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METALLOPROTEINASE BINDING PROTEINS:

Dyax Corp. WO 2009/097397 A2

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Abstract

Matrix metalloproteinases (MMPs) are a group of metalloendopeptidases whose major role is in extracellular matrix (ECM) catabolism under physiological and pathological conditions. ECM catabolism is often associated with different diseases, and accumulated studies support the causal relationship between MMPs and different diseases especially with cancer and arthritis. Therefore, MMPs have been considered as therapeutic target molecules. However, multiple failures of MMP inhibitor drugs in clinical trials from late 1990s to early 2000s resulted in the consideration that wide inhibitory spectrum inhibitors of metalloproteinases may not be desirable for treatment of diseases. On the other hand developing low molecular weight selective inhibitor(s) targeting the active site is not a very easy task. One way to overcome this problem is to develop biologic inhibitors, namely antibodies. In this patent application, Dyax Corp reported that a binding protein (such as an antibody) against metalloproteinases, especially membrane-type 1 MMP (MMP-14) can be used as a therapeutic drug against different diseases including cancer. They succeeded in generating a selective MMP-14 inhibitory antibody with a K_i of 0.6 nM, which showed significant suppression of different tumour growth in mice. Further development of such an antibody as a drug may fulfil an unmet area of disease treatment targeting uncontrolled cell invasion and tissue destruction.

Keywords

Matrix metalloproteinase; MMP; MT1-MMP; antibody; Phage display

1. Introduction

1.1. Matrix metalloproteinase and their inhibitor drugs

In humans, there are 23 MMPs1-3. MMPs are a family of structurally related enzymes sharing common domains of a signal peptide, a prodomain, a catalytic domain, a hinge (linker-1), a hemopexin domain1-3. There are some exceptions for example MMP-7 and MMP-26 which do not possess a hemopexin domain making them the smallest MMPs in the family. MMP-23 has a transmembrane domain inserted in the position of a signal peptide, and an additional cysteine array and immunoglobulin-like domains are present instead of a hinge and a hemopexin domain. MMP-2 and MMP-9 have an insertion of three repeats of the fibronectin type II domain, making them collagen binding proteinases1-3. There is also sub group of six membrane-type MMPs (MT-MMPs) which have an additional membrane anchoring domain at their C-terminus, of which 4 are transmembrane-type and 2 are glycosylphosphatidylinositol (GPI)-anchored enzymes. Many of these enzymes cleaves

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ECM macromolecules, but some of them, including MMP-23, MMP-11, MT4-MMP, do not cleave ECM molecules as effectively as the others¹⁻³.

Many MMP genes are upregulated in different diseases and given their biochemical properties (e.g. ECM degradation), MMPs have been considered as target molecules for the therapy of diseases in particular for cancer and arthritis^{4, 5}. Many pharmaceutical companies thus developed MMP inhibitor drugs^{1, 4, 5}. Pre-clinical animal studies indicated that these inhibitors showed significant inhibition of tumor progression if co-administered with cytotoxic drugs, and many of us investigating MMPs were astonished to see such efficacy. However, these MMP inhibitor-drugs were unexpectedly unsuccessful in clinical trials showing no clinical efficacy^{4, 5}. Retrospectively we now think that these failures may have been caused by at least two problems^{1, 4, 5}. The first problem is a design of the clinical trials. Given the function of MMPs in cancer progression, they are not expected to show significant efficacy in late stage cancer patients. The second problem relates to the specificity of the inhibitors. All the inhibitors developed were active site-orientated small molecule inhibitors containing a zinc-chelating moiety such as hydroxamic acid. Although some of inhibitors are said to be relatively more specific towards certain MMPs than others, they generally have quite broad target specificity. There are 23 MMPs in humans, and the structural topology of their catalytic sites are also similar to other metalloproteinases including the 12 ADAM (a disintegrin and metalloproteinase) proteinases, 20 ADAMTS (ADAM with thrombospondin motif) proteinases, and 10 astacin family proteinases⁶. Not all of these metalloproteinases are biochemically characterized and many of them have not been recombinantly expressed for in vitro characterization. In some cases, there is currently no assay available to test their activity. Therefore although some inhibitors have a preference for some MMPs over others, none of the drugs have been tested against all of the metalloproteinases, and due to their inhibitory manner it was expected that these inhibitors would broadly inhibit many, if not all metalloproteinases⁷. Recently it has been getting clearer that there are not only “bad” MMPs which promote disease progression, but also “good” MMPs which enhances self defence mechanisms to prevent disease progression. Also many metalloproteinases are thought to play a role in physiological processes⁷. Therefore, administration of broad specificity MMP inhibitor drugs would upset cellular physiology, which may be the cause of the unexpected side effects of these drugs such as muscular pain. Taken together, identifying target enzyme(s) and anti-target enzyme(s) in each disease would be key to developing the next generation of MMP inhibitor drugs for different diseases.

1.2. MT1-MMP as a target enzyme for Cancer and different diseases

Among MMPs, one of the most characterized enzymes is MT1-MMP/MMP-148. MT1-MMP is the first MT-MMP to be discovered, and has been shown to be expressed in different cancer cells and to promote their invasiveness⁹. It degrades various components of the extracellular matrix including collagen I, II, III, fibronectin, vitronectin, laminin 1 and 5, tenascin, nidogen, fibrin, and aggrecan core protein. It activates other MMPs namely proMMP-2 and proMMP-13, broadening the proteolytic repertoire on the cell surface. It also cleaves several membrane proteins including CD44, α_v integrin, low density lipoprotein receptor-related protein, and tissue transglutaminase⁸. These activities have been shown to contribute to the cell invasion promoting activity of the enzyme¹⁰. MT1-MMP is highly expressed in a wide variety of cancers including breast carcinoma, squamous cell carcinoma¹¹, fibrosarcoma¹², glioma¹³, prostate cancer¹⁴ and others¹⁵. MT1-MMP is expressed not only by cancer cells, but also by mesenchymal cells¹⁵, macrophages and endothelial cells within the tumor microenvironment^{15, 16}. It has been shown that co-innoculation of MT1-MMP null fibroblasts with cancer cells, mimicking tumor stromal cells, showed significantly reduced tumor growth in comparison to fibroblasts with the

MT1-MMP gene¹⁷. MT1-MMP expressed in cancer cells promotes not only their invasiveness but also plays an important role in tumor growth¹⁸, especially in a collagen-rich environment. Considering these facts, MT1-MMP is a good molecular target in cancer. MT1-MMP was also shown to play a crucial role in cartilage invasion by synovial tissue in rheumatoid arthritis¹⁹, and smooth muscle cell migration into neointima region in atherosclerosis²⁰. These findings provided a potential opportunity to develop novel therapy targeting MT1-MMP for these diseases.

2. Chemistry

As the inhibitor(s) described in this patent is a biologic agent, there is not much chemistry to be described. The methodology to generate biologic inhibitor(s) is based on phage display library screening which is quite a common method and many publications have already described this²¹. After screening for their binding ability, the antibodies are produced as human IgGs and tested for their inhibitory activity against different members of MMPs.

To increase the stability and retention in circulation, the authors described that the binding proteins can be conjugated with a non-antigen polymer such as polyalkylene oxides, polyethylene oxides, polyethylene glycols, polyoxyethylenated polyols, and others, which is again quite a common approach.

3. Biology and action

Although the authors describe application of MT1-MMP inhibitory antibodies to a number of different diseases including cancer, rheumatoid arthritis, osteoarthritis, diabetes, and Alzheimer's disease, a causal relationship between MT1-MMP and these diseases has not been validated for all of them. However, MT1-MMP is most widely accepted to be involved in cancer, acting at different levels of the disease, and inhibition of MT1-MMP activity would have significant impact on tumour growth¹⁸, angiogenesis²², invasion and metastasis⁹. Dyax Corp has developed an MT1-MMP inhibitor antibody with K_i of 0.6 nM, which they refer to as DX-2400 or M0038F01. This is a significant achievement as it is the most selective and potent inhibitor for MT1-MMP to date. It does not inhibit even very closely related enzymes, namely MT2-, MT3-, and MT5-MMPs. It completely inhibited spontaneous proMMP-2 activation detected in HT1080 human fibrosarcoma cells at 66 nM. Strikingly, growth of MDA-MB231 breast cancer cells inoculated into the mammary fat pad of female Balb/c mice was significantly suppressed by intraperitoneal injection of the inhibitor at 10 mg/kg on alternating days (Q2d) for five weeks starting at week 5 after inoculation of the cancer cells. At the end of the period, tumour volume of control mice was 800-900 mm³ whereas the drug treated tumour was 200 mm³ in size. This is a significant suppression given the fact that it is a result of inhibition of only one enzyme. The authors have also shown significant inhibition of growth and lung metastasis of B16F1 melanoma cells and growth of PC3 prostate cancer and BT74 breast cancer cells in mice, and these data are also published recently²³. One of the unique characteristics of DX-2400 is that it only binds to active MT1-MMP, and does not bind to the enzyme already inhibited by physiological inhibitors such as TIMP-2, TIMP-3 and TIMP-4. Thus it targets only "live" MT1-MMP.

4. Expert opinion

The trend in MMP inhibitor drugs is certainly going towards the development of selective inhibitors^{4, 24-27}, and this should be the way forward. The MT1-MMP antibody inhibitor invented by Dyax Corp will answer the question of whether MMP inhibitors really can be successful drugs. In terms of selectivity, no other inhibitor can be better at moment and this is one of the big advantages of biologic drugs. Also the target molecule, MT1-MMP, is one

of the most highly validated target MMPs in cancer. It will be a big test and further development of this drug will be a worthy to follow. There are, however, a few things that need to be considered. MT1-MMP is a cell surface-bound enzyme, and half-life of MT1-MMP on the cell surface is less than 30 min as the enzyme is constantly endocytosed by both clathrin- and caveolae-dependent pathway²⁸⁻³¹. On the other hand, newly synthesized MT1-MMP constantly appears on the cell surface. Enzyme bound and inhibited by DX-2400 would be internalized and newly synthesized MT1-MMP has to be bound and inhibited by another DX-2400 molecule. So, it is expected that maintaining high DX-2400 concentrations in the pericellular region is crucial to controlling MT1-MMP activity. Another issue that needs to be considered is the potential side effects of MT1-MMP inhibition. MT1-MMP is certainly a good target molecule since it accelerates disease progression. However, MT1-MMP activity is a double-edged sword and its role in physiological processes cannot be underestimated. MT1-MMP null mice exhibit the most significant developmental phenotype of all the MMP null mice generated to date^{32, 33}. They exhibit dwarfism, fibrosis, defects in skeletal development, hyper activation of osteoclasts, defects in mammary gland, submandibular gland, and lung development³²⁻³⁴. Although these are developmental phenotypes and may not be applicable to physiological process occurring in adulthood, they need to be taken into account when considering the safety of treatment. MT1-MMP is has also been implicated in wound healing³⁵ and angiogenesis^{16, 22, 33}. If MT1-MMP activity is blocked for long periods of time, one would expect that wound healing may be affected. Nevertheless, these issues are associated with the development of any new drugs, and only time will tell the extent of these side effects.

If selective inhibitors for different MMPs or even for other proteinases become available in the future, it may open up an opportunity for co-administration of these specific drugs which may be useful to strengthen the efficacy of treatment if additional target enzymes are present. However, this will be possible only when these 'specific' inhibitors become available. In conclusion, Dyax's invention is a significant step towards the development of further specific MMP inhibitory drugs in the future.

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