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A way to invade
A story of ErbB2 and lysosomes

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Overexpression of the ErbB2 (Her2/neu) receptor tyrosine kinase is implicated in the induction and progression of many different types of human cancers. It is particularly frequent in breast cancer, where up to 25% of patients overexpress ErbB2. The ErbB2-positive cancers exhibit extremely malignant and invasive behavior, which is associated with increased disease recurrence and worsened prognosis. Although many of the signaling pathways activated by ErbB2 are well characterized, the understanding of its downstream effectors that can drive cancer cell invasiveness has just begun to emerge.

With expression array or tissue microarray analysis of over 1,000 primary breast cancer samples and healthy controls, we recently showed that the expression of cathepsin B and L genes (CTSB and CTSL1, respectively) correlate positively with the ErbB2 status in breast cancer patients.[1] This finding supports earlier observations showing that in malignant tumors, lysosomal cathepsin B and L expression and activity are increased. This increase is connected to altered lysosomal distribution, upon which lysosomes migrate from their "normal" perinuclear location and adapt pericellular location, accumulating toward the invasive tumor front.[2] This process is assumed to facilitate secretion of the lysosomal contents into the extracellular space, where it will promote matrix degradation, cancer cell migration, invasion, angiogenesis and metastasis. Several in vivo studies using different murine cancer models support this by showing that increased cathepsin activity, especially cathepsin B, is important for tumor invasion and metastasis.[3-5]

We next asked the question whether ErbB2 could regulate the cathepsin B and L levels in breast cancer cells. Indeed this was the case.[1] Moreover, we found out that activation of ErbB2 signaling utilizing an inducible expression system led to pericellular localization of lysosomes and made cancer cells invasive, as measured with a three-dimensional Matrigel invasion assay model system. This all could be reversed by depletion of cathepsins B and L. Employing high-throughput RNA-interference-based screens; we identified a signaling network that consists of several regulators of ErbB2-induced cathepsin B and L expression that is needed for the invasiveness of ErbB2-positive breast cancer cells. This network included protein kinases that we found functioning downstream of ErbB2, such as p21-activated protein kinase 4 (PAK4), cdc42 binding protein kinase β (Cdc42bpβ), the protein kinase Cα (PKCα) and extracellular-regulated kinase 2 (ERK2). These ErbB2-regulated kinases can increase the activity of transcription factors ETS1 and myeloid zinc finger-1 (MZF1), leading to increased expression of CTSB and CTSL1. All of these components were found crucial for the invasion process.

Our work on ErbB2 and cathepsins B and L led us to identify a signaling network that regulates a previously unidentified, invasive arm of the ErbB2 signaling and could thus contain potential targets (or markers) for the specific inhibition of ErbB2-induced invasion.[1] Of the components discovered that were particularly interesting are the serine-threonine kinase Cdc42bpβ and the transcription factor MZF1. Very little information exists of either of them in respect to cancer, making them attractive and promising research targets. Of these, a recent study shows that MZF1 binds to the gene encoding receptor tyrosine kinase Axl, increasing its expression and leading to enhanced migration and invasion of colorectal and cervical cancer cells.[6] We showed that MZF1 activates CTSB expression by binding to an ErbB2-responsive enhancer element in the first intron of CTSB in vivo.[1] In respect to this, it would be interesting to investigate if Axl could be an additional target of ErbB2 signaling.

Another recent study has identified the transcription factor EB (TFEB) as a master regulator of lysosomal biogenesis.[7] In response to cellular degradative needs, TFEB can upregulate the expression of several lysosomal genes, including cathepsins B and L, thus increasing the amount and activity of lysosomes. TFEB-activated gene program can also facilitate lysosomal secretion, also known as lysosomal exocytosis, promoting cellular clearance and well-being.[8] Interestingly, activated ERK2 and mTORC1 phosphorylate TFEB at Serine 142, blocking its transport to nucleus,[9,10] meaning that in cancers harboring active mTORC1 or ERK signaling, such as ErbB2-positive cancers, TFEB could not enter the nucleus or induce lysosomal biogenesis or secretion, but would, instead, be sequestered to cytosol. On the other hand, another recent study has shown that the activation of mTORC1 leads to the phosphorylation of TFEB at its serine-rich region between amino acids 462 and 469, inducing its nuclear-transport.[11] All in all these studies indicate that the nuclear entry and biological function of TFEB is under complex regulation, an understanding of which has just begun to emerge. Supporting the previous studies, we did not observe an involvement of TFEB in the ErbB2-induced invasion pathway,[1] although TFEB and MZF1, in

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principle, can activate similar lysosomal processes, suggesting that at least some oncogene-induced malignant changes in the lysosomal function may occur independent of TFEB. These studies altogether show that cysteine cathepsin-mediated invasion involving increased cysteine cathepsin activity, and lysosomal translocation is actually a tightly controlled and complex process.

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