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The cathepsin family and their role in colorectal cancer

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Abstract

Cathepsins are a class of globular proteases, initially described as intracellular peptide hydrolases, although several cathepsins also have extracellular functions. Cathepsins B, C, F, H, L, K, O, S, V, W, and X are cysteine proteases of the papain family, and represent the largest and best-known class of the cathepsins. Cathepsin G is a serine carboxypeptidases, and cathepsins D and E are aspartic proteases. Cathepsins are synthesized as inactive proenzymes and processed to become mature and active enzymes. Endogenous protein inhibitors, such as cystatins and some serpins, inhibit active enzymes. As primarily lysosomal proteases, cathepsins play important roles in proteolysis during physiological processes, as well as in several diseases. On the basis of their ability to degrade extracellular matrix proteins, cathepsins have been implicated to play a role in invasion and metastasis of colorectal cancer. In the present review, the role of cathepsins in the disease process of colorectal cancers and the correlation of cathepsin expression and activity with clinicopathological features is discussed. Furthermore, we give an overview of the recent developments of cathepsins in animal models and in *in vitro* experiments of colorectal disease, and provide information on inhibitors of cathepsins as possible therapeutics.

Keywords: Cathepsins; Proteases; Colorectal cancer; Invasion; Budding

Introduction

Cathepsins are *lysosomal peptidases* which belong to the cysteine, serine, or aspartic protease classes. Some cathepsins are ubiquitously expressed, such as cathepsins B, L, H, and C, whereas different newly found cathepsins like cathepsins K, W, and X were expressed by specific cells and tissues [9,8,23,41,42,44,85,86]. Historically, cathepsins were described as a group of intracellular hydrolases participating in general protein turnover in the lysosome. However, in the last 15 years, using gene knockout models, a number of discrete functions were found for different members of the cathepsin familiy. Cathepsin S was shown to be important for the MHC-II mediated antigen presentation [69]; cathepsin L is implicated in processes of keratinocyte differentiation [59], heart functions [76], and reproduction [57]; cathepsin K is a major factor in bone remodeling [62], and cathepsin C activates granzymes and mast cell proteases. Mutations in cathepsin K and cathepsin C genes result in hereditary disorders, in pycnodysostosis, and Papillon–Lefevre syndrome, respectively (for review see Ref. [83]).

In addition to their involvement in different *physiological processes*, several cathepsins participate in *inflammatory processes*, e.g. periodontitis, rheumatoid arthritis, atheriosclerosis, pancreatitis and gastritis. However, the main focus is the functional role of cathepsins in *tumor progression* and *metastasis* [53,58].

Abbreviations: ECM, extracellular matrix; CRC, colorectal cancer.

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Enhanced production and release of cathepsins in tumor cells leads to tumor cell growth, invasion, and metastasis. Cathepsin D has mitogenic properties, stimulates the release of basic fibroblastic growth factor, and degrades many intracellular and endocytosed proteins. Cathepsin D participates in limited proteolysis, activates procathepsins B and L, and also degrades and inactivates their active forms [5,81]. Cathepsins B, L, and D are capable of degrading extracellular matrix (ECM) molecules, including collagen, fibronectin, proteoglycans, and elastin [49], as well as basement membrane components, including Type-IV-collagen and laminin [74,73].

In tumor cells, *regulation* at one or more of the levels changing cathepsin biosynthesis leads to an upregulation, membrane association, and secretion of the particular cathepsin. Extensive studies on cathepsin expression in different cancer types including melanoma and carcinomas of the lung, colon, prostate, and breast revealed increased expression of cathepsin B, L, or D [83]. In some of these tumor entities, cathepsins and their *inhibitors* reached prognostic significance. Furthermore, the localization of cathepsins B and L alters as the grade of malignancy of human tumors increases. Proenzymes and/or mature cathepsins can be secreted, can be found to be associated with the plasma membrane, or can be redistributed to vesicular compartments. Immunohistochemical studies found cathepsins at the invasive edges.

Expression of cathepsins in intestinal mucosa

Cathepsins B, L, D, H, F, X, and E were detected in the normal intestinal mucosa even at the mRNA level of different cell populations. Electron immunocytochemistry localized cathepsin E to endosomal vesicles and endoplasmic reticulum of specialized M cells in human ileum, indicating a possible role for cathepsin E in the processing of macromolecules and microorganisms [19]. Northern blots showed a widespread distribution of cathepsins X and F, including normal colon and colon tumors [64,84]. Cathepsin H protein was primarily localized to epithelial cells as a punctuate pattern typical of lysosomal localization [16]. However, there is no doubt that cathepsins D, B, and L are the most extensively analyzed cathepsins in colorectal diseases. Mice deficient in cathepsin D are normally born but showed progressive atrophy of the intestinal mucosa in the third week of postnatal development, indicating that cathepsin D is essential for regulating cell growth and tissue homeostasis of colon epithelium [61]. Hausmann and Menzel et al. performed subtractive hybridization screenings and mRNA analysis between normal intestinal macrophages and inflammation-associated macrophages, and found a dramatic induction of tissue degrading enzymes, such as cathepsins D, B, and L [24,52]. In contrast to cathepsin D mRNA, which was expressed only in inflammation-associated macrophages, mRNA of cathepsins B and L was even found in normal intestinal macrophages, but besides cathepsin D, only cathepsin L was significantly upregulated by inflammation. Immunohistochemistry revealed macrophages as cathepsin-expressing cells, and showed a significant subepithelial accumulation of cathepsin-positive cells close to the crypts in areas of mucosal ulcerations in cases of ulcerative colitis and Crohn's disease. In an experimental colitis model, inhibition of all three cathepsins resulted in an amelioration of DSS-induced colitis. The inhibitors (CA074-cathepsin B; Z-Phe-Tyr-aldehyde-cathepsin L; pepstatin A–cathepsin D) reduced the severity of colitis in all parameters tested [24,52].

As early as 1985, cathepsin B was shown for the first time to be expressed in ganglion cells and macrophages of the small intestinal mucosa; the epithelium in appendix and colon displayed weak expression [30]. Later, cathepsin B and L activities were detected in normal colon mucosa with intermediate and low levels compared to other tissues. In the early 1990s, the studies of cathepsin in normal tissues were extended to analyze these enzymes in malignancy. The results concerning the role of different cathepsins in cancer progression, survival rates, and metatstasis are partly contradictory. However, cathepsins B, L, and D were generally overexpressed in colorectal carcinomas.

Detection of cathepsins in colorectal carcinoma and its precursors lesions

Several investigations have confirmed a significantly higher mRNA content, antigen level, enzymatic activity, and immunohistochemical protein expression of cathepsins D, L, H, and, in particular, cathepsin B in the cytosol and homogenat in tissue of colorectal carcinoma (CRC) compared to matched controls of unchanged colorectal mucosa [1,22,51,55,54,66,70,78]. This observation clearly indicated that these cathepsins might be involved in CRC development and growth. Usually, epithelial cells of normal mucosa show negative or minimal positive immunohistochemical staining of cathepsins B, D, H, and L [10,34,50,78].

Regarding precancerous changes of CRC, data are scarce. In *colorectal adenoma*, immunohistochemical studies revealed no or limited staining for cathepsins B and D, unrelated to the degree of dysplasia [3,10,33,34,70,78]. Staining intensity, as well as antigen and activity levels for cathepsins B and D increased with the transition from adenoma to adenocarcinoma, suggesting that elevated cathepsin levels may be a sensitive marker of progression from premalignant colorectal adenoma to invasive CRC [39,70,78]. Insterestingly, the *subcellular distribution* pattern of the cathepsins changes during the adenoma–carcinoma