the journal (Diabetes 2013), I created abstracts for all of the articles, despite abstracts not being included in the STs. In consideration of the abstract's function to inform about the contents of the RA, I opted to create an informative abstract which reports on the entire paper in an abbreviated format, reflective of the IMRD structure (Lorés 2004: 282; Englander 2014: 53). Given that the abstract is also in the IMRD structure, it was imperative to write these after restructuring of the RAs. Abstracts are typically 200-250 words (Andrade 2011: 172) and have a clear rhetorical structure, which includes an Introduction, Methods, Results and Discussion (Lorés 2004: 281). The 'Introduction' functions to contextualise and indicate the gap in the research (Andrade 2011: 173) (T1P1L5-7; T2P1L5-9; T3P1L5-9) and the 'Methods' is typically the shortest (Andrade 2011: 173) (T1P1L7-10; T2P1L9-12; T3P1L9-11). In contrast, the 'Results' is typically the longest (Andrade 2011: 174) as this section has the most important message to convey: the research findings. Accordingly, the 'Results' constitute the largest section of each of the abstracts (T1P1L10-17; T2P1L12-19; T3P11-14) and is supplemented by the 'Discussion' which contextualises the results in terms of how it contributes to the field (T1P1L17-19; T2P1L19-21; T3P1L14-17). The addition of an abstract not only acts to fulfil the requirements for manuscript submission, but also assists to fulfil the persuasive-informative textual function, as it functions to persuade the reader that the information contained within the paper is significant, without having to commit to reading the entire paper. The abstract also assists in fulfilling the referential-informative function, as it provides the reader with a summary of the contents of the RA so that they can make an informed decision as to whether to read it.

3. Critical Analysis: Chapter 2

The next area for analysis is the complex negotiation of making claims. When considering publishing, the researcher must make a decision about the level of claim to be made: too high a claim may contradict a lot of existing literature but too low a claim will offer no contribution to the field (Swales 1990: 117). The aim for a researcher is to have the greatest level of certainty and the broadest generalisation admissible (Englander 2014: 29).

The use of metadiscourse is an important tool in making claims. Metadiscourse is one of the ways that writers negotiate their relationship with the information they are presenting and with the scientific community. Metadiscourse helps to organise the text and demonstrate the author's stance to the information presented (Hyland 2010: 126). Therefore, as an RA not only represents an offer of information, but also the academic's reputation within the scientific community, metadiscourse is an important tool in negotiating these considerations: both presenting a credible representation of research findings and negotiating the social relations within the disciplinary community (Hyland 2010: 127). However, metadiscourse is linked to the norms of cultures and professional groups, meaning that as writing is 'culturally situated' (Hyland 1998: 438), these writing norms can vary cross-culturally. For metadiscourse to be effective, there must be appropriate observation of TA generic conventions. Academic writing is a persuasive endeavour (Hyland and Jiang 2015: 529) and when submitting an RA to an academic journal, there are two main reasons for rejection of the RA. The first is failing to meet adequacy conditions, meaning that is it fails to meet discipline-specific rhetorical conventions, epistemological understandings or the intertextual norms of presentation of ideational material (Hyland 1998: 440). The second is failing to address acceptability conditions, meaning that the RA failed to adopt a professionally acceptable tenor which is consistent with disciplinary norms (Hyland 1998: 440). Metadiscourse, used correctly, is a tool which allows the reader to meet both adequacy and acceptability conditions.

3.1 Adequacy Conditions

The first element to be explored is adequacy conditions. In order to be able to effectively make a claim, adequacy conditions must be met, namely, the rhetoric of the argument must be clearly established using disciplinary-specific generic conventions. The argumentation structure of expository writing, such as is seen in an RA, is formed on the basis of a logical

order. However, this logic differs cross-culturally (Hinds 1983: 79). It is well established that Japanese to English translation often requires the reorganisation of information (Hinds 1990: 90). This is often due to a difference in argumentation style; English academia tends to favour deductive writing and Japanese tends to prefer inductive writing (Hinds 1990: 89; Fukuoka and Spyridakis 2002: 99; Okamura 2006: 62). Deductive writing is considered key to Anglophone text coherence, thus Japanese STs can give the appearance of disorganisation in English translations (Hinds 1990: 89). Deductive writing moves from general information to specific information, whilst inductive texts first outline supporting ideas and end with a general statement (Fukuoka and Spyridakis 2002: 99). The influence of the argumentation style is significant. This is due to reader's sense of structural schemata, which are frameworks for rhetorical structures that determine expectations for information type and location and facilitate reader comprehension (Fukuoka and Spyridakis 2002: 100). Studies have found that the comprehension and recall of native English-speakers is better when reading deductively organised paragraphs rather than inductively organised paragraphs (Fukuoka and Spyridakis 2002: 100). In contrast, native Japanese-speakers found inductively organised paragraphs more readable (Fukuoka and Spyridakis 2002: 101). This has significant implications for the publication of RAs as a lack of comprehension will lead to a failure of adequacy conditions and rejection by the scientific community.

Japanese argumentation style is considered to be indirect and tentative, showing a preference for ambiguity and avoidance of direct disagreement (Uysal 2014: 181). This can be problematic when it comes to making claims, due to a reliance on the reader to make their own conclusions in a form of indirect hedging. This tendency toward an inductive and ambiguous style of argumentation means that Japanese is considered a 'reader-responsible language'. A reader-responsible language is one which requires the reader to find the topic link between paragraphs and the main theme (Okamura 2006: 62), the relationship between parts is not explicit and transition devices are subtle (Hinds 1987: 67). Whilst English writing, considered a 'writer-responsible language', favours a hierarchal structure that has a dominant topic sentence (Hinds 1983: 8), prioritises unity and the use of transition statements to guide the reader (Hinds 1987: 67), objective language and avoids ambiguity and circumlocution (Bennett 2007: 161). In translation, this necessitates intervention by the translator in order to convert a reader-responsible text to a writer-responsible text, by using cohesive devices to

guide the reader and make thematic connections clearer, so that claims can be made. The main ways in which I made thematic connections clearer was through the use of context frames (CFs), which are sentence initials and finals that contextualise a sentence (Gosden 1992: 211), and frame markers, which label elements such as text stages, goals or topic shifts (Hyland 2010: 129). There are many types of CFs that are used to create textual cohesion and to help establish the theme of the clause (Gosden 1992: 215). The use of these is particularly important as, in Japanese discourse, once the topic is established it tends not to be repeated, which results in the ellipsis of a topic where there would be one in English (Yamaguchi 2007: 114-116).

Due to the different functions of IMRD sections, different CFs were necessary in each. The Introduction generally requires contrastive CFs to indicate a problem to be solved (Gosden 1992: 217). Contrastive CFs were added in all three TTs (T1P2L22-24; T2P2L20-21;T3P2L21) in order to explicitly indicate a gap in knowledge, headed with the adversatives 'however' and 'despite' to guide the reader for a change in discourse direction. A location in space CF (Gosden 1992: 212) was also added in order to set the internal parameters of the research (T1P3L7-11; T1P3L15-17;T3P2L24-28) indicated by 'the current study/paper' or 'this paper'.

The Methods deals with real world temporal sequences and purpose (Gosden 1992: 217). Accordingly, CFs to introduce the purpose (Gosden 1992: 213) were added within the Methods such as in (T1P4L2; T1P4L5-7; T1P4L9; T2P4L6) which serve to explain the aim of the methods that follow. The Results uses internal validation CFs in order to help pinpoint the research's contribution (Gosden 1992: 218). In the TTs, this included the insertion of phrases such as 'these results indicate that' (T1P5L25) or 'using the previous research as a basis' (T1P6L18). Causative CFs were also used to direct the reader in terms of the significance of the research. This involved the insertion of phrases such as 'this is indicative that' 'is significant as' and 'is beneficial in' (T2P5L19-20; T2P5L21; T2P5L22).

The Discussion has the greatest need for skilled rhetorical manipulation and thus requires the largest proportion of CFs (Gosden 1992: 218). In general, the CFs inserted within the Discussion were to make the implications of the research more explicit 'thus our results also support' (T1P7L6-7),'this could represent an important step' (T1P7L13-14), 'offers further insight' (T2P6L6-9), 'this is a result which' (T2P6L11-13),'from these results',(T2P6L20-21),'the aforementioned results have contributed'(T2P6L25-26), 'it was therefore suggested that'

(T3P6L14), ‘this means that’ (T3P6L17) and ‘our research suggests that’ (T3P6L23). All of the aforementioned CFs function to explicitly establish a territory and emphasise that it is their research which made further insights possible.

Another method employed for increasing the cohesion of the text was specification, a form of reformulation (Hyland 2007: 274). Specification is a technique frequently used within the biological sciences which involves restricting how the reader can interpret a text by adding further detail to clauses thereby restricting the reader’s interpretation (Hyland 2007: 276-7). Therefore, specification is an important tool in converting a reader-responsible text to a writer-responsible text. In T2 this involved the addition of the phrases labelling the AGE-RAGE interaction as a ‘potential contributor’ (T1P2L17) to highlight its contextual significance, referring to AGE breakers as ‘another option explored’ (T1P2L29) to group it with AGE inhibitors and referring to the two objects of research as ‘two promising forms’ (T1P3L4) to distinguish them from the previous research attempted. Specification therefore assisted in making links between ST sentences which would seem disparate to an Anglophone reader clearer.

Specification was also used to limit the ambiguity of what is being referred to. Misinterpretation in Japanese can often occur due to the writer providing too few overt cues or omitting relevant information (Hinds 1985: 9). Even in formal writing the ellipsis of the main verbal elements or subject is common and does not require the ‘it’ placeholder (Hinds 1982: 313). For example, it was necessary to specify ‘研究材料’ [research materials] (ST2P1L12) as being ‘for investigation into beta cell function and insulin resistance’ (T2P2L11-12) and ‘他の細胞’ [other cells] (ST2P2L13) are specified as ‘other cells types within the islets of Langerhans’ (T2P4L23). Additionally, in T3 I specified the full terms for acronyms when first mentioned as is conventional (T3P2L1-2; T3P2L11; T3P3L4; T3P3L29). This helps to avoid ambiguity when referring to the acronyms.

Scientific texts are formed on the basis of a chain of reasoning, which needs to succinctly refer back to set up the next move (Behnam 2013: 148). Therefore, the addition of anaphoric reference was necessary as Japanese tends to establish continuity through subject ellipsis (Maynard 1998: 105). Thus, the phrases ‘using this technique’ (T1P1L23) and ‘using the aforementioned screening method’ (T1P4L17) and ‘by building upon this research’ (T3P2L16)

were added for anaphoric reference as without explicit statement the relation to the preceding sentence would be unclear in English.

The above additions of CFs, specification and anaphoric reference all function to reduce ambiguity for the Anglophone reader, establish a clear argument structure and limit communicative suffering.

3.2 Acceptability Conditions

Next, in order to be able to make an effective claim, acceptability conditions must be met. Thus, the writer needs to consider the TA to be able to anticipate and respond to potential negation of their arguments (Hyland 2010: 128) and establish a tenor which conforms to disciplinary norms (Hyland and Tse 2004: 170). This involves a complex balance between the researcher's authority as a field expert and the researcher's humility as a servant of the discipline (Hyland 1998: 440). The negotiation of these two roles is mainly achieved through hedging. Hedges are the most commonly used metadiscoursal tool; hedges mark statements as provisional and thus seek to involve the reader in the ratification (Hyland 1998: 444-446). Hedges mark the author's reluctance to present their findings as categorical (Hyland 2010: 129), thereby presenting findings whilst still showing deference to the scientific community. In English academic writing, hedges have three important functions: firstly, they identify the factual from the potential, secondly, they assist in protecting the researcher's reputation by avoiding personal responsibility and thirdly, they indicate an appropriate amount of deference thereby showing awareness of the reader (Uysal 2014: 182).

The first function of hedges, identifying the factual from the potential, is important in fulfilling the referential-informative textual function as hedging clearly identifies claims as discrete from established disciplinary knowledge. Hedging also assists in fulfilling the expressive-evaluative textual function as it makes it possible to critically discuss the actual and potential contribution of the research. The second and third functions of hedges help to fulfil the persuasive-informative textual by assisting in negotiating the reader-writer relationship (Hyland and Tse 2004: 170).

Inappropriate use of hedging is a common issue in Japanese to English translation (Okamura 2006: 66) as what is considered appropriate is culture-specific (Uysal 2014: 179). However, since it has been noted that appropriate levels of hedges are an important aspect of the socio-

pragmatic success of an English academic text (Uysal 2014: 181), it is important to ensure that both manner and level of hedging conforms to the TA's disciplinary-specific conventions.

In order to ensure that the TTs were of an appropriate tenor, several aspects had to be adapted. First, although common in Japanese discourse (Uysal 2014: 188), use of rhetorical questions is discouraged in Anglophone scientific discourse as it is considered to be of an inappropriate tenor (Uysal 2014: 189). Thus, the rhetorical question in T1 'では[…]は何か？' (ST1P2L1) [(13) So, what is the primary factor which induces characteristic changes in every type of vascular cell in those presenting with diabetes?] was changed to 'the primary factor that induces the characteristic changes observed in every type of vascular cell in those with diabetes had not been identified.' (T1P2L1). This conserves the meaning of the sentence, that the primary factor is unidentified, whilst still being of an appropriate objective tenor. T1 also employs a direct quote including a rhetorical question '脊椎動物への進化の[…]のではなかろうか' (ST1P2L7-9) [In the stages of evolution in vertebrates […] wouldn't developing diabetes be an inevitable complication?]. Not only is the use of rhetorical questions considered to be of an inappropriate tenor, but direct quotation within an RA is also unconventional. This sentence was therefore adapted to 'as diabetes is characterised by a hyperglycaemic state, it has been noted that it would not be surprising to find that AGE formation is associated with diabetes and is an evitable complication of the disease[7]' (T1P2L10-12). This adaptation maintains that the statement is attributable to somebody else through the reference, but by omitting the quotation, avoids unnecessarily marked dialogic intervention. The final adaptation made in order to conform to the expected tenor was the conversion of results in a list form (ST2P2L6-10) to full sentences (T2P4L15-20). These adaptations served to help the TTs conform to acceptability norms through the use of an appropriate and professional tenor.

Next, differences in styles of hedging were addressed. There are four main ways that claims are modulated: probability hedges, generalisation hedges, distancing hedges and lexical selection (Englander 2014: 52). Below I discuss three hedging strategies applied in the TTs in order to make claims whilst meeting acceptability conditions. Probability hedges is one of the most common hedging strategies, accordingly the use of the modal 'could' was employed frequently (T1P7L13; T1P7L13; T1P7L24; T2P6L8; T3P2L15; T3P2L18; T3P2L27; T3P4L29; T3P5L15 T3P5L17; T3P6L24). Other ways probability hedges were employed was as an

adjective in ‘possible/potential’ (T1P5L23; T2P6L23; T1P7L12) as well as in the adverbial modifier ‘likely’ (T3P5L23-24).

Lexical selection is used as a method of hedging by strategically selecting reporting verbs, which affirm but do not confirm a result. For example, ‘our results support’ (T1P7L6) or ‘our research suggests’ (T2P6L7; T3P5L15; T3P6L23). The use of the above two forms of hedging allows for the writer’s interpretation to be presented, whilst negotiating their role as a member of the scientific community by presenting their results as open to ratification, thereby showing deference to the scientific community.

The final hedging strategy employed was distancing. The most common form of distancing hedging is the use of the passive (Englander 2014: 52). The passive is used to downplay the role of the author, emphasise the object of research (Hyland 2008: 11) and suggest objectivity (Fujii 2008: 41). However, Japanese sentences can omit the agent without using the passive (Fujii 2008: 43), thus avoiding the requirement to use the passive to de-emphasise the agent (Fujii 2008: 45). This can result in an asymmetrical relationship between the passive and active voice in Japanese and English (Fujii 2008: 40), which is particularly apparent in the increasingly abstract RAs (Fujii 2008: 44). As the passive voice foregrounds the research activity rather than the researcher, the passive voice is particularly useful in the Methods and Results (Englander 2014: 33). Accordingly, the Methods of all three TTs were translated in the passive voice. When the passive voice is used, narrative continuity is maintained through the omission of the agent which can be assumed to be the researcher. However, in T1 there were various researchers cited by name when describing the methods employed. Therefore, the first step in translating the TTs was establishing whether the author had contributed to that section of the research. As objectivity is achieved through reducing the personal elements of the text (Behnam 2013: 152), when the author participated in the research, colleagues’ names were omitted and the passive voice was used for narrative continuity. Thus, all the actions listed under Yamamoto et al. (ST1P2L15), which is discussed in T1P3L14-17, Tanaka et al. (ST1P3L2) discussed in T1P3L17-18, Han et al. (ST1P3L10-11) discussed in T1P3L19-27, Yonekura et al. (ST1P4L20) discussed in T1P4L2-8 and Munesue et al. (ST1P5L18-19) discussed in T1P4L16-18, are translated omitting the subject and employing the passive voice. The inclusion of another researcher as a subject in either the Methods or Results of an Anglophone RA would have the

implication that the author was not involved within the research and thus undermine the author's claim.

Some of the most frequent adaptations to passive voice in the Methods were 'を解析した' [(I) analysed] (ST2P2L3; ST1P2L17; ST2P3L22) to 'was analysed' (T2P3L26-27; T1P3L17; T2P4L11; T2P4L4; T3P3L16) and 'を作成した' [(I) created] (ST2P3L21-22; ST1P2L16; ST3P4L17) to 'was created' (T2P4L9; T1P3L15; T3P3L18). The use of the passive voice gives the Methods a conventionally expected sense of objectivity, thereby helping to meet acceptability conditions and strengthening the author's claim.

The passive voice is employed in the Results in order to give the findings a sense of objectivity. In the TTs, this involved converting reporting verbs to the passive voice such as translating 'を見出した' [(I) discovered] (ST2P3L15; STP2L22; ST3P3L5) as 'it was discovered' (T2P3L23; T2P4L1; T2P5L7) and 'が明らかになった' [revealed] (ST2P2L10; ST2P3L18-19; ST3P4L10; ST3P4L21) as 'it was found that' (T2P4L17; T2P5L12; T3P4L15; T3P4L26). Moreover, in order to maintain this objective tone, certain phrases which contained personal elements such as 留学中の成果 [results from my time studying abroad] (ST2P2L17-18) were omitted and verbs showing the author's perspective such as '気がついた' (ST2P2L13-14) [I realised that] were changed to the passive voice becoming 'it became clear that' (T2P4L21). The use of an objective voice serves to distance the author from the results, adding a sense of objectivity and validity to the author's claim.

The final distancing hedging strategy applied within the TTs was the use of stance nouns and mood structures (Martínez 2003: 111). Stance nouns and mood structures are a way for the authors to foreground their attitude to the findings (Koutsantoni 2004: 164), convincing the reader to take the information presented as given, forestalling disagreement (Hyland and Jiang 2015: 532). A common form of stance noun and mood structure used within the sciences is the anticipatory-it pattern (Hyland 2008: 11). In the TTs, this included 'it is thought that' (T1P6L9; T1P7L19; T2P3L14; T2P6L17; T3P6L21), 'it is possible that' (T1P6L2), 'it has become evident that' (T2P3L8-9), 'it is likely that' (T2P3L9) and 'it is clear that' (T2P6L4). This hedging strategy allows the author to strengthen their claim by presenting their opinion as objective fact. However, in the Discussion, once the results have been stated, there is a need to emphasise the author's relationship to the research findings in order to claim new knowledge.

This is mainly emphasised through the use of the pronoun ‘we’ (Englander 2014: 34). As Japanese tends to use active sentences without a personal pronoun (Uysal 2014: 186), this involved the insertion of personal and possessive pronouns throughout the Discussion. For example, ‘our’ (T1P6L25; T1P7L6; T1P7L12; T2P6L4; T2P6L23; T3P5L10; T3P6L23) and ‘we’ (T1P6L26; T2P6L3). This allows the author to highlight their contribution and therefore clearly outline their claim, whilst meeting acceptability conditions by conforming to disciplinary-specific conventions.

4. Conclusion

To conclude, this critical analysis offers a broad overview of some of the translation strategies employed to adapt the TTs to Anglophone disciplinary-specific conventions. The aim of the translations was to produce TTs of an acceptable register to submit to a journal editor. The main difficulties in achieving this aim were an ambiguous and indirect argument style, which would lead to a lack of comprehension by the Anglophone reader, and differences between the TTs and disciplinary- specific Anglophone conventions in terms of RA macrostructure, organisational schemata and hedging. In order to produce TTs of an appropriate register, the STs had to be adapted to fulfil three textual functions. Namely, to be referential-informative, expressive-evaluative function and persuasive-informative.

The major elements which contributed to the achievement of the referential-informative function were the adaptations made in order to fulfil adequacy conditions. For the TT to be referential-informative, the ideational material must be successfully conveyed. The application of CFs and explicit anaphoric reference assisted in making thematic connections clearer, thereby facilitating reader comprehension. Specification was also useful in fulfilling the referential-informative function by making sure that referents were explicit, consequently reducing ambiguity. These strategies assisted in clearly conveying the information within the STs and therefore made the thematic thread clearer. Finally, the adaptation of the text into an IMRD structure also assisted in fulfilling the referential-informative function because reader’s comprehension is facilitated by conforming to conventional organisational schemata.

The expressive-evaluation function was mainly achieved through the application of Swales’ CARS model in the Introduction and Swales’ 8 move scheme in the Discussion. The use of these models provided a framework in which to make critical evaluations of relevant previous

research as well as the research presented in the paper. An evaluation of previous research was not explicit in the STs perhaps due to being considered a Face Threatening Act (Brown and Levinson 1999: 313), a phenomenon unable to be discussed within the scope of this paper. The use of M2 of the CARS model in the Introduction served to explicitly highlight a gap within disciplinary knowledge, justifying the need for the author's research as an explicit evaluation. Moreover, move 7 of Swales' 8 move scheme requires the author to evaluate their own research in order to be able to give its wider implications, thereby fulfilling the expressive-evaluative function.

The persuasive-informative textual function was achieved through an accumulation of all the translation strategies used to make the TTs conform to disciplinary-specific conventions because the RA is more likely to be understood and therefore accepted by conforming to conventions. However, the adaptations that had particular influence upon the persuasive-informative aspect were the strategies used to meet acceptability conditions. The adaptation to an appropriate tenor, including the use of omissions, hedging and strategic use of pronouns, made the register more formal, thus making it more likely to be accepted. The application of the two models by Swales were also significant in achieving the persuasive-informative function by explicitly outlining the contribution of the research. Moreover, these adaptations assisted the TTs to meet the TA's criteria of representing 'a significant advance in diabetes research' (Diabetes 2013).

Achieving the above textual functions served to produce a text that would be considered socio-pragmatically successful by the TA. Beyond the scope of the three TTs, this dissertation has helped further the understanding of the issues of Japanese to English translation and analysed translation strategies that could be applied to Japanese to English scientific translation and could therefore facilitate the dissemination of scientific knowledge.

Words: 7,997

Dissertation Total Word Count: 14,583

Bibliography

Andrade, Chittaranjan (2011) 'How to Write a Good Abstract for a Scientific Paper or Conference Presentation', *Indian Journal of Psychiatry* 53(2): 172–175.

Bazerman, Charles (1988) *Shaping Written Knowledge: the genre and activity of the experimental article in science*, Wisconsin: The University of Wisconsin Press Wisconsin.

Behnam, Biook, Naser Ghafoori and Bahram Kazemian (2013) 'Ideational Grammatical Metaphor in Scientific Texts: A Hallidayan perspective', *International Journal of Linguistics* 5(4): 146-162.

Bennett, Karen (2007) 'Epistemicide!', *The Translator* 13(2): 151-169.

Brown, Penelope and Stephen C. Levinson (1999) 'Politeness: Some universals in language', in Nikolas Coupland and Adam Jaworski (eds) *The Discourse Reader*, London: Routledge, 311-323.

Diabetes (2013) 'Diabetes Instructions for Authors' [style guide] http://diabetes.diabetesjournals.org/site/misc/ifora.xhtml [last accessed: 27 July 2016]

Englander, Karen (2014) *Writing and Publishing Science Research Papers in English a Global Perspective*, New York: Springer.

Fujii, Yasunari (2008) 'The Asymmetrical Relationship Between the Active and Passive Voice: Implications for teaching Japanese-to-English translation of specialized scientific texts', *The Linguistics Journal* 3(1): 40-74.

Fukuoka, Waka and Jan Spyridakis (2002) 'The Effect of Inductively Versus Deductively Organized Text on American and Japanese Readers', *IEEE Transactions on Professional Communication* 45(2): 99-114.

Gosden, Hugh (1992) 'Discourse Functions of Marked Theme in Scientific Research Articles', *English for Specific Purposes* 11(3): 207-224.

----- (1995) 'Success in Research Article Writing and Revision: A Social-Constructionist Perspective', *English for Specific Purposes* 14(1): 37-57.

Hinds, John (1976) 'A Taxonomy of Japanese Discourse Types', *Linguistics* 184: 45-54.

----- (1982) 'Japanese Conversational Structures', *Lingua* 52: 301-326.

----- (1983) 'Linguistics and Written Discourse in Particular Languages. Contrastive studies: English and Japanese', *Annual Review of Applied Linguistics* 3: 78-84.

----- (1985) 'Misinterpretations and Common Knowledge in Japanese', *Journal of Pragmatics* 9: 7-19.

----- (1987) 'Reader versus Writer Responsibility: A new typology', in Tony Silva and Paul Kei Matsuda (eds) *Landmark Essays on ESL Writing: Volume 17* New York: Routledge, 63-75.

----- (1990) 'Inductive, Deductive, Quasi-inductive: Expository writing in Japanese, Korean, Chinese and Thai', in U. Connor and A. Johns (eds) *Coherence in writing* Alexandria: TESOL, 89-109.

Hyland, Ken (1998) 'Persuasion and Context: The pragmatics of academic metadiscourse', .*Journal of Pragmatics* 30(4): 437-455.

---- (2007) 'Applying a Gloss: Exemplifying and reformulating in academic discourse', *Applied Linguistics* 28(2): 266-285.

---- (2008) 'As Can Be Seen: Lexical bundles and disciplinary variation', *English for Specific Purposes* 27(1): 4-21.

---- (2010) ‘Metadiscourse: Mapping interactions in academic writing’ *Nordic Journal of English Studies* 9(2): 125-143.

Hyland, Ken and Polly Tse (2004) ‘Metadiscourse in Academic Writing a Reappraisal’, *Applied Linguistics* 25(2): 156-177.

Hyland, Ken and Feng Jiang (2015) “The Fact That': Stance nouns in disciplinary writing’, *Discourse Studies* 17(5): 529-550.

Ishihara, Hisamitsu (2009) ‘2 型糖尿病発症における膵 b 細胞障害の分子機構’ [The molecular mechanism of pancreatic beta cell damage in the pathogenesis of Type 2 diabetes], *糖尿病 [Diabetes]* 52(11): 884-866. (Text 2)

Kawase, Tomoyuki (2015) ‘Metadiscourse in the Introductions of Phd Theses and Research Articles’, *Journal of English for Academic Purposes* 20: 114-124.

Koutsantoni, Dimitra (2004) ‘Attitude, Certainty and Allusions to Common Knowledge in Scientific Research Articles’, *Journal of English for Academic Purposes* 3: 163–182.

Lorés, Rosa (2004) ‘On RA Abstracts: From rhetorical structure to thematic organisation’, *English for Specific Purposes* 23: 280-302.

Martínez, Iliana (2003) ‘Aspects of Theme in the Method and Discussion Sections of Biology Journal Articles in English’, *Journal of English for Academic Purposes* 2: 103-123.

Maynard, Senko (1998) *Principles of Japanese Discourse: A handbook*, Cambridge: Cambridge University Press.

Montgomery, Scott (2009) ‘English and Science: Realities and issues for translation in the age of an expanding lingua franca’, *The Journal of Specialised Translation* 11: 6-16.

Nord, Christiane (2006) 'Loyalty and Fidelity in Specialized Translation', *Confluências – Revista de Tradução Científica e Técnica* 4: 29-41.

Okamura, Akiko (2006) 'Two Types of Strategies Used by Japanese Scientists, when Writing Research Articles in English', *System* 34(1): 68-79.

Sionis, Claude (1995) 'Communication Strategies in the Writing of Scientific Research Articles by Non-native Users of English', *English for Specific Purposes* 14(2): 99-113.

Swales, John (1990) *Genre Analysis*, Cambridge: Cambridge University Press.

Teufel, Simone (1998) 'Meta-discourse Markers and Problem-structuring in Scientific Articles', in M. Stede, L. Wanner, and E. Hovy (eds) *Proceedings of the Workshop on Discourse Relations and Discourse Markers at the 17th International Conference on Computational Linguistics,* ACL: Montreal, 43-49.

Uysal, Hacer (2014) 'A Cross-Cultural Study of Indirectness and Hedging in the Conference Proposals of English NS and NNS Scholars', in Andrzej Lyda and Krystyna Warcha (eds) *Occupying Niches: Interculturality, cross-culturality and aculturality in academic research.* Switzerland: Springer International Publishing, 179-195.

Watada, Hirotaka (2009) '膵 b 細胞容積調節機構に関する研究' [The Clinical Application of the Mechanism for Regulating Beta Cell Mass in the Treatment of Type 2 Diabetes], *糖尿病 [Diabetes]* 52(11): 881-883. (Text 3)

Vermeer, Hans (1989/2000) '*Skopos and Commission in Translational Action*', in Lawrence Venuti (ed.) *The Translation Studies Reader*, London: Routledge: 221–232.

Yamaguchi, Toshiko (2007) *Japanese Language in Use: An introduction*, London: Continuum.

Yamamoto, Hiroshi (2014) '糖尿病合併症の成因・病態・克服に関する基礎的研究' [Fundamental Research on the Causes, Pathology and Subjugation of Diabetes Complications], *糖尿病 [Diabetes]* 57(10): 765-711. (Text 1)

Appendices

Appendix A: Bilingual table of translations

Text 1

Yamamoto, Hiroshi (2014) '糖尿病合併症の成因・病態・克服に関する基礎的研究' [Fundamental Research on the Causes, Pathology and Subjugation of Diabetes Complications], *糖尿病 [Diabetes]* 57(10): 765-711.

Source segment	Target segment
<51/><54/><57>ハーゲドーン賞受賞講演</57>	<51/><54/>The Hagedorn Prize
糖尿病合併症の成因・病態・克服に関する基礎的研究	Fundamental Research on the Causes, Pathology and Subjugation of Diabetes Complications
山本　博	Hiroshi Yamamoto
はじめに	Introduction
<83>本賞に冠せられた Hagedorn の名にはじめて接した のは，須藤憲三金澤醫科大學醫化學初代教授の著書「醫 化學的微量測定法」</83><101>１）</101><108>を紐解いたときであった．</108>	The first time I heard the name Hagedorn, the namesake of the Hagedorn Prize, was in the book 'The method for measuring microdosing in medicinal chemistry'<Subscript>1)<Subscript></Subscript></Subscript>which my first professor of medicinal chemistry at Kanazawa Medical University, Kenzou Sutou authored.
須藤先 生は，尿糖測定法を確立するなど，わが国糖尿病研究 の草分け的存在で「，栄養」の命名者としても知られる．	Among the methods established by Sutou was the method for measuring the glucose concentration in urine. Sutou was such a significant pioneer in the field of diabetes research in Japan that he was known by the name 'Professor Nutrition'.
「醫化學的微量測定法」では「一二滴の血液を用いて容 易に且つ正確に」血糖を 定量できる測定として Hagedorn-Jensen 法が紹介されている．	In 'The method for measuring microdosing in medicinal chemistry' the Hagedorn-Jensen Method was introduced as a method to both easily and accurately measure blood glucose concentration using a fixed quantity of one or two drops of blood.

私は恩師岡本宏先生の下で糖尿病研究に着手し，富 山医科薬科大学および前任の東北大学時代には，主と して膵ランゲルハンス島の分子生物学的研究に取り組 んだ.	I engaged in diabetes research under the guidance of Professor Hiroshi Okamoto specialising in research into the molecular biology of the pancreatic Islets of Langerhans whilst researching at Toyama Medical and Pharmaceutical University and at my previous post in Tohoku University.
<147>アロキサンとストレプトゾトシンが膵ランゲル ハンス島の DNA 鎖を切断することの発見</147><174>２）</174><178>や“，Mo- lecular biology of the islets of Langerhans”</178><226>３）</226><229>の編纂 に関わった．</229>	I was involved in the discovery that alloxan and streptozotocin induce DNA strand breaks in the pancreatic Islets of Langerhans<Subscript>2)</Subscript></Subscript>and in the edited volume ‘Molecular Biology of the Islets of Langerhans’<Subscript>3)</Subscript>.
血管生物学研究から糖尿病合併症研究へ	From research in vascular biology to research into the complications of diabetes
<253>1990 年金沢大学赴任後，血管生物学に取り組み，血 管構成細胞種の共存培養系を確立して，周皮細胞が内 皮細胞の増殖を抑制し，プロスタサイクリン産生能を 保持するとともに，過酸化脂質による内皮細胞障害を 防止することを明らかにした</253><265>４）</265><272>．</272>	After appointment to a new research post at Kanazawa University in 1990, I started researching into vascular biology. The co-cultivation of the types of constituent vascular cells was established and it was found that pericytes inhibited the proliferation of endothelial cells and preserved the production of prostacyclin in addition to preventing endothelial cell damage by lipid peroxides.
これにより，糖尿病網 膜症ではなぜ周皮細胞喪失に伴って血管新生が起こる かの一端が説明されるようになった.	This discovery gave an insight into why neovascularization accompanies pericyte loss in cases of diabetic retinopathy.
では，糖尿病状態で各種血管細胞に特徴的な変化を 来す primary の要因は何か?	So, what is the primary factor which induces characteristic changes in every type of vascular cell in those presenting with diabetes?
<281>この問いに答えるため， 周皮細胞や内皮細胞の純培養系を用い，種々の環境要 因を探索した結果，同定された因子が advanced glyca- tion endproducts（以下，AGE）であった</281><314>５， ６</314><318>（</318><321>）</321><327>Fig. 1）．</327>	In order to answer this question, we used an axenic culture of endothelial cells and pericytes in an investigation using a variety of environmental factors and as a result, identified the factor as advanced glycation end products (hereafter: AGE). (Figure 1)
<336/><361><339>AGE は，糖のカルボニル基と蛋白のアミノ基とが非酵素的に反応する結果，不可逆的に形成される産物の総 称である．</339></361>	<336/> AGE is the term used to denote the irreversible products formed through a non-enzymatic reaction between the carbonyl group on a sugar and the amino group on a protein.

AGE の形成，蓄積は慢性的な高血糖状態で 加速的に進行する．	The process of AGE formation and accumulation is accelerated by a chronic hyperglycaemic state.
ブドウ糖は有効な燃料分子である が，糖化という負の側面をもつ．	Despite glucose being an efficacious source of energy, it still has the issue of glycation.
<339>大阪大学の垂井清一 郎先生は「，脊椎動物への進化の段階で遊離のブドウ糖 を血糖として採用し閉鎖循環系のなかを循環させるに 至ったのが，そもそも糖尿病の個体が出現するきっか けであり，糖尿病は宿命的に合併症発現のリスクを 担った疾患なのではなかろうか」と記されている</339><360>７）</360><367>．</367>	Professor Seiichiro Tarui of Osaka University noted “In the stages of evolution in vertebrates the release of glucose to be used within a closed circulatory system as blood sugars led to blood circulation. However, this means that the chance of individuals with diabetes presenting was possible from inception and that the risk of developing diabetes was an inevitable complication <Subscript>7)</Subscript>.
AGE-RAGE は糖尿病合併症の一成因―遺伝子 改変動物を用いた証明	Proof that AGE-RAGE is the cause of diabetes complications using genetically modified animals
AGE の血管細胞作用の少なくとも一部は，特異受容 体 receptor for AGE（以下，RAGE）を介する．	At least a part of the action that AGE has on vascular cells is achieved through the specific receptor for AGE (hereafter RAGE).
RAGE は，パターン認識受容体に分類され，さまざまな病原 体関連分子パターン pathogen-associated molecular pattern（PAMP）や傷害関連分子パターン damage- associated molecular pattern（DAMP）をリガンドとして認識する（Fig. 2A）．	RAGE is classified as a pattern recognition receptor and is recognised as a ligand by various pathogen-associated molecular patterns (PAMP)and damage- associated molecular patterns (DAMP).(Figure 2a)
<451>教室の山本靖彦准教授ら</451><454>８，９）</454><467>は，血管細胞で RAGE を過剰に発現するトランスジェニックマウス，内在性 RAGE 遺伝子を欠損したマウスを作製し，糖尿病を誘 発して，合併症を解析した．</467>	Associate Professor Yasuhiko Yamamoto and his team<Subscript>8,9</Subscript> created transgenic mice that over expressed RAGE within their vascular cells and mice that endogenously lacked the RAGE gene. Yamamoto then induced diabetes within the mice and analysed the resulting complications.
<467>すると，RAGE 過剰発現 マウスは糖尿病腎症</467><497>８）</497><503>および網膜症</503><506>１０）</506><512>指標の増悪を示し（Fig. 2 B and C），他方，RAGE 欠損マウスは糖尿病 腎症を発症しなかった</512><551>９</551><554>（</554><557>） </557><563>Fig. 2D）．</563>	It was found that index for diabetic nephropathy<Subscript>8)</Subscript> or diabetic retinopathy<Subscript>10)</Subscript> in the mice made to overexpress RAGE showed signs of worsening (Figure 2B and 2C). Whilst the RAGE deficient mice did not develop any symptoms of diabetic nephropathy<Subscript>9)</Subscript> (Figure 2D).

以上の結果は，糖尿病合併症の発症に AGE と RAGE の相互作用が機能的に関わっていることを示す．	These results indicate that there is a functional interaction between the pathogenesis of diabetes complications and AGE and RAGE.
ヒト RAGE 遺伝子の転写調節機構	The Transcriptional Regulatory Mechanism for the human RAGE gene
<1027>教室の Tanaka ら</1027><1030>１１）</1030><1034>は，ヒト RAGE 遺伝子の転写調節機構を調べた．</1034>	Tanaka<Subscript>11)</Subscript> and his team investigated the transcriptional regulatory mechanism for the human RAGE gene.
<1056/><1057/><1067><1063>B（NF-</1063><1068/><1072/><1078>B）を活性化して RAGE 遺伝子の転写を活性化する，という positive なフィードバックの仕組みが見い出された（Fig. 3）．</1078></1067>	As a result it was discovered that it is AGE itself which activates the transcription factor nuclear factor-<1056/><1057/>B (NF-<1068/><1072/>B) and activates the transcription of the RAGE gene forming a positive feedback loop.
これが，糖尿病状態で認められる見かけ上 constitutive な RAGE 発現や AGE-RAGE 共局在の分子的基礎と考えられた．	This research forms the molecular basis for the apparent constitutive RAGE expression and AGE-RAGE colocalization in the observed diabetic state.
<1067><1078>RAGE 後の多様なシグナル経路の中でも糖尿病では とくに NF-</1078><1154/></1067><1155/><1156/><1162>B を介する経路が重要と考えられる．</1162>	Among the numerous RAGE-flanking signalling pathways the NF-<1154/><1155/><1156/>B intermediary pathway is considered to be particularly important in regard to diabetes.
<1175><1171>AGE-RAGE は膵</1171><1176/></1175><1177/><1181/><1190><1187>細胞不全にも関わる</1187></1190>	The involvement of AGE-RAGE in pancreatic<1176/><1177/><1181/> cell deficiency
<1193>2 型糖尿病の進行に伴い膵ランゲルハンス島</1193><1201/><1204> </1204><1207>細胞の機能不全や</1207><1211/><1215/><1221>細胞塊の減少が起こることが知られている．</1221>	The cell dysfunction in pancreatic <1201/> cells within the islets of Langerhans and the decline in <1211/><1215/> cell mass which accompanies the progression of Type 2 diabetes is well established.
この現象に AGE-RAGE が関係していないかどうかが教室の Han ら<1243><1239>１２）</1239><1242>によって調べられた．</1242></1243>	Han et al. have carried out investigations to find out whether there is a connection between AGE-RAGE and the aforementioned aspects of Type 2 diabetes pathogenesis.
<1243><1242>すると，意外なことに，正常な</1242></1243><1256/><1257/><1285><1263>細胞では細胞表面に RAGE 蛋白は検出されなかった．</1263></1285>	Surprisingly, Han et al. found that the RAGE protein was not detected on the cell surface of normal <1256/><1257/> cells.

<1285><1263>ところが，2 型糖尿病 モデル動物である </1263><1284>ob</1284><1288>ƒ</1288><1291>ob </1291><1294>マウスや </1294><1297>db</1297><1300>ƒ</1300><1303>db </1303><1309>マウスでは加 齢に伴って膵 </1309><1313/></1285><1314/><1333><1320>細胞の RAGE 蛋白陽性率が増大し た． </1320></1333>	However, in animal models of type 2 diabetic mice, the ratio of RAGE protein positive<1313/> <1314/> cells within ob/ob mice and db/db mice increased with age.
<1333><1320>そこで，</1320><1332>db</1332><1336>ƒ</1336><1339>db </1339><1342>マウスと RAGE 欠損マウスを交配 させると，糖尿病の進行に伴う耐糖能異常とアポトー シスによる </1342><1367/></1333><1368/><1420><1374>細胞塊の減少が改善されることが見い 出された（Fig. 4）． </1374></1420>	It was discovered that when the db/db RAGE deficient mice were then crossbred, the apoptosis induced reduction in <1367/><1368/> cell mass and impaired glucose tolerance associated with advancement of diabetes improved (Figure 4).
さらに，MIN-6 細胞を用いた解析 で，遊離脂肪酸とレプチン受容体アンタゴニストの投 与により細胞表面 RAGE 蛋白発現が誘導され AGE 曝露による細胞死がもっとも顕著になることが観察さ れた．	In addition, the most remarkable thing observed was the cell death due to AGE exposure. MIN-6 cells were used in this analysis, free fatty acids and leptin receptor antagonists were then administered to these cells, causing them to induce the expression of RAGE proteins on the cells' surface, cell death then occurred when exposed to AGE.
<1374>以上の結果から，2 型糖尿病に伴う膵 </1374><1421/><1425/><1431>細胞不全 のメカニズムとして従来想定されてきた lipotoxicity と glucotoxicity の実体の少なくとも一部は遊離脂肪酸と AGE によって担われているものと考えられた</1431>	The above results are in line with the prevailing theories on the mechanism behind the deficiency in pancreatic <1421/><1425/> cells which is associated with Type 2 diabetes which postulate that non-esterified fatty acids and AGE at least play a part in the lipotoxicity and glucotoxicity contributing to the mechanism.
RAGE が関係するその他の病態	How RAGE relates to other pathologies
<1482>東北大学久保裕司博士ら</1482><1485>１３，１４） </1485><1498>との共同研究で，RAGE が，亜急性炎症モデルで上皮間葉移行に関わることや， phosphatidylserine を特異的に認識してアポトーシス 細胞の貪食に関わることが見い出された． </1498>	In a collaborative study with Dr. Hiroshi Kubo of Tohoku University<Subscript>13,14<Subscript>, </Subscript></Subscript>there were various discoveries concerning RAGE and its relationship with to epithelial-mesenchymal transition<Subscript> </Subscript>using a subacute inflammation model. In addition there were discoveries concerning RAGE specifically recognising phosphatidylserine and its involvement in the phagocytosis of apoptopic cells.
また，金沢大学における学際的研究により，アミロイド <1520/><1530><1523>1-42 </1523><1529>ペプ チドの脳内への移行が	Moreover, it was demonstrated in interdisciplinary research at Kanazawa University that both a RAGE deficiency and the

RAGE 欠損や後述する可溶型 RAGE 蛋白の過剰発現で有意に抑制されることが明らかにされた</1529><1563>１５）</1563><1569>. </1569></1530>	overexpression of the aforementioned soluble RAGE protein significantly suppresses the uptake of amyloid <1520/><Subscript>1-42</Subscript> peptide into the brain<Subscript>15</Subscript>.
AGE-RAGE ターゲッティング	AGE-RAGE Targeting
以上述べてきた知見から，AGE-RAGE は糖尿病とその合併症および各種ヒト疾患の治療標的候補と考えられる.	From the research mentioned thus far, AGE-RAGE can be considered to be a possible target for treatment for all kinds of human diseases including diabetes and the complications associated with diabetes.
理論上考えうる主な AGE-RAGE 標的療法の方針と手段を Table 1 に示す.	Table 1 lists the theoretically conceivable strategies for the main AGE-RAGE therapeutic targets
第一は，AGE 形成の阻害である.	The first strategy is the inhibition of the formation of AGE.
が，これまで開発されてきた AGE 形成阻害剤の多くは AGE 形成中間体への共有結合を作用機構としており，中間体に対して等モル近い薬剤を必要とするという化学量論的な問題がある.	However, the mechanism of action for the majority of currently developed AGE inhibitors is to target the covalent bonds of the AGE formation intermediate compound. This intermediate compound requires close to an equimolar of drugs resulting in a stoichiometric problem.
<1623>興味深いことに，angiotensin receptor blocker（ARB）が AGE 形成阻害活性をもつことが報告されている</1623><1725>１６）</1725><1729>. </1729>	Interestingly, it has been reported that angiotensin receptor blocker (ARB) has AGE formation inhibiting activity.
第二は，すでに形成された AGE を分解する薬物である.	The second option is a drug to break down already formed AGE.
このカテゴリーに属する薬物は AGE breaker とよばれる.	This type of drug belongs to a category of drugs called AGE breakers.
が，未だ効率よく AGE を分解できる breaker の開発には至っていない.	However, an AGE breaker which is efficacious at breaking down AGE is yet to be developed.
可溶型 RAGE	Soluble RAGE
第三は，AGE を細胞外で補足し血管細胞を保護するデコイ受容体である（Fig. 5）.	The third option is the addition of an extracellular decoy receptor for AGE that protects the vascular cells.
教室の Yonekura（現金	Yonekura
1.	1.
AGE をつくらせない：阻害剤，ARB 2.	Prevent AGE formation: inhibitors, ARB 2
AGE を壊す：Breaker	Break down AGE: Breakers
3.	3.

AGE をつかまえる：<1961>デコ</1961>イ	Seize AGE: Decoys
4.	4.
RAGE 作用を抑える：RAGE 拮抗剤	Inhibit the action of RAGE: RAGE antagonists
<1988>沢医科大学教授）ら</1988><1991>１７）</1991><1998>は，ヒト血管細胞ポリソームのスクリーニングでオルタナティブ RNA スプライシングにより生成されるデコイ RAGE 蛋白を同定し，esRAGE（endogenous secretory RAGE）と命名した．</1998>	of Kanazawa Medical University identified a decoy RAGE protein which was named endogenous secretory RAGE (esRAGE) by screening of human vascular cell polysomes and alternative RNA splicing.
<1998>Motoyoshi ら</1998><2061>１８）</2061><2067>は，細胞内サイクリック AMP 濃度が上昇すると，MMP9 によるエクトドメインシェディングにより，膜結合型 RAGE 蛋白から可溶型 RAGE 蛋白への転換が誘導されることを示した．</2067>	Motoyoshi et al.<Subscript>18</Subscript> demonstrated that it possible to induce membrane-bound RAGE proteins to convert into soluble RAGE proteins through MMP9 induced ectodomain shedding when the concentration of intracellular cyclic AMP is increased.
RAGE アンタゴニストの開発と食品 AGE の評価	Evaluation of the advent of RAGE antagonists and AGE in food products
第四は，RAGE アンタゴニストの開発である．	The fourth option is the invention of a RAGE antagonist.
RAGE 抗体を用いた fluorescence resonance energy transfer	When analysis of fluorescence resonance energy transfer (FRET)
（FRET）解析を行うと，リガンド刺激前後で RAGE monomer に由来すると考えられる蛍光の強度は変化せず，オリゴマーに由来すると考えられる蛍光の強度が増大した．	has been carried out using RAGE antibodies, at around the same time as ligand stimulation, without any change in fluorescence intensity originating from what is believed to be a RAGE monomer, the fluorescence intensity believed to be originating form an oligomer increased .
細胞内に情報を送るアゴニスティックなリガンドは RAGE 受容体をオリゴマー化するものと考えられる．	It is thought that the agonist ligands which send intracellular signals make the RAGE receptors oligimerize.
そこで，分子量 300 ほどの低分子 AGE	So, when a pharmacological assessment was carried out
<2226/><2266><2229>を調製し，薬理学的な評価を行ったところ，低分子 AGE は RAGE に対してアンタゴニスト活性を示すことが見い出された</2229><2265>１９）</2265><2269>．</2269></2266>	<2226/>in which low-molecular weight AGE of a maximum molecular weight of 300 was prepared, it was discovered that low-molecular weight AGE showed signs of RAGE antagonist activity<Subscript>19</Subscript>.
<2272>大阪大学小林祐次名誉教授のグループとの共同研究で，ヒト RAGE 蛋白の三次元構造を決定した</2272><2287>２０）</2287><2291>．</2291>	In collaborative research with Kobayashi et al. we undertook research to determine the three-dimensional structure of the human RAGE protein<Subscript>20</Subscript>.

構造情報に立脚した低分子化合物の in silico スクリーニングと，その後の薬理学的な評価により，RAGE 拮抗活性を示す数種の候補物質も得られた．	After screening low-molecular weight compounds based on their structural information in silico, several potential agents which showed RAGE antagonist activity were obtained through subsequent pharmacological assessment.
ある種の食品は AGE に富み，色，香り，味などの風味の一部は AGE に由来する．	Some varieties of food products are rich in AGE. These food product's taste partially originates from AGE including the colour, aroma and flavour.
<2345>摂取した食品に含まれる AGE の約 10％が循環血中に回収され，48 時間後には 70％が体内に留まる</2345><2423>２１）</2423><2430>．</2430>	Of the intake of food derived AGE, approximately 10% is absorbed into the blood stream. Yet after 48 hours 70% of the AGE consumed remains within the body.
欧米では従来，食品中の AGE を有害視する考え方が支配的であったが，食品 AGE のもつ生物学的作用についてはなお検証の必要があると考えられる．	Conventionally in the Western world, the dominant view has been that food derived AGE is regarded as harmful to health. However, it is also thought that further investigation into the biological effects of AGE in food products is required.
<2430>Munesue ら</2430><2460>１９）</2460><2466>は RAGE アゴニズム f アンタゴニズムの観点から醤油，コーヒー，赤ワイン，コーラをモデルとした評価を行った．</2466>	Munesue et al.<Subscript>19</Subscript> carried out an assessment of RAGE as a agonist/antagonist using the following food within their assessments: soy sauce, coffee, red wine and cola.
その結果，醤油，コーヒー，赤ワインは高分子 AGE の RAGE アゴニスト活性を中和することが見い出され，このアンタゴニスト活性は低分子画分に回収された．	It was found that soy sauce, coffee and red wine neutralised the RAGE agonist activity of the high molecular weight AGE and that it was low-molecular weight fractions that reversed this into antagonist activity.
まとめ	Summary
<2536><2520>AGE を含むリガンドと RAGE との相互作用は，糖尿病における血管障害および</2520><2537/></2536><2538/><2544>細胞不全の成因の一つと考えられ，糖尿病の一次・二次・三次予防上の標的となると考えられる．</2544>	The interaction between AGE, its ligands and RAGE is considered to be one of the causes of diabetic angiopathy and <2537/> <2538/> cell failure in diabetes, Accordingly they are considered to be the primary, secondary and tertiary targets for prevention of these disorders.
RAGE アンタゴニスト薬やデコイバリアント産生誘導法の開発により糖尿病合併症	There is great reason to hope for the elimination of diabetes complications through the development of
を克服できる日が来ることを期待したい．	a RAGE antagonist drug or a method of inducing the production of a decoy variant of the RAGE protein will become a reality in the near future.

<2685>著者の COI（conflicts of interest）開示</2685>：特になし <2699><2696>謝　辞</2696></2699>	Conflicts of interest (COI): none to declare Acknowledgements
厳しく，また，温かく，私の糖尿病研究をお見守りいた	I would like to express my profound gratitude to my respected emeritus professor Hiroshi Okamoto
だきました恩師岡本宏東北大学名誉教授に深甚の謝意を表します.	Of Tohoku University for his stern but warm guidance concerning my diabetes research.
折にふれ encourage いただきました金沢大学名誉 教授竹田亮祐先生に心より感謝申し上げます.	I would also like to express heartfelt thanks to emeritus Professor Ryousuke Takeda of Kanazawa University for his frequent encouragement.
今回の受賞は真に共同研究者各位との共同研究の賜物であります.	This prize truly is a result of the collaborative research efforts of every member involved.

Text 2

Ishihara, Hisamitsu (2009) '2 型糖尿病発症における膵 b 細胞障害の分子機構' [The molecular mechanism of pancreatic beta cell damage in the pathogenesis of Type 2 diabetes], *糖尿病 [Diabetes]* 52(11): 884-866.

Source segment	**Target segment**
リリー賞受賞講演	The Lilly Prize
<5>2 型糖尿病発症における膵</5><11>b</11><15>細胞障害の分子機構</15>	The molecular mechanism of pancreatic beta cell damage in the pathogenesis of Type 2 diabetes
石原寿光	Hisamitsu Ishihara
はじめに	Introduction
2 型糖尿病は，膵 b 細胞からのインスリン分泌障害と骨格筋<36>・</36><39>脂肪組織や肝臓でのインスリン抵抗性が複</39>雑に絡み合って，発症<45>・</45><48>進展する疾患である．</48>	Type 2 diabetes is a complex disease where an impaired insulin secretion from the pancreatic beta cells complicated by insulin resistance in the skeletal muscle, adipose tissue and liver results in the pathogenesis and progression of the disease.
<48>私が糖</48>尿病を専門とすることを決意し，医局の研究室で研究を始めた当初，私のまわりではインスリン抵抗性に関する研究がより盛んに行われていた．	When I first decided to make my specialism diabetes and started my research in the laboratory of medical office, I was surrounded by people engaging in research into insulin resistance as a research topic amounting academic interest at the time.
膵島あるいは膵 b 細胞は単離することが簡単ではなく，研究材料を豊富に得られないことが，研究を進めるうえで足かせとなっていた．	Isolating the islets of Langerhans or pancreatic beta cells is not a simple matter. If it is not possible to obtain plentiful amounts of research materials then this becomes a major stumbling block for progressing the research.
b 細胞の機能の研究—engineeringofnutrient-stimulatedinsulinsecretion	Research into beta cell function: engineering of nutrient-stimulated insulin secretion
幸運なことに，1990 年に，今日最も代表的なインスリン分泌細胞株として世界中で広く使用されている MIN6 細胞が樹立された．	Fortunately, in 1990 the MIN6 cell, which is still currently widely used global and considered the most typical insulin-secreting cell line, was created.
これによって，研究材料が得にくいという困難さはかなり解消された．	This solved the issue of the difficulty obtaining research materials.

2 型糖尿病の発症初期あるいは耐糖能異常の段階において，インスリン分泌応答はしばしば遅延過大反応と記述されるように，絶対量の低下は認められない.	As it is noted that there is frequent latent excessive response to insulin secretion at the stage of initial pathogenesis of Type 2 diabetes or at the stage of impaired glucose tolerance, it is not possible to observe the absolute amount of decline of beta cells,
このため，1990 年代前半，b 細胞の異常は量の低下ではなく，むしろグルコース濃度認識機構の異常であると考えられていた.	As such, in the first half of the 1990s, the prevailing view became that rather than researching the decline in amount of beta cells resulting in abnormalities, that instead the research target was the abnormalities in the mechanism for recognising glucose concentration.
私も，b 細胞のグルコース濃度認識機構の詳細を解明することが，2 型糖尿病におけるインスリン分泌異常の治療，ひいては 2 型糖尿病の治療に役立つと考えた.	Thus, I considered this research to not only elucidate the details of the mechanism for recognising glucose concentration in beta cells but also to assist in the treatment of type 2 diabetes through the treatment of insulin secretion disorders.
そこで，MIN6 細胞を用い，遺伝子工学の技術を取り入れて，どのような遺伝子を導入した場合にグルコースによるインスリン分泌応答が変化するかを解析していった.	Therefore, I carried out analysis using MIN6 cells and incorporating genetic engineering technology, testing out how the insulin secretion response changed upon introduction of different kinds of genes.
グルコースなどの栄養源(nutrient)によるインスリン分泌を engineering して検討するという方法であった.	The method employed used genetic engineering in order to investigate the insulin secretions to a variety of nutrients including glucose.
1.グルコース応答性インスリン分泌機構	Glucose-stimulated mechanism of insulin secretion
まず，解糖系の酵素に注目して取り組み，グルコース取り込みを行うグルコース輸送担体 GLUT1 とグルコースリン酸化を行うヘキソキナーゼ I の過剰発現の効果を解析した 1).	First, I focused research efforts on the enzymes in the glycolytic pathway. Then, I analysed the efficacy of over expressing the glucose transporter GLUT1 which uptakes glucose and hexokinase I which carries out glucose phosphorylation<Subscript>1</Subscript>.
また，解糖系とミトコンドリア代謝との連携のうえで重要なグリセロール 3 リン酸脱水素酵素 2)や乳酸脱水素酵素 3)，ミトコンドリアでのアデノシン 5'-三リン酸(ATP)産生を抑制する脱共役タンパク UCP14)の強制発現の効果を検討した.	In addition, taking into account the link between the glycolytic pathway and mitochondrial metabolism, the efficacy of the forced expression of uncoupling protein 14 (UCP14) was analysed. UCP14 suppresses the production of glycerol-3-phosphate dehydrogenase<Subscript>2</Subscript>, lactic dehydrogenase<Subscript>3</Subscript> and adenosine 5'-triphosphate (ATP).

それらの結果をまとめると，①グルコースリン酸化過程が b 細胞における解糖系の律速過程であり，グルコース代謝流量を規定しグルコースセンサーとしての役割を担っていること，②b 細胞においては解糖系とミトコンドリア代謝の連関効率が高いこと，そして，③ミトコンドリア代謝が ATP をはじめインスリン分泌のシグナル形成に重要な役割を果たしていること，が明らかとなった．	To summarise the results of this investigation: 1) The rate-determining step of the glycolytic pathway in pancreatic beta cells was determined as glucose phosphorylation process and the role of the glucose sensor as regulating glycolytic flux were discovered. 2) It was found that there is a highly functional connection between the glycolytic pathway and mitochondrial metabolism. 3) It was clarified that mitochondrial metabolism not only fulfils an important role for ATP, but also in the formation of insulin secretion signalling.
2.インスリン分泌のグルコース特異性	The glucose specificity of insulin secretion
Nutrient によるインスリン分泌機構を engineering する過程で，b 細胞がインスリン分泌をグルコースに限定して起こすために，他の細胞と異なった特徴を有していることに気がついた．	In the process of engineering an insulin secretion mechanism which responds to nutrients that causes beta cell insulin secretion to be in restricted to responding to glucose it became clear that these cells possessed different features to other cells.
すなわち，b 細胞はグルコース以外の nutrient によってインスリン分泌を起こさないために，それらに対する細胞膜上の輸送担体をもっていないことが明らかとなった．	Namely, it became clear that there were no transporters on the cell membranes of these cells in order to ensure that beta cells do not secrete insulin in response to nutrients other than glucose.
実際，TCA サイクルの中間体であるジカルボン酸を細胞に取り込むジカルボン酸輸送担体を発現させたところ，これらの分子に対してインスリンを分泌するようになった 5)．	In fact, when the expression of dicarboxylic acid transporters, which uptake the intermediary product of the TCA cycle, dicarboxylic acid, was forced, these molecules began to secrete insulin<*Subscript*>5</*Subscript*>.
また，留学中の成果であるが，ピルビン酸輸送担体を発現させることにより，膵島がピルビン酸に対してインスリン分泌を起こすようになることも観察された 3)．	In addition, in the results of an international study it was observed that pyruvic acid caused the islets of Langerhans to secrete insulin due to the forced expression of a carrier for pyruvic acid<*Subscript*>3</*Subscript*>.
これは，ピルビン酸逆説の解明に繋がる興味深い結果であった．	This was an interesting result that paradoxically tied pyruvic acid to the explanation of the mechanism of insulin secretion.
b 細胞量の維持機構の研究	Research into the mechanism of maintaining beta cell mass
1.b 細胞の生存と小胞体ストレスジュネーブ大学での留学を終え，岡芳知教授のもとで，同教授が山口大学の谷澤幸生教授らとともに発見したウオルフラム症候群原因遺伝子 Wfs1 の解析に携わった．	After completing a period of research abroad concerning beta cell survivals and endoplasmic reticulum stress in the University of Geneva, I began studying under Dr Yoshitomo Oka who was coworkers with Dr

	Yukio Tanizawa. I then participated in a study into Wfs1, a gene causing Wolframin's Syndrome, a disease which Tanizawa et al. discovered.
Wfs1 遺伝子破壊マウスの膵島では，グルコースによるインスリン分泌応答の異常が生じ，その過程に b 細胞内でのカルシウム動態の異常が関与していることを明らかにした 6).	In the islets of Langerhans of wfs1 gene disrupted mice, it was found that dysfunction in insulin secretion in response to glucose developed and that that process is connected to a dysfunction in calcium movement within beta cells<Subscript>6</Subscript>.
また，同時に，WFS1 タンパクが欠損する状態では，b 細胞での小胞体ストレス応答の亢進が認められ，b 細胞がアポトーシスに陥りやすくなっていることが明らかとなった 7,8).	However, at the same time, it has been observed that there is acceleration of the endoplasmic reticulum stress response in conditions in which there is a lack of WFS1 proteins. It has become evident that in these conditions it becomes likely that beta cell apoptosis will be incurred<Subscript>7,8</Subscript>.
小胞体ストレス応答亢進の一因は，b 細胞内でのカルシウム動態の異常であると考えられる.	It is believed that one of the causes of acceleration in the endoplasmic reticulum stress response is dysfunction of calcium movement within beta cells.
すなわち，Wfs1 遺伝子破壊マウスの膵島では，インスリン分泌機能の低下とともに，アポトーシスが亢進する結果，膵 b 細胞量の低下も起こり，個体としてのインスリン分泌不全に陥って糖尿病が発症すると考えられた.	In other words, in the islets of Langerhans of Wfs1 gene deleted mice, as well as decline in the functionality of insulin secretion, there is a decrease in pancreatic beta cell mass due to accelerated apoptosis. It is thought that this leads to hyposecretion of insulin which in turn results in the pathogenesis of diabetes.
2.b 細胞のストレス応答と翻訳制御	2. The Beta Cell Stress Response and Translation Regulation
さらに，Wfs1 遺伝子破壊マウス膵島における小胞体ストレス誘導アポトーシスの分子機構を検討する過程で，翻訳開始因子(eIF)4E 結合蛋白 1(eIF4E-bindingprotein1:4E-BP1)の発現が増加していることを見出した 9).	Whilst analysing the molecular mechanism of endoplasmic reticulum stress induced apoptosis in the islets of Langerhans of wfs1 gene deleted mice, it was discovered that the expression of translation initiation factor eIF4E-binding protein1: 4e-bp1) increased<Subscript>9</Subscript>.
4E-BP1 の増加は，Wfs1 遺伝子破壊マウス膵島に限ったものではなく，インスリン分子の異常による小胞体ストレス亢進によって糖尿病を発症する Akita マウスの膵島でも認められた.	The increase in 4E-BP1 was not only observed in the islets of Langerhans of Wfs1 gene deleted mice but was also observed in the islets of Langerhans of Akita mice where pathogenesis of diabetes was generated through endoplasmic reticulum stress caused by insulin secretion dysfunction.

この小胞体ストレス応答における 4E-BP1 の発現増加は，ストレス応答のマスター転写因子 ATF4 による 4E-BP1 の転写活性化によることを明らかにし，4E-BP1 遺伝子のイントロン 1 の内部に ATF4 の結合領域を見出した.	Evidence that the increased expression of 4E-BP1 that occurs as a endoplasmic reticulum stress response is caused by the transcription activation of 4E-BP1 by the master transcription factor for the stress response, ATF4. The binding region of ATF4 was found within intron 1 of the 4E-BP1 gene.
Wfs1 遺伝子破壊マウスや Akita マウスにおける 4EBP1 誘導の意義を解析するため，Akita マウスあるいは Wfs1 遺伝子破壊マウスと 4E-BP1 遺伝子破壊マウスを交配して 2 重変異マウスを作製し，解析した.	In order to analyse the significance of 4EBP1 induction in WFS1 gene deleted mice and Akita mice, a second generation cross was created by cross-breeding either the Akita mice or Wfs1 deleted mice with the 4E-BP1 gene deleted mice. The F2 mice were then analysed.
Akita マウスおよび Wfs1 遺伝子破壊マウスのいずれにおいても，4E-BP1 の欠損が b 細胞障害を進行させ，耐糖能障害を悪化させることが観察された.	It was observed that in either case, whether crossbred with a Akita mouse or WFS1 gene deletion mouse, the lack of 4E-BP1 caused advancing beta cell damage and worsened glucose intolerance.
小胞体ストレス下の b 細胞では，タンパク合成を抑制しておくことが長期的な生存にとっては有利であり，その役割を 4E-BP1 が担っているものと考えられた.	The regulation of protein synthesis in beta cells under endoplasmic reticulum stress is beneficial to long-term survival and it is thought that 4E-BP1 fulfils this role.
おわりに	Conclusion
b 細胞障害が 2 型糖尿病発症において不可欠であることは疑いがなく，そこには，b 細胞量の低下と機能不全の両者が存在するものと思われる.	There is no doubt that beta cell damage is essential to Type 2 diabetes pathogenesis. However, it is thought that there are two factors contributing to this: a decrease in beta cell mass as well as beta cell dysfunction.
Wfs1 遺伝子の変異による細胞内カルシウム動態の異常が，インスリン分泌を低下させるとともにアポトーシスを亢進させるように，ある 1 つの細胞機能の異常は，細胞の生存と高度に分化した機能であるインスリン分泌の両者に多かれ少なかれ影響を与えると考えられる.	Dysfunction in calcium movement within the cell due to Wsf1 gene mutation causes a decrease in insulin secretion alongside an acceleration of apoptosis. It is thought that just one type of cell dysfunction will have an effect on both cell survival and also the highly specialised function of insulin secretion to a greater or lesser extent.
b 細胞のインスリン分泌と生死のメカニズムの全貌を解明し，2 型糖尿病におけるその障害を明らかにして，糖尿病の治療に役立てられるよう取り組んでいきたい.	For future research, I would like to endeavour to elucidate a comprehensive view of the mechanisms of insulin secretion and cell death in beta cells, further the understanding of the disorder of type 2 diabetes in order to use this knowledge to treat diabetes.
謝辞	Acknowledgments

このたびのリリー賞受賞にあたり，糖尿病の臨床，教育ならびに基礎研究のすべてにわたって，これまでご指導いただきました東北大学大学院医学系研究科糖尿病代謝科岡芳知教授に深謝いたします.	Upon this occasion of being awarded the Lilly Prize, I would like to express my sincere gratitude to Professor Yoshitomo Oka of the Diabetes Metabolism department of Tohoku University School of Medicine for all his guidance across all of the clinical diabetes research, eduction and fundamental research.
また，長い間，b 細胞研究の全般にわたってご教示いただきました朝日生命新人病研究所菊池方利所長，分子生物学の初歩からご指導いただきました MIN6 細胞の樹立者である大阪大学医学部幹細胞制御分野宮崎純一教授，また，留学当時から今日まで貴重なご助言を下さった Geneve 大学 ClaesB.Wollheim 教授に心から感謝いたします.	Additionally, I'd like to express my appreciation for the teaching I received throughout all of my research into beta cells in general from the director of the Asahi Life institute for research into new and emerging diseases Mr. Masatoshi Kikuchi. I'd also like to express my sincere gratitude to Professor Junichi Miyazaki who specialises in the field of stem cell management in Osaka University's Faculty of Medicine and established the MIN6 cell, for his guidance during my initial stages of molecular biological research. Equally, I'd like to express my sincere thanks to Professor Claes B. Wolheim of Genevia University for all his valuable advice from my time studying abroad up until today.
さらに，深夜まで実験*<600>*・*</600><603>*研究をともに*</603>*行って下さった多くの先生方に厚くお礼申し上げます.	Lastly, I would like to express my sincere gratitude to all of my numerous mentors who even went as far as to help me perform experiments and research late into the night.

Text 3

Watada, Hirotaka (2009) '膵 b 細胞容積調節機構に関する研究' [The Clinical Application of the Mechanism for Regulating Beta Cell Mass in the Treatment of Type 2 Diabetes], *糖尿病 [Diabetes]* 52(11): 881-883.

Source segment	**Target segment**
*<27/><30>*リリー賞受賞講演*</30>*	The Lilly Research Award
膵*<36>*β*</36>*細胞容積調節機構に関する研究	The Mechanism for Regulating B-Cell Mass
綿田裕孝	Hirotaka Watada
IPF-1	IPF-1
研究開始時の私共の疑問は，なぜ，インスリンは，ほぼ膵 β 細胞に限局して発現するのかということであった.	Our initial research question was whether insulin expression was confined to pancreatic b-cells.
この疑問の解決のための第一歩として，インスリン遺伝子の転写調節機構の解明に携わりたいと考えた.	The first step to answer this question was to investigate the mechanism for the regulation of insulin gene transcription.
そのためにインスリン遺伝子エンハンサー領域に結合する転写因子 IPF-1 に焦点を当て実験することとした.	Accordingly, we decided to focus the investigation on Insulin Promoter Factor-1 (IPF-1), a transcription factor which binds to the insulin gene promoter region.
その結果，IPF-1 が膵 β 細胞のブドウ糖センサーである膵 β 細胞型グルコキナーゼ遺伝子および IAPP 遺伝子プロモーターに結合し，それぞれの遺伝子の転写活性化を行うことを見出した 1).	We found that IPF-1 binds to the glucose sensor of the b-cells, the pancreatic beta cell glucokinase gene or the IAPP gene promoter region and activates the transcription of the respective genes.*<Subscript>*1)*</Subscript>*
ただし，当時の遺伝子発現調節メカニズムの検討は，主にレポーター遺伝子アッセイやゲルシフトアッセイなどを用いて行っており，IPF-1 が本当にゲノムに存在するそれぞれの遺伝子のプロモーターに結合し，遺伝子発現を活性化させるのかということに関しては，さらなるデータが必要と考えた.	However, the investigation into the mechanisms of regulating gene expression at the time was carried out mainly using reporter gene assays and gel shift assays. However, in order to investigate whether IPF-1 actually does bind to the respective gene promoters which are present on the genome and cause this activation of the gene expression further data was necessary.
そこで，膵 a 細胞株 aTC1 細胞に外来性に IPF-1 遺伝子を発現させた.	Therefore, we forced the IPF-1 gene to be expressed exogenously in a pancreatic a cell line: aTC1.

すると，Betacellulin 存在下で極めて低レベルではあるものの，インスリン，グルコキナーゼ，IAPP という膵 β 細胞特異的遺伝子の発現が誘導されることを見出した 2).	Once expressed, we discovered that, albeit in the presence of extremely low levels of Betacellulin, this induced the expression of genes specific to pancreatic b cells: insulin, glucokinase and IAPP.<Subscript>2)</Subscript>
この結果は，当初の研究目的のとおり，IPF-1 が各膵 β 細胞特異的遺伝子の発現を直接活性化するという強い証拠となったが，同時に，筆者らは，この実験結果を受け，内因性インスリン遺伝子が発現している細胞を類膵 β 細胞と仮に呼ぶとすれば，本実験の結果は，IPF1 遺伝子発現が非膵 β 細胞を類 β 細胞化したと解釈できるかもしれないと考えた.	This means that, in accordance with our initial research objectives, this provides strong evidence that IPF-1 directly activates the expression of each type of pancreatic b cell specific genes. For example, if endogenous cells which express the insulin gene can be called to be varieties of pancreatic b cells, then in addition, in consideration of the result of this experiment it could be interpreted that the expression of IPF-1 causes non-pancreatic b cells to become a variety of pancreatic b cells.
もし，そうだとすると，転写因子を用いて内因性遺伝子発現を変化させる分化誘導法は，将来的には，糖尿病患者に不足している膵 β 細胞を補充する新規治療法の開拓につながるのではないかと考えた.	If we assume the aforementioned, then in the near future, the method of inducing differentiation using transcription factors to change the endogenous gene expression could be used in a trailblazing new method to treat diabetes sufferers with insufficient levels of b cells by replenishing the b cells.
そのためには，細胞内での遺伝子発現パターンを膵 β 細胞にできるだけ近似させなければならないわけであり，その目的のためには，生理的な膵 β 細胞の発生過程を解明し，その過程を模倣することで膵 β 細胞分化誘導法を考案することが重要ではないかと考えた.	In order to achieve this, it is necessary to approximate the intracellular gene expression patterns of pancreatic β cells as closely as possible. To achieve this objective, it can be considered imperative to elucidate the physiological developmental process of pancreatic β cells and then imitate this process in order to devise a method of inducing pancreatic β cell differentiation.
なお，これらの研究結果を報告する前後で，IPF-1 の統一呼称名が Pdx1 となり，MODY の原因遺伝子であることも報告された.	Furthermore, at around the same time that these research results were announced, the term IPF-1 was consolidated into being synonymous with the term Pdx1 and it was announced that Pdx1 is a causative gene of MODY.
膵 β 細胞発生分化過程と転写因子カスケード	The pancreatic β cell development and differentiation process and the transcription factor cascade

そこで，筆者は，膵β細胞発生過程を調節している転写因子カスケードの解明に携わった.	Next, the author came to be involved in research into the elucidation of the transcription factor cascade which regulates that pancreatic β cell developmental process.
膵臓は，発生学的には一層の内胚葉上皮細胞に由来する.	The pancreas is derived from one embryological germ layer, namely the epithelial cells of the endoderm.
この一部の細胞が膵内分泌前駆細胞となり，膵内分泌前駆細胞から数々の分化ステップを経て，成熟した膵β細胞ができる.	This section of cells become pancreatic endocrine precursor cells. These pancreatic endocrine precursor cells then goes through numerous steps of differentiation to become a mature pancreatic β cell.
膵前駆細胞には Pdx1 が発現しており，内胚葉上皮細胞から，膵前駆細胞への分化に大きな役割を果たすと考えられている.	It is thought that the expression of Pdx1 fulfils a considerable role in the development of pancreatic precursor cells through the differentiation of the pancreatic endoderm epithelial cells into pancreatic precursor cells.
膵前駆細胞から膵内分泌前駆細胞への分化に関わる転写因子が Neurogenin3(Ngn3)である.	One of the transcription factors which is involved in the differentiation of pancreatic precursor cells into endocrine cells is Neurogenin-3 (Ngn3).
筆者らは，Ngn3 遺伝子の発現調節機構を解明し，HNF3β や HNF6 などの内胚葉に発現する転写因子，Notch シグナル，Activin や HGF シグナルなどが極めて複雑に Ngn3 の遺伝子発現に関与していることを見出した 3).	Our team elucidated the mechanism for regulating the expression of theNgn3 gene. It was discovered that transcription factors which are expressed in the endoderm such as HNF3β and HNF6 as well numerous signalling pathways such as Notch signalling, Activin signalling and HGF signalling have an extremely complex involvement in the gene expression of Ngn3<Subscript>3</Subscript>.
一方，膵β細胞分化因子，Pax4，Nkx2.2 遺伝子の発現調節機構を検討すると，Ngn3 が発現すると自動的にこれらの転写因子が発現するかのように，Ngn3 と HNF 転写因子群との協調作用により遺伝子発現が調節されていることが明らかになった 4).	On the other hand, upon the examination of the mechanism for regulating the gene expression of the pancreatic β cell differentiation factors Pax4 and Nkx2.2 it seemed that when Ngn3 was expressed these transcription factors were also automatically expressed. It was thus shown that the Ngn3 and HNF groups of transcription factors regulate gene expression through synergistic action<Subscript>4.</Subscript>
一方，Nkx2.2 遺伝子の下流に存在する転写因子 Nkx6.1 の発現調節機構は，転写後発現調節機構も含めて，極めて複雑に調節されていることを見出した 5,6).	On the other hand, it was discovered that the mechanism for gene regulation, including the mechanism of post transcriptional regulation of the transcription factor Nkx6.1 which lies downstream from

	transcription factor Nkx2.2, is extremely intricately regulated<Subscript>5,6</Subscript>.
なお，Nkx6.1 の下流に MafA という強力なインスリン遺伝子転写活性化因子が存在することは，明らかにされていた.	Furthermore, it was disclosed that the potent insulin gene transcription activator MafA lies downstream of Nkx6.1.
そこで，発現調節機構が複雑で，かつ膵 β 細胞分化に重要な転写因子群として，Pdx1，Ngn3，Nkx6.1 を選別し，非膵 β 細胞から膵 β 細胞への分化誘導を試みた.	Thereupon, we attempted to induce differentiation of non-pancreatic β cells into pancreatic β cells through this complex mechanism for regulating gene expression and by classifying the crucial transcription factors in pancreatic β cell differentiation Pdx1. Ngn3 and Nkx6.1.
膵前駆細胞のモデル細胞株である AR42J-B13 細胞は Pdx1 をもともと発現している.	The cell line model for pancreatic precursor cells AR42J-B13 cells expressed Pdx1 from the offset.
この細胞に Ngn3 を強制発現させると，Nkx2.2 や Pax4 の発現が認められた.	The expression of Nkx2.2 and Pax4 was observed when the AR42J-B13 cells were forced to express Ngn3.
そこに，Nkx6.1 を発現させてもインスリンの発現は認められなかったが，代わりに MafA を強制発現させるとインスリンの発現が著明に認められた 7).	Insulin expression was not observed when there was forced expression of Nkx6.1. Instead, it was when there was forced expression of MafA that insulin expression was clearly observed<Subscript>7</Subscript>.
ちょうどこの論文を報告したとき，Melton らのグループは膵外分泌細胞に Pdx1，Ngn3，MafA を強制発現することで，膵 β 細胞への分化誘導に成功したことを Nature 誌に報告した.	At precisely the same time as the publication of this paper, Melton et al. published a paper in Nature that they succeeded in inducing differentiation into pancreatic β cells by forcing the expression of Pdx1, Ngn3 and MafA in exocrine cells.
これらの結果から，膵 β 細胞の発生分化機構を解明し，それらの知識を集積させると，将来的な新規膵 β 細胞分化誘導法の確立に役立つ可能性が強く示唆された.	From these results it is strongly suggested that the elucidation of the mechanism for the development and differentiation of pancreatic β cells and the gathering of extensive information on this mechanism could be useful in the establishment of a new future technique to induce differentiation into pancreatic β cells.
既存の膵 β 細胞の容積を増加させるために	Increasing the cell mass of existing pancreatic β cells
—膵 β 細胞容積に影響を与える因子の解明—(Fig.)	- Explanation of the factors influencing pancreatic β cell mass-
以上のような膵 β 細胞分化誘導法は，膵 β 細胞容積が低下している糖尿病の将来の治療として有用である.	Methods of inducing pancreatic β cell differentiation such as the above are valuable in the development of a future therapy to treat cases of diabetes where the pancreatic β cell mass is in decline.

膵β細胞容積増加のためのその他の戦略としては，膵β細胞容積に影響を与える因子を解明し，その因子が2型糖尿病状態下で作用低下しているのであれば，それを補うようにすれば，2型糖尿病の新規治療法の確立が可能と考えられる.	Another strategy for increasing pancreatic β cell mass involves elucidating of the factors which influence pancreatic β cell mass. Then, if these factors act as an agent to decrease the diabetic state then it is thought that it would be possible to establish a new therapeutic technique for type 2 diabetes through supplementing these factors.
そこで，まず着目したのが，膵ランゲルハンス島の血管構築である.	Accordingly the first thing that attracted research attention was the angioarchitecture of the pancreatic islets of Langerhans.
健常者では膵β細胞容積増加時に膵ラ氏島の血管密度が増加し，逆に2型糖尿病では，膵ラ氏島の血管密度が減少することが知られている.	It is established that when pancreatic β cell mass increases in healthy individuals then the vascular density of the islets of Langerhans increases, whilst under the same conditions, the vascular density of the islets of Langerhans decreases in individuals with type 2 diabetes.
すなわち，膵ラ氏島血管と膵β細胞機能とには明確な相関があるが，これが原因か，ただの相関であるのかは明らかでなかった.	In other words, although there is an evident correlation between the islets of Langerhans vasculature and pancreatic β cell function, it is not clear whether this pertains to a causative relationship or whether it is just mere correlation.
そこで，膵ラ氏島の血管不全モデルとして，膵β細胞特異的血管内皮細胞増殖因子(VEGF)-Aノックアウトマウスを用いて，膵β細胞機能を検討した.	Thus, using a model of vascular insufficient islets of Langerhans we investigated pancreatic β cell function through the use of pancreatic β cell specific vascular endothelial growth factor (VEGF)-A knockout mice.
その結果，膵β細胞の血管構築は正常な膵β細胞機能に必須であるが，定常状態の膵β細胞容積には無関係であること，骨髄移植の膵β細胞容積増加機能には必須であるが，インスリン抵抗性による膵β細胞容積増加機構には正常の血管構築は必須ではないことが明らかになった8,9).	<Italic>As a result it was shown that although the angioarchitecture of the pancreatic β cells is crucial in normal pancreatic β cell function, it is unrelated to pancreatic β cell mass in a steady state. Moreover, although essential for an increase in the function of bone marrow transplanted pancreatic β cell mass normal angioarchitecture was not required for the mechanism of increasing pancreatic β cell mass because of insulin resistance</Italic><Subscript><Italic>8,9.</Italic> </Subscript>
次に，着目したのはオートファジー機構である.	The next focus of research was the mechanism of autophagy.
オートファジーは不要な蛋白を除去する細胞内浄化という点で重要である.	Autophagy has an important function in the process of intracellular cleaning by removing unnecessary proteins.
膵β細胞におけるオートファジーの状態を検討すると，インスリン抵抗性がオートファジーを誘導するものの，dβ/dβマウスなどの糖尿病モデ	Although, insulin resistance induces autophagy, in analysing the state of autophagy within pancreatic β cells, results were obtained that

ルマウスの膵β細胞ではオートファジー不全を示唆する結果が得られた.	suggested that there was an autophagy deficiency in the pancreatic β cells of diabetic mouse models including db/db mice.
次に，膵β細胞におけるオートファジーの意義を検討する目的でオートファジー機構に必須な ATG7(autophagy-specificgene7)を膵β細胞特異的にノックアウトした膵β細胞特異的 ATG7 ノックアウトマウスを作成した.	Next, in order to investigate the significance of autophagy in pancreatic β cells, pancreatic β cell specific autophagy-specific gene 7 (ATG7) knockout mice were created by knocking out the pancreatic β cell specific form of ATG7, which is essential in the mechanism of autophagy.
その結果，膵ラ氏島における恒常的オー'トファジー不全は，ミトコンドリアでのアデノシン 5-三リン酸(ATP)産生能の低下を介して，ブドウ糖応答性インスリン分泌低下をもたらすことが示唆された.	As a result, it was suggested that a deficiency of constitutive autophagy causes a reduction in insulin secretion in response to glucose through a reduction in mitochondrial adenosine-5-triphosphate (ATP) productivity.
また，インスリン抵抗性による誘導性オートファジーは，膵β細胞増殖促進とアポトーシスの抑制を介して，膵β細胞容積を増加させるのに必須な機構であることが明らかになった 10).	Moreover, it was found that induction of autophagy triggered by insulin resistance is a necessary mechanism for increasing pancreatic β cell mass through the promotion of the proliferation of pancreatic β cells and the suppression of apoptosis<Subscript>10</Subscript>.
以上をあわせると，2 型糖尿病モデルマウスで認められる膵β細胞におけるオートファジー不全は，ブドウ糖応答性インスリン分泌低下の原因となり，また，インスリン抵抗性による膵β細胞容積増加不全の原因となりうることが明らかになった.	To summarise the above, it was proven that the autophagy deficiency observed in the pancreatic β cells of type 2 diabetic mouse models causes the reduction in insulin secretion in response to glucose. It was also proven that an autophagy deficiency could also cause the insulin resistance-induced deficiency in increasing pancreatic β cell mass.
今後，この膵β細胞オートファジー不全を改善させることが出来れば，2 型糖尿病の根本治療に役立つかもしれない.	If it is possible to improve pancreatic β cell autophagy deficiencies then this research could be applied to a radical cure for type 2 diabetes in future.
さいごに	Conclusion
今後も，リリー賞受賞を励みに，糖尿病における膵β細胞の病態解明，糖尿病治療につながる研究を行ってゆきたい.	Under the encouragement offered to me by the award of the Lilly prize, I will continue to engage in research related to finding a cure for diabetes and the elucidation of the pathology of pancreatic β cells in individuals with diabetes.
謝辞	Acknowledgments
今回の受賞は，関係の諸先生方，同僚の皆さま方のご指導，ご鞭撻なしにはなし得なかったことで，この場を借りて深謝いたします.	I would like to take this opportunity of being awarded the Lilly Prize to express my sincere thanks to all the professors involved in my research

	and all of my colleagues. I couldn't have done it without all your guidance and encouragement.
特に終始お世話になった恩師の河盛隆造先生に厚く御礼申し上げたいと存じます.	I would particularly also like to express my deep gratitude to Dr. Ryuzo Kawamori who oversaw my research from conception to completion.
さらに，大阪大学第一内科で直接ご指導頂いた梶本佳孝先生，留学中，UCSF にてご指導頂いた MichaelS.German 先生をはじめとする諸先生方，さらにこれまで共同研究などをしてくださった大変多くの先生方にも厚く御礼申し上げたいと思います.	Finally, I'd like to express deep gratitude to all of the various mentors who guided me including Dr. Yoshitaka Kajimoto who directly supervised me in Osaka University's Internal Medicine department and Dr. Michael S. German who supervised me during my research abroad at UCSF and also to the numerous colleagues who assisted me with collaborative research.

Appendix B: Texts with numbered sentences before restructuring

Text 1: Yamamoto, Hiroshi (2014) '糖尿病合併症の成因・病態・克服に関する基礎的研究' [Fundamental Research on the Causes, Pathology and Subjugation of Diabetes Complications], *糖尿病 [Diabetes]* 57(10): 765-711.

(1) The Hagedorn Prize: **Fundamental Research on the Causes, Pathology and Subjugation of Diabetes Complications**

(2) Hiroshi Yamamoto

(3) Introduction

(4) The first time I heard the name Hagedorn, the namesake of the Hagedorn Prize, was in the book 'The method for measuring microdosing in medicinal chemistry'$_1$ which my first professor of medicinal chemistry at Kanazawa Medical University, Kenzou Sutou authored.

(5) Among the methods established by Sutou was the method for measuring the glucose concentration in urine. Sutou was such a significant pioneer in the field of diabetes research in Japan that he was known by the name 'Professor Nutrition'.

(6) In 'The method for measuring microdosing in medicinal chemistry' the Hagedorn-Jensen Method was introduced as a method to both easily and accurately measure blood glucose concentration using a fixed quantity of one or two drops of blood.

(7) I engaged in diabetes research under the guidance of Professor Hiroshi Okamoto specialising in research into the molecular biology of the pancreatic Islets of Langerhans whilst researching at Toyama Medical and Pharmaceutical University and at my previous post in Tohoku University.

(8) I was involved in the discovery that alloxan and streptozotocin induce DNA strand breaks in the pancreatic Islets of Langerhans$_2$ and in the edited volume 'Molecular Biology of the Islets of Langerhans'$_3$.

(9) **From research in vascular biology to research into the complications of diabetes**

(10) After appointment to a new research post at Kanazawa University in 1990, I started researching into vascular biology.

(11) The co-cultivation of the types of constituent vascular cells was established and it was found that pericytes inhibited the proliferation of endothelial cells and preserved the production of prostacyclin in addition to preventing endothelial cell damage by lipid peroxidates[4].

(12) This discovery gave an insight into why neovascularization accompanies pericyte loss in cases of diabetic retinopathy.

(13) So, what is the primary factor which induces characteristic changes in every type of vascular cell in those presenting with diabetes?

(14) In order to answer this question, we used an axenic culture of endothelial cells and pericytes in an investigation using a variety of environmental factors and as a result, identified the factor as advanced glycation end products (hereafter: AGE)[5,6]. (Figure 1)

(15) AGE is the term used to denote the irreversible products formed through a non-enzymatic reaction between the carbonyl group on a sugar and the amino group on a protein.

(16) The process of AGE formation and accumulation is accelerated by a chronic hyperglycaemic state.

(17) Despite glucose being an efficacious source of energy, it still has the issue of glycation.

(18) Professor Seiichiro Tarui of Osaka University noted “In the stages of evolution in vertebrates the release of glucose to be used within a closed circulatory system as blood sugars led to blood circulation. However, this means that the chance of individuals with diabetes presenting was possible from inception and that the risk of developing diabetes was an inevitable complication”[7].

(19) **Proof that AGE-RAGE is a cause of diabetes complications using genetically modified animals**

(20) At least a part of the action that AGE has on vascular cells is achieved through the specific receptor for AGE (hereafter RAGE).

(21) RAGE is classified as a pattern recognition receptor and is recognised as a ligand by various pathogen-associated molecular patterns (PAMP)and damage- associated molecular patterns (DAMP).(Figure 2a)

(22) Associate Professor Yasuhiko Yamamoto and his team[8,9] created transgenic mice that over-expressed RAGE within their vascular cells and mice that endogenously lacked the RAGE gene.

(23) Yamamoto then induced diabetes within the mice and analysed the resulting complications.

(24) It was found that index for diabetic nephropathy[8] or diabetic retinopathy[10] in the mice made to overexpress RAGE showed signs of worsening (Figure 2B and 2C).

(25) Whilst the RAGE deficient mice did not develop any symptoms of diabetic nephropathy[9] (Figure 2D).

(26) These results indicate that there is a functional interaction between the pathogenesis of diabetes complications and AGE and RAGE.

(27) **The Transcriptional Regulatory Mechanism for the human RAGE gene**

(28) Tanaka[11] and his team investigated the transcriptional regulatory mechanism for the human RAGE gene.

(29) As a result it was discovered that it is AGE itself which activates the transcription factor nuclear factor-κB（NF-κ B）and activates the transcription of the RAGE gene forming a positive feedback loop.

(30) This research forms the molecular basis for the apparent constitutive RAGE expression and AGE-RAGE colocalization in the observed diabetic state.

(31) Among the numerous RAGE-flanking signalling pathways the NF-κ B intermediary pathway is considered to be particularly important in regard to diabetes.

(32) The involvement of AGE-RAGE in pancreatic β cell deficiency

(33) The cell dysfunction in pancreatic β cells within the islets of Langerhans and the decline in β cell mass which accompanies the progression of Type 2 diabetes is well established.

(34) Han et al. have carried out investigations to find out whether there is a connection between AGE-RAGE and the aforementioned aspects of Type 2 diabetes pathogenesis.

(35) Surprisingly, Han et al.[12] found that the RAGE protein was not detected on the cell surface of normal β cells.

(36) However, in animal models of type 2 diabetic mice, the ratio of RAGE protein positive pancreatic β cells within ob/ob mice and db/db mice increased with age.

(37) It was discovered that when the db/db RAGE deficient mice were then crossbred, the apoptosis induced reduction in cell mass and impaired glucose tolerance associated with advancement of diabetes improved (Figure 4).

(38) In addition, the most remarkable thing observed was the cell death due to AGE exposure.

(39) MIN-6 cells were used in this analysis, free fatty acids and leptin receptor antagonists were then administered to these cells, causing them to induce the expression of RAGE proteins on the cells' surface, cell death then occurred when exposed to AGE.

(40) The above results are in line with the prevailing theories on the mechanism behind the deficiency in pancreatic β cells which is associated with Type 2 diabetes which postulate that free fatty acids and AGE at least play a part in the lipotoxicity and glucotoxicity contributing to the mechanism.

(41) How RAGE relates to other pathologies

(42) In a collaborative study with Dr. Hiroshi Kubo of Tohoku University[13,14] there were various discoveries concerning RAGE and its relationship to the epithelial-mesenchymal transition using a subacute inflammation model.

(43) In addition there were discoveries concerning RAGE specifically recognising phosphatidylserine and its involvement in the phagocytosis of apoptotic cells.

(44) Moreover, it was demonstrated in interdisciplinary research at Kanazawa University that both a RAGE deficiency and the overexpression of the aforementioned soluble RAGE protein significantly suppresses the uptake of amyloid β 1-42 peptide into the brain[15].

(45) **AGE-RAGE Targeting**

(46) From the research mentioned thus far, AGE-RAGE can be considered to be a possible target for treatment for all kinds of human diseases including diabetes and the complications associated with diabetes.

(47) Table 1 lists the theoretically conceivable strategies for the main AGE-RAGE therapeutic targets.

(48) The first option is the inhibition of the formation of AGE.

(49) However, the mechanism of action for the majority of currently developed AGE inhibitors is to target the covalent bonds of the AGE formation intermediate compound.

(50) This intermediate compound requires close to an equimolar of drugs resulting in a stoichiometric problem.

(51) Interestingly, it has been reported that angiotensin receptor blocker (ARB) has AGE formation inhibiting activity[16].

(52) The second option is a drug to break down already formed AGE.

(53) This type of drug belongs to a category of drugs called AGE breakers.

(54) However, an AGE breaker which is efficacious at breaking down AGE is yet to be developed.

(55) **Soluble RAGE**

(56) The third option is the addition of an extracellular decoy receptor for AGE that protects the vascular cells.

(57) Yonekura of Kanazawa Medical University[17] identified a decoy RAGE protein which was named endogenous secretory RAGE (esRAGE) by screening of human vascular cell polysomes and alternative RNA splicing.

(58) Motoyoshi et al. [18] demonstrated that it possible to induce membrane-bound RAGE proteins to convert into soluble RAGE proteins through MMP9 induced ectodomain shedding when the concentration of intracellular cyclic AMP is increased.

(59) **Assessment of AGE in food products and the advent of RAGE antagonists**

(60) The fourth option is the invention of a RAGE antagonist.

(61) When analysis of fluorescence resonance energy transfer (FRET) has been carried out using RAGE antibodies, at around the same time as ligand stimulation, without any change in fluorescence intensity originating from what is believed to be a RAGE monomer, the fluorescence intensity believed to be originating form an oligomer increased.

(62) It is thought that the agonist ligands which send intracellular signals make the RAGE receptors oligimerize.

(63) So, when a pharmacological assessment was carried out in which low-molecular weight AGE of a maximum molecular weight of 300 was prepared, it was discovered that low-molecular weight AGE showed signs of RAGE antagonist activity[19].

(64) In collaborative research with Kobayashi et al. we undertook research to determine the three-dimensional structure of the human RAGE protein[20].

(65) After screening low-molecular weight compounds based on their structural information *in silico*, several potential agents which showed RAGE antagonist activity were obtained through subsequent pharmacological assessment.

(66) Some varieties of food products are rich in AGE.

(67) These food product's taste partially originates from AGE including the colour, aroma and flavour.

(68) Of the intake of food derived AGE, approximately 10% is absorbed into the blood stream. Yet after 48 hours 70% of the AGE consumed remains within the body[21].

(69) Conventionally in the Western world, the dominant view has been that food derived AGE is regarded as harmful to health.

(70) However, it is also thought that further investigation into the biological effects of AGE in food products is required.

(71) Munesue et al.[19] carried out an assessment of RAGE as a agonist/antagonist using the following food within their assessments: soy sauce, coffee, red wine and cola.

(72) It was found that soy sauce, coffee and red wine neutralised the RAGE agonist activity of high molecular weight AGE and that it was low-molecular weight fractions that reversed the activity into being antagonistic.

(73) **Summary**

(74) The interaction between AGE, its ligands and RAGE is considered to be one of the causes of diabetic angiopathy and β cell failure in diabetes.

(75) Accordingly they are considered to be the primary, secondary and tertiary targets for prevention of these disorders.

(76) There is great reason to hope for the elimination of diabetes complications through the development of a RAGE antagonist drug or a method of inducing the production of a decoy variant of the RAGE protein will become a reality in the near future.

(77) **Conflicts of interest (COI)**: none to declare

(78) **Acknowledgements**

(79) I would like to express my profound gratitude to my respected emeritus professor Hiroshi Okamoto of Tohoku University for his stern but warm guidance concerning my diabetes research.

(80) I would also like to express heartfelt thanks to emeritus Professor Ryousuke Takeda of Kanazawa University for his frequent encouragement.

(81) This prize truly is a result of the collaborative research efforts of every member involved.

Text 2: Ishihara, Hisamitsu (2009) '2 型糖尿病発症における膵 b 細胞障害の分子機構' [The molecular mechanism of pancreatic beta cell damage in the pathogenesis of Type 2 diabetes], *糖尿病 [Diabetes]* 52(11): 884-866.

(1) The Lilly Prize: The molecular mechanism of pancreatic beta cell damage in the pathogenesis of Type 2 diabetes

(2) Hisamitsu Ishihara

(3) **Introduction**

(4) Type 2 diabetes is a complex disease where an impaired insulin secretion from the pancreatic beta cells complicated by insulin resistance in the skeletal muscle, adipose tissue and liver results in the pathogenesis and progression of the disease.

(5) When I first decided to make my specialism diabetes and started my research in the laboratory of medical office, I was surrounded by people engaging in research into insulin resistance as a research topic amounting academic interest at the time.

(6) Isolating the islets of Langerhans or pancreatic beta cells is not a simple matter.

(7) If it is not possible to obtain plentiful amounts of research materials then this becomes a major stumbling block for progressing the research.

(8) **Research into beta cell function: engineering of nutrient-stimulated insulin secretion**

(9) Fortunately, in 1990 the MIN6 cell, which is still currently widely used global and considered the most typical insulin-secreting cell line, was created.

(10) This solved the issue of the difficulty obtaining research materials.

(11) As it is noted that there is frequent latent excessive response to insulin secretion at the stage of initial pathogenesis of Type 2 diabetes or at the stage of impaired glucose tolerance, it is not possible to observe the absolute amount of decline of beta cells,

(12) As such, in the first half of the 1990s, the prevailing view became that rather than researching the decline in amount of beta cells resulting in abnormalities, that instead the research target was the abnormalities in the mechanism for recognising glucose concentration.

(13) Thus, I considered this research to not only elucidate the details of the mechanism for recognising glucose concentration in beta cells but also to assist in the treatment of type 2 diabetes through the treatment of insulin secretion disorders.

(14) Therefore, I carried out analysis using MIN6 cells and incorporating genetic engineering technology, testing out how the insulin secretion response changed upon introduction of different kinds of genes.

(15) The method employed used genetic engineering in order to investigate the insulin secretions to a variety of nutrients including glucose.

(16) **Glucose-stimulated mechanism of insulin secretion**

(17) First, I focused research efforts on the enzymes in the glycolytic pathway.

(18) Then, I analysed the efficacy of over expressing the glucose transporter GLUT1 which uptakes glucose and hexokinase I which carries out glucose phosphorylation[1].

(19) In addition, taking into account the link between the glycolytic pathway and mitochondrial metabolism, the effect of the forced expression of uncoupling protein 1[4] (UCP1) was analysed.

(20) It was found that UCP1[4] has a suppressive role in the production of glycerol-3-phosphate dehydrogenase[2], lactic dehydrogenase[3] and adenosine 5'-triphosphate (ATP).

(21) To summarise the results of this investigation: 1) The rate-determining step of the glycolytic pathway in pancreatic beta cells was determined as glucose phosphorylation process and the role of the glucose sensor as regulating glycolytic flux were discovered.

(22) 2) It was found that there is a highly functional connection between the glycolytic pathway and mitochondrial metabolism.

(23) 3) It was clarified that mitochondrial metabolism not only fulfils an important role for ATP, but also in the formation of insulin secretion signalling.

(24) **The glucose specificity of insulin secretion**

(25) In the process of engineering an insulin secretion mechanism which responds to nutrients that causes beta cell insulin secretion to be restricted to responding to glucose it became clear that these cells possessed different features to other cells.

(26) Namely, in order to ensure that beta cells do not secrete insulin in response to nutrients other than insulin, it became clear that there were no transporters on the cell membranes of these cells.

(27) In fact, dicarboxylic acid, an intermediary product of the TCA cycle forced the expression of dicarboxylic acid transporters and insulin secretion was observed in response to dicarboxylic acid[5]

(28) In addition, in the results of an international study pyruvic acid-stimulated insulin secretion was observed in the islets of Langerhans due to the forced expression of a transporter for pyruvic acid[3].

(29) This was an interesting result that paradoxically tied pyruvic acid to the explanation of the mechanism of insulin secretion.

(30) **Research into the mechanism of maintaining beta cell mass**

(31) After completing a period of research abroad concerning beta cell survivals and endoplasmic reticulum stress in the University of Geneva, I began studying under Dr Yoshitomo Oka who was a co-worker of Dr Yukio Tanizawa.

(32) I then participated in a study into Wfs1, a gene causing Wolframin's Syndrome, a disease which Tanizawa et al. discovered.

(33) In the islets of Langerhans of wfs1 gene disrupted mice, it was found that dysfunction in glucose stimulated insulin secretion developed and that that process is connected to a dysfunction in calcium movement within beta cells[6].

(34) Moreover, at the same time, it has been observed that there is acceleration of the endoplasmic reticulum stress response in conditions in which there is a lack of WFS1 proteins.

(35) It has become evident that in these conditions it becomes likely that beta cell apoptosis will be incurred[7,8].

(36) It is believed that one of the causes of acceleration in the endoplasmic reticulum stress response is dysfunction of calcium movement within beta cells.

(37) In other words, in the islets of Langerhans of Wfs1 gene deleted mice, as well as decline in the functionality of insulin secretion, there is a decrease in pancreatic beta cell mass due to accelerated apoptosis.

(38) It is thought that this leads to hyposecretion of insulin which in turn results in the pathogenesis of diabetes.

(39) 2. **The Beta Cell Stress Response and Translation Regulation**

(40) Whilst analysing the molecular mechanism of endoplasmic reticulum stress induced apoptosis in the islets of Langerhans of wfs1 gene deleted mice, it was discovered that the expression of translation initiation factor eIF4E-binding protein1: 4e-bp1) increased[9].

(41) The increase in 4E-BP1 was not only observed in the islets of Langerhans of Wfs1 gene deleted mice but was also observed in the islets of Langerhans of Akita mice where pathogenesis of diabetes was generated through endoplasmic reticulum stress caused by insulin secretion dysfunction.

(42) Evidence that the increased expression of 4E-BP1 that occurs as an endoplasmic reticulum stress response is caused by the transcription activation of 4E-BP1 by the master transcription factor for the stress response, ATF4.

(43) The binding region of ATF4 was found within intron 1 of the 4E-BP1 gene.

(44) In order to analyse the significance of 4EBP1 induction in WFS1 gene deleted mice and Akita mice, a second generation cross was created by cross-breeding either the Akita mice or Wfs1 deleted mice with the 4E-BP1 gene deleted mice.

(45) The F2 mice were then analysed.

(46) It was observed that in either case, whether crossbred with an Akita mouse or WFS1 gene deletion mouse, the lack of 4E-BP1 caused advancing beta cell damage and worsened glucose intolerance.

(47) The regulation of protein synthesis in beta cells under endoplasmic reticulum stress is beneficial to long-term survival and it is thought that 4E-BP1 fulfils this role.

(48) **Conclusion**

(49) There is no doubt that beta cell damage is essential to Type 2 diabetes pathogenesis.

(50) However, it is thought that there are two factors contributing to this: a decrease in beta cell mass as well as beta cell dysfunction.

(51) Dysfunction in calcium movement within the cell due to Wsf1 gene mutation causes a decrease in insulin secretion alongside an acceleration of apoptosis.

(52) It is thought that just one type of cell dysfunction will have an effect on both cell survival and also the highly specialised function of insulin secretion to a greater or lesser extent.

(53) For future research, I would like to endeavour to elucidate a comprehensive view of the mechanisms of insulin secretion and cell death in beta cells, further the understanding of the disorder of type 2 diabetes in order to use this knowledge to treat diabetes.

(54) **Acknowledgments**

(55) On this occasion of being awarded the Lilly Prize, I would like to express my sincere gratitude to Professor Yoshitomo Oka of the Diabetes Metabolism department of Tohoku University School of Medicine for all his guidance across all of the clinical diabetes research, education and fundamental research.

(56) Additionally, I'd like to express my appreciation for the teaching I received throughout all of my research into beta cells in general from the director of the Asahi Life institute for research into new and emerging diseases Mr. Masatoshi Kikuchi.

(57) I'd also like to express my sincere gratitude to Professor Junichi Miyazaki who specialises in the field of stem cell management in Osaka University's Faculty of Medicine and established the MIN6 cell, for his guidance during my initial stages of molecular biological research.

(58) Equally, I'd like to express my sincere thanks to Professor Claes B. Wolheim of Genevia University for all his valuable advice from my time studying abroad up until today.

(59) Lastly, I would like to express my sincere gratitude to all of my numerous mentors who even went as far as to help me perform experiments and research late into the night.

Text 3: Watada, Hirotaka (2009) '膵 b 細胞容積調節機構に関する研究' [The Clinical Application of the Mechanism for Regulating Beta Cell Mass in the Treatment of Type 2 Diabetes], *糖尿病 [Diabetes]* 52(11): 881-883.

(1)The Lilly Research Award

(2)The Mechanism for Regulating B-Cell Mass

(3) Hirotaka Watada

(4) IPF-1

(5) Our initial research question was whether insulin expression was confined to pancreatic b-cells.

(6) The first step to answer this question was to investigate the mechanism for the regulation of insulin gene transcription.

(7) Accordingly, we decided to focus the investigation on Insulin Promoter Factor-1 (IPF-1), a transcription factor which binds to the insulin gene promoter region.

(8) We found that IPF-1 binds to the glucose sensor of the b-cells, the pancreatic beta cell glucokinase gene or the IAPP gene promoter region and activates the transcription of the respective genes 1).

(9) However, the investigation into the mechanisms of regulating gene expression at the time was carried out mainly using reporter gene assays and gel shift assays.

(10) However, in order to investigate whether IPF-1 actually does bind to the respective gene promoters which are present on the genome and cause this activation of the gene expression further data was necessary.

(11) Therefore, we forced the IPF-1 gene to be expressed exogenously in a pancreatic a cell line: aTC1.

(12) Once expressed, we discovered that, albeit in the presence of extremely low levels of Betacellulin, this induced the expression of genes specific to pancreatic b cells: insulin, glucokinase and IAPP2).

(13) This means that, in accordance with our initial research objectives, this provides strong evidence that IPF-1 directly activates the expression of each type of pancreatic b cell specific genes.
(14) For example, if endogenous cells which express the insulin gene can be called to be varieties of pancreatic b cells, then in addition, in consideration of the result of this experiment it could be interpreted that the expression of IPF-1 causes non-pancreatic b cells to become a variety of pancreatic b cells.
(15) If we assume the aforementioned, then in the near future, the method of inducing differentiation using transcription factors to change the endogenous gene expression could be used in a trailblazing new method to treat diabetes sufferers with insufficient levels of b cells by replenishing the b cells.
(16) In order to achieve this, it is necessary to approximate the intracellular gene expression patterns of pancreatic β cells as closely as possible.
(17) To achieve this objective, it can be considered imperative to elucidate the physiological developmental process of pancreatic β cells and then imitate this process in order to devise a method of inducing pancreatic β cell differentiation.
(18) Furthermore, at around the same time that these research results were announced, the term IPF-1 was consolidated into being synonymous with the term Pdx1 and it was announced that Pdx1 is a causative gene of MODY.

(19) The pancreatic β cell development and differentiation process and the transcription factor cascade

(20) Next, the author came to be involved in research into the elucidation of the transcription factor cascade which regulates that pancreatic β cell developmental process.
(21) The pancreas is derived from one embryological germ layer, namely the epithelial cells of the endoderm.
(22) This section of cells become pancreatic endocrine precursor cells.
(23) These pancreatic endocrine precursor cells then goes through numerous steps of differentiation to become a mature pancreatic β cell.
(24) It is thought that the expression of Pdx1 fulfils a considerable role in the development of pancreatic precursor cells through the differentiation of the pancreatic endoderm epithelial cells into pancreatic precursor cells.

(25) One of the transcription factors which is involved in the differentiation of pancreatic precursor cells into endocrine cells is Neurogenin-3 (Ngn3).

(26) Our team elucidated the mechanism for regulating the expression of theNgn3 gene.

(27) It was discovered that transcription factors which are expressed in the endoderm such as HNF3β and HNF6 as well numerous signalling pathways such as Notch signalling, Activin signalling and HGF signalling have an extremely complex involvement in the gene expression of Ngn3[3].

(28) On the other hand, upon the examination of the mechanism for regulating the gene expression of the pancreatic β cell differentiation factors Pax4 and Nkx2.2 it seemed that when Ngn3 was expressed these transcription factors were also automatically expressed.

(29) It was thus shown that the Ngn3 and HNF groups of transcription factors regulate gene expression through synergistic action[4].

(30) On the other hand, it was discovered that the mechanism for gene regulation, including the mechanism of post transcriptional regulation of the transcription factor Nkx6.1, which lies downstream from transcription factor Nkx2.2, is extremely intricately regulated[5,6].

(31) Furthermore, it was disclosed that the potent insulin gene transcription activator MafA lies downstream of Nkx6.1.

(32) Thereupon, we attempted to induce differentiation of non-pancreatic β cells into pancreatic β cells through this complex mechanism for regulating gene expression and by classifying the crucial transcription factors in pancreatic β cell differentiation Pdx1, Ngn3 and Nkx6.1.

(33) The cell line model for pancreatic precursor cells AR42J-B13 cells expressed Pdx1 from the offset.

(34) The expression of Nkx2.2 and Pax4 was observed when the AR42J-B13 cells were forced to express Ngn3.

(35) Insulin expression was not observed when there was forced expression of Nkx6.1.

(36) Instead, it was when there was forced expression of MafA that insulin expression was clearly observed[7].

(37) At precisely the same time as the publication of this paper, Melton et al. published a paper in Nature that they succeeded in inducing differentiation into pancreatic β cells by forcing the expression of Pdx1, Ngn3 and MafA in exocrine cells.

(38) From these results it is strongly suggested that the elucidation of the mechanism for

the development and differentiation of pancreatic β cells and the gathering of extensive information on this mechanism could be useful in the establishment of a new future technique to induce differentiation into pancreatic β cells.

(39) Increasing the cell mass of existing pancreatic β cells

(40) - Explanation of the factors influencing pancreatic β cell mass-

(41) Methods of inducing pancreatic β cell differentiation such as the above are valuable in the development of a future therapy to treat cases of diabetes where the pancreatic β cell mass is in decline.

(42) Another strategy is increasing pancreatic β cell mass involves elucidating of the factors which influence pancreatic β cell mass.

(43) Then, if these factors act as an agent to decrease the diabetic state then it is thought that it would be possible to establish a new therapeutic technique for type 2 diabetes through supplementing these factors.

(44) Accordingly the first thing that attracted research attention was the vasculature of the pancreatic islets of Langerhans.

(45) It is established that when pancreatic β cell mass increases in healthy individuals then the vascular density of the islets of Langerhans increases, whilst under the same conditions, the vascular density of the islets of Langerhans decreases in individuals with type 2 diabetes.

(46) In other words, although there is an evident correlation between the islets of Langerhans vasculature and pancreatic β cell function, it is not clear whether this pertains to a causative relationship or whether it is just mere correlation.

(47) Thus, using a model of vascular insufficient islets of Langerhans we investigated pancreatic β cell function through the use of pancreatic β cell specific vascular endothelial growth factor (VEGF)-A knockout mice.

(48) As a result it was shown that although the vasculature of the pancreatic β cells is crucial in normal pancreatic β cell function, it is unrelated to pancreatic β cell mass in a steady state.

(49) Moreover, although essential for an increase in the function of bone marrow transplanted pancreatic β cell mass normal vasculature was not required for the mechanism of increasing pancreatic β cell mass because of insulin resistance[8,9].

(50) The next focus of research was the mechanism of autophagy.

(51) Autophagy has an important function in the process of intracellular cleaning by removing unnecessary proteins.
(52) Although, insulin resistance induces autophagy, in analysing the state of autophagy within pancreatic β cells, results were obtained that suggested that there was an autophagy deficiency in the pancreatic β cells of diabetic mouse models including db/db mice.
(53)Next, in order to investigate the significance of autophagy in pancreatic β cells, pancreatic β cell specific autophagy-specific gene 7 (ATG7) knockout mice were created by knocking out the pancreatic β cell specific form of ATG7, which is essential in the mechanism of autophagy.
(54) As a result, it was suggested that a deficiency of constitutive autophagy causes a reduction in glucose stimulated insulin secretion through a reduction in mitochondrial adenosine-5-triphosphate (ATP) productivity.
(55) Moreover, it was found that induction of autophagy triggered by insulin resistance is a necessary mechanism for increasing pancreatic β cell mass through the promotion of the proliferation of pancreatic β cells and the suppression of apoptosis[10].
(56) To summarise the above, it was proven that the autophagy deficiency observed in the pancreatic β cells of type 2 diabetic mouse models causes the reduction in insulin secretion in response to glucose.
(57) It was also proven that an autophagy deficiency could also cause the insulin resistance-induced impairment in increasing pancreatic β cell mass.
(58) If it is possible to improve pancreatic β cell autophagy deficiencies then this research could be applied to a radical cure for type 2 diabetes in future.

(59) Conclusion

(60) Under the encouragement offered to me by the award of the Lilly prize, I will continue to engage in research related to finding a cure for diabetes and the elucidation of the pathology of pancreatic β cells in individuals with diabetes.

(61) Acknowledgments

(62) I would like to take this opportunity of being awarded the Lilly Prize to express my sincere thanks to all the professors involved in my research and to all of my colleagues.
(63) I couldn't have done it without all your guidance and encouragement.

(64) I would particularly also like to express my deep gratitude to Dr. Ryuzo Kawamori who oversaw my research from conception to completion.
(65) Finally, I'd like to express deep gratitude to all of the various mentors who guided me including Dr. Yoshitaka Kajimoto who directly supervised me in Osaka University's Internal Medicine department and Dr. Michael S. German who supervised me during my research abroad at UCSF and also to the numerous colleagues who assisted me with collaborative research.

Appendix C: Restructured texts including original sentence numbers and additions/editing in red

Text 1: Yamamoto, Hiroshi (2014) '糖尿病合併症の成因・病態・克服に関する基礎的研究' [Fundamental Research on the Causes, Pathology and Subjugation of Diabetes Complications], *糖尿病 [Diabetes]* 57(10): 765-711.

(2) Winner of the Hagedorn Prize: Hiroshi Yamamoto

(1) **Fundamental Research on the Causes, Pathology and Subjugation of Diabetes Complications**

Abstract

The decline in β cell mass and vascular complications associated with Type 2 diabetes is well established. Although no viable therapies are currently available, a primary factor associated with these changes, the AGE-RAGE pathway, has become a promising therapeutic target. In this paper we use transgenic mice that overexpress or lack the RAGE gene to investigate the relationship between the AGE-RAGE interaction and diabetic complications. We explore two potential therapies through the creation of a decoy RAGE protein and a RAGE antagonist. Our results show a definite relationship between the AGE-RAGE interaction and diabetic complications with RAGE contributing to the apoptotic-induced reduction in β cell mass and impaired glucose tolerance associated with Type 2 diabetes. In identifying a potential therapy, we successfully promoted the solubilisation of membrane-bound RAGE by increasing cAMP concentration. We also successfully identified potential RAGE antagonists in the form of low-molecular weight RAGE, using this information to assess the agonist/antagonist activity of AGE-derived food products. The results presented in this paper offer highlights the viability of using AGE-RAGE as a therapeutic target to develop a potential therapy to treat Type 2 diabetes and its complications.

(3) Introduction

(10) In the 1990s(11) the co-cultivation of constituent vascular cell types was established within vascular biology. Using this technique, it was found that pericytes inhibit the

proliferation of endothelial cells while preserving the production of prostacyclin and preventing endothelial cell damage by lipid peroxides[4]. (12) This discovery gave an insight into why neovascularization and pericyte loss are observed simulatenously in cases of diabetic retinopathy. However, the primary factor (13) that induces the characteristic changes observed in every type of vascular cell in those with diabetes had not been identified. (14) In order to determine this instigator, an axenic culture of endothelial cells and pericytes was used to test the effect of a variety of environmental factors. This investigation identified the factor as advanced glycation end products (AGE)[5,6]. (Figure 1)

(15) AGE is the term used to describe the product formed following an irreversible non-enzymatic reaction between the carbonyl group on a sugar and the amino group on a protein impairing its functionality. (17) Glucose despite being an efficacious source of respiratory derived energy still has the issue of glycation. (16) Accordingly, the process of AGE formation and accumulation is accelerated by a chronic hyperglycaemic state. (18 replaced) As diabetes sufferers are characterised by a hyperglycaemic state it is not surprising to find that AGE formation is associated with diabetes and is an evitable complication of the disease.

(20) It is thought that a degree of AGE derived complications associated with vascular cells is caused by interaction with the specific receptor for AGE (RAGE). (21) RAGE is classified as a pattern recognition receptor which is recognised as a ligand by various pathogen-associated molecular patterns (PAMP) and damage- associated molecular patterns (DAMP) (Figure 2a). The AGE-RAGE interaction is considered to be a potential contributor to (33) the established cell dysfunction in pancreatic β cells within the islets of Langerhans and the decline in β cell mass which accompanies the progression of Type 2 diabetes. Thus firstly, this paper seeks to further clarify the relationship between AGE-RAGE, decline in beta cell mass and consequent diabetes complications.

Despite the hypothesis that AGE-RAGE interaction is a potential therapeutic target in the treatment of Type 2 diabetes there have been no viable therapies developed as of yet. It is proposed that the first theoretically conceivable strategy is to inhibit (48) the formation of AGE. (51) Interestingly, it has been reported that angiotensin receptor blocker (ARB) has AGE inhibiting activity[16]. (49) However, the mechanism of action for the majority of currently developed AGE inhibitors is to target the covalent bonds of intermediate compounds generated during AGE formation. (50) These intermediate compounds requires therapeutic

concentrations close to an equimolar which results in stoichiometric issues. Another option that has been explored is the development of (52) a drug to break down already formed AGE. (53) This type of drug belongs to a category of drugs called AGE breakers. (54) However, an AGE breaker which is sufficiently efficacious at breaking down AGE is yet to be developed.

Two promising forms of targeting the AGE-RAGE interaction are firstly, the addition of (58) an extracellular decoy receptor for AGE that protects the vascular cells and secondly (62) the invention of a RAGE antagonist.

The current paper will firstly further analyse and investigate the role of the AGE-RAGE interaction and its associated pathways in the decline of beta cell mass and the appearance of diabetes complications. Secondly, this paper will investigate and critically analyse the potential of proposed therapeutic targets for disrupting the AGE-RAGE interaction including decoy RAGE targets and RAGE antagonists.

Method

2.1

(22) Transgenic mice that over-expressed RAGE within their vascular cells and mice that endogenously lacked the RAGE gene were created in order to investigate the relationship between AGE-RAGE and diabetic complications. (23) Diabetes was then induced within the mice and the resulting complications analysed.**(28)** Next, the transcriptional regulatory mechanism for the human RAGE gene was investigated[11].

Following the investigation of the transcriptional regulatory mechanism for the human RAGE gene, an (34) investigation was carried out to find out whether there is a connection between the AGE-RAGE interaction and Type 2 diabetes pathogenesis. The cell surface of normal pancreatic β cells were analysed in animal models of type 2 diabetic mice including ob/ob mice and db/db mice for RAGE proteins. An age variable was also introduced. The db/db RAGE deficient mice were then crossbred. (39) Non-esterified fatty acids and leptin receptor antagonists were then administered to MIN-6 cells, causing them to induce the expression of RAGE proteins on the cells' surface. This was followed by a study into the relation of RAGE to other pathologies using a subacute inflammation mechanism.

2.2 Next ways of targeting AGE-RAGE interaction were investigated. The first therapeutic target investigated was the invention of a decoy RAGE protein. A (59) decoy RAGE protein was identified and named endogenous secretory RAGE (esRAGE) by screening of human vascular cell polysomes. It was then formed through alternative RNA splicing[17]. Next, the feasibility of creating soluble RAGE was investigated. This was achieved through (60) MM9 induced ectodomain shedding by increasing the concentration of intracellular cyclic AMP[18]. This process induced membrane-bound RAGE proteins to convert into soluble RAGE proteins

The next option explored was (62) the invention of a RAGE antagonist. (63) Analysis of fluorescence resonance energy transfer (FRET) was carried out using RAGE antibodies, whilst undergoing ligand stimulation. Then, (65) a pharmacological assessment was carried out in which low-molecular weight AGE of a maximum molecular weight of 300 was prepared and investigated for signs of antagonist activity[19]. Following this analysis (65) using the three-dimensional structure of the human RAGE protein established in earlier research[20]. (66) low-molecular weight compounds were screened in silico by their structural information. A pharmacological assessment ensued in order to identify potential agents. Finally, (72) an assessment of RAGE as an agonist/antagonist was carried out using the aforementioned screening method. Soy sauce, coffee, red wine and cola were used within this assessment.

Results

3.1 Analysis of the transgenic mice for diabetic complications (24) revealed that both the indexes for diabetic nephropathy[8] and diabetic retinopathy[10] increased in species that overexpress RAGE (Figure 2B and 2C) (25) whilst RAGE deficient mice did not develop any symptoms (Figure 2D). (26) These results indicate that there is a functional interaction between the pathogenesis of diabetes complications and the AGE- RAGE interaction.

Investigations into **(28)** the transcriptional regulatory mechanism of the human RAGE gene (29) showed that AGE activates the transcription factor nuclear factor-κB（NF-κ B）as well as promoting the transcription of the RAGE gene establishing a positive feedback loop. (30)

This research forms the molecular basis for the apparent constitutive RAGE expression and AGE-RAGE colocalization in the observed diabetic state. (31) The NF-κ B intermediary pathway is considered to be particularly important in regard to diabetes among the numerous RAGE-flanking signalling pathways.

In analysing (34) the connection between AGE-RAGE and the aforementioned aspects of Type 2 diabetes pathogenesis, (35) the surprising discovery was that the RAGE protein was not detected on the cell surface of normal β cells [12]. (36) However, in animal models of type 2 diabetic mice, the ratio of RAGE protein positive pancreatic β cells within ob/ob mice and db/db mice was observed and increased with age. (37) It was discovered that when the db/db RAGE deficient mice were crossbred, the apoptosis induced reduction in β cell mass and impaired glucose tolerance associated with diabetes improved (Figure 4).

(38) However, the most remarkable observation was cell death due to AGE exposure. (39) In this analysis, free fatty acids and leptin receptor antagonists were administered to MIN-6 cells which caused the MIN-6 cells to induce the expression of RAGE proteins on the cells' surface, and promoted cell death when exposed to AGE.

(42) There were various discoveries concerning RAGE and its relationship to the epithelial-mesenchymal transition using a subacute inflammation model[13,14] (43) in addition to discoveries concerning RAGE specifically recognising phosphatidylserine and its involvement in the phagocytosis of apoptotic cells. (44) Moreover, interdisciplinary research demonstrated that both RAGE deficiency and soluble RAGE overexpression significantly suppresses the uptake of amyloid β 1-42 peptide into the brain[15].

(46) This supports the hypothesis that AGE-RAGE can be considered as a possible target for treatment for all kinds of human diseases including diabetes and the complications associated with diabetes.

3.2 Initial efforts to identify potential AGE-RAGE therapeutic treatments (59) focused on establishing a decoy RAGE protein. An endogenous secretory RAGE (esRAGE) was created by screening the polysomes for human vascular cells and splicing it with alternative RNA. (60) It demonstrated that it possible to cause membrane-bound RAGE proteins to become soluble

through ectodomain shedding by MMP9 when the concentration of intracellular cyclic AMP is increased.

The second method investigated was (62) the invention of a RAGE antagonist. (63) Analysis of fluorescence resonance energy transfer (FRET) was carried out using RAGE antibodies. There was no change in fluorescence intensity originating from what is believed to be a RAGE monomer. However, at the same approximate time of ligand stimulation, the fluorescence intensity, which is believed to originate from an oligomer, increased. (64) It is thought that the intracellular signals sent from agonist ligands make the RAGE receptors oligimerize. (65) So, when a subsequent pharmacological assessment was carried out in which low-molecular weight AGE of a maximum molecular weight of 300 was prepared, it was discovered that low-molecular weight AGE showed signs of RAGE antagonist activity[19]. Next, (65) the three-dimensional structure of the human RAGE protein established in earlier collaborative research, was used in order to screen low-molecular weight compounds *in silico* based on their structural information[20]. Through subsequent pharmacological assessment, (66) several potential agents which showed RAGE antagonist activity were obtained.

Finally, using the previous research as a basis, (72) an assessment of RAGE as an agonist/antagonist was carried out. (73) It was found that soy sauce, coffee and red wine neutralised the RAGE agonist activity of the high molecular weight AGE and that it was low-molecular weight fractions that reversed the activity into becoming that of a RAGE antagonist.

Discussion

4.1 Our investigations found that (36) the ratio of RAGE protein positive pancreatic β cells within ob/ob mice and db/db mice increased with age. Moreover we found that (37) when **the db/db RAGE deficient mice were crossbred, the apoptosis induced reduction in β cell mass and impaired glucose tolerance that is associated with advancement of diabetes showed signs of improvement (Figure 4).**

(40) The above results are in line with prevailing theories on the mechanism behind pancreatic β cell deficiency which is associated with Type 2 diabetes. These theories postulate that free fatty acids and AGE at least play a part in the lipotoxicity and glucotoxicity contributing to the

mechanism of β cell deficiency. Thus these results also support the targeting of AGE-RAGE in order to treat type 2 diabetes.

4.2 (75) The interaction between RAGE and its ligands, including AGE is now considered to be one of the causes of diabetic angiopathy and β cell failure in diabetes. (76) Accordingly they are considered to be the primary, secondary and tertiary targets for prevention of these disorders.

In our study, several potential agents which showed RAGE antagonist activity were obtained. This could represent an important step in the development of an AGE-RAGE therapeutic target.

(70) Conventionally, the dominant view has been that food derived AGE is regarded as harmful to health. (67-68) The taste of varieties of food products which are rich in AGE partially originates from AGE itself. This includes the colour, aroma and flavour of AGE rich food. (69) Of the intake of food derived AGE, approximately 10% is absorbed into the blood stream. Yet after 48 hours 70% of the AGE consumed remains within the body[21]. (71) Thus, it is also thought that further investigation into the biological effects of AGE in food products is required before the development of an RAGE antagonist.

However, to conclude, (77) there is great reason to hope that the development of a RAGE antagonist drug or a method of inducing the production of a decoy variant of the RAGE protein will soon become a reality. These developments could mean the elimination of diabetes complications in the near future.

(78) **Conflicts of interest (COI)**: none to declare

(79) **Acknowledgements**

(80 I would like to express my profound gratitude to my respected emeritus professor Hiroshi Okamoto of Tohoku University for his stern but warm guidance concerning my diabetes research.

(81) I would also like to express heartfelt thanks to emeritus Professor Ryousuke Takeda of Kanazawa University for his frequent encouragement.

(82) This prize truly is a result of the collaborative research efforts of every member involved.

Text 2: Ishihara, Hisamitsu (2009) '2 型糖尿病発症における膵 b 細胞障害の分子機構' [The molecular mechanism of pancreatic beta cell damage in the pathogenesis of Type 2 diabetes], *糖尿病 [Diabetes]* 52(11): 884-866.

(1) Winner of the Lilly Prize: (2) Hisamitsu Ishihara

(1b) **The molecular mechanism of pancreatic beta cell damage in the pathogenesis of Type 2 diabetes**

Abstract

Type 2 diabetes is a complex disease caused by impaired insulin secretion by the pancreatic beta cells, complicated by insulin resistance in the skeletal muscle, adipose tissue and liver. There has been mounting academic interest into insulin resistance. However, there is still not a comprehensive view of the mechanism of insulin secretion in response to glucose, or the mechanism of beta cell apoptosis and its association with endoplasmic stress. We analysed the insulin secretion response upon the introduction of different genes using genetically engineered MIN6 cells. *wfs1* disrupted mice were analysed and crossbred to investigate the beta cell stress response. We found that the forced expression of necessary transporter proteins resulted in insulin secretion in response to nutrients other than glucose. Pyruvic acid was also found to be intertwined within the explanation of the mechanism of insulin secretion and its glucose specificity. We found that expression of eIF4E-binding protein 1: 4e-bp1 increased under endoplasmic reticulum stress-induced apoptosis and a lack of 4E-BP1 caused advancing beta cell damage and worsened glucose intolerance. This indicated that 4E-BP1 is beneficial to long-term survival of beta cells, as it regulates protein synthesis in beta cells under endoplasmic reticulum stress. Our research provides new perspectives on the mechanisms of insulin secretion and cell death in beta cells and how these factors contribute to diabetes pathogenesis, thereby offering potential new therapeutic targets.

1. (3) **Introduction**

1.1

(4) Type 2 diabetes is a complex disease in which insulin secretion from the pancreatic beta cells is impaired. It is complicated by insulin resistance in the skeletal muscle, adipose tissue

and liver which results in the pathogenesis and progression of the disease. (5) Since the 1990s there has been mounting academic interest into insulin resistance. In order to research insulin resistance islets of Langerhans need to be isolated. However the process of (6) isolating the islets of Langerhans or pancreatic beta cells is extremely complicated. (7) A lack of these resources in large quantities can become a major stumbling block for the progression of diabetic research. (9) Fortunately, in 1990 the MIN6 cell, which is still currently used globally and considered the most typical insulin-secreting cell line, was created. (10) This solved the issue of obtaining sources of research materials for investigation into beta cell function and insulin resistance.

(11) It has been noted that at the stage of initial pathogenesis of Type 2 diabetes, or at the stage of impaired glucose tolerance, there is frequent latent excessive response to insulin secretion. This has meant that it is not possible to observe the absolute amount of beta cell decline. (12) Thus, since the first half of the 1990s, there has been a shift in research interest. The prevailing view became to make the research target the abnormalities in the mechanism for recognising glucose concentration, rather than researching the decline in amount of beta cells resulting in abnormalities.

Despite this shift in research interest, there is still not a comprehensive view of the mechanism of insulin secretion in response to glucose. (13) The present study seeks not only to assist in elucidating the details of the mechanism for glucose concentration recognition in beta cells but also assist in the treatment of type 2 diabetes through the treatment of insulin secretion disorders.

1.2

(32) Wolframin's Syndrome, a disease which Tanizawa et al. discovered, is caused by a mutation in the *wfs1* gene. It is linked with defects in the endoplasmic reticulum stress response and a reduction in beta cell mass. A study which analysed (33) the islets of Langerhans of *wfs1* disrupted mice found that glucose stimulated insulin secretion became dysfunctional and that the process of insulin secretion is connected to a dysfunction in calcium movement within beta cells[6]. (34) Moreover, it was also observed that there is acceleration of the endoplasmic reticulum stress response in conditions in which there is a lack of the wfs1 protein. (35) From this, it has become evident that under wfs1 deficient

conditions, it is likely that beta cell apoptosis will be induced[7]. (36) It is now hypothesised that one of the causes of this acceleration in the endoplasmic reticulum stress response is the dysfunction of calcium movement within the beta cells of *wfs1* disrupted mice. (37) In other words, as well as a decline in the functionality of insulin secretion in the islets of Langerhans of *wfs1* disrupted mice, there is also a decrease in pancreatic beta cell mass due to accelerated apoptosis. (38) It is thought that these two factors lead to hyposecretion of insulin, which in turn results in the pathogenesis of diabetes. The current study seeks to further elucidate the details of the mechanism of beta cell endoplasmic reticulum stress induced apoptosis in order to attempt to prevent hyposecretion of insulin.

Method

2.1

(15) In order to investigate the mechanisms of insulin secretion in response to a variety of nutrients including glucose, techniques of genetic engineering were applied. (14) Analysis using MIN6 cells which incorporate genetic engineering technology was carried out. This analysis tested how the insulin secretion response changed upon introduction of different kinds of genes. (17) First, research efforts were focused on the enzymes in the glycolytic pathway before (18) the efficacy of over expressing the glucose transporter GLUT1 which uptakes glucose was analysed. Subsequently the overexpression of hexokinase I, which carries out glucose phosphorylation, was tested[1]. (19) Finally, taking into account the link between the glycolytic pathway and mitochondrial metabolism, the effect of forcing uncoupling protein 1[4] (UCP1) expression, (20) which has a suppressive role in the production of glycerol-3-phosphate dehydrogenase[2], lactic dehydrogenase[3] and adenosine 5'-triphosphate (ATP), was analysed.

2.2

In order to examine (39) **the beta cell stress response and the regulation of translation,** (40) the molecular mechanism of endoplasmic reticulum stress induced apoptosis in the islets of Langerhans of *wfs1* disrupted mice was analysed. Upon increased expression of translation initiation factor eIF4E-binding protein1: 4e-bp1), (44) a second generation cross was created

by cross-breeding either the Akita mice or *wfs1* disrupted mice with the *4e-bp1* deleted mice, (45) the F2 mice were then analysed (44) in order to ascertain the significance of 4e-bp1 induction.

Results

3.1

Investigations into the mechanisms of insulin secretions using the forced expression of UCP1 discovered that (21) the rate-determining step of the glycolytic pathway in pancreatic beta cells was determined as the glucose phosphorylation process. It also determined the role of the glucose sensor as regulating glycolytic flux. Furthermore, (22) it was found that there is a highly functional association between the glycolytic pathway and mitochondrial metabolism. (23) It was clarified that mitochondrial metabolism not only fulfils an important role for ATP, but it also plays a pivotal position in the formation of insulin secretion signalling. (25) In the process of engineering a nutrient-stimulated insulin secretion mechanism, it became clear that in order to ensure that beta cells only responded to glucose, beta cells possess different characteristics to other cells types within the islets of Langerhans. (26) Namely, it became apparent that there were no transporters on the cell membranes of beta cells for types of nutrients other than glucose in order to ensure that beta cells do not secrete insulin in response to nutrients other than glucose. (27) In fact, dicarboxylic acid, an intermediary product of the TCA cycle, forced the expression of dicarboxylic acid transporters. Upon expression of the transporters, insulin secretion was observed in response to dicarboxylic acid[5]. (28) Pyruvic acid-stimulated insulin secretion was also observed in the islets of Langerhans due to the forced expression of a transporter for pyruvic acid[3]. This was an interesting result which has tied pyruvic acid to the mechanism of insulin secretion.

3.2

(40) Upon analysing the molecular mechanism of endoplasmic reticulum stress induced apoptosis in the islets of Langerhans of *wfs1* disrupted mice, it was discovered that the expression of translation initiation factor eIF4E-binding protein 1: 4e-bp1 increased[9]. (41) The increase in 4e-bp1 was not only observed in the islets of Langerhans of *wfs1* disrupted mice

but was also observed in the islets of Langerhans of Akita mice. Pathogenesis of diabetes was generated in the Akita mice through endoplasmic reticulum stress caused by insulin secretion dysfunction.

(42) Evidence was found that the increased expression of 4e-bp1, which occurs in the endoplasmic reticulum stress response, is caused by the transcription activation of *4e-bp1* by ATF4, the master transcription factor for the stress response. Moreover, (43) the binding site for ATF4 was discovered within intron 1 of *4e-bp1*.

After creating two second generation mice crosses by cross-breeding either the Akita mice or *wfs1* disrupted mice with the 4E-BP1 gene deleted mice in order to analyse the significance of 4e-bp1 induction, (46) it was observed that the lack of 4e-bp1 caused advancing beta cell damage and worsened glucose intolerance in the F2 mice of both crosses. This is indicative that (47) *4e-bp1* fulfils a role in the regulation of protein synthesis in beta cells under endoplasmic reticulum stress. This regulation of protein synthesis is significant as it is beneficial to the long-term survival of beta cells and therefore beneficial in the prevention of beta cell apoptosis.

Discussion

4.1

(25-26) From our results it is clear that beta cells possess different characteristics to other cells within the islets of Langerhans, due to their lack of transporters in the cell membrane. The resulting insulin secretion from forced expression of membrane transporters offers further insight into mechanisms of inducing insulin secretion and suggests that cells which are normally unresponsive to certain nutrients could be activated by expressing the protein needed for the metabolism of that nutrient. (28) This result is further compounded by the unexpected observation that the forced expression of a carrier for pyruvic acid caused the islets of Langerhans to secrete insulin in response to pyruvic acid. This is a result which has paradoxically tied pyruvic acid to an explanation of the mechanism of insulin secretion (24) and its glucose specificity.

4.2

(49) There is no longer any doubt that beta cell damage is essential to Type 2 diabetes pathogenesis. (50) However, it is now thought that there are in fact two factors contributing to this: a decrease in beta cell mass as well as beta cell dysfunction. As discussed, (51) dysfunction in calcium movement within the cell due to *wsf1* gene mutation causes a decrease in insulin secretion, in addition to an acceleration of apoptosis caused by a lack of 4e-bp1. From these results, (52) it is now thought that just one type of cell dysfunction will have an effect to a greater or lesser extent on not only cell survival but also on the highly specialised function of insulin secretion. These results thus provide a potential therapeutic target for treating type 2 diabetes in *4e-bp1*.

The aforementioned research has contributed to advancing the understanding of mechanism of insulin secretion, loss of beta cell mass and the pathogenesis of type 2 diabetes. (53) Moving forward, I would like to endeavour to elucidate a comprehensive view of the mechanisms of insulin secretion and cell death in beta cells, to further the understanding of type 2 diabetes and then apply this knowledge in the treatment of type 2 diabetes.

(54) **Acknowledgments**

(55) On this occasion of being awarded the Lilly Prize, I would like to express my sincere gratitude to Professor Yoshitomo Oka of the Diabetes Metabolism department of Tohoku University School of Medicine for all his guidance across all of the basic research, clinical diabetes research and education undertaken. (56) Additionally, I'd like to express my appreciation for the mentoring I received throughout all of my research into beta cells in general from the director of the Asahi Life institute for research into new and emerging diseases Mr. Masatoshi Kikuchi. (57) I'd also like to express my sincere gratitude to Professor Junichi Miyazaki who specialises in the field of stem cell management at Osaka University's Faculty of Medicine and established the MIN6 cell, for his guidance during my initial stages of molecular biological research. (58) Equally, I'd like to express my sincere thanks to Professor Claes B. Wolheim of Genevia University for all his valuable advice given since my time studying abroad up until today. (59) Lastly, I would like to express my sincere gratitude to all of my numerous mentors who all went above and beyond, even going to the extent of helping me perform experiments and research late into the night.

Text 3: Watada, Hirotaka (2009) '膵 b 細胞容積調節機構に関する研究' [The Clinical Application of the Mechanism for Regulating Beta Cell Mass in the Treatment of Type 2 Diabetes], *糖尿病 [Diabetes]* 52(11): 881-883.

(1)Winner of the Lilly Research Award: (3) Hirotaka Watada

(2)The Clinical Application of the Mechanism for Regulating Beta Cell Mass in the Treatment of Type 2 Diabetes

Abstract

Diabetes, a disease caused by a lack of insulin secretion, is among the top-ten causes of death globally. However, a viable method of increasing insulin secretion is yet to be established. In order to establish this method, the gene expression patterns of pancreatic β cells must be established by elucidating their developmental process, allowing this process to be imitated to induce pancreatic β cell differentiation and increased beta cell mass. We analysed AR42J-B13 cells and various mouse models in order to identify crucial transcription factors which control pancreatic β cell differentiation and influence cell mass. We found and classified the mechanism of insulin gene regulation and used it to transform non-pancreatic β cells into pancreatic β cells. We also established the roles of the vasculature of the pancreatic β cells and autophagy in increasing β cell mass. The results discussed in this paper provide a method of inducing pancreatic β cell differentiation which is likely to have clinical applications in the development of a therapy to treat diabetes and also provide possible therapeutic targets for increasing β cell mass which could offer a radical cure for type 2 diabetes.

Introduction

Diabetes is a disease defined by the hypo- or complete impairment of insulin secretion, which results in hyperglycaemia. This lack of insulin secretion causes various complications, making diabetes one of the top ten leading causes of death globally. This in turn has resulted in an influx of research interest into the establishment of a method for increasing insulin secretion. In order to establish a method of promoting insulin secretion, previous research has addressed whether (5) insulin expression is confined to pancreatic β cells (6) by investigating the regulatory mechanism of insulin gene transcription. (7) Accordingly, the focus of investigation was on Insulin Promoter Factor-1 (IPF-1), a transcription factor which binds to the insulin gene promoter region. (18) The term IPF-1 has since been replaced by the term Pancreatic and Duodenal Homeobox 1 (Pdx1). (8) It was found that IPF-1/Pdx1 binds to the promoter regions of the glucokinase (a glucose sensor in the pancreatic β cells) or the promoter region of islet amyloid polypeptide (IAPP) genes stimulating their transcription. [1]. However, at the time of this study (9) investigation into the mechanisms of regulating gene expression was mainly carried out using reporter gene and gel shift assays. (10) However, in order to investigate whether IPF-1/Pdx1 actually does bind to the respective gene promoters present on the genome and cause this activation of the gene expression, further data was necessary. (11) Therefore, the exogenous expression of IPF-1/Pdx1 was forced in a pancreatic **α** cell line, **α**TC1, and examined. (12) It was discovered that, once IPF-1/Pdx1 was expressed, albeit in the presence of extremely low levels of Betacellulin, this induced the expression of genes specific to pancreatic β cells: insulin, glucokinase and IAPP[2]. (13) This research, in addition to the discovery that (18) IPF-1/Pdx1 is a causative gene of Maturity Onset Diabetes of the Young (MODY) provided strong evidence that IPF-1/Pdx1 directly activates the expression of each type of pancreatic β cell specific genes. (14) For example, if endogenous cells which express the insulin gene can be induced into a type of pancreatic β cell, then in consideration of the results of the aforementioned research, it could be interpreted that the expression of IPF-1/Pdx1 can induce non-pancreatic β cells to become a type of pancreatic β cell. (15) By building upon this research, it is conceivable that in the near future, this method of inducing differentiation using transcription factors to change the endogenous gene expression could be used in a trailblazing new method to treat diabetes sufferers with insufficient levels of β cells by replenishing the β cells. (16) In order to achieve this, it is necessary to approximate the intracellular gene expression patterns of pancreatic β cells as closely as possible. However, in order to approximate pancreatic cell gene expression

patterns (17), it is imperative to elucidate the physiological developmental process of pancreatic β cells. Then this developmental process would need to be imitated in order to devise a method of inducing pancreatic β cell differentiation. This paper seeks to first establish (19) the pancreatic β cell development and differentiation process, identify the associated transcription factor cascade and then establish the factors which (39) increase the cell mass of existing pancreatic β cells. These discoveries could then be applied in a novel treatment for type 2 diabetes where there is a decline in β cell mass.

Methods

(20) Initial research focused on elucidating the transcription factor cascade which regulates that pancreatic β cell developmental process. Upon (32) classifying the crucial transcription factors in pancreatic β cell differentiation Pdx1, Neurogenin-3 (Ngn3) and NK6 homeobox 1 (Nkx6.1) differentiation of non-pancreatic β cells into pancreatic β cells was attempted using this mechanism for regulating gene expression in AR42J-B13 cells (33), the cell line model for pancreatic precursor cells.

Next, research into (42) the factors which influence pancreatic β cell mass was carried out in order to increase pancreatic β cell mass. This involved research into (44) the vasculature of the pancreatic islets of Langerhans. (47) A model of vascular insufficient islets of Langerhans was used in order to investigate pancreatic β cell function. This model used pancreatic β cell specific vascular endothelial growth factor (VEGF)-A knockout mice.

Next, (50) the mechanism of autophagy was investigated. (52) The state of autophagy within pancreatic β cells of diabetic mouse models, including db/db mice, was analysed. Then, (53) in order to investigate the significance of autophagy in pancreatic β cells, pancreatic β cell specific autophagy-specific gene 7 (*atg7*) knockout mice were created. These mice were created by knocking out the pancreatic β cell specific form of *atg7* which is essential in the mechanism of autophagy.

Results

(26) The mechanism for regulating the expression of the Ngn3 gene was elucidated. (27) It was discovered that transcription factors such as HNF3β and HNF6, which are expressed in the endoderm, as well numerous signalling pathways such as Notch signalling, Activin signalling and Hepatocyte growth factor (HGF) signalling have an involvement in the gene expression of Ngn3[3] which is extremely complex. (28) Upon examination of the mechanism for regulating the gene expression of the pancreatic β cell differentiation factors Paired box gene 4 (Pax4) and NK2 Homeobox 2 (Nkx2.2), it was found that Pax4 and Nkx2.2 were automatically expressed when Ngn3 was expressed. (29) Thus it was shown that the Ngn3 and Hepatocyte nuclear factors (HNF) groups of transcription factors regulate gene expression through synergistic action[4].

(30) It was also discovered that the mechanism for gene regulation, including the mechanism of post transcriptional regulation of the transcription factor Nkx6.1 which lies downstream from transcription factor Nkx2.2, is extremely intricately regulated[5,6]. (31) Furthermore, it was proven that the potent insulin gene transcription activator MafA lies downstream of Nkx6.1.

(32) After classifying the crucial transcription factors Pdx1, Ngn3 and Nkx6.1 in terms of pancreatic β cell differentiation, differentiation of non-pancreatic β cells into pancreatic β cells was attempted in (33) the cell line model for pancreatic precursor cells AR42J-B13. It was found that the AR42J-B13 cells expressed Pdx1 from the offset. (34) The expression of Nkx2.2 and Pax4 was observed when the AR42J-B13 cells were forced to express Ngn3. (35) Insulin expression was not observed when there was forced expression of Nkx6.1. (36) Instead, insulin expression was clearly observed when there was forced expression of MafA[7].

Through an investigation using (VEGF)-A knockout mice(48) it was shown that although the pancreatic β cells vasculature is crucial in normal pancreatic β cell function, it is unrelated to pancreatic β cell mass in a steady state. (49) Moreover, it was found that although normal vasculature is essential for an increase in the function of pancreatic β cells derived from a bone marrow transplant, normal vasculature is not required for the mechanism of increasing pancreatic β cell mass when due to insulin resistance[8,9].

(52) In analysing the state of autophagy within pancreatic β cells, results were obtained that suggested that there was an autophagy deficiency in the pancreatic β cells of diabetic mouse models including db/db mice. (53) In order to investigate the significance of autophagy in pancreatic β cells, pancreatic β cell specific autophagy-specific gene 7 (ATG7) knockout mice were analysed. This analysis (54) suggested that a deficiency of constitutive autophagy causes a reduction in glucose stimulate insulin secretion through a reduction in mitochondrial adenosine-5-triphosphate (ATP) productivity. (55) Moreover, it was also found that induction of autophagy triggered by insulin resistance is a necessary mechanism for increasing pancreatic β cell mass through the promotion of the proliferation of pancreatic β cells and the suppression of apoptosis[10]. (57) It was also proven that an autophagy deficiency could also cause the insulin resistance-induced impairment of the process of generating pancreatic β cell mass.

Discussion

In order to establish a method of promoting insulin secretion, it is necessary to understand the pancreatic β cell development and differentiation process and identify the associated transcription factor cascade. (21) The pancreas is derived from one embryological germ layer, namely the epithelial cells of the endoderm. (22) This section of cells become pancreatic endocrine precursor cells. (23) These pancreatic endocrine precursor cells then go through numerous steps of differentiation involving various transcription factors to become a mature pancreatic β cell. (24) It has long been thought that the expression of Pdx1 fulfils a considerable role in the development of pancreatic precursor cells through the differentiation of the pancreatic endoderm epithelial cells into pancreatic precursor cells. Our research elucidated both the mechanisms regulating the expression of the Ngn3 gene and its relationship with Pax4 and Nkx2.2. It also proved that the potent insulin gene transcription activator MafA lies downstream of Nkx6.1. This classification of the crucial transcription factors in the pancreatic β cell process meant that differentiation of non-pancreatic β cells into pancreatic β cells could be attempted. (38) The results of this attempt strongly suggest that the elucidation of the mechanism for the development and differentiation of pancreatic

β cells and the gathering of extensive information on this mechanism could be practically applied in the establishment of a new future technique to induce differentiation into pancreatic β cells.

Moreover, (37) at precisely the same time as the publication of this paper, Melton et al. published a paper in Nature that they also succeeded in inducing differentiation into pancreatic β cells by forcing the expression of Pdx1, Ngn3 and MafA but in exocrine cells. Therefore (41) methods of inducing pancreatic β cell differentiation, such as those attempted in this paper, are likely to be valuable in the development of a future therapy to treat cases of diabetes where pancreatic β cell mass is in decline.

(42) Another strategy for increasing pancreatic β cell mass involves elucidating the factors which influence pancreatic β cell mass. (44) The first area that attracted research attention was the vasculature of the pancreatic islets of Langerhans. (45) It is already established that when pancreatic β cell mass increases in healthy individuals then the vascular density of the islets of Langerhans also increases. However under the same conditions, the vascular density of the islets of Langerhans decreases in individuals with type 2 diabetes. (46) In other words, although there is an evident correlation between the islets of Langerhans vasculature and pancreatic β cell function, it is not clear whether this pertains to a causative relationship or whether it is just mere correlation. In this paper it was found that although normal vasculature is essential for an increase in the function of pancreatic β cells from a bone marrow transplant it is not required for the mechanism of increasing pancreatic β cell mass when due to insulin resistance.

(50) Next therefore the focus of research was the mechanism of autophagy. (51) Autophagy has an important function in the process of intracellular cleaning by removing unnecessary proteins. (52) Although, insulin resistance induces autophagy, in analysing the state of autophagy within pancreatic β cells, (54) results were obtained that showed a deficiency of constitutive autophagy causes a reduction in glucose stimulated insulin secretion through a reduction in mitochondrial adenosine-5-triphosphate (ATP) productivity. It was therefore suggested that (55) induction of autophagy triggered by insulin resistance is a necessary

mechanism for increasing pancreatic β cell mass through the promotion of the proliferation of pancreatic β cells and the suppression of apoptosis. (56) This means that the autophagy deficiency observed in the pancreatic β cells of type 2 diabetic mouse models causes a reduction in glucose stimulated insulin secretion and a reduction in the generation of beta cell mass.

(43) It is thought that it would be possible to establish a new therapeutic technique for type 2 diabetes through supplementing the factors which influence pancreatic β cell mass. (58) Therefore this research suggests that if we are able to improve pancreatic β cell autophagy deficiencies then this could offer a radical new cure for type 2 diabetes in the future.

(60) In consideration of the encouragement offered to me by the award of the Lilly prize and the promising research outlined above, I will continue to engage in research into finding a cure for diabetes and into the elucidation of the pathology of pancreatic β cells in individuals with diabetes.

(61) Acknowledgments

(62) On this occasion of being awarded the Lilly Prize, I would like to take the opportunity to express my sincere thanks to all the professors involved in my research and to all of my colleagues. (63) I could not have accomplished this without all your guidance and encouragement. (64) I would also particularly like to express my deep gratitude to Dr. Ryuzo Kawamori who oversaw my research from conception to completion.

(65) Next, I'd like to express deep gratitude to all of the various mentors who guided me throughout my research, including Dr. Yoshitaka Kajimoto who directly supervised me in Osaka University's Internal Medicine department and Dr. Michael S. German who supervised me during my research abroad at UCSF. Finally, I'd like to thank the numerous colleagues who assisted me in collaborative research.