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Sounding Board

Do Proteases Play a Role in Cancer Invasion and Metastasis?

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Abstract—*The main difference between a benign and malignant tumor is the ability of the malignant form to invade normal tissue and spread or metastasize to distant sites throughout the body. It is the ability to form metastasis which makes cancer such a difficult disease to treat. Evidence suggesting that proteolytic enzymes are involved in cancer spread is as follows: proteases are involved in normal destructive events and tissue remodelling, correlations exist between different protease activities and metastatic potential in model tumor systems, inhibitors and antibodies against proteases inhibit metastasis in model systems and the finding of highest levels of protease activity at the invading front in tumors. The most likely mechanism by which proteases could mediate metastasis is by catalyzing degradation of the extracellular matrix and basement membranes. It is concluded that if proteases could be proved to play a role in the spread of human cancers, inhibition of these enzymes could open up new therapeutic approaches for the control of malignancy.*

THE primary difference between malignant and benign tumors is the ability of the former to metastasize, i.e. spread to distant sites throughout the body and establish secondary growths or metastases. It is the production of metastasis which makes cancer such a difficult disease to treat and is ultimately responsible for the death of the patient. The reasons why cancer cells metastasize are currently unknown. It is known, however, that not all malignant cells within a tumor have the capacity to form distant colonies and some malignancies such as basal cell carcinomas of the skin rarely metastasize. Clearly therefore, the properties of cancer cells which enable them to form metastasis are different from those responsible for carcinogenesis.

Characteristics of cancer cells which may be important in invasion and metastasis include: increased mobility compared with normal cells, altered cell membrane structure, production of angiogenic molecules and increased synthesis of proteolytic enzymes. The purpose of this paper is to critically review the role played by proteases in cancer invasion and metastasis. Firstly, the main steps in cancer metastasis will be described.

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MAIN STEPS IN METASTASIS

The formation of metastasis is a multistep process. The main steps thought to be involved were recently summarized by Sloane and Honn [1] and are likely to include the following:

1. Invasion of the primary malignancy into adjacent normal tissue.
2. Release of tumor cells from the primary source.
3. Passage of malignant cells into bloodstream or lymphatic system.
4. Interaction of tumor cells in bloodstream with fibrin and platelets.
5. Dissemination of tumor cells throughout the body via the blood.
6. Extravasation of tumor cells through the blood vessel wall into a secondary organ.
7. Formation of a metastatic deposit.

Details of these steps are reviewed in Refs [2, 3].

PROTEASES IMPLICATED IN INVASION AND METASTASIS

The various proteases postulated to play a role in invasion and metastasis are listed in Table 1. Of these, most attention has focussed on the collagenases, plasminogen activator (PA) and cathepsin B.

The main properties of these 3 hydrolases are summarized in Table 2. It is important to remember that, for at least collagenase and PA, multiple forms or isoenzymes exist and that not all of the forms may be involved in cancer spread. For example, the urokinase-type PA (UK–PA), and not the tissue-type PA (t–PA), appears to be the isoenzyme of PA active in metastasis, see below.

EVIDENCE THAT PROTEASES ARE INVOLVED IN CANCER INVASION AND METASTASIS

1. *Proteases play a role in normal destructive events*

Proteases, such as PA, are involved in a number of normal physiological actions where tissue destruction and remodelling occurs. These events include mammary gland involution after lactation, prostate gland involution after castration, disruption of the ovarian follicle during ovulation and blastocyst implantation (for reviews see Refs [4, 5]). These events partly resemble the invasive growth of cancer, for example during both mammary gland involution and cancer invasion, basement membranes are destroyed. Thus, if proteases play a role in normal destructive processes they may also be involved in pathological destructive events such as cancer invasion.

2. *Correlation between protease activities and metastatic potential in model tumor systems*

In a number of different model tumor systems correlations have been found between metastatic potential and levels of different proteases. Much of this type of work has been done with mouse melanoma cell sublines known as B16–F1 and B16-F10. These melanoma sublines have very different metastatic potential following injection into mice, the B16-F10 cells form lung metastases with high frequency whereas the B16-F1 cells rarely form secondary colonies. In separate studies the levels of both PA (6), and cathepsin B [7] have been shown to correlate with metastatic potential in these sublines.

Table 1. Proteases thought to play a role in cancer invasion and metastasis

Plasminogen activator
Collagenase
Cathepsin B-like enzyme
Elastase
Trypsin-like enzyme
Thrombin
Proteoglycan-degrading enzyme
Heparin sulfate endoglycosidase

The relationship between cathepsin B and metastatic potential of these cells has been studied in relative detail. Cathepsin B activity was almost completely confined to viable tumor cells with little activity found in contaminating host cells [7]. In contrast to cathepsin B, other hydrolytic enzymes such as cathepsin D, β-*N*-acetylglucosaminidase β-glucuronidase and acid phosphatase showed no relationship with metastatic potential in these cells [7]. More recently, cathepsin B activity has been shown to correlate with lung colonization potential in 3 additional B16 sublines [1].

Correlations between protease activities and metastatic capacities have also been found in other animal tumors. These include collagenase in frog renal carcinomas [8] and mouse mammary tumors [9], PA in both rat mammary [10] and Lewis lung carcinomas [11] and cathepsin B in Lewis lung metastatic variants [12].

In a further attempt to seek a correlation between protease levels and metastatic ability, Liotta and colleagues [13] studied the levels of collagenase IV in a number of cell lines and hybrids derived from fusion of these cell lines. In both the parent and hybrid lines a correlation was found between the amount of collagenase IV secreted and metastatic capacity. All hybrids with an ability to metastasize expressed collagenase IV. On the contrary in the hybrids unable to metastasize, collagenase IV was suppressed. Thus, in these systems, the synthesis of

Table 2. Main properties of PA, cathepsin B and collagenases

Protease	Type	Main forms	Main substrate
PA	Serine	Tissue-type Urokinase-type	Plasminogen
Cathepsin B	Cysteine		Not known
Collagenase	Metallo	Form I	Stroma collagen
		Form II	Cartilage collagen
		Form III	Stroma collagen
		Form IV	Basement membrane collagen
		Form V	Placental collagen

collagenase IV appears to be genetically linked to the metastatic ability [13].

There are, however, a number of animal malignancies where no relationships have been found between levels of specific protease activity and metastatic ability. For example, McLaughlin *et al.* [14] found no correlation between cathepsin B activity and metastatic ability in variants of methylcholanthrene-induced sarcomas in mice. Nicolson *et al.* [15] could find no difference in PA activity between melanoma sublines of different metastatic capacity including the F10 and F1 cells mentioned above. These results of Nicolson *et al.* [15] could be due to the fact that insufficient cells were cultured [6]. In another study, Whur *et al.* could find no difference in PA levels in cultures from primary and metastatic Lewis lung carcinomas [16]. It should be noted in this study, however, that relatively large numbers of the tumor cells died soon after establishment of culture and the cultured cells were atypical of the parent tumors.

If proteases could be shown to play a role in cancer invasion and metastasis, measurement of their levels in human tumors might provide useful prognostic information. However, as with animal malignancies, data on the relationship between protease levels and either invasive or metastatic potential in human cancers are contradictory.

Abramson *et al.* [17] showed a relationship between collagenase secretion from head and neck tumors and survival of patients. In another study, Corasanti *et al.* [18] found a significant correlation between PA activity and local invasion and metastasis in colonic carcinomas. Other studies have shown significant relationships between high levels of fibrinolysis and local metastasis in both breast [19] and epidermoid lung cancers [20]. In contrast to these findings, no significant correlation was found between PA levels and local invasion in either lung [21] or breast cancer [22].

From the above it is clear that consistent relationships between levels of different proteases and local invasion or metastasis do not exist. Some possible reasons for the lack of correlation are as follows:

(*i*) *Failure to separate total enzyme activity into its individual forms.* It was mentioned above that proteases such as collagenase and PA have multiple enzyme forms. These different forms or isoenzymes may have different biological functions in both normal and neoplastic tissues. Furthermore, not all the forms may be involved in the spread of cancer. For example, we have shown that while total PA was not signficantly higher in malignant breast tumors compared with benign tumors, the UK form of PA was significantly higher in the carcinomas compared with the benign group [23]. On the other hand, the t–PA form of PA tended to be lower in the carcinomas than in the benign specimens. In another study it was shown that while antibodies against UK–PA inhibited metastasis, antibodies against t–PA had no such effect (see below).

(*ii*) *Variable contamination of tumors with white cells.* Solid tumors are composed not only of malignant cells but also of normal cells such as endothelial, fibroblasts and various types of white cells. The relative amounts of these groups of cells varies from tumor to tumor. White cells, such as macrophages and neutrophils, are well known to contain different proteases. In most biochemical investigations the contaminating white cells are not separated from the neoplastic cells. Thus, the enzyme activity measured is derived from all of the cells in the tumor and not just the malignant cells. Of course, proteases derived from white cells could also play a part in invasion and metastasis. However, more meaningful data might be available if the relative amounts of enzyme activities derived from the different cell types within a tumor were known.

In order to overcome some of these problems of mixed cell populations attempts have been made to culture malignant cells and monitor their *in vitro* production of proteases.

However, this approach is also not without criticism. Although cell culture of malignant cells eliminates interference from host cells, the cell lines eventually produced may show little similarity to the cells *in vivo*. Cell selection is well known to occur during culture and it is impossible to know how representative the growing cells are of the tumor as a whole. In addition, the cellular environment in culture is different from that *in vivo*. Appropriate hormones and growth factors may be absent *in vitro*. Also, the interaction between different cell types which may be important in controlling protease production [5] is missing. Another disadvantage of culturing cells is that protease production does not occur at a steady state during these conditions [24]. Liu *et al.* [25] have found that production of PA by cell lines of neoplastic origin was inversely proportional to cell density. When taking all these factors into consideration it is not surprising that discrepancies exist between levels of protease produced by cell cultures and metastasis *in vivo*.

(*iii*) *Different levels of endogenous inhibitors.* Naturally occurring inhibitors appear to exist for all proteases. As with white cell infiltration, the amount of endogenous inhibitor may vary in different tumors. Since most protease assays carried out on tumors have relied on activity measurement rather than mass, they would be susceptible to the effect of endogenous inhibitors. Therefore, inaccurate

results on enzyme activity levels could be obtained. In one study on tumors, however, where t–PA was measured both by activity and immunoassay, a reasonable correlation was found between the 2 types of assay ($r_s = 0.650$, $P < 0.001$) [23].

(*iv*) *Failure to assay appropriate protease.* Different proteolytic enzymes may be involved in metastasis in different malignancies. As shown in Table 1, a variety of different proteases are claimed to catalyze the formation of metastasis. Most studies have concentrated on either collagenase, PA or cathepsin B. However, other enzymes apart from these may be involved. In Walker carcinoma cell lines the protease most likely to be involved in metastasis was neither collagenase or PA but a trypsin-like enzyme [26]. In Dunning Rat prostatic carcinomas the proteases that correlated best with metastatic ability was elastase and a chymotrypsin-like enzyme [27]. Collagenase, PA, cathepsin B and trypsin-like protease showed no correlation with metastasis in this model system.

(*v*) *Heterogeneity of tumors.* Studies from some tumors have shown that less than 1% of cells released from the primary malignancy eventually produce metastasis [28]. If this is the true situation for all tumors, it is not surprising that measurement of the total enzyme activity within a malignancy does not always correlate with invasion or metastasis.

(*vi*) *Protease production may not be a rate-limiting step.* The formation of metastasis involves many steps, each possibly requiring different biological activities. Protease action could be involved in some of these steps but unless their function is a rate-limiting one in the metastatic cascade, a correlation between protease levels and metastatic potential should not be expected [29].

(*vii*) *Tumors secreting high levels of PA.* Evers *et al.* [22] have suggested that if malignant tumors synthesized high levels of a protease and rapidly secreted it, little activity would be found in tumor cell-free extracts. This could happen for neoplasms occurring close to serous membranes or epithelial cavities. With these tumors, proteases secreted could be washed away with normal body fluids [22]. Measurement of protease levels in cell-free extracts of tumors would clearly give misleading results in these situations.

3. *Prevention of metastasis by protease inhibitors*

A number of studies have shown that the administration of protease inhibitors has prevented metastasis in experimental animals. One study showed that the serine protease inhibitors tranexamic acid and epsilon-amino caproic acid inhibited tumor growth and metastasis of breast carcinomas in syngeneic mice [30]. In another report, administration of UK–PA was found to enhance spontaneous pulmonary metastasis but in the presence of tranexamic acid the enhancing effect of UK–PA was abolished [31]. Other inhibitors such as leupeptin and aprotonin have also been reported to inhibit metastasis in experimental systems (for reviews, see Refs [32, 33]).

Preliminary data suggests that protease inhibitors may also have a beneficial therapeutic role in human carcinomas. Tranexamic acid has been reported to be of value in the treatment of advanced ovarian cancer [34].

Although these results with protease inhibitors are consistent with the hypothesis that protease plays a role in tumor spread, it has to be pointed out that the mode of action of these compounds *in vivo* is unclear. These chemicals may have pharmacological action in addition to inhibiting proteolytic enzymes. It should also be said that reports have appeared where no inhibitory effects of proteases inhibitors on metastasis was observed and even reports where these compounds were found to enhance metastasis [32, 33].

In contrast to protease inhibitors which usually lack specificity, antibodies against specific proteases are potentially more valuable tools in investigating the role of these enzymes in cancer spread. At this stage only one report has appeared describing an inhibitory effect of a protease antibody on metastasis. In 1983, Ossowski and Reich [35] showed that antibodies against UK–PA, but not against t–PA, decreased the formation of lung metastasis following transplantation of the human HEp-3 tumor into chick embryos. Control experiments showed that the anti-metastatic effect of the UK antibody was due to inhibition of UK–PA activity and not due to a generalized immunological reaction. This study is the best evidence yet that a proteolytic enzyme plays a role in metastasis, in at least some systems. It also shows the importance of concentrating on the individual forms of enzymes. Whether the formation of metastasis from slowly growing human tumors is similar to that in the chick embryo system remains to be shown.

4. *Presence of proteases at invasive fronts in tumors*

If proteases are involved in cancer invasion they should be found at highest levels in the invasive front, i.e. where degradation of normal tissue is occurring. This is indeed the area where proteases are primarily located. Skriver *et al.* [36] have shown that immunoreactive UK–PA was mostly concentrated in areas of invasive growth in Lewis lung tumors. UK–PA was mostly found in extracellular sites or attached to the cell membrane. Lesser

amounts were found in the cytoplasm of tumor cells. In parts of the tumor where there was no invasive growth and degradation of normal tissue, UK–PA was not detected.

Cathepsin B-like activity has also been detected at the invasive front, at least in rabbit V2 carcinomas [37]. In this tumor the enzyme was detected only in host fibroblasts and leukocytes and not in carcinoma cells. Cathepsin B-like enzyme was also present in the extracellular matrix surrounding clusters of tumor cells. The production of the cathepsin B-like enzyme by the host cells may have been stimulated by factors originating within the tumor cells [37].

As with PA and cathepsin B-like activity, collagenase has also been localized primarily at tumor invasive fronts [38]. The cell types synthesizing the different forms of collagenase remain to be established.

The finding of proteases at the invasive fronts in tumors, while suggestive, does not prove that they are active in mediating destruction of the extracellular matrix. The next step is to show that the proteases found at the front are biologically active.

MECHANISMS BY WHICH PROTEASES COULD PLAY A ROLE IN INVASION AND METASTASIS

Although the precise role played by proteolytic enzymes in cancer spread is still unknown, a number of possible mechanisms by which they could mediate metastasis have been suggested. One of the most popular theories is that proteases released from either tumor or host cells degrade natural barriers allowing cancer cells to penetrate through the extracellular matrix and basement membrane. The extracellular matrix is composed mostly of collagen, proteoglycans, glycoproteins and elastin. Basement membranes in addition contain a form of collagen which is structurally and genetically different from stroma collagen. This unique type of collagen is referred to as Type IV collagen.

Collagenase I and III can catalyze the hydrolysis of stroma collagen while basement membrane collagen can be broken down in the presence of collagenase IV [39]. Like a number of proteolytic enzymes, collagenase is initially synthesized as an inactive form, at least in some cells. This zymogen form can, however, be activated in presence of cathepsin B or the PA/plasmin system [40, 41]. Both PA and cathepsin B have also been found to catalyze the degradation of non-collagenous molecules of the extracellular matrix and basement membrane [42, 43]. All these reactions are based on *in vitro* studies. Whether a similar series of reactions occurs *in vivo* remains to be shown.

Proteases, however, can catalyze additional reactions which may be important in the spread of cancer. These include their ability to modify cell membranes [44], stimulate cellular migration [45], act as procoagulants [46] digest fibrin surrounding tumors [47], stimulate tumor cell–platelet aggregation [48], reduce the adhesiveness of malignant cells [49], or mediate the action of angiogenic factors [50]. Finally, at least one protease, i.e. PA, has been shown to have amino acid sequence homology with the growth factor EGF [51]. Growth factors, such as EGF, are thought to act by stimulating cellular proliferation and excessive action may be partly responsible for carcinogenesis. Like growth factors, proteases has also been found to enhance cell division [52]. It is possible that proteases, such as PA, may also increase mitogenesis and this indirectly might lead to an increased probability of invasion and metastasis.

CONCLUSION

Definite evidence that proteases are involved in the metastasis of clinically important tumors is not yet available. However, from the evidence presented above, it would be surprising if proteases were not involved in some steps of the metastatic cascade. To prove the 'protease' theory of metastasis, further experiments have still to be done. It is essential when assaying proteases in tumor tissue to measure individual forms of enzymes, such as PA and collagenase. Assays should be based on both activity and mass measurements in order to eliminate possible interference due to endogenous inhibitors in activity assays. It is also important to identify the cell type, i.e. tumor or host cell, which contains the enzyme and whether these cells are found at the invading front or are more centrally located.

Experiments relating levels of proteases to metastatic potential of tumors will only provide indirect evidence that these hydrolases play a role in metastasis. To obtain more direct evidence for such a function, suitable model systems for studying the processes of metastasis will have to be developed. With such a system, the effects of specific protease inhibitors and protease antibodies on metastasis, could be investigated. The work of Ossowski and Reich [35] showing inhibition of metastasis in the chick embryo using antibodies to UK–PA is promising. However, this type of experiment needs to be repeated using different model systems and antibodies against different proteases.

Another approach to see if proteases take part in the spread of cancer is to study the genes responsible for this process. Recently, the metastatic phenotype was transferred to cells using DNA from a human metastatic tumor [53]. The metastatic phenotype was associated with a discrete DNA sequence [53]. The identity of the proteins coded by these genes is eagerly awaited.

Finally, if it can be proved that proteases are

responsible for metastasis, new approaches to the management of cancer patients could be opened up. Firstly, measurement of protease levels in primary tumors might be able to predict the metastatic potential of the tumor. Secondly, new ways of preventing metastasis, e.g. using protease inhibitor or antibodies, could become available. Clearly therefore, proving or eliminating the 'protease' hypothesis of cancer invasion and metastasis is urgently required.

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