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Cathepsin B as a Cancer Target

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Abstract

Cathepsin B is of significant importance to cancer therapy as it is involved in various pathologies and oncogenic processes in humans. Numerous studies have shown that cathepsin B overexpression is correlated with invasive and metastatic phenotypes in cancers. Studies have shown that abnormal regulation of cathepsin B causes cells to acquire an oncogenic character. Cathepsin B is normally associated with the lysosomes involved in autophagy and immune response, but its aberrant expression has been shown to lead to cancers. The proteolytic nature of cathepsin B has been attributed to the infiltrative nature of tumor cells, and it has been shown that cathepsin B is secreted into the extracellular matrix (ECM), thereby facilitating its destruction. Studies have demonstrated that suppression of the proteolytic activity of cathepsin B retards the infiltrative behavior of tumor cells. Further suppression of the expression of cathepsin B via antisense or RNAi also resulted in suppression of tumor growth. In addition, studies have shown that combined suppression of various molecules, such as proteases and protease receptors, including cathepsin B, can significantly suppress tumor growth. Therefore, targeting the activity or expression of cathepsin B is of significant therapeutic importance for cancer treatment.

Keywords

Cathepsin B; cancer therapy; cancer target; ECM; Cathepsin B signaling

Introduction

Cathepsins are lysosomal proteases that belong to the papain family. The name “cathepsin” is derived from ancient Greek “kata” meaning down and “hepsein” meaning to boil. More than a dozen cathepsins have been identified in various organisms (e.g., cathepsin A [1], cathepsin B [2], cathepsin C [3], cathepsin D [4], cathepsin E [5], cathepsin F [6], cathepsin G [7], cathepsin H [8], cathepsin J [9], cathepsin K [9], cathepsin L [10], cathepsin O [11], cathepsin S [12], cathepsin T [13], cathepsin V [14], cathepsin W [15], cathepsin Y [16], and cathepsin Z [17]). Of all the cathepsins, studies have shown that cathepsin B is of significant importance as it is involved in various pathologies and oncogenic processes. In normal physiological conditions, cathepsin B is tightly regulated in a well-coordinated manner at multiple levels. However, during malignant transformation, the regulation of cathepsin B can be altered at multiple levels and thereby resulting in the overproduction of cathepsin B. Overexpression of cathepsin B has been observed in various malignancies, including brain [18], lung [19], prostate [20, 21], breast [22], and colorectal cancer [23]. The altered regulation of cathepsin B can occur at various levels [24, 25]. Cathepsin B has been mapped to chromosome 8p22 [26–28], and interestingly, the importance of this region as a tumor suppressor has also been identified in many studies [29–31]. One of the prominent features of oncogenic cathepsin B is its alternate splicing [32]. The cathepsin B gene, also known as the *CTSB* gene, consists of 13 exons, which include exons 2a and b. The portion that codes for cathepsin B is approximately 1 kb in size with the overall gene spanning at

least 27 kb [26]. A detailed description of the alternate splicing of cathepsin B is discussed by Baici, et al. [32]. In addition, cathepsin B has been postulated to have many non-oncogenic roles, including being anti-amyloidogenic and a neuroprotective protease. Cathepsin B was found to be associated with amyloid plaques in Alzheimer's disease brains and has been suspected to increase A-beta production. Mueller-Steiner, et al. [33] have demonstrated that cathepsin B actually reduces levels of A-beta peptides, especially the aggregation-prone species A-beta1-42 through proteolytic cleavage. Researchers have also demonstrated that cathepsin B may be involved in reducing mutant huntingtin protein levels through macro-autophagy and that it functions as a neuro-protector [34]. It is known that cathepsin B is an important molecule involved in autophagy [35], and under normal conditions, plays an important role in the maintenance of physiological function and also as a scavenger [36]. Cathepsin B has been shown to be involved in invasion and migration [37, 38]. It appears that the abnormal regulation of cathepsin B causes cells to acquire an oncogenic character.

Oncogenic role of cathepsin B

Numerous studies have shown that cathepsin B overexpression is correlated with invasive and metastatic cancers [39–41]. Cathepsin B is known to interact with cystatins [42, 43] and annexin II tetramer (p11), which is also known as S100A10 [44]. These interactions place cathepsin B at crucial positions for the proteolytic activation of ECM components, thereby enabling ECM degradation. It has been observed that the promoter of cathepsin B contains a GC-rich region including many SP1 sites, similar to a housekeeping gene [45]. These SP1 sites are known to increase in tumor cells [46]. Interestingly, it has been observed that cathepsin B is also involved in autophagy and cannibalism, as researchers have shown that tumor cannibalism is advantageous in tumor malignancy and is possibly involved in specific immune resistance [47], enabling tumor cells to recycle nutrients and maintain a proliferative and infiltrative phenotype. This could also explain why the cores of highly infiltrative tumors are necrotic. Various studies have shown that cathepsin B has an active role in initiating the proteolytic cascade involving uPA, plasminogen and plasmin, and it has also been shown to activate latent TGF- β [48]. Cathepsin B appears to support a pro-malignant phenotype by enabling the maintenance of active proteases by actively suppressing their inhibitors. For example, cathepsin B enhances the activity of the matrix metalloproteinases (MMPs) by destroying their inhibitors (e.g., TIMP1 and TIMP2) in human articular chondrocytes and maintains a high level of MMPs, thereby promoting ECM degradation and angiogenesis [49]. Overall, cathepsin B appears to have both pro- and anti-oncogenic roles.

Regulation of cathepsin B

Most malignant tumors show increased transcriptional activation of cathepsin B. More than a decade ago, Qian, et al. identified that increased expression of cathepsin B in B16a melanoma cells is due to a specific increase in the amount or activity of a transcriptional activator of the cathepsin B gene [50]. Later, the research group of Sloane, et al., found that more than one promoter could direct expression of human cathepsin B as shown by multiple transcript species that were detected in their studies. This could result in the production of alternative splicing in the 5'- or 3'-untranslated regions, and possibly the use of alternative promoter regions, and also indicate that membrane association and secretion of cathepsin B is not a random process in the tumor cell, but is part of a tightly controlled system [24, 51]. Further, it was also reported that differentiating agents, such as inducers of monocytic differentiation agent PMA, calcitriol (D3), and sodium butyrate (NaB), and inducers of granulocytic differentiation [all-trans retinoic acid (RA) and 9-cis retinoic acid (9-cis RA)] increase cathepsin B mRNA levels in a dose-dependent manner [52]. As newer molecular

techniques developed, it was further determined that the regulation of cathepsin B transcription is by Sp1 and Sp1-related factors, which is mediated through multiple GC boxes on its promoter [53]. Other researchers have also come to the same conclusion that variation of cathepsin B expression is largely due to different levels of Sp1 presence [54]. Moreover, it has also been shown that myogenic transcription factor mediates the activation of cathepsin B expression during myogenic differentiation [55] and that an E-box at nucleotides -7 to -2 of the cathepsin B promoter is critical to the expression of cathepsin B.

Additionally, the binding of USF-1 and USF-2 to this E-box can regulate cathepsin B promoter activity [56], thus highlighting its role in differentiation. Interestingly, it was further shown that USF2c, an isoform of USF2, regulates the expression of cathepsin B by binding to the E-box element in the cathepsin B promoter functioning as a repressor [57]. Further, it was also recently shown that the EMT activator ZEB1 (zinc finger E-box binding homeobox 1) is a crucial promoter of metastasis and stemness, which functions by suppressing stemness and mediates inhibition of microRNAs involved in tumor regulation [58]. These findings make it clear that cathepsin B may also be involved in EMT and may also be regulated by various EMT and stemness-related factors. All of these findings highlight the fact that cathepsin B is a multifaceted molecule and is regulated at multiple levels with diverse function, some of which are still not clear.

Cathepsin B cascade

The aberrant expression of cathepsin B, which is normally an intracellular lysosomal protease, has been observed in the ECM of malignant tumors. Cathepsin B is secreted as a pro-enzyme [59] and is activated to its active form via various molecules. The mechanism of secretion of cathepsin B to the pericellular surface of cancer cells is still unclear. It has been observed that human lung macrophages express four of the known cathepsins – cathepsins B, H, L, and S [60]. It is also known that brain macrophages of microglial origin possess high levels of cathepsin B and L, and thus, these cells are potentially cytotoxic. These studies indicate that these cells can play a universal role in the defense of the lung tissue [60] and the neural parenchyma [61]. It is also known that cathepsin B is involved in bone resorption [62]. All of these data indicate that cathepsin B is an important part of the immune defense system and plays a vital role in healing.

Interestingly, cathepsin B has previously been identified as a lysosomal thiol proteinase that may have additional extralysosomal functions [63]. In cancer cells, activation of the cathepsin B gene is mediated by extracellular signals, which are probably mediated via the integrin-uPAR-uPA and the CD44-MMP complex supermolecular complex. This activation results in the overproduction of cathepsin B mRNA, which in turn, is processed to pro-cathepsin B. This pro-cathepsin B is transported to the cell surface where it localizes on the annexin-p11 complex. Activation of pro-cathepsin B is via numerous mechanisms such as CathD, tPA and uPA, and we suspect that alternate splice variants can also auto-activate it. Cathepsin B is also known to activate pro-MMPs, which in turn, targets the ECM, causes ECM degradation and facilitates metastasis (Fig. 1). Interaction of MMPs, specifically MMP-2 and MMP-9, with CD44 on the cell surface occurs via the hemopexin domain [64]. It is speculated that these MMPs are activated or shed into the ECM via the action of cathepsin B and uPA. In other words, cathepsin B plays an important role in orchestrating the protease cascade and directing it towards the invasive fronts of metastatic cells. The activation of various proteases is usually mediated by plasmin, a broad spectrum protease. Other than plasmin, the only other known efficient activator of MMPs is cathepsin B [65]. Further, cathepsin B was also observed to activate trypsinogen-1 in pancreatitis [66], and pancreatic inflammation appears to increase the risk of pancreatic cancer [67]. Researchers have also found that infections from viruses such as Influenza-A virus elevates active

cathepsin B in primary murine dendritic cells [68], contributing to the hypothesis that prolonged infections can lead to carcinogenesis [69]. Further, it was also recently observed that TLR3 signaling and cleavage are largely dependent on cathepsin B [70]. It has been shown that TLR3 detects double-stranded RNA produced during the replication cycle of most viruses and leads to the activation of the transcription factors NF- κ B and interferon regulatory factor (IRF) 3, thus further leading to the production of type I interferons (IFNs) and pro-inflammatory cytokines, such as IL-6 and IL-8 [71]. Further, activation of the TLR3 pathway is also known to induce apoptosis in tumor cells via the activation of caspases 8 and 9 [72]. As such, cathepsin B may inactivate this TLR3-mediated apoptotic pathway, leading to the survival of tumor cells. It is also known that the pro-apoptotic activity of TLR3/TRIF/caspase 8 in melanoma cells is under the control of inhibitors of apoptosis (IAPs) [73]. It is also known that TLR3 signaling triggers apoptosis and induces growth arrest of LNCaP cells partially via the inactivation of the PI3K/Akt pathway and that treatment-associated autophagy plays a cyto-protective role, thereby leading one to speculate on the pro-oncogenic role of cathepsin B [74]. Interestingly, several systems have shown the involvement of cathepsin B in apoptosis regulation. The release of cathepsin B from the lysosomes may activate apoptotic pathways [75]. This dual nature of cathepsin B is interesting as studies show that cathepsin B serves as a pro-oncogenic molecule involved in ECM degradation, angiogenesis and metastatic induction; as an anti-apoptotic molecule suppressing the TLR3 pathway and maintaining a pro-survival state; and as a pro-apoptotic molecule involved in autophagy and immune response. The catalytic function of cathepsin B is dependent on the pH. It is known that lysosomes have a low pH and provide the ideal environment for cathepsin B activation [76]. A similar type of environment is observed around the invasive front of tumor cells, as such tumors are considered to be more acidic than normal cells [77]. This leads one to speculate that the tumor microenvironment shows high cathepsin B activity. All of these aspects, such as the tumor microenvironment, secretion of cathepsin B and the activation of various proteases contribute to a malignant phenotype. This raises another question as to the mechanism of tumor cell survival under low pH conditions. It is known that cancer cells can survive under low nutrient conditions when compared to normal cells [78], but the mechanism of tolerance to low pH is still not clear. Cathepsin B is known to be localized in the lysosomes and to facilitate autophagy and immune responses by trafficking TNF-alpha-containing vesicles to the plasma membrane in macrophages [60, 79]. Cathepsin B is also known to be localized in the mitochondria [80] where it is thought to initiate cell death [81]. Interestingly, cathepsin B is also known to be localized to the nucleus where it is found to be associated with the nuclear scaffold [82] and is thought to be involved in cell division. A recent report suggests that cytoplasmic cathepsin B might be able to enter the cell nucleus and that cell death associated with cathepsin B is not triggered by its regular enzymatic activity but rather by an unknown mechanism of truncated cathepsin B [83]. A recent study was done to determine the trafficking of cathepsin B in thyroid carcinoma cells [84]. This study employed the use of GFP-tagged cathepsin B and showed that sub-cellular localization of proteolysis is a crucial step in regulation of tissue homeostasis. This study also concluded that any interference with protease trafficking resulting in altered regulation of the proteolytic events could lead to the onset and progression of cancer. These findings indicate that the normal regulation and distribution of cathepsin B is vital for cellular functioning and any aberrant expression of cathepsin B resulting from altered splicing or change in expression levels can significantly alter the homeostasis of cells and result in a malignant phenotype. These studies suggest that besides the N-terminal signal peptide, other cathepsin B domains may contain patterns that are responsible for differentiated targeting to the mitochondria, the nucleus, or vesicles. These diverse functions of cathepsin B and its multifaceted role illustrate that cathepsin B as a molecule is still shrouded in mystery and many of its functions still remain unknown, but its role in the progression of cancer remains undisputed. Identification of cathepsin B as a

prominent contributor to the malignant behavior of tumor cells has helped identify it as a possible target for cancer therapy.

Therapeutic potential of targeting cathepsin B

Numerous studies have shown that infiltrative tumors express high levels of proteases including cathepsin B [85–87]. Various therapeutic strategies have been developed to suppress proteolytic activity of proteases in an attempt to curb metastatic infiltration mediated by proteases. Among the strategies developed, chemical inhibitors, antibodies, and gene therapy approaches have shown promising developments. Inhibitors of cathepsin B have been isolated from various sources such as the marine bacteria *Pseudomonas*, marine sponges and other organisms [88]. Researchers have also observed that non-peptidic compounds, such as flavonoids, also showed strong cathepsin B inhibition properties. However, even with numerous compounds being isolated and identified, none have really shown any significant therapeutic potential. Recently, Shree, et al. [89] have demonstrated that cathepsin B expression in breast tumors plays a tumor protective role, and they showed that a combination approach to cathepsin B inhibition, which involved use of the compound JPM and the chemotherapy agent Taxol, significantly retarded tumor development. Researchers have also tried development of various hybrid inhibitors to suppress cathepsin B activity. For example, a bifunctional inhibitor has been developed that binds to cathepsin B and uPAR [90]; it behaves as a tight binding inhibitor of cathepsin B as well as an effective uPA receptor antagonist. Recently, a potato cysteine proteinase inhibitor (PCPI 8.7) was demonstrated to inhibit invasion in murine B16 melanoma cell by 21% [91]. Further, it has been demonstrated in leukemia cells that Imatinib-mediated apoptotic induction of CML cells is through LMP and cathepsin B-specific degradation of BCR-ABL, which shows that cathepsin B may also play a role as a cancer suppressor under appropriate conditions [92]. Parker, et al. have demonstrated that expression of Stefin A (also known as cystatin A) reduces distant metastasis in breast cancer, and they proposed that this may be due to the inhibition of cathepsin B [93]. Cystatin A is a well known inhibitor of cathepsin B [94], and its possible use as an inhibitor of cathepsin B is not new. Li, et al. [95] have demonstrated that overexpression of cystatin A has possible therapeutic implications in human esophageal squamous cell carcinoma cells. The evolution of cathepsin B inhibition from chemical inhibitors to biological inhibitors has now progressed to the use of gene therapy. As has been demonstrated by numerous researchers, cathepsin B is overexpressed in various cancers [87] and seems to play an important part in maintenance of the cancer phenotype. Targeting the expression of cathepsin B could have significant therapeutic implications. We have previously shown that cathepsin B expression is greatest in highly malignant astrocytomas, especially in glioblastomas, and is correlated with the malignant progression of astrocytomas [96]. Using this as a starting point, we attempted to downregulate cathepsin B using the anti-sense approach [97]. We observed that intracerebrally implanted SNB19 stable cathepsin B antisense cells resulted in reduced tumor formation in nude mice. Further, to determine whether suppressing the activity of cathepsin B by cystatin A would achieve the same result, we used cystatin A overexpression using sense-transfected cells and observed that these cells did not form tumors in nude mice upon intracerebral implantation [98]. Not only did suppression of cathepsin B retard tumor formation, other aspects of tumor malignancy, such as angiogenesis, were also reduced [99]. In most cases though, targeting cathepsin B alone did not significantly reduce tumor burden. Therefore, we asked whether using a combination approach would be the next logical approach. We simultaneously downregulated the expression of cathepsin B with one other molecule known to be associated with tumor invasion and migration, such as uPAR, and observed that the simultaneous downregulation of uPAR and Cathepsin B using the antisense approach inhibited tumor growth, invasion, and angiogenesis [100]. It is known that for antisense to function, an equal molar ratio of the antisense molecule to the target

mRNA is required. However in the case of RNAi, such a ratio is not required as the siRNA-associated RISC is recycled and almost has a catalytic function [101–103]. Therefore, we attempted to use the RNAi approach to target cathepsin B. We used the RNAi approach to simultaneously downregulate MMP-9 and cathepsin B and in the direct intratumoral injections of plasmids expressing hpRNA targeting MMP-9 and cathepsin B. We observed that this approach significantly inhibited established glioma tumor growth and invasion in intracranial tumors *in vivo* [104]. Using a similar approach, we simultaneously downregulated uPAR and cathepsin B using the RNAi approach where intratumoral injections of plasmids expressing hpRNA targeting uPAR and cathepsin B resulted in the regression of pre-established intracranial tumors. Further, RNAi for uPAR and cathepsin B inhibited cell proliferation, reduced the levels of pERK and pFAK [105], and altered the cellular cytoskeleton, which retarded motility and migration of tumor cells [106] and initiated a partial extrinsic apoptotic cascade accompanied by the nuclear translocation of AIF [107]. In addition to our studies in gliomas, we also observed that RNAi-mediated downregulation of cathepsin B and MMP-9 led to decreased tumor growth, invasion and angiogenesis in malignant meningioma cell line [108]. These studies show the effectiveness of simultaneously downregulating two tumor-associated molecules. Simultaneous downregulation of two molecules—one of which is cathepsin B—has shown to be effective at retarding tumor burden and tumor-associated phenomena, such as angiogenesis. Similar results were also observed in prostate cancer cells [109] where targeting MMP-9, uPAR, and cathepsin B inhibited invasion, migration and activated apoptosis. Clearly, the simultaneous downregulation of cathepsin B along with one other tumor-associated molecule appears to have a synergistic effect when compared to downregulating cathepsin B alone.

Understanding the mechanisms associated with tumor suppression mediated by cathepsin B downregulation will enable the development of treatment strategies aimed at treating cancers where cathepsin B overexpression is observed. It was observed that downregulation of uPAR and cathepsin B initiates a caspase-dependent mitochondrial apoptosis in U251 cells and caspase-independent mitochondrial apoptosis in 5310 cells, thereby indicating that the mechanism of apoptotic induction via suppression of uPAR and cathepsin B can vary from cell type to cell type [110, 111]. The addition of another component to this combinational approach is the use of radiation. It has been observed that collateral radiation damage [112] affects normal cells differently than tumor cells. The next logical strategy would be to sensitize these cancer cells to radiation without severely affecting normal cells. This was partially achieved by suppressing cathepsin B where the simultaneous downregulation of uPAR and cathepsin B (pUC) in combination with radiation was shown to have a greater potential application for the treatment of human meningioma [113]. It was also shown that the simultaneous downregulation of uPAR and cathepsin B inhibits radiation-induced PKC-integrated integrin signaling to the cytoskeleton of glioma-initiating cells [114]. All of these studies show that the suppression of cathepsin B has high therapeutic significance. A recent screening of ongoing clinical trials has revealed that, at present, no study is in progress that directly targets cathepsin B as a therapeutic target. Expression of cathepsin B is only being used as a marker to study the effect of various chemotherapy and bio-therapy products being investigated. From all the previous research reports, we see that targeting cathepsin B alone would not be sufficient to eliminate tumors. A multifaceted approach either targeting multiple molecules, including cathepsin B, or using a combination of chemotherapy or radiation along with targeting cathepsin B, seems to be a more appropriate approach.

Conclusion

From the review of literature, it is evident that cathepsin B is a versatile molecule with numerous biological roles. The role of cathepsin B in cancer progression and the metastatic

process is clear. Therefore, targeting cathepsin B would have significant clinical relevance in the treatment of various cancers.

Expert Opinion

Cathepsin B has been linked to tumor progression and metastasis. The broad influence of cathepsin B on various aspects of tumor survival and growth makes it an attractive target for cancer therapy. It is clear that targeting cathepsin B has significant therapeutic benefit. Moreover, it is evident that targeting cathepsin B alone does not completely diminish or suppress tumor progression. As researchers have shown, it is possible that cathepsin B can influence signaling events both at the cytoplasmic and nuclear levels and may also be involved in mitochondrial signaling, thereby placing it at a higher level in the signaling cascade than previously thought. Whether the expression of cathepsin functions as a signal initiator or is the product of a signaling event associated with tumor progression still remains unclear. As seen in immune cells, such as macrophages, which have high levels of lysosomal cathepsin B but no pericellular localization of cathepsin B as seen in tumor cells, it appears that the cellular location of cathepsin B plays an important part in its functioning. It is not clear whether global suppression in normal cells of cathepsin B activity or expression would produce unintended effects or cause the activation of or suppression of unwanted pathways and this still remains to be studied. A localized approach to target the expression of cathepsin B would be more relevant.

Moreover, suppression of cathepsin B at the cellular level *in vitro* cannot model the effects *in vivo*. More robust and clinically relevant studies need to be performed to highlight the importance of targeting cathepsin B. However, targeting cathepsin B alone does not abolish tumor growth, which is probably because cathepsin B appears to have diverse functions and influence numerous pathways. Therefore, a more prudent approach would be to target multiple molecules to achieve a clinically relevant outcome.

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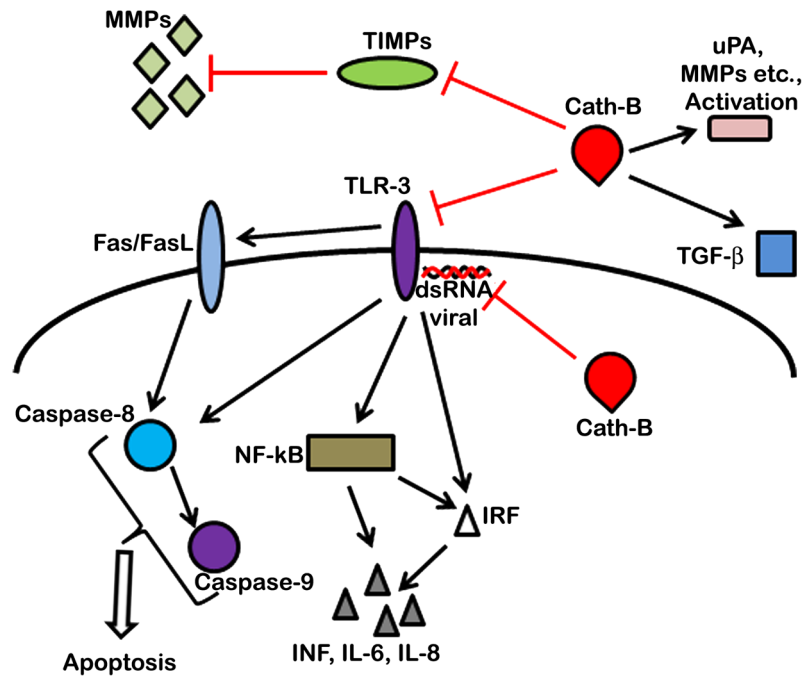


Figure 1. Schematic representation showing the signaling and protease cascade events mediated by cathepsin B on tumor cells.

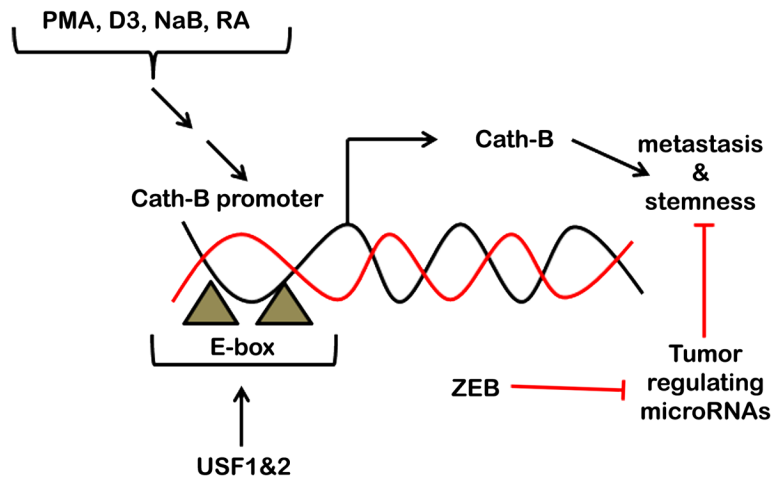


Figure 2.

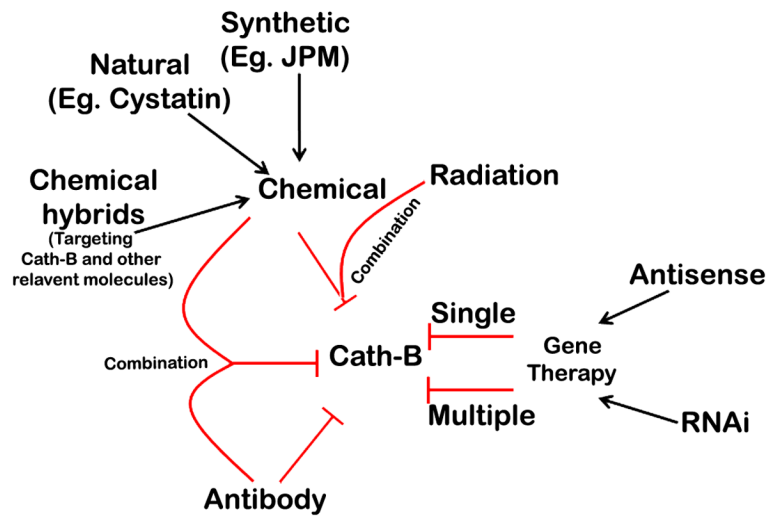


Figure 3.