

Claudin Promotes Activation of Pro-MMP-2 Mediated by Membrane-Type Matrix Metalloproteinases.

H. Miyamori, T. Takino, Y. Kobayashi and H. Sato

Genes associated with regulation of membrane-type matrix metalloproteinase-1 (MT1-MMP)-mediated pro-MMP-2 processing was screened in 293T cells by a newly developed expression cloning method. One of the gene products which promoted processing of pro-MMP-2 by MT1-MMP was claudin-5, a major component of endothelial tight junctions. Expression of claudin-5 not only replaced TIMP-2 in pro-MMP-2 activation by MT1-MMP but also promoted activation of pro-MMP-2 mediated by all MT-MMPs and MT1-MMP mutants lacking the transmembrane domain (Δ MT1-MMP). A C-terminal deletion mutant of pro-MMP-2 (pro Δ MMP-2) was processed to an intermediate form by MT1-MMP in 293T cells, and was further converted to an activated form by introduction of claudin-5. In contrast to the stimulatory effect of TIMP-2 on pro-MMP-2 activation by MT1-MMP, activation of pro-MMP-2 by Δ MT1-MMP in the presence of claudin-5 and pro Δ MMP-2 processing by MT1-MMP were both inversely repressed by expression of exogenous TIMP-2. These results suggest that TIMP-2 is not involved in claudin-5-induced pro-MMP-2 activation by MT-MMPs. Stimulation of MT-MMP-mediated pro-MMP-2 activation was also observed with other claudin family members; claudin-1, claudin-2 and claudin-3. Amino acid substitutions or deletions in ectodomain of claudin-1 abolished stimulatory effect. Direct interaction of claudin-1 with MT1-MMP and MMP-2 was demonstrated by immunoprecipitation analysis. MT1-MMP was co-localized with claudin-1 not only at cell-cell borders, but also at other parts of the cells. TIMP-2 enhanced cell-surface localization of MMP-2 mediated by MT1-MMP, and claudin-1 also stimulated it. These results suggest that claudin recruits all MT-MMPs and pro-MMP-2 on the cell surface to achieve elevated focal concentrations, and consequently enhances activation of pro-MMP-2.

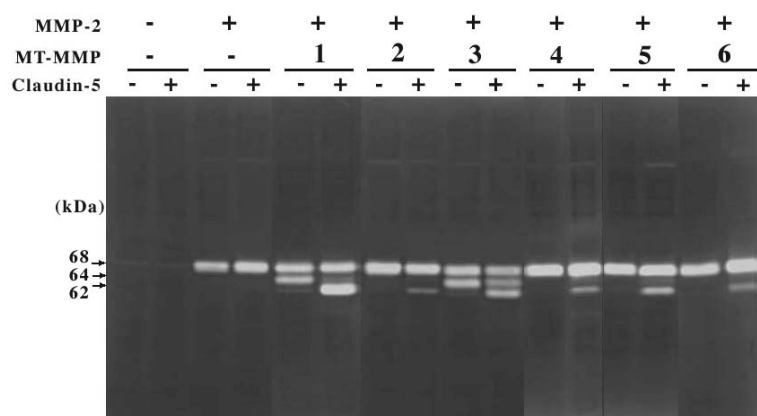


Fig. 1 Claudin-5 Promotes pro-MMP-2 Processing by MT-MMPs. Control (pSG5) plamid or claudin-5 plasmid was co-transfected into 293T cells with plasmids of MMP-2 and each MT-MMPs as indicated. At 48 h post-transfection, the cells were harvested and analyzed by gelatin zymography.

Reference:

Miyamori, H., Takino, T., Kobayashi, Y., Tokai, H., Itoh, Y., Seiki, M., and Sato, H. (2001) Claudin promotes activation of Pro-MMP-2 mediated by Membrane-Type Matrix Metalloproteinases. *J. Biol. Chem.*, 276, 28204-28211.