

Regulatory role of cathepsin D in apoptosis

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Abstract: Cathepsin D (CTSD, EC 3.4.23.5) is well known aspartyl protease. Among different role in cell physiology, a new function of this enzyme is examined. Cathepsin D is an important regulator of apoptotic pathways in cells. It acts at different stage of intrinsic and extrinsic pathway of apoptosis. Cathepsin D can either induce apoptosis in presence of cytotoxic factors, but in certain studies an inhibitory role in apoptosis was also reviewed. Detailed review of involvement of cathepsin D in cell apoptosis is a purpose of this paper.

Keywords: Cathepsin D - CTSD - Apoptosis - Regulation of apoptosis

Introduction

Programmed cell death plays crucial role in many physiological processes from fetal development and in adult tissues. Apoptosis is strictly regulated through many molecular mechanisms, including proteolytic enzymes such as cathepsin D (CTSD). Cathepsin D is a glycoprotein, member of A1 family of aspartyl proteases. To A1 family of aspartyl proteases belong most of aspartyl endopeptidases present in human body *e.g.* cathepsin E, pepsin A or renin. Aspartyl proteases hydrolyze peptic bond inside polypeptide chains. In physiology, cathepsin D is localized inside lysosomal compartment. Beside catalytic functions, new function of this protease are recently described *i.e.* regulation of programmed cell death. This function is expressed on various steps of apoptotic pathways. In this short review we will concentrate on regulation of certain apoptotic processes by cathepsin D.

Cathepsin D (EC 3.4.23.5)

The gene of human cathepsin D is located on short arm of chromosome 11 in region p15 in vicinity of H-ras oncogene [1,2,11]. Overexpression of *CTSD* gene leads to increased synthesis of proenzyme, overloads

transport pathways to lysosomes and is observed as extracellular secretion of procathepsin D [4,14]. Human cathepsin D consists of 412 aminoacidic residues, with pre-part that is 20 aminoacids long and pro-part that is 44 aminoacids long [8]. During its cell life cathepsin D undergoes various proteolytic transformations. However, posttranslational glycosylation is observed only in cathepsin D, cathepsin E and memapsin [36]. Other aspartyl proteases does not present sugar moieties.

Sugar moiety is consist of two oligosaccharide chains connected by N-glycosyde bond with protein element [5,6]. Terminal mannose residues are phosphorylated to mannose 6-phosphate. Differences in sugar moiety structure is thought to be responsible for heterogeneity of the enzyme. Cathepsin D is synthesized as preproenzyme, transformed into catalytically inactive proenzyme. Proteases are necessary for transformation of procathepsin D (52 kDa) into active intermittent form (48 kDa) and mature double-chained enzyme (34 kDa and 14 kDa). Pro-sequence removed during maturation of enzyme is described in literature as propetide or activating peptide. Activating peptide is usually 40-60 aminoacids long. Cleavage of prosequence can be also autocatalytic. Detailed studies on maturation of cathepsin D revelead, that during autoactivation only, the so-called pseudo-cathepsin D is produced, containing C-terminal part of activating peptide connected to mature enzyme [9,36]. Full activation of cathepsin D can be achieved only through involvement of other lysosomal peptidases.

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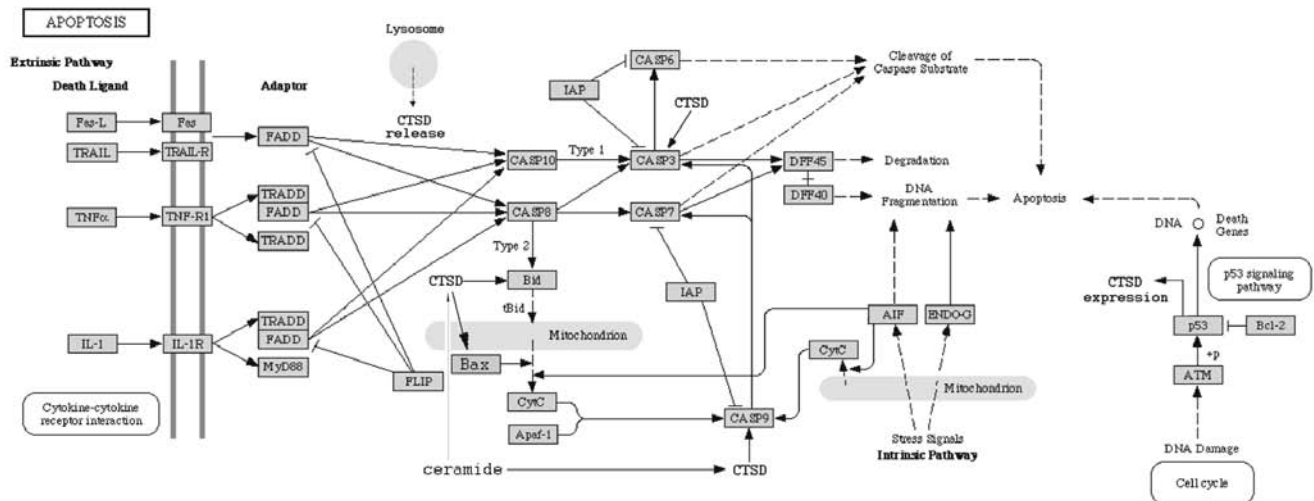


Fig. 1. Involvement of cathepsin D (CTSD) in apoptosis regulation. Solid line presents induction of process; broken line represents efflux of enzymes (e.g. cathepsin D from lysosomes, cytochrome c from mitochondrion). Abbreviations used: CTSD, cathepsin D; CytC, cytochrome c; CASP, caspases; Apaf-1, apoptotic protease activating factor-1; TRADD, tumor necrosis factor receptor-associated death domain; FADD, Fas-associated death domain; Bid, Bcl-2-interacting protein; tBid, truncated Bid; IAP, inhibitors of apoptosis proteins; DFF40, caspase-activated DNase/DNA fragmentation factor; DFF45, inhibitor of caspase-activated DNase. Figure modified basing on open source KEGG database apoptosis pathway (<http://www.genome.jp/kegg/pathway/hsa/hsa04210.html>).

Important feature of cathepsin D is its selective localization inside acidic compartments of cell. At least two mechanisms of localization of cathepsin D to lysosomes are known. In first mechanism the terminal end of oligosaccharide is marked with mannose 6-phosphate and along with appropriate concentration of mannose 6-phosphate receptor (M6Pr) mature enzyme is transported from Golgi apparatus into endosomes. In endosomes acidic milieu causes dissociation of this complex and released receptor is comes back to Golgi apparatus [6,10,36]. In second mechanisms that is independent from M6Pr, procatepsin D interacts with prosaposines. These two particles form a multipart, connected through N-terminal part of pseudocathepsin D (in other words C-terminus of activating peptide), which is transported to primary lysosomes [36,37,39]. These complexes were also observed extracellularly.

Mammalian cells does not contain known inhibitors of cathepsin D, excluding its propeptide detached during activation of the enzyme [17]. According to Shibata *et al.*, the activity of cathepsin D can be also inhibited through DNA fragments. The higher melting temperature (T_m) the oligonucleotide have, the stronger is inhibition of the enzyme [18].

Functions of cathepsin D in cells

Cathepsin D is endopeptidase. It cleaves peptide bonds preferably in hydrophobic aminoacids. The catalytic center of cathepsin D consists of two asparaginian acid residues Asp33 i Asp231 [16]. It cleaves used intracellular proteins and proteins in endosomes. During lim-

ited proteolysis it activates certain proenzymes, pro-hormones and precursor of biologically active peptides; it inactivates their active forms [7]. Cathepsin D inactivates many inhibitors of proteolytic enzymes [8,9]. It also takes part during mitosis, regeneration, activation of leukocytes and regulation of vassal wall permeability. The proteolytic activity of the enzyme is regulated by various intralysosomal factors such as pH, products of metabolism, hormones, growth factors and specific inhibitors [10,11]. Hormones, e.g. estrogens and progesterone can regulate the synthesis of cathepsin D and its receptor mannose 6-phosphate.

In physiology, only trace amounts of procatepsin D can be observed. Majority (ca. 90%) is transformed into intermediate form and then into mature enzyme [3]. Cathepsin D can be found extracellularly what was confirmed using specific antibodies [12,13]. To intercellular environment and body fluids cathepsin D is actively excreted or released from dead cells. After binding by alpha-2-macroglobuline cathepsin D is eliminated from blood by mononuclear phagocytosing cells.

Cathepsin D in apoptosis

On every stage of apoptotic cascade cooperation of many different proteins is necessary. Most important effectory proteins of apoptosis are caspases. The activity of caspases is strictly regulated by inhibitors of apoptosis proteins (IAP). Moreover, other groups of proteins are also responsible for regulation of apoptosis at different stages. Main regulator of apoptosis are:

Bcl-2 protein family, TNF and p53 [40]. Deiss *et al.* presented for the first time that aspartyl protease cathepsin D is also involved in regulation of apoptosis [24,25].

Depending on environment cathepsin D can induce or inhibit apoptosis, acting through different mechanisms. CTSD-mediated apoptosis can be induced by cytotoxic factors [24-34,38] or prevent apoptosis, which was discussed in animal models [19-23].

Cathepsin D and inducing of apoptosis

Since mid-nineties regulatory role of cathepsin D in inducing apoptotic pathways is indispensable [24,25]. Apoptosis is most frequent cause of death of immunologic cells. This process can be initiated by two major pathways: intrinsic and extrinsic. Extrinsic pathway begins at surface of the cell by connection of appropriate ligand with surface receptor and that leads to activation of caspase-8, -10 and -3 [41]. Activation of caspase-3 as terminal effector of apoptosis can be enhanced by additional activation of caspase-9 and -3 through intrinsic pathway [42]. Intrinsic pathway is initiated by dysfunction of mitochondrions, which leads to release of caspase activators (e.g. cytochrome c) and that leads to activation of caspase-9 and in consequence caspase-3. Both pathways combine when caspase-8 induces proteolysis of Bid to truncated Bid (tBid). tBid is transported through mitochondrion membrane where it augments oligomerization of Bax and induces intrinsic pathway of apoptosis [43].

Mature form (34 kDa) of cathepsin D regulates intrinsic apoptosis pathway through stimulation of cytochrome c (CytC) release from mitochondrion [27-29,30,32,34]. Heinrich *et al.* presented that Bid is cleaved *in vitro* at pH=6.2 [38] and that Bax can be activated independently from Bid cleavage [45]. Pepstatine A, an *in vitro* cathepsin D inhibitor, restrains apoptosis induced by cathepsin D and reactive oxygen species (ROS) [27-29]. Many anti-tumor chemotherapeutics can induce apoptosis through cathepsin D *i.e.* adriamycine, etoposide, cisplatin and 5-fluorouracil [27-29,30,32,34]. Erdal *et al.* examined the effect of twenty different drugs on inducing apoptosis in tumor cells [44]. They concluded that seven of examined agents induced apoptosis through activation of p53 and increased lysosome membrane permeabilization (LMP). Only three drugs (NSC651079, NSC687852, gold Cl-3-ethylphosphosine) induced DNA damage that lead to apoptosis and LMP, but LMP was observed before DNA was damaged. The decrease of cytosol pH essential for cathepsin D activation was a consequence of LMP and lysosomal proton efflux, what was confirmed in a study in U937 cell lines treated with TNF-alpha [48,51].

The activation of cathepsin D can be also regulated through sphingolipids, sphingosine and ceramide, which are produced during hydrolysis of sphingomyelin by acidic sphingomyelinase (A-SMase) [27,38,46]. Produced during that type of activation byceramide cathepsin D, hydrolyses procaspase-9 and procaspase-3 into their active forms caspase-9 and -3 [38]. Cathepsin D is then a missing link between apoptosis induced by ceramide production during TNF stimulation and apoptotic effectors caspase-9 and 3 [38]. Baumgartner *et al.* presented an alternative, CTSD-independent pathway of capase-9 activation [47]. In cases when induction of apoptosis through activation of procaspase-8 is not possible, lysosomes are activated and cathepsin D is released into cytosol. The reversal situation is also possible, in cases when activation of lysosomes is inhibited [48,49]. Kashkar *et al.* observed that conformational change of Bax can be independent on its activation by caspase 8 or cathepsin D - A-SMase can be the sole activator [50].

Inhibition of apoptosis by cathepsin D

Koike *et al.* observed that neurons of cathepsin D deficient mice accumulate subunit c of mitochondrial ATP synthase and saposine in lysosomes [20]. Neurons without cathepsin D presented similar features to those observed in neuronal ceroid lipofuscinosis. In these animals atrophy of cells in internal and external nuclear layer of retina was also observed. The atrophy of retina was caused by apoptosis through activation of caspase-9 and -3 [22]. Similar changes were not observed in retinas of cathepsin B and L deficient mice. Stftig *et al.* in the series of studies showed that cathepsin D is indispensable for normal development of mucus membrane of jejunum in newborn mice [19,21]. In newborns of cathepsin D knockout mice (CTSD^{-/-}) normal growth was observed until second week of life. Since third week loss of weight was observed. In 26 day of live newborns died in state of anorexia. In small intestine walls massive necrosis of cells was present with creation of thrombembolia in small vessels of intestine and small coronary arteries. Berchem *et al.* observed that 3Y1-Ad12 cell lines transfected with cathepsin D gene showed overexpression of CTSD, which can prevent apoptosis independently from catalytic function of cathepsin D [35]. However the same tumor cells treated with antitumor agent undergo apoptosis also separately from catalytic function of cathepsin D [23].

Conclusion

Cathepsin D plays various function in cells which are not fully recognized yet. Recent publications indicate that CTSD plays important role in apoptosis. Many

question concerning transduction of apoptotic signal are yet to be answered. From evolutionary point of view presence of different way of inducing apoptosis should protect cells from cancerous transformation. This can explain relatively low prevalence of cancer considering very high number of mutations and cell division errors during life of every organism. Increasing knowledge of caspase-independent apoptotic pathways can bring vital clues for designing novel antitumor therapies, in which proteolytic enzymes will be the target (52).

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