Cysteine cathepsins (proteases)—On the main stage of cancer?

Cysteine cathepsins are involved in degradation of extracellular matrix, facilitating growth, invasion, and metastasis of tumor cells, in tumor angiogenesis, in apoptosis, and in events of inflammatory and immune responses. In this issue of *Cancer Cell*, Joyce et al. (2004) demonstrate association of increased cathepsins activity with angiogenic vasculature and invasive fronts of carcinomas during tumorigenesis in transgenic mouse models using activity-based chemical probes and in vivo imaging. Moreover, this study shows that a broad-spectrum cysteine cathepsin inhibitor effectively blocks several stages of tumorigenesis in the RIP1-Tag2 transgenic mouse model, offering new therapeutic opportunities in cancer treatment.

For more than thirty years, proteases have been known to be critically involved in a number of steps in tumor progression, such as tumor growth, invasion, migration and metastasis (Koblinski et al., 2000). Although ~500–600 proteases have been found to exist in human and mouse genomes (Puente et al., 2003), not all of them have been found to be linked with cancer. The main stage has been primarily reserved for the metalloproteases from the MMP family (matrix metalloproteases) and, to a smaller extent, to the serine protease uPA (urokinase-type plasminogen activator). Although evidence for their involvement in cancer progression has been accumulating for more than two decades, cysteine cathepsins have always somehow lagged behind (Rao et al., 2003; Koblinski et al., 2000; Kos and Lah, 1998). However, the failure of broad-spectrum MMP inhibitors in clinical trials (Coussens et al., 2002) has opened the door for other proteases to be considered as relevant drug targets in anticancer therapies.

In this issue of *Cancer Cell*, Joyce et al. (2004) present data showing that increased cysteine cathepsin levels and activities in tumors are associated with the angiogenic vasculature and invasive fronts of pancreatic neuroendocrine carcinomas in the RIP1-Tag2 transgenic mouse model, accompanied by differential expression of cathepsins in immune and endothelial cells. Support for the generality of these findings comes from a pilot study in a second mouse model, mimicking human cervical carcinogenesis (K14-HPV/E_2). These are the first transgenic mouse models of cancer in which cathepsins have been studied, and they offer a major advantage over cell culture and xenograft models. Moreover, transgenic mouse models offer the possibility to study the full ontogeny of cancer development, including the early premalignant steps; by contrast, xenotransplants and human clinical studies typically only involve the later stages, where the disease has already advanced. However, genetically engineered mouse models cannot necessarily be directly translated to human disease, nor do we have mouse models for all types of cancer, suggesting that both old and new model systems will continue to be instructive.

So how are cysteine cathepsins, normally involved in intracellular turnover, immune response, protein processing, and other important cellular processes, connected to cancer? There are 11 cysteine cathepsins present in the human genome (B, C, F, L, K, V, S, X/Z, H, W, and O) and 19 in mouse (10 of them are orthologs), each with different expression patterns, levels, and specificities, all of which contribute to their differential physiological roles. Some, such as cathepsins B, L, and H, are very abundant; none are completely specific, and most are highly active but differently stable at neutral pH—collectively making these proteases potentially harmful if transposed outside of their normal endosomal/lysosomal localization (Turk et al., 2001). One situation where trouble could occur is in the degradation of the extracellular matrix (ECM), a proteolytic event associated both with early tumor development, affecting tumor cell proliferation and angiogenesis, and with dissemination of malignant cells from primary tumors. Although ECM degradation has largely been attributed to MMPs, it is now clear that different classes of proteases can also make a contribution, with cathepsins being involved either directly in the degradation of components of ECM, such as laminin, fibronectin, and collagen, or through the modulation of protease-sensitive regulatory networks, involving other proteases as well as non-proteases, such as annexin II, found at the cellular surface of cancer cells (Koblinski et al., 2000; Roshy et al., 2003).

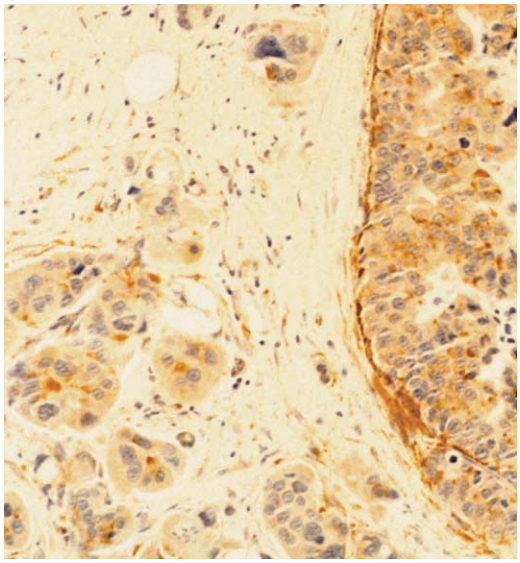

Figure 1. Expression of cathepsin B in human invasive ductal breast carcinoma

The carcinoma is on the right and normal tissue, including tumor cell nests, is on the left. Cathepsin B (brown color revealed by peroxidase staining) is located at the invasive front of the tumor and involved in the basement membrane degradation.

The common belief is that cathepsin-mediated degradation of the ECM is primarily extracellular at the invasive front of tumor cells, also observed by Joyce et al. (2004). However, analyses of proteolytic degradation of quenched fluorescent protein substrates in living cells showed that cells differ in their sites of matrix remodeling, which can be extracellular, intracellular, or even a combination of both (Roshy et al., 2003; Premzl et al., 2003). Furthermore, inhibitors capable of blocking both intra- and extracellular fractions of cathepsin B were the most effective in reducing the invasive potential of tumor cells (Premzl et al., 2003). It is thus the activity of a protease, often connected with altered cellular localization, that usu-

ally makes the critical switch between harmless and harmful, and not the differential expression pattern. That is precisely one of the important points made by Joyce et al. (2004) by using small cell permeable active-site directed probes: they were able to measure and image protease activities in living cells, which is becoming a key parameter of analysis in cancer, and in other biological systems.

The involvement of cathepsins in regulation of angiogenesis reveals yet another distinct role for cathepsins in tumor progression (Joyce et al., 2004), adding to previous results of Felbor et al. (2000) implicating cathepsin L in generation of the angiogenesis inhibitor endostatin, as well as to more recent data suggesting a role for cathepsin S in wound healing (Shi et al., 2003). Thus, new functions of cathepsins in tumor progression are emerging, and we can expect more to be revealed.

Despite differential expression of 6 cathepsins in the development of pancreatic islet carcinomas (Joyce et al., 2004), their individual functions have not yet been assigned. Nevertheless, these alterations in cathepsin expression levels (primarily of cathepsins B and L), processing, and localization, which have been observed in various tumors (breast, lung, brain, colon, and head and neck tumors), when compared to their normal and benign tissue counterparts, have also made cysteine cathepsins valuable prognostic and diagnostic markers (Kos and Lah, 1998).

An important breakthrough achieved by Joyce et al. (2004) was the use of an analog of the irreversible cathepsin binding scaffold E-64 as a broad spectrum pharmacological inhibitor of cysteine cathepsins. This compound, JPM-OEt, had profound effects on tumor growth, invasiveness, and angiogenic switching, disrupting both early and late stages of tumorigenesis, in contrast to MMP inhibitors, which have not proved effective in the later stages of the disease (Coussens et al., 2002). Interestingly, the cathepsin inhibitor was not found to be toxic at the dosage used, which led the authors to suggest its potential use in cancer treatment. Despite these promising results (Joyce et al., 2004), one should be very careful when suggesting use of nonselective inhibitors in treatment of a disease such as cancer, where the roles of individual players, e.g., cysteine cathepsins, are not fully resolved. Recall the failed clinical trials targeting late stage tumors with broad-spectrum MMP inhibitors, which showed beneficial effects in mouse models (Coussens et al., 2002), albeit at earlier stages.

A number of other proteases, such as proteasome and histone deacetylases, are emerging as potential drug targets in cancer, and new selective inhibitors of MMPs are being developed. Another interesting approach in anticancer therapy is based on protease activation of apoptosis (Schimmer et al., 2004). Instead of blocking protease activity, they suppressed the activity of XIAP, an endogenous inhibitor of another family of cysteine proteases, the caspases, thereby enabling caspase activation, selectively sensitizing a number of different cancer cells to chemotherapeutic drugs or even directly inducing cancer cell apoptosis.

Future research should reveal which of the protease-based approaches will be the most beneficial for targeting different forms of cancer. Given the variety of cancers, with distinctive molecular and cellular anatomy, there is not likely to be one ideal therapy for all of them. Specific protease inhibitors, likely in combination with conventional anticancer agents, will probably prove to have value for certain forms of cancer. It is now becoming clear that cysteine cathepsins should be seriously considered as potential targets in cancer treatment.

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