Assays for determination of matrix metalloproteinases and their activity

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Matrix metalloproteinases (MMPs) are involved in many physiological and pathological processes. Due to their ability to cleave and to remodel components of surrounding tissues, MMPs may affect cell migration, differentiation, growth, inflammatory processes, neovascularization, wound healing, apoptosis, the uterine cycle and many other actions within the body, including those needed for tumorigenesis and other diseases.

MMPs can therefore be used as potential markers for detecting various cancers, neurodegenerative, and immune and cardiovascular diseases. Numerous MMP assays were developed for clinical and research purposes, but far more attention has been devoted to understanding their biological functions.

Due to differences in methodology, results obtained in various laboratory settings are difficult to compare because of the lack of standards and analytical methods of validation. Limits of detection of particular methods used for identifying MMPs are also disputable.

Enzymatic, immunochemical and fluorimetric methods are particularly suitable for clinical use. *In-vivo* imaging methods offer many potential advantages in cancer research and diagnostics. Other methods are subject to investigation [e.g., phage display, multiple-enzyme/multiple-reagent assay system (MEMRAS) and activity-based profiling].

Keywords: Activity-based profiling; Cancer; Disease marker; Enzymatic method; Fluorimetric method; Immunochemical method; *In-vivo* imaging; Matrix metalloproteinase (MMP); Multiple-enzyme/multiple-reagent assay system (MEMRAS); Phage display

Abbreviations: ABPP, Activity-based proteomic probes; Adp, D,L-2-amino-3-(6,7-dimethoxy-4-coumaryl)propionic acid; Amp, D,L-2-amino-3-(7-methoxy-4-coumaryl)propionic acid; APMA, p-aminophenylmercuric acetate; CPSA, Chronopotentiometric stripping analysis; Dabcyl, 4-(4-methylaminophenylazo)benzoic acid; Dnp, Dinitrophenol; ECM, Extracellular matrix; EDANS, Tryptophan, 5-((2-aminoethyl)amino)naphthalene-1-sulfonic acid; ELISA, Enzyme-linked immunosorbent assay; FITC, Fluorescein isothiocyanate; fTHP, Fluorescently-labeled triple-helical peptide; ICAT, Isotope-coded affinity tag; iTRAQ, Isobaric tag for relative and absolute quantification; MCA, 7-methoxycoumarin; MEMRAS, Multiple-enzyme/multiple-reagent assay system; MMP, Matrix metalloproteinase; MRI, Magnetic resonance imaging; MT-1-MMP, Membrane type 1 matrix metalloproteinase; MS, Mass spectrometry; NMA, N-methylanthranyl acid; OIM, optical imaging; PET, Positron-emission tomography; pNA, p-nitroaniline; proUKCOL, pro-urokinase; SDS, Sodium-dodecylsulfate; SDS-PAGE, Sodium-dodecylsulfate polyacrylamide gel electrophoresis; SPECT, Single-photon emission computed tomography; TAPI-2, TNF-alpha protease inhibitor 2; TIMP, Tissue inhibitors of matrix metalloproteinases; TNBSA, 2,4,6-trinitrobenzensulfonic acid

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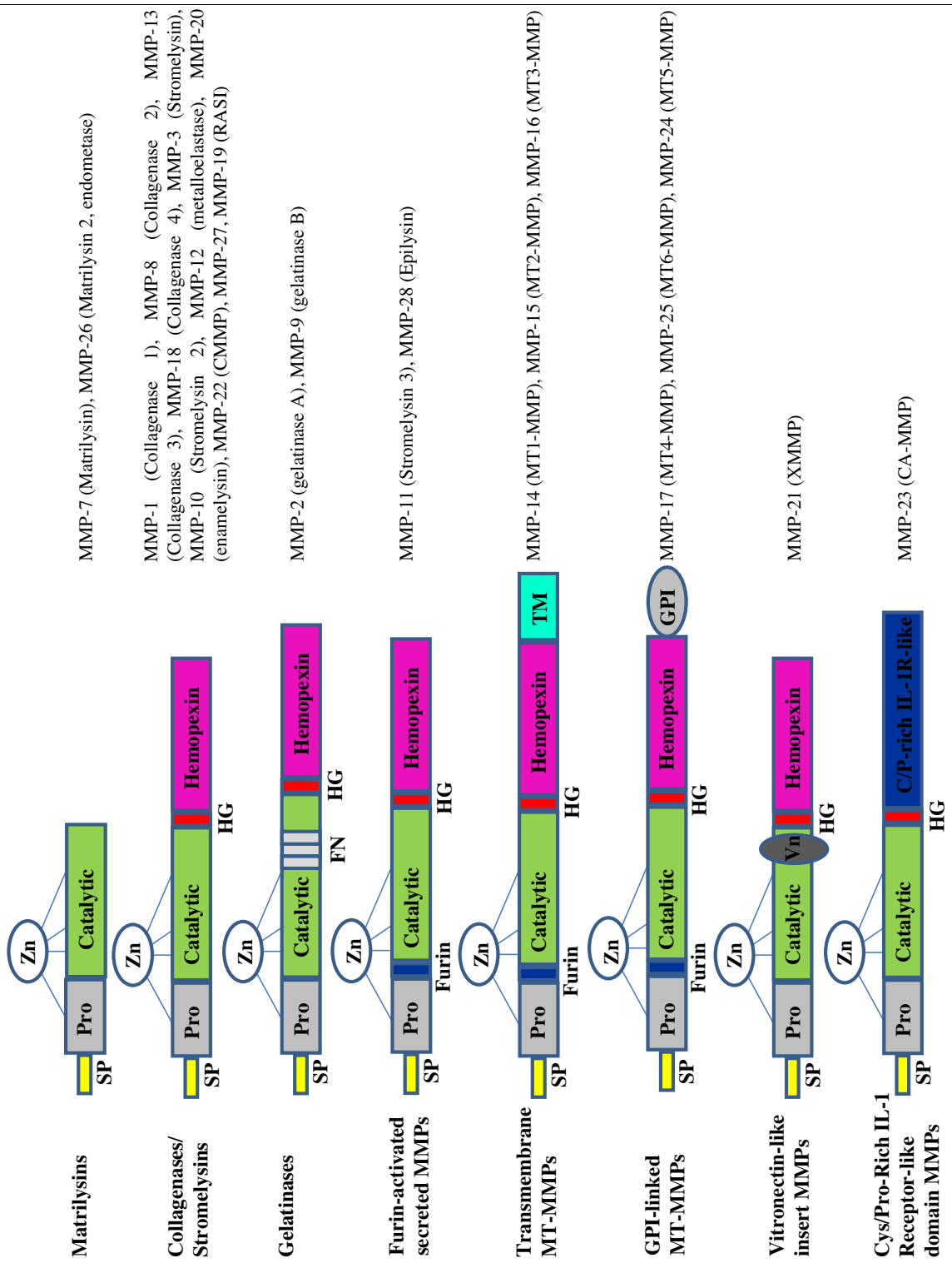

Figure 1. Classification and domain structure of matrix metalloproteinases (MMPs). Most MMPs contain a signal peptide (necessary for secretion), a propeptide [a catalytic domain that binds zinc (Zn^{2+})], a hinge region (HG), and a hemopexin carboxy (C)-terminal domain. In the catalytic domain, MMP has a Zn^{2+} binding site and a binding site for the specific substrate. Matrilysins lack a hemopexin domain. Gelatinases contain fibronectin type II modules (FN) that improve the efficiency of collagen and gelatine degradation. Furin-activated secreted MMPs (MMP-11 and MMP-28) have a recognition motif for furin-like serine proteinases within their catalytic domain for intracellular activation. This motif is also found in the vitronectin (Vn)-like insert MMPs (MMP-21) and the MT-MMPs. MT-MMPs have an additional transmembrane-binding domain (TM). Most MMPs are secreted, but six membrane-type MMPs (MT-MMPs) have been identified and they are anchored by a transmembrane domain or a glycosyl-phosphatidylinositol (GPI) linker.