INDIAN INSTITUTE OF TECHNOLOGY GUWAHATI
SHORT ABSTRACT OF THESIS

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Thesis Title: **Understanding the role of ERK signaling pathway and mechanotransduction in breast cancer**

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SHORT ABSTRACT

Breast cancer is currently the most prevalent cancer affecting the lives of a large number of people, worldwide. One of the major signaling pathways crucially regulating breast cancer cell proliferation, migration, metastasis, stemness and angiogenesis is the ERK signaling pathway. Aberrant activity of its downstream signaling kinases are the forerunners of malignancy. Thus, targeting the ERK signaling pathway at different levels can provide insights into the mechanism by which oncogenic properties like stem-ness, chemoresistance, anoikis resistance, epithelial to mesenchymal transitions or EMT, are inhibited by and large. In this study two cell lines MCF7 and MDA-MB-231 have been used as a model for *in-vitro* studies on human breast cancer. The first aspect of the study focuses on the treatment of breast cancer cells with Raf inhibitor Sorafenib and ERK1/2 specific inhibitor BVD-523. *Invitro* treatment of breast cancer cells MCF7 and MDA-MB-231 with Sorafenib showed effective inhibition of proliferation, migration, stemness and downregulation of other tumorigenic pathways. Treatment with BVD-523 similarly inhibited proliferation, migration and self-renewal ability in breast cancer cells, thereby suggesting potential role of ERK signaling pathway in regulating breast cancer cell behavior. As compared to other cancers, breast cancer shows lesser frequency of mutation in MAP-K pathway but its dysregulation causes poor prognosis in patients. Further studies can pave way for better therapeutic targets specific to ERK signaling pathway. The second aspect of the study focuses on the progressive behavior of tumor with alterations in the mechanochemical changes in the extracellular matrix of breast cancer. Matrix stiffness has been identified as a critical factor in the progression of cancer along with

PIEZO1, a mechanosensitive ion channel. Yoda1 mediated activation of PIEZO1 downregulated proliferation and migration of breast cancer cells whereas its antagonist Dooku1 failed to reverse the effect of Yoda1. Re-evaluation of Dooku1 as an allosteric inhibitor of PIEZO1 is required. In order to downregulate PIEZO1 gene, lentiviral method of silencing PIEZO1 in breast cancer cells was done. As ERK1/2 pathway is one of the key signaling pathways modulated by PIEZO1 modification, the effect of ERK1/2 inhibitor BVD523 on the growth of MCF7 and MDA-MB-231 as 3D spheroid was determined. BVD523 treatment significantly reduced the growth of MCF7 cells regardless of PIEZO1 expression levels, however, MCF7^{shPIEZO1} cells were less sensitive to BVD523 treatment unlike the control cells. MDAMB-231 cells on the other hand, exhibited higher spheroid size on BVD523 treatment, however, PIEZO1 silencing significantly inhibited the spheroid growth, suggesting a context dependent role of PIEZO1 in breast cancer cells of different subtypes. A differential role in proliferation was observed in breast cancer cells upon PIEZO1 silencing. PIEZO1 downregulation, although did not impact the proliferation, it plays a role in modulating the self-renewal ability of MDA-MB-231. cells. PIEZO1 silencing significantly inhibited the migration potential of both MCF7 and MDA-MB-231 cells. A significant downregulation was observed in NOTCH2, β-CATENIN (B-Cat) and FZD2 (Frizzled-2) genes in MDA-MB-231^{shPIEZO1} cells indicating suppression of Wnt signaling pathway. Further, cancer stemness and self-renewal genes such as OCT4, NANOG, SOX2**.** ALDH1-A3 were significantly downregulated, correlating with the reduced colony forming ability observed in PIEZO1 silenced cells. PIEZO1 silencing reduces the mechanosensing ability, enhancing the survival of the cells under stress conditions specifically, the highly metastatic MDA-MB-231 breast cancer cells compared to MCF7 cells. PIEZO1 silencing also downregulated the expression of both CD49B and CD49E in cells cultured under shear stress and adherent conditions as well as the expression of RHOA and β-CATENIN in MCF7 cells. In agreement with the increased survival and colony forming ability observed in PIEZO1 silenced MDA-MB-231 cells, MDA-MB-231^{shPIEZO1} cells had significantly higher expression of survival factors BCL2, RHOA and phosphoERK1/2. Thus, PIEZO1 silencing differentially modulates the breast cancer cells with reduced PIEZO1 favoring the survival and self-renewal of metastatic breast cancer cells MDA-MB-231 by enhancing the expression of survival genes BCL2 and pERK1/2. Therefore, it can be said that PIEZO1 expression can serve as a prognostic marker and should be assessed while considering the use of ERK pathway inhibitors in breast cancer patients. Together the findings obtained from the study, provide necessary information that may be relevant to the field of breast cancer therapeutics with newer strategies.