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Importance of Matrix Metalloproteinases (MMP) in cancer Progression with production of Human Monoclonal antibodies: A short review

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Abstract
Cancer is one of the leading causes of disease and mortality worldwide. As a result, the past two decades of biomedical research have yielded an enormous amount of information on the molecular events that take place during carcinogenesis and the signaling pathways participating in cancer progression. Matrix metalloproteinases (MMPs) consist of a multigene family of zinc-dependent extracellular matrix (ECM) remodeling endopeptidases implicated in pathological processes, such as carcinogenesis. In this regard, their activity plays a pivotal role in tumor growth and the multistep processes of invasion and metastasis, including proteolytic degradation of ECM, alteration of the cell–cell and cell–ECM interactions, migration and angiogenesis. MMPs are therefore amenable to therapeutic intervention by synthetic and natural inhibitors, providing perspectives for future studies.

Keywords: matrix metalloproteinases (MMP), human monoclonal antibodies

Introduction
The ability of cancer cells to invade other tissues and spread to distant organs is an often-fatal characteristic of malignant tumours. Proteolytic enzymes play a fundamental role in cancer progression. Providing an access for tumour cells to the vascular and lymphatic systems, which support tumour growth and constitute an escape route for further dissemination [1]. MMPs have been considered as potential diagnostic and prognostic biomarkers in many types and stages of cancer. Studies conducted over more than 40 years have revealed mounting evidence supporting that extracellular matrix remodeling proteinases, such as matrix metalloproteinases (MMPs), are the principal mediators of the alterations observed in the microenvironment during cancer progression.

Physiological functions of MMPs
Embryonic growth and tissue morphogenesis are fundamental events that require disruption of ECM barriers to allow cell migration and matrix microenvironment remodelling. The ability of MMPs to degrade structural components of ECM and basement membranes has supported their direct implication in these processes. MMPs are implicated in a variety of physiological processes, including wound healing, uterine involution and organogenesis, as well as in pathological conditions, such as inflammatory, vascular and auto-immune disorders, and carcinogenesis [2].

Roles of MMPs in cancer progression
Pivotal roles of MMPs in cancer progression. Cancer progression involves different stages, including tumor growth and the multistep processes of invasion, metastasis and angiogenesis, all of which can be modulated by MMPs. The expression of MMPs in the tumor microenvironment depends not only on the cancer cells, but also on the neighboring stromal cells. MMPs exert their proteolytic activity and degrade the physical barriers, facilitating angiogenesis, tumor cells invasion and metastasis. Tumor growth and angiogenesis also depend on the increased availability of signaling molecules, such as growth factors and cytokines, by MMPs making these factors more accessible to the cancer cells and the tumor microenvironment. This occurs by liberating them from the ECM (IGF, bFGF and VEGF) or by shedding them by from the cell surface (EGF, TGF-a, HB-EGF). Angiogenesis is also tightly modulated by the release of negative regulators of angiogenesis, such as angiostatin, tumstatin, endostatin and endorepellin. MMPs also modulate the cell–cell and cell–ECM interactions by processing E-cadherin and integrins, respectively, affecting both cell phenotype (EMT) and increasing cell migration [3].

Human monoclonal antibodies
Since the development of hybridoma technology over three decades ago, numerous monoclonal antibodies have been produced and the use of monoclonal antibodies has become one of the major breakthroughs in medicine. Significant progress has been made on new technologies for generating human monoclonal antibodies. The first success in generating human mAbs (hmAbs) with predefined specificity was conducted through the fusion of human spleen cells from patients with human myelomas. Since then, several major methods have been established to generate hmAbs, including 1) immortalization of antigen-specific human B cells; 2) acquisition of antigen-specific human B cells via phage display technology; 3) the production of hmAbs from transgenic mice; and 4) single human B cell cloning techniques to directly clone and express immunoglobulin (Ig) genes *in vitro* from antigen-specific B cells. This review serves as an introduction to the immortalization of antigen-specific human B cell and hybridoma technologies, phage display platform, the use of transgenic mice, and the generation of monoclonal antibodies from single B cells. A

monoclonal antibody (MAb) is derived from a single clone of cells and recognizes a unique antigenic determinant. Since 1975, when Keller and Milstein developed hybridoma technology, technological strides towards the production of antibodies have been made. The first success in generating human mAbs (hmAbs) with predefined specificity was conducted in 1980 through the fusion of human spleen cells from patients with human myelomas. Since then, several major methods have been established to generate hmAbs, including 1) immortalization of antigen-specific human B cells; 2) acquisition of antigen-specific human B cells via phage display technology; 3) the production of hmAbs from transgenic mice; and4) single human B cell cloning techniques to directly clone and express immunoglobulin (Ig) genes *in vitro* from antigen-specific B cells [4].

Immortalization of antigen-specific human B cell and hybridoma technology

Hybridoma technology has contributed to virtually all areas of biology and medicine and has been greatly refined since its introduction in 1975. In the early days, approaches to produce hmAb included the hybridoma technique, based on the fusion of antibody-producing human B lymphocytes with either mouse or human myeloma or lymphoblastoid cells; or Each method has its advantages and drawbacks. Antibodysecreting hybridomas are derived from a fusion of myeloma cells that can grow indefinitely and an immune B lymphoblast that expresses a specific antibody gene. Attempts to use the hybridoma technology for generating hmAbs have been hampered by the lack of a suitable human myeloma cell line. The best results were obtained using heteromyelomas (mouse × human hybrid myelomas) as fusion partners [5].

Phage display

Phage display technology, as one type of display platform, was first developed in 1985 and has been used to produce large numbers of peptides and proteins on the bacteriophage. It is the next method being successfully used to select antigen-specific variable region genes and to express functional antibody fragments with unique specificity. To isolate human antibodies, the library of diverse human immunoglobulin-heavy chain variable (VH) gene and lightchain variable (VL) gene segments are prepared by reversetranscription of mRNA from B cells and PCR amplification. The gene encoding single chain variable fragment (scFv) can be created by randomly combining VH and VL gene segments using PCR. The large antibody repertoires can be generated using the process of combinatorial infection and *in vivo* recombination, to display scFv on the surface of the phage [6, 7, 8, 9].

Transgenic mice

The transgenic mouse platform is another technology available to generate hmAbs. The engineered transchromosomal mice comprised of disruption of endogenous mouse Ig-heavy chain and Igκ-light chain loci together with the introduction of transgenes encoding human Ig-heavy chain and Igκ-light chain genes. Over the past few decades, progress has been made to express additional V gene segments by transgenic mice and to expand the potential repertoire of the recovered antibodies. Transgenic mice producing hmAbs with various heavy-chain isotypes have also been generated to tailor effector functions. Although immune responses in transgenic mice are sometimes less robust than those observed in wild type mouse strains that are used to generate mouse mAbs, the expression of human Ig in transgenic mice prevents human anti-mouse antibody responses and maintains the advantages of mouse hybridoma technology for the production of antibodies for potential clinical uses [10,11].

Generation of mAbs from single human B cells by Ig gene cloning and expression *in vitro*

A new platform to generate mAbs from single human B cells by single cell RT-PCR and expression vector cloning has been developed. This strategy is based on the combination of immunoglobulin (Ig) gene repertoire analysis and Ig reactivity profiling at the the single cell level. The light-chain and heavy-chain genes coding for variable fragments of antibody in each cell are separately amplified by RT-PCR and then combined with the sequences by an overlapping PCR technique. For each cell, Ig heavy- and corresponding Ig light-chain gene transcripts are amplified by nested RT-PCR and cloned into mammalian expression vectors to produce hmAb with defined specificity *in vitro* Recently, novel linear Ig heavy- and light-chain gene expression cassettes to express Ig V(H) and V(L) genes isolated from single B cells as IgG1 antibody without a cloning step have been developed. The cassettes contain all essential elements for transcriptional and translational regulation, including CMV promoter, Ig leader sequences, constant region of IgG1 heavy- or Ig light-chain, poly(A) tail, and substitutable V(H) or V(L) genes. These Ig gene expression cassettes constitute a highly efficient strategy for rapid expression of Ig genes for high-throughput screening and analysis without cloning [12].

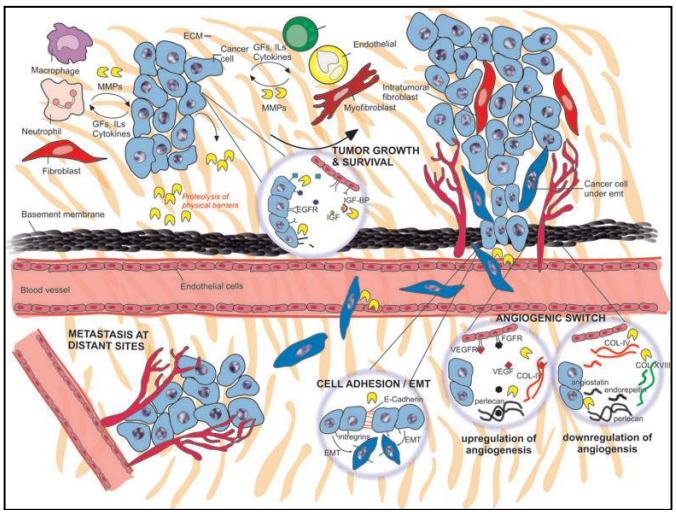

Fig 1: MMPs as potential targets in malignancy [2].

Conclusions and perspectives

The overproduction of MMPs in cancer has long been correlated with tumour progression and metastasis. Therefore, it is not surprising that over the last years MMPs have been the focus of multiple anticancer trials. This has prompted the development of a variety of strategies aimed to

block the proteolytic activities of these enzymes. The recent recognition of the complex roles that these enzymes play during physiological and pathological conditions may explain the lack of success of the first generation of MMPIs. Accordingly, the increased knowledge on this proteolytic system may lead to the development of new strategies of MMP inhibition, based on targeting any of the three major levels of endogenous regulation of these enzymes: transcription, activation and inhibition. The advent of MAb technologies, human monoclonal antibodies have been widely used for development of therapeutics and diagnostics and have been used in research to dissect various humoral immune responses in humans. As a result of their natural roles and no inherent toxicity *in vivo*, human monoclonal antibodies have been considered as natural drugs and more than 20 therapeutic monoclonal antibodies, including humanized mouse mAb with human complementarity-determining regions, have been approved for clinical use in the past few decades. There are many other monoclonal antibodies at different stages of preclinical and clinical development/pipelines of pharmaceutical companies and development programs. Therefore, human MAb technologies are not only extremely useful strategic research tools but are also of enormous values for future health economics.

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