Cathepsin S as a cancer target

Minireview

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Cathepsin S is a lysosomal enzyme that belongs to the papain family of cysteine proteases. Multiple studies indicate that cathepsin S acts not only as a crucial role in antigen presentation but also as an important protagonist of cancers. Cathepsin S has been shown to participate in dissolution and remodelling of connective tissue and basement membranes, resulting in the process of tumor growth, invasion, metastasis and angiogenesis. Therefore, cathepsin S has been suggested as a potential therapeutic target in cancer therapy. In the present study, we review the rapidly advancing field of cathepsin S in cancer therapy, highlighting the specific roles of cathepsin S in cancer.

Key words: cathepsin S, cancer, combined chemotherapy, inhibitors

Cancer is a broad group of diseases involving unregulated cell proliferation. In cancer, cells divide and grow uncontrollably, form malignant tumors and invade surrounding tissues. Cancer cells may also spread to more distant parts of the body through the lymphatic system or bloodstream. Despite decades of concerted efforts and the advances in surgery, radiotherapy and chemotherapy, the prognosis of some cancer patients remains poor. Currently, many new therapeutic strategies have been developed for the treatment of cancer. Of these therapeutic strategies, lysosomal cysteine cathepsins inhibitors seem to be extremely attractive.

Lysosomal cysteine cathepsins are proteases found in all animals as well as other organisms. There are 11 members of this family, which are distinguished by their structure, catalytic mechanism and the proteins they cleave. These cathepsins have been implicated in a range of physiological processes including bone remodelling, antigen presentation, and diseases such as cancer, osteoporosis and arthritis [1]. Of these lysosomal cysteine cathepsins, cathepsins B and L have been studied most thoroughly in cancers. Overexpression of cathepsin B has been observed in various malignancies, including lung cancer [2-4], prostate cancer [5-7], breast cancer [8-10], colorectal cancer [11-13] and gliomas [14-16]. Cathepsin L was also emphasized to have increased activity and expression in malignant tumors including breast cancer [9, 17, 18], lung cancer [3, 19, 20], gastric cancer [21-23], colorectal cancer [13, 24], melanomas [25-27], and gliomas [28-30]. However, cathepsin B has widespread tissue expression, raising off-target toxicity concerns [31] and cathepsin L has been shown to possess tumor suppressor properties [32]. Therefore, new targets are urgently needed.

Recently, cathepsin S is drawing the attentions of researcher and its importance is gradually developed. This protein is predominantly expressed by antigen presenting cells including macrophages, B-lymphocytes, dendritic cells and microglia. Moreover, cathepsin S is expressed in some epithelial cells and malignant tumor cells. In contrast, cortical thymic epithelial cells and normal epidermal keratinocytes do not express cathepsin S [33-37]. Cathepsin S is distinguished from other cathepsin members as evidenced by: 1. Cathepsin S remains catalytically active under the neutral pH and has pH optimum between the pH values 6.0 and 7.5. However the pH optima of many other lysosomal proteases are acidic; 2. Most of other lysosomal proteases are trapped inside the lysosome due to a problem with their stability. Unlike them, cathepsin S remains stable and it has a physiological role outside the lysosome. Although cathepsin S is primarily localized in lysosomes, it can be translocated to the cell surface and subsequently secreted.
into the extracellular milieu, leading to the degradation of various extracellular matrix proteins such as laminin, fibronectin, elastin, osteocalcin and some collagens, resulting in the promotion of tumor cell invasion and metastasis [38-41].

In the last decade, researchers were focused substantially on the crucial role of cathepsin S in class II MHC antigen presentation [42-45] and some physiological and pathological states [46-51]. Apart from these roles, cathepsin S has recently been demonstrated to be involved in multiple types of cancer, including colorectal cancer [41, 52-54], lung cancer [54, 55], gastric cancer [56], prostate cancer [57, 58], hepatocellular carcinomas [59, 60], melanomas [54, 61], and gliomas [62-65]. The abnormal expression of cathepsin S has been shown to be involved in cancer progression, angiogenesis, cell invasion and migration [38, 66, 67]. Therefore, cathepsin S has been suggested as a potential therapeutic target against cancers. In this review, we aim to investigate the value of cathepsin S inhibition as anti-cancer therapy (Supplementary Table I).

The role of cathepsin S in cancers

In vitro role of cathepsin S

Apoptosis. Apoptosis is the process of programmed cell death (PCD) that may occur in multicellular organisms [68]. Biochemical events lead to characteristic cell changes (morphology) and death. These changes include blebbing, cell shrinkage, nuclear fragmentation, chromatindensification, and chromosomal DNA fragmentation [69]. Due to the high hydrolase content, lysosomes are key organelles in the process of apoptosis. Some apoptosis stimuli, such as lysosomotropic drugs, ultraviolet (UV) irradiation and oxidative stress, can lead to damage of the lysosomal membrane and result in leakage of lysosomal content to the cytosol [70-76]. The executors of lysosome-mediated apoptosis are mostly cathepsins. A critical step in the mediation of apoptotic signalling by cathepsins is the release of cathepsins to the cytosol, a process known as lysosomal membrane permeabilization (LMP) [77].

As a member of lysosomal cathepsins, cathepsin S also plays an important role in the apoptosis of cancer cells. The apoptosis induced by targeting cathepsin S was reportedly attributed to different apoptotic pathways. Generally, there are two distinct apoptotic pathways, including the mitochondrial death pathway (intrinsic pathway of apoptosis) and the death receptor pathway (extrinsic pathway of apoptosis). The intrinsic apoptotic pathway is controlled by members of the B-cell lymphoma-2 (Bcl-2) family, such as Bcl-2 and Bcl-2-associated death promoter (Bad). It is characterized by permeabilisation of the mitochondria and release of cytochrome c into the cytoplasm, cytochrome c then forms a multi-protein complex known as the 'apoptosome' and initiates activation of the caspase cascade through caspase-9. By contrast, the extrinsic apoptotic pathway is activated by death receptors on the plasma membrane such as tumour necrosis factor receptor 1 (TNFR1) and Fas/CD95. As ligands bind to these receptors, the death inducing signaling complex (DISC) is formed leading to initiation of the caspase cascade through caspase-8. It has been demonstrated that targeting cathepsin S by 6mer induced cleavage of both caspase-3 and poly ADP ribose polymerase (PARP), down-regulate anti-apoptotic molecules Bcl-2 and B-cell lymphoma-extra large (Bcl-XL) and induce mitochondria membrane de-polarization, resulting in significant apoptosis in nasopharyngeal carcinoma cells. Moreover, the pan-caspase inhibitor, Z-VAD-FMK, rescued the apoptosis induced by 6mer [40]. This data indicates that targeting cathepsin S induced apoptosis through the intrinsic apoptotic pathways. Conversely, although targeting cathepsin S by Z-Phe-Gly-NHO-Bz induced apoptosis in human leukemia and lymphoma cells, bcl-2-associated X protein (Bax) was not required for Z-Phe-Gly-NHO-Bz-induced apoptosis [78], indicating that targeting cathepsin S-enhanced death signaling is independent of intrinsic pathways. The difference may be due to the intricate connections between cathepsin S and cell apoptosis, depending on cell types and stimulation manners. In addition, targeting cathepsin S could also induce apoptosis in hepatocellular carcinoma cells [59] and colorectal carcinoma cells [54]. However, the specific molecular mechanisms are unclear.

Although studies on the relationship between cathepsin S and apoptosis are rare, since cathepsin S is overexpressed in many cancers, it could be speculated that targeting cathepsin S in some other cancers such as lung cancer, gastric cancer and prostate cancer could also induce apoptosis. However, it remains to be clarified.

Autophagy. Autophagy is a cell death process whereby cells remove cytosolic proteins and organelles and degrade themselves [79]. During autophagy, targeted cytosolic proteins and organelles are isolated from the rest of the cell within the autophagosomes, which are then fused with lysosomes and degraded or recycled [80]. Autophagy can be stimulated by various stress situations, such as oxidative stress and nutrient depletion. During these situations, autophagy ensures the synthesis, degradation and recycling of cellular components, which ensure cellular survival by maintaining cellular energy levels [81, 82]. However, extensive or inappropriate activation of autophagy can lead to cell death (autophagic cell death or type II PCD). Therefore, autophagy may be considered as a double-edged sword when responding to environmental stress [83].

Due to the relationship between lysosome and cathepsin S, there have been reports showing that cathepsin S was associated with autophagy in cancer cells. For example, cathepsin S was found to be overexpressed in tumor-associated macrophages (TAMs) and its overexpression in TAMs was required for not only autophagic flux but also for the fusion processes of autophagosomes and lysosomes. More importantly, cathepsin S-activated autophagy contributed to tumor development by regulating the M2 phenotype of TAMs [84]. This study provided strong evidence for a protective and tumor-promoting
role of cathepsin S-mediated autophagy. Conversely, cathepsin S inhibition could also induce autophagy in cancer cells. It has been demonstrated that targeting cathepsin S by 6r induced autophagy in nasopharyngeal carcinoma cells. In this case, autophagy induced by 6r promoted apoptosis, as evidenced by 3-MA, an autophagy inhibitor, rescued cell death caused by 6r [40]. Furthermore, in glioblastoma cells, targeting cathepsin S by specific inhibitors yet induced autophagy. In this situation, autophagy also played a pro-apoptosis role [85]. In addition, targeting cathepsin S could induce autophagy in many other cancer cells including nasopharyngeal, colon adenocarcinoma cells, oralepidermoid carcinoma cells, alveolar basal epithelial cells and human squamous carcinoma cells [40], suggesting that cathepsin S inhibition-induced autophagy was not cell-specific. In most cases, reactive oxygen species (ROS) acts as an upstream of autophagy [86-90]. However, cathepsin S inhibition-induced autophagy can serve as an upstream of early ROS production. As mentioned before, targeting cathepsin S by 6r induced autophagy-related apoptosis in nasopharyngeal carcinoma cells. Moreover, 6r also induced ROS production. Inhibition of autophagy by either wortmannin or 3-MA reduced 6r-mediated ROS production. Therefore, ROS acts as a mediator between autophagy and apoptosis in this case.

Autophagy is a widespread phenomenon in cells, and the relationship between autophagy and cathepsin S has been fully clarified in nasopharyngeal carcinoma and glioblastoma cells. Therefore targeting cathepsin S may induce autophagy in other cancers such as gastric, breast and lung cancers. Moreover, autophagy is a double-edged sword, so although autophagy acts as an upstream of apoptosis in nasopharyngeal carcinoma cells, it may served a protective role in other cancer types. Thus, whether the anti-cancer activity of cathepsin S inhibition in some malignancies is related to autophagy and whether autophagy plays a protective role or not remained to be explored.

Angiogenesis. Angiogenesis is the physiological process through which new blood vessels are formed from pre-existing vessels. Angiogenesis is a normal and vital process in growth and development, as well as in wound healing and in the formation of granulation tissue. However, it is also a crucial part of tumor development [91]. When a tumor reaches a size of approximately 1–2 mm in diameter, it requires stimulation of angiogenesis for further growth [92]. Furthermore, it is a fundamental step in the transition of tumors from a benign state to a malignant one. Thus, disruption of tumor angiogenesis has been extensively investigated for the development of novel anti-tumor strategies. Nowadays, antibodies targeting the vascular endothelial growth factor (VEGF), such as bevacizumab, have proved therapeutically effective [93-95]. However, due to a lack of efficacy, together with resistance and toxicity in some patients [96], alternative anti-angiogenesis strategies are required.

Cathepsin S has been recently shown to play a key role in angiogenesis. For example, in human umbilical vein endothelial cells (HUVEC), while VEGF alone was shown to stimulate the branch point formation as measured by HUVEC capillary tube formation assays, the addition of α-ketoamides inhibited angiogenesis. In glioblastoma cells, targeting cathepsin S by small interfering RNA (siRNA) markedly suppressed VEGF secretion and restrained HUVEC tube formation in human hepatocellular carcinoma compared with controls [59]. These studies have highlighted the potential of targeting cathepsin S in the tumor microenvironment in vitro. However the molecular mechanisms between cathepsin S and angiogenesis have not been yet explained clearly. Recently, one in vivo study has suggested some anti-angiogenic peptides which may be involved in the effects of cathepsin S on angiogenesis [38] and we will discuss later.

Invasion and migration. Invasion and migration are two pivotal processes in the prognosis of cancer [98]. To invade surrounding tissue and metastasize, malignant cancer cells break away from the primary tumor, attach to and degrade proteins that make up the surrounding extracellular matrix (ECM), which separates the tumor from adjoining tissues [99]. By degrading these proteins, cancer cells are able to breach the ECM, escape the original tumor site and migrate to other parts of the body via the bloodstream, the lymphatic system, or by direct extension [100]. After tumor cells come to rest at another site, they re-penetrates the vessel or walls and continue to multiply, eventually forming another clinically detectable tumor. This new tumor is known as a metastatic (or secondary) tumor.

Cathepsin S was first reported to be related to invasion in glioma cells [64]. In this study, targeting cathepsin S by LHVS29 significantly decreased cells invading through matrigel. Subsequently, cathepsin S inhibition demonstrated anti-invasion efficacy in various cancer models. It has been reported that in umbilical vein endothelial [66], lung adenocarcinoma [97] and colorectal carcinoma cells [41], targeting cathepsin S by α-ketoamides or Fsm0503, blocked cell invasion obviously. In addition, cathepsin S also plays a critical role in cell migration. Silencing of cathepsin S by the specific siRNAs not only inhibited the invasion of gastric cancer cells but also led to a reduction on the ability of cells to cross scratch wound compared to control cells [56]. The same occurred in hepatocellular carcinoma [59], lung adenocarcinoma, and skin melanoma cells [101], suggesting that cathepsin S might be a potential target for inhibition of cell invasion and migration.

The mechanisms underlying the inhibition of invasion and migration are extremely complicated, include the down-regulation of receptor tyrosine kinases such as c-Met, peptidases such as cathepsin D, a disintegrin and metalloproteinase (ADAM) metallopeptidases, matrix metallopeptidases (MMPs) and kallikrein families, chemokines/ cytokines such as interleukin 11 (IL11) and C-X-C motif chemokine ligand 16 (CXCL16) and cytoskeletal and adhesion proteins such as...
cortactin and integrins [41, 56, 59, 66]. Depending on different cell types, there may be different molecules participating in the process of invasion and migration. In figure 1 and figure 2, we provide an overview of cathepsin S inhibition in cancer biology.

**Prognosis.** Several studies have demonstrated that cathepsin S is related to the prognosis of cancers. It has been reported that in gliomas, higher levels of cathepsin S were associated with higher grade of tumor and shorter survival of patients [63]. Moreover, overexpressed cathepsin S in lung cancer and colorectal cancer were also related to tumor progression and poor outcome [53, 55]. Therefore, cathepsin S may serve as a useful prognostic indicator and potential target for anticancer therapy.

**In vivo role of cathepsin S.** In addition to the in vitro activity, cathepsin S also shows therapeutic effect in animal models. For example, in a murine model of sporadic pancreatic carcinogenesis (RIP1-Tag2), cathepsin S knockout could down-regulate anti-angiogenic peptides such as canstatin, arresten, tumstatin and generate pro-angiogenic γ2 fragments from laminin-5, resulting in a significant reduction of tumor-associated angiogenesis switching of neovascularization [38]. In addition, in mouse colon adenocarcinoma and murine melanoma models, cathepsin S knockout mice showed significant reduction in tumor growth; the CD34-positive mean vessel number (MVN), which was usually used to examine the neovascularization, was evidently decreased in cathepsin S knockout mice; cathepsin S depletion also resulted in reduced proliferation and increased apoptosis in tumors as measured by Ki67 and TUNEL staining, respectively [54]. Furthermore, in nude mice with human colorectal carcinoma cell xenografts, targeting cathepsin S by Fsn0503 blocked tumor neovascularization and tumor develop-
ment, as evidenced by a significant reduction in tumor burden and mean vessel area [41, 66]. Collectively, these findings support the role of cathepsin S in promotion of tumor growth and neovascularization both in vitro and in vivo, and highlight it as a target for cancer therapy.

Chemotherapy combined with cathepsin S inhibition

Surgery, radiation therapy and chemotherapy are the three major options in the treatment of cancer. Chemotherapy is the treatment of cancer with one or more cytotoxic antineoplastic drugs as part of a standardized regimen. It is often used in conjunction with other cancer treatments, such as radiation therapy and surgery. However, despite various chemotherapy drugs, the prognosis of cancer patients is not too optimistic. One of the factors contributing to the poor outcomes is the development of chemotherapy resistance following several rounds of chemotherapy. Drug resistance is considered as a multifactorial phenomenon and results from a variety of factors including genetic as well as epigenetic differences in tumors [102]. Several researches have reported the mechanisms of drug resistance, including altered expression or mutation of transporter proteins that decreased drug permeability and/or increase drug efflux from cancer cells, increased repair of DNA damage and decreased sensitivity due to induction of apoptosis, and acceleration of drug metabolism [103-105]. It is generally accepted that multiple molecular mechanisms contribute to chemotherapy resistance and that a single mechanism is unlikely to account for tumor response to chemotherapy [106]. In addition, single chemotherapy drug may also cause serious adverse effects. Thus, combination therapy with different anticancer drugs seems to be an effective strategy for overcoming drug resistance, reducing side effects and achieving better outcomes in chemotherapy of cancers.

Recently, some molecular-targeted agents such as signal-transduction inhibitors and anti-angiogenesis drugs have been developed. Of which, the cathepsin S inhibitions appear to be effective and may have synergistic effects with other chemotherapy drugs. For example, in RIP1-Tag2 mice, combined cyclophosphamide with a broadspectrum cysteine cathepsin inhibitor (JpM-Oet), could significantly reduce tumor burden [107]. However, as JpM-Oet inhibits all cathepsins, it is impossible to draw conclusions concerning the exact role of cathepsin S in the observed effect. In addition, in HUVeC, Fsn0503 or anti-VeGf antibody alone caused a decrease in new vessel. Moreover, the combination of Fsn0503 and anti-VeGf antibody further blocked new vessel development [66]. Furthermore, cathepsin S expression levels were significantly increased in colorectal carcinoma upon treatment with irinotecan (CPT-11), a traditional chemotherapy drug for colorectal carcinoma. Moreover, mice receiving CPT-11, in combination with Fsn0503, had significantly smaller tumors than those receiving CPT-11 alone. Microvessel density (MVD) and the number of small and large vessels, which were used to assess the tumor neovascularization, were also significantly reduced in CPT-11 and Fsn0503 treated groups in comparison to the CPT-11 alone treated groups [52].

Interestingly, when colorectal carcinoma cells and breast carcinoma cells were exposed to 5-Fu, another chemotherapy drug, cathepsin S expression levels were also increased [52], however the combined effects of cathepsin S inhibition and 5-Fu were unclear. Thus it seems that cathepsin S expression levels could be induced in various different tumor types by exposure to distinct chemotherapeutic agents. But whether the synergistic effects presented in colorectal carcinoma would occur in other cancers remains to be determined.

Figure 2. Role of cathepsin S inhibition in tumorigenesis: effects on apoptosis, autophagy, angiogenesis, invasion and metastasis.
Cathepsin S inhibitors used in cancer patients

For its role in various diseases such as cancer, Alzheimer’s disease, neuropathic pain, rheumatoid arthritis and multiple sclerosis, small molecule inhibitors of cathepsin S have been pursued for approximately the past 20 years. Until now, many broad-spectrum and selective cathepsin S inhibitors have been developed, including carbonyl compounds, nitrile compounds, non-covalent inhibitors and so on [108]. However, most of the inhibitors are used in diseases such as rheumatoid arthritis, multiple sclerosis, Alzheimer’s disease, atherosclerosis and obesity. The inhibitors used in cancers are limited. Here, we discuss some broad-spectrum and selective cathepsin S inhibitors, which have been suggested to be effective in cancer and may have a greater potential for therapeutic use (Supplementary Table II).

Broad-spectrum cathepsin inhibitors. The effect of a broad-spectrum cathepsin inhibitor Z-Phe-Gly-NHO-Bz has been studied by De-Min Zhu et al. In this study, they showed that Z-Phe-Gly-NHO-Bz, selective inhibitor of cysteine cathepsins, could induce apoptosis in neck squamous carcinoma, breast adenocarcinoma, prostate adenocarcinoma, glioblastoma and cervix carcinoma cells. In contrast, Z-Phe-Gly-NHO-Bz was not cytotoxic to normal peripheral blood mononuclear cells [109]. Another broad-spectrum irreversible cathepsin inhibitor, JPM-OEt, was also studied on cancer therapy. For example, when using JPM-OEt in mice that had already developed tumors, mice treated with JPM-OEt showed significantly decline in angiogenesis and invasion as compared to controls [110]. There are also some other broad-spectrum cathepsin inhibitors such as E-64 [111, 112] and JPM-565 [113, 114], which showed therapeutic activity in cancers. These trials lead to the conclusion that cathepsins may play a pivotal role for the survival of cancer cells, and therefore, represent a suitable molecular target for therapeutic interventions. However, no distinction can be made between effects of these inhibitors on cathepsin S and other cathepsins.

Selective cathepsin S inhibitors. In addition to broad-spectrum cathepsin inhibitors, several inhibitors which are specific for cathepsin S are available. One of these inhibitors is Fsn0503, a murine anti-cathepsin S monoclonal antibody. Fsn0503 has been reported to block angiogenesis and endothelial cell capillary tube formation, inhibit migration and invasion of cells in various cancer models both in vitro and in vivo [41, 52]. 4-Morpholineurea-Leu-HomoPhe-vinylsulfphone (LHVS) is an irreversible inhibitor of cathepsin S. In glioblastoma cells, LHVS could reduce invasion and induce apoptosis [64, 85]. Z-FL-COCHO (ZFL), a slow, tight-binding reversible inhibitor of cathepsin S, has shown preclinical efficacy in cancer models. It has been demonstrated that in nasopharyngeal carcinoma and glioblastoma cells, ZFL could induce autophagy-related apoptosis [83, 85].

Recently, synthetic α-ketoamide compounds such as 6n, 6r and 6w have been discovered for their specific inhibition of cathepsin S. α-ketoamide compounds have shown antitumor efficacy in various cancer models, including those of human nasopharyngeal carcinoma cells, human lung adenocarcinoma and skin melanoma cells and HUVEC cells. The anticancer effects of α-ketoamide compounds are reportedly attributed to their role in inducing autophagy and apoptosis, inhibiting angiogenesis, invasion and migration [40, 83, 97, 101]. Of note, most compounds had low IC_{50} values and had no obvious cytotoxicity on normal cells at concentrations as high as 50μM [97]. Therefore, the newly synthesized α-ketoamide cathepsin S inhibitors may give rise to alternative drug therapies to prevent the proliferation, invasion and migration of malignant tumor cells.

It should be mentioned that in macrophages, when inhibiting cathepsin S with inhibitors, its function may be compensated by cathepsin F [115]. Thus, whether this phenomenon would occur in cancer remains to be clarified.

In conclusion, studies using cathepsin S inhibitors show a promising outcome in multiple types of cancer. However, these results from in vitro and in vivo experiments may not be found when using cathepsin S inhibitors in humans. Since broad-spectrum cathepsin inhibitors seem to have more potential than inhibitors specific for cathepsin S and the role of cathepsin S may be compensated by other cathepsins. Therefore, we recommend monotherapeutic inhibition as a promising therapeutic option.

Conclusion

Cathepsin S is a lysosomal cysteine protease belonging to the papain family, which is expressed in spleen, antigen presenting cells, such as B cells, macrophages and dendritic cells. The major role of cathepsin S is the degradation of the class II major histocompatibility complex (MHC) associated invariant chain, which is essential for the normal functioning of the immune system. Cathepsin S is thus an attractive therapeutic target for the treatment of autoimmune disorders. However, over the past ten years, cathepsin S has emerged as a key player in cancer therapy. Targeting cathepsin S has shown preclinical antitumor efficacy in various cancer models, including those of gliomas, prostate cancer, lung cancer, gastric cancer and hepatocellular carcinomas. In these malignancies, the anticancer effects of targeting cathepsin S are reportedly attributed to its role in inducing apoptosis and autophagic cell death, reducing angiogenesis, invasion and migration. For example, cathepsin S was found to be overexpressed in lung cancer and its overexpression in lung cancer was associated with tumor progression and poor outcome. Moreover, targeting cathepsin S by α-ketoamide compounds suppressed migration, invasion and angiogenesis of lung cancer cells. In another case, cathepsin S was reported to remain a high level in colorectal cancer. Targeting cathepsin S by the selected cathepsin S antibody, Fsn0503, could induce cytotoxicity, inhibit invasion and angiogenesis of colorectal cancer cells. These observations make cathepsin S an attractive therapeutic target for developing alternative treatment protocols, and, pos-
sibly, for combining with other anticancer drugs to overcome drug resistance and achieve better outcomes. Nowadays, there have been many cathepsin S inhibitors used in caners and the progress of these inhibitors is very fast. Furthermore, some novel cathepsin S inhibitors have been developed. However, in our opinion, the α-ketoamide compounds seem to be the most promising drug in these inhibitors. They have been widely used in cancer models including nasopharyngeal carcinoma, lung adenocarcinoma, skin melanoma cells and HUVEC cells. And importantly, their usages in cancers have not been determined. In addition, most compounds had low IC₅₀ values and had no obvious cytotoxicity on normal cells. Thus the α-ketoamide compounds may be the most attractive cathepsin S inhibitor in the future. However, further studies are needed to clarify whether they are effective in clinical use. Besides α-ketoamide compounds, LHVS and ZFL were recently reported to be used in glioblastoma cells. They were found to induce apoptosis and autophagic cell death [85]. But up to now, LHVS was just found to be used in gliomas and ZFL was found to be used in gliomas and nasopharyngeal carcinoma, therefore their application in cancer is not widespread. Recently, some novel cathepsin S inhibitors such as compound19-(S) appeared [116]. They have showed promising in vitro/vivo pharmacological activities and properties as a selective cathepsin S inhibitor. However since they have just been developed and have not been widely used in experimental researches, so it is uncertain whether they could be used in cancer therapy. Taken together, it is clear that further studies are required to elucidate the specific role of cathepsin S on cancers and develop cathepsin S inhibitors that can be used in practice. Ultimately, effective inhibitions will be developed and may hold promise for clinical cancer therapy.

Supplementary information is available in the online version of the paper.

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Supplementary Information

Cathepsin S as a cancer target

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Supplementary Table

Supplementary Table I. The role of cathepsin S in cancers.

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LHVS, 4-Morpholineurea-Leu-HomoPhe-vinylsulphone; ZFL, Z-FL-COCHO; ROS, reactive oxygen species; siRNA, small interfering RNA; shRNA, short-hairpin RNAs.
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<td>E-64</td>
<td>All cathepsins except C</td>
<td>Irreversible binding</td>
<td>111, 112</td>
</tr>
<tr>
<td>JPM-OEt</td>
<td>All cathepsins</td>
<td>Irreversible binding</td>
<td>107, 110, 114</td>
</tr>
<tr>
<td>JPM-565</td>
<td>All cathepsins</td>
<td>Irreversible binding</td>
<td>113, 114</td>
</tr>
</tbody>
</table>

LHVS, 4-Morpholineurea-Leu-HomoPhe-vinylsulphone; ZFL, Z-FL-COCHO.