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Matrix metalloproteinases and metastasis

Abstract Metastatic disease is responsible for the majority of cancer-related deaths, either directly due to tumor involvement of critical organs or indirectly due to complications of therapy to control tumor growth and spread. An understanding of the mechanisms of tumor cell invasion and metastasis may be important for devising therapies aimed at preventing tumor cell spread. Matrix metalloproteinases (MMPs) are a family of zinc-dependent endoproteinases whose enzymatic activity is directed against components of the extracellular matrix (ECM). In humans, 16 members of this family have been identified by cloning and sequencing. These proteinases are linked by a core of common domain structures and by their relationship to a family of proteinase inhibitors called the tissue inhibitors of metalloproteinases (TIMPs). Four members of the TIMP family have been cloned and sequenced in humans and they inhibit MMPs by forming tight-binding, noncovalent associations with the active site of the MMPs. MMPs facilitate tumor cell invasion and metastasis by at least three distinct mechanisms. First, proteinase action removes physical barriers to invasion through degradation of ECM macromolecules such as collagens, laminins, and proteoglycans. This has been demonstrated in vitro through the use of chemoinvasion assays and in vivo by the presence of active MMPs at the invasive front of tumors. Second, MMPs have the ability to modulate cell adhesion. For cells to move through the ECM, they must be able to form new cell–matrix and cell–cell attachments and break existing ones. Using a cell transfection system that altered the ratio of MMP-2 to TIMP-2 we have demonstrated significant variation in the adhesive phenotype of tumor cells. Finally, MMPs may act on ECM components or other proteins to uncover hidden biologic activities. For example, the angiogenesis inhibitor angiostatin may be produced from plasminogen by MMP action and laminin-5 is specifically degraded by MMP-2 to produce a soluble chemotactic fragment. Thus MMPs play multiple key roles in facilitating the metastasis of tumor cells. Therapies designed to interfere with specific MMP actions may be useful in the control of metastatic disease.

Key words Matrix metalloproteinases · Metastasis

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Introduction

For any individual patient, the battle against cancer is often won or lost in the opening round, with the success or failure of the initial surgical resection and the clinical evaluation for metastatic disease. In the USA, about one-third of patients will present with metastatic disease. In these patients, surgical therapy will be only a prelude to systemic chemotherapy. Of the remaining patients, half will be cured by the initial surgical procedure and will remain free of disease, either because there were never metastases present or because adjuvant therapy successfully treated the micrometastases that were clinically undetectable at presentation. The other 50% of patients will relapse with clinically detectable metastatic disease, which then must be treated by systemic therapy, since surgery has only palliative value for the treatment of metastases.

It is clear from this description that the development, detection, and therapy or prevention of metastatic disease is the most significant problem facing oncologists. Traditional forms of cytotoxic chemotherapy often fail when bulky metastatic disease is present. Thus the study

of the basic biological mechanisms of metastasis is critical for finding solutions to the problems faced by oncologists when they are confronted by patients who relapse with intractable metastatic disease. In our laboratory, investigations into this field have made use of a scientific model that postulates that tumor cells must be able to accomplish three goals successfully to invade locally and metastasize systemically [29–31]. These are the ability to degrade proteins in the extracellular matrix (ECM), to attach and detach from ECM proteins, and to move through defects in the ECM. These steps occur simultaneously and continuously as epithelial tumor cells first break out through their basement membrane, invade through tissue into local lymphatics and capillaries, and finally break out of vascular structures at distant sites to establish new tumors. Others have embellished this basic model with other characteristics, such as the ability to stimulate new blood vessel growth and to evade the immune system, but such characteristics are also shared by benign tumors and are not peculiar to malignancy, which may spread to distant sites with impunity.

Using this three-step hypothesis as a guide, we and many others have carefully examined tumor cells for distinctive phenotypes that permit ECM degradation, adherence, and motility. One family of enzymes stands out from others as playing a key role in the phenotype of ECM degradation [37, 47, 58]. This is the matrixin family of matrix metalloproteinases (MMPs) (Table 1) and their corresponding family of specific inhibitors, the tissue inhibitors of metalloproteinases (TIMPs). As more is known about the significance of protease action in tumor cell invasion and metastasis, it has become clear that MMPs not only play a role in ECM modification, but also have effects on tumor cell adhesion and motility.

Characteristics of MMPs and TIMPs

Currently, 16 members of the MMP family have been cloned and sequenced in humans (Table 1). However, MMPs have been cloned from a wide variety of species, including mammals, birds, amphibians, plants [36, 43],

Table 1 The matrixin family of metalloproteinases

MMP number	Common name	Molecular weight (kDa)[a] full/pro/active	Substrates	Genbank accession no.[b]	Reference[c]
1	Interstitial collagenase	54.0/51.8/42.6	Types I, II, III, VII, and X collagen	X05231, X54925, M13509	18, 62, 64
2	Gelatinase A	73.9/71.0/62.1	Gelatin, types I, IV, V, and X collagen, laminin V	J03210	7, 8, 22
3	Stromelysin 1	54.0/52.2/42.8	Types III, IV, IX, and X collagen, gelatin, proMMP-1, laminin, proteoglycans	J03209, X05232	52, 64, 65
7	Matrilysin	29.7/27.9/19.1	Gelatin, fibronectin, proMMP-1	X07819, Z11887, S41832	13, 35, 39
8	Neutrophil collagenase	53.4/51.1/42.2	Types I, II, III, VII, and X collagen	J05556	13, 35, 39
9	Gelatinase B	78.4/76.3/66.6[d] 94/92/82	Gelatin, types I, IV, V, and X collagen	J05070	23, 66
10	Stromelysin 2	54.2/52.3/43.0	Types III, IV, IX, and X collagen, gelatin, proMMP-1, laminin, proteoglycans	X07820, Y00728	39
11	Stromelysin 3	54.6/51.1/44.3	Alpha-1-antiprotease	X57766	3
12	Metalloelastase	54.0/ 52.3/ 42.8	Elastin	L23808	54
13	Collagenase 3	53.8/51.7/42.2	Types I, II, III, VII, and X collagen	X75308	12
14	MT1-MMP	65.9/63.8/53.9	proMMP-2, gelatin, collagens	D26512, Z48481, X83535, U41078, X90925	42, 51, 61, 67
15	MT2-MMP	75.8/71.2/61.2	proMMP-2	Z48482, D86331	67
16	MT3-MMP	69.5/65.8/55.7	proMMP-2	D83646, E12862, E12884, D50477, D83647, D85511, AB009303	55, 60
17	MT4-MMP	?/61.7/53.7	?	X89576	46
18/19	RASI-1	57.4/54.7/46.5	?	Y08622, U37791, X92521	9, 27, 45, 53
20	Enamelysin	54.4/52.1/42.6	Amelogenin	AJ003144, Y12779	33

[a] Molecular weights are given for full-length, proenzyme, and active forms based on amino acid sequence
[b] Genbank accession numbers are for full-length or near full-length cDNA sequences
[c] References are given for full-length sequencing papers
[d] MMP-9 is significantly glycosylated. The first set of molecular weights is for the unglycosylated forms, the latter set for the glycosylated forms

and *Caenorhabditis elegans* [63]. They have been linked to a wide range of physiological and pathological processes outside the field of tumor biology, including normal wound healing, bone remodeling, uterine resorption, trophoblast implantation, angiogenesis, and chronic inflammatory and degenerative diseases of all types. The ubiquitous nature and importance of the MMPs has led to intense interest on the part of both basic scientists and pharmaceutical companies who view knowledge of MMP action as a key to understanding and manipulating the natural history of these processes.

The MMPs share common structural and functional characteristics [5, 26]. All of the family members sequenced to date have at least three domains (Fig. 1): a prodomain which contains a conserved cysteine residue and which is lost on enzyme activation; a catalytic domain of 106 to 119 residues which contains conserved structural metal-binding sites; and a highly conserved zinc-binding active site domain of 52 to 58 amino acids. Among the human MMPs, all but MMP-17 (MT4-MMP) have a signal peptide sequence and all but MMP-7 have a C-terminal domain with homology to the protein hemopexin. Two subclasses of MMPs have additional domains that set them apart from the rest of the family. The two gelatinases, MMP-2 and MMP-9, each have a gelatin-binding domain inserted between the catalytic domain and the active site domain, and there are four membrane-associated MMPs, MMP-14, -15, -16, and -17, which have transmembrane domains added on to the C-terminal domain. The membrane-associated enzymes, along with MMP-11 (stromelysin 3), are further distinguished by a short sequence inserted between the prodomain and the catalytic domain that has been postulated to be a furin-sensitive cleavage site important in the activation of these enzymes.

MMPs have two key structural motifs which are highly conserved among all of the members of the family (Fig. 2) and which set them apart from other metalloproteases. The first is the zinc-binding active site domain, a highly conserved stretch of 50 to 55 amino acids which contains three histidines occupying three of the coordination sites of the active site zinc ion [34]. Across the 64 unique and complete MMP sequences found in the Genbank repository, the sequence HE-GH–G–H——M is completely conserved, with several other amino acids being conserved in more than 90% of the known sequences. The second key structural motif is the conserved sequence in the prodomain of the protein that contains a cysteine residue which is responsible for maintaining latency in proMMPs by occupying the fourth zinc coordination site with its sulphydryl group [4, 57]. Activation of proMMPs must, at some point in the mechanism, destabilize and break the zinc–sulfur bond. This is usually followed by cleavage of the cysteine-containing sequence from the now active enzyme [26].

Most MMPs appear to be secreted from cells in their inactive proforms, making activation a key step in regulating the amount of degradative activity outside the cell. The activation mechanisms postulated for this family usually involve cleavage of the prodomain on the amino side of the conserved cysteine, followed by opening of the active site through breakage of the zinc–cysteine bond and loss of the remaining portion of the prodomain through further proteolytic cleavage. For most MMPs, including MMP-1, -3, -7, and -9, serine

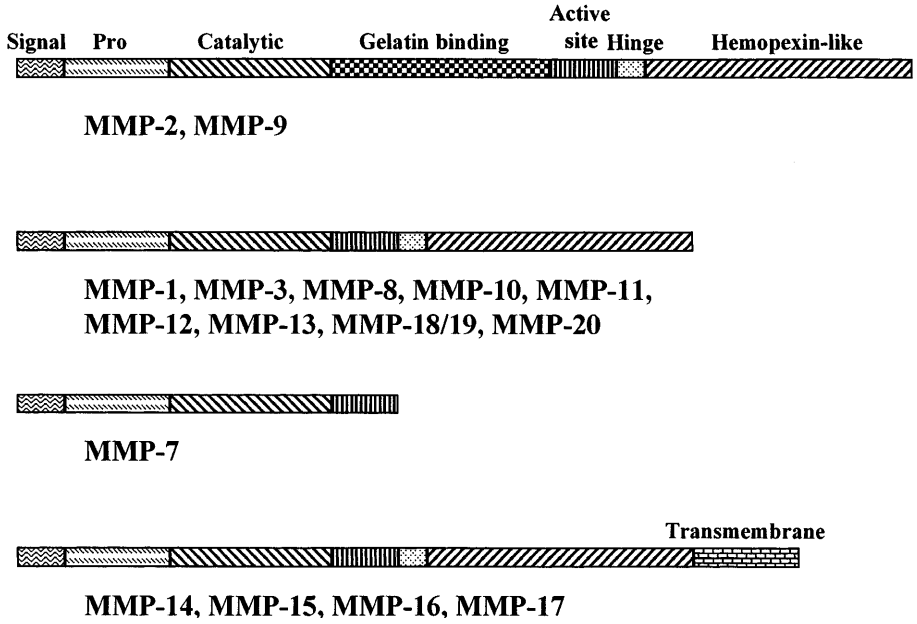

Fig. 1 Schematic domain structure of the matrixins. The basic domain structure of the family of MMPs is shown, with MMPs having similar domain stuctures grouped together. The length of each segment is roughly proportional to the number of amino acids which comprise the domain

proteases such as plasmin and urokinase-type plasminogen activator have been shown to initiate activation [5, 26]. Some MMPs may activate other members of the family. For example, the membrane-bound MMPs MMP-14, -15, and -16 can activate MMP-2 [51, 60, 67], and MMP-2 and -3 have been shown to be able to activate MMP-9 [41]. The five MMPs with furin cleavage sites are postulated to be activated by furin, possibly prior to secretion from the cell or display on the cell surface [50].

The other major control point in the regulation of active enzyme is inhibition of active forms by the TIMP family of inhibitors. These are small, 21–28-kDa proteins with a highly conserved set of six intramolecular disulfide bonds. All have the ability to form tight-binding, noncovalent inhibitory complexes with multiple members of the MMP family [5]. Recent structural work has demonstrated that during inhibition the terminal amino group of the TIMP fills the fourth coordination site of the active site zinc [19]. The three-dimensional structure of the protein at this location is

Fig. 2 Aligned sequences of the known human MMPs. The 16 known human MMPs have been aligned to show areas of significant homology. The Genbank sequences of MMP-18 and -19 are identical, so these have been shown together as a single sequence, MMP 18/19. Where some of the MMPs have large insertions, the corresponding "empty" sequences of the other MMPs have been left out of the figure. This is the case for the gelatin-binding domain of MMP-2 and -9 and the transmembrane domains of MMP-14 to -17. The two key functional motifs described in the text are highlighted. Asterisks mark the location of the three active site histidine residues and the two cysteine residues in the C-terminal domain that form the only disulfide bond which is conserved in all members of the family

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MMP-2       --------ME ALMARGALTG PLRALCLLGC LLSHAAAAPS PIIKFPGDVA P-KTDKELAV QYLNTFYGCP KESCN-----
MMP-9       ---------- ----MSLWQP LVLVLLVLGC CFAAPRQRQS TLVLFPGDLR TNLTDRQLAE EYLYRYGYTR VAEMRG---E
MMP-7       ---------- ---------- --MRLTVLCA VCLLPGSLAL PLPQEAGGMS E--LQWEQAQ DYLKRFYLYD SETKN-----
MMP-1       ---------- ---------- MHSFPPLLLL LFWGVVSHSF PATLETQEQ- ----DVDLVQ KYLEKYYNLK NDGRQVEKRR
MMP-8       ---------- ---------M FSLKTLPFLL LLHVQISKAF PVSSKEK--- ----NTKTVQ DYLEKFYQLP SNQYQSTRKN
MMP-3       ---------- ---------- --MKSLPILL LLCVAVCSAY PLDGAARGED T---SMNLVQ KYLENYYDLE KDVKQFVRRK
MMP-10      ---------- ---------- --MMHLAFLV LLCLPVCSAY PLSGAAKEED S---NKDLAQ QYLEKYYNLE KDVKQFRR-K
MMP-12      ---------- ---------- ---MKFLLIL LLQATASGAL PLNSSTSLEK N---NVLFGE RYLEKFYGLE INKLPVTKMK
MMP-13      ---------- ---------- MHPGVLAAFL FLSWTHCRAL PLPSGGDEDD LSEEDLQFAE RYLRSYYHPT NLAGILKENA
MMP-20      ---------- -------MKV LPASGLAVFL IMALKFSTAA PSLVAASPRT WRN-NYRLAQ AYLDKYYTNK EGHQIGEMVA
MMP-14      ---------- -------MSP APRPSRCLLL PLLTLGTALA SLGSAQS--- ----SSFSPE AWLQQYGYLP PGDLRTHTQR
MMP-15      MGSDPSAPGR PGWTGSLLGD REEAARPRLL PLLLVLLGCL GLGVAAED-- ----AEVHAE NWLRLYGYLP QPSRHMSTMR
MMP-16      ---------- MILLTFSTGR RLDFVHHSGV FFLQTLLWIL CATVCGTE-- ----QYFNVE VWLQKYGYLP PTDPRMSVLR
MMP-11      ---------- ---------- ---MAPAAWL RSAAARALLP PMLLLLLQP- ----PPLLAR ALPPDVHHLH AERRG-----
MMP-17      ---------- ---------- ---------- ---------- ---------- ---------- EWLSRFGYLP PADPTTG-QL
MMP-18/19   ---------- ---------- --MNCQQLWL GFLLPMTVSG RVLGLAE--- -----VAPVD YLSQYGYLQK PLEGSNN-FK

MMP-2       LFV--LKDTL KKMQKFFGLP QTGDLDQNTI ETMRKPRCGN PDVAN----- ------YNFF PRKPKWDKNQ ITYRIIGYTP D--LDPETVD
MMP-9       SKS--LGPAL LLLQKQLSLP ETGELDSATL KAMRTPRCGV PDLGR----- ------FQTF EGDLKWHHHN ITYWIQNYSE D--LPRAVID
MMP-7       ANS--LEAKL KEMQKFFGLP ITGMLNSRVI EIMQKPRCGV PDVAE----- ------YSLF PNSPKWTSKV VTYRIVSYTR D--LPHITVD
MMP-1       NSG-PVVEKL KQMQEFFGLK VTGKPDAETL KVMKQPRCGV PDVAQ----- ------FVLT EGNPRWEQTH LTYRIENYTP D--LPRADVD
MMP-8       GTN-VIVEKL KEMQRFFGLN VTGKPNEETL DMMKKPRCGV PDSGG----- ------FMLT PGNPKWERTN LTYRIRNYTP Q--LSEAEVE
MMP-3       DSG-PVVKKI REMQKFLGLE VTGKLDSDTL EVMRKPRCGV PDVGH----- ------FRTF PGIPKWRKTH LTYRIVNYTP D--LPKDAVD
MMP-10      DSN-LIVKKI QGMQKFLGLE VTGKLDTDTL EVMRKPRCGV PDVGH----- ------FSSF PGMPKWRKTH LTYRIVNYTP D--LPRDAVD
MMP-12      YSGNLMKEKI QEMQHFLGLK VTGQLDTSTL EMMHAPRCGV PDLHH----- ------FREM PGGPVWRKHY ITYRINNYTP D--MNREDVD
MMP-13      ASS--MTERL REMQSFFGLE VTGKLDDNTL DVMKKPRCGV PDVGE----- ------YNVF PRTLKWSKMN LTYRIVNYTP D--MTHSEVE
MMP-20      RGSNSMIRKI KELQAFFGLQ VTGKLDQTTM NVIKKPRCGV PDVAN----- ------YRLF PGEPKWKKNT LTYRISKYTP S--MSSVEVD
MMP-14      SPQ-SLSAAI AAMQKFYGLQ VTGKADADTM KAMRRPRCGV PDKFGAEIKA NVRR--KRYA IQGLKWQHNE ITFCIQNYTP K--VGEYATY
MMP-15      SAQ-ILASAL AEMQRFYGIP VTGVLDEETK EWMKRPRCGV PDQFGVRVKA NLRRRRKRYA LTGRKWNNHH LTFSIQNYTE K--LGWYHSM
MMP-16      SAE-TMQSAL AAMQQFYGIN MTGKVDRNTI DWMKKPRCGV PDQTRGSSKF HIRR--KRYA LTGQKWQHKH ITYSIKNVTP K--VGDPETR
MMP-11      -PQ----PWH AALPSSPAPA PATQEAPRPA SSLRPPRCGV PDPSDG---L SARNRQKRFV LSGGRWEKTD LTYRILRFPW Q--LVQEQVR
MMP-17      QTQEELSKAI TAMQQFGGLE ATGILDEATL ALMKTPRCSL PDLPVLTQAR ---R--RRQA PAPTKWNKRN LSWRVRTFPR DSPLGHDTVR
MMP-18/19   PED--ITEAL RAFQEASELP VSGQLDDATR ARMRQPRCGL EDPFN----- ----QKTLKY LLLGRWRKKH LTFRILNLPS T--LPPHTAR

MMP-2       DAFARAFQVW SDVTPLRFSR IH-------- DGEADIMINF G-RWEHGDGY PFDGKDGLLA HAFAPGTG-V GGDSHFDDDE LWTLGEGQVV
MMP-9       DAFARAFALW SAVTPLTFTR VY-------- SRDADIVIQF G-VAEHGDGY PFDGKDGLLA HAFPPGPG-I QGDAHFDDDE LWSLGKGVVV
MMP-7       RLVSKALNMW GKEIPLHFRK VV-------- WGTADIMIGF A-RGAHGDSY PFDGPGNTLA HAFAPGTG-L GGDAHFDEDE RWTDGSSLGI
MMP-1       HAIEKAFQLW SNVTPLTFTK VS-------- EGQADIMISF V-RGDHRDNS PFDGPGGNLA HAFQPGPG-I GGDAHFDEDE RWTNNFR---
MMP-8       RAIKDAFELW SVASPLIFTR IS-------- QGEADINIAF Y-QRDHGDNS PFDGPNGILA HAFQPGQG-I GGDAHFDAEE TWTNTSA---
MMP-3       SAVEKALKVW EEVTPLTFSR LY-------- EGEADIMISF A-VREHGDFY PFDGPGNVLA HAYAPGPG-I NGDAHFDDDE QWTKDTT---
MMP-10      SAIEKALKVW EEVTPLTFSR LY-------- EGEADIMISF A-VKEHGDFY SFDGPGHSLA HAYPPGPG-L YGDIHFDDDE KWTEDAS---
MMP-12      YAIRKAFQVW SNVTPLKFSK IN-------- TGMADILVVF A-RGAHGDFH AFDGKGGILA HAFGPGSG-I GGDAHFDEDE FWTTHSG---
MMP-13      KAFKKAFKVW SDVTPLNFTR LH-------- DGIADIMISF G-IKEHGDFY PFDGPSGLLA HAFPPGPN-Y GGDAHFDDDE TWTSSSK---
MMP-20      KAVEMALQAW SSAVPLSFVR IN-------- SGEADIMISF E-NGDHGDSY PFDGPRGTLA HAFAPGEG-L GGDTHFDNPE KWTMGTN---
MMP-14      EAIRKAFRVW ESATPLRFRE VPYAYIREGH EKQADIMIFF A-EGFHGDST PFDGEGGFLA HAYFPGPN-I GGDTHFDSAE PWTVRNED--
MMP-15      EAVRRAFRVW EQATPLVFQE VPYEDIRLRR QKEADIMVLF A-SGFHGDSS PFDGTGGFLA HAYFPGPG-L GGDTHFDADE PWTFSSTD--
MMP-16      KAIRRAFDVW QNVTPLTFEE VPYSELENGK -RDVDITIIF A-SGFHGDSS PFDGEGGFLA HAYFPGPG-I GGDTHFDSDE PWTLGNPN--
MMP-11      QTMAEALKVW SDVTPLTFTE VH-------- EGRADIMIDF A-RYWHGDDL PFDGPGGILA HAFFPKTH-R EGDVHFDYDE TWTIGDDQ--
MMP-17      ALMYYALKVW SDIAPLNFHE VAG------- -STADIQIDF S-KADHNDGY PFDARR-HRA HAFFPGHHHT AGYTHFNDDE AWTFRSSD--
MMP-18/19   AALRQAFQDW SNVAPLTFQE VQ-------- AGAADIRLSF HGRQSSYCSN TFDGPGRVLA HADIPELG-- --SVHFDEDE FWTEGTYR--

MMP-2       RVKYGNADGE YCKFPFLFNG KEYNSCTDTG RSDGFLWCST TYNFEKDGKY GFCPHEALFT MGGNAEGQPC KFPFRFQGTS YDSCTTEGRT
MMP-9       PTRFGNADGA ACHFPFIFEG RSYSACTTDG RSDGLPWCST TANYDTDDRF GFCPSERLYT RDGNADGKPC QFPFIFQGQS YSACTTDGRS

MMP-2       DGYRWCGTTE DYDRDKKYGF CPETAMSTVG G-NSEGAPCV FPFTFLGNKY ESCTSAGRSD GKMWCATTAN YDDDRKWGFC
MMP-9       DGYRWCATTA NYDRDKLFGF CPTRADSTVM GGNSAGELCV FPFTFLGKEY STCTSEGRGD GRLWCATTSN FDSDKKWGFC
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MMP-2       PDQGYSLFLV AAHEFGHAMG LEHSQDPGAL MAPIYTYTK- ---NFRLSQD DIKGIQELYG ASPDIDLG-- ---------- -----TGP--
MMP-9       PDQGYSLFLV AAHEFGHALG LDHSSVPEAL MYPMYRFTE- ---GPPLHKD DVNGIRHLYG PRPEPEPRPP TTTTPQPTAP PTVCPTGPPT
MMP-7       -----NFLYA ATHELGHSLG MGHSSDPNAV MYPTYGNGDP --QNFKLSQD DIKGIQKLYG KRSNSRKK-- ---------- ----------
MMP-1       ---EYNLHRV AAHELGHSLG LSHSTDIGAL MYPSYTFSG- ---DVQLAQD DIDGIQAIYG ---------- ---------- ---------R
MMP-8       ---NYNLFLV AAHEFGHSLG LAHSSDPGAL MYPNYAFRET S--NYSLPQD DIDGIQAIYG ---------- ---------- ---------L
MMP-3       ---GTNLFLV AAHEIGHSLG LFHSANTEAL MYPLYHSLTD L-TRFRLSQD DINGIQSLYG PPPDSPETP- ---------- ---------L
MMP-10      ---GTNLFLV AAHELGHSLG LFHSANTEAL MYPLYNSFTE L-AQFRLSQD DVNGIQSLYG PPPASTEEP- ---------- ---------L
MMP-12      ---GTNLFLT AVHEIGHSLG LGHSSDPKAV MFPTYKYVDI N--TFRLSAD DIRGIQSLYG DP-------- ---------- ----------
MMP-13      ---GYNLFLV AAHEFGHSLG LDHSKDPGAL MFPIYTYTGK S--HFMLPDD DVQGIQSLYG P--------- ---------- ----------
MMP-20      ---GFNLFTV AAHEFGHALG LAHSTDPSAL MYPTYKYKNP Y--GFHLPKD DVKGIQALYG PR--KVFLG- ---------- ---------K
MMP-14      -LNGNDIFLV AVHELGHALG LEHSSDPSAI MAPFYQWMDT E--NFVLPDD DRRGIQQLYG GESG------ ---------F PTKMPPQP-R
MMP-15      -LHGNNLFLV AVHELGHALG LEHSSNPNAI MAPFYQWKDV D--NFKLPED DLRGIQQLYG TPDGQPQPT- -------QPL PTVTPRRPGR
MMP-16      -HDGNDLFLV AVHELGHALG LEHSNDPTAI MAPFYQYMET D--NFKLPND DLQGIQKIYG PPDKIPPPT- -------RPL PTVPPHRS-I
MMP-11      ---GTDLLQV AAHEFGHVLG LQHTTAAKAL MSAFYTFRYP ----LSLSPD DCRGVQHLYG QPWPTVTSR- ---------- -TPALGPQAG
MMP-17      -AHGMDLFAV AVHEFGHAIG LSHVAAAHSI MRPYYQGPVG DPLRYGLPYE DKVRVWQLYG VRESVSPTAQ ---------- -------PEE
MMP-18/19   ---GVNLRII AAHEVGHALG LGHSRYSQAL MAPVYEGYRP ---HFKLHPD DVAGIQALYG KKSPVIRDE- ---------- ------EEEE

                                                        *
MMP-2       -----TPTL- GPVTP----- ---------- ---------- --EICKQDIV FDGIAQIRGE IFFFKDRFIW RTVTPR-DKP MGPLLVATFW
MMP-9       VHPSERPTA- GPTGPPSAGP TGP--PTAGP STATTVPLSP VDDACN-VNI FDAIAEIGNQ LYLFKDGKYW RFSEGRGSRP QGPFLIADKW
MMP-1       SQNPVQPI-- GPQTP----- ---------- ---------- --KACDSKLT FDAITTIRGE VMFFKDRFYM RTNPFY-PE- VELNFISVFW
MMP-8       SSNPIQPT-- GPSTP----- ---------- ---------- --KPCDPSLT FDAITTLRGE ILFFKDRYFW RRHPQL-QR- VEMNFISLFW
MMP-3       VPTEPVPP-- EPGTP----- ---------- ---------- --ANCDPALS FDAVSTLRGE ILIFKDRHFW RKSLRK-LE- PELHLISSFW
MMP-10      VPTKSVPS-- GSEMP----- ---------- ---------- --AKCDPALS FDAISTLRGE YLFFKDRYFW RRSHWN-PE- PEFHLISAFW
MMP-12      KENQRLPNP- DNSEP----- ---------- ---------- --ALCDPNLS FDAVTTVGNK IFFFKDRFFW LKVSER-PK- TSVNLISSLW
MMP-13      GDEDPNPK-- HPKTP----- ---------- ---------- --DKCDPSLS LDAITSLRGE TMIFKDRFFW RLHPQQ-VD- AELFLTKSFW
MMP-20      PTLPHAPHH- KPSIP----- ---------- ---------- --DLCDSSSS FDAVTMLGKE LLLFKDRIFW RRQVHL-RTG IRPSTITSSF
MMP-14      TTSRPSVPD- KPKNP----- ---------- --------TY GPNICDGN-- FDTVAMLRGE MFVFKERWFW RVRNNQ-VMD GYPMPIGQFW
MMP-15      PDHRPPRPP- QPPPPGGKPE RPPKPGPPVQ PRATERPDQY GPNICDGD-- FDTVAMLRGE MFVFKGRWFW RVRHNR-VLD NYPMPIGHFW
MMP-16      PPADPRKND- RPKPPRPPTG RPS------- ------YPGA KPNICDGN-- FNTLAILRRE MFVFKDQWFW RVRNNR-VMD GYPMQITYFW
MMP-11      IDTNEIAPL- EPDAP----- ---------- ---------- -PDACEAS-- FDAVSTIRGE LFFFKAGFVW RLRGGQ-LQP GYPALASRHW
MMP-17      PPLLPEPPDN RSSAPPRK-- ---------- ---------D VPHRCSTH-- FDAVAQIRGE AFFFKGKYFW RLTRDRHLVS LQPAQMHRFW
MMP-18/19   TELPTVPPVP TEPSP----- ---------- ---------- MPDPCSSEL- DAMMLGPRGK TYAFKGDYVW TVSDSG---P GPLFRVSALW

MMP-2       PELP---EKI DAVYEAPQEE KAVFFAGNEY WIYSASTLER GYPKPLTS-L GLPPDVQRVD AAFNWSK-NK KTYIFAGDKF WRYNEVKKKM
MMP-9       PALP---RKL DSVFEEPLSK KLFFFSGRQV WVYTGASVLG --PRRLDK-L GLGADVAQVT GALRSGR-GK -MLLFSGRRL WRFDVKAQMV
MMP-1       PQLP---NGL EAAYEFADRD EVRFFKGNKY WAVQGQNVLH GYPKDIYSSF GFPRTVKHID AALSEEN-TG KTYFFVANKY WRYDEYKRSM
MMP-8       PSLP---TGI QAAYEDFDRD LIFLFKGNQY WALSGYDILQ GYPKDISN-Y GFPSSVQAID AAVFYR---S KTYFFVNDQF WRYDNQRQFM
MMP-3       PSLP---SGV DAAYEVTSKD LVFIFKGNQF WAIRGNEVRA GYPRGIHT-L GFPPTVRKID AAISDKE-KN KTYFFVEDKY WRFDEKRNSM
MMP-10      PSLP---SYL DAAYEVNSRD TVFIFKGNEF WAIRGNEVQA GYPRGIHT-L GFPPTIRKID AAVSDKE-KK KTYFFAADKY WRFDENSQSM
MMP-12      PTLP---SGI EAAYEIEARN QVFLFKDDKY WLISNLRPEP NYPKSIHS-F GFPNFVKKID AAVFNPR-FY RTYFFVDNQY WRYDERRQMM
MMP-13      PELP---NRI DAAYEHPSHD LIFIFRGRKF WALNGYDILE GYPKKISE-L GLPKEVKKIS AAVHFED-TG KTLLFSGNQV WRYDDTNHIM
MMP-20      PQLM---SNV DAAYEVAERG TAYFFKGPHY WITRGFQMQ- GPPRTIYD-F GFPRHVQQID AAVYLRE-PQ KTLFFVGDEY YSYDERKRKM
MMP-14      RGLP---ASI NTAYERKD-G KFVFFKGDKH WVFDEASLEP GYPKHIKE-L GRGLPTDKID AALFWMP-NG KTYFFRGNKY YRFNEELRAV
MMP-15      RGLP---GDI SAAYERQD-G RFVFFKGDRY WLFREANLEP GYPQPLTS-Y GLGIPYDRID TAIWWEP-TG HTFFFQEDRY WRFNEETQRG
MMP-16      RGLP---PSI DAVYENSD-G NFVFFKGNKY WVFKDTTLQP GYPHDLIT-L GSGIPPHGID SAIWWED-VG KTYFFKGDRY WRYSEEMKTM
MMP-11      QGLP---SPV DAAFEDAQ-G HIWFFQGAQY WVYDGEKPVL G-PAPLTE-- -LGLVRFPVH AALVWGPEKN KIYFFRGRDY WRFHPSTRRV
MMP-17      RGLPLHLDSV DAVYERTSDH KIVFFKGDRY WVFKDNNVEE GYPRPVSD-- -FSLPPGGID AAFSWAH-ND RTYFFKDQLY WRYDDHTRHM
MMP-18/19   EGLP---GNL DAAVYSPRTQ WIHFFKGDKV WRYINFKMSP GFPKKLNR-- ---VE-PNLD AALYWPL-NQ KVFLFKGSGY WQWDELARTD

                                                                                *
MMP-2       DPGFPKLIAD AWNAIPDNLD AVVDLQGGG- HSYFFKGAYY LKLENQS--L KSVKFGSIKS DWLGC----- ---------- ----------
MMP-9       DPRSASEVDR MFPGVPLDTH DVFQYR--E- KAYFCQDRFY WRVSSRSELN QVDQVGYVTY DILQCPED-- ---------- ----------
MMP-1       DPGYPKMIAH DFPGIGHKVD AVFMKDG--- FFYFFHGTRQ YKFDPKT--- KRILTLQKAN SWFNCRKN-- ---------- ----------
MMP-8       EPGYPKSISG AFPGIESKVD AVFQQEH--- FFHVFSGPRY YAFDLIA--- QRVTRVARGN KWLNCRYG-- ---------- ----------
MMP-3       EPGFPKQIAE DFPGIDSKID AVFEEFG--- FFYFFTGSSQ LEFDPNA--- KKVTHTLKSN SWLNC----- ---------- ----------
MMP-10      EQGFPRLIAD DFPGVEPKVD AVLQAFG--- FFYFFSGSSQ FEFDPNA--- RMVTHILKSN SWLHC----- ---------- ----------
MMP-12      DPGYPKLITK NFQGIGPKID AVFYSKN--K YYYFFQGSNQ FEYDFLL--- QRITKTLKSN SWFGC----- ---------- ----------
MMP-13      DKDYPRLIEE DFPGIGDKVD AVYEKNG--- YIYFFNGPIQ FEYSIWS--- NRIVRVMPAN SILWC----- ---------- ----------
MMP-20      EKDYPKNTEE EFSGVNGQID AAVELNG--- YIYFFSGPKT YKYDTEK--- EDVVSVVKSS SWIGC----- ---------- ----------
MMP-14      DSEYPKNIK- VWEGIPESPR GSFMGSD-EV FTYFYKGNKY WKFNNQKLKV EPGYPKSALR DWMGCPSGGR PDE------- -------GTE
MMP-15      DPGYPKPIS- VWQGIPASPK GAFLSND-AA YTYFYKGTKY WKFDNERLRM EPGYPKSILR DFMGCQEHVE PGPRWPDVAR PPFNPHGGAE
MMP-16      DPGYPKPIT- VWKGIPESPQ GAFVHKE-NG FTYFYKGKEY WKFNNQILKV EPGYPRSILK DFMGCDGPTD RVKEG----- -------HSP
MMP-11      DSPVPRRAT- DWRGVPSEID AAFQDADG-- YAYFLRGRLY WKFDPVKVKA LEGFPRLVGP DFFGCAEPAN TFL------- ----------
MMP-17      DPGYPAQSP- LWRGVPSTLD DAMRWSDG-- ASYFFRGQEY WKVLDGELEV APGYPQSTAR DWLVCGDSQA DGSVAAG--- ------VDAA
MMP-18/19   FSSYPKPIKG LFTGVPNQPS AAMSWQD--G RVYFFKGKVY WRLNQQLR-V EKGYPRNISH NWMHCRPRTI DTTPSGGNTT PSGTGITLDT

MMP-14      EETEVIIIEV DE-------- ---EGGG--- --------AV SAAAVVLPVL LLLLVLAVGL AVFFFRRHGT PRRLLYCQRS LLDKV
MMP-15      PGADSAEGDV GDGDGDFGAG VNKDGGSRVV VQMEEVARTV NVVMVLVPLL LLLCVLGLTY ALVQMQRKGA PRVLLYCKRS LQEWV
MMP-16      PDDVDIVIKL DN-------- ---TAS---- --------TV KAIAIVIPCI LALCLLVLVY TVFQFKRKGT PRHILYCKRS MQEWV
MMP-17      EGPRAPPGQH DQSRSEDGYE VCSCTSGASS PPGAPGPLVA ATMLLLLPPL SPGALWTAAQ ALTL------ ---------- -----
MMP-18/19   TLSATETTFE Y--------- ---------- ---------- ---------- ---------- ---------- ----------
```

very rigid due to the presence of two disulfide bonds nearby, and this may contribute significantly to the very tight interactions between inhibitor and enzyme (K_Ds range down to 10–50 pM). Two of the TIMPs are known to bind tightly to particular latent MMPs: TIMP-2 binds tightly to proMMP-2 and TIMP-1 binds tightly to proMMP-9. The precise significance of these relationships is unclear, but TIMP-2 may be necessary for MMP-2 to bind to MMP-14 for the purpose of activation [59].

```
                                                1  2           3
TIMP-1  ------MAPF EPLASGILLL LWLIAPSRAC TCVPPHPQTA FCNSDLVIRA
TIMP-2  ---MGAAART LRLALGLLLL ATLLRPADAC SCSPVHPQQA FCNADVVIRA
TIMP-3  -----MTPWL G-LIVLLGSW SLGDWGAEAC TCSPSHPQDA FCNSDIVIRA
TIMP-4  MPGSPRPAPS WVLLLRLLAL LRPPGLGEAC SCAPAHPQQH ICHSALVIRA


TIMP-1  KFMGSPEINE -------TTL YQRYKIKMTK MLKGFKAVGN AADIRYAYTP
TIMP-2  KAVSEKEVDS GNDIYGNPIK RIQYEIKQIK MFKGPE---- -KDIEFIYTA
TIMP-3  KVVGKKLVKE ------GPFG TLVYTIKQMK MYRGFTKM-- -PHVQYIHTE
TIMP-4  KISSEKVVPA SA-DPADTEK MLRYEIKQIK MFKGFEKV-- -KDVQYIYTP

             1                                  2
TIMP-1  VMESLCGYAH KSQNRSEEFL ITGRLR-NGN LHISACSFLV PWRTLSPAQQ
TIMP-2  PSSAVCGVSL DVGG-KKEYL IAGKAEGDGK MHITLCDFIV PWDTLSTTQK
TIMP-3  ASESLCGLKL EVN--KYQYL LTGRVY-DGK MYTGLCNFVE RWDQLTLSQR
TIMP-4  FDSSLCGVKL EANS-QKQYL LTGQVLSDGK VFIHLCNYIE PWEDLSLVQR

                   3  4    5     5        6
TIMP-1  RAFSKTYSAG CGVCTVFPCL SIPCKLESDT HCLWTDQVLV GSED-YQSRH
TIMP-2  KSLNHRYQMG CE-CKITRCP MIPCYISSPD ECLWMDWVTE KNINGHQAKF
TIMP-3  KGLNYRYHLG CN-CKIKSCY YLPCFVTSKN ECLWTDMLSN FGYPGYQSKH
TIMP-4  ESLNHHYHLN CG-CQITTCY TVPCTISAPN ECLWTDWLLE RKLYGYQAQH

          6        4
TIMP-1  FACLPRNPGL CTWRSLGAR- ----------
TIMP-2  FACIKRSDGS CAWYRGAAPP KQEFLDIEDP
TIMP-3  YACIRQKGGY CSWYRGWAPP DKSIINATDP
TIMP-4  YVCMKHVDGT CSWYRGHLPL RKEFVDIVQP
```

Fig. 3 Aligned sequences of the known human TIMPs. The four known human TIMPs have been aligned to show areas of homology. The numbers above the sequence mark pairs of cysteine residues that participate in disulfide bonds

MMPs have been associated with the invasive and metastatic behavior of essentially all types of malignancies. However, an exhaustive review of all the literature is beyond the scope of this paper; therefore it focuses on both the typical and novel roles these enzymes play in tumor cell invasion, using MMP-2 and TIMP-2 as the archetypal MMP and inhibitor.

Role 1: Removal of physical barriers to invasion

The classic role of ECM-degrading enzymes is to degrade components of the ECM. The ECM is not a static collection of structural proteins that merely provide compartmentalization, but a dynamic matrix of structural proteins, growth factors, and latent enzymes which affects the way cells interact with each other and with the matrix. Basement membranes and other matrix structures undergo constant remodeling and MMPs have a central function in maintaining the integrity of the ECM by removing undesired proteins. As a group, MMPs can degrade essentially all of the structural components of the ECM (Table 1). Degradation of the ECM, and in particular the basement membrane, is viewed as one of the critical phenotypes necessary for tumor cell invasion. It was the search for a protease capable of degrading basement membrane collagens which led Liotta and colleagues to identify MMP-2, then known as the 72-kDa type IV collagenase for its ability to degrade native type IV collagen [32, 49].

Experimentally, the necessity for protease action in tumor cell invasion can be demonstrated using the chemoinvasion assay. This assay is a variation of the Boyden chamber chemotaxis assay where the porous membrane separating the two chambers is coated with ECM proteins, either purified or in a complex mixture such as Matrigel. Invasion of cells such as HT 1080 fibrosarcoma cells can be enhanced by the addition of active MMP-2 or inhibited by zinc-chelating agents, TIMP-2, or antibodies that have MMP-2-inhibiting activity [2]. In vitro evidence of the importance of MMP-2 and other MMPs in removing ECM barriers to invasion has been supplemented by in vivo assays and correlative studies. In a pair of bladder cancer transfection studies, Kawamata et al. have shown that transfection of MMP-2 into MYU3L cells enhances their metastatic potential, while transfection of TIMP-2 into the highly metastatic LMC19 cell line reduces its metastatic potential [24, 25]. In correlative studies done using clinical tumor tissue samples from patients, the presence of greater amounts of activated MMP-2 has been associated with clinically aggressive breast and lung carcinomas [6, 10]. Studies such as these have led to the simplistic conclusion that as the ratio of active MMPs to TIMPs increases, the associated tumors or tumor cell lines have increased metastatic potential. However, while malignant tumors do induce greater local degradation of the ECM, the role of MMPs in tumor cell invasion and metastasis is more complicated than a "more is better" paradigm would suggest.

Role 2: Modulation of cell adhesion by MMPs

Any scientist who has detached cells from a tissue culture plate using trypsin knows that protease action can have dramatic affects on cell adhesion. However, to demonstrate that MMPs may affect cell adhesion in a physiologically relevant system is a complex undertaking. The first experiments in this area focused on the addition of exogenous agents to a tissue culture system and observing changes in numbers of adherent cells. Anti-MMP-2 antibodies with inhibitory action were able to increase adhesion of A2058 cells to tissue culture plastic dramatically. Using A2058 cells stably transfected with retroviral TIMP-2 sense and antisense constructs, Ray et al. were able to demonstrate increased adhesion of cells with increased secretion of TIMP-2 (sense transfectants) and reduced adhesion with decreased secretion of TIMP-2 (antisense transfectants) [48]. However, both sense and antisense transfectants showed reduced motility in a chemotaxis assay, which could be corrected by the addition of MMP-2 or TIMP-2 to bring the ratio of enzyme to inhibitor closer to that of the parent cell line. Ray and colleagues hypothesized that since attachment to matrix is a necessary part of cell migration, cells which attached poorly were less motile and cells which attached too tightly were unable to detach and move. Thus because an optimal invasive phenotype requires cells to be able to attach, detach, and move on the ECM, the balance between active MMP-2 and available TIMP-2 is critical. Either too much or too little degradation can adversely affect the invasive phenotype.

Role 3: Protease action by MMPs reveals hidden activities of proteins

In the activation of the complement cascade during inflammation, two of the complement factors, C3 and C5, are cleaved by convertases into major and minor components. While the major components bind to cell membranes and ultimately lead to cell killing, the minor components have chemotactic and immunostimulatory capabilities that are important for the inflammatory process to proceed. Thus proteolytic cleavage has uncovered hidden properties of C3 and C5.

Similarly, MMPs have recently been shown to uncover hidden properties of two proteins, plasminogen and laminin-5. In 1994, Folkman and coworkers isolated angiostatin, a protein with powerful antiangiogenic properties [40]. This protein was identified as part of a search for factors secreted by primary tumors that had the ability to inhibit the growth of metastases. Sequencing of angiostatin revealed it to be a fragment of plasminogen without proteolytic activity. Subsequently, MMP-3, -7, -9, and -12 have all been shown to degrade plasminogen into angiostatin-like fragments [11, 28, 44]. Thus MMPs may have significant affects on angiogenesis through mechanisms other than those of direct basement membrane degradation by endothelial cells.

Another hidden activity revealed by MMP action was discovered during an investigation into the mechanism of haptotaxis. Haptotaxis is the phenomenon in which apparently insoluble proteins may attract cells at a distance. Giannelli and coworkers studied the migration of tumor cells on laminin-5 in the presence of different proteases and discovered that a soluble fragment of laminin-5 was the actual chemoattractant [14]. Further investigation showed that of the proteases tested only active MMP-2 was able to stimulate chemoattraction by specifically cleaving laminin-5 between the long and short arms of the $\gamma 2$ chain. Using immunoblots they demonstrated the presence of cleaved laminin-5 in tissues undergoing both physiological and pathological tissue remodeling, but not in quiescent tissues. Thus MMP-2 was shown to uncover a hidden activity of a structural ECM protein that may be critical in attracting inflammatory cells or in the autostimulation of tumor cells to migrate.

Clinical relevance of MMPs and conclusions

This discussion has focused on the activities of one of the MMP family members to demonstrate the varied roles that this enzyme alone may fill. MMPs are clearly important enzymes in normal and abnormal processes involving ECM remodeling or degradation. They have been implicated as key proteases for the removal of ECM barriers to cell migration. Increased MMP activity appears to be a necessary part of tumor cell invasion and metastasis, but because MMPs may have direct actions on cell adhesion and migration the amount of ECM degradation is probably tightly controlled by balancing the available amounts of active proteases and inhibitors. In addition, the presence of membrane-bound MMPs suggests the ability of cells to regulate tightly the spatial dimension of degradation. Finally, recent work in several laboratories has demonstrated the ability of MMPs to uncover heretofore unknown activities of both proenzymes, in the case of plasminogen, and structural ECM proteins, in the case of laminin-5.

However, all of these basic studies would have little meaning for oncologists or cancer patients if we could not take advantage of this knowledge in a clinical setting. Numerous clinical and pathological studies have demonstrated an increase in particular MMPs or TIMPs in various solid tumors. Although the pattern of enzyme expression may vary from tumor to tumor, the general observation is that particular MMPs have increased activity and expression in tumor cells and peritumoral stromal cells and that increased levels of TIMP expression also occur, generally in stromal cells and in the desmoplastic ECM around tumors. Recent clinical studies have focused more on the prognostic significance of changes in MMP or TIMP levels and on the testing of inhibitors of MMPs as potential antineoplastic agents.

As prognostic markers, Gohji and coworkers have examined the utility and predictive value of measuring the MMP-2/TIMP-2 ratio in the sera of patients with invasive bladder cancer [15, 17]. Patients with high ratios of MMP-2 to TIMP-2 have earlier recurrence and more aggressive disease courses. Compatible with this finding is the observation that high expression of MMP-2, TIMP-2, and MMP-14 (an activator of MMP-2) in primary invasive bladder cancers is also predictive of poor survival [20]. The prognostic importance of MMP-2 in prostate cancer [16], MMP-11 in breast cancer [1], and MMP-2 and -9 in gastric cancer [56] has also been described. Elevated TIMP levels have been associated with poor prognosis in bladder and gastric cancer [20, 38]. These studies suggest that MMPs and TIMPs might not only be good targets for antineoplastic therapy but may have clinical utility in identifying subgroups of patients at increased risk for recurrence. Future trials might be designed to take advantage of the prognostic information provided by these markers of biologic aggressiveness.

In summary, the MMPs are significant for the multiple roles they play in the process of tumor cell invasion and metastasis, affecting all three of the key phenotypes of the invasive process: ECM degradation; attachment to ECM components; and cellular motility. In addition, by degradation of ECM or other proteins they may release peptide fragments with hidden cytokine properties. Finally, many studies have demonstrated the relevance of MMPs to virtually every neoplastic process and some measures of MMP activity may have independent prognostic value. For all of these reasons, pharmacological intervention which seeks to modulate the activity of MMP action may have significant effects on the ability of tumor cells to spread within the body.

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