INDIAN INSTITUTE OF TECHNOLOGY GUWAHATI
SHORT ABSTRACT OF THESIS

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SHORT ABSTRACT

The most perilous attribute of malignant tumors is to metastasize, which directly impacts survival. Cystatin A (CSTA) is an endogenous inhibitor of cathepsins, which play a significant role in the malignant progression of tumors. Loss of CSTA expression shifts the balance towards cathepsins resulting in extensive ECM remodeling, tumor invasion, and metastasis. The precise mechanism behind the loss or regulation of CSTA expression in breast tumors is not known. In this study, we elucidated the mechanisms of regulation of CSTA and its clinical significance in breast cancer. Analysis of TCGA data revealed the subtype-dependent effect of CSTA on the survival of breast cancer patients. CSTA expression was inversely associated with the histopathological marker, estrogen receptor α (ERα). Estrogen, an endogenous ligand of ERα, regulates CSTA expression in breast cancer cell lines. Chromatin immunoprecipitation analysis showed that ERα binds to intron-2 of CSTA upon stimulation with estrogen. As an exception, in T47D cells, estrogen does not regulate CSTA. Since CSTA expression was significantly less in breast tumors than breast tissues, we also investigated the role of epigenetic mechanisms in the silencing of CSTA expression. CSTA expression was inversely associated with DNA methylation in the upstream and intron-2 region in breast tumors and breast cancer cells. Interestingly, ERα binding site was located amidst the three differentially methylated intron-2 CpGs. Therefore, we looked for the probable connection between DNA methylation and estrogen regulation of CSTA. Global demethylation restored the estrogen regulation of CSTA in T47D cells. This revealed that the estrogen regulation of CSTA in breast cancer is an integrated result of an interplay between ERα binding and DNA methylation in the intron-2 region. This study also attempted to understand the possible role of CSTA in breast cancer using stable cell lines. Overexpression of CSTA in breast cancer cells reduced migration and invasion of breast cancer cells without affecting proliferation. Taken together, this work offers novel insights into the regulation of CSTA expression in breast cancer. This is the first study to provide detailed molecular insights into the estrogen mediated regulation of CSTA. Further, it indicates that DNA methylation is the probable reason for the loss of CSTA expression in breast tumors. This work also proposes the potential interplay between ERα binding and DNA methylation in the regulation of CSTA expression.