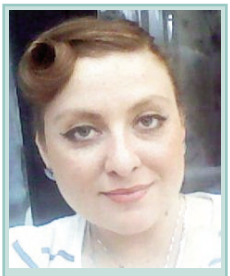
Protease Enzymes and Cancer Metastasis



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A major cause of patient mortality after breast cancer is the development of secondary, disseminated cancers, metastases. The contribution of extracellular proteolysis to the process of tumour invasion and metastasis has been recognised for more than 20 years [1]. Proteolysis is a process in which enzymes degrade proteins into peptides and amino acids by cleavage of peptide bonds. This results in the production of smaller disconnected substances - analogous to the reduction of a wall to simple rubble. This is of particular relevance when we consider that metastasis is the process of cancer cell movement around the body despite the presence of multiple protein based, physiological barriers [2,3].

There are a diverse range of proteases which are produced in eukaryotic cells. Amongst these the matrix metalloproteinases (MMPs) have been the subject of considerable interest. The MMPs are a related (and homologous) family of approximately twenty zinc containing proteolytic enzymes. MMPs were first identified using in vitro and in vivo models of cancer, during the 1970s. At this time they were shown to be involved in the degradation of the fibrous basement membranes and supportive extracellular matrix (ECM) protein components surrounding cancer cells [4,5]. Experiments identified MMP activation cascades and cell surface proteins were shown to be key initiators of MMP activity [6]. Evidence from a number of laboratories showed that MMPs play a key role in mediating cancer cell degradation of the ECM, enabling space to be created to allow cancer cell migration and movement. This knowledge led to millions of pounds and hours of investment into pre-clinical research and the development of a broad range of MMP inhibitors [7,8,9]. The general consensus was that if methods for the inhibition of pericellular proteolysis could be developed, these would have application in preventing an essential step in the metastatic cascade. There are many events which are required for successful development of metastases and cancer cell invasion is a key part of the process. Invasion requires the cancer cell, or group of cancer cells, to break through the basement membrane material from the bulk of the primary tumour [10,11].

In conjunction with reduced levels of adhesion molecules, such as cadherins that normally serve to keep cells in situ, cancer cells secrete MMPs through invasive protrusions called invadopodias. These reconstruct the ECM, allowing the cancer cells to forge a pathway through the ECM in the first step of cancer progression proper. Predicting if, or when, cancer cells will invade into the surrounding tissue is a pertinent problem of particular relevance to patients with in situ carcinoma such as that associated with the breast (DCIS or LCIS).

Once the cancer cell (or bolus of cancer cells) has separated from the primary tumour, it must gain access to either the lymphatics or the blood-stream; this is achieved through the secretion of enzymes such as MMP-9 which have been shown to degrade the endothelial cell membranes [12]. The cancer cell needs to travel undetected by immune cells, through the blood-stream until it arrests at a site of metastasis. Here it will attach to the endothelial cell wall, or gap in the endothelial cell wall, typically in a capillary in the organ of metastasis. Once this process has been achieved, again, secreted MMP-9 mediates cancer cell movement, this time out of the vessel and into the secondary site, by degradation of the surrounding tissues [13].

Clinical trials

Early studies supported the hypothesis that metastasis formation could be delayed by treatment of cancer cells with protease inhibitors [14]. Subsequent clinical trials, however, failed to demonstrate a significant reduction in metastasis formation [15].

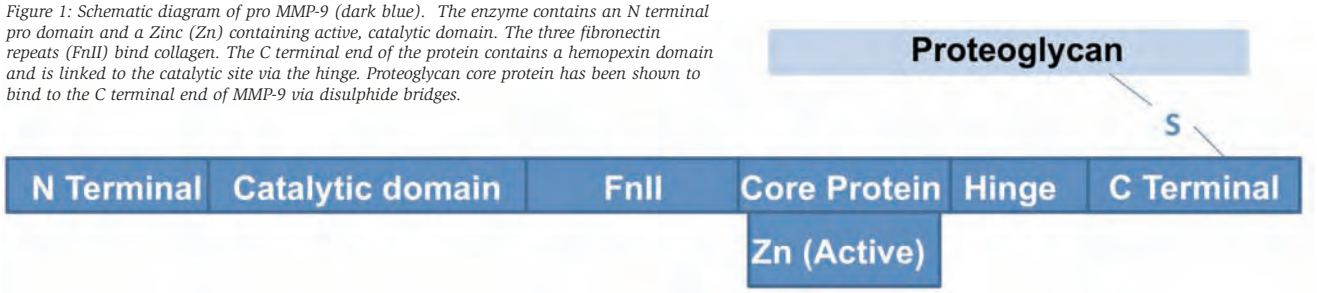
The first generation MMP inhibitors were designed to mimic natural MMP substrates. These include compounds with a peptide-like backbone and a hydroxamate moiety (for example Batimastat) which served to inhibit MMPs via hydrogen bonding and through interactions with zinc in the active site. The relatively poor solubility and bioavailability proved an obstacle to efficacy of these compounds and they were not continued beyond 1998. Second-generation compounds including non-peptide, hydroxamate substances were found to induce musculoskeletal pain and inflammation in long term use and this led to a discontinuation of these inhibitor compunds in clinical trials [16].

Given the lack of results in terms of the inhibitors prolonging survival, reducing time to progression, or reducing tumour burden, scientific attention turned away from protease biology and inhibition of matrix degradation as a means of preventing tumour metastasis [17]. Instead the focus was on other areas of scientific endeavour, including protease expression pattern profiling – which is being researched as a potential diagnostic and prognostic biomarker opportunity [18].

Glycosidase enzymes and metastasis

Glycobiology is the biology of complex carbohydrates attached to proteins or lipids. Such carbohydrates are associated with the majority of proteins (glycoproteins) in the human body, including those of the ECM. It has been demonstrated that the process of carbohydrate attachment to these proteins occurs either while (co-translationally) or after (post translationally) they are created before they are secreted from their founding cell [20]. During the last decade there have been substantial advancements in

Figure 1: Schematic diagram of pro MMP-9 (dark blue). The enzyme contains an N terminal pro domain and a Zinc (Zn) containing active, catalytic domain. The three fibronectin repeats (FnII) bind collagen. The C terminal end of the protein contains a hemopexin domain and is linked to the catalytic site via the hinge. Proteoglycan core protein has been shown to bind to the C terminal end of MMP-9 via disulphide bridges.



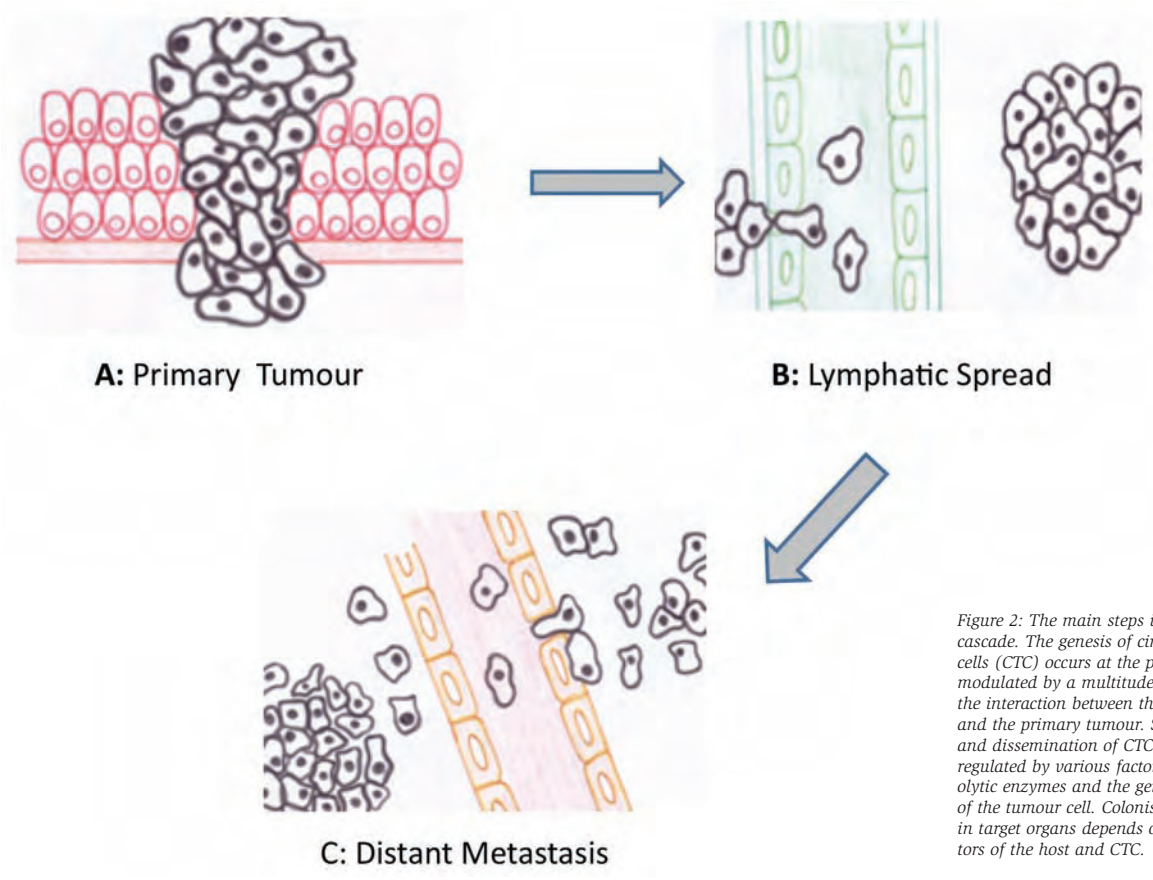


Figure 2: The main steps in the metastatic cascade. The genesis of circulating tumour cells (CTC) occurs at the primary site and is modulated by a multitude of factors including the interaction between the reactive stroma and the primary tumour. Shedding, survival and dissemination of CTC to distant organs is regulated by various factors including proteolytic enzymes and the genetic determinants of the tumour cell. Colonisation and growth in target organs depends on angiogenic factors of the host and CTC.

our understanding of the intracellular mechanisms responsible for protein glycosylation. Specifically we are able to identify different forms of glycosylation, including N-linked and O-linked types [21].

Cells also produce and secrete proteins with huge functional variability, some of which become components of the ECM. Proteins such as collagen, laminin and fibronectin are flanked by sugars which mask the protein backbone and arguably might affect the function of proteolytic enzymes. Sugar degrading glycosidase enzymes may first need to trim back the carbohydrate residues to make proteins available for proteolysis [19] - thereby mediating metastatic cancer cell movement and invasion.

Future cancer therapies

The possible role of secreted exoglycosidases has previously been overshadowed by research into intracellular endoglycosidases [22]. Research using an ovarian cancer cell line showed that secreted glycosidase (β-N-acteylglucosaminidase, β-NAG) [23] was implicated in the degradation of the ECM. In addition, simple sugar analogues, applied to the space surrounding the ovarian cancer cells, resulted in a reduction in ECM degradation. Recent research using β-NAG inhibitors systematically investigated the effect of enzyme function in an in vitro model of breast cancer invasion [24]. β-NAG was elevated in breast cancer cells with a metastatic phenotype and was linked to abnormalities in the secretory pathway, particularly the location of intracellular lysosomes, which correlated with increased enzyme secretion.

Combined protease and glycosidase inhibition reduced cancer cell migration through Matrigel™ matrix (a model of the ECM) [25,26].

The presence of carbohydrate polymers as well as glycoproteins within the ECM, coupled with evidence of glycosidase involvement in ECM degradation suggests that further research into simultaneous protease and glycosidase inhibition is a worthwhile endeavour. Improvements in our understanding of the role of enzymes in cancer cell migration through the ECM, may reignite the idea of preventing matrix degradation in order to reduce metastatic potential.

Conclusion

MMP inhibitors trialled in the 1980's were generally disappointing, failing to significantly inhibit metastasis. Subsequently the role of protease inhibition is beginning to be considered in conjunction with carbohydrate degrading glycosidase enzymes. The results so far are encouraging and reflect the complex physiology of the cancer cell – ECM milieu. When cells were treated with a cocktail of protease inhibitors along with the β-NAG inhibitor molecule, a reduction of up

to 70% cell migration through Matrigel™ was recorded [20]. This research represents a potential novel treatment pathway, highlighting the functional role of both glycosidases and proteases in cancer metastasis and the concept offers the tantalising possibility for future high efficacy anti-metastasis combination therapies. ■

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