INDIAN INSTITUTE OF TECHNOLOGY GUWAHATI
SHORT ABSTRACT OF THESIS

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Thesis Title: The G-protein coupled estrogen receptor in breast tumors positively associates with ERalpha, and constitutes a clinically significant genomic target of estrogen in breast cancer cells.

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SHORT ABSTRACT

Estrogen exerts its effects on target cells, and tissues via genomic, and non-genomic pathways. The genomic effects of estrogen are mediated by the canonical estrogen receptors, namely ERα and ERβ. These are ligand-dependent transcription factors encoded by the ESR1 and ESR2 genes, respectively. The non-genomic effects of estrogen are mediated by membrane-tethered canonical estrogen receptors, ERα36, a splice variant of ERα, or the non-canonical G-protein coupled estrogen receptor (GPER).

GPER is the most recent entry into the list of membrane-associated ERs (mER). It was originally cloned by independent investigating groups with unrelated research interests. Sequence analysis using bioinformatic tools revealed that GPER codes for a G-protein coupled receptor with a predicted molecular weight of 42.2 kDa, which shares 28% sequence identity with angiotensin II 1A and interleukin 8A receptors. An orphan receptor then, this protein was called GPR30. Its recognition as an estrogen receptor has its origin in the substantial works of Filardo et al. (2000) and others, which demonstrated specific estrogen-GPER interaction, and subsequent downstream effects. Both short-term non-genomic, and long-term genomic effects on gene expression were demonstrated. A large body of work indicates that GPER activation leads

to increased cyclic adenosine monophosphate (cAMP) levels. However, with contradictory reports, this issue is debatable. Recently, the role of GPER as an estrogen receptor has also been questioned. Nevertheless, GPER is relevant in different physiological systems, such as immune, reproductive, cardiovascular, neuroendocrine, urinary, and musculoskeletal systems. GPER is aberrantly expressed in endocrine and non-endocrine tumors, and significantly associated with various clinicopathological markers.

The rising popularity of GPER in the field of breast cancer research stems from the large volumes of data that have revealed- a) its association with clinicopathological variables, b) its role in epidermal growth factor (EGF) - like effects of estrogen, c) its potential as a therapeutic target or a prognostic marker, and d) tamoxifen agonism and endocrine resistance. GPER cross-talks with ERα. However, the literature portrays ambivalence in the nature of their association. Besides, the significance of their association in mammary epithelial cells, or breast tumors, is not clear. Despite the known regulation of GPER by hormones, the mechanism of estrogen-mediated regulation of GPER in breast cancer cells is not completely understood. The estrogen receptor α (ERα) is a decisive variable that governs the mode of breast cancer treatment. It influences breast cancer prognosis, and tumor phenotype. ERα expression in breast tumors has been consistently linked to indolence, favorable prognosis, and prediction of response to endocrine therapy, while its absence suggests a more aggressive tumor behavior. However, the underlying genetic and molecular determinants of these differences and their relationship with ERα expression remain poorly understood. A potential avenue for exploration lies within the ERα co-expression network, involving genes that interact with or are regulated by ERα. Unraveling the intricate interactions and functions of these genes may provide insights into the mechanisms underlying ERα-associated outcomes.

In line with this supposition, Carmeci et al. (1997) employed differential screening of cDNA libraries from two breast cancer cell lines: MCF-7 (ERα-positive) and MDA-MB-231 (ERα-negative). Their objective was to identify genes associated with ERα expression. They identified a gene called GPCR-Br, which encodes an orphan G-protein-coupled receptor (GPCR). They found that GPCR-Br was abundantly expressed in ERα-positive MCF-7 cells but not in ERα-negative MDA-MB-231 cells. This finding suggests a potential involvement of

GPCR-Br with ERα signaling or function. However, the precise role of GPCR-Br (now known as GPER) and its relationship with ERα expression in breast cancer is yet to be fully elucidated.

The clinical import of ERα-GPER co-expression in the aforementioned cell culture models is of value, but warrants due attention in the face of the inconsistencies and knowledge gaps. Since its discovery, the co-expression, or association of GPER with ERα in breast tissue specimen was examined by several investigators. While a positive association was reported by some; negative, or no association was reported by others. More independent investigations across different cohorts are needed to better understand the relationship between GPER-ERα co-expression in breast tumors. Furthermore, the clinical significance of GPER-ERα co-expression has remained elusive. The prognostic implications of GPER expression in the presence, or absence, of ERα, if any, would be clinically valuable.

Clinical investigations have indicated lower GPER expression in breast tumors compared to normal tissues, and its expression was found to be negatively associated with tumorigenesis. Based on these findings, GPER is predicted as a potential tumor suppressor. The epigenetic silencing of tumor suppressors, and its association with tumorigenesis, is a well known. Recent studies show that DNA methylation is associated with the loss of GPER expression in breast and colorectal cancer cells. In a study on breast cancer cell lines, GPER expression showed an inverse relationship with methylation in the upstream CpG island (upCpGi) in the GPER locus. Given either negative or positive association between GPER and ERα expression in breast cancer, the current investigation examines the association between upCpGi methylation and ERα expression in breast tumors.

The mechanistic basis of GPER-ERα co-expression is unknown. Hormonal regulation of GPER, particularly by estrogen, has been touched upon in several published studies. However, the data are confusing, and do not address the role of hormone receptors, particularly ERα. The impact of hormonal regulation of GPER on cells' responsiveness towards GPER activating ligands, and its clinical import, is yet to be determined.