



# Proteases and Tumour Microenvironment

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Extracellular proteases are key players in the regulation of the cellular environment, acting as major effectors of both cell-cell and cell-extracellular matrix (ECM) interactions, essentially as 'signalling scissors'.

Epithelial tumours evolve in a multi-step manner, involving both inflammatory and mesenchymal cells. Although intrinsic factors drive malignant progression, the microenvironment of neoplastic cells is a major feature of tumourigenesis. Our premise – that proteases are integral to the regulation of extrinsic effectors – is the basis for our work and for our plans to dissect events at the cellular and molecular level, as well as proceeding to complex tumour models addressing tumour-stromal interactions. Based on our findings we are developing and evaluating novel approaches to the regulation of proteases in tumour systems.

### Understanding the roles of proteases in tumour biology

The successful development of tumours is determined by the tissue environment in which the 'host' participates in the induction, selection and expansion of the neoplastic cells. Malignant tumour cells recruit vasculature and stroma through the production of stimulatory factors. The locally activated host environment (both cells and extracellular matrix) in turn modifies the proliferative and invasive behaviour of tumour cells. The nature and function of the activating factors involved and the subsequent effectors are important areas of basic biological research in the field of cancer studies. Extracellular proteases are major effectors of both cell-cell and cell-extracellular matrix (ECM) interactions,

modifying ECM integrity, growth factor availability and the function of cell surface signalling systems, with consequent effects on cellular differentiation, proliferation and apoptosis. Early data from screens of cancer tissues have shown that different patterns of proteinase elevation occur and that the relationship of expression to tumour progression and the contribution of individual cell types – tumour cells, fibroblasts, endothelial cells and inflammatory cells – requires detailed dissection. A major aim of the drive to understand protease biology within a specific tumour environment relates to the need to assess potential targets within the interface between tumour cells and the 'host' cells that may be appropriate for therapeutic intervention. It is anticipated that the understanding and the manipulation of proteinase function will give clear leads as to the critical stages in the breakdown of the normal tissue-cell 'society' that occurs in neoplasia.

Within this remit our research is focussed on cell surface associated forms of the zinc-dependent proteases, notably the aminopeptidase N (CD13), the membrane type matrix metalloproteinase-1 (MMP14) and members of the disintegrin-type metalloproteinases (ADAMs), as well as the tissue inhibitors of metalloproteinases, TIMPs. We aim to elucidate how these metalloproteases and inhibitors function in the regulation of extrinsic effectors at the cellular and molecular level, as well as proceeding to complex tumour models addressing tumour-stromal interactions. The fundamental data accrued will drive the development of novel reagents for disease therapy and diagnosis.

### Membrane associated metalloproteases

The aminopeptidase CD13 is a homodimeric 140-150 kDa type II transmembrane metallo-ectopeptidase that

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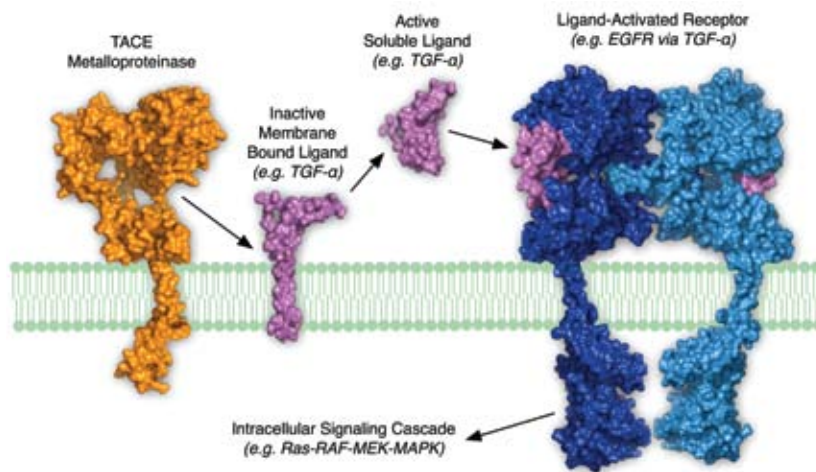


Figure 1. ADAM17, a TNF $\alpha$  converting enzyme, is a type I membrane bound metalloprotease which acts as the primary generator of soluble EGFR ligands such as TGF $\alpha$ , a process known as 'shedding'. ADAM17 activity is regulated by cell receptors such as G protein coupled receptors, as well as other extracellular proteins. It is therefore a critical regulator of both autocrine and paracrine signalling within the tumour environment and the elucidation of its regulation and significance in cancer is a major goal of our work.

preferentially removes neutral or basic amino acids from the unblocked N-termini of bioactive peptides or proteins. It also participates in cell migration and invasion of extracellular matrix and is important in both angiogenesis and tumour progression. We are studying its role as a mediator of membrane remodelling in both tumour cells and endothelial cells and some aspects of its regulation, notably in relation to cellular trafficking. Future work will focus on the mechanisms of CD13 action at the cell surface.

The membrane type I matrix metalloproteinase MT1 MMP plays a major role in tumorigenesis, including tumour cell migration, aspects of stromal cell function and angiogenesis. As a potentially key target for therapeutic approaches to cancer we are addressing its involvement in intracellular signalling with a focus on the role of its different domains in important interactions. We have elucidated novel roles for the MT1 MMP cytoplasmic domain in its regulation by cellular trafficking and have identified several intracellular and extracellular interaction partners. We are evaluating their significance and roles in (among others) VEGF receptor regulation, collagen degradation and cell migration. Future studies will look at the effects of MT1 MMP on gene transcription. The characterisation of scFv antibodies to MT MMPs that we have isolated is being used to address the question of the importance of exosite interactions in the proteolytic capacity of MT1 MMP. We are also investigating the use of the scFv antibodies as targeting or imaging agents for both tumour and endothelial cells and this will continue to be a focus of our work.

The disintegrin metalloproteinases are also regulators of cellular signalling and we are studying ADAM10 and 17 in

this respect. Projects on the role of different ADAM domains in the proteolytic 'shedding' of cell surface proteins are in progress. We are particularly interested in the generation of soluble EGF receptor ligands (Figure 1) which are key drivers of a number of different tumour types. The significance of ADAM activity is being investigated using siRNA techniques and novel antibody tools that we have recently developed. ADAM regulation by G protein coupled receptors and redox mechanisms will be investigated over the course of the year.

### TIMPs

Protein engineering of members of the tissue inhibitor of metalloproteinase (TIMP) family has given us insights into their enzyme target specificity and how to modify them for the development of potential therapeutics. We are able to engineer forms of these inhibitors that distinguish to some extent between the MMPs and ADAM proteinases, and between individual ADAMs. Furthermore, the motifs determining the extracellular matrix binding capabilities of TIMP-3 have been identified and the transfer of this binding capacity to the soluble TIMPs demonstrated, giving us the capacity to generate both cell/matrix bound and soluble inhibitors. With this toolbox of TIMP mutants their efficacy in cell models of proteolysis is being evaluated. With our collaborators we have prepared adenoviral and lentiviral delivery systems for studies in cell and animal models of cancer. Alongside siRNA techniques the TIMPs are good tools to assess the role of individual proteinases in specific proteolytic events and we will continue to use them in cancer models *in vitro* and *in vivo*.

### 3D *in vitro* models

In order to carry out molecular studies and inhibitor screens on the complexity of cells within tumour tissue we have set up several more complex 3D models (tumour cells, endothelial cells and fibroblasts) for the evaluation of metalloproteinase function and eventual therapeutic abrogation. Such models are being used to study the role of fibroblasts in angiogenesis and in the development of chemoresistance by small cell lung cancer, and the mechanisms behind fibrosarcoma cell migration through collagen matrices.

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