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ENZYMES AS TARGETS FOR CANCER THERAPY

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ABSTRACT: Cancer is a deliberating disease affecting people worldwide. Treatment of cancer has become a number one priority among medical specialists and different modes of treatment are being pursued. Various factors are involved in the proliferation of cancer thereby complicating its treatment. Certain enzymes or protein products within the cell can be up-regulated or down-regulated as the cancer progresses inside the tissue. In order to treat cancer, enzymes which play a key role in the disease can be targeted. These enzymes include cysteine cathepsins and matrix metalloproteinases. Cysteine cathepsins are a group of enzymes that are involved in degradation of the surrounding cell environment of cancer cells. The surrounding healthy cells are thereby invaded and destroyed by cancer cells. Hence they cause localized spreading and growth of cancer cells. Matrix metalloproteinase are mainly involved in metastasis of these cancer cells. They help in angiogenesis surrounding the cancer tissue thus providing nutrition to cells and proliferation via the blood stream. Hence both enzymes has a vital role in proliferation of cancer cells into body. These enzymes can be targeted for treatment of cancer and can act as a supplementary process to the cancer Containment, which in turn can increase the chances of survival. Various drugs are under trial for this form of therapy.

INTRODUCTION: Cancer is a group of disease, where there is abnormal cell growth which have the potential to invade other parts of the body. Cancer cells can form a tumour which invades the local tissue and cancer has been one of the leading causes of death. The most common cancers are breast cancer, lung and bronchus cancer, prostate cancer, colon and rectum cancer, bladder cancer, melanoma of the skin, non-Hodgkin lymphoma, thyroid cancer, kidney and renal pelvis cancer, leukaemia, endometrial cancer, and pancreatic cancer.

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There are over 100 different known cancers that affect humans [1]. In 2012 about 14.1 million new cases of cancer occurred globally. It caused about 8.2 million deaths or 14.6% of all human deaths [2]. The cells of a tumour descend from a common ancestral cell that at some point became mutant. This program of multiple mutations, accumulate throughout the life of the host and each mutation is passed on to the daughter cells. For cancer to rise, a series of critical mutations must occur, usually through mutations in specific classes.

To control normal functioning of a cell, regulatory functions are required. However in a cancer cell, these regulatory functions are diminished or lost altogether. Because of their altered regulation, the cells have distinguished features other than that of normal cells and produce different proteins altogether. The loss of such regulation is mainly due to gene alteration in these cells.

These mutations can arise from various factors such as viruses, oncogenes, mutagenic chemical exposure, carcinogens and environmental factors. It is because of such mutations that the formation and regulation of certain proteins can be altered leading to higher levels of same in a cancer cell. Various proteins involved in cancer include matrix metalloproteinase, cysteine cathepsins and serine proteases.

Matrix Metalloproteinase: The MMPs are a family of zinc-containing endopeptidases that degrade various components of the extracellular matrix (ECM) [3]. They are part of larger family of proteases [4]. MMPs include the requirement of zinc in their catalytic site for activity and their synthesis as inactive zymogens that generally needed to be proteolytically cleaved to be active. Normally the MMPs are expressed only when and where needed for tissue remodelling. MMP expression and activity are regulated at several levels. In most cases, MMPs are not synthesized by the cell until needed. Transcription can be induced by various signals including cytokines, growth factors, and mechanical stress [3]. However, uncharacteristic expression of various MMPs has been correlated with pathological conditions such as periodontitis, rheumatoid arthritis tumour cell invasion and metastasis [5].

20 different members of the MMP family have been identified, and they are sub-grouped based on their structures [3]. Each member consists at least of a signal peptide, prodomain, band catalytic domain. MMPs containing only the minimal domain are referred to as matrilysins (MMP-7 and -26). They are classified based on the presence of different domains. The most common structures for secreted MMPs are collagenases, and stromelysins (MMP-1, -3, -8, -10, -12, -13, -19, and -20) and the gelatinases (MMP-2 and -9) [6].

MMPs participate in angiogenesis by degrading basement membrane and other ECM components, allowing endothelial cells to detach and migrate into new tissue. In addition, MMP degradation of ECM components generates fragments with accessible integrin binding sites, triggering integrin intracellular signalling. By directly binding to 3, MMP-2 may itself initiate integrin signalling and thereby contributing to endothelial cell survival and proliferation [7]. MMPs have both pro- and antiangiogenic functions. On the whole, however, MMPs are required for angiogenesis [8].

Role of Matrix Metalloproteinase in Cancer: During development of cancer, tumour cells interact with the tumour microenvironment in ECM, such as the growth factors and cytokines associated with ECM, as well as surrounding endothelial cells [9]. Four traits of cancer that include migration, invasion, metastasis and angiogenesis are dependent on this surrounding microenvironment. MMPSs play an important role as they degrade various cell adhesion molecules, thereby increasing cell–cell and cell–ECM interactions.

Expression of MMPs has been found to be upregulated in all type of human cancer and correlates with advanced stage, invasive and metastatic [10, 11]. Different MMPs have different roles at various stages of cancer. For cancer cells to continue to grow and start migrating, it is necessary to form new blood vessels. The first step in this process is to eliminate the physical barriers by ECM degradation and, subsequently, to generate pro-angiogenic factors. Up-regulation of MMP expression, in particular the gelatinases, degrade basement membrane components, thereby permitting the tumour cells to invade into the adjacent stroma and to break down the basement membranes associated with capillaries and lymphatic vessels allowing tumour cells to enter the circulation [12]. MMPs are involved in cell migration by removing sites of adhesion which exposes new binding sites by cleaving cell-cell or cell-matrix receptors [61]. MMP-9 participates in the angiogenic switch because it increases the bioavailability of important factors in this process, such as the vascular endothelial growth factor (VEGF), which is the most potent mediator of tumour vasculature, and basic fibroblast growth factor (bFGF), by degradation of extracellular components, such as collagen type IV, XVIII and perlecan, respectively [13, 14].

Members of the MMP and ADAM families can release the cell-membrane- precursors of several growth factors, such as insulin-like growth factors (IGFs) and the epidermal growth factor receptor (EGFR) ligands that promote proliferation. Several MMPs (MMP-1, -2, -3, -7, -9 -11 and -19) and

ADAM12 cleave IGF-binding proteins that regulate the bioavailability of the growth factor [15]. EGFR, mediator of cell proliferation, is implicated in cancer progression because it is over expressed in more than one-third of all solid tumours [16].

Matrix-degrading enzymes confer both apoptotic and anti-apoptotic actions. MMPs and ADAMs, especially MMP-7 and ADAM10, confer anti-apoptotic signals to cancer cells by cleaving Fas ligand, a transmembrane stimulator of the death receptor Fas, from the cell surface. This proteolytic activity inactivates Fas receptor and induces resistance to apoptosis and chemo-resistance to the cancer cells or promotes apoptosis to the neighbouring cells depending on the system [17].

Moreover, proteolytic shedding of tumour associated major histocompatibility proteins complex class-I related proteins by ADAM17 may suppress natural killer (NK) cell-mediated cytotoxicity toward cancer cells [18]. Notably, MMPs may contribute to the anti-apoptotic effect by activating indirectly the serine ⁄ threonine kinase Akt ⁄ protein kinase B through the signalling cascades of EGFR and IGFR [16, 17]. MMPs also promote apoptosis, most likely indirectly by changing the ECM composition; for example, by cleaving laminin, which influences integrin signalling [19].

Cysteine Cathepsins: Cysteine cathepsins are enzymes which belong to the papain subfamily of cysteine proteases [20]. They function at a lower pH and are distinguished by their structure, catalytic mechanism, and which proteins they cleave. They are mostly endopeptidases located in endolsysomal vesicles intracellularly.

However some do function extracellularly like cathepsins K which is secreted into the resorptive pit between osteoclasts and bone [21]. There are 11 human cysteine cathepsins, which primarily function as endopeptidases within endolysosomal compartments. Specific cysteine cathepsins have extracellular functions, for example, cathepsin S in MHC (major histocompatibility complex) class II antigen presentation [22]. Multiple mechanisms increase cysteine cathepsin expression in tumours, including amplification of the cathepsin B gene and alternative splicing of cathepsin L and B transcripts. Increases in expression occur both in tumour cells and tumour-associated cells such as macrophages, endothelial cells and myo-epithelial cells. In tumours these enzymes can be secreted, bind to specific regions on the cell membrane and are localized in endolysosomal vesicles. Their substrates and functions differ depending on their location. Causal roles for cysteine cathepsins in cancer have been demonstrated by pharmacological and genetic techniques.

This includes functional down regulation of cysteine cathepsin activity by increasing expression of endogenous inhibitors and administration of small-molecule cysteine protease inhibitors. Contributory roles of cysteine cathepsins in cancer have also been identified in regard to intracellular matrix degradation following endocytosis of collagens by urokinase plasminogen activator receptor-associated protein (uPARAP). Contributory roles for specific cysteine cathepsins in cancer have been demonstrated by down regulating their expression or crossing mouse models of cancer with mice in which the cysteine cathepsin has been genetically ablated. These studies have identified roles for cysteine cathepsins in both tumour cells and tumour-associated cells such as endothelial cells and macrophages [23].

Cysteine cathepsins were thought to function intracellularly within lysosomal vacuoles. However cathepsin K, B and L are found to be present both intracellularly and extracellularly. Studies on mice that are deficient in these 3 cathepsins have shown that all three function, both extracellularly and intracellularly to liberate thyroglobulin [24].

Functions of these cysteine cathepsinsin cancers are not yet well defined nor are their roles in tumour cells and the tumour-associated cells that contribute to neoplastic progression are known Since cysteine cathepsins are secreted and localized in lysosomal vesicles their enzymatic substrates and functions might change along with their localization. Cysteine cathepsins are used cleavage of extracellular matrix proteins such as laminin [25, 26], type IV collagen62 and tenascin C64, cell-adhesion proteins such as E-cadherin [27], activation of pro-enzymes such as pro-urokinase plasminogen activator (prouPA) [28, 29]. Degradation of extracellular matrix protein such as collagens [30, 31] also occurs intracellularly.

Up Regulation During Cancer: As the genes coding for various proteins get mutated in cancer various proteins get up regulated while others get down regulated. Gene amplifications may take place for the gene coding of cysteine cathepsin, for example over expression of cathepsin B in adenocarcinomas of the oesophagus [32] and gastric cardia [33].

Transcript variants are tumour cells for cathepsins B and L, arising from the use of alternative promoters [34, 35] and alternative splicing [36, 37]. In these cells shorter transcripts are formed which are easily translated and hence lead to their over expression [38, 39]. These transcripts are however regulated by various factors and require further study. For example low levels of expression of cathepsin L in a B-cell lymphoma line are due to methylation of a CpG island in the cathepsin L promoter [40].

However up regulation mechanism is not known for all types of cathepsins. Higher levels of cathepsin V is used as a diagnosis tool for detection of breast carcinomas [41]. The over expression of a certain cysteine cathepsins is different in different types of cell. This over expression is found mostly in tumour cells and tumour associated cells. For example high levels of cysteine cathepsins in myoepithelial cells, associated with ductal carcinoma in situ, for cathepsins F, K and L [42], and in the endothelial cells for cathepsin B [43].

Role of Cysteine Cathepsins within Cells: Cysteine cathepsins are mainly involved in proteolysis. However in neoplastic progression it is known that pericellular proteolysis occurs. Inactive precursor forms of a number of cysteine cathepsins are secreted from both transformed and tumour cells [44, 45]. Active forms are also released from cells [46] and activated macrophages [47, 48]. Proteolysis by such cathepsins leads to tumour invasion to neighbouring cells. Intracellular proteolysis in tumour cells, just like pericellular proteolysis, involves both cysteine cathepsins and uPA/uPAR. The two proteolytic systems are networked through uPARAP (urokinase plasminogen activator receptor-associated protein)/Endo180, a member of the macrophage mannose receptor family [49] uPARAP forms a trimolecular complex with pro-uPA and uPAR and is essential for the cellular uptake of collagen and its subsequent degradation in lysosomes by cysteine cathepsins [50, 51]. This is an important pathway of extracellular matrix degradation during mammary tumour progression [31]. Intracellular degradation of collagens in tumours has also been observed within tumour cell lysosomes, for example, in colon tumour cells [52].

Individual cathepsin is found in higher levels in different tumour cells [53]. For example, cathepsin B has been shown to be assisting in migration and invasion of human osteosarcoma and glioblastoma cells [54, 55]. Cathepsin L is found to have a role in migration and invasion of human osteosarcoma cells [56] and cathepsin X in migration of human gastric carcinoma cells [57].

These indicate role of cathepsin B in tumour development and progression [58], but also indicate smaller roles of cathepsin L and X. Cathepsins B and S have shown to affect the ability of tumours to induce angiogenesis. This has been shown for cathepsin B in glioblastomas [59], cathepsins B and S in RIP1–Tag2 pancreatic tumours [60, 61] and cathepsin S in brain tumor [62].

Cysteine cathepsins also have roles in tumour associated cells. Genetic ablation of various cathepsins has been found to contribute to metastasis. Genetic ablation of cathepsin B in the MMTV-PyMT model revealed a role for macrophage cathepsin B in experimental lung metastasis of these tumours [63].

Functional down regulation of cysteine cathepsin activity by increasing the expression of the endogenous inhibitors of these enzymes has confirmed roles proteases in cancer. Studies in which cystatin C, cystatin M and stefin/cystatin A were over expressed in tumour cell lines [64, 65] have shown that cysteine cathepsins have functional roles in growth, invasion and metastasis of tumour cells of epithelial and mesenchymal origins. Alternatively, silencing expression of inhibitor cystatin M in a metastatic oral cancer cell line results in increased migration and invasion as it caused increase in cysteine cathepsin activity [66]. These studies indicate the proteolytic activity of the cysteine cathepsin family is involved in the malignant phenotype of tumour cells and tumour-associated endothelial cells.

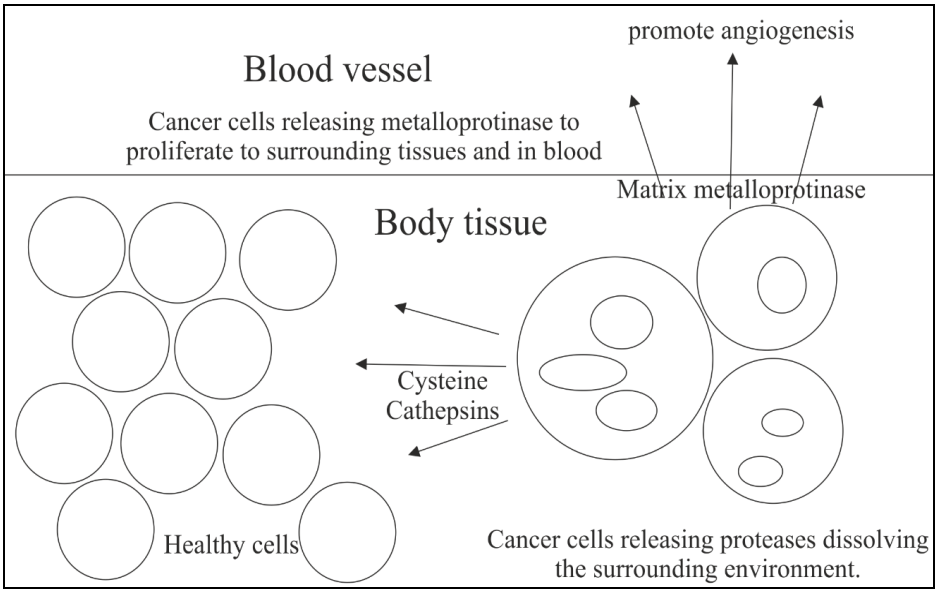

FIG. 1: THE FIGURE DENOTE THE WORKING OF THE TWO ENZYMES IN CONJUNCTION IN A TISSUE AND HOW THEY PROMOTE CANCER. CATHEPSINS DISSOLVE THE SURROUNDING CELLS AND OCCUPY THEIR PLACE. METALLOPROTEINASE ENSURE THE PROLIFERATION AND RELEASED IN BLOOD VESSELS TO INCREASE THE BLOOD FLOW TO CANCER TISSUE BY ANGIOGENESIS

Current Strategies for Treatment: All the enzymes mentioned can be targeted for treatment of cancer. Although it would be ineffective as sole treatment, but when provided as secondary treatment it can increase the chances of survival. Currently there need to be more study available on such treatment regimes. Recent advances in these fields seem promising.

Several generations of synthetic MMPIs were tested in phase III clinical trials in humans, including peptidomimetics, nonpeptidomimetics inhibitors and tetracycline derivatives, which target MMPs in the extracellular space [67]. In addition, various natural compounds have been identified as inhibiting MMPs [68]. The first generation of MMPIs introduced comprised the peptidomimetic. These pseudopeptide derivatives mimic the structure of collagen at the MMP cleavage site, functioning as competitive inhibitors, and chelating the zinc ion present at the activation site [11]. These include drugs like batimastat and marismastat. To improve specificity and oral bioavailability, the nonpeptidomimetic MMPIs were synthesized which include prinomastat. However musculoskeletal toxicity has also been reported in clinical trials with the drug [69]. Another generation of MMPIs, tetracycline derivatives, inhibit both the enzymatic activity and the synthesis of MMPs *via* blocking gene transcription.

Currently only Food and Drug Administration approved MMPI are used for the prevention of periodontitis, whereas metastat has entered phase II trials for Kaposi's sarcoma and brain tumors [70].

Merck and Co., has developed a Cathepsin K inhibitor which is currently in Phase 3 clinical trials by the name of Odanacatib[71]. Velcura Therapeutics, Inc. has also developed a highly selective cathepsin K inhibitor named VEL-0230, which is in on-going trials in humans and rats [72]. Folkman and colleagues have targeted intracellular cathepsin B in neovessels endothelial cells in order to increase the efficacy of angiogenesis inhibitors [73].

Therapeutic agents designed for activation at the tumour cell surface by cathepsin B have proved efficacious. Two groups have conjugated cathepsin B-cleavable linkers to pore-forming toxins from staphylococcal α-haemolysin [74] and a sea anemone [75]. A similar strategy has been used to develop prodrugs of doxorubicin [76].

CONCLUSION: Although there is no definitive treatment option available for cancer, different options are available. Targeting of these enzymes alone cannot help treat the cancer. However these treatment options can be used in conjunction with current treatment options to provide higher chances of cure and recovery. These enzymes show a promising target for cancer treatment and can be

used effectively. However further testing needs to be done to understand the underlying mechanism of the enzymes. Data and research need to be generated on the safety and efficacy of the drugs using this route. With greater efforts the future of cancer treatment can be brighter.

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