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Overexpression and Activation of Histamine N-methyltransferase inducing Tumorigenesis in Human Breast Epithelial Cells

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Histamine N-methyltransferase (HNMT) is a histamine-degrading enzyme. The microarray data showed reported that HNMT was highly expressed in most breast cancer cell lines (HCC1954, HCC1500, MCF-7, MDA-MB-157, MDA-MB-231, SUM-225, SK-BR-3, MDA-MB-175, BT-483, BT-474, and ZR75-1). Through this study, we will understand and clarify the role and molecular mechanisms of HNMT gene in tumorigenesis of breast cancer and provide information for combined molecular targeted therapy for breast cancer in the future. This study we demonstrated HNMT expression profiles by RT-PCR. Total mRNAwas isolated from both human cell lines and breast tissue samples (n=370) using TRIzol reagent according to their manufacturer's protocol. For real-time PCR analysis, a LightCycler thermocycler was used. The HNMT mRNA fluorescence intensity was measured and normalized to GUS expression using the built-in software. The HNMT protein localization in breast tumor tissues was further assessed by immunohistochemistry. We also detected the tumorigenic ability, for example, cell growth, wound-healing, anchorage-independent cell growth, invasion assay, and etc. The interaction of HER2 and HNMT proteins was detected by immunoprecipitation (IP), fluorescence life-time imaging microscopy (FLIM), fluorescence resonance energy transfer (FRET), and the split luciferase assay. We investigated whether HNMT and HER2 interact by Deconvolution Microscope. We used xenografted tumor model to verify the importance of HNMT (HNMT inhibition/HNMT overexpression) in breast cancer growth. The cells (1 × 107) were implanted subcutaneously into 6-week-old nude mice (n=4).During the experiment, tumor size was measured using calipers, and the tumor volume was estimated using the following formula: tumor volume (mm^3) = ½ × L × W2, where L is the length and W is the width of the tumor. We demonstrated that high HNMT levels were preferentially detected in HER2+ (human epidermal growth receptor 2+) tumor tissues (8.25-fold, n=61, $p< 0.001$) and HER2+ tumor cells isolated by laser capture microdissection (LCM) (90.5-fold, n=5, $p< 0.001$). HNMT inhibition (SiRNA) suppressed tumorigenesis in vitro and in vivo. In contrast, HNMT overexpression promoted tumorigenesis in vitro and in vivo. We found that after Herceptin (200g/mL) treatment, HNMT membrane translocation was detected after 30 min, and the interaction of HER2 and HNMT proteins was detected by immunoprecipitation (IP), fluorescence life-time imaging microscopy (FLIM), fluorescence resonance energy transfer (FRET), and the split luciferase assay. We also found that HNMT translocated to the nucleus and that the HNMT/HER2 complex could be detected in the nucleus after 72 hours by FRET assay. A 3-dimensional imaging analysis was performed, and nuclear localization of HNMT/HER2 complex was detected at 72 hours after Herceptin treatment using a deconvolutuon microscope. HNMT acted as a transcriptional co-regulator of HER2 expression, as demonstrated by chromatin immunoprecipitation (ChIP) and ChIP sequencing analysis. This study demonstrated that HNMT is important for tumorigenesis. HNMT is a potential predictive biomarker that could help clinicians to more accurately select responders or non-responders to targeted therapy.