Dynamics of Internalization and Recycling of the pro-Metastatic Membrane Type 4-Matrix Metalloproteinase (MT4-MMP) in Breast Cancer cells

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1. ABSTRACT

Keyword(s): matrix metalloproteinase; trafficking; breast cancer cells; protein-protein interaction

1.1 Introduction

MT4-MMP (MMP17) is a glycosyl-phosphatidylinositol-anchored membrane-type MMP expressed at the cell surface of human breast cancer cells. MT4-MMP emerged recently as a key driver of breast cancer growth and metastasis in vivo [1, 2]. Pro-angiogenic and pro-metastatic effects of MT4-MMP were abrogated by an inactivating mutation (E249A) in the catalytic site of the enzyme [2]. Aside its proteolytic activity, MT4-MMP affects breast cancer cell proliferation by interacting with epidermal growth factor receptor (EGFR) and enhancing its activation in response to ligand [3].

While trafficking of MT-MMPs was extensively investigated and mainly focused on the membrane associated MT1, 2 and 3-MMP, the internalization of MT4-MMP is unknown. Understanding mechanisms regulating MT4-MMP availability at the cell surface is important for the designing approaches to interfere with its function.

1.2 Results and conclusions

Dynamics of MT4-MMP at the cell surface was investigated by two technics using cell surface protein biotinylation and antibody feeding assay with confocal microscopy analysis. Our data revealed that the internalization of MT4-MMP appeared early at 15 minutes and reached its maxim at 45 min (Figure 1). In MT4-E249A cells, internalized MT4-MMP was visible as green spots in the cytoplasm. In the absence of BB94, only few spots were detectable in MT4-WT expressing cells, even after 60 minutes. In contrast, MT4-MMP degradation was completely prevented by the MMP inhibitor BB94. These data demonstrate that MT4-MMP is internalized and partially auto-degraded, a process that is prevented by the inactivation of its catalytic domain or the treatment with BB94.
Figure 1: MT4-MMP is internalized and auto-degraded. Antibody feeding assay on coverslips of MT4-MMP or its inert form MT4-E249A in MDA-MB-231 cells after 0, 30 and 60 minutes using an Alexa Fluor 488 coupled anti-MT4 antibody (green). Cells expressing active form of MT4-MMP (MT4-WT) were incubated or not with BB94 24h before the assay. At 30 minutes, internalization of the MT4-MMP has started but the majority of the protease is still next to the plasma membrane. After 60 minutes, MT4-MMP is completely internalized and majority is degraded in WT form, but protected from degradation in the inactive form (MT4-E249A). Incubation with the use of BB94, block the autocleavage of MT4-WT in the intracellular vesicles.

We next demonstrated that the MT4-MMP was internalized in Early Endosomes and that intact enzyme can be recycled at the cell surface. Cell surface biotinylation experiments showed that half amount of the internalized MT4-MMP was recycled at cell surface after 30 min (Figure 2). Through its recycling, MT4-MMP could be repeatedly used by cancer cells during cancer cell invasion and metastatic dissemination.

Figure 2: MT4-MMP is recycled at the cell surface. A) Schematic representation of the recycling assay of MT4-MMP. After cell surface biotinylation (black spots), cells were incubated for 45 minutes at 37°C to allow its internalization. A first treatment with MESNA removed biotin remaining at the cell surface and was followed by a second incubation, at 37°C for 30 minutes, to allow the recycling of biotinylated enzyme. A second MESNA treatment cleaved all the biotinylated proteins re-exposed on the surface. Difference between the second treatment or not with MESNA determines the amount of
recycled enzyme at the cell surface. Protein extracts were immunoprecipitated with streptavidin beads and separated on an SDS-PAGE to analyze the expression of MT4-MMP by immunoblotting. B) Western blot analysis of MT4-MMP expression after recycling assay. The difference between lane 3 versus 4 or lane 7 versus 8, represents the amount of proteins recycled to cell surface during the second incubation at 37°C. After 30 min, half of the internalized MT4-MMP returned to cell membrane.

To reveal the endocytic pathway used by MT4-MMP for its internalization, we studied the clathrin- and caveolin-mediated endocytosis pathways. While MT4-MMP was found to be localized with caveoline-1, its internalization was not inhibited by caveolin pathway inhibitor neither inhibitor of clathrin dependent pathways. We next investigated the CLIC/GEEC pathway ("Clathrin Independent Carriers" / "GPI-AP Enriched Endosomal Compartments") which is dependent on RhoGTPase like RhoA and cdc42. FACS analysis showed that MT4-MMP availability at the cell surface was increased after knockdown of RhoA and cdc42 expression by siRNA in cancer cells. These data suggest that CLIC/GEEC pathway is involved in MT4-MMP internalization, a mechanism that is distinct from other MT-MMPs members, like MT1-MMP that uses clathrin-dependent pathway.

Finally, we showed that MT4 MMP can be found as monomeric, dimeric and oligomeric forms using co-immunoprecipitation and non-reducing Western blots experiments. MT4-MMP oligomerization was not inhibited neither by broad-spectrum MMP inhibitors (GM6001 and BB94) nor TIMP-2 a physiological MMP inhibitor. Moreover, a single mutation in key cystein residues C564 or C566 into serine residues (C564S and C566S respectively) did not inhibit MT4-MMP dimerization, whereas a double mutation lowered the formation of dimers without its complete inhibition (Figure 3). MT4-MMP molecules contribute to the formation of covalent dimers by intermolecular disulfide bonds and suggest the existence of other non-covalent interactions. These latter may involve hydrophobic or polar interaction between the hemopexin domain of adjacent MT4-MMP molecules.

Our data demonstrate that MT4-MMP bioavailability at the surface is regulated through its internalization, degradation and recycling in human breast cancer cells. Overall, we provide a new mechanistic insight on the regulatory mechanisms of MT4-MMP in human breast cancer cells, which may have implication in the design of novel therapeutic strategies for metastatic breast cancer.
REFERENCES

