Lysosomes as Targets for Cancer Therapy

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Abstract

Tumor invasion and metastasis are associated with altered lysosomal trafficking and increased expression of the lysosomal proteases termed cathepsins. Emerging experimental evidence suggests that such alterations in lysosomes may form an “Achilles heel” for cancer cells by sensitizing them to death pathways involving lysosomal membrane permeabilization and the release of cathepsins into the cytosol. Here, we highlight recent results on cancer-related changes in the composition and function of lysosomes, focusing on possible implications for the development of novel cancer therapeutics that target tumor cell lysosomes. (Cancer Res 2005; 65(8): 2993-5)

Cathepsins Have Multiple Functions

The lysosomal compartment is responsible for the controlled recycling of cellular organelles and macromolecules (1). Both heterophagic and autophagic cargos find their final destiny in lysosomes, where they are broken down by numerous hydrolyses. Proteases of the cathepsin family are among the best studied lysosomal hydrolases. Cathepsins can be divided into three subgroups according to their active-site amino acid [i.e., cysteine (B, C, H, F, K, L, O, S, V, W, and X/Z), aspartate (D and E), and serine (G) cathepsins (2)]. They are optimally active in the acidic pH of lysosomes (pH 4-5) but can also function in neutral pH outside the lysosomes, albeit with less efficacy. Apart from their function in general protein turnover, certain cathepsins perform also more specific functions, for example in the control of cell-cycle progression, antigen presentation, epidermal homeostasis, and hair follicle morphogenesis (3). Furthermore, emerging evidence indicates that cathepsins have interesting functions outside the lysosomal compartment (i.e., degradation of extracellular matrix when secreted to the extracellular space and execution of programmed cell death when released to the cytoplasm; refs. 4, 5).

Several members of the cathepsins have been implicated in cancer progression, in particular the cysteine cathepsins B and L and the aspartate cathepsin D. High expression levels of these cathepsins offer a reliable diagnostic marker for poor prognosis (6). Oncogenic activation may contribute to altered lysosomal function, as shown in murine fibroblasts and breast epithelial cells transformed with ras oncogene (7, 8). In cancer cells, especially those at the invasive edges of tumors, the localization of lysosomes is often altered from perinuclear to peripheral regions and the lysosomal contents are commonly secreted into the extracellular space (4). Together with matrix metalloproteases and the plasminogen activator system, secreted cathepsins have been suggested to participate in the degradation of extracellular matrix, thereby enabling enhanced cellular motility, invasion, and angiogenesis.

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Immortalization and Transformation Sensitize Cells to the Lysosomal Death Pathway

The overexpression of antiapoptotic proteins (e.g., Bcl-2, Bcl-xL, Akt/PKB, and inhibitors of apoptotic proteins) as well as mutations in prosapoptotic proteins (e.g., Bax, Apaf-1, and p53) favor tumor development by blocking classic apoptosis pathways and by interfering with efficient caspase activation (5). However, it should be noted that although cancer cells may all harbor one or several defects in the classic death machinery, cancer cells are nonetheless still able to undergo programmed cell death. Indeed, particularly during early stages of tumorigenesis, cells are sensitized to numerous death stimuli and often undergo spontaneous cell death, possibly due to activation of oncogenes such as Myc or Ras, which can be sufficient to trigger cell death themselves or to sensitize cells to various cytotoxic drugs or death-receptor activation events (13).

The marked changes that occur in the lysosomal compartment during the cell transformation process and the involvement of lysosomal enzymes in death pathways that remain functional in advanced tumor cells suggest that the lysosomal death pathway could contribute significantly to the programmed cell death sensitivity of cancer cells (Fig. 1). For example, recent findings from our laboratory show that immortalization and transformation sensitize murine embryonic fibroblasts to the lysosomal cathepsin-mediated death pathway triggered by tumor necrosis factor (TNF; ref. 14). By itself, spontaneous immortalization in this system,

Two recent reports based on in vivo tumor models provide strong experimental evidence supporting this hypothesis. In a mouse model of pancreatic cancer, pharmacologic inhibition of cysteine cathepsin activity impaired angiogenic switching in progenitor lesions and inhibited tumor growth, vascularity, and invasiveness (9). In line with these data, genetic suppression of cathepsin B and matrix metalloprotease-9 expression significantly reduced tumor cell invasion, tumor growth, and angiogenesis in a mouse glioblastoma model (10).

Akin to tumor progression, cathepsins B, L, and D have been implicated as mediators of the lysosomal cell death pathway (summarized below and in refs. 5, 11). A wide variety of death stimuli such as death-receptor activation, p53 activation, microtubule-stabilizing agents, oxidative stress, growth factor deprivation, and staurosporine can induce partial lysosomal membrane permeabilization and the release of cathepsins into the cytosol (5, 11). Depending on the extent of the lysosomal membrane permeabilization and the amount of active cathepsins released into the cytoplasm, a variety of death morphologies from classic apoptosis to necrosis can be triggered. Cathepsins activate the classic apoptosis pathway possibly by cleaving the proapoptotic Bcl-2 family member Bid (12). From the cancer point of view, however, it is most exciting to note that cathepsins are able to mediate caspase- and mitochondrion-independent programmed cell death, allowing cell death to occur even in cancer cells with multiple defects in the classic apoptosis pathway (5).
which is linked to inactivation of the p53 pathway, was sufficient to sensitize murine embryonic fibroblasts over 1,000-fold to TNF-induced cell death. This sensitization was highly dependent on cathepsin B activity because immortalized murine embryonic fibroblasts derived from cathepsin B–deficient mice displayed the TNF-resistant phenotype that is characteristic of wild-type primary murine embryonic fibroblasts. Importantly, the TNF resistance of cathepsin B–deficient immortalized murine embryonic fibroblasts could not be explained by differences in growth rate, ploidy, p53 mutations, p19Arf status, or TNF-receptor expression or functionality. Furthermore, reintroduction of cathepsin B into immortalized cathepsin B–deficient murine embryonic fibroblasts was sufficient to resensitize them, whereas inhibition of cysteine cathepsin activity by pharmacologic inhibitors or RNA interference significantly protected immortalized wild-type murine embryonic fibroblasts against TNF-induced cell death. Lastly, transformation of immortalized murine embryonic fibroblasts with v-Ha-ras or c-Src enhanced the expression of cysteine cathepsins (B and L) by severalfold and further sensitized cells to TNF-induced lysosomal cell death. Human breast epithelial cell lines are also sensitized to chemotherapy-induced death by oncogenic transformation (15).

Whereas cell transformation was associated with increased expression of cysteine cathepsins, cell immortalization had no effect on the intracellular level of these enzymes. Thus, the mechanism by which immortalization sensitizes murine embryonic fibroblasts to TNF-induced lysosomal membrane permeabilization remains unclear. The volume of individual lysosomes as well as that of the total lysosomal compartment increases during replicative aging of fibroblasts. This phenomenon may be relevant because larger lysosomes have been suggested to be more susceptible to rupture than small lysosomes (16). Alternatively, lysosomal membranes may be destabilized by the oxidative stress produced by the increased lysosomal accumulation of iron, due to Fenton chemistry, which is in turn produced by the increased protein turnover that occurs in rapidly growing cells (17). Finally, it is possible that immortalization may affect a specific signaling event between the TNF receptor and the lysosome. We propose that this last option is plausible because immortalization sensitized cells much more than other stimuli to induction of the TNF-induced lysosomal death pathway. However, it should be noted that lysosomal death pathways could be very different from each other, either at the level of the mechanism or sensitivity of lysosomal membranes to permeabilization or at levels downstream of this.

1 Unpublished observations.
step on, the basis of results from immortalized murine embryonic fibroblasts derived from various genetic backgrounds. For example, whereas the cathepsin B–dependent TNF-induced lysosomal death pathway functioned normally in cathepsin D–deficient cells, cell death induced by staurosporine depended on cathepsin D, but not on cathepsin B (14). In closing, there remains much to learn about the mechanisms underlying lysosomal death pathways that use cathepsins, as well as the modifier pathways that may dictate sensitivity to this general form of cell death in immortalized and transformed cells.

Lysosomes as Targets for Future Cancer Therapy

Emerging evidence argues that both classic apoptosis pathways and lysosomal death pathways must be suppressed for effective development and progression of cancer (5). The increased activity of phosphatidylinositol-3-kinase, which is characteristic of many cancers, may contribute to the stability of tumor cell lysosomes. Among its many other functions, phosphatidylinositol-3-kinase controls the maturation, size, and activity of lysosomes (18), and the inhibition of phosphatidylinositol-3-kinase in certain settings can shift the TNF-induced death pathway from caspase dependent to cathepsin dependent (19). Tumor cells may also protect themselves against lysosomal leakage by translocation of heat shock protein 70 into the lysosomal membrane (20). Cells that have heat shock protein 70 on their lysosomal membranes contain larger and more stable lysosomes, and the depletion of heat shock protein 70 triggers a tumor cell–specific lysosomal death program. Finally, expression of cathepsin inhibitors in the cytosin (21) or serpin families (22) may increase the viability of cancer cells with enhanced lysosomal activity. Thus, drugs that inhibit the phosphatidylinositol-3′-kinase pathway, the lysosomal localization of heat shock protein 70, or the activity of certain cathepsin inhibitors are likely to sensitize tumor cells to cancer drugs by facilitating lysosomal membrane permeabilization.

The study of lysosomal changes in tumor progression and treatment is still very young, but the recent great advances in this field promise rapid progress in the near future. A more defined role for each of the cathepsins is awaited by dissecting their nonredundant functions in tumor progression and cell death. Important lessons can also be learnt from wound-healing models, where changes in lysosomal trafficking similar to those seen in invasive tumors give rise to cell migration and subsequent wound closure (23). Because tumor cells exhibit rather specific characteristics in their increased cysteine cathepsin levels, altered lysosomal trafficking, and shifts in different endolysosomal populations, drug screens to identify molecules that induce lysosomal membrane permeabilization may prove effective in cancer treatment. A very recent study on the screening of a small molecule drug library identified 175 compounds that induced death in cultured HCT116 colon cancer cells. Importantly, over half of the 11 compounds that induced significant cell death in p53-null cells triggered lysosomal membrane permeabilization and cathepsin-mediated killing of tumor cells (24). Taken together, the recent progresses in lysosome research prompt additional investments into the detailed elucidation of tumor-associated lysosomal changes, lysosomal death pathways, and mechanisms controlling lysosomal membrane stability.

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