HTLV-1 belongs to a group of retroviruses, and is thought to be the cause of adult T-cell leukemia in humans. This virus is apparently responsible for some other diseases as well, such as the nervous system disorder known as tropical spastic paraparesis, or HTLV-1 associated myelopathy (TSP/HAM). HTLV1-Tax is an oncogenic viral protein, responsible for most of the pathogenicity of the virus. Research has demonstrated that the Tax protein affects many cellular processes within the host, and has considerable potential to affect cellular function. Among these, the Tax protein affects the activity of various transcription factors, inhibits DNA repair mechanisms, interferes with cell cycle arrest, and inhibits apoptosis. These activities can lead, over time, to the transformation of a normal cell into a malignant cell.

BRCA1 is a tumor suppressor protein with range of effects on cell processes, including regulation of transcription factors, DNA repair mechanisms, acceleration of ubiquitination, cell cycle arrest, and induction of controlled apoptosis. Defects in BRCA1 function can lead to development of breast cancer, and in fact, women who are carriers of mutant BRCA1 exhibit an 85% risk for breast or ovarian cancer.

Comparison of the Tax protein and BRCA1 reveals that they exhibit opposing activity to one another. Therefore, in this study, we hypothesized that expression of HTLV1-Tax protein in epithelial cells of breast tissue will hinder the activity and possibly also the expression of BRCA1, resulting in highly increased vulnerability to malignant change from environmental carcinogens.

Researchers have discovered that breast milk from women infected with HTLV-1 contains high levels of T-cells infected with the virus. Additionally, infected T-cells transmit the virus to epithelial cells in breast milk, which may in turn infect nearby epithelial cells. We supposed that in women infected with HTLV-1, the virus is transmitted to breast tissue cells from breast milk rich in infected T-cells, and that these women who also breastfeed are at higher risk for breast cancer. The goal of this study was to provide molecular evidence to confirm these hypotheses.

In order to confirm our hypothesis, we first examined whether Tax and its mutant forms are expressed and active in breast cells in-vitro. Our results indicate that the Tax protein and its mutants are in fact expressed in these cells, and display effects on CREB and NF-κB pathways, similar to the expression and effects on their natural target cells.

Results from the following experiments indicate that Tax very strongly inhibits estrogen (E2)-induced expression of BRCA1 in breast cells. In order to understand the inhibitory mechanism of Tax protein on BRCA1 expression, we investigated whether this inhibition acts through known pathways of Tax (primarily the CREB or NF-κB pathways), or through other pathways. In order to accomplish this, we examined the effects of mutant variants of Tax protein, TaxV89A, TaxM47, and TaxM22, on the expression of BRCA1 in breast cells. The results of our experiment indicated that the mechanism of inhibition of BRCA1 expression is not mediated through the CREB or NF-κB pathways – rather, it is related to cofactors p300/CBP.

We then further investigated the mechanism in which Tax alters BRCA1 expression. We examined the effects of Tax on E2 receptors (known as ERα), which functions as a transcription factor responsible for activating BRCA1 expression. We simultaneously tested the effects of Tax on cofactors p300/CBP, which are required for the activity of transcription factor ERα. Our results indicated an increase in cellular levels of p300 and CBP, which in turn greatly reduced inhibition of BRCA1 expression by Tax.

In light of these findings, we supposed that the Tax protein, which has high affinity for CBP and p300, binds to these cofactors, decreasing their availability to bind to ERα, thereby interfering with the effects of ERα on transcription. To our surprise, we discovered that Tax does not hinder binding of p300/CBP to ERα; rather, it simultaneously binds to the p300/CBO complex and to ERα, creating a large quaternary structure.

We hypothesized that since the p300/CBP complex possesses a large number of binding sites, it is more likely that Tax fuses with the p300/CBP-ERα complex by directly binding to p300/CBP rather than to ERα. In support of this hypothesis, our previous experiments demonstrated that increased expression of these cofactors leads to a reduction in the inhibition of BRCA1 expression by Tax. Moreover, our data indicated that in contrast to the inhibitory effects of Tax on BRCA1 expression, Tax protein (via the ERα-E2 complex) accelerated the basal and induced expression of genes containing ERE sequences in their promoter regions. Therefore, it appears there is a synergistic effect of ERα and Tax on the activation of genes containing ERE sequences. Interestingly, some of these genes accelerate cellular reproduction, exposing them to development of various mutations.

Since ERα binds to the promoter region of BRCA1 via cofactors p300/CBP, the binding of Tax to these cofactors inhibits their binding of ERα to the BRCA1 promoter. With the Chip method, this study demonstrated that Tax binding to the ERα complex inhibited its binding to the BRCA1 promoter. However, because ERα directly binds to ERE sequences, without the help of other cofactors, it is possible that binding of Tax to the ERα complex does not interfere with binding of ERα to ERE sequences. Our results confirm our hypothesis, indicating that in fact Tax does not interfere with ERα binding to ERE,.

From our study we can infer that ERα binding to other cofactors necessary for the transcription process occurs in the nucleus, before ERα binds to promoters of BRCA1 or promoters containing ERE sequences. This finding conflicts with previous research which indicates that the binding of various cofactors to transcription factor ERα occurs after its binding to the promoter region. It is possible that some of the cofactors bind to ERα after it binds to promoters, however this implies that not all cofactors that bind to ERα are required for its function.

In summary, we conclude from this study that the protein Tax strongly inhibits BRCA1 expression induced by ERα in breast epithelial cells. Consequently, if HTLV-1 succeeds in infecting breast cells in women who are carriers of the virus, they may be at increased risk for breast cancer, since Tax not only accelerates reproduction of these cells and exposes them to possible mutation by promoting activation of ERα transcription factors regulated by ERE sequences (including genes that accelerate cell reproduction), but it also inhibits BRCA1 expression, which is induced by ERα, leaving these cells vulnerable to various carcinogenic factors.