Identification and analysis of MMP substrates using proteomic approach

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Matrix metalloproteinases (MMPs), which are a family of zinc-containing endopeptidases, cleave extracellular matrix components as well as a variety of functional proteins. MMPs are involved in normal physiological processes as well as in a number of pathological processes including tumor growth, metastasis, atherosclerosis, rheumatoid and osteoarthritis, and periodontal disease. Identification of substrates of MMPs is important for understanding of normal biological and pathological roles of MMPs. To rapidly screen MMP substrates we have used degradomic approaches using basic proteomic techniques. Diverse samples were analyzed for screening for MMP substrates: a complex protein mixture (e.g. plasma proteins) treated with or without an MMP; conditioned culture media of cells transfected with or without an MMP or the cells with or without stable knockdown of an MMP; and soluble digests of the cell surface proteins treated with or without an MMP. The proteins were differentially displayed in SDS gels or 2-D gels. The interested bands or spots were subjected to in-gel digestion, mass spectrometry of the resultant peptides, and protein identification. The identified proteins include several known substrates for the MMP, indicating that our approaches are valuable tools to identify MMP substrates, and proteins which are not reported for MMP substrates. Cleavage sites of target proteins were determined by C-terminal labeling of MMP-digested fragments with isotope ($^{18}$O:$^{16}$O=1:1) and identification of the doublet fragments or peptides showing 2-Da difference by mass spectrometry, along with N-terminal sequencing of the fragments. Then, we analyzed whether proteolytically cleaved proteins lose their own functions and are involved in MMP-related diseases. From these studies, we demonstrated that our approaches to identify MMP substrates would be valuable tools to unravel roles of MMPs in various complex diseases.