Abstract

ErbB2-positive breast cancers exhibit highly malignant and invasive behavior, which is associated with increased disease recurrence and poor prognosis. We have previously identified a novel signaling network that links overexpression of ErbB2 to elevated expression of the lysosomal proteases, cysteine cathepsin B and L, enhanced breast cancer cell invasiveness and a distribution of lysosomes to the cellular periphery e.g. into structures resembling invadosomes. The identified signaling network increases cathepsin B amount and activity by activating the transcription of the cathepsin B gene (CTSB) via the transcription factor myeloid zinc finger-1 (MZF1) leading to invasion. Depletion of MZF1 results in a nearly complete inhibition of the invasive growth of breast cancer cells overexpressing a constitutive active truncated form of ErbB2, as well efficient inhibition of invasion in both the ErbB2 positive cell lines, SK-BR-3 and MDA-MB-453, showing that MZF1 is necessary for the invasive capabilities of breast cancer cells overexpressing ErbB2.

Here we show that let-7 microRNA (miRNA), a well-established tumor suppressor, regulates MZF1 levels in breast cancer cells. Our results indicate that MZF1 is a direct target of specific let-7 miRNAs, which by downregulating MZF1 abrogates the invasive cellular phenotype of different breast cancer cells. Supportively, the analysis of breast cancer patient data from almost 600 primary breast cancer tumors showed that MZF1 expression correlates negatively with the expression of the specific let-7 family members. Thus, this study shows that the oncogenic function of MZF1 in breast cancer can be regulated by let-7 miRNA and suggests that loss of let-7 can increase the malignancy of breast cancer cells via mechanism that involves increased expression of MZF1.

The biogenesis of let-7 is blocked by the RNA-binding proteins Lin28A and Lin28B, regulators of growth and developmental timing. Aberrant activation of Lin28A/B expression has been reported in several cancers and Lin28A/B overexpression in primary breast tumors has been reported to be a powerful predictor of poor prognosis. Blocking the biogenesis of the let-7 family miRNAs, and thereby promoting malignancy has been thought to underlie the role of Lin28A/B in cancer. Here we show that LIN28A contains several predicted MZF1 bindings sites its proximal promoter region, suggesting transcriptional regulation by MZF1 and a putative MZF1-Lin28A-let-7-axis. Analysis of data from mRNA expression microarray of more than 500 primary breast cancer patient samples and analysis of tissue micro-arrays (TMA) comprising 225 breast cancer patient samples both revealed a positive correlation between MZF1 and Lin28A expression. However, we are unable to identify a direct regulation of Lin28A expression by MZF1.

Oncogenic transformation of cells has been shown to severely affect the lysosomal compartment, including changes in the proteolytic content and redistribution to the periphery of the cells. Similarly, increased activity ErbB2 increases proteolytic content and induces redistribution of lysosomes into cellular protrusions. Cancer cells can activate and utilize lysosomes and their degradative enzymes to promote invasion and metastasis through exocytosis. The transcription factor TFEB controls the biogenesis and exocytosis of lysosomes under normal conditions. In breast cancer cells TFEB was found not to be involved in the ErbB2-induced
increased expression of CTSB. Instead, the CTSB transcription activating function of TFEB was taken over by MZF1. Here, we aim to identify the transcriptional program activated by MZF1 in response to ErbB2-signaling, as it is very likely that cathepsin B is not the only gene contributing to invasiveness, which is activated by MZF1 in response to ErbB2. To identify ErbB2/MZF1 regulated genes we have performed chromatin-immunoprecipitation (ChIP) sequencing and compare the genome-wide binding of MZF1 in cells overexpressing constitutive active ErbB2 and vector control cells. The results of the ChIP sequencing might elucidate whether MZF1 is involved in cancer-induced lysosomal changes and exocytosis.