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Review

Cathepsin D expression levels in nongynecological solid tumors: Clinical and therapeutic implications

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Abstract

Cathepsin D is a lysosomal acid proteinase which is involved in the malignant progression of breast cancer and other gynecological tumors. Clinical investigations have shown that in breast cancer patients cathepsin D overexpression was significantly correlated with a shorter free-time disease and overall survival, whereas in patients with ovarian or endometrial cancer this phenomenon was associated with tumor aggressiveness and a degree of chemoresistance to various antitumor drugs such as anthracyclines, cis-platinum and vinca alkaloids. Therefore, a lot of research has been undertaken to evaluate the role and the prognostic value of cathepsin D also in other solid neoplasms. However, conflicting results have been generated from these studies. The discrepancies in these results may, in part, be explained with the different methodological approaches used in order to determine the levels of expression of the enzyme in tumor tissues and body fluids. Further investigations using well-standardized techniques may better define the clinical significance of cathepsin D expression in solid tumors. Nevertheless, evidence emerging from these studies indicates that this proteinase seems to facilitate early phases of tumor progression such as cell proliferation and local dissemination. These findings support the concept that cathepsin D may be a useful marker for identifying patients with highly malignant tumor phenotypes who may need more aggressive clinical treatment; this enzyme may also be considered as a potential target for a novel therapeutic approach in the treatment of solid neoplasms.

Abbreviations: cAMP – cyclic adenosine monophosphate; BCC – basal cell carcinoma; CB – cathepsin B; CD – cathepsin D; CL – cathepsin L; CNS – central nervous system; CRC – colorectal cancer; EGFR – epidermal growth factor receptor; GC – gastric cancer; HCC – hepatocellular carcinoma; H&N – head and neck; HIF-1 – hypoxia–inducibile factor 1; IGF – insulin-like growth factor; IGFBP – insulin-like growth factor binding protein; LSCC – laryngeal squamous cell carcinoma; MMP-2 – matrix-metalloproteinase-2; MMP-9 – matrix-metalloproteinase-9; NSCLC – non small cell lung cancer; PCNA – nuclear proliferating antigen; PSA – prostatic specific antigen; SCC – squamous cell carcinoma; SCLC – small cell lung cancer; TGF-β – transforming growth factor beta; TSH – thyroid stimulating hormone; uPA – urokinase-type plasminogen activator

Introduction

Structure and biological functions of cathepsin D

Cathepsin D (CD) (EC 3.4.23.5) is a lysosomal acid proteinase which, in the range of pH 2.8–5.0, can degrade structural and functional proteins, peptides, peptide precursors and hormones [1–4]. However, it has been shown that the biological functions of this enzyme are not only confined to the metabolic degradation of intracellular proteins but also comprise some other important biological processes (Table 1) [1–17]. In humans, the gene coding for CD has been located in chromosome 11p15 and contains 9 exons [18]. Mice deficient in this gene present alterations in the small intestine (i.e., necrosis associated with thrombosis of small vessels) and a large-scale destruction of lymphoid cells in the spleen and timus [17]. These findings further confirm the active role of CD in the renewal, remodelling and in apoptosis of a wide variety of tissues [1, 5–8, 11, 12, 17]. This enzyme is synthetized as an inactive 52kDa precursor form [19]. Its activation is processed intracellularly by sequential proteolytic cleavage which first involve the removal of the 44-amino-acid propeptide. This cleavage yelds an active 48-kDa single chain molecule which is then cleaved by cysteine proteinases into two active chain forms of 34 kDa (heavy chain) and 14 kDa (light chain) [19]. CD is ubiquitously present in animal and human tissue, with qualitative and quantitative differences in its distribution among different

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organs or cell types of the same organ [1, 4, 11, 12, 14–16, 20–23]. This uneven distribution seems to be related to the different biological function carried out by this enzyme in tissue [1].

Cathepsin D and cancer

CD has also been shown to be widely distributed also in human tumors. However, many of these tumors present altered processing, secretion and activity levels of this enzyme as compared to normal tissues [4, 22–30]. These phenomena, which may also be associated with the aggressive behavior of tumors, suggest that CD, in concert with other proteolytic enzymes involved in the metastatic process such as cysteine proteinases cathepsins B (CB) and L (CL), serine-proteinase urokinase-type plasminogen activator (uPA) and matrix-metalloproteinases-2 (MMP-2) and -9 (MMP-9), may promote the malignant progression of neoplastic diseases [25–36]. The mechanism(s) by which CD may facilitate this process has still not been fully elucidated. However, *in vitro* studies carried out mainly on human breast or ovarian cancer cell lines evidenced that this proteinase may stimulate tumor cell proliferation, invasion and metastasis by various mechanism(s) summarized in Figure 1 (reviewed by Rochefort et al. in [19, 26, 27]). As a consequence, several studies have been undertaken in order to evaluate its clinical significance in breast cancer and in other gynecological neoplasms. These studies showed that, in node-negative breast cancer patients, CD overexpression was associated with an increased risk of recurrence and death [26, 37, 38]. These findings were not confirmed in male breast cancer, which seems to be biologically different from female breast tumor [39, 40]. Conflicting results were also obtained in other gynecological malignancies [42–54]. However, these investigations additionally highlighted a close association between altered expression levels of CD and the degree of aggressiveness and chemoresistance of ovarian or endometrial tumors [42–55]. In this context, several investigations have also been undertaken to assess the clinical significance of CD expression in other nongynecological solid tumors. Extensive updated reports on the results of these studies are still lacking in literature. This review article summarizes these data and discusses their clinical implications.

Cathepsin D in central nervous system tumors

CD is commonly found in animal and human central nervous system (CNS) tissue [1, 20, 22–24]. Although its biological role in CNS is still not well defined, experimental evidence suggests that this proteinase, along with other proteolytic enzymes of the apoptotic process, namely caspases [56], may be involved in the regulation of neuronal cell death, survival and differentiation [57]. Therefore, altered expression levels of this enzyme may result in detrimental effects on the biological and physiological functions of neuronal cells which may lead to severe degenerative disorders of CNS such as Alzheimer's disease or CNS neoplasms [22, 24, 58–62]. This hypothesis is currently supported by several clinical observations which have shown that, in the human brain, altered CD levels can be frequently associated with these diseases [21–23, 57–62]. The mechanism(s) by which CD may promote the progression of CNS tumors are still unknown. However, certain *in vitro* studies showed that anti-CD antibodies, in a dose-dependent fashion, significantly reduced the invasive potential of human glioblastoma cells. These findings indicated that this proteinase may facilitate the adhesion and the subsequent invasion of these tumor cells to the extracellular matrix of host tissue [63, 64]. The potential role of CD in the modulation of the invasive activity of CNS tumors has been further supported by some immunohistochemical investigations which demonstrated that the invading cells of astrocytomas, glioblastomas, oligodendrogliomas and mixed gliomas expressed CD, MMP-2 and MMP-9, and that the switching to an invasive phenotype of these tumors was followed by an increase in CD expression levels [64]. More recently, Castino et al. [65] have hypothesized that this proteinase, in association with CB, can modulate some proteolytic occurrences of caspase-dependent apoptosis which are essential for neuroblastoma cell survival. This may, in part, explain the cytotoxic effect induced by pepstatin A, a naturally occurring inhibitor of CD, on neuroblastoma cells after a 72-h incubation period [65, 66]. These findings further showed that CD may act at different steps of the growth of CNS neoplasms and suggested a potential clinical role of this proteinase as an indicator of aggressiveness and prognosis of these tumors. However, little research has been undertaken so far to test this hypothesis. Some biochemical studies have shown, at least in human gliomas, a close relationship between up-regulation of this enzyme and malignant progression of this tumor [63]. Recently, Castilla et al. [62] reported a significant association between an increased immunocytochemical expression of CD in meningiomas and recurrence, whereas no correlation with patients' outcome was noted. These observations indicate that the evaluation of CD expression may be useful in identifying more aggressive forms of CNS neoplasms, and may be seen as an attractive target for innovative therapeutic approaches to these tumors [63–65]. However, due to the lack of extensive clinical studies, its prognostic value remains to be determined.

Cathepsin D in head and neck tumors

Immunohistochemical and immunoenzymatic studies have shown that CD expression levels may be found to be altered in different tumors of the head and neck (H&N) including those of the oral cavity, parotid gland, salivary glands, oro- and hypo-pharinx and larynx [67–73]. The biological significance of such alterations has still not been properly understood. However, there is a lot of experimental and clinical evidence to suggest that this phenomenon may be related to the dissemination of H&N neoplasms, and ultimately to the onset of more aggressive forms of these tumors [68–75]. This latter observation is further supported by recent *in vitro* studies which have shown that, in human laryngeal

Table 1. Main biological functions in which cathepsin D appears to be involved.

	Reference number
Metabolic degradation of intracellular proteins	[1]
Processing, activation and degradation of polypeptide hormones, growth factors and receptors	[1–4]
Biological regulation of programmed cell death	[5–10]
Tissue remodelling and renewal	[1, 11, 12, 17]
Activation of latent precursor forms of other proteolytic enzymes	[13]
Activation of neutrophils and leukocytes	[14, 15]
Monocyte-mediated fibrinolysis	[16]

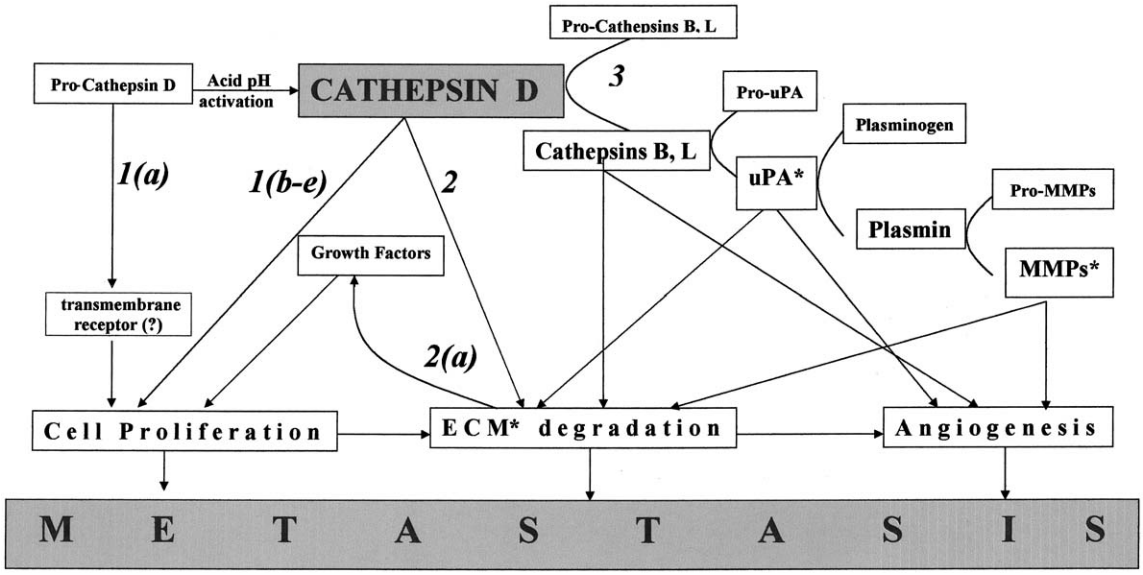

Figure 1. Step 1. Cathepsin D may promote tumor cell proliferation: ***a)*** by acting as an autocrine mitogen through the interaction with a transmembrane receptors (?) [19, 26, 34, 35, 140, 141]; ***b)*** by activating growth factors [19, 26, 34, 35]; ***c)*** by degrading growth factors inhibitors [36]; ***d)*** by interacting with growth factor receptors [19, 26, 27, 138–141]; ***e***) by affecting the apoptotic process [5, 34, 36, 57, 65, 113–115]. *Step 2.* Cathepsin D may directly degrade extracellular matrix (ECM) and facilitate tumor cell invasion [19, 25, 26, 63]: ***2a***) this phenomenon may additionally induce the release of biologically active forms of growth factors preincorporated in the ECM which, in turn, may stimulate tumor cells to proliferate [19, 26]. *Step 3*. Cathepsin D may trigger off a proteolytic cascade, by activating latent precursor forms of other proteolytic enzymes and chemiotactic factors which may facilitate ECM degradation, invasion, angiogenesis and metastasis formation [13, 24-26, 35, 102, 116, 127, 161, 170]. *MMPs – matrix-metalloproteinases; uPA – urokinase-type plasminogen activator; ECM – extracellular matrix

carcinoma (LSSC) cell lines, increased intracellular levels of CD and uPA, were associated with the onset of resistance to different classes of antitumor agents such as doxorubicin, cis-platinum and vincristine [76]. The mechanisms by which CD may induce the progression H&N tumors remain to be clarified. However, clinical evidences suggest that CD may well aid the early phases in this process by facilitating the proliferation, adhesion and local dissemination of tumor cells. In fact, immunohistochemical studies by Goussa et al. [72] on 39 tissue samples from invasive LSCC showed a significant correlation between the expression levels of CD and the hyaluronic acid receptor CD44 whereas, in 97 patients with oral cancer, Vigneswaran et al. [73] observed a significant association between increased CD expression and proliferation rate, histological grade and presence of metastasis. Moreover, Kawasaki et al. [74] recently demonstrated in 78 patients with oral squamous cell carcinoma (SCC), a close relationship between the CD expression and the pattern of invasion, clinical stage, nodal status, nuclear proliferating antigen (PCNA) and shorter survival time. The hypothesis that CD may play a role in the early phases of H&N cancer progression has been further corroborated by some of our recent clinical observations, which showed that, unlike in early clinical stage (I/II) LSCC, CD levels were not increased in locally advanced tumors (stage III/V) as compared to normal adjacent mucosa [67, 77]. In addition, our data also highlighted significantly higher uPA levels in tumor tissues when compared to its normal counterpart [77]. A comparative analysis of the distribution of CD and uPA in stage III/IV LSCC showed that CD was more noticeably expressed only in tumors with a high proliferation rate, as compared to those with a low proliferation index, whereas uPA was more noticeably expressed in node-positive tumors than in node-negative tumors, in stage-IV tumors than stage-III tumors and in aneuploid multiclonal tumors than in aneuploid-monoclonal or diploid tumors. [77]. These results further indicated that, at least in the case of LSCC progression, CD seems more likely to be involved in tumor cell proliferation and local growth whereas uPA seemed to be implicated in subsequent events in this process such as

the invasion and further dissemination of tumor cells. Although these findings might suggest a prognostic role for CD in LSCC, conflicting results have emerged from studies aimed at evaluating this hypothesis. Immunohistochemical investigations by Resnick et al. [78] on 88 LSCC patients did not find any significant relationship between CD expression, degree of nodal involvement, disease-free survival or overall survival time. Similar studies by Seiwerth et al. [79] in 61 untreated LSCC patients, in part, confirmed these findings. However, these authors reported a significant correlation between CD immunoreactivity in both epithelial and stromal cells and patient survival. On the other hand, Lazaris et al. [80] observed by immunocytochemistry that, among 64 LSCC patients, those with CD positive lymph nodes were at higher risk of relapse. The positive relationship between CD expression and nodal involvement was further confirmed by the immunoradiometric studies of Maurizi et al. [81] on 63 patients with primary LSCC. In addition these authors showed that, at the cut-off level considered (13.8 pmol/mg of protein), CD tumor content significantly correlated also with metastasis-free survival and overall survival. However, other immunoradiometric or immunoenzymatic studies, including our own investigations, failed to find any correlation between CD tumor levels and the biological and clinical parameters of progression or patients' survival [77, 82]. The discrepancies in these results may be, in part, explained by the different assay methods and/or antibody used and/or by the cut-off limits considered. However, it also cannot be ruled out that the different criteria of selection, the number of patients, the different anatomical site considered and the different follow-up periods may also account for these conflicting results. Although the prognostic significance of CD remains controversial, evidence emerging from these studies suggests that this proteinase may be useful as as a biological marker for identifying patients with primary tumor at increased risk of recurrence and onset of resistence to therapeutic treatment. Further studies with standardized techniques may better define the clinical role of CD in H&N cancer.

Cathepsin D in thyroid tumors

In the human thyroid gland CD is one of the proteolytic enzymes involved in the metabolism of thyreoglobulin [3, 4]. In some pathological conditions, such as Graves' disease and toxic adenoma, increased activity or content levels of this proteinase have been shown to be associated with a hyperfunctional thyroid [4, 83]. This phenomenon appears to be induced by TSH as these pathological conditions are related to a permanently stimulated cAMP transduction pathway which mediates the effects of TSH on thyroid cells including the synthesis and secretion of CD [4, 84, 85]. Therefore, a number of investigations have also been carried out to assess the clinical significance of CD expression in thyroid tumors. Early biochemical studies by Sinadinovic et al. [86] reported that the patients' papillary thyroid carcinoma tissue presented an enhanced proteolytic activity of lysosomal acid proteases as compared to normal thyroid tissue. The authors speculated that this phenomenon was probably the result of metabolic disturbances in the catabolic degradation of thyreoglobulin in tumor tissue. Further immunoenzymatic investigations by Métayé et al. [83] carried out on a small number of patients ($n = 12$) showed that CD content levels were significantly higher in thyroid carcinoma, toxic adenoma or Graves' disease, than in normal tissue or benign nodules. However, no difference was observed between thyroid carcinoma and Graves' disease or toxic adenoma. Interestingly, these studies and later investigations of the same group carried out on 34 patients evidenced a significant association between CD levels in primary tumors and tumor size [83, 87]. Additional observations of these authors showed that the activity levels of this enzyme in different human thyroid tissues were 3.0, 2.3 and 1.3 times higher in cancer tissue or toxic adenoma, in Graves'disease, and in cold benign nodule respectively, than in normal thyroid tissue [4]. In addition, in a small number of samples, it was also noted that mean CD activity levels were higher in anaplastic carcinomas than in well differentiated thyroid carcinomas [4]. These findings indicated the existence of a possible correlation between degree of CD expression and aggressive behavior of thyroid tumors, and suggested that CD could play a role in their propensity to metastasize. This hypothesis was supported by some studies by Ruhoy et al. [88], who showed that CD immunostaining was higher in follicular carcinomas than in follicular adenomas and that this phenomenon was greatest in extensively invasive follicular carcinomas. These observations suggested that this proteinase could be of clinical interest as a prognostic marker in thyroid tumors [83, 88]. To date, however, no extensive clinical studies to assess this hypothesis have yet been undertaken. A single study on 44 patients with medullary carcinoma reported a weak correlation between the immunohistochemical expression of CD and poor prognosis [89]. Thus, additional clinical investigations to establish the prognostic significance of this enzyme are needed. However, the results of the existing studies indicated that CD may be useful as a specific marker to identify tumors endowed with a greater invasive and metastatic potential. This findings may greatly improve the therapeutic approach and the prognosis of these tumors.

Cathepsin D in lung cancer

The role of CD and its clinical significance in lung cancer is still controversial. Some biochemical and immunoenzymatic studies have questioned its direct involvement in the growth and advance of at least of some histological types of lung cancer. For instance, Ledakis et al. [90] reported that, in non-small-cell lung cancer (NSCLC), CD activity or content levels, had not increased, unlike CB and CL, as compared with adjacent normal lung tissue. Moreover, other immunohistochemical studies by Fontanini et al. [91] undertaken on 108 NSCLC patients showed that, CD expression levels was associated with smaller size (< 3 cm), less advanced tumors (T1), more differentated (G1-2) tumors and non-squamous histotypes. In addition, in non-squamous histotypes, CD immunoreactivity was associated with early staged (S1) and

lymph node-negative (N0) tumors whereas no correlation was observed with proliferation indices such as DNA ploidy, S-phase fraction, PCNA or Ki-67. On the contrary, studies on patients with small cell lung cancer (SCLC) evidenced that a low staining for CD or its absence was associated with a prolonged survival [92]. Moreover, Higashiyama et al. [93] observed that, in patients with stage I lung adenocarcinoma, the subcellular localization of CD in the basal or infranuclear side of the cytoplasm in tumor cells, as well as its presence in stromal cells within the tumor tissues, was associated with a worse prognosis. These results suggested that CD may play a differential role in the regulation of the growth and differentiation of various histological types of lung cancer. As a consequence, its prognostic impact in these neoplasms might be strongly affected by the histological type of the tumor and/or its status in stromal cells. Therefore, the clinical significance of CD expression in different histotypes of lung cancer needs to be better defined through precisely conceived studies and standardized immunohistochemical methods. Furthermore, studies have been recently undertaken to evaluate the clinical utility of serum-CD activity levels in the therapeutic monitoring of lung cancer patients [94]. These investigations, carried out on 21 patients with stage-II/III SCLC, showed that the activity levels of this enzyme were significanty higher in SCLC patients than in healthy subjects. These levels markedly declined after surgery albeit remained three times higher than those determined in the control group [94]. These observations suggested that serum CD activity may be potentially useful for the therapeutic monitoring of lung cancer patients undergoing surgical and/or clinical treatments. However, extensive clinical studies with a larger number of patients are needed to assess this hypothesis.

Cathepsin D in tumors of the gastrointestinal tract

Gastric cancer

Immunohistochemical analysis of CD distribution in gastric tissues has shown that this proteinase is widely present in different cell types of normal gastric mucosa, as well as in benign and malignant gastric diseases [21, 95–103]. However, its specific role in the malignant progression of gastric cancer (GC) has still not been clarified. Early immunocytochemical studies did not evidence any significant difference in the distribution of this proteinase between normal mucosa and inflammatory gastric diseases [95]. Moreover, these studies showed the absence of any immunoreactivity to CD in intestinal metaplasia or dysplasia or in well differentiated gastric adenocarcinoma. However, a strong and diffuse staining for CD was observed in poorly differentiated adenocarcinomas and in signet ring cell carcinoma [95]. These findings suggested a correlation between CD expression levels and degree of differentiation of gastric cancer but did not clarify whether this enzyme might have a specific role in the progression of this tumor. However, clinical observation supports the hypothesis that CD may play a key role in this process, probably, by facilitating tumor cell invasion. In fact, some immunohistochemical studies have described a differential immunostaining pattern of CD expression in primary gastric carcinoma tissue [96]. The most intense staining was noted in tumor cells at the advancing margin of the tumor. This peculiar localization was significantly correlated with certain clinical parameters of GC progression, such as the clinical stage and occurrence of lymph-node metastasis [96]. Other biochemical and immunohistochemical studies further confirmed the relationship between CD activity and the invasive potential of GC [97, 98]. Therefore, several clinical investigations were carried out to evaluate the clinical significance of this proteinase, mainly in patients with curatively resected cancer, that was at risk of recurrence. Immunohistochemical studies by Allgayer et al. [99] on 203 consecutive patients showed that CD expression levels were significantly associated with overall survival and a shorter disease-free interval. Multivariate analysis identified CD as an independent parameter for disease-free interval [99]. In contrast, some of our immunoenzymatic studies undertaken on 57 patients with operable GC showed, that CD levels in GC tissue, unlike CB and CL, did not increase as compared to paired normal gastric mucosa nor did they correlate with some of the clinicobiological parameters of progression of this tumor including the patients' outcome [100]. It is likely that the different methods and antibodies used, and/or the different number of patients investigated may account for these conflicting results. On the other hand, some recent immunohistochemical studies by Goishi et al. [101] showed that, in 136 patients with tumors invading the submucosa and muscularis propria, CD expression correlated significantly with the increasing incidence of lymph-node metastasis. These observations were in agreement with those of Ikeguchi et al. [102] who showed, in 160 patients with early GC, a significant correlation between CD expression and occurrence of micro-lymph-node metastasis. Further observation by the same group of a larger number of patients ($n = 478$) evidenced that the percentage of CD-positive cancer cells was higher in diffuse-type carcinoma than in intestinal-type carcinoma [103]. These findings were also confirmed by the immunoenzymatic studies of Garcia et al. [104]. Furthermore, Ikeguchi et al. [103] reported that, in both diffuse and intestinal type of carcinoma, CD expression levels were associated with depth of tumor invasion and a worse disease-specific five-year survival rate whereas CD levels in stromal cells were associated with either depth of invasion or with a worse five-year survival rate only in the intestinal type carcinoma [103]. These findings indicated that the CD of stromal cells seems to play an active role in the invasion of this type of tumor and suggested that it may strongly influence the prognostic significance of this enzyme. Although these studies did not fully clarify the mechanisms by which CD may induce the growth and spread of GC, they provided evidence for the clinical utility of CD as a marker of lymph node involvement and indicate that this proteinase may be a good candidate as prognostic parameter for predicting the clinical outcome of GC patients.

Colorectal cancer

Several investigations have shown that CD levels may be found to be altered in colorectal cancer (CRC) and that this phenomenon is frequently associated with CRC progression (105–112). These data indicate that CD may have a role in the growth and spread of CRC. However, the mechanisms by which CD may facilitate this process are not well understood. *In vitro* studies showed that HT-29 human colon carcinoma cell lines presented an altered secretion of CD which was associated with a less differentiated state of these cells [113]. This phenomenon, which appears to facilitate the degradation of the extracellular matrix by tumor cells, seems to be related to altered levels of cell associated ceramide, an important mediator of the apoptotic process [114, 115]. As CD seems to be involved in the biological regulation of programmed cell death [5–10, 57, 65, 113–115] it is conceivable that its altered secretion may also result in disturbances of the normal apoptotic process which may facilitate tumor cell growth. However, other experimental observation showed that CD may induce the growth and spread of this tumor by other mechanisms such as the activation of latent precursor forms of other proteinases involved in the metastatic process. This latter hypothesis is supported by some studies of van der Stappen et al. [13] who observed that, in an *in vitro* model of CRC progression, the conversion of non tumorigenic adenoma derived cell lines to a highly tumorigenic phenotype was associated with an eight-fold increase of pro-CB and to an enhanced secretion of CD. Moreover, these studies also evidenced that the activation of CB was mediated by CD. This phenomenon may, in turn, trigger off the proteolytic cascade which leads to the degradation of extracellular matrix and the subsequent invasion of host tissues by malignant cells (Figure 1). The potential involvement of CD in CRC invasion has been further sustained by recent observation which has shown that, in human HCT116 colon carcinoma cells, the overexpression of hypoxia-inducibile factor 1 (HIF-1) stimulates the expression of genes encoding for several factors, including CD, which contribute to extracellular-matrix invasion [116]. These experimental findings suggest that CD may have a potential clinical role as marker of aggressiveness and poor prognosis in CRC patients. Several clinical studies have been performed to assess this hypothesis. Our early investigations on 68 matched paired sets of CRC and normal tissue sample homogenates showed significantly increased CD activity levels in tumor tissues as compared to adjacent paired normal mucosa and it was demonstrated for the first time that this activity was significantly higher in early clinical stage CRC (i.e., Dukes' stage A) than in later clinical stages (i.e, Dukes' B, C and D) [105]. These latter observations, which further indicated an involvement of CD in the early stages of CRC growth and progression, were not confirmed by subsequent studies from other authors [106]. These discrepant results might be due to the different number of patients considered. On the contrary, our later immunoenzymatic studies on 21 matched paired CRC and normal mucosa samples did not evidence any significant difference in CD content between tumor and paired normal tissue [107]. However, unlike the described enzyme activity, a significant correlation was observed between CD tumor content and tumor size or grade [107]. These findings further underline that different results may be obtained in accordance to the methodological approaches and/or number of patients used. For instance immunohistochemical analysis of CD expression carried out by Theodoropoulos et al. [108] on 60 surgical CRC samples showed that the presence of CD immunoreactivity in stromal cells was associated with a more invasive phenotype, while Kanber et al. [109] showed that stromal CD expression was also related to tumor stage. On the other hand, Arao et al. [110] reported that the immunostaining pattern of CD in tumor cells, but not the incidence of CD positive tumors, was associated significantly with lymphatic invasion. Furthermore, Oh-e et al. [111], following the immunocytochemical evaluation of the intracellular pattern of expression of CD in 254 invasive CRC, observed a significant correlation between this parameter or positive expression of CD in stromal cells, and incidence of lymph-node metastasis. These findings indicate that, as previously described for GC, CD stromal cells appear to influence the invasive potential of CRC tumors. Therefore, analysis of CD expression in both tumor and stromal cells can be regarded as a useful predictor for lymph-node metastasis, and consequently, may have a clinical relevance in predicting the clinical outcome of CRC patients. These observations indicate that the immnunohistochemical evaluation of CD expression is more reliable than other methods for investigating the clinical significance of this proteinase in CRC. In conclusion, experimental and clinical findings indicate that CD appears to promote the progression of CRC, probably by affecting the apoptotic process of tumor cells and/or by facilitating tumor cell invasion, and that this enzyme may be clinically relevant as a predictive marker of lymph-node involvement and poor clinical outcome in CRC patients.

Liver cancer

Several experimental and clinical studies support the hypothesis that CD may also be implicated in the onset and progression of liver tumors. For instance, *in vitro* and *in vivo* observations evidenced that the extremely fast-growing Morris hepatoma 777 cells presented altered, intracellular processing and increased secretion of a precursor form of CD whereas the ascitic fluid and the plasma of rats transplanted with Yoshida AH-130 hepatoma presented elevated levels of CD activity [118, 119]. Interestingly, increased CD activity levels were also observed in human hepatoma tissues as compared to its normal counterpart [120]. These experimental observations were further confirmed by clinical findings, which showed that CD activity or content levels were significantly elevated in sera of patients with chronic liver diseases, such as active hepatitis, cirrhosis and/or hepatocellular carcinoma (HCC), as compared to healthy subjects [121–124]. These findings suggest an active involvement of CD in the malignant progression of liver tumors. Experimental studies undertaken to investigate the mechanisms by which CD may trigger this pathological process suggested that this proteolytic enzyme seems to facilitate the prolifera-

tion and dissemination of hepatic tumor cells. In fact, some *in vivo* studies in mice showed that intraperitoneal injections of purified preparation of CD stimulated DNA synthesis and mitosis in the intact liver of these mice [125, 126]. As CD seems to promote *in vitro* tumor cell proliferation, it can be hypothesized that this proteinase may stimulate tumor cells to proliferate through its mitogenic activity [19, 26]. However, experimental evidence to support this hypothesis has not yet been forthcoming. Furthermore, other *in vitro* studies showed that human hepatoma cell line PLC/PRF/5 actively secretes CD in addition to transforming growth factor β (TGF-β) and fibronectin [127]. The authors of these studies speculated that the secreted CD may activate latent forms of TGF-β which, in turn, regulates the secretion of fibronectin, a strong inducer of chemiotaxis. These events may lead to a further migration and invasion of surrounding tissues by tumor cells. However, in this case too, experimental and clinical evidences which supports this mechanism are still lacking. The hypothesis that CD may play an active role in the onset and progression of liver tumors have led several studies to assess its clinical significance in these tumors as well as in some premalignant liver diseases such as cirrhosis. Our studies and those of Brouillet et al. [123, 124] showed that, in patients with cirrhosis, CD antigen levels were significantly higher than those determined in HCC patients. In addition, our data also showed that patients with steatosis had significantly higher CD serum levels as compared to healthy subjects, but these levels were significantly lower than those measured in patients with liver cirrhosis and/or HCC [124]. These findings and other experimental data indicated that CD, in concert with other proteolytic enzymes, could be involved in the process of tissue remodelling which occurs during the evolution of cirrhosis [121, 128–130]. As this process may be associated with the malignant transformation of liver tissue, it can be speculated that CD may contribute also to the onset of malignant lesions in cirrhotic tissue [123, 124, 131]. These observations suggest that CD may be potentially useful as a biochemical marker for identifying those patient with cirrhosis who risk developing HCC. On the other hand its prognostic value in these tumors remains to be assessed. Unfortunately, to date few clinical studies have been carried out toward this aim. A recent immunohistochemical study on 85 HCC patients showed that CD expression, was correlated with the histological grade but the prognostic value of this enzyme to predict the clinical outcome for these patients was not assessed [132].

Pancreatic cancer

There are not very many studies into the role, and the clinical significance, of CD in pancreatic cancer. In 1986 Yamaguchi and Kawai [133] first reported that human pancreatic tumor cell line HPC-YT actively secreted *in vitro* a 'Cathepsin-D-like enzyme' which was different from that present in normal pancreas. The authors suggested that this form might be responsible for the degradation of the host extracellular matrix and that it might facilitate the invasion of this tumor. However, no further studies to confirm this hypothesis have been carried out. On the basis of these observations we tried to assess the clinical significance of the serum levels of CD, and also of CB and CL in patients with pancreatic carcinoma or pancreatitis. Our results showed that in cancer patients, unlike observed for CB and CL, CD serum levels were lower than those measured in normal subjects nor were the enzyme levels correlated with any of the biological and clinical parameters of progression of this tumor [134]. These findings confirmed previous immunocytochemical observations by Nakata et al. [135], who demonstrated a lack of correlation between CD expression in pancreatic tumor cells and the presence of metastatic foci in lymph nodes and in other organs. However, our data also showed that CD serum concentrations were significantly more elevated in patients with acute or chronic pancreatitis as compared to healthy subjects or cancer patients [134]. This phenomenon was noted also for CB, but not for CL. These observations indicate that CB and CL might well appear to be more relevant than CD as prognostic markers in pancreatic cancer. This hypothesis was recently confirmed by immunohistochemical studies by Niedergethmann et al. [136] who reported a significant correlation between the expression levels of these proteinases and an unfavorable clinical outcome in patients with operable pancreatic cancer. These findings seem to rule out a direct role for CD in the progression of pancreatic cancer. However, the different serum pattern of this proteinase in pancreatic cancer and pancreatitis might be a useful additional parameter in the differential diagnosis of these diseases.

Cathepsin D in tumors of the genitourinary tract

Prostatic cancer

At present the role of CD in prostatic cancer is not well known. However, experimental evidence indicates that this proteinase may likely stimulate the growth of this tumor by interacting with hormone receptors or growth factor receptors and/or through its mitogenic activity. In fact, *in vitro* studies suggested that CD seems to facilitate the proliferation of prostatic tumor cells induced by insulin-like growth factor (IGF), by proteolytic degradation of IGF-binding proteins (IGFB) or, in the case of androgen-dependent tumor cells, by hydrolizing androgen receptors [137–139]. Interestingly, this latter phenomenon did not occur in normal prostatic tissue. On the other hand, Vétvićka et al. [140] showed that several human prostatic cancer cells secreted an enzymatically inactive pro-CD, containing an activation peptide localized in the N-terminal amino-acid region 27–44, which induces tumor cell proliferation and motility, probably, by interacting with an unknown transmembrane receptor [140, 141]. This hypothesis was corroborated by some experimental *in vivo* observations which demonstrated that the administration of anti-27–44 peptide antibodies to nude mice transplanted with LNCaP human prostatic cancer cells or MDA-MB-231 breast cancer cells inhibited the growth of these tumors [140, 141]. However, Konno et al. [142] showed that the antibiotic brefeldin A inhibited *in vitro*

Table 2. Clinical significance of cathepsin D expression in nongynecological solid tumors.

Tumor	Reference	Assay methods	No. of patients	Correlation with	
				Clinicopathological[b] parameters	Survival[c]
CNS					
Neuroblastomas	24	IHC	13	G	NA
Meningiomas	62	IHC	86	G	DFS
Gliomas	64	IHC	45	I	NA
Head and neck					
Different	69	IRMA	53	No	NA
anatomical sytes	70	IRMA	92	No	NA
	71	IRMA	111	G	NA
	75	IHC	34	N	NA
Salivary gland	68	IHC	44	Histological type	NA
Oral cavity	73	IHC	97	P, G, M	NA
	74	IHC	78	N, S, I, PCNA	OS
	75	IHC	34	N	NA
Laryngeal SCC	77	EIA	57	No	No
	78	IHC	88	No	No
	79	IHC	61	No	OS
	80	IHC	64	No	DSF
	81	IRMA	63	N	DFS, OS
Thyroid					
	4	EA/IHC	107	G	NA
	83	IRMA	14	T	NA
	87	IRMA	32	T	NA
	88	IHC	34	G	NA
	89	IHC	44	S	No
Lung					
	91	IHC	108 (NSCLC)	T, N, G, S[d]	NA
	92	IHC	13 (SCLC)	No	OS[d]
	93	IHC	152 (AdenoK, Stage I)	Number of scars	OS
Gastrointestinal tract					
Oesophageal SCC	182	IHC	154	I, p53, Ki-67	No
Gastric	95	IHC	21	G	–
	96	IHC	44	N, S	NA
	97	EA	42	I, N, G	NA
	98	IHC	29 + 15 adenomas	I, N	NA
	99	IHC	203	G	OS
	100	EIA	57	No	No
	101	IHC	136	N	NA
	102	IHC	160	N	No
	103	IHC	478	I	OS
Colorectal	105	EA	68	S[d]	NA
	106	EA	27	No	NA
	107	EIA	21	T, G	NA
	108	IHC	60	I, S	OS
	109	IHC	34 + 24 adenomas	I	NA
	110	IHC	254	G, I, N	NA
	111	IHC	31 + 29 adenomas	S	NA

Table 2. Continued.

Tumor	Reference	Assay methods[a]	No. of patients	Correlation with	
				Clinicopathological[b] parameters	Survival[c]
Liver	123	IRMA/ serum	27	No	NA
	124	EIA/ serum	56	No	NA
	132	IHC	85	G	NA
Pancreas	134	EIA serum	22	No	NA
	135	IHC	21	No	No
Genitourinary tract					
Prostate	146	EIA	20	No	NA
	147	IRMA	15	No	NA
	148	IHC	69	S	NA
	149	IHC	102	Gleason score	NA
	150	IHC	61	Gleason, ploidy	NA
	151	IHC	105	No	No
	152	IHC	71	No	No
	153	EIA/density[e]	80	S, M	No
	154	EIA	72	I, S, M	NA
Bladder	155	IHC	77	Rb, CD44, G[d], S[d], P53[d]	NA
	156	IHC	105	G, S[d]	DFS[d], OS[d]
	157	IHC	60	G, S[d]	DFS[d]
	158	IHC	177	G, I, EGRF, P53, S-phase	OS
	159	IHC	20 (T1)	No	No
	160	IRMA	93	No	No
	161	IHC	32	No	No
	167	IHC/Western blot	23	I	No
Melanoma and other skin tumors					
Melanoma	169	IRMA	51	NA	DFS
	171	IHC	147	I	DFS
	172	EIA/plasma	108	M[d]	No
SCC, BCC, Bowen disease	173	IHC	46	I	NA
SCC	174	IHC	53	I, M	DFS

[a]IHC – immunohistochemistry; IRMA – radioimmunoassay; EIA – enzyme immunoassay; EA – enzyme activity.
[b]T – tumor size; G – tumor grade; N – nodal involvement; S – stage; M – metastasis; P – proliferation rate; I – depth of invasion.
[c]DFS disease-free survival; OS – overall survival; NA – not assessed.
[d]Inversely correlated with cathepsin D expression.
[e]Cathepsin D density: ratio Cathepsin D serum content/prostate volume.

the proliferation of LNCaP, PC-3 and DU-145 human prostatic cancer cells. These effects, which were more marked in LNCaP cells, were associated with an overexpression of pro-CD induced by a blocking of maturation (i.e., activation) of CD caused by this antibiotic. These results seem to be in contrast with those reporting a stimulating effects of pro-CD on proliferation of breast or prostatic cancer cells. However, as LNCaP are androgen-dependent cancer cells, and brefeldin A has been shown to induce a dramatic reduction (> 90%) in the level of expression of androgen receptors [143], it might conceivably be hypothesized that the inhibiting effects of this antibiotic on cell growth are the consequence of a down-regulation of these receptors which may also mediate the proliferating effects of pro-CD. On the other hand, the inhibiting effects of brefeldin A on androgen-indipendent PC-3 and DU-145 prostatic tumor cells seemed to be induced by different mechanisms involving the cell-cycle regulatory retinoblastoma protein (pRB) and cyclin-dependent kinase inhibitor WAF1 (p21) respectively [142]. These findings suggest that CD may have a differential role in modulating the growth of androgen-dependent and independent prostatic cancer cells. This hypothesis is supported by recent *in vitro* studies which show that pro-CD secreted by androgen-independent PC-3 human prostatic carcinoma cell lines, following its conversion to pseudo-CD, could generate angiostatin from plasminogen and that this phenomenon might prevent angiogenesis-dependent growth of the metastasis [144]. However, evident proof that CD may modulate also *in vivo* the growth of prostatic cancer by these mechanisms is still lacking. On the other hand, clinical studies aimed at assessing the role and the prognostic impact of this enzyme in prostatic cancer have generated conflicting results, according also to the method used. Immunoblot analysis by Cherry et al. [145] reported that human prostatic

cancer tissue expressed a mature form of CD, with a higher catalytic activity, whereas normal or benign prostatic hyperplasia predominantly expressed an inactive precursor form of the enzyme. However, immunoenzymatic studies by Yang et al. [146] which analyzed the distribution of CD in the cytosol fractions of 22 samples of hyperplastic tissue and 20 of prostate cancer tissue, showed no difference in enzyme content between these tissues nor any correlation between CD tumor levels and other clinicobiological parameters such as degree of differentiation and expression of sex hormones while other prognostic parameters were not considered. On the contrary, immunoradiometric studies by Chambon et al. [147] on 15 human prostate cancer tissue samples showed that cystosolic CD concentrations were more elevated in tumor tissues than in normal prostate or prostatic hyperplasia. However, when the authors further analyzed by immunohistochemistry, the distribution of CD in these tissues, they observed, according to their score system, a higher expression of CD in benign prostatic hyperplasia [147]. On the other hand, other immunohistochemical studies by Makar et al. [148] on 69 cases of primary adenocarcinoma of the prostate showed, according to the score method reported by these authors, a significant correlation with the pathological stage but not with the Gleason grade. Unlike these observations, later studies by Maygarden et al. [149] showed in a larger number of patients ($n = 102$) a significant correlation between CD expression and Gleason's combined score. Furthermore, quantitative immunohistochemical studies by Ross et al. [150] on 61 prostatic carcinoma biopsies further confirmed this latter finding. In addition, these authors also demonstrated a significant correlation between CD expression and DNA-ploidy, but not with serum prostate specific antigen (PSA) levels, pathological stage or post resection disease recurrence [151]. The failure of CD to the predict clinical outcome for patients with clinically localized prostatic carcinoma was further supported by other immunohistochemical studies [151, 152]. These findings suggest that in prostatic cancer CD appeared to be of clinical relevance as an indicator of disease progression but not as a prognostic parameter. This hypothesis has been corroborated by recent immunoenzymatic studies by Hara et al. [153] who have shown that serum CD and its density (i.e., ratio CD serum levels/prostate volume) were significantly higher in patients with metastatic disease as compared to those without metastasis. However, either CD serum levels, or its density did not significantly correlate with patients' survival rate. Moreover, Miyake et al. [154] have recently observed that serum CD combined with systemic biopsy and/or PSA levels may be useful as predictive marker of extraprostatic extension of the tumor in patients who have undergone radical prostatectomy. These results indicated that CD may be an additional marker to identify patients with more aggressive forms of prostatic cancer needing specific therapeutic treatment. However, the use of well standardized methodologies are needed to better assess the clinical role of CD in the management of this tumor.

Bladder cancer

The pattern of CD expression in normal and pathological bladder tissue and its clinical significance in bladder cancer has been extensively investigated mainly by immunohistochemical methods [21, 155, 156]. Early studies by Dickinson et al. [156] on 105 samples of transitional bladder carcinoma showed that CD was expressed in 100% of normal urothelium whereas only 51% of tumors were CD positive. In addition, these studies evidenced a significant inverse correlation between CD expression and tumor morphology, tumor stage or grade whereas no correlation with DNA ploidy was observed. Univariate analysis showed that negative staining for CD was associated with a poor prognosis, while multivariate analysis failed to demonstrate any correlation between CD expression and overall survival. These results tallied with other immunohistochemical studies which reported an inverse correlation between CD score and tumor grade [157]. Furthermore, these studies showed that patients with a high PCNA labelling index and CD-negative tumors had a significantly poorer prognosis compared to those with a low PCNA index and highly CD-positive group while multivariate analysis indicated that both these parameters were not independent prognostic factors [157]. Therefore, CD was considered a useful tool for identifying the malignant potential of bladder transitional cell carcinoma, and may provide additional information for predicting survival when stratifying for tumor grade. In this context, immunohistochemical studies by Lipponen [158] on 177 patients reported that the strong expression of CD detected in 40% of bladder tumor specimens was associated with tumor grade 2–3, S-phase fraction, muscle invasive growth and overexpression of EGFR. These findings, in part, clash with the previous ones. These conflicting data may partially be explained by the different number of patients considered. Interestingly, this author also showed that CD was expressed in macrophage-like cells at the invasion front of the tumor which were infiltrated by inflammatory cells and tumor cells overexpressing EGFR or p53 protein. Multivariate analysis showed that the presence of CD-positive tissue macrophage, in addition to other variables, was an independent prognostic factor. These studies indicated that, similarly as reported for other neoplasms, stromal CD, may have a role in modulating the invasive activity of this tumor and may influence its prognostic significance. On the contrary, Ozer et al. [159] did not find any prognostic value of CD immunostaining in 20 patients with high-grade T1-stage primary bladder cancer. Furthermore, imunoenzymatic studies by Salman et al. [160] reported that CD content levels, detemined from 93 bladder tumor tissue samples, did not correlate with the clinical parameters of progression considered or with prognosis. More recently, immunohistochemical studies by Carrascosa et al. [161] on 32 patients with invasive bladder carcinoma further confirmed these observations. These findings render the clinical role of CD in bladder cancer controversial. It appears likely the conflicting results are due to different methods and numbers of patients used in these studies. Moreover, as CD in stromal cells seems to modulate the invasive activity of this cancer

[156, 158] this phenomenon should be taken into account in evaluating the prognostic significance of this enzyme. These observations indicate that among the analytical methods used to assess the clinical role of CD in bladder cancer immunohistochemical methods which can separately assess the prognostic impact of CD expression in tumor cells or stromal cells appear to be the more reliable than other methods. Further investigations with well standardized immunocytochemical methods may better clarify the clinical role of CD in bladder carcinoma.

Cathepsin D in melanoma and other skin tumors

There is a clear evidence that a number of intracellular and extracellular proteolytic enzymes may play a major role in the onset and progression of melanoma and other skin tumors [162–165]. In fact several *in vitro* studies have shown that human melanoma cells may release different proteinases, including CD, which may cooperate to degrade the extracellular matrix, thus facilitating the invasion of this tumor [31–33, 162, 166]. Immunohistochemical, immunoenzymatic and biochemical studies have shown that CD is markedly expressed either *in vitro* in human metastatic melanoma cells or *in vivo* in primary and metastatic melanoma tissue [166–171]. Interestingly, some of these investigations evidenced, by immunohistochemistry and Western blot analysis, that CD was always present in dysplastic nevi but only in 18% of nevocellular nevi while it was absent in normal melanocytes [166, 167]. These observations were further indication that the presence of this enzyme seemed to be associated with melanoma development and progression and that it might be of clinical interest as a prognostic marker. This hypothesis has been confirmed by a number of clinical investigations which have reported a significant correlation between CD expression levels, determined by immunocytochemistry, in primary melanoma tissue and poor clinical outcome [169–171]. On the contrary, other biochemical studies have shown that CD plasma levels were not of clinical value for identifying patients with malignant melanoma at high risk of recurrence. The correlation between CD expression and aggressive behavior of tumors has been observed also in other skin tumors. Immunohistochemical studies by Kawada et al. [173] showed that CD expression increased in SCC, but not in patients with Bowen's disease, seborrhoic keratosis or basal cell carcinoma (BCC). More recently, Goldmann et al. [174], by analyzing the immunostaining pattern of CD expression in 53 specimens from primary SCC of the skin, noted that CD and type-IV collagenase were significantly overexpressed at the invading front of metastasized tumors, as compared to those which were not. These findings suggested that CD, in concert with other proteolytic enzymes, may be also involved in the SCC skin invasion. These results suggest that CD may be of value as biochemical marker to identify highly aggressive forms of these tumors and therefore to identify high-risk patients for adjuvant therapy [162, 170, 171].

Cathepsin D as therapeutic target in cancer treatment

The experimental and clinical findings suggesting a role for CD in tumor progression, imply that the modulation of its biological activity by the use of its specific inhibitors or antibodies may have a clinical relevance in the treatment of solid neoplasms. Experimental *in vitro* and *in vivo* studies showed that anti-CD antibodies significantly reduced, in a dose-dependent fashion, the invasive potential human glioblastoma cells, whereas antibodies raised against the 27–44 activation peptide of pro-CD administered to nude mice with human breast or prostatic tumors inhibited the growth of these tumors [63, 140, 141]. However, further investigations to evaluate the potential clinical applications of these antibodies in cancer treatment have not yet been pursued. On the other hand, most experimental studies have been carried out to evaluate the therapeutic activity of specific inhibitors of CD. These investigations have been mainly undertaken with pepstatin A, a naturally occurring inhibitor of CD and other aspartyl proteinases [1, 66]. *In vitro* studies by Castino et al. [65] evidenced that this inhibitor, at 100 μM concentration, was cytotoxic for some human neuroblastoma cell lines. These authors hypothesized that the cytotoxic effects induced by pepstatin A may be the consequence of the inhibition of CD activity which, in these tumor cells, seems to modulate caspase-dependent apoptosis [9, 57, 65]. On the other hand, our previous *in vivo* studies showed that the intraperitoneal administration of pepstatin A induced a significant reduction in the number of spontaneous lung or liver metastases in mice transplanted with Lewis Lung carcinoma, MCa mammary carcinoma or M5076 ovarian reticulum cell sarcoma but not in B16 melanoma or L1210-tumor bearing mice [66, 175]. Interestingly, the administration of pepstatin A in combination with an antitumor agent with a broad spectrum of activity such as doxorubicin, to Lewis Lung or M5076-tumor-bearing mice, resulted in an additive effect on metastasis formation as compared to that induced by each single agent [66]. The inhibiting effects of pepstatin A on metastasis formation did not seem to be due to the direct cytotoxic activity of this agent on tumor cells, as the growth of primary tumors in mice was not affected by its administration. Moreover, our preliminary *in vitro* experiments showed that this inhibitor, at concentrations of up to $1\times\ 10^{-5}$ M, (i.e., 10 times lower than that tested by Castino et al. [65] on human neuroblastoma cells), induced in Lewis Lung or M5076 tumor cells, a marked inhibition of intracellular CD activity, but no citotoxic effects. It could, thus, be speculated that the inhibition of CD activity may also account for the therapeutic activity of pepstatin A. However, other unknown pharmacological effects induced by this inhibitor as well as the inhibition of other aspartyl proteinases which may account for its antimetastatic activity cannot be ruled out. Nevertheless, these findings suggest a potential role of pepstatin A and its analogs in the adjuvant therapy of solid tumors [25, 65, 66, 176]. However, more detailed information about the pharmacological and toxicological profile of these substances, as well as on their range of activity

is needed before assessing their potential clinical value in cancer treatment.

Conclusions

Experimental and clinical observation indicate that CD may facilitate the growth and spread of solid tumors by acting at various phases of this process (Figure 1). Other evidence suggests that CD may also be involved in upstream events of tumor progression such as carcinogenesis. This latter hypothesis is supported by some *in vitro* studies which reported that the intracellular expression levels of CD were significantly altered during the oncogenic transformation of murine fibroblasts, or during the conversion of nontumorigenic colon adenoma-derived cells to a highly tumorigenic phenotype [177, 178]. These results might also, in part, account for the increased serum levels of this enzyme noted in certain pre-malignant conditions [121, 123, 124, 128, 129]. Therefore, the evaluation of CD expression levels in tumor tissues, and/or body fluids, is proposed as prognostic parameter for predicting the clinical outcome for cancer patients [179]. Although, there is, to date, a general consensus on the prognostic role of CD in female breast cancer [19, 26, 37, 38, 179], conflicting results have been obtained in other gynecological tumors, as well as in other solid neoplasms (Table 2). These discrepant results have been, in part, explained with: i) the different methods used to determine CD expression: it is well known that, biochemical or immunoenzymatic assay methods, unlike immunohistochemical methods, measure total CD activity or content from tumor cells, stromal cells and other non-tumor cells. The presence of CD in stromal cells, which may influence the aggressive behaviour of tumors, may affect the concentration of this enzyme in tumor homogenates and consequently its prognostic significance [80, 91, 93, 103, 109–111, 155, 158, 179, 180]; ii) with the different monoclonal or polyclonal antibodies used, as they may detect single or multiple variations of form of enzyme or may also be unable to detect other altered forms of the enzyme secreted by tumor cells [179]; iii) with the subjective methods of scoring to evaluate the staining intensity of the enzyme; iv) with different cut-off levels considered [179]; v) with the different number and selection of patients; vi) with the different follow-up periods considered. Therefore, further clinical studies, with more appropriate standardized techniques, may better assess the prognostic significance of CD in solid tumors. Nevertheless, these investigations provided evidence that CD expression in some tumors may significantly correlate to more aggressive forms of solid neoplasms. These findings are further corroborated by observation which reported that altered levels of the proteinase in tumor cells may be associated, either *in vitro* or *in vivo*, with the onset of resistance, or degree of chemosensitivity to antitumor drugs [43, 50, 55, 76]. Moreover, a number of experimental studies reported that, in some tumors, CD appears to mediate the apoptotic effects induced by some cytokines, antitumor drugs or differentiating agents [10, 181]. The mechanisms involved in these processes are still controversial, though recent findings indicate that CD may modulate p53-mediated apoptosis and cell chemosensitivity to antitumor agents [182]. Clinical studies which have shown a significant correlation between CD and P53 overexpression in different tumors, support this hypothesis [81, 117, 155, 158, 183]. These findings suggest that CD may be also viewed as a potential attractive target for the drawing-up of new therapeutic strategies in the treatment of solid tumors, and in circumventing the onset of antitumor drug-induced resistance.

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