Goldy Family Cancer Research Fund

Targeting RNF4, a possible oncogene in Cancer Development

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**Introduction**: Cancer cells, working with oncogenes, have the ability to rewire genetic networks that contribute to tumor growth. In healthy cells, negative functioning triggers the cellular stress response that forces the cell to shut down or die. However, oncogenes can turn off that healthy response, enabling the tumor to grow and survive. Recent drug research has focused on targeting these oncogenes in order to deactivate them. Various modifications can act on the gene DNA when it is replicating itself, thereby mutating the resulting processes. Our research is examining whether STUbLs/ RNF4, a gene that generates a Ubiquitin Ligase protein, could be such an oncogene and, therefore, an effective target for new drug therapies in cancers such as breast, colon, osteosarcoma and melanoma.

**Research Aims:**

1. **Examining the presence and role of RNF4 proteins in epithelial cells**

**Background:** Changes in the translation of the gene, specifically in regards to nuclear oncoproteins, play an important role in cancer. Alterations in signalling pathways and ubiquitin and similar proteins, such as small ubiquitin-like modifier (SUMO), is crucial and is known to affect oncogenic activity in the cell. Identifying how these changes (like phosphorylation, ubiquitylation, and SUMOylation) are connected and act in concert to regulate transcriptional activity, has become an important research area. Understanding these mechanisms will also help us understand how to prevent them from dysregulating the healthy cell and generating cancer.

**Results**: We found that RNF4 is able to directly stabilize otherwise short-lived oncogenic transcription factors, increasing the likelihood of cancer protein activity. We also found that RNF4 increased the tumorous properties of cancer cells. Significantly, RNF4 protein levels were elevated in 30% of human colon adenocarcinomas, and RNF4 was essential for the survival and colony formation in cancer cells.

1. **Identifying the role of RNF4 in melanoma**

**Background**: Malignant melanoma is a highly aggressive tumor and its prevalence is consistently rising. In advanced stages of the disease, treatment options are sparse and drug resistance is a major challenge. Therefore, deciphering signalling pathways and molecular mechanisms that are crucial for melanoma survival, may lead to the identification of novel molecular targets. The role of STUbLs/RNF4 in melanoma is still unknown.

Dr. Emily Avitan-Hersh MD, a senior dermatologist, joined our laboratory and together with Prof. Ze’ev Ronai, an expert in melanoma research, we identified RNF4 as a central gene in melanoma.

**Results**: In 50% of human melanoma samples, we found increased presence of high levels of RNF4, while we did not find any such increase in regular mole cells. In another subset of 28 melanomas, high RNF4 protein levels in the tumors were connected to both poor prognosis and resistance to multiple chemotherapeutic agents. Studying melanoma cell lines, we found that quieting RNF4 slowed down cell growth, effective cell colony development and the migration of melanoma cells. RNF4 suppression also restored chemotherapy sensitivity in previously drug resistant melanoma cells. Collectively, our work suggests that RNF4 keeps melanoma cells alive and plays an important role in chemo-resistance as well. We are further perusing this set of experiments.

1. **The role of RNF4 in mesenchymal stem cell differentiation of healthy bone and osteosarcoma cells**

 **Background**: Primary malignant bone tumors (mostly osteosarcoma (OS) accounts for approximately 6% of all childhood malignancies. Currently, aggressive treatment with high-dose chemotherapy and surgery leads to a cure of 70-75% in non-metastatic disease and less than 20% survival in metastatic or recurrent tumors. Moreover, the current chemotherapy protocols have grave short-term toxicity, and survivors are at risk for serious late effects. Thus, it is essential to identify molecular targets that would be the basis for more effective and less toxic treatment. Our preliminary results strongly suggest that the RNF4-related network may be highly relevant for bone differentiation and OS treatment.

**Results**: In order to determine RNF4’s role in osteosarcoma development, we first needed to understand its role in healthy bone development. Through a series of unique experiments, we found that RNF4 is highly present in mesenchymal cells in bone tissue, and that when RNF4 is silenced, bone growth halts completely. We also found that RNF4 is required for the degradation of substances that inhibit cell differentiation. Accordingly, we predict that RNF4 is a key protein involved in bone growth; this offers an opportunity for the discovery of new molecular targets that may serve as a basis for therapies for OS, via the regulation of RNF4 bone growth properties.

After we understood the overall mechanism, we also examined RNF4’s effects on OS cell development. We found that silencing RNF4 inhibited OS cell growth in culture, induced spontaneous cell death and impaired the mobility of OS cells. Moreover, quieting RNF4 prevented the OS cells from colonizing. Currently, we are further testing the role of RNF4 OS tumor growth in both cell cultures and mouse models.

**Summary**: Our results support our hypothesis that RNF4 is an essential oncogene implicated in multiple cancers and associated with poor prognosis. We are studying the impact of genetic inactivation and over expression of RNF4 in cancer cells, both in culture and human samples. In the near future we will screen and identify RNF4 inhibitors, thus benefitting the research community and bringing effective treatment closer to the patients.