Functional Analysis of Mel-18 and BMI-1 in Different Breast Cancer Sub-types

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Breast cancer (BC) is the most common cause of cancer-related death in women globally. Polycomb group genes (PcGs) are essential for cancer stem cell (CSC) self-renewal, inhibit senescence through inhibition of p16, and influence tumorigenesis. PcGs are organized in multimeric Polycomb Repressive Complexes (PRCs), which catalyze histone post-translational modifications, thereby silencing specific genes. PRC1 can play an oncogenic or tumor-suppressor role depending upon the cellular context. BMI-1 and Mel-18 are PRC1 components. BMI-1 influences BC progression by repressing anti-metastatic and anti-apoptotic genes. Mel-18 may function as a tumor-suppressor by competing with BMI-1 and reactivating p16 expression. We have previously demonstrated that BMI1 cooperates with RAS to increase tumor aggressiveness and significantly increase metastases. Mel18 expression will modulate tumor aggressiveness and inhibit metastases in breast cancer. Gene expression analysis using the Oncomine database (oncomine.com) was performed to determine whether expression of Mel-18 is associated with molecular subtypes of BC. We evaluated Mel-18 and BMI-1 mRNA expression by Real-Time PCR and protein expression by western blot in a panel of BC cell lines. We performed Mel-18 Knockdown (KD) in cells with high Mel-18 expression using short hairpin-RNA (shRNA) in luminal type cells MDA-MB-453 and MCF7, and the basal BMI1/Mel18-overexpressing cell type MCF10A+BMI-1. We over-expressed Mel-18 in the low-Mel-18 expressing TNBC cell line MDA-MB-231. Proliferation, migration and invasion assays evaluated the growth rates and the invasive potential of BC cell lines with Mel-18 KD or over-expression. Further experiments investigating the potential role of Mel-18 and BMI-1 in the identification of BC-CSCs markers and the evaluation of primary tumors and metastasis development are being conducted.Analysis of the Oncomine database revealed that low expression of Mel-18 is associated with a shorter time-to-tumor-recurrence after mastectomy in all BC sub-types ($p<0.001$, fold-change: 2.043). Mel-18 is selectively silenced in TNBC compared to other histologic subtypes ($p=0.004$, fold-change: 2.238). Mel-18 expression is high in Luminal-type BC cell lines MCF-7, MDA-MB-453, and MCF10A cells overexpressing BMI-1 and barely detectable in the TNBC cell lines MDA-MB-231, HS578T, HCC38 and BT549 cells. BMI-1 expression increased in several BC cell lines following Mel-18 KD. However, BMI-1 expression increased in MDA-MB-231 cells that overexpress Mel-18. Rates of proliferation were increased in MCF7-sh-Mel18 and MCF10A-BMI1-sh-Mel18 cells, whereas migration increased in MCF7-sh-Mel-18 cells and in MCF10A-BMI1-sh-Mel18 cells compared to control cells. Interestingly, proliferation and migration was reduced in MDA-MB-231 cells overexpressing Mel-18 compared to the control. Mel-18 expression is generally high in luminal type BC and is associated with better prognosis and expression is lowest in TNBC. Depending on the cellular context, Mel-18 can alter proliferative and invasive properties and lead to the down-regulation or up-regulation of BMI-1. Further studies are being conducted to evaluate the biologic consequences of Mel18 expression and mechanisms of its action. This may lead to additional insights that may be of value for designing innovative treatment strategies.