Involvement of MMPs in Tumour Cell Invasion and Metastasis
- Prospects for Synthetic Inhibitor Therapy

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Tumour cell invasion and metastatic spread indicates a poor prognosis in cancer and the localised hydrolysis of basement membranes by matrix metalloproteinases (MMPs) is thought to be one of the steps that occur during this process. The MMPs are a family of eleven structurally related endopeptidases and at physiological pH these multi-domain enzymes can degrade most of the important structural components of the extracellular matrix. It is therefore not surprising that the activity of MMP family members such as collagenase, stromelysin and gelatinase, in healthy tissues is tightly regulated. In addition to MMP gene transcription being controlled by inflammatory mediators such as IL.1 and TNF, these enzymes are regulated extracellularly by being secreted from cells as latent proenzymes, that are then processed to active species through the autoproteolytic removal of a propeptide. Even when activated the MMPs are susceptible to inhibition by tight non-covalently binding natural inhibitors called TIMPs (tissue inhibitors of metalloproteinases). In addition to binding to the active forms of the enzymes, TIMP.2 is found bound to the C-terminal domain of gelatinase-A and TIMP.1 is found associated with gelatinase-B. In this capacity TIMP.2 is able to further regulate gelatinase-A activity by preventing enzyme activation.

The initiation of MMP activation requires propeptide perturbation and for most of the enzymes in vivo this probably occurs by the propeptide being clipped by proteases such as plasmin. Gelatinase-A is unlike the other MMPs in that its activation cannot be initiated by plasmin. A clue to its in vivo activation mechanism comes from the finding that it can be activated by cells that have been stimulated by effectors such as concanavalin A, cytochalasin D, phorbolesters, TGFβ, or by cells that have been grown on collagen. Cell mediated activation involves an interaction between the gelatinase-A C-terminal domain and the cell surface, and evidence for the existence of gelatinase-A binding sites on a human breast tumour line has been reported. Interestingly a newly isolated, membrane associated MMP (MT-MMP) has been shown to confer gelatinase-A activating properties on cells, and may in fact play a role, together with TIMP.2, in binding gelatinase-A to the cell surface.

The existence of gelatinase-A binding sites on tumour cells might explain recent reports that show that although MMPs can be immunolocalised to tumour cells, the corresponding mRNA was found to be made by surrounding stromal cells. In these cases it is possible that malignant cells are responsible for switching on the stromal synthesis of proteolytic enzymes, perhaps via agents such as tumour derived collagenase stimulatory factor (TCSF). Since active gelatinase-A can be localised to the invadopodia of malignant cells, these observations suggest that the activation of enzyme sequestered on the cell surface, may permit the focal hydrolysis of extracellular matrix at sites of cell invasion. Such proteolytic activity is likely to have profound local effects on the resident cell population, either through modulation of cell attachment sites, or through the release of matrix bound growth factors and cytokines.

In order to explore these ideas in a biological setting we transfected cell lines with representative members of each of the main classes of MMP and compared their ability to invade a reconstituted basement membrane in vitro and to give rise to lung colonies in vivo. Only gelatinase-A was found to convey an invasive and metastatic phenotype on the transfected cells, and this was dependent on both the catalytic properties of the enzyme, and on properties determined by its non-catalytic C-terminal domain. Knowing the specificity of the autoproteolytic cleavages that occur during MMP activation we were able to design highly potent (K's <10⁻¹⁰M) and selective gelatinase inhibitors (typically 10000-fold over collagenase). These compounds inhibit the gelatinases found in the cytosols of human breast tumours, and when long acting orally bioavailable analogues are administered to mice injected with the gelatinase-A transfecteds, or a syngeneic colorectal tumour cell line, they reverse the metastatic phenotype. Our further finding that they can delay both angiogenesis and the subcutaneous growth of human xenografts in nude mice points to their potential for treating cancer in man.