**Interaction of IGF2 and PTEN in (Malignant) Breast Tissues**

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**Background:** Breast Cancer (BC) is one of the leading malignancies affecting women worldwide. Epigenetic mechanisms regulate gene expression playing an important role in the pathophysiology of cancer. In the present study IGF2 and PTEN genes in AKT pathway were selected for evaluation.

**Objective:** To investigate the role of methylation and interaction of IGF2 and PTEN and in the pathoetiology of BC.

**Methods:** Paraffin-embedded archival breast tumor and adjacent normal tissue samples were used for carrying out PCR-based methylation assay, genomic PCR, immunohistochemistry and qRT-PCR.

**Results:** In-Silico study indicated the absence of hormone responsive elements in the promoters of the selected genes. Methylation results indicated significant loss of methylation in IGF2 exon 9 CpG cluster and significant gain of PTEN promoter methylation in tumors. Immunohistochemistry revealed enhanced cytoplasmic expression of IGF2 protein (p< 0.0001) and decreased nuclear localization of PTEN protein (p=0.0069) in the breast tumors. RT-PCR results indicated an increased IGF2 (p=0.024) and decreased PTEN transcripts (p<0.0001) in the tumors.

**Conclusion:** Increased IGF2 in normal tissues increases PTEN which acts as a negative regulator of AKT pathway in the cytoplasm controlling excessive proliferation while in tumors this regulation is lost. PTEN acts as a negative regulator of MAPK pathway in the nucleus, plays an important role in cell cycle arrest in normal breast tissue. Reduction of PTEN in tumor tissue affects this pathway leading to cell survival. IGF2 and PTEN have a role in breast cancer and these molecular factors can be used for targeting therapy in future.

**Key Words:** IGF2, PTEN, Breast Cancer.
INTRODUCTION

Breast Cancer (BC) is one of the leading malignancies affecting one of nine women in their lifetime; the increasing incidence of BC in developing countries including India is alarming. India reports roughly 1, 00,000 new cases annually\(^1\). BC is familial or sporadic, and upto 5–10% are due to the inheritance of mutation in BRCA1 or BRCA2\(^2\). Earlier studies from our group did not find any association of BRCA1 mutations with sporadic BC.

BC has multifactorial causes; a complex network of relationships between genes and/or proteins, including epigenetic interactions governs the neoplastic processes. Steroid hormones have been shown to play an active role in breast epithelial cell growth, development and in lactation\(^3\). Since these hormones and their receptors regulate normal breast tissue, it is possible that malignant cells arising from breast tissue might also express receptors for many of these hormones and might retain some degree of hormonal dependence.

The role of epigenetics as a distinct and crucial mechanism to regulate a variety of genes (both oncogenes and tumor suppressor genes) in a tissue-specific manner has emerged as an important mechanism in cancer pathology\(^4\). Loss of imprinting of Insulin-like growth factor 2 (IGF2, maternally imprinted), a peptide hormone with growth promoting, mitogenic and anti-apoptotic functions and its subsequent over-expression have been associated with different cancers\(^5\). Further, disrupted tumor suppressive activity by epigenetic alteration of genes like Phosphatase and tensin homolog (PTEN) leads to cell proliferation and survival via the protein kinase B pathway in the cytoplasm or faulty cell cycle arrest mechanism in the nucleus via cyclin D and pMAPK pathways.

Since evidence from previous reports pointed that hormonal factors probably played a role in the etiology of breast cancer, it was of interest to know if the above mentioned genes had direct interaction with the steroid hormones. Hence, the \textit{in silico} method to evaluate the presence of steroid responsive elements in the promoter regions of IGF2 and PTEN was employed.

The drugs for the treatment of cancers are selected on the basis of their ability to interfere with specific molecules to limit the progression to cancer. The identification of appropriate drug targets depends on the detailed understanding of molecular interactions in causing cancer. The present study also focuses on understanding the interactions between the two candidate genes IGF2 and PTEN, which probably are relevant in the aetiopathogenesis of BC.

MATERIAL AND METHODS

Ninety three paired breast tissue samples (breast tumor and adjacent non-tumorous breast tissue) containing more than 70% tumor tissue, from both fresh mastectomy and archival samples were processed for methylation studies, immunohistochemistry and quantitative RT-PCR and data on the two candidate genes were obtained as described earlier\(^8\).

\textit{In silico} method was used to evaluate the presence of steroid responsive elements in the promoter regions of IGF2 and PTEN using the MAST website, reached via the same URL as the MEME website \url{http://meme.sdsc.edu/meme4_4/cgi-bin/mast.cgi}. Estrogen responsive element (ERE\(-5’-AGGTCANNNTGACCT-3’\)) and progesterone responsive elements (PRE \(-5’-GATCTGGTACAAACTGTTCTA-3\)) in the promoter sequences of IGF2 (NP_000603) and PTEN (NP_000305.3) were evaluated.

RESULTS

It was found that neither ERE nor PRE were present in the promoter regions of the IGF2 and PTEN gene. Nuclear PTEN protein expression was reduced in the breast tumor tissue as compared to the adjacent normal breast tissue which was due to the reduced transcription of PTEN gene in the tumor tissue. The reduced transcript in tumor was attributed to PTEN promoter methylation in breast tumors. On the other hand, there was an increase in the IGF2 levels in breast tumor.

DISCUSSION

Cancer arises from the consecutive acquisition of genetic alterations that, in general, produces the loss of function or transcriptional down-regulation of tumor suppressor genes and the activation or transcriptional up-regulation of
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Figure 1: Proposed mechanism of IGF2 and PTEN in breast tumor tissue

IGF2 binds to IGF1 receptor present on the cell membrane resulting in activation of intracellular signaling AKT pathway, which favors proliferation. This pathway is negatively regulated by PTEN. Few molecules of PTEN are translocated into the nucleus by MVP, a putative nucleocytoplasmatic transport protein. In the nucleus PTEN negatively regulates MAPK pathway, thereby bringing cell cycle arrest. In breast tumors reduced PTEN may be responsible for removing the check from both the pathways resulting in abnormal proliferation. p53 down regulates PTEN protein levels by promoting caspase-mediated degradation of PTEN and polyubiquitylation by NEDD4-1 also promotes its degradation in the cytoplasm. In the tumor cells due to increased degradation of PTEN, its function of negatively regulating the two important pathways (AKT and MAPK) are lost resulting in uncontrolled cell proliferation.

In breast cancers, IGF2-binding protein was found to regulate PTEN expression according to some workers. The observed increase in IGF2 expression with a reduced PTEN in tumor tissue indicates failure of IGF2 to upregulate PTEN and/or PTEN degradation in the present study. That is, in spite of an increased IGF2, absence of PTEN expression could point to increased IGFBP-2 resulting in low IGF2 being available for PTEN activation. This could account for the reduction in the levels of PTEN in breast tumors studied. This may be happening in addition to the p53 downregulation of PTEN and its caspase-mediated degradation. Insulin-like growth factors are known to stimulate IGF2 expression, which may be playing a role in the observed high levels of IGF2 in breast tumors.
factor-2 (IGF-2) regulates survival genes that protect the mitochondria and promote chemoresistance\textsuperscript{14}. IGF-2 interacts with ER-\(\alpha\) and ER-\(\beta\), and modulates their translocation to the nucleus, membrane organelles, and the mitochondria. As BC progresses to estrogen-independent growth, the insulin-like growth factor-1 receptor (IGF-1R) and the ER interact and result in enhanced activation of both receptors’ signaling cascades\textsuperscript{15}. The estrogen receptor (ER) is a primary target for breast cancer treatment. Estrogen response element (ERE) is considered to be the major regulatory element in genes regulated by estrogen receptors\textsuperscript{16}. IGF-2 is a downstream target of prolactin signaling that lies upstream of cyclin D1 transcription and this signaling pathway is known to result in the proliferation of mammary epithelial cells\textsuperscript{17}. Absence of the ERE and PRE on the candidate genes was also backed by recent findings of Richardson et al\textsuperscript{14}, who suggest that IGF2 interacts with the ER-\(\alpha\) and \(\beta\) via IGF-1R and modulates their translocation to the nucleus, membrane organelles and the mitochondria.

CONCLUSION

Most of the current knowledge of cancer susceptibility, progression and treatment has been generated by traditional approaches, in which small numbers of genes or proteins are characterized in depth to study the molecular mechanisms of neoplastic processes. These alterations in the oncogene and tumor suppressor gene studied, may be associated with the etiology and may be responsible for the malignant changes related to breast cancers. The current report highlights the importance and potential benefits of identifying genetic interactions at the human genome level for creating a better understanding of cancer susceptibility and progression and developing novel effective anticancer therapies.

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