Membrane-type matrix metalloproteinases in pericellular proteolysis and melanoma cell invasion

Olga Tatti

Departments of Pathology and Virology,
Haartman Institute and
Helsinki University Hospital,
and
Translational Cancer Biology Research Program,
Biomedicum Helsinki,
University of Helsinki
Finland

ACADEMIC DISSERTATION

To be publicly discussed with the permission of the Faculty of Medicine, University of Helsinki, in Auditorium 3, Biomedicum Helsinki, Haartmaninkatu 8, on May 31st 2013, at 12 o'clock

Helsinki 2013

Supervised by:

Docent Kaisa Lehti, Ph.D.
Genome-Scale Biology Research Program
Biomedicum Helsinki
University of Helsinki
Helsinki, Finland

and

Professor Jorma Keski-Oja, M.D., Ph.D
Departments of Pathology and Virology, Haartman Institute
Helsinki University Hospital
Translational Cancer Biology Research Program
Biomedicum Helsinki
University of Helsinki
Helsinki, Finland

Reviewed by:

Professor Tuula Salo, D.D. Sc.
Department of Diagnostics and Oral Medicine
Institute of Dentistry
University of Oulu
Oulu, Finland

and

Docent Sanna Pasonen-Seppänen, Ph.D.
Institute of Biomedicine
University of Eastern Finland
Kuopio, Finland

Opponent:

Professor Cornelia Mauch, M.D., Ph.D., Dr. rer. nat.
Department of Dermatology and Venereology
University of Cologne
Cologne, Germany

Contents

ORIGINAL PUBLICATIONS

This thesis is based on the following original publications which are referred to by their roman numerals in the text:

I.   **Tatti O.**, Vehviläinen P., Lehti K. and Keski-Oja J.: MT1-MMP releases latent TGF-β from endothelial cell extracellular matrix via proteolytic processing of LTBP-1. Exp Cell Res. 314**,** 2501-2514, 2008.

II.   **Tatti O.**, Arjama M., Ranki A., Weiss SJ., Keski-Oja J. and Lehti K.: Membrane-type-3 matrix metalloproteinase (MT3-MMP) functions as a matrix composition-dependent effector of melanoma cell invasion. PLoS One. 6(12):e28325, 2011.

III.   **Tatti O.**, Pekkonen P., Holopainen T., Maliniemi P., Lohi J., Rantanen V., Hautaniemi S., Ranki A., Alitalo K., Ojala P., Keski-Oja J. and Lehti K.: Membrane-type-3 matrix metalloproteinase controls a proteolytic switch between lymphatic and blood vascular invasion of melanoma cells. Manuscript submitted, 2013.

ABBREVIATIONS

3D	3-dimensional
ADAM	adamalysin-related metalloproteinase
ADAMTS	ADAM with thrombospondin type I motifs
APP	amyloid precursor protein
BEC	blood endothelial cell
BM	basement membrane
BMP	bone morphogenic protein
BVI	blood vessel invasion
CAM	chorioallantoic membrane
cDNA	complementary deoxyribonucleic acid
C-terminal	carboxy terminal
DNA	deoxyribonucleic acid
EC	endothelial cell
ECM	extracellular matrix
Eph	erythropoietin-producing hepatocellular carcinoma tyrosine kinase
EMT	epithelial to mesenchymal transition
EGF	epidermal growth factor
ER	endoplasmic reticulum
ERK	extracellular signal-regulated kinase
FGF	fibroblast growth factor
FGFR	FGF receptor
GAG	glycosaminoglycan
GAPDH	glyceraldehyde-3-phosphate dehydrogenase
GPI	glycosyl-phosphatidyl inositol
HGF	hepatocyte growth factor
HUVEC	human umbilical vein endothelial cells
IGF	insulin-like growth factor
kDa	kilodalton
L1CAM	neural cell adhesion molecule L1
LAP	latency associated peptide
LEC	lymphatic endothelial cell
LLC	large latent complex
LTBP	latent TGF-β binding protein
LVI	lymphatic vessel invasion
LYVE-1	lymphatic vessel hyaluronan receptor-1
MAGP	microfibril associated glycoprotein
MAPK	mitogen activated protein kinase
MEK	MAPK kinase
MEM	minimum essential medium
MCAM	melanoma cell adhesion molecule
MMP	matrix metalloproteinase
mRNA	messenger RNA
MT-MMP	membrane-type matrix metalloproteinase

NC	noncollagenous domain
NM	nodular melanoma
N-terminal	amino terminal
PAGE	polyacrylamide gel electrophoresis
PAR-1	protease-activated receptor-1
PBS	phosphate buffered saline
PCR	polymerase chain reaction
PFA	paraformaldehyde
PG	proteoglycan
PI-3K	phosphatidylinositol-3-kinase
PKC	protein kinase C
PMA	phorbol 12-myristate 13-acetate
RNA	ribonucleic acid
Prox-1	prospero-related homeobox-1
RGP	radial growth phase of melanoma
RT-PCR	reverse transcriptase PCR
SDS	sodium dodecyl sulfate
shRNA	short hairpin RNA
SLC	small latent complex
SLN	sentinel lymph node
SSM	superficially spreading melanoma
TGF-β	transforming growth factor-β
TβRI	TGF-β receptor I
TIMP	tissue inhibitor of metalloproteinases
TNF-α	tumor necrosis factor-α
uPA	urokinase type plasminogen activator
uPAR	uPA receptor
UV	ultraviolet
VEGF	vascular endothelial growth factor
VEGFR	VEGF receptor
VGP	vertical growth phase of melanoma
ZO-1	zonula occludens-1

ABSTRACT

Tumor microenvironment comprised of extracellular matrix (ECM) and non-malignant cells has profound effects on cancer progression. Membrane-type matrix metalloproteinases (MT-MMPs) expressed by both tumor and stroma cells are involved in the modulation of tumor microenvironment and thereby regulate tumor cell proliferation, invasion and dissemination. MT1-MMP is a prototype of MT-MMP family, which is overexpressed in many types of cancer, where it promotes tumor cell invasion through collagen-rich tissues. The biological functions of another member of the MT-MMP family, MT3-MMP, have remained largely unknown. Unlike MT1-MMP, MT3-MMP cannot cleave native collagen type I. MT3-MMP is expressed in the adult brain, as well as various cancer types such as brain tumors and nodular melanoma. The purpose of this thesis was to elucidate the functions of MT1-MMP and MT3-MMP in melanoma cell invasion. To understand the pericellular growth regulation, we searched for endogenous enzymes which could release latent TGF-β from endothelial cell extracellular matrix.

Neovessel formation (angiogenesis) is a prerequisite for tumor growth. Pericellular modulation of the ECM by MT1-MMP releases matrix-associated growth factors and bioactive peptides, which further affect angiogenesis and tumor cell biology. We found that MT1-MMP mRNA expression and activity were induced after morphological activation of endothelial cells, which mimics the initial phases of angiogenesis. MT1-MMP modulated subendothelial extracellular matrix, and cleaved latent TGF-β binding protein-1 (LTPB-1), with subsequent release of latent TGF-β complexes from the ECM. TGF-β can both promote and inhibit endothelial cell proliferation, and the opposing effects of TGF-β depend on its concentration. Thus, MT1-MMP-mediated LTBP-1 cleavage provides a mechanism for the tightly controlled release of matrix-associated TGF-β at the sites of neovessel formation.

To elucidate the functions of MT-MMPs in melanoma cell invasion, we analyzed the expression of MT1-MMP and MT3-MMP from biopsies of normal human skin, benign nevi, and melanoma metastases. MT3-MMP was upregulated in lymph node metastases of human melanoma, while MT1-MMP expression was comparable in all biopsies. By culturing melanoma cells in 3D collagen and fibrin matrices, we found that MT3-MMP was associated with expansive melanoma growth in 3D collagen, but promoted their sprouting growth in 3D fibrin. In *in vivo* xenograft experiments, MT3-MMP expressing melanoma xenografts grew slowly, while MT3-MMP silencing enhanced tumor growth rate by over twofold. Interestingly, high MT3-MMP expression in murine xenografts and a human melanoma tumor was associated with prominent lymphatic vessel invasion but negligible blood vessel invasion of melanoma cells. Silencing of MT3-MMP reduced lymphatic invasion but facilitated blood vessel invasion of melanoma cells over ~10-fold. MT3-MMP reduced cell surface MT1-MMP *in vitro* and *in vivo*, resulting in limited collagen invasion in vitro and collagen accumulation *in vivo*. This suggested that low collagenolytic ability of MT3-MMP-expressing melanoma cells resulted in decreased blood vascular invasion. These cells invaded instead into more permissive lymphatic vessels. Since lymphatic vessel invasion is associated with metastatic spread in melanoma, MT3-MMP expression may serve as a new prognostic factor in this disease.

TIIVISTELMÄ

Kasvaimet koostuvat syöpäsolujen lisäksi soluväliaineesta ja useista muista solutyypeistä, jotka myötävaikuttavat syövän kasvuun. Solukalvoon kiinnittyneet matriksin metalloproteinaasit (MT-MMP:t), jotka ilmentyvät sekä syöpäsoluissa että muissa strooman soluissa, pilkkovat solukalvon ja soluväliaineen proteiineja. Näin ollen ne muokkaavat syöpäsolujen proliferaatiota ja invaasiota. MT1-MMP on tämän entsyymiperheen prototyyppi. Se osallistuu luuston ja verisuoniston kehitykseen sekä kollageenin normaaliin kiertoon aikuisessa elimistössä. MT1-MMP:tä ilmennetään monessa syövässä, joissa se lisää syöpäsolujen invaasiota kollageenipitoisten kudosten läpi. Tämä tehostaa etäpesäkkeiden muodostumisen. MT3-MMP on MT1-MMP:n läheinen homologi, joka ei pysty pilkkomaan tyypin I kollageenia, mutta sen sijaan pilkkoo fibriiniä. Sitä ilmennetään aikuisen aivoissa ja muutamissa syöpätyypeissä, kuten aivokasvaimissa ja nodulaarisessa melanoomassa. Väitöskirjatyöni tarkoituksena on ollut selvittää MT1-MMP:n ja MT3-MMP:n vaikutuksia melanoomasolujen invaasioon. Tähän liittyen selvitimme mitkä endogeeniset entsyymit voivat vapauttaa latenttia transformoivaa kasvutekijä-betaa (TGF-β) endoteelisolujen soluväliaineesta.

Soluväliaineen proteiinien pilkkominen voi vapauttaa niihin kiinnittyneitä kasvutekijöitä ja muita bioaktiivisia peptidejä. Tuloksemme osoittavat että MT1-MMP pilkkoo latenttia TGF-β:aa sitovaa proteiinia (LTBP-1) verisuonia verhoavien, eli endoteelisolujen, soluväliaineesta. LTBP-1 sitoo TGF-β:aa ja kiinnittyy soluväliaineen proteiineihin. LTBP-1:n pilkkominen vapauttaa TGF-β -komplekseja soluväliaineesta, mikä myötävaikuttanee tämän latentin kasvutekijän aktivaatioon. TGF-β:n vaikutukset muodostuvaan verisuontistoon riippuvat sen pitoisuudesta. TGF-β:n vapauttaminen ja aktivoiminen soluväliaineesta MT1-MMP:n välityksellä voi säädellä uusien verisuonien muodostumista.

Melanooma on yleistymässä oleva ihosyöpätyyppi, ja levinneen melanooman hoitomahdollisuudet ovat rajalliset. MT3-MMP:aa ilmennetään nodulaarisessa melanoomassa, joka on huonoennusteisin melanoomatyyppi. Analysoimme tässä MT3-MMP:n vaikutuksia melanoomasolujen invaasiotapahtumaan käyttäen *in vitro* invaasiokokeita ja hiirikokeita. MT3-MMP:n ilmentyminen oli lisääntynyt ihmisen melanooman imusolmuke-etäpesäkkeissä. Melanoomasolujen kasvattaminen kolmiulotteisissa geeleissä osoitti, että MT3-MMP esti solujen invasiivista kasvua kollageenissa edistämällä pyöreiden pesäkkeiden kasvua. Fibriinissä MT3-MMP sen sijaan edisti invasiivista kasvua. Vastaavasti MT3-MMP:tä ilmentävät melanoomaksenograftit kasvoivat hitaasti hiirten subkutaanisissa kudoksissa, kun taas MT3-MMP:n hiljentäminen nopeutti niiden kasvua noin kaksinkertaiseksi. MT3-MMP vähensi solupinnalla olevan MT1-MMP:n tasoja *in vitro* ja *in vivo*, mikä johti vähentyneeseen kollageenin pilkkomiseen ja kollageenin määrän nousuun kasvainten sisällä. Havaitsimme MT3-MMP:tä ilmentävissä ksenografteissa ja ihmisen melanoomassa kasvainsolujen voimakkaan invaasion imusuoniin. MT3-MMP:n hiljentäminen puolestaan esti imusuoni-invaasiota ja lisäsi verisuoni-invaasiota noin kymmenkertaiseksi. Ihmisen melanoomissa, matala MT1-MMP ja korkea MT3-MMP liittyivät melanoomasolujen kohesiiviseen kasvuun pyöreinä pesäkkeinä, kun taas korkea MT1-MMP yhdistettynä matalaan MT3-MMP:hen liittyi diffuusiin invasiiviseen kasvuun. Koska melanoomasolujen imusuoni-invaasio liittyy etäpesäkkeiden muodostumiseen, MT3-MMP:n ilmentyminen voi osoittautua melanooman huonon ennusteen merkkiaineeksi.

АННОТАЦИЯ

Опухолевая микросреда состоит из внеклеточного матрикса и нераковых клеток, которые влияют на развитие рака. Мембрано-ассоциируемые матриксные металлопротеиназы (МТ-ММП) являются протеолитическими ферментами, способствующими метастазированию раковых клеток. МТ1-ММП экспрессируется во многих видах опухолей. В отличие от МТ1-ММП, МТ3-ММП не способен расщеплять коллаген I-типа. Этот фермент экспрессируется в тканях головного мозга человека, а так же в некоторых видах опухолей, таких как рак головного мозга и узловая меланома. В этой диссертации была исследована роль МТ1-ММП и МТ3-ММП в инвазии клеток меланомы. Также были изучены ферменты способствующие освобождению трансформирующего ростового фактора бета (ТРФбета-1) из внеклеточного матрикса эндотелиальных клеток.

Образование новых сосудов является необходимым условием для роста опухоли. МТ1-ММП модулирует внеклеточный матрикс, освобождая из него факторы роста и биологически активные пептиды, которые в свою очередь влияют на развитие кровеносных сосудов и рост раковых клеток. Мы обнаружили, что МТ1-ММП освобождает ТРФбета-1 из внеклеточного матрикса посредством расщепления латентного ТРФбета-связывающего белка (LTBP-1), с которым ТРФбета-1 ассоциирован. ТРФбета-1 может как индуцировать, так и ингибировать пролиферацию эндотелиальных клеток, и эти противоположные эффекты ТРФбета-1 зависят от его концентрации. Таким образом, МТ1-ММП с помощью расщепления LTBP-1 регулирует концентрацию активного ТРФбета в местах образования новых сосудов.

Для выяснения функций МТ3-ММП в инвазии клеток меланомы, мы проанализировали экспрессию МТ3-ММП в биопсиях здоровой кожи человека, доброкачественных родинок, и метастаз меланомы. Экспрессия МТ3-ММП была повышена в метастазах меланомы в лимфатических узлах. Путем культивирования клеток меланомы в 3-х мерном коллагене и фибрине мы обнаружили, что МТ3-ММП ингибировал инвазию клеток меланомы в коллагене, но способствовал их инвазии в фибрине. В привитых мышам опухолях меланомы, опухоли экспрессирующие МТ3-ММП росли в 2 раза медленнее чем опухоли, из которых МТ3-ММП был удален. Любопытно, что экспрессия МТ3-ММП была связана со значительной инвазией клеток меланомы в лимфатические сосуды, а удаление МТ3-ММП привело к инвазии в кровеносные сосуды. МТ3-ММП значительно уменьшил количество МТ1-ММП на клеточной мембране клеток меланомы, что привело к замедлению инвазии в 3-х мерном коллагене *in vitro* и аккумуляции коллагена *in vivo*. Таким образом, в данной работе показано, что клетки меланомы экспрессирующие МТ3-ММП обладают слабой способностью расщеплять коллаген, что приводит к неэффективной инвазии в кровеносные сосуды. Тем не менее, эти клетки растут расширяющимися колониями и способны эффективно инвазировать в лимфатические сосуды с более тонкими стенками. Ввиду того, что инвазия лимфатических сосудов связана с метастазированием меланомы, МТ3-ММП может послужить новым маркером для определения прогноза меланомы и выявления пациентов нуждающихся в агрессивной терапии уже на ранних стадиях.

REVIEW OF THE LITERATURE

1. Introduction

Cancer cells utilize matrix metalloproteinases (MMPs) to grow, traverse interstitial tissue, and disseminate to distant organs via lymphatic or blood vessels. In order to get access to oxygen and nutrients, growing tumors induce new blood vessel formation (angiogenesis), a process where MMPs are also involved. Membrane-type matrix metalloproteinases (MT-MMPs) are cell surface proteases which mediate both pericellular proteolysis and cleavage of other cell surface proteins. Malignant melanoma is one of the numerous cancer types in which MT-MMPs are associated with the disease progression. MT1-MMP is specifically expressed at the invading edge of melanoma tumors, and is linked to metastatic form of the disease. MT3-MMP is overexpressed in the most aggressive melanoma subtype, nodular melanoma. Since metastatic form of melanoma is highly resistant to current treatment modalities, it is essential to unravel the factors which lead to the acquirement of the metastatic ability by melanoma cells. Furthermore, new prognostic markers which can be assessed from the primary tumor and can predict metastatic progression are needed in order to more accurately select patients who would benefit from sentinel lymph node biopsy and early aggressive treatment. In this thesis we analysed the functions of MT1-MMP in endothelial cells, and assessed the functions of MT3-MMP in melanoma cell invasion, growth and intravasation. These results will help to understand the role of these MT-MMPs in pericellular proteolysis, growth factor release from the extracellular matrix, and melanoma progression.

2. Melanoma

2.1 Classification

Malignant melanoma is not the most frequent skin cancer, but beyond doubt the most lethal one. Every year ~700 Finns acquire this disease. The incidence of melanoma is rising around the world. From 1953 through 2003, the incidence of skin melanoma increased in Finland from 1.5 to 12.8 per 100,000 among men and from 1.8 to 10.4 per 100,000 among women (Stang et al., 2006). The median age of patients at diagnosis is about 57, which is 10 years less than that of cancer patients in general (Rager et al., 2005). The mean 5-year survival of melanoma patients in Finland is 83% for men and 88% for women (http://www.cancer.fi/syoparekisteri/tilastot/).

The four most common clinicopathological subtypes of cutaneous melanoma are superficially spreading melanoma (SSM, 70%), nodular melanoma (NM, 15%), lentigo maligna (13%), and acral lentiginous melanoma (2-3%) (Bradford et al., 2009). SSM grows first radially in the epidermis (radial growth phase, Breslow index <0.75 mm, Fig. 1), gradually gaining the ability to grow vertically into the dermis and further metastasize

(vertical growth phase, Fig. 1). Histologically SSM is characterized by single cells or nests of atypical melanocytes seen in all layers of epidermis and dermis, with no sharp lateral margins (Smoller, 2006). Mitotic activity, lack of full maturation, vesicular nuclei with prominent nucleoli, and inflammatory response are features which help to differentiate malignant melanoma of this type from benign nevi (Smoller, 2006). ABCD-criteria (asymmetry, border, color, and diameter) have been introduced to help the diagnosis of SSM. NM grows in the dermis, often pushing epidermis up forming a clear nodule, and is frequently amelanotic (Chamberlain et al., 2002). This subtype lacks radial growth phase. Instead, these tumors invade deeper dermis at the early stage, and have often metastasized at the time of diagnosis. The diagnosis of NM might be delayed due to the lack of typical ABCD-features of melanoma (Lallas et al., 2012). Therefore, additional EFG-criteria (elevation, firmness, and growth) have been introduced, but their diagnostic specificity remains unknown (Kelly et al., 2003). The presence of both blue and black regions as observed by dermoscopy may predict the malignant nature of the nodular tumor (Lallas et al., 2012). Usually NM affects middle-aged people, men more often than women, and occurs most frequently on trunk (Smoller, 2006). In contrast to SSM, these tumors are sharply circumscribed (Smoller, 2006).
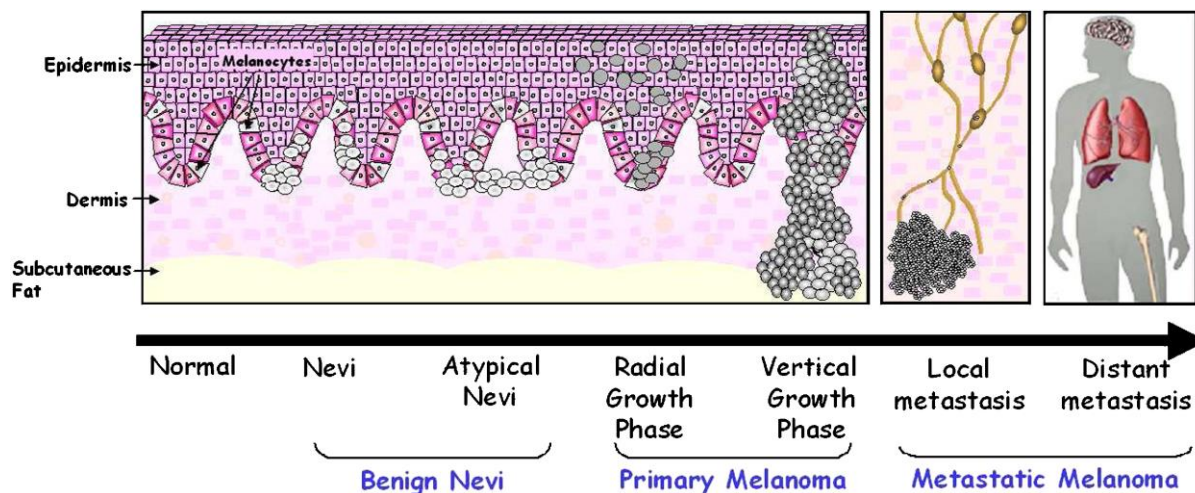
Figure 1. Phases of melanoma progression. Modified from Gremel et al., 2009.

2.2 Staging and prognosis

In 1970 Alexander Breslow developed a still widely used method for estimating melanoma prognosis by measuring tumor thickness under the microscope (Breslow, 1970). Breslow thickness is the most significant feature of primary tumor which affects survival (Balch et al., 2009). The next important predictors are the number of mitoses and ulceration status. Thus, 10-year survival for T1 (≤1 mm) non-ulcerated melanoma is 92%, while for T4 melanoma (>4 mm) it is ~40% or ~55%, depending on the ulceration status (Balch et al., 2009). Breslow thickness also predicts sentinel lymph node metastasis. Thus, in T1 melanoma 1% of patients

and in T4 melanoma 35.5% of patients have micrometastasis in the sentinel lymph nodes (Lens et al., 2002).

The 7th version of the TNM classification and staging system introduced by American Joint Committee on Cancer (AJCC) is presented in Table 1 (Balch et al., 2009). When the disease has spread, the stage is the most powerful prognostic factor (Balch et al., 2009). Staging is defined by combining tumor thickness, ulceration status, mitosis rate per mm^2, involvement of regional and distant lymph nodes, as well as the presence of distant metastases. The most powerful predictors for prognosis of spread disease (stage III) are the number of metastatic lymph nodes, micro- vs. macrometastasis, Breslow thickness, and ulceration status (Balch et al., 2009).

Melanoma metastases are detected first in the sentinel lymph node, which is the first lymph node receiving lymphatic liquid from the area of the tumor. Sentinel lymph node mapping and biopsy has been introduced in 1992, and is currently a common practice worldwide (Morton et al., 1992). Sentinel lymph node biopsy is the most important prognostic procedure in melanoma patients with T2-4 primary tumors (Vuylsteke et al., 2003; Yee et al., 2005). Thus, 5-year survival of SLN-negative patients is 90-92%, and for SLN-positive – 56-67% (Vuylsteke et al., 2003; Yee et al., 2005). If SLN is positive, all regional lymph nodes are commonly evacuated. After the resection of the regional lymph nodes, 34% of patients have no recurrence during 25 years of follow-up, 44 % have distant recurrence, and 22% local or regional recurrence (White et al., 2002).

Table 1. TNM classification and staging by AJCC. Modified from Balch et al., 2009.

Stage	T	Thickness	Ulceration status
0	T in situ		
IA	T1a	≤ 1.0 mm	- and mitoses $<1/mm^2$
IB	T1b		+ or mitoses $\geq 1/mm^2$
IB	T2a	1.01-2.0 mm	-
IIA	T2b		+
IIA	T3a	2.01-4.0 mm	-
IIB	T3b		+
IIB	T4a	> 4.0 mm	-
IIC	T4b		+

Stage	N	No of metastatic nodes	Ulceration status	Nodal metastatic mass
IIIA	N1a	1 node	-	micrometastasis
	N2a	2-3 nodes	-	micrometastasis
IIIB	N1a	1 node	+	micrometastasis
	N2a	2-3 nodes	+	micrometastasis
	N1b	1 node	-	macrometastasis
	N2b	2-3 nodes	-	macrometastasis
	N2c	2-3 nodes	-	in transit/satellite w/o metastatic nodes
IIIC	N1b	1 node	+	macrometastasis
	N2b	2-3 nodes	+	macrometastasis
	N2c	2-3 nodes	+	in transit/satellite w/o metastatic nodes

Stage	M	Site	Ulceration status	Serum LHD
	N3	4 or more nodes	Any	
IV	M1a	Distant skin, subcutaneous or distant nodes	Any	Normal
	M1b	Lung	Any	Normal
	M1c	All other visceral	Any	Normal
	M1c	Any	Any	Elevated

Clark invasion level has not been included to the newest version of TNM classification and staging, but is still widely used method to depict invasion depth of primary melanoma (Clark et al., 1969). Thus, in level I melanoma tumor cells have not crossed the basement membrane, in level II melanoma cells are detected in the upper part of the papillary dermis, in level III they are detected across the whole thickness of papillary dermis, in level IV – in the reticular dermis, and in level V – in the subcutaneous tissue.

2.3 Genetic aberrations in melanoma

Melanoma arises from a benign or dysplastic naevus, or directly de novo without a precursor lesion. Ultraviolet (UV) radiation is the strongest factor predisposing to melanoma. It induces both melanocyte proliferation and DNA damage. Mutations in the Ras-Raf-MAPK pathway are among the most frequent mutations observed in melanoma (Davies et al., 2002). In this pathway, activation of receptor tyrosine kinases triggers guanosine triphosphate (GTP) loading of Ras, which then recruits serine threonine Raf kinases to the cell membrane. Activated Raf phosphorylates MEK1 and MEK2 kinases which activate Erk1/2 kinases. These translocate into the nucleus where they induce transcription of the genes involved in cell-cycle progression (Pearson et al., 2001). Ras can signal also through phosphatidylinositol-3 kinase (PI-3K) or phospholipase (PLC) pathways (Cullen, 2001; Katz and McCormick, 1997). The Ras family includes N-ras, H-ras, and K-ras kinases. The Raf family consists of three isoforms, Raf-1 (C-Raf), A-Raf, and B-Raf. *BRAF* mutation is seen in 50-81% of benign nevi (Wu et al., 2007), but additional mutations are needed for malignant transformation. *NRAS* mutation is detected in 21% of SSM, in 31% of NM (Platz et al., 2008), in 9% of acquired benign nevi, and in 35% of congenital nevi (de Snoo and Hayward, 2005). Mutations in Ras-Raf-MAPK pathway induce constitutive activation of ERK1/2 kinases, which induces melanoma cell proliferation, tumor invasion and metastasis. 66% of melanomas have mutated *BRAF* (Davies et al., 2002), and 80% display mutations in either *BRAF* or *NRAS* (Platz et al., 2008). The V600E accounts for 90% of *BRAF* mutations detected in human melanoma (Platz et al., 2008). Interestingly, this mutation induces the senescence of naevus cells (Michaloglou et al., 2005).

One of the pathways proposed to be responsible for the eventual malignant transformation of melanocytes is the cyclin-dependent kinase inhibitor 2A (CDKN2A) pathway. CDKN2A is a tumor suppressor gene, which encodes two distinct products, p16[INK4a] and p14[ARF]. The p16[INK4a] inhibits cyclin-dependent kinases CDK4 and CDK6, which promote cell cycle progression by phosphorylating Retinoblastoma (Rb) protein. High proportion of

melanoma cell lines exhibit inactivation of CDKN2A gene or activating mutations of CDK4 gene, which both lead to uncontrolled CDK4 and CDK6 activities (Walker et al., 1998). Reduced expression of p16[INK4a] in melanoma has been associated with higher Clark invasion levels, melanoma progression, and increased risk of the disease relapse (Straume et al., 2000; Talve et al., 1997).

PTEN (phosphatase and tensin homolog deleted on chromosome 10) is a tumor suppressor which dephosphorylates PIP3 (phosphatidylinositol-4,5,6-triphosphate). Loss of PTEN leads to increased PIP_3 activity, which further activates PI3K/Akt signaling pathway, leading to increased cell proliferation and survival. Loss of PTEN is observed in 70% of melanoma cell lines, and it is epigenetically silenced in 30-40% of metastatic melanomas (Mehnert and Kluger, 2012).

Molecules involved in the transition from RGP to VGP melanoma phases include MMP-1 (Blackburn et al., 2009), αvβ3 integrin (Seftor, 1998), PAR-1 (Tellez and Bar-Eli, 2003), VEGF (Streit and Detmar, 2003), and loss of AP-2 transcription factor (Bar-Eli, 2001). Loss of AP-2 is associated with the upregulation of its target genes, such as MCAM and MMP-2, both of which have been implicated in melanoma progression (Tellez and Bar-Eli, 2003). In contrast, Haqq et al (2005) observed only the downregulation of some genes during the RGP/VGP transition, such as cadherin-3, MMP10, integrin α2, and laminin γ2 (Haqq et al., 2005). Maspin is a serine protease inhibitor that inhibits angiogenesis and is also downregulated in RGP/VGP transition (Chua et al., 2009).

3. Extracellular matrix

All tissues in the body are composed of cells and their extracellular matrix. Extracellular matrix was earlier considered merely as structural support to organs, but later has been recognized as an active component of tissues. It stores growth factors and other bioactive molecules (Taipale and Keski-Oja, 1997), acts as a barrier for cell invasion or a scaffold for cell migration along its fibers, and provides signalling cues to the cells via cell surface receptors (Lu et al., 2012). Perturbations in ECM deposition, composition, or turn-over may lead to such devastating diseases as fibrosis and cancer. Extracellular matrices can be roughly divided to interstitial matrices and basement membranes (Lu et al., 2012). Provisional matrix is a temporary matrix formed at the sites of tissue injury, or tumor/host interface.

3.1 Interstitial matrix

Interstitial matrix is composed of a hydrated gel-like ground substance and fibers (collagens and elastic fibers) embedded in it.

3.1.1 Ground substance

Ground substance is an amorphous material composed of glycosaminoglycans (GAGs), proteoglycans and glycoproteins (Dudas and Semeniken, 2012). The water in hydrated ground substance permits the rapid exchange of nutrients and waste products. GAGs are polysaccharides composed of chains of repeating disaccharide units. Almost all GAGs are sulfated, except hyaluronan, including keratin sulfate, heparan sulfate, heparin, and chondrotin-4-sulfate. GAGs are usually covalently linked to proteins to form proteoglycans. Hyaluronan contributes to cell migration, and also promotes malignant progression of several cancers (Bharadwaj et al., 2009; Pasonen-Seppänen et al., 2012; Sironen et al., 2011).

Proteoglycans are large macromolecules which look like bottle brush, with the protein core resembling the stem, and GAGs projecting from its core resembling the bristles of the brush. Examples of proteoglycans are aggrecan, which is found in cartilage and other connective tissue, perlecan, found in vascular ECM, biglycan, found in bone and cartilage, and decorin, which modulates collagen fibril formation as well as affects the bioactivity of TGF-β (Danielson et al., 1993). Several aggrecan molecules can attach to a single, up to 20 μm long hyaluronan molecule non-covalently. This complex can reach the molecular weight of several hundred million daltons. Proteoglycans resist the compression of the tissue and retard the movement of bacteria or metastatic cells. Many bacteria, such as Staphylococcus aureus, secrete hyaluronidase, which cleaves hyaluronan and presumably helps the spread of the bacteria (Makris et al., 2004).

Syndecans are an example of cell membrane proteoglycans. They modulate the adhesion of integrins to various ECM molecules (Beauvais and Rapraeger, 2003). Syndecan-1 inhibits tumor cell invasion by reducing MT1-MMP mRNA (Vuoriluoto et al., 2011), while MT1-MMP induces breast carcinoma cell proliferation and fibrosarcoma cell migration by cleaving syndecan-1 (Endo et al., 2003; Su et al., 2008). Syndecan-4 is a component of focal adhesions and a co-receptor of α5β1 integrin (Bass et al., 2008; Woods et al., 2000). It drives focal adhesion formation by binding to fibronectin and activating PKC-α (Woods et al., 2000).

Glycosylated proteins are called glycoproteins. Fibronectin is a large dimeric glycoprotein composed of two polypeptides, which are attached to each other at their carboxyl ends by disulfide bond and forming thus V-shaped molecule. It has binding sites for collagens, heparin, heparan sulfate, hyaluronan, and integrins (Engvall et al., 1978). Fibronectin is abundant in plasma, interstitial matrix and basement membrane (Ruoslahti and Vaheri, 1974). It promotes lung metastasis in xenograft models by incorporating into the blood clots around the tumor emboli and stimulating tumor cell invasion via αvβ3 integrin (Malik et al., 2010).

3.1.2 Collagens

Collagens are large extracellular molecules with various functions in maintaining tissue structure. Collagens and their cleavage products have also signalling functions, for example in angiogenesis. A total of 28 collagen proteins have been described in human to date. All collagens consist of three α-chains, each of which contains collagenous domain with series of Gly-X-Y sequences. Non-collagenous domain (NC) is important for collagen fiber assembly. Three α-chains are each coiled into a left-handed helix, and then get twisted around a common

axis to form a right-handed triple helix. Glycine at every third position is important for packing structure. Proline is often found in the X position and 4-hydroxyproline in the Y position (Myllyharju and Kivirikko, 2004). Collagen-like triple helical domains can be found in 20 other proteins, for example subcomponent of complement C1q (Heino, 2007). Structurally collagens can be divided to 8 groups. We will restrict this review to the groups which are expressed in the skin.

Fibril-forming collagens (type I, II, III, V, XI, XXIV and XXVII) provide tissues with tensile strength. Collagen type I is the most abundant protein in the body. It is expressed in skin, bone, and tendons. It is a heterotrimer consisting of two α1 and one α2 chains. Collagen type III is a homotrimer and, in addition to skin, is found in the spleen, liver, cardiovascular system and lung. Type II and XI are expressed in the cartilage. Collagen type II is a homotrimer consisting of three α1 chains. Collagen fibrils can contain more than one collagen type. Thus, collagen type I fibrils can contain type III, V and XII collagen fibrils (Myllyharju and Kivirikko, 2004). Fibril-forming collagens are secreted as procollagens with propeptides at both ends, which are cleaved by procollagen peptidases. Then, the collagen molecules self-assemble in head-to-tail direction into fibrils. Lysyl oxidase catalyzes covalent crosslinking of collagen molecules inside the fibrils. Subsequently, the fibrils arrange into fibers. Mutations in type I collagen cause osteogenesis imperfecta and Ehlers-Danlos syndromes, and mutations in type III collagen are behind the Ehlers-Danlos syndrome and arterial aneurysms (Myllyharju and Kivirikko, 2004).

Type VII collagen is a homotrimer and the main component of anchoring fibrils that connect basement membranes to the papillary dermis. The mutations in COL7A1 account for dystrophic epidermolysis bullosa (Ryynanen et al., 1991), and in epidermolysis bullosa acquisita autoantibodies against collagen type VII decrease the number of anchoring fibrils, making the skin fragile and predisposed to blistering (Lapiere et al., 1993).

3.1.3 Elastic fibers

Elastic fibers provide mechanical elasticity to the organs, such as the lung, skin, and the large arteries. Under electron microscopy they appear as twisted or straight strands (Ushiki, 2002). Elastic fibers can be stretched to one and a half of their length and, when released, return to their resting length. They are produced by fibroblasts and smooth muscle cells of blood vessels. The core of these fibers composed of elastin is surrounded by microfibrils. Tropoelastin is a soluble precursor of elastin, which is deposited on the preformed network of microfibrils, and becomes cross-linked by lysyl oxidase (Kielty et al., 2002). Microfibrils are mainly composed of fibrillins, members of fibrillin/LTBP family. Three fibrillins have been identified to date. They are large glycoproteins (~350 kDa), mainly composed of EGF-repeats and 8-cysteine motifs, which can bind to integrins via RGD motif (Robinson and Godfrey, 2000). Microfibril associated glycoprotein-1 (MAGP-1) is a 31 kDa glycoprotein, which is covalently linked to the microfibrils. Moreover, it binds tropoelastin and collagen type VI, and may thus mediate tropoelastin deposition as well as the association of collagen type VI and microfibrils, seen in soft connective tissue (Robinson and Godfrey, 2000).

Mutations in fibrillin-1 gene account for the Marfan syndrome and Marfan-like abnormalities (Robinson and Godfrey, 2000). Marfan syndrome is an autosomal dominant disease characterized by ocular, skeletal and cardiovascular defects. Its typical features

include scoliosis, chest wall deformity, tall stature, hypermobility of the joints and ligaments, mitral valve prolapse, and aortic aneurysms (Robinson and Godfrey, 2000). Mutations in fibrillin-2 cause congenital contractural arachnodactyly, or Beal's syndrome, characterized by marfanoid appearance, long fingers, contractures of elbows, knees and hips, and underdeveloped muscles (Viljoen, 1994).

The composition and organisation of elastic fibers vary in different tissues. In the aorta they fuse to form sheets, in the lungs elastic fibers form a thin branched network, in the reticular dermis they are organized horizontally, while in the papillary dermis elastic fibers are perpendicular merging with oxytalan fibers which connect them to the basement membrane (Kielty et al., 2002; Ushiki, 2002). Oxytalan fibers are microfibrils composed of fibrillins, which also connect lymphatic vessels to underlying elastic fibers, preventing lymphatic vessels from collapsing (Gerli et al., 1990).

3.2 Basement membranes

Basement membranes (BMs) are amorphous sheet-like structures, which consist of about 50 proteins, but collagens comprise ~50% of their protein mass. BM regulates cell polarity of various cell types, including epithelial, endothelial, muscle cells, and peripheral nerves, separating them from underlying collagenous tissue. Main components of BM are collagen type IV, laminins, nidogen, proteoglycan perlecan which has heparan sulfate side chains, fibronectin, and collagen type XV and XVIII (Kalluri, 2003).

Collagen type IV heterotrimers do not form fibrils, because their propeptides do not get removed. Instead, heterotrimers assemble into hexamers via their NC1 domains, which further associate into sheet-like meshwork via 7S domains (Kalluri, 2003). Six different genes encode six α-chains (α1- α6), which can form at least seven different heterotrimers (Kalluri, 2003). Collagen type IV is found mainly in basement membrane, but can be also accumulated in tumor ECM. Mutations in collagen type IV lead to Alport syndrome with defective glomerular basement membrane (Ortega and Werb, 2002). In Goodpasture syndrome, autoantibodies are produced against α3 chain of collagen type IV, resulting in severe glomerulonephritis and pulmonary haemorrhage (Hudson et al., 1993). Tumstatin, the NC1 domain of collagen type IV α3 chain, inhibits angiogenesis (Petitclerc et al., 2000). Another anti-angiogenic collagen IV peptide derived from NC1 domain of α1 chain, arresten, inhibits in addition to angiogenesis tumor growth in mice and tumor metastasis (Colorado et al., 2000; Nyberg et al., 2008). Interestingly, the NC1 domain of α4 chain, tetrastatin, reduces the expression of membrane-type-1 matrix metalloproteinase (MT1-MMP), which is involved in tumor cell invasion. This leads to reduced proliferation and invasion of melanoma xenografts (Brassart-Pasco et al., 2012).

Collagens type XV and XVIII are proteoglycans called multiplexins, and participate in the stabilization of the basement membrane. They are interesting in the regards that cleavage products of their NC domains generate restin (Ramchandran et al., 1999) and endostatin (O'Reilly et al., 1997; Wickstrom et al., 2002), respectively, which both display anti-angiogenic activities. Mutation in α1 chain of collagen XVIII results in Knobloch syndrome, characterized by abnormalities in retinal vasculature, blindness, and neural tube closure defects (Seppinen and Pihlajaniemi, 2011).

The self-assembly of BM begins when integrins initiate laminin polymer deposition. Then, collagen type IV connects to laminin polymers via nidogen bridging (Kalluri, 2003). Laminin is a large glycoprotein composed of three polypeptide chains, α, β, and γ, which can assemble into at least 15 heterotrimers making up cross-like, Y-shaped, or rod-shaped structures (Miner, 2008). Laminin binds to heparan sulfate, type IV collagen, nidogen, and cell membrane. The variety of existing isoforms of laminin, collagen type IV and proteoglycans permits the diversity in the BM composition in different organs, supporting tissue plasticity and different functions (Kalluri, 2003).

3.3 Provisional matrix

Provisional matrix is a temporary connective tissue formed at the sites of injury, such as skin wound. Furthermore, it can be observed around the growing tumor. The initial response to tissue injury is hemostasis, or coagulation response. Exposed collagen activates platelets which adhere to ECM, secrete clotting factors, and aggregate with each other (Reinke and Sorg, 2012). Activated coagulation cascade results in the activation of thrombin, which converts circulating fibrinogen to fibrin. Fibrin forms a meshwork between the platelets, which further strengthens their aggregation. Formed provisional matrix is composed of fibrin, fibronectin, vitronectin, and thrombospondins. It acts as a scaffolding matrix for invasion of leukocytes, keratinocytes, fibroblasts and endothelial cells. Platelets and leukocytes release inflammatory cytokines and growth factors, such as interleukins, TNF-α, bFGF, TGF-β, VEGF-A, HIF-1α and IGF-1 (Reinke and Sorg, 2012). This results in transformation of fibroblasts to myofibroblasts, induction of collagen secretion, angiogenesis, and re-epithelialization. The provisional matrix is then replaced by the acute granulation tissue, which is composed of collagen, fibroblasts, and inflammatory cells, and is highly vascular. As a last phase of wound healing, granulation tissue is matured to avascular and acellular scar (Reinke and Sorg, 2012).

Fibrinogen is a hexamer which consists of two copies of its three chains: Aα, Bβ, and γ, $(A\alpha B\beta\gamma)_2$. Three chains of human fibrinogen are encoded by three different genes (Fish and Neerman-Arbez, 2012). They are expressed by hepatocytes, which secrete glycosylated fibrinogen hexamers (~340 kDa) into the circulation. Blood plasma contains between 1.5 to 3.5 g/l of fibrinogen. Activated thrombin cleaves Aα and Bβ chains, exposing specific sites which initiate rapid fibrinogen polymerization. This leads to the formation of fibrin fibers (Standeven et al., 2005). The determinants of fibrin structure are pH and various molecules, such as calcium, chloride ions, albumin, and collagen, among others (Standeven et al., 2005). Activation of coagulation cascade sets into motion a fibrinolytic cascade, which will limit the size of a blood clot. Fibrin promotes the conversion of plasminogen to plasmin by tissue plasminogen activator (t-PA) (Standeven et al., 2005). Plasmin cleaves fibrin and interferes with its polymerization. Fibrin degradation products act also as anticoagulants, providing an additional mechanism to modulate fine coagulation/anticoagulation balance at the site of tissue injury.

3.4 ECM-sequestered growth factors

The targeting of growth factors to the ECM provides means for their rapid activation in a spatially and temporally regulated manner. Growth factors such as VEGFs, IGFs, FGFs, HGF, and TGF-βs can associate with ECM molecules or heparan sulfate (Taipale and Keski-Oja, 1997). We will restrict the current review to TGF-β, the release of which from the ECM has been addressed in this thesis.

3.4.1 Structure of TGF-βs

Transforming growth factor β and its receptors are expressed ubiquitously in all metazoans, and in humans in almost all cell types. The effects TGF-β exerts on the cells are very complex, sometimes opposing, and depend on growth factor concentration, cell type and microenvironmental factors (Massague, 2012). TGF-β was first identified as an activity which induced anchorage-independent growth of fibroblasts in soft agar, a phenomenon closely associated with the malignant transformation (de Larco and Todaro, 1978). TGF-β precursor was cloned in 1985 (Derynck et al., 1985), and the receptor for TGF-βs was identified in 1982 (Massague et al., 1982). TGF-β belongs to a large TGF-β superfamily of at least 30 members including bone morphogenic proteins (BMPs), growth differentiation factors (GDFs), Nodal, and inhibins/activins (Massague, 2012).

Three different TGF-β proteins have been identified and cloned in mammals. Their sequences share 71-76% similarities, and they have different expression patterns and affinity to TGF-β receptors. TGF-β1 knockout mice die postnatally at about day 20 from the multifocal inflammation, tissue necrosis and organ failure (Kulkarni et al., 1993; Shull et al., 1992). TGF-β2 knockout mice die perinatally and exhibit cardiac, lung, craniofacial, limb, spinal column, eye, inner ear and urogenital defects (Sanford et al., 1997). TGF-β3 knockout mice die within 20 h from birth, and exhibit defects in lung maturation and cleft palates with 100% penetrance (Kaartinen et al., 1995).

TGF-β1 is a prototype of TGF-β family. It is translated as a 390-amino acid precursor protein, which is cleaved by furin generating 112-amino-acid TGF-β1 and 249-amino-acid latency associated peptide (LAP), to which TGF-β1 is non-covalently linked forming small latent TGF-β1 complex (SLC, Fig. 2, Dubois et al., 1995; Gentry et al., 1988). Both TGF-β1 and LAP are disulfide-linked dimers. Further, SLC is covalently linked to latent TGF-β1 binding protein (LTBP) by disulfide links forming large latent TGF-β complex (LLC, Fig. 2, Miyazono et al., 1988; Tsuji et al., 1990). LTBP facilitates the secretion of the complex and targets it to the ECM (Miyazono et al., 1991).
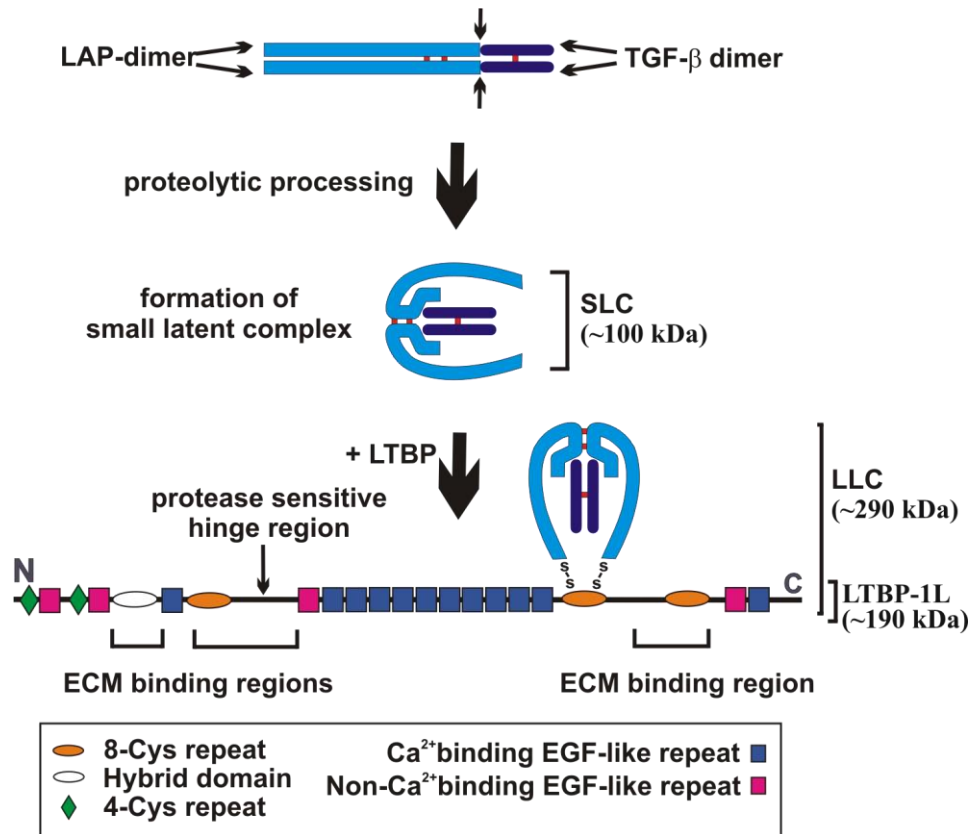
Figure 2. Formation of large latent complex of TGF-β. LAP, latency associated peptide, SLC, small latent complex, LLC, large latent complex. Modified from Hyytiäinen et al., 2004.

3.4.2 Structure of LTBPs

LTBPs are members of LTBP-fibrillin family consisting of LTBPs 1-4 and fibrillins 1-3 (Fig. 3). LTBPs contain 15-20 EGF-like repeats, and four 8-cysteine repeats (including hybrid domain), which are found only in LTBP-fibrillin family. LAP is covalently bound to the third 8-cys repeat of LTBPs 1, 3 and 4 in ER (Saharinen et al., 1999). EGF-like repeats mediate protein-protein interactions, and N-terminal region and parts of C-terminal region of LTBPs associate with ECM (Taipale et al., 1994; Unsöld et al., 2001).

LTBP-1 isolated first from platelets was characterized as a 125-160 kDa protein, which is bound by disulfide bonds to LAP (Miyazono et al., 1988). LTBP-1 cloned from fibroblasts was larger, 170-190 kDa protein, which was named LTBP-1L, the long form (Kanzaki et al., 1990). LTBP-1L is extended in its N-terminal domain (Fig. 3), and assembles with ECM more efficiently than LTBP-1S (Olofsson et al., 1995). The expression of LTBP-1S (short form) and LTBP-1L are driven by different promoters (Koski et al., 1999). LTBP-1L is expressed mainly in the heart, kidney, placenta and prostate, and LTBP-1S is detected in addition in the lung, spleen, stomach, ovary, testis and skeletal muscles (Olofsson et al., 1995). LTBP-1 facilitates the secretion of TGF-β and correct folding of SLC in certain cell types (Miyazono et al., 1991), but some cells, such as glioblastoma cells and osteoblasts, secrete TGF-β mainly without LTBP-1, as SLC (Dallas et al., 1994; Olofsson et al., 1992). Over 90% of LTBP-1 is secreted without TGF-β, indicating that it has also TGF-β -

independent functions (Kwak et al., 2005). Thus, LTBP-1 is expressed in the bone, where it colocalizes with microfibrils (Dallas et al., 2000). In cell culture, LTBP-1 is first co-localized with fibronectin, and later with fibrillin. *In vivo*, LTBP-1 and fibrillin-1 colocalize in tendons (Isogai et al., 2003). However, LTBP-1 is not an integral part of microfibrils of elastic fibers (Isogai et al., 2003). LTBP1 is located in the skin only around hair follicles, while fibrillin-1 is abundant in human dermis (Isogai et al., 2003). The C-terminal parts of both LTBP-1 and LTBP-4 can associate with fibrillin-1. Overexpression of LTBP-1 has been observed in pathological conditions, such as IgA nephropathy, chronic C hepatitis, idiopathic pulmonary fibrosis, and ovarian carcinoma (Kwak et al., 2005). LTBP-1 knockout mice are fertile and lack gross abnormalities. However, they exhibit shortened skull and abrogated surgery-induced fibrotic reaction in the liver, suggestive of perturbed TGF-β signalling (Drews et al., 2008).
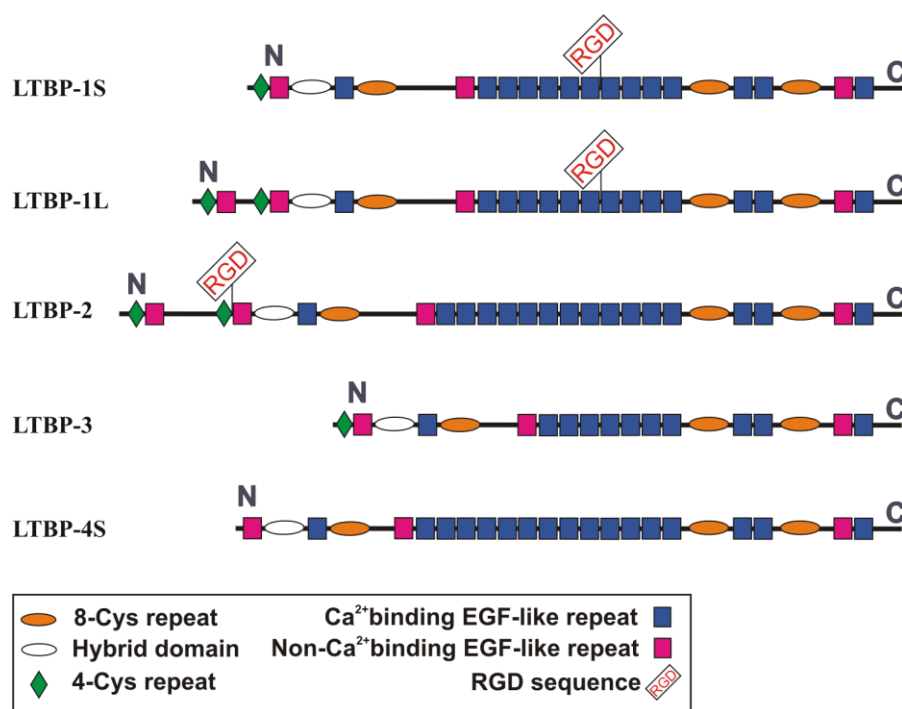
Figure 3. The domain structures of LTBPs. Modified from Saharinen et al., 1999.

LTBP-2 differs from the other family members in the respect that it does not bind SLC. Instead, it has functions in cell adhesion to the matrix, and its importance is underlined by the fact that LTBP-2 knockout embryos die between embryonic days 3.5 and 6.5 possibly due to disrupted implantation (Shipley et al., 2000). LTBP-2 is expressed in the lung, heart, placenta, liver and skeletal muscle. It is associated with elastic fibers. Melanoma cells adhere to LTBP-2 via β1, α3 and α6 integrins (Vehviläinen et al., 2003). In cell culture, LTBP-2 deposition to ECM depends on preformed fibronectin and fibrillin-1 matrices (Vehviläinen et al., 2009).

LTBP-3 is expressed in the skeletal muscle, heart, prostate, ovaries, testis, and small intestine (Penttinen et al., 2002). It exists as two forms, with the longer form containing additional calcium binding EGF-repeat in C-terminal domain (Penttinen et al., 2002). LTBP-3 knockout mice develop cranio-facial abnormalities, kyphosis, osteosclerosis and osteoarthritis (Dabovic et al., 2002).

LTBP-4 was identified from human heart cDNA library by using 3rd 8-Cys repeat sequence of LTBP-1 (Saharinen et al., 1998). LTBP-4 has several splice variants, one of which lacks the 3rd 8-Cys repeat required for binding SLC (Koli et al., 2001; Saharinen et al., 1998). LTBP-4 is expressed in the aorta, heart, uterus, small intestine, skeletal muscle and ovaries (Giltay et al., 1997; Saharinen et al., 1998). LTBP-4 binds to fibronectin via its N-terminal region, and the disruption of its association to ECM leads to increased TGF-β activity (Kantola et al., 2008). LTBP-4 has long and short transcripts, which are regulated by two distinct promoters and different expression patterns (Kantola et al., 2010). Thus, LTBP-4S is expressed in the heart, skeletal muscle, lung and small intestine, while LTBP-4L is expressed in the heart, skeletal muscle, and liver (Kantola et al., 2010). LTBP-4 knockout mice develop lung emphysema, cardiomyopathy, and colorectal cancer (Sterner-Kock et al., 2002). Tissues of the knockout mice exhibit defects in elastic fibers, and reduced TGF-β signalling (Sterner-Kock et al., 2002).

3.4.3 Activation and signalling of TGF-β

To be able to bind to its receptor, in other words to become activated, TGF-β has to be released from LAP. This can occur through chemical alterations of the environment, such as heat, acid, and urea treatment, by proteases, such as plasmin, thrombin, BMP1-like proteinase, MT1-MMP, calpain, and cathepsin G, by conformation change of LAP induced by thrombospondin-1 or integrins, such as αvβ6, αvβ8, and by shear force (Ribeiro et al., 1999; Saharinen et al., 1999; Sato et al., 1993; Taipale et al., 1992). The LAP of TGF-β2, however, lacks RGD sequence and cannot be activated via integrins (Sheppard, 2005).

Correct targeting of LLC to ECM is essential for the regulation of TGF-β activation. Increased TGF-β activation accounts for the part of symptoms in Marfan syndrome, a disease caused by mutation in fibrillin-1 gene (Neptune et al., 2003). Since LTBPs associate with fibrillin-1 (Dallas et al., 2000; Kantola et al., 2008), this suggests that disrupted fibrillin assembly in Marfan patients prevents LLC targeting to ECM, leading to the pathologic TGF-β activation.

The cleavage of LTBP-1 in its hinge-region for example by mast cell chymase, leukocyte elastase, and BMP-1 releases cleaved LLC from ECM, which may be the first step in TGF-β activation (Ge and Greenspan, 2006; Taipale et al., 1995). In addition, the secretion of active TGF-β into the conditioned medium can be induced by treating the keratinocyte cultures with retinoids or vitamin D_3, and cancer cells with antiestrogens and glucocorticoids (Koli and Keski-Oja, 1993; Saharinen et al., 1999). Coculture of endothelial cells with pericytes produces activated TGF-β, which exerts inhibitory effect on endothelial cell migration (Antonelli-Orlidge et al., 1989; Sato and Rifkin, 1989). Activation in this coculture system occurs at the cell surface and is prevented by inhibitors of plasmin (Sato and Rifkin, 1989) and antibodies against cation-independent mannose-6-phosphate receptor (CI-MPR) and LTBP-1 (Dennis and Rifkin, 1991; Flaumenhaft et al., 1993).

TGF-β receptors are transmembrane serine/threonine kinases and are divided to type I and type II receptors. First active TGF-β binds to type II receptor. The ligand/receptor complex is recognized by type I receptor, which binds to type II receptor. The receptor complex consists of two type I receptors and two type II receptors bound to TGF-β dimer (Massague, 2012). Seven type I and five type II receptors have been identified in man. TGF-β

binds to only one type II receptor, TβRII, and three type I receptors, ALK1, ALK2 and ALK5. Most of the TGF-β signalling occurs via ALK5, ALK-1 being important for TGF-β signalling in endothelial cells. Upon receptor complex activation, type II receptors phosphorylate type I receptors, which can then bind to and phosphorylate R-Smads (receptor activated Smads). ALK5 binds and phosphorylates Smad2 and -3, while ALK1 activates Smad1 and -5. Phosphorylated R-Smads bind to Co-Smad, Smad4, and translocate into the nucleus, where they regulate gene expression (Attisano and Wrana, 2002). Inhibitory Smads (Smad-6 and -7) counteract the effects of R-Smads.

3.4.4 Functions of TGF-β in melanoma

The importance of TGF-β1 as a strong modulator of the immune system is underlined by the profound immune cell infiltration of the tissues in TGF-β1 knockout mice, which die shortly after birth (Shull et al., 1992). In cancer, TGF-β1 promotes tumor growth by inhibiting immune surveillance. Unlike other cancers, no mutations in TGF-β pathway have been found in melanoma (Rodeck et al., 1999). Nevertheless, the production of TGF-β is increased during the progression of melanoma (Berking et al., 2001), and, like many other cancers, melanoma is resistant to growth-restricting signals of TGF-β (Krasagakis et al., 1999; Rodeck et al., 1999). TGF-β1 and -3 are expressed by both melanocytes and melanoma cells (Hussein, 2005), but TGF-β2 is expressed specifically by melanoma cells and is associated with melanoma invasion level (Reed et al., 1994). TGF-β may induce melanoma progression by upregulating MMP-9, integrins β1 and β3, and by downregulating E-cadherin (Janji et al., 1999). Further, TGF-β induces melanoma cell adhesion to endothelial cells (Teti et al., 1997). TGF-β overexpressing melanoma xenografts produce more stroma, exhibit less apoptosis and increased lung metastasis (Berking et al., 2001). These effects are mediated by TGF-β stimulation of stromal fibroblasts to produce collagens type VI, XV, XVIII, fibronectin and tenascin, which may provide scaffolding for migration of melanoma cells (Berking et al., 2001).

4. Composition of the skin

Skin is the largest organ of our body. It provides not only mechanical protection, but has functions in heat regulation, sensation, excretion from sweat glands, absorption of ultraviolet radiation for the synthesis of vitamin D, and anti-microbic surveillance. It consists of upper layer, epidermis, and lower layer, dermis, which are separated by basement membrane. Epidermis is derived from ectoderm and is composed of stratified squamous keratinized keratinocytes. Its thickness is between 0.07 and 0.12 mm, but on the soles it can be as much as 1.4 mm. Keratinocytes are constantly renewed through proliferation in the basal layer of epidermis, from where older keratinocytes are pushed upwards, differentiate and accumulate keratin filaments, lose their nuclei and die (Fuchs, 2008). The life cycle of a keratinocyte lasts from 20 to 30 days. Psoriasis is a disease where keratinocyte proliferation is increased (about 7-fold), leading to patchy lesions, especially on the scalp, knees and elbows. Basal cell

carcinoma and squamous cell carcinoma arise from the keratinocytes (Gartner and Hiatt, 2001).

Melanocytes reside in the basal layer of epidermis or upper dermis, with their long processes extending to the upper layers of epidermis. They are originally derived from the neural crest. Tyrosinase produced in these cells is packed to melanosomes by the Golgi apparatus. When it is activated by ultraviolet light, tyrosinase catalyzes the reaction of transition of tyrosine to melanin. Melanosomes travel to the tip of the process of melanocytes. The tip then penetrates into the cytoplasm of a keratinocyte, and becomes pinched off. This process is called cytocrine secretion. Within keratinocytes, melanosomes are settled in the supranuclear region, protecting nuclear DNA from ultraviolet rays. Eventually, melanin is degraded by lysosomes of the keratinocyte. The number of melanocytes ranges from 800 to 2300/mm^2 and is almost the same in all races. The darkness of the skin depends in turn on tyrosinase activity (Gartner and Hiatt, 2001; Marks and Seabra, 2001).

Epidermis contains also other cells, such as Merkel cells (acting as mechanoreceptors or neuroendocrine cells and derived from neural crest, Halata et al., 2003), and Langerhans cells (antigen presenting cells). Unlike melanocytes, which proliferate after exposure to UV radiation, Langerhans cells decrease in number in response to UV radiation, which might contribute to carcinogenesis.

The dermis is derived from mesoderm and consists of collagen types I and III, which form 85-90% and 10-15% of total skin collagen, respectively, and co-exist in the same fibers (Vitellaro-Zuccarello et al., 1992). With aging, collagen fibrils become enzymatically degraded, resulting in decreased adhesion of fibroblasts to collagen, and reduced production of new collagen type I by fibroblasts (Quan et al., 2012).

Upper layer of dermis, the papillary dermis, contains thin type I/III collagen fibers and elastic fibers. Anchoring fibrils, composed of collagen type VII, bind epidermis to dermis. Papillary dermis is rich in cells, such as macrophages, fibroblasts and mast cells. Capillary loops of the papillary layer nourish epidermal cells and regulate body temperature. Meissner corpuscles of papillary dermis are mechanoreceptors sensitive to light touch and probably act also as nociceptors (Widera et al., 2012).

Reticular dermis is the lower layer of the dermis, which is composed of thick collagen type I/III fibers lying parallel to the skin surface, thick elastic fibers which are intermingled with collagen fibers, and proteoglycans (Watt and Fujiwara, 2011). Reticular dermis contains fewer cells, which include fibroblasts, mast cells, lymphocytes and macrophages. Sweat glands, sebaceous glands and hair follicles, though being of epidermal origin, invade the dermis and subcutaneous tissue during embryogenesis. Arrector muscles are inserted into the hair follicles and contract when the body is exposed to cold, creating "goose bumps". Pacinian receptors which are sensitive to pressure and vibration, and ruffini corpuscles which respond to tensile forces, are located also in the reticular dermis.

The subcutaneous tissue is composed of fat, sweat glands, blood and lymphatic vessels, and cutaneous nerves. It protects the skin from the underlying bony prominences. Skin ligaments (L. retinacula cutis) extend from the deep fascia to the dermis and prevent the excess of skin mobility over the deep structures (Gartner and Hiatt, 2001).

5. Matrix metalloproteinases

Matrix metalloproteinases form a family of enzymes closely involved in various pathological conditions of the skin, such as psoriasis, atopic dermatitis, as well as skin cancer including melanoma (Buommino et al., 2012; Harper et al., 2010; Hofmann et al., 2005). Matrix metalloproteinases belong to the metzincin family, which is in turn part of metalloproteinase superfamily (Gomis-Ruth, 2003). They were originally discovered in vertebrates as collagenolytic enzymes involved in tail resorption during frog metamorphosis (Gross and Lapiere, 1962). The term matrix metalloproteinase was introduced at the first international MMP conference in Destin, Florida (Brinckerhoff and Matrisian, 2002). MMPs have been described in all organisms, from archaebacteria to worms and plants. They function in the events such as blastocyst implantation, embryogenesis, tissue remodelling, bone resorption, wound healing, and a plethora of pathological conditions. MMPs can degrade all ECM proteins. In addition, they cleave cell surface receptors, growth factors, chemokines, and other MMPs. The ADAMs (a disintegrin and metalloproteinase) and ADAMTSs (a disintegrin and metalloproteinase with thrombospondin motifs) are a related group of zinc-dependent transmembrane proteases of metzincin family. They act primarily as sheddases, cleaving the ectodomains of cell surface proteins and receptors. Similarly to MMPs, ADAMs are upregulated during inflammation, and are implicated in several other pathological conditions, including melanoma (Huovila et al., 2005).

5.1 Structures of MMPs

The human MMP family consists of 23 zinc-dependent enzymes (Chernov and Strongin, 2011), 7 of which are produced with a membrane anchor. Secreted MMPs can be divided into collagenases, which cut collagen triple helix generating ¾ and ¼ fragments, gelatinases, capable of cleaving denatured collagens, and stromelysins and matrilysins, which can cleave various ECM and non-ECM substrates and proteoglycans.

Starting at the N-terminus, a signal peptide, a propeptide, a catalytic domain, a hinge region, and a hemopexin-like (PEX) domain are found in almost all MMPs (Fig. 4). All MMPs are synthesized as latent zymogens, and their latency is maintained by an interaction of a cysteine-sulphydryl group in the propeptide and the zinc ion of the catalytical domain. The N-terminal propeptide prodomain must be cleaved upon their activation (Sternlicht and Werb, 2001).

The membrane-type matrix metalloproteinase family comprises 6 members, four of which (MT1-, MT2-, MT3- and MT5-MMPs) have stalk region, transmembrane and cytosolic domains (type I transmembrane), while two of them (MT4- and MT6-MMPs) are glycophosphotidyl inositol (GPI)-anchored to the cell membrane. ProMMP-23 contains a potential membrane spanning region (type II transmembrane) in its N-terminus. The cleavage of MMP-23 propeptide both activates it and releases the soluble enzyme from the cell membrane (Pei et al., 2000).
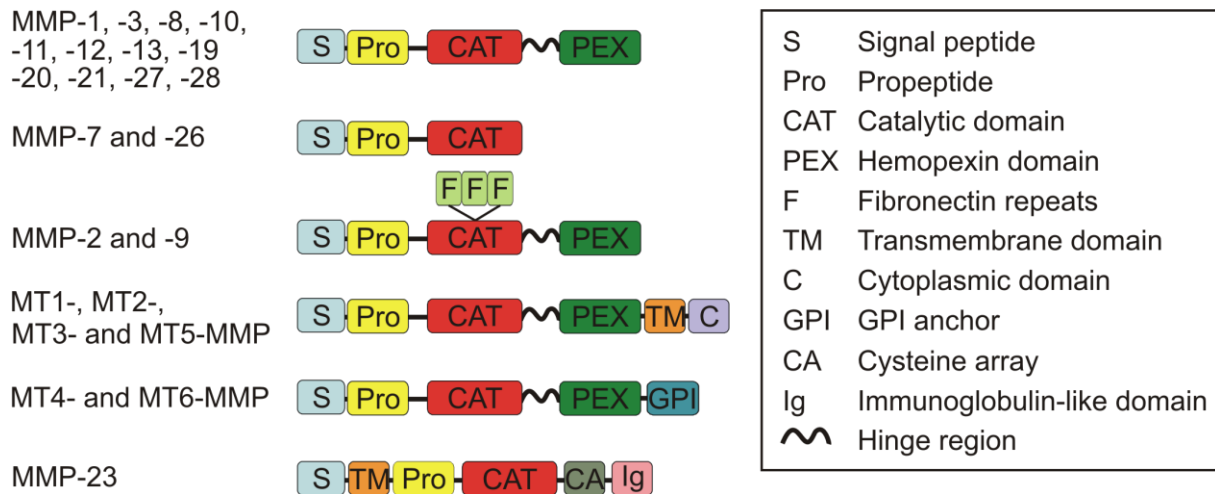
Figure 4. Domain structures of MMPs. Modified from Radisky and Radisky, 2010.

PEX domain is found in all MMPs except MMP-7, MMP-23, and MMP-26, and is essential for the substrate specificity of MMPs. For example, PEX domain is required for unwinding collagen type I triple helix by MMP-1, MMP-13 and MT1-MMP (Knäuper et al., 1997; Manka et al., 2012; Tam et al., 2002), for substrate specificity of MMP-8 (Gioia et al., 2002), MMP-9 binding to gelatin (Roeb et al., 2002), MMP-2 binding to fibrinogen (Monaco et al., 2007), for MT1-MMP homodimerization and MMP-2 activation (Itoh et al., 2001; Lehti et al., 2002), for heteromerization of MT1-MMP with CD44, MT1-MMP localization to lamellipodia, and MT1-MMP-mediated directional migration (Mori et al., 2002). Interestingly, MT1-MMP PEX domain is not necessary for 3D collagen invasion, but is required for fibrin invasion (Li et al., 2008). However, MT1-MMP with deleted PEX degrades collagen type I less efficiently than wild-type MT1-MMP (Li et al., 2008). MT1-MMP mutant with the PEX from MT3-MMP, in turn, cannot confer cells with collagenolytic activity, but drives cell invasion through fibrin and activates MMP-2 as efficiently as the wild type MT1-MMP (Jiang and Pei, 2003; Li et al., 2008). The contrasting results that MT1-MMP PEX domain is not required for MMP-2 activation but is necessary for mediating 3D collagen invasion have been reported (Wang et al., 2004), which may be due to different overexpression systems, cell types and collagen degradation/invasion assays used (Li et al., 2008).

Gelatinases (MMP-2 and MMP-9) contain additional fibronectin type II repeats, which are required for collagen binding and cleavage (Steffensen et al., 1995).

The cytosolic domain of MT1-MMP is required for the enzyme targeting to the cell membrane and invadopodia, and for clathrin-mediated endocytosis (Jiang et al., 2001; Lehti et al., 2000; Nakahara et al., 1997), but not for proMMP-2 activation or collagen invasion (Hotary et al., 2003; Lehti et al., 2000). It is further required for the MT1-MMP-induced ERK phosphorylation and cell migration (Gingras et al., 2001; Gingras and Beliveau, 2010). The generation of proteolytically active soluble MT1-MMP by cleaving its ectodomain close to the cell membrane has been reported (Toth et al., 2005), but the anchoring of MT1-MMP to the cell membrane is essential for tightly regulated pericellular proteolysis and collagen type I invasion (Hotary et al., 2000).

5.2 Membrane-type matrix metalloproteinases

MT1-MMP is a prototype of the MT-MMP family, widely expressed in connective tissue, vasculature, bones, lung, liver, kidneys, and placenta (Apte et al., 1997; Holmbeck et al., 1999; Shofuda et al., 1997; Takino et al., 1995). It is essential for bone and connective tissue homeostasis, collagen type I turnover, angiogenesis, wound healing, and tooth development (Hiraoka et al., 1998; Holmbeck et al., 1999; Itoh and Seiki, 2006; Okada et al., 1997). The MT1-MMP knockout mice are born small, with fibrosis of soft connective tissue, delayed bone ossification, defects in the vasculature, and they die soon after birth (Holmbeck et al., 1999; Zhou et al., 2000). Like other MMPs, MT1-MMP is synthesized as a zymogen, and its N-terminal propeptide needs to be cleaved for activation (Nagase, 1997). This takes place mainly inside the Golgi apparatus by furin and other proprotein convertases (Mazzone et al., 2004; Sato et al., 1996; Yana and Weiss, 2000). In addition, plasmin can cleave recombinant soluble MT1-MMP propeptide (Okumura et al., 1997), but not the membrane anchored wild-type MT1-MMP (Monea et al., 2002). MT1-MMP is upregulated in tumor and stromal cells of many cancers, such as breast, prostate, colon, gastric, cervical, pancreatic cancer, osteosarcoma, head and neck carcinoma, melanoma, and is often associated with cancer progression and poor prognosis (Iacobuzio-Donahue et al., 2002; Jiang et al., 2006; Mimori et al., 2008; Nomura et al., 1995; Okada et al., 1995; Riker et al., 2008; Uchibori et al., 2006; Zhai et al., 2005).

MT2-MMP is also expressed in some cancer cells, and has been found to prevent apoptosis of tumor cells (Abraham et al., 2005). The expression of MT2-MMP in tumor cells can be elevated in response to tumor hypoxia via HIF-1α, which can serve as a mechanism by which tumor cells evade apoptosis triggered by hypoxia (Zhu et al., 2011).

MT3-MMP is expressed in microglial cells of both white and grey matter of adult brain, in heart, lung, placenta, bone, and smooth muscle cells (Shi et al., 2008; Shofuda et al., 1997; Takino et al., 1995; Yoshiyama et al., 1998). MT3-MMP is expressed in corneal epithelium during development, where it cleaves CD44 and modulates ECM to allow effective invasion of neural crest cells (Huh et al., 2007). MT3-MMP is also expressed in fetal brain, suggesting that it might contribute to brain development (Shofuda et al., 1997). Consistently, the loss of MT3-MMP gene as a result of chromosomal translocation has been associated with human autism (Borg et al., 2002). MT3-MMP is also overexpressed in brain malignancies (Nakada et al., 1999; Xia et al., 2009), and malignancies derived from neural crest or neuroectoderm, such as melanoma, medulloblastoma, and neuroblastoma (www.genesapiens.org, Kilpinen et al., 2008). In addition, MT3-MMP is expressed in hepatocellular carcinoma, and a bladder cancer cell line (Arai et al., 2007; Takino et al., 1995; Xu et al., 2008). MT3-MMP knockout mice are fertile, but display slightly retarded skeletal growth (Shi et al., 2008). MT1-MMP/MT3-MMP double knockout mice have more severe skeletal defects than MT1-MMP knockout mice, suggesting that MT3-MMP may also participate in skeletal development (Shi et al., 2008). The alternative splicing of MT3-MMP mRNA produces its soluble form expressed in human ovary (Matsumoto et al., 1997). Instead of transmembrane and intracellular domains, this form possesses a novel sequence of 50 amino acids at its C-terminus. Soluble MT3-MMP can activate MMP-2 and cleave collagen type III and fibronectin (Matsumoto et al., 1997).

MT4-MMP is expressed in the brain, colon, ovary, testis, and macrophages (English et al., 2000). It has been reported to promote breast cancer metastasis (Chabottaux et al., 2006).

The purified catalytic domain of MT4-MMP can activate MMP-2 (Wang et al., 1999), but the overexpressed mouse MT4-MMP does not cleave proMMP2 to its activated form (English et al., 2000). MT4-MMP knockout mice appear normal, but have diminished thirst (Srichai et al., 2011).

MT5-MMP is expressed in brain, kidney, pancreas and lung. Its expression is further upregulated in brain tumors (Llano et al., 1999).

MT6-MMP is expressed in the lung, spleen, and neutrophils, and it facilitates transendothelial migration of neutrophils (Nie and Pei, 2003; Pei, 1999). MT6-MMP is also overexpressed in the brain, urothelial, prostate and colon tumors (Sohail et al., 2008; Sun et al., 2007; Velasco et al., 2000).

5.2.1 Substrates of MT-MMPs

The substrate range of the MMP family members is extremely wide. They can cleave virtually any ECM component and many other extracellular and cell surface proteins (Table 2). Since MT-MMPs are membrane-anchored, they engage in pericellular proteolysis, a process where proteolytic activity is concentrated at the cell surface and especially at the invading structures of the cell. By cleaving pericellular native collagen type I, MT1-MMP and MT2-MMP endow the cells with the ability to invade through collagen rich tissues (Sabeh et al., 2004). In addition, MT1-MMP cleaves collagen type II, III, IV, laminin, fibronectin, tenascin, fibrin, and many other ECM proteins (Sternlicht and Werb, 2001, Table 2). MT3-MMP can cleave e.g. collagen type III, IV, fibronectin, fibrin, laminin, and vitronectin (Table 2).

The first proteolytic activity to be described for MT1-MMP was cleaving and activating proMMP-2 (Sato et al., 1994). MT1-, MT2-, MT3-, MT5- and, to some extent, MT6-MMP can cleave the 72 kDa proMMP-2 to its 64 kDa intermediate form, which is then autocatalytically cleaved to the active 62 kDa form (Atkinson et al., 1995; English et al., 2000; Nie and Pei, 2003). Tissue inhibitor of metalloproteinases 2 (TIMP-2) is required for MMP-2 activation by MT1-MMP. The N-terminal domain of TIMP-2 binds to the catalytic domain of MT1-MMP, and its C-terminal domain binds to the PEX domain of proMMP-2 (Butler et al., 1998; Strongin et al., 1995). Homotypic oligomerization of MT1-MMP via PEX and cytoplasmic domains facilitates MMP-2 activation (Itoh et al., 2001; Lehti et al., 2002). Plasmin can also activate MMP-2 in the presence of MT1-MMP, independently of MT1-MMP proteolytic activity (Monea et al., 2002).

MT1-MMP can cleave a number of cell surface proteins and receptors (Tomari et al., 2009, Table 2). For example, it cleaves CD44, ADAM9, syndecan-1, αv, α3, and α5 integrin subunits, among others (Endo et al., 2003; Kajita et al., 2001; Ratnikov et al., 2002; Chan et al., 2012). MT3-MMP has also been described to cleave several cell surface proteins, such as the amyloid precursor protein (APP), CD44, endogenous Nogo-66 receptor 1 (NgR1), tissue transglutaminase (tTg), and syndecan-1, but the biological functions of these cleavages have largely remained unknown (Ahmad et al., 2006; Belkin et al., 2001; Endo et al., 2003; Ferraro et al., 2011; Kajita et al., 2001).

Name	ECM substrates	Cell-surface/other substrates

Collagenases

Name	ECM substrates	Cell-surface/other substrates
MMP-1 (collagenase-1)	Col I, II, III, VII, VIII, X, XI, gelatin, tenascin, nidogen, MBP, aggrecan, perlecan, IGFBP-2, -3, fibronectin, laminin, vitronectin, fibrin	ProMMP-1, -2, casein, α2M, A1AT, α1AC, proTNFα, CTGF, dickkopf-1, DJ-1, neuron specific enolase, Niemann-Pick C2, pentraxin-3, progranulins, C1q
MMP-8 (collagenase-2)	Col I, II, III, gelatin, aggrecan, tenascin	ProMMP-8, α2M, A1AT, DJ-1, iduronate-2-sulfatase, neuron specific enolase, Niemann-Pick C2, progranulins
MMP-13 (collagenase-3)	Col I, II, III, VI, IX, X, XIV, gelatin, aggrecan, tenascin, osteonectin, fibrin, fibrillin, fibronectin	ProMMP-9, -13, α2M α1AC, PAI, casein

Gelatinases

Name	ECM substrates	Cell-surface/other substrates
MMP-2 (gelatinase A, 72-kDa gelatinase)	Gelatin, elastin, fibronectin, Col I, IV, V, VII, X, XI, laminin, aggrecan, vitronectin, decorin, MBP, IGFBP-3/5, nidogen, fibrillin, osteonectin, tenascin, IGFBP-4, -6, osteopontin, fibrin	ProMMP-1, -2, -13, plasminogen, casein, α2M, A1AT, α1AC, proTNFα, proTGFβ2, proIL-1β, MCP3, FGFr1, APOE, CTGF, cystatin C, DJ-1, follistatin-like-1, -3, CX3CL-1, galectin-1, -3, HSP-90α, iduronate-2-sulfatase, neuron specific enolase, Niemann-Pick C2, pentraxin-3, pleiotrophin, PCPE, progranulins, S100A8, S100A9, C1q
MMP-9 (gelatinase B, 92-kDa gelatinase)	Gelatin, Col IV, V, VII, XI, XIV, XVII, elastin, fibrillin, fibronectin, aggrecan, fibrin, MBP, decorin, laminin, osteonectin, vitronectin, IGFBP-4	Plasminogen, casein, α2M, A1AT, proTNFα, proTGFβ2, proIL-1β, APP, integrin β2, dickkopf-1, DJ-1, follistatin-like-3, LIF, neuron specific enolase, nexin-1, Niemann-Pick C2, pentraxin-3, progranulins, Ym-1, galectin-3, C1q

Other secreted MMPs

Name	ECM substrates	Cell-surface/other substrates
MMP-3 (stromelysin-1)	Fibronectin, laminin, gelatin, Col III, IV, V, VII, IX, X, XI, elastin, decorin, nidogen, perlecan, aggrecan, tenascin, fibrin, fibrillin, vitronectin, IGFBP-3	ProMMP-1, -3, -7, -8, -9, -13, plasminogen, casein, α2M, A1AT, α1AC, proTNFα, E-cadherin, proIL-1β, VEGF-A, proHB-EGF, CTGF, C1q
MMP-10 (stromelysin-2)	Fibronectin, laminin, gelatin, COL II, III, IV, V, IX, X, XI, decorin, elastin, nidogen, fibrin, fibrillin, tenascin, vitronectin, aggrecan	ProMMP-1, -8, -10, casein
MMP-11 (stromelysin-3)	Laminin, fibronectin, aggrecan, IGFBP-1	α2M, A1AT, galectin-1
MMP-7 (matrilysin-1)	Fibronectin, laminin, Col IV, gelatin, aggrecan, decorin, nidogen, elastin, fibrillin, laminin, MBP,	ProMMP-2, -7, casein, A1AT, pro α-defensin, FasL, integrin β4, E-cadherin, plasminogen, proTNFα, APOE,

	osteonectin, tenascin, vitronectin	syndecan-1
MMP-26 (matrilysin-2)	Col IV, gelatin, fibronectin, fibrin	Pro-MMP9, casein, A1AT
MMP-12	Col IV, elastin, fibronectin, fibrin, laminin, nidogen, fibrillin, vitronectin	Plasminogen, casein, α2M, A1AT, proTNFα
MMP-19	Col IV, gelatin, fibronectin, tenascin, aggrecan, COMP	Casein
MMP-20	Amelogenin, aggrecan, COMP	
MMP-21	ND	
MMP-23	Gelatin	
MMP-27	ND	
MMP-28 (epilysin)	ND	Casein

Membrane-type MMPs

MMP-14 (MT1-MMP)	Col I, II, III, IV, gelatin, fibronectin, laminin-1, -5, vitronectin, aggrecan, tenascin, nidogen, perlecan, fibrin, fibrillin, laminin, Cyr61	ProMMP-2, -13, α2M, A1AT, CD44, CXCL12, proTNFα, tTg, integrin αv, APOE, death receptor 6, dickkopf-1, DJ-1, follistatin-like-3, galectin-1, HSP-90α, iduronate-2-sulfatase, IL-8, neuron specific enolase, Niemann-Pick C2, pentraxin-3, progranulins, SLPI, thrombospondin-1, EMMPRIN, APP, α2-MRAP, syndecan-1, Sem3C, PTK7, ADAM9
MMP-15 (MT2-MMP)	Col I, IV, gelatin, fibronectin, laminin-1, vitronectin, aggrecan, tenascin, nidogen, perlecan, fibrin	ProMMP-2, tTg
MMP-16 (MT3-MMP)	Col III, IV, gelatin, casein, fibronectin, laminin-1, vitronectin, aggrecan, fibrin	ProMMP-2, CD44, APP, NgR1, syndecan-1, tTg
MMP-17 (MT4-MMP)	Gelatin, fibrillin, fibronectin	proTNFα, ADAMTS4
MMP-24 (MT5-MMP)	Heparan and chondroitin sulfate proteoglycans, gelatin, fibronectin	ProMMP-2, APP
MMP-25(MT6-MMP)	Col IV, gelatin, fibrin, fibronectin, vitronectin	A1AT, MBP

Table 2. The substrates of MMPs. Modified from Ahmad et al., 2006; Belkin et al., 2001; Chan et al., 2012; Endo et al., 2003; Ferraro et al., 2011; Golubkov and Strongin, 2012; Koziol et al., 2012; McCawley and Matrisian, 2001; Morrison et al., 2009; Patwari et al., 2005; Prudova et al., 2010; Shiryaev et al., 2009; Sternlicht and Werb, 2001.

Abbreviations: α1AC, alpha1 antichymotrypsin; α2M, alpha2 macroglobulin; α2-MRAP, alpha2 macroglobulin associated protein; A1AT, α1-antitrypsin; APOE, apolipoprotein E; APP, amyloid β A4 protein; C1q, complement C1q; Col, collagen; COMP, cartilage oligomeric matrix protein; CTGF, connective tissue growth factor; Cyr61, cystein-rich 61; EGF, epidermal growth factor; EMMPRIN, extracellular matrix metalloproteinase inducer; HSP-90α, heat shock protein 90 kDa alpha; IGFBP, insulin-like growth factor binding protein; IL, interleukin; MBP, myelin basic protein; ND, not determined; NgR1, Nogo-66 receptor-1; PAI, plasminogen

activator inhibitor; PCPE, procollagen C-endopeptidase enhancer 1; proHB-EGF, proheparin- binding EGF-like growth factor; PTK-7, protein-tyrosine pseudokinase 7; Sem3C, semaphorin 3C; SLPI, secretory leukocyte peptidase inhibitor; tTg, tissue transglutaminase

5.2.2 Regulation of MT-MMP activity

The activity of MT-MMPs is regulated mainly by posttranslational modifications/processes, such as autocatalytic cleavage and endocytosis, and inhibition by endogenous inhibitors. In addition, some transcriptional regulation occurs. Many MMP promoters contain elements interacting with such transcription factors as AP-1, NF-kB, and PEA3, suggesting coordinated transcription in response to growth factors or cytokines (Yan and Boyd, 2007). Unlike most MMPs, the promoters of both MMP-2 and MT1-MMP lack a conserved TATA box and AP-1 binding sites (Huhtala et al., 1990; Lohi et al., 2000). MT1-MMP promoter contains overlapping binding sites for transcription factors Sp1 and Egr-1, while MMP-2 promoter contains binding site only for SP-1 (Haas and Madri, 1999). AP-2 inhibits the transcription of MMP-2 and MMP-9 (Davidson et al., 2004; Frisch and Morisaki, 1990). Wnt/β-catenin pathway upregulates MT1-MMP via TCF transcription factor in colon carcinoma cells (Hlubek et al., 2004). In melanoma, β-catenin in turn upregulates the microphthalmia-associated transcription factor (MITF), which suppresses MT1-MMP expression, with subsequent inhibition of melanoma cell invasion in collagen (Arozarena et al., 2011). β-catenin upregulates also MT3-MMP in gastric cancer through unknown transcription factor (Lowy et al., 2006).

MT1-MMP transcription can be triggered by PMA, concavalin A and TNF-alpha (Lewalle et al., 1995; Migita et al., 1996), but not by bFGF or TGF-β1 (Lohi et al., 1996). Granulocyte-macrophage colony stimulating factor (GM-CSF) upregulates MT1-MMP mRNA via MEK in endothelial cell spheroids (Krubasik et al., 2008). Homeobox D10 inhibits MT1-MMP expression in endothelial cells (Myers et al., 2002). miR-10b in turn inhibits HOXD10, thus inducing MT1-MMP activity and glioma invasion (Sun et al., 2011). MT3-MMP can also be inhibited by microRNAs, namely miR-146a and miR-146b, leading to reduced cell motility of cultured glioma, colon cancer, and pancreatic cancer cells (Astarci et al., 2012; Lin et al., 2011; Xia et al., 2009). The translation of MT3-MMP is, in addition, tightly regulated by the formation of G-quadruplex in the 5'UTR region of its mRNA, which inhibits gene translation by more than half (Morris and Basu, 2009).

EMMPRIN, a member of the immunoglobulin family, induces MT1-MMP mRNA expression, the invasion of breast epithelial and carcinoma cells, and the formation of invadopodia, where MT1-MMP colocalizes with EMMPRIN (Grass et al., 2012). Tumor cell EMMPRIN is further cleaved by MT1-MMP, and its shed fragment can induce MMP-2 expression in fibroblasts (Egawa et al., 2006).

Interestingly, ECM itself and its mechanical properties regulate the transcription of MT1-MMP. Cultivation of cells inside 3D collagen type I but not on thin collagen or plastic induces MT1-MMP transcription and MMP-2 activation through the induction of MAPK and expression of Egr-1 transcription factor mRNA (Azzam and Thompson, 1992; Boyd et al., 2005; Haas et al., 1998; Montesano et al., 1999). This effect is mediated by integrin α2β1 and α3β1 clustering upon binding to collagen, which leads to Src phosphorylation and Egr-1 upregulation (Barbolina et al., 2007). Integrin clustering induces the localization of MT1-

MMP to the integrin complexes on the cell surface, allowing specific targeting of pericellular proteolysis to the invading edge of the cell (Ellerbroek et al., 2001; Munshi and Stack, 2006; Wolf et al., 2003).

Posttranslational regulation of MT-MMP activity involves autocatalytic cleavage, endocytosis and degradation, phosphorylation, and inhibition of activity by endogenous tissue inhibitors of metalloproteinases (TIMPs). MT1-MMP activity correlates with accumulation of its inactive autocatalytic 43-45 kDa fragments (Ellerbroek et al., 2001; Lehti et al., 1998; Lehti et al., 2000; Lohi et al., 1996). This cleavage occurs mainly *in trans* by an adjacent molecule of MT1-MMP. In addition, the induction of the MT1-MMP cleavage to its 44 and 37 kDa fragments by incubation with active MMP2, MMP13 and soluble MT1-MMP has been described (Stanton et al., 1998). The 44 kDa truncated fragment of MT1-MMP can prevent its collagenolytic activity, providing a feedback mechanism for regulation of MT1-MMP activity (Tam et al., 2002).

Internalization of MT1-MMP is another mechanism to regulate the net amount of the active enzyme at the cell surface. It occurs via clathrin or caveolae-dependent endocytosis, for example in response to concavalin A treatment (Annabi et al., 2001; Jiang et al., 2001; Uekita et al., 2001). Internalized MT1-MMP may be targeted for lysosomal degradation, or recycled back to the cell surface (Remacle et al., 2003). The internalization-defective mutant MT1-MMP does not promote cell invasion, emphasizing the importance of MT1-MMP endocytosis for continuous recycling of the active enzyme to the invading cell edge during cell invasion (McAllister et al., 1994). Collagen type I, fibronectin, and fibrinogen inhibit MT1-MMP internalization in endothelial cells, increasing its cell surface expression in cell-cell contacts, where it colocalizes with β1 integrin (Galvez et al., 2002). MT1-MMP trafficking to the invadopodia via Rab8- and VAMP-7-dependent exocytosis is essential for collagen invasion ability of the cells (Bravo-Cordero et al., 2007; Steffen et al., 2008).

MT1-MMP activity is regulated also by the Src dependent tyrosine-573 phosphorylation of its C-cytoplasmic domain (Nyalendo et al., 2007; Nyalendo et al., 2008). Phosphorylation defective mutant accumulates on the cell surface, leading to epithelial-to-mesenchymal transition and enhanced collagen invasion of ovarian carcinoma cells (Moss et al., 2009). In addition, growth factor receptors regulate MT1-MMP activity. EGFR activation by EGF leads to increased MT1-MMP mRNA expression, MT1-MMP phosphorylation, and internalization (Moss et al., 2009; Van Meter et al., 2004). Fibroblast growth factor receptor-4 (FGFR4) R388 polymorphism induces MT1-MMP phosphorylation and decreases its lysosomal degradation, leading to enhanced tumor cell invasion (Sugiyama et al., 2010a; Sugiyama et al., 2010b).

5.2.3 Tissue inhibitors of metalloproteinases

Endogenous tissue inhibitors of metalloproteinases (TIMPs) are secreted proteins which inhibit MMP activity by non-covalent binding to their catalytically active sites (Baker et al., 2002). Four different TIMPs have been described in man (Table 3). They are composed of N-terminal domains, and smaller C-terminal domains, each domain being stabilized by three disulfide bonds. N-terminal domain of TIMPs binds to the catalytical sites of MMPs even in the absence of their C-terminal domains (Baker et al., 2002). However, C-terminal domain of TIMP-2 is essential for the ternary complex formation during MMP-2 activation, as it binds to

C-terminal domain of proMMP-2 (Butler et al., 1998; Strongin et al., 1995). In addition, the C-terminal domain of TIMP-3 mediates its tight association with ECM (Brew et al., 2000). TIMPs exhibit different selectivity towards MMPs and ADAMs (Table 3). Both pro- and antitumorigenic effects have been described for TIMPs (Baker et al., 2002). TIMP-2, -3, and -4 inhibit endothelial tube formation. TIMP-1, -2, and -3 knockout mice are viable and fertile. Female TIMP-1 knockout mice have shorter reproductive period (Nothnick, 2001). TIMP-2 knockout mice appear normal, despite perturbed proMMP-2 activation in the tissues (Caterina et al., 2000). This is consistent with almost normal phenotype of MMP-2 deficient mice, which exhibit only slight growth reduction (Itoh et al., 1997). TIMP-3 knockout mice develop emphysema-like condition starting from two weeks after birth, which correlates with increased collagen degradation in their lungs (Leco et al., 2001).

Inhibitor	Size	Localization	MMPs inhibited	ADAMs inhibited	Gene expression upregulated by
TIMP-1	28 kDa	Soluble	Secreted MMPs, MT4-MMP, MT6-MMP	ADAM10	TGF-β, bFGF, EGF, TNF-α, PDGF, IL-1, IL-6, PMA, retinoic acid, progesterone, oncostatin M
TIMP-2	21 kDa	Soluble/cell surface	Secreted MMPs, MT-MMPs	None	cAMP, LPS, retinoic acid, progesterone
TIMP-3	24/27 kDa	ECM	Secreted MMPs, MT-MMPs	ADAM10, -12, -17, -19, ADAMTS-4, -5	TGF-β, PMA
TIMP-4	22 kDa	Soluble/cell surface	Secreted MMPs, MT-MMPs	None	

Table 3. Tissue inhibitors of metalloproteinases. Modified from Amour et al., 2000; Baker et al., 2002; Henriet et al., 1999; Will et al., 1996.

6. Tumor cell invasion

6.1 Types of invasion

Cell migration and invasion are required for various physiological processes, e.g. gastrulation, neural crest migration, immune cell surveillance, wound closure, and angiogenesis. In cancer, the ability of tumor cells to invade confers them with the lethal metastatic potential. Tumor cells can use interchangeable methods to invade across interstitial tissues depending on the microenvironment (Alexander et al., 2008). Cell invasion is divided to single cell and collective invasion, and single cell invasion can be further classified to amoeboid and mesenchymal-type invasion (Fig. 5, Friedl, 2004).

In single cell invasion the contacts between the invading cells are lost. Amoeboid invasion received its name from the invasive phenotype of amoeba *Dictyostelium discoideum.* Its fast movement is characterised by bleb-like protrusions, weak non-integrin interactions with surrounding ECM, and the lack of MMP-dependent ECM degradation (Friedl, 2004). In man, leukocytes and some tumor cells engage in amoeboid movement, whereby the cells squeeze between the fibers in the pre-existing spaces of the ECM. Protease-independent amoeboid invasion is possible only when matrix pore size and rigidity permits cell squeezing through the pores. This requires the ability to modify cell shape, and to push the surrounding ECM to displace its fibers instead of degrading them (Wolf and Friedl, 2011). Amoeboid invasion is fast, 2-25 μm/min (Brabek et al., 2010). Small GTPases are closely involved in the mediating signals from the ECM to cytoskeleton, a process required for the cell morphology changes during different types of invasion. Thus, RhoA activation is linked to amoeboid type invasion, but in 2D environment it mediates stress fiber formation and cell spreading (Friedl, 2004). In amoeboid invasion, RhoA induces cortical actin polymerization, generating cortical tension, stiffness and roundish cell morphology (Friedl, 2004).
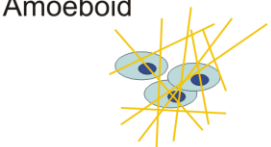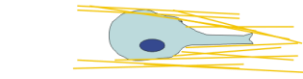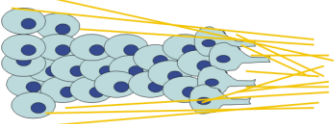
	Integrins	Proteases	Cadherins
Amoeboid	-/+	-/+	-
Mesenchymal	+	+	-
Collective	++	++	++

Figure 5. Types of invasion. Modified from Friedl, 2004.

Fibroblasts and most tumor cells engage in slower (0.1-2 μm/min) mesenchymal-type movement, where the cells are elongated, have invadopodia/filopodia, integrin-mediated adhesions to the ECM, and MMP-dependent matrix degradation activity (Friedl, 2004). This type of invasion is often associated with epithelial-to-mesenchymal transition (EMT), where epithelial cells downregulate proteins associated with epithelial phenotype, such as E-cadherin, upregulate mesenchymal markers, such as N-cadherin and vimentin, lose their cell-cell contacts, and move in a single cell manner. Mesenchymal invasion involves tentatively five different steps. First, cell polarization to cell front and rear occurs, which enables invadopodia formation at the invading edge (step 1). At the cell front membrane protrusions attach to the ECM via focal adhesions, mediated by integrins (step 2), where MT1-MMP,

urokinase-type plasmin activator (uPA) and its receptor (uPAR) are also recruited for initiation of pericellular proteolysis (step 3, Belkin et al., 2001; Wei et al., 1996; Wolf et al., 2007). Then, myosin II binds to actin filaments and generates actomyosin contraction (step 4). The posterior cell body loses its focal contacts with the ECM, and the trailing tail of the cell moves forward (step 5, Friedl and Wolf, 2003; Lauffenburger and Horwitz, 1996). Small GTPases Rac1 and Cdc42 are activated during mesenchymal type invasion. They induce lamellipodia and filopodia formation, actin assembly, and cell elongation (Friedl, 2004). Tumor cells can shift their motility mode between mesenchymal and amoeboid types, called mesenchymal-amoeboid transition (MAT), for example when MMP activity is reduced by inhibitors (Friedl, 2004; Wolf et al., 2003).

Recently it has been reported that when cells are confined to move through 3 μm channels, they do not use actin polymerization, actomyosin contraction, or small GTPases. Instead, this type of movement depends on microtubule polymerization (Balzer et al., 2012).

Collective invasion has been noticed in the histopathological sections of various human cancers, such as melanoma and breast cancer (Friedl et al., 2012; Silye et al., 1998). Vascular sprouting can also be considered as an example of collective invasion. In this type of movement, multicellular polarization occurs with the leading edge acquiring mesenchymal-type phenotype with MMP-activity, integrin-dependent ECM adhesion, and invadopodia formation, while the cells in the stalk region retain their epithelial-like phenotype and cell-cell contacts. Cell-cell contacts are mediated by adhesion junction proteins, such as cadherins, by integrins, and by other cell-cell contact proteins, such as L1CAM (Friedl and Gilmour, 2009). The invasion type used by tumor cells is dictated by microenvironment. For example, in loose connective tissue, HT-1080 cells invade mainly as single cells, whereas in areas with pre-existing structures, such as collagen fibers, muscles and lymphatic vessels, HT-1080 cells engage in collective invasion moving along these structures (Alexander et al., 2008).

6.2 Three-dimensional (3D) matrix models for studies of cell invasion

Cell signalling and expression of adhesion receptors is different in 2D and 3D systems (Cukierman et al., 2002). Therefore, 3D models recapitulate better *in vivo* environment than 2D migration assays. Both single cell invasion and collective invasion can be analyzed using 3D models (Friedl and Gilmour, 2009). Interstitial tissue features which affect tumor invasion include molecular composition, fiber density, assembly and cross-linking, pore size, stiffness, and liner vs. non-linear elasticity (Petrie et al., 2012; Wolf and Friedl, 2011). *In vivo*, connective tissues are rather heterogeneous, with loose and dense areas, gaps, and fiber bundles of various thicknesses (Wolf et al., 2009). The most common 3D models which mimic some of these features and can be used for cell invasion studies *in vitro* are the hydrogels of collagen type I, fibrin, and a basement membrane analogue Matrigel. The other ECM components, such as fibronectin, vitronectin, hyaluronan, laminin, or stromal cells, such as fibroblasts, may be added to the hydrogels (Wolf et al., 2009).

Collagen type I remains soluble after extraction by acidic pH, and needs to be neutralized for polymerization to occur. Acid-extracted collagen contains nonhelical telopeptides at the N- and C–terminal ends, which covalently crosslink collagen molecules together (Sabeh et al., 2009). It is rapidly polymerized upon neutralization, with the eventual pore size of 1-2 μm. Pepsin-extracted collagen lacks telopeptides and polymerizes more

slowly, with the pore size of 3-5 µm (Wolf et al., 2009). However, Demou et al. (2005) reported that the pore size of pepsin-treated collagen is smaller than that of telopeptide-intact collagen, suggesting that eventual pore size depends also on the experimental conditions (Demou et al., 2005). Matrigel is the trade name for a basement membrane analogue extracted from Engelbreth-Holm-Swarm (EHS) mouse sarcoma tumors. It consists mainly of laminin, collagen type IV, heparan sulfate proteoglycan and nidogen. Matrigel, however, differs from *in vivo* basement membranes, since its components are much less cross-linked. Subsequently, the invasion through Matrigel, unlike the invasion through *in vivo* basement membrane, does not require MMP activity (Hotary et al., 2006; Sodek et al., 2008).

The composition of 3D collagen gels, the degree of collagen crosslinking, and pore size affect the speed of the invading cells, dictates the requirement of MT-MMPs in cell invasion, and determines whether the cells can invade in the hydrogel in amoeboid fashion without matrix degradation (Sabeh et al., 2009; Wolf et al., 2009). For the cell to move through a certain pore without proteolytic degradation, the diameter of the pore must be bigger than what the cell nucleus can fit by deformation. The nucleus is the most rigid organelle of the cell, being a rate-limiting feature in amoeboid migration (Sabeh et al., 2009; Wolf and Friedl, 2009). The diameter of the cell nucleus is usually between 5 and 15 µm, while pore size *in vivo* can range from less than 1 µm in the basement membrane to 30 µm in the loose connective tissue (Friedl et al., 2011). Myosin II, an ATP-dependent motor protein required for actin filament contraction, is required for nucleus deformation during cell migration through narrow spaces, since myosin II silenced glioma cells cannot migrate through the pores smaller than their nuclear diameters (Beadle et al., 2008). In 3D collagen hydrogels, the mechanistic properties of the formed collagen depend, in addition to collagen concentration, on the experimental conditions during collagen polymerization. Thus, pH and the temperature of collagen solution during polymerization inversely correlate with the eventual collagen fiber diameter and pore size of the hydrogel (Raub et al., 2007; Raub et al., 2008; Wolf et al., 2009).

Further, chicken chorioallantoic membrane (CAM) is widely used to mimic tumor cell microenvironment. It is composed of a chorionic epithelium of ectodermal origin, an intermediate mesenchyme, and an endodermal allantoic epithelium. Both epithelium layers are separated from the mesenchyme by BM (Li et al., 2008; Ota et al., 2009). In addition, organotypic models have been introduced to better mimic *in vivo* tumor stroma which, in addition to ECM proteins, contains various non-malignant cells (Jenei et al., 2011). In human leiomyoma organotypic model, tumor cells are cultured atop the disks of myoma tissues, which are composed of stromal ECM proteins, fibroblasts, endothelial and inflammatory cells (Nurmenniemi et al., 2009). The invasion of myoma tissue by tumor cells is suitable for studying MMP functions in tumor invasion since it is MMP-dependent. In addition, mouse tissue explants, for example from mouse ears, can be used to study cell invasion (Petrie et al., 2012).

6.3 MT-MMPs in tumor cell invasion

MT1-MMP is required for tumor cell invasion through 3D native cross-linked type I collagen or CAM membrane. Despite the fact that several secreted proteases can cleave collagen type I, only membrane-tethered enzymes can promote cell movement through this type of collagen

(Sabeh et al., 2004). This may be due to the requirement of targeted pericellular proteolysis at the invading edge, or the importance of physical interactions with signalling receptors mediated by C-terminal domain of MT-MMPs. In addition, cell surface localization of MT-MMPs can activate intracellular signalling which promote cell migratory functions. Indeed, the activation of ERK signalling by MT1-MMP promotes cell invasion in Matrigel (Gingras et al., 2001). MT2-MMP can also drive tumor cell invasion through collagen type I and CAM. MT1-, MT2- and MT3-MMP, in turn, can each mediate cell invasion through basement membrane and fibrin (Hotary et al., 2006; Hotary et al., 2002). Epithelial-to-mesenchymal transition –like changes are often seen in the invading front of the carcinomas. The process is regulated by Snail, Slug and Twist transcription factors. Snail-1 induces MT1-MMP and MT2-MMP expression, with subsequent induction of CAM invasion (Ota et al., 2009).

MT1-MMP is recruited to focal adhesions, where it colocalizes with $\beta 1$ integrin (Wolf et al., 2007). Further, MT1-MMP accumulates in invadopodia, where it colocalizes with cortactin (Artym et al., 2006; Poincloux et al., 2009). MT1-MMP promotes cell motility also by cleaving hyaluronan receptor CD44, which is widely expressed in tumor cells, cell surface proteoglycan syndecan-1, and several integrins (Kajita et al., 2001; Endo et al., 2003). Small GTPase Rac1 induces MT1-MMP expression and collagen invasion (Itoh et al., 2001; Zhuge and Xu, 2001). Thus, MT1-MMP promotes tumor invasion by several mechanisms, including ECM matrix degradation, activation of intracellular signalling cascades, regulating invadopodia formation, activation of other MMPs, and the cleavage of cell surface proteins, which are involved in cell invasion (Fig. 6). Although the expression of the other MT-MMPs has been described in several cancers, their functions in cancer cell invasion remain largely unknown.
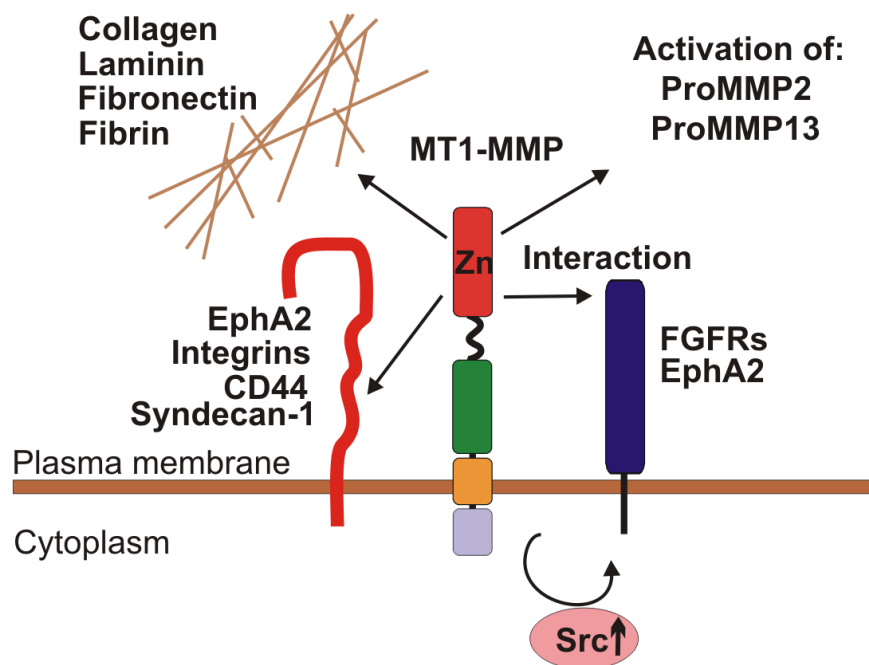

Figure 6. The functions of MT1-MMP which promote cell invasion. Modified from Itoh and Seiki, 2006, Sugiyama et al., 2010a.

7. Tumor microenvironment

Solid tumors are increasingly considered to be not just a mass of transformed cells, but new kind of complex organs with interstitial tissue and non-malignant cells which affect tumor progression (Hanahan and Weinberg, 2011). The tumor microenvironment consists of ECM, growth factors, cytokines, non-malignant cells, blood and lymphatic vessels, nutrients, and oxygen (Postovit et al., 2006). Hypoxia can promote both malignant transformation and tumor growth by upregulating several signalling pathways, such as HIF-1α (Bedogni et al., 2005; Harris, 2002). Interestingly, ECM conditioned by metastatic melanoma cells induces malignant transformation of normal melanocytes (Seftor et al., 2005). Furthermore, exposing melanoma cells to the embryonic environment (e.g. by injecting them into the chick embryo) can reprogram melanoma cells to more benign differentiated phenotype (Kulesa et al., 2006; Postovit et al., 2006).

7.1 Tumor ECM

The physical properties of ECM, such as stiffness, porosity, elasticity, and organization, directly affect cell proliferation and invasion (Alexander et al., 2008; Lu et al., 2012). ECM stiffness, for example, is sensed by cells via integrins and focal adhesion components which link ECM to the cell cytoskeleton (Lu et al., 2012). ECM serves both as a barrier for tumor invasion, and provides anchorage for tumor invasion along its fibers (Alexander et al., 2008). Further, ECM binds many growth factors and cytokines, and limits their free diffusion and accessibility. Biochemical properties of the ECM also affect tumor cell proliferation and invasion. In tumors, ECM production, organization, and degradation are deregulated. Tumors are often stiffer than surrounding tissues (Lopez et al., 2011). Mammographically dense breast tissue correlates with higher incidence of breast cancer (McCormack and dos Santos Silva, 2006). Consistently, Col1A1 overexpressing transgenic mice develop more breast adenocarcinomas than control mice, and the developed tumors are more invasive and metastatic (Provenzano et al., 2008). Overexpression of lysyl oxidase induces collagen crosslinking and tumor invasiveness (Levental et al., 2009). The production of many ECM components and their receptors, such as collagens type I, II, III, V, IX, CD44, is increased in cancer (Lu et al., 2012). In addition, the secretion of MMPs by tumor cells and stromal cells is induced (Kessenbrock et al., 2010).

In addition to collagen, also the tenascin-C glycoprotein has also been implicated in carcinogenesis. Thus, it is upregulated in melanoma, and correlates with melanoma progression and metastasis (Tuominen and Kallioinen, 1994). Tenascin-C promotes stem cell-like phenotype of melanoma cells and growth of melanoma spheres (Fukunaga-Kalabis et al., 2010).

Fibrinogen is often detected in tumors around blood vessels and at the tumor/host tissue interface and is associated with tumor progression (Costantini and Zacharski, 1992; Hotary et al., 2002). Xenograft tumors form significantly less distant metastases when grown in fibrinogen deficient mice compare to control mice, probably due to the lack of fibrinogen deposition around the circulating tumor cell emboli, which may promote tumor cell adhesion to the capillary walls of distant organs (Palumbo et al., 2002).

7.2 Non-malignant cells in tumor stroma

Tumor cells affect non-malignant cells in tumor stroma, which differentiate from tumor-suppressing to tumor-supporting cells. Cancer associated fibroblasts (CAFs) are irreversibly activated cells probably derived from normal fibroblasts, epithelial and endothelial cells, or cancer cells themselves (Petersen et al., 2003; Xing et al., 2010). They express α-smooth muscle actin (α-SMA), reminding the phenotype of myofibroblasts contributing to wound healing (Xing et al., 2010). CAFs secrete fibrin, MMPs, hyaluronan, and promote both tumor cell and stromal cell invasion through multiple signalling pathways (Pasonen-Seppänen et al., 2012; Xing et al., 2010). For example, IL-1α and bFGF secreted by melanoma cells induce CAFs to secrete MMP-1, which in turn promotes ECM degradation and melanoma cell invasion (Löffek et al., 2005). Furthermore, the interaction of melanoma cells with CAFs mediated by ADAM9 facilitates melanoma cell invasion (Zigrino et al., 2011).

Another group of non-malignant cells in tumor stroma are the bone marrow derived cells (BMDCs), including macrophages, neutrophils, mast cells, myeloid cell-derived suppressor cells (MDSCs), and mesenchymal stem cells (Joyce and Pollard, 2009). In the past the presence of immune cells was considered to reflect host immune reaction against developing tumor. However, tumor cells can escape immune surveillance, and in addition can render immune cells to become tumor promoting (Joyce and Pollard, 2009).

Tumor associated macrophages (TAMs) have complex pro- and antitumorigenic effects. TAM density often correlates with poor prognosis in breast cancer and melanoma (Makitie et al., 2001; Zhang et al., 2012b). High macrophage count in melanoma is associated with ulceration, Breslow thickness, mitosis rate, lymphocyte infiltration, vascular density and lymphatic vessel invasion (Chen et al., 2011; Storr et al., 2012). Tumor cells secrete chemokines which attract TAMs, which in turn secrete immunosuppressive cytokines, interleukin-10, TGF-β, and VEGF-A, further affecting tumor cell proliferation and angiogenesis (Balkwill, 2004). Macrophage colony–stimulating factor (CSF-1) promotes macrophage infiltration and tumor invasion in breast cancer transgenic model (Lin et al., 2001). However, some report the positive correlation between TAM infiltration and patient survival (Bingle et al., 2002). Further, VEGF-A produced by macrophages is known to contribute to abnormal tumor vasculature and to inhibit tumor growth (Stockmann et al., 2008), suggesting that the net effect of TAM infiltration may differ depending on tumor type, experimental conditions and other factors in tumor microenvironment (Bingle et al., 2002).

7.3 Tumor angiogenesis

Angiogenesis is the formation of new blood vessels from pre-existing ones. In the adult organs, it occurs during wound healing and menstruation cycle. Excessive or deregulated angiogenesis contributes to various pathological conditions, such as arthritis, inflammation, diabetic retinopathy, and cancer. In diseased tissues, angiogenesis is initiated if the balance between pro- and antiangiogenic factors is tilted towards stimulators, a phenomenon called "angiogenic switch" (Carmeliet, 2005). Endothelial cells (ECs) of an adult organism are mostly quiescent, and their turnover time is hundreds of days (Kalluri, 2003). During embryogenesis, angioblasts assemble to create primitive vascular plexus. This process is called vasculogenesis. During angiogenesis, in turn, endothelial cells sprout from pre-existing vessels after receiving signals from growth factors. The sprouting begins when one cell

becomes a tip cell, while the cells next to it become stalk cells and start to proliferate (Gerhardt, 2008).

Tumor angiogenesis is a prerequisite for tumor growth beyond the size of ~1 mm (Carmeliet and Jain, 2000). Melanoma cells secrete many proangiogenic molecules, such as VEGF, bFGF, IL-8, PDGF and PlGF (Streit and Detmar, 2003). The proteoglycans of endothelial basement membrane (BM) sequester VEGF and bFGF, which are released by MMP-9 and MMP-2 derived from stromal and immune cells during tumor angiogenesis (Bashkin et al., 1989; Gengrinovitch et al., 1999; Kalluri, 2003). MMP-9 cleaves collagen type IV, causing the disorganization of BM. As MMP-dependent degradation of BM proceeds, both pro-angiogenic (early stages) and anti-angiogenic (later stages) cryptic sites or peptides are exposed (Kalluri, 2003; Petitclerc et al., 2000; Xu et al., 2001). The imbalance of pro- and antiangiogenic signals in tumors leads to abnormal vasculature. Tumor vessels are often fragile, hyperpermeable, and have gaps in pericyte lining. This leads to tumor hypoxia despite of high vessel density, and release of proangiogenic factors, which results in even more abnormal vessels (Ziyad and Iruela-Arispe, 2011).

Blocking tumor angiogenesis as a cancer treatment option was proposed already in 1971 (Busam et al., 1995). However, the prognostic value of blood vessel density in melanoma remains controversial. Some studies report inverse correlation of vascular density with survival (Graham et al., 1994; Kashani-Sabet et al., 2002; Vlaykova et al., 1999), while others report no correlation (Busam et al., 1995; Carnochan et al., 1991), or even positive correlation (Ilmonen et al., 1999). Vascular density is positively correlated with melanoma stage (Storr et al., 2012). Furthermore, the expression of miRNAs which target ApoE, an angiogenesis inhibitor, is positively correlated with melanoma metastasis (Pencheva et al., 2012). In addition, decreased tumor angiogenesis in MMP-13 knockout mice is associated with reduced melanoma xenograft growth as well as reduced lung and liver metastasis (Zigrino et al., 2009). Serum levels of proangiogenic factors, such as VEGF, bFGF and IL-8, correlate with decreased patient survival (Ugurel et al., 2001). Overexpression of VEGF in melanoma cells induces tumor growth, vascularization, and lung colonization in mice (Claffey et al., 1996), though the effects of VEGF vary depending on its isoform (Yu et al., 2002). The expression of VEGF165 isoform in melanoma xenografts induces higher level of vascularization than expression of VEGF121, but the tumor growth rate remains comparable (Yu et al., 2002). Further, melanomas usually express VEGF121, which leads to moderate vascularization, suggesting that moderate vascularization would be sufficient or even optimal for melanoma growth (Yu et al., 2002). In addition, melanoma cells extracted from hypoxic areas and injected back to mice grow faster than melanoma cells extracted from the vicinity of a blood vessel (Yu et al., 2001). Consistently, the induction of tumor hypoxia can even promote tumor invasiveness and metastasis (Harris, 2002), which may partly explain the lack of clear correlation between vessel density, tumor growth and prognosis. An interesting feature of some aggressive melanomas is vascular mimicry, a phenomenon where melanoma cells are organised in fluid-conducting tubes which are not lined with endothelial cells (Hendrix et al., 2003; Ribatti et al., 2012).

The importance of tumor angiogenesis has led to the development of antiangiogenic therapy for cancer treatment. Antibody against VEGF-A (bevacizumab) and kinase inhibitors which target VEGFR (sorafenib, sunitinib) have been approved for treatment of several human cancers in combination with chemotherapy (Bergers and Hanahan, 2008). These inhibitors often demonstrate initial clinical benefit in many cancer types, but tumors rapidly

adapt and develop mechanisms to overcome the effects of angiogenesis inhibitors (Bergers and Hanahan, 2008). These mechanisms may include the secretion of other proangiogenic factors, such as FGF, angiopoetins, and ephrins, the recruitment of immune cells which can stimulate angiogenesis, and the recruitment of bone marrow-derived cells (BMDCs) which can form new vessels. In addition, hypoxia may induce tumor cell invasion which facilitates the co-option of pre-existing vasculature, leading to distant organ colonizaion (Bergers and Hanahan, 2008; Casanovas et al., 2005).

7.4 Tumor lymphangiogenesis

Lymphangiogenesis has been much less explored than angiogenesis, since until recently no specific markers for lymphatic vessels have been known. During the last few years, however, this area of research has gained increasing attention, and especially evaluation of the functions of the lymphatic vessels in tumor progression has become possible. Markers currently used for detecting lymphatic vessels include LYVE-1 (Prevo et al., 2001), podoplanin (Kahn et al., 2002; Weninger et al., 1999), VEGFR-3 (Kaipainen et al., 1995), and PROX-1 (Wigle and Oliver, 1999). Lymphangiogenesis is generally induced by VEGF-C and VEGF-D, which bind to their receptor VEGFR-3 expressed by lymphatic endothelial cells (LECs) (Stacker et al., 2002). VEGF-C can also bind to VEGFR-2 expressed by blood vessel endothelial cells (BECs) and LECs (Stacker et al., 2002). Some report that in tumors with high interstitial pressure no lymphangiogenesis or no functional intratumoral lymphatics may exist, and tumor cells are spreading via peritumoral lymphatics (Padera et al., 2002; van der Schaft et al., 2007). However, there is strong evidence for the existence and functionality of intratumoral lymphatics (Beasley et al., 2002; Skobe et al., 2001; Van der Auwera et al., 2005). This discrepancy can be explained by different cancer types examined and models used (Alitalo and Detmar, 2012; Beasley et al., 2002; Van der Auwera et al., 2005). Even the tumor growth pattern can affect lymphangiogenesis. Thus, although lymphatic density is higher in infiltrating breast tumors growing around the host structures, lymphangiogenesis is stronger in breast tumors which grow in an expansive manner destroying pre-existing lymphatics (Van der Auwera et al., 2005). Nowadays it is widely accepted that at least in tumors which spread primarily through lymphatics, such as breast cancer, prostate cancer, and melanoma, the intratumoral lymphatics are functional (Alitalo and Detmar, 2012; Burton et al., 2008). Tumor cells induce lymphangiogenesis also inside sentinel lymph node metastasis, which further promote the formation of postsentinel metastasis (Kerjaschki et al., 2011). Interestingly, tumors induce lymphangiogenesis also in the sentinel lymph nodes even before metastatic cells arrive there (Hirakawa et al., 2005).

Tumor lymphangiogenesis can be induced by VEGF-C secreted by both tumor cells and immunity cells, such as macrophages (Alitalo and Detmar, 2012; Moussai et al., 2011). The lymphatic density of the tumors correlates with lymph node metastases and decreased patient survival in breast cancer and melanoma (Alitalo and Detmar, 2012; Choi et al., 2005; Dadras et al., 2003; Dadras et al., 2005; Massi et al., 2006; Mohammed et al., 2009; Nakamura et al., 2005; Ran et al., 2010). Inhibition of VEGFR-3 can restrict lymphangiogenesis and lymph node metastasis in mouse xenograft assays (Alitalo and Detmar, 2012; Burton et al., 2008; He et al., 2005). The increased ratio of peritumoral/intratumoral lymphatic vessels appears to predict lymph node metastasis better than the overall lymphatic vessel density (LVD, Dadras

et al., 2005; Shayan et al., 2012). Others have reported that intratumoral LVD is a more significant factor predicting sentinel lymph node metastasis than peritumoral LVD (Massi et al., 2006). Tumor lymphatics express specific markers, which can be used to home the cytotoxic peptides to the tumors (Laakkonen et al., 2008).

7.5 Vascular and lymphatic intravasation of tumor cells

Metastatic spread both to local sites (satellite metastasis/ in transit metastasis) or distant sites occurs via vascular or lymphatic system. The first step of this process is intravasation of tumor cells into intra- or peritumoral blood and lymphatic vessels. Some cancers, such as melanoma or breast cancer, metastasize in the early stages to the regional lymph nodes, while others, such as soft tissue sarcoma, engage almost solely in haematogenous spread (Leong et al., 2011).

To date the mechanisms of intravasation have remained largely unknown. It is unclear if tumor cells actively invade into vessels, or whether they are passively pushed (Bockhorn et al., 2007). Intratumoral imaging techniques have revealed that breast cancer cells actively orient toward the blood vessels before entering the circulation (Wyckoff et al., 2000). Blood vessel invasion seems to require active permeabilization of blood vessels by tumor cells (Stoletov et al., 2007), while lymphatic vessels may permit passive tumor cell passage since they have discontinuous basement membrane and intraendothelial channels (Azzali, 2007). Further, VEGF-C and VEGF-D released by tumor cells induce peritumoral lymphatic vessel dilation and sprouting, which makes them even more permissive for passive tumor cell entry (He et al., 2005).

The factors which affect the preference of tumor cells to intravasate into blood or lymphatic vessels are getting better understood. Several studies have indicated that circulating tumor cells are nonviable, but it remains to be seen whether they die in the circulation, or whether dead cells are passively shed into the circulation. Consistently, the number of circulating tumor cells does not directly correlate with the amount of metastatic colonies (Butler and Gullino, 1975; Fidler, 2003). In contrast, the presence of tumor cells in the regional lymph nodes predicts the risk of distant metastasis. Further, the ability of tumor cells to invade into lymphatic vessels, but not blood vessels, correlates with lung metastases in breast cancer xenografts (Giampieri et al., 2009). In addition, malignant cells often express endothelial adhesion receptors, which may facilitate their adhesion and extravasation. Some tumors also express the same chemokine receptors as immunity cells, which, when stimulated by chemokines released from lymphatic endothelial cells, can guide tumor cells to the draining lymph nodes (Muller et al., 2001; Wiley et al., 2001). Thus, some melanomas express CCR10, CCR7, and CXCR4. Inhibition of CCL12/CXCR4 signalling prevents breast cancer cell metastasis to the regional lymph nodes (Muller et al., 2001), and overexpression of CCR7 in B16 melanoma cells induces lymph node metastasis in mice (Wiley et al., 2001). Interestingly, lymphatic endothelial cells, but not blood endothelial cells, secrete CCL21, a ligand for CCR7 receptor often expressed by melanoma cells (Kriehuber et al., 2001).

Lymphatic vessel invasion (LVI) has been detected in 16-63% of melanoma biopsies (Storr et al., 2012). It correlates strongly with sentinel lymph node status and decreased patient survival in breast cancer (Mohammed et al., 2009; Ran et al., 2010; Van der Auwera et al., 2005; van der Schaft et al., 2007) and melanoma (Egger et al., 2011; Niakosari et al.,

2008; Petersson et al., 2009; Rose et al., 2011). In melanoma, LVI also correlates with the nodular subtype (Storr et al., 2012). Macrophages may promote LVI in melanoma (Storr et al., 2012). Shields et al. (2004) proposed the use of Shields index, which combines lymphatic density, Breslow thickness and LVI, and may predict overall survival even more accurately than Breslow thickness alone (Emmett et al., 2010; Shields et al., 2004).

Blood vessel invasion (BVI) has been detected in 3-43% of melanoma biopsies, but it does not correlate with the disease stage or patient survival (Storr et al., 2012). In addition to melanoma, LVI is also more frequently observed than BVI in breast cancer (Ran et al., 2010). According to some reports, BVI also correlates with disease progression. HIF-1α, angiopoietin-like 4, and L1CAM in breast cancer cells promote transendothelial migration, BVI, and lung metastasis (Zhang et al., 2012a).

7.6 MT-MMPs in angiogenesis and tumor progression

MT-MMPs have been implicated in several key aspects of tumor progression, including tumorigenesis, local spreading, angiogenesis, tumor cell intravasation and extravasation. The upregulation of all six MT-MMP family members has been reported in various cancers (see 5.2, Membrane-type matrix metalloproteinases).

The fine balance between proteinases and their inhibitors tightly control the processes of angiogenesis, such as sprouting, vessel stabilization and regression. MT1-MMP is essential in several processes during angiogenesis. It induces both new vessel sprouting as well as pericyte recruitment (Lehti et al., 2005; Yana et al., 2007). MT1-MMP is expressed in developing large arteries during mouse embryogenesis (Apte et al., 1997). MT1-MMP deficient mice exhibit severe defects in angiogenesis (Zhou et al., 2000). For example, reduced vascularization of forming bones perturbs normal bone ossification in these mice. Further, in cornea angiogenesis assay, no formation of new blood vessels occurs in response to FGF treatment in MT1-MMP deficient mice (Zhou et al., 2000). VEGF and FGF -induced tube formation in 3D fibrin or collagen type I requires MT1-MMP but not MMP-2 activity (Chun et al., 2004; Lafleur et al., 2002). Melanoma cell -derived VEGF-A induces MT1-MMP expression in cultured endothelial cells, leading to increased tube formation by ECs grown on 3D fibrin (Löffek et al., 2006). MT1-MMP in turn induces VEGF-A transcription via Src phosphorylation (Deryugina et al., 2002; Sounni et al., 2004). Further, MT1-MMP promotes tumor vascularization when overexpressed in MCF7 breast cancer cells, which do not normally express MT1-MMP (Sounni et al., 2002a). The expression of MT1-MMP is associated with BVI and lung metastasis, but not with LVI, in breast cancer (Perentes et al., 2011). However, there are contrasting reports demonstrating that MT1-MMP downregulation does not affect blood vessel intravasation of fibrosarcoma cells (Deryugina et al., 2005). MT3-MMP is expressed together with MT1-MMP in endometrial microvascular endothelial cells, where it promotes tube formation in 3D fibrin (Plaisier et al., 2004).

In addition to being involved in angiogenesis and tumor cell invasion, MT1-MMP can promote tumorigenesis by inducing malignant transformation of some cells. Thus, overexpression of MT1-MMP in non-malignant Madin-Darby canine kidney epithelial (MDCK) cells leads to the formation of invasive xenograft tumors in mice (Soulie et al., 2005). MT1-MMP expression is also increased upon MDCK cell transformation by v-src or erbB2 (Kadono et al., 1998). Intracellular proteolytic activity of MT1-MMP against

pericentrin enhances chromosomal instability and may be one of the mechanisms how MT1-MMP promotes tumor progression (Golubkov et al., 2005).

The functions of MT-MMPs in tumor progression *in vivo* have been analyzed using transgenic mice, xenograft tumors, and *in vivo* metastasis assays (Deryugina and Quigley, 2006). MT1-MMP overexpression in transgenic mice under the MMTV promoter results in mammary gland abnormalities, such as lymphocytic infiltration, periductal fibrosis, epithelial hyperplasia, and adenocarcinomas (Ha et al., 2001). Glioma xenografts overexpressing MT1-MMP grow more rapidly, and exhibit higher angiogenesis and VEGF expression than control tumors (Deryugina et al., 2002). Similar results were obtained using breast adenocarcinoma and melanoma cells as a model (Sounni et al., 2002a; Sounni et al., 2002b). Moreover, MT1-MMP promotes fibrotic reactions in pancreatic cancer transgenic mice via the induction of TGF-β activity (Krantz et al., 2011).

Following the findings of MMP functions in tumor progression, MMP inhibitors have been developed for cancer treatment. They have reached phase III studies. However, while MMP inhibitors displayed marked inhibition of tumor progression in mice, the results of clinical studies have remained disappointing. This may be due to several factors. Broad-spectrum MMP inhibitors lack specificity, which can be essential for successful treatment. Some MMPs, such as MMP-3 and MMP-8, have namely protecting properties in tumor progression (Agarwal et al., 2003; Balbin et al., 2003; McCawley et al., 2004). Inhibition of all MMPs also increases the frequency of various side effects, since most MMPs are required for normal tissue homeostasis. Further, drug concentration may have remained suboptimal due to adverse side effects. In addition, patients recruited for these studies have usually had late-stage metastatic disease, while the biggest benefit from MMP inhibitors is anticipated during initial stages of cancer, such as tumorigenesis, local invasion of primary tumor, as well as intravasation and extravasation processes of metastatic cells (Overall and Kleifeld, 2006; Sabeh et al., 2009). Therefore, better understanding of both protumorigenic and antitumorigenic functions of specific MMPs is needed for developing precisely targeted therapies.

AIMS OF THE STUDY

The general purpose of this study was to elucidate the potential functions of MT-MMPs in melanoma invasion and pericellular proteolysis. The information of MT3-MMP and its biological functions and substrates *in vivo* is currently relatively scant. Since MT3-MMP was found to be associated with the aggressive nodular subtype of melanoma, we set our goal to elucidate the functions of MT3-MMP in melanoma cell invasion, and further on in melanoma growth *in vivo*. Furthermore, although many proteolytic enzymes can activate TGF-β when overexpressed or added ectopically, the roles of endogenous matrix-degrading proteases in the activation of TGF-β have remained poorly characterized.

The specific aims included:

1. To assess which endogenous enzymes release ECM-bound TGF-β in cultured endothelial cells

2. To analyze the functions of MT3-MMP in melanoma invasion in different 3D matrices

3. To evaluate the effect of MT3-MMP/MT1-MMP interactions on melanoma progression in *in vivo* xenografts

MATERIALS AND METHODS

1. Cells lines

The cell lines used have been listed below with their American Type Culture Collection (ATCC, Manassas, VA, USA) reference number or reference. Endothelial cell lines were grown in endothelial cell growth medium MV containing 2% of fetal calf serum (PromoCell), containing 50 µg/ml gentamicin. Other cell lines were grown in Eagle's minimal essential medium (MEM) containing 10% heat-inactivated fetal calf serum (GIBCO), 100 IU/ml penicillin, and 50 µg/ml streptomycin. For stable overexpression, transfected Bowes cells were selected using G418 (400 µg/ml; Calbiochem).

Cell line	Description	Reference number or reference
HUVEC	human umbilical vein endothelial cell line	PromoCell, Heidelberg, Germany
CCL-137	human embryonic lung fibroblasts	ATCC; CCL-137
Bowes	human melanoma cell line, derived from RGP of melanoma	ATCC; CRL-9607
COS-1	African green monkey kidney epithelial cells, SV40 transformed	ATCC; CRL-1650
G361	human melanoma cell line, derived from primary tumor	ATCC; CRL-1424
WM164	human melanoma cell line, derived from NM metastasis	Wistar melanoma, Wistar Institute, USA
WM165	human melanoma cell line, derived from SSM metastasis	Wistar melanoma, Wistar Institute, USA
WM293	human melanoma cell line, derived from SSM metastasis	Wistar melanoma, Wistar Institute, USA
WM739	human melanoma cell line, derived from VGP of SSM	Wistar melanoma, Wistar Institute, USA
WM852	human melanoma cell line, derived from NM skin metastasis	Wistar melanoma, Wistar Institute, USA
HDBEC	primary juvenile foreskin lymphatic endothelial cell line	PromoCell
LEC	primary juvenile foreskin lymphatic endothelial cell line	PromoCell
293FT	human embryonal kidney cells, SV40 transformed	Life Technologies, Carlsbad, Ca, USA

2. Antibodies

Antibodies used are listed below. Antibodies have been produced against human immunogens unless stated otherwise.

Name	Immunogen	Description	Use	Source
	LTBP-1	mouse (MAB388)	IB (I)	R&D Systems, Minneapolis, MN USA
	β1-LAP	goat (AF-246-NA)	IB (I)	R&D Systems
Ab39	LTBP-1	rabbit	IB, IF (I)	Dr. C.-H. Heldin, Ludwig Institute for Cancer Research Uppsala, Sweden
	LTBP-2	rabbit	IB, IF (I)	(Hyytiäinen et al., 1998)
	Fibronectin	rabbit	IF (I)	Sigma, St. Louis, MO, USA
	Collagen IV	goat	IF (I)	Chemicon, Temecula, CA, USA
	Laminin	rabbit	IF (I)	Chemicon
680	β1-LAP	rabbit	IB (I)	(Taipale et al., 1994)
	ERK1/2	rabbit	IB (I)	Promega, Madison, WI, USA
	P-p44/42	rabbit	IB (I)	Cell Signalling, Beverly, MA, USA
Ab-2	MT1-MMP, cytoplasmic tail	rabbit	IB, IP (II)	(Lehti et al., 1998)
RP1	MT1-MMP, hinge domain	rabbit	IF (II)	Triple Point Biologics, Forest Grove, OR, USA
	MT1-MMP, Ab6004	rabbit	IB, IP (II)	Chemicon
	MT1-MMP, catalytic domain, LEM2-15/8	mouse	IB, IF, IHC (II, III)	Millipore, Billerica, MA, USA
	Hemagglutinin	mouse	IB (II)	Covance, Princeton, NJ, USA
	MT3-MMP	rabbit, ab38968	IB, IP (II)	Abcam, Cambridge, UK
Ab318	MT3-MMP, hinge domain	rabbit	IB, IP (II)	Dr. R. Fridman, Wayne State University, Detrot, USA
	mouse Lyve-1	rabbit	IHC (III)	(He et al., 2005)
	collagen type I	goat	IF (III)	Millipore
	mouse CD31	rat	IHC (III)	BD Biosciences, Franklin Lakes, NJ, USA
	mouse collagen type I	rabbit	IF (III)	Millipore
	mouse collagen type IV	rabbit	IF (III)	Millipore
	fibrinogen	rabbit	IHC (III)	Daco, Glostrup, Denmark
	S100	rabbit	IHC (III)	Daco
	human CD31	mouse	IF (III)	Daco
	human Prox-1	goat	IF (III)	R&D Systems

3. Other reagents

Reagent	Source	Used in
PMA	Sigma	I
TAPI-1 (MMP inhibitor)	Millipore	I
GM6001 (MMP inhibitor)	Millipore	I, II, III
AEBSF (serine protease inhibitor)	Millipore	I
LY294002 (PI-3K inhibitor)	Millipore	I
Bisindoylmaleimide-1 (PKC inhibitor)	Millipore	I
PD98059 (MEK inhibitor)	Millipore	I
DRB (casein kinase-II inhibitor)	Enzo Life Sciences, Farmingdale, NY, USA	I
Aprotinin (serine protease inhibitor)	Sigma	I, II, III
UO126 (MEK inhibitor)	Promega	I
Phalloidin-TRITC	Sigma	II, III
Collagen type I rat tail, acid-extracted	Sigma	II, III
Bafilomycin A1 (lysosomal inhibitor)	Millipore	II
Human recombinant HGF	R&D Systems	II
Fibrinogen	Millipore	II, III
Thrombin	Sigma	II, III

4. RNA interference

293FT producer cells were cotransfected with the packaging plasmid (pCMVdr8.74), envelope plasmid (pMD2-VSVG) and shRNA-plasmid (hairpin-pLKO.1 vector) or control plasmid (scrambled) using Lipofectamine 2000. The viral supernatants were harvested for 48 h, filter sterilized and transferred to target cells. After 16 h infection the supernatants were replaced with complete media for the subsequent assays. The knockdown efficiency was assessed by quantitative PCR (qPCR) after 48 h. For stable shRNA expression cells were selected using puromycin (5 µg/ml). Small interfering RNAs (siRNA) were transfected using Lipofectamine™ 2000 (Life Technologies).

Name	Source	Used in
Scrambled shRNA in pLKO.1 vector	Open Biosystems	I, II, III
MT1-MMP shRNA, Cstr-1	Open Biosystems, TRCN0000050854	I
MT1-MMP shRNA, Cstr-2	Open Biosystems, TRCN0000050855	I
MT1-MMP shRNA, Cstr-3	Open Biosystems, TRCN0000050856	I
MT1-MMP shRNA, Cstr-4	Open Biosystems, TRCN0000050857	I
Negative control siRNA	Qiagen, 1027280	II, III
MT1-MMP siRNA	Qiagen, SI03648841	II, III
MT1-MMP siRNA	Qiagen, MMP14sIR1Q	II
MT3-MMP siRNA	Qiagen, SI00083006	II, III
MT3-MMP siRNA	Qiagen, MMP16sIR1Q-1	II
MT3-MMP shRNA, shMT3-1	Open Biosystems, TRCN0000052249	II

MT3-MMP shRNA, shMT3-2	Open Biosystems, TRCN0000052250	II, III
MT3-MMP shRNA, shMT3-3	Open Biosystems, TRCN0000052251	II

5. Expression constructs (II)

The following expression vectors were transfected using FuGENE (Roche, Penzberg, Germany) or TransIT-2020 (Mirus, Madison, WI, USA): MT3-MMP, MT1-MMP, MT1-MMP with inactivating E240A point mutation (MT1E/A), HA-tagged MT1-MMP (Hotary et al., 2002; Lehti et al., 2000; Li et al., 2008), inactive MT3-MMP E247A construct (MT3E/A) kindly provided by Dr. D. Pei (Kang et al., 2000). Rescue constructs for MT3-MMP with double silent mutations in shMT3-2 binding sequence rescMT3-1 (T822C-T825C) and rescMT3-2 (T831C-A836C) were generated using QuikChange® Site-Directed Mutagenesis Kit (Agilent Technologies, Santa Clara, CA, USA).

6. Immunofluorescence analysis (I, II, III)

The cells on glass coverslips were fixed with 4% PFA for 5 min at +4°C followed by blocking and permeabilization in 5% BSA/0.1% Triton X-100/PBS for 30 min RT, and staining with the indicated primary antibodies (1 h) and Alexa Fluor-labeled secondary antibodies (30 min, Life Technologies). The coverslips were finally mounted on glass slides using Vectashield anti-fading reagent (Vector Laboratories) and examined using the Axioplan microscope (Zeiss). For immunofluorescence staining of the mouse tumor frozen sections, sections were post fixed with cold methanol-acetone, blocked with 15% fetal calf serum/0.3% Triton X-100/Dulbecco for 1 h, followed by staining with the indicated primary antibodies (1 h) and Alexa Fluor-labeled secondary antibodies (30 min). The sections were then mounted with Vectashield anti-fading reagent.

7. Isolation and plasmin digestion of ECM-associated proteins (I)

The cell cultures were washed once with PBS and lysed by 0.5% sodium deoxycholate (NaDOC) in 10 mM Tris–HCl buffer, pH 8.0, containing 150 mM NaCl and 1% NP-40, and clarified by centrifugation at 8000 ×g for 10 min (Taipale et al., 1994). The NaDOC insoluble material was then washed once with ice-cold PBS and digested with 0.3 U/ml of plasmin (Sigma) in PBS containing 1mM $MgCl_2$, 1 mM $CaCl_2$ and 0.1% n-octyl-β-D-glucopyranoside (Sigma) at 37°C for 2 h. Protease inhibitors (Complete® protease inhibitor cocktail, Roche) were then added, and the supernatants were clarified by centrifugation. Subsequently, the soluble proteins were characterized by SDS-PAGE and immunoblotting.

8. SDS-PAGE and immunoblotting (I, II)

Cells were lysed with RIPA buffer (150 mM Tris-HCl pH 7.5, 150 mM NaCl, 1% Triton X-100, 0.5% sodium deoxycholate, 0.1% SDS) containing Complete® protease inhibitor cocktail (Roche). Equivalent quantities of proteins were size-fractionated by gradient (4–20%) SDS-PAGE followed by transfer to polyvinylidene difluoride membranes. The non-specific protein binding sites were saturated with 5% non-fat milk in TTBS-buffer (TBS containing 0.05% Tween-20) for 30 min. The membranes were washed with TTBS and incubated with primary antibodies overnight, and with HRP-conjugated secondary antibodies (Dako) for 30 min, and the bound antibodies were detected using enhanced chemiluminescence (Promega).

9. Gelatin zymography (I, II)

Polypeptides of the conditioned media were separated under nonreducing conditions using 10% SDS-polyacrylamide gels containing 1 mg/ml of gelatin. After that the gels were washed twice with 50 mM Tris-HCl buffer, pH 7.6, containing 5 mM $CaCl_2$, 1 μM $ZnCl_2$ and 2,5% Triton X-100 for 15 min to remove SDS, followed by a brief rinsing in washing buffer without Triton X-100. Next, the gels were incubated at +37 °C overnight in the developing buffer, 50 mM Tris-HCl, pH 7.6, containing 5 mM $CaCl_2$, 1 μM $ZnCl_2$, 1% Triton X-100 and 0.02% $NaNO_3$. The gels were then stained with Coomassie Blue for 2 h and destained with a solution containing 10% methanol and 10% acetic acid.

10. Isolation of RNA from cultured cells and human biopsies, reverse transcription and qPCR (I, II, III)

Total cellular mRNA was isolated using RNeasy Mini kit (Qiagen) followed by reverse transcription with Random hexamer primers (Life Technologies) and Superscript III reverse transcriptase (Life Technologies). Total mRNAs from representative microdissected samples of archival paraffin-embedded biopsies of benign intradermal or compound melanocytic nevi, melanomas , and of adjacent normal skin were extracted using High Pure RNA Paraffin Kit (Roche). The biopsies had been removed for diagnostic purposes and the histological diagnosis had been confirmed by the University Hospital dermatopathologist. The cDNAs were amplified on GeneAmp 7500 Sequence Detector thermal cycler (Applied Biosystems) using TaqMan Universal PCR Master Mix and validated primers (MT1-MMP; Hs 01037006_gH, MT2-MMP; Hs 00233997_m1, MT3-MMP; Hs 00234676_m1K; human col1A1, Hs00164004_m1; mouse col1A1, Mm00801666_g1; mouse col3A1, Mm01254476_m1; Applied Biosystems). The expression levels were normalized with TATA-binding protein (TBP, Applied Biosystems) or GAPDH. TissueScan Melanoma Tissue qPCR Arrays I and II (OriGene Technologies) containing cDNAs from human tissue biopsies of normal skin (n=6) and melanoma metastases (n=77) were used for the quantification of the MT-MMP transcripts.

11. Cell invasion and growth in three-dimensional collagen and fibrin (II, III)

Cell invasion was assessed essentially as described (Hotary et al., 2000; Illman et al., 2006). Briefly, rat tail type I collagen (4.8 mg/ml) was mixed with equal amount of 2xMEM, and pH was adjusted to ~7.4 by NaOH. Collagen was cast in the upper chambers of Falcon cell culture inserts in 24 well plates and incubated at 37°C for 1 h to allow gelling. Cross-linked fibrin gels were cast in the inserts by combining 75 μl plasminogen-free human fibrinogen (6 mg/ml) in Hank's Balanced Salt Solution (HBSS), and 75 μl HBSS (pH 7.4) containing 4 U/ml human thrombin and 400 μg/ml aprotinin. The cells were seeded atop the matrix in the upper chamber in medium containing 1% FCS. HGF (25 ng/ml) and medium containing 10% FCS served as chemoattractants in the lower chambers, with medium changed every third day. After a 3-5 d culture period, the cells were fixed with 4% PFA, and paraffin sections were stained with hematoxylin and eosin (HE-staining). Sections were photographed, and the invaded cells were counted from 8 random sections of each sample. Each assay was performed in triplicate. For 3D growth/invasion assays, collagen (2.4 mg/ml) and fibrin were prepared as above. The cells (5000) were suspended in 40 μl hydrogel, the suspension transferred to a 24-well plate, and incubated at 37°C for 1 h to allow complete gelling. After 5 d incubation in complete growth medium, cultures were fixed and photographed using Axiovert 200 microscope (Carl Zeiss). Alternatively, collagen or fibrin gels were stained with phalloidin-TRITC to visualize filamentous actin, and photographed using Axioplan upright epifluorescence microscope (Carl Zeiss). The percentages of invasive colonies were calculated from 20 random phase-contrast or epifluorescence images of 6 separate gels.

12. Northern hybridization (II)

Total cellular RNA (10 μg) was fractioned by formaldehyde-agarose gel electrophoresis and transferred to a Zeta probe membrane (Bio-Rad) in 20 x SSC (1 x SSC: 150 mM NaCl, 15 mM sodium citrate, pH 7.0) and fixed by UV crosslinking. cDNA probes corresponding to full-length MT1-MMP and MT3-MMP cDNA were labelled with [32P] dCTP by the random priming method (Amersham Pharmacia Biotech). The membranes were hybridized in ExpressHyb hybridization solution (Clonetech) at 58°C for 16-24 h. Washing was carried out in 0.2 x SSC containing 0.1 % SDS at 63°C. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as an internal standard.

13. Immunoprecipitation (II)

Endogenous or recombinant MT1-MMP and MT3-MMP were immunoprecipitated from cell lysates by incubation with specific rabbit polyclonal and G protein Sepharose (GE Healthcare) at 4°C for 2 h. The Sepharose particles were then pelleted by centrifugation and washed extensively with PBS. The proteins were eluted with the sample buffer and separated for immunodetection by SDS-PAGE.

14. Cell surface labeling (II)

Cell surface biotinylation was performed as described (Lehti et al., 1998). Briefly, cells were rinsed twice with PBS and incubated with 0.5 mg/ml biotin (Pierce) in PBS on ice for 1 h. The reaction was terminated by washing 3 times for 10 min each with 150 mM glycine/TBS. The cells were then lysed and subjected to immunoprecipitation with rabbit polyclonal MT1-MMP or MT3-MMP antibodies. The immunoprecipitated material was resolved by SDS-PAGE, and detected with horseradish-peroxidase-conjugated streptavidin (Daco).

15. *In vivo* xenografts in mice (III)

Experiments were approved by the State Provincial Office of Southern Finland. WM852 cells were lentivirally transduced with scrambled shRNA and shRNA targeting MT3-MMP (shMT3-2), and stable pools were selected by puromycin (Sigma). Cells were also retrovirally transduced with a Renilla luciferase – green fluorescent protein fusion reporter protein. Cell pools (1.3×10^6 or 2.0×10^6) were implanted into the abdominal subcutis of ICR-severe combined immunodeficient (SCID) female mice. Tumor volumes were measured with a caliper and calculated according to the formula V = width × height × depth / 2, derived from the formula for the volume of an ellipsoid (Laakkonen et al., 2007). After ~8 weeks, the tumors, the lymph nodes and organs were collected, fixed with 4% PFA, and embedded in paraffin or processed for frozen sections.

16. Immunohistochemistry (III)

Mouse xenograft tumors and lymph nodes were fixed with 4% paraformaldehyde, dehydrated, and embedded in paraffin. Alternatively, PFA-fixed tumors were incubated in 25% sucrose/PBS for 4 h, washed with PBS, and embedded in OCT Compound (Tissue-Tek) for frozen sections. For immunohistochemistry, tissue sections were de-paraffinized in TissueClear (Tissue-Tek) and re-hydrated in graded ethanol series. After antigen retrieval by boiling in sodium citrate buffer (10 mM sodium citrate, 0.05% Tween-20, pH 6), the sections were incubated for 10 min in 0.6% (v/v) hydrogen peroxide. The tissue sections were subsequently rinsed and incubated with 2.5% Normal Horse Serum (blocking buffer, Vector Laboratories) for 30 min. Primary antibodies were incubated o/n in blocking buffer, followed by incubation with peroxidase conjugated anti-mouse Ig/anti-rabbit Ig for 30 min. Detection was performed using Vectastain DAB kit according to manufacturer's instructions. Alternatively, TSA™ Biotin System Kit (PerkinElmer) was used. After washes slides were dehydrated and mounted using Cytoseal mounting medium (Thermo Scientific). Images were taken using Leica DM LB microscope (Leica).

17. Melanoma cell co-culture with BEC and LEC spheroids in 3D fibrin (III)

HDBEC and LEC cells (PromoCell) were cultured in Endothelial Cell Growth Medium MV (PromoCell). Endothelial cell spheroids were formed in non-adherent conditions by culturing 4000 cells/well in agarose coated 96-well round bottom plate o/n. Ten to twelve endothelial cell spheroids were then mixed with 5000 melanoma cells pretreated with green fluorescent Vybrant® CFDA SE Cell Tracer (Life Technologies), and 50 µl 3mg/ml fibrin was formed as described above. After gelling at 37˚C for 30 min, Endothelial Growth Medium MV supplemented with 100 ng/ml aprotinin (Sigma-Aldrich) was added to the cultures. After 3 d, the cells were fixed with 4% PFA and subjected to immunofluorescence staining with anti-CD31 (Dako) antibodies. The spheroids were then mounted with Vectashield (Vector Laboratories) and imaged using Leica TSC SP5 confocal microscope (Leica).

18. Cell adhesion assay (III)

Melanoma cells treated with green fluorescent Vybrant® CFDA SE Cell Tracer were seeded on lymphatic (LEC) or blood vessel (HUVEC) cell monolayers in 96-well plates. After 1 h unadhered cells were removed by washing 3 times with PBS. The adherent cells were fixed with 4% PFA. Nuclei were then stained with Hoechst (Sigma). The number of adhered melanoma cells/well was analyzed using Cellomics View software (Thermo Scientific).

19. Transendothelial migration assay (III)

Melanoma cells pretreated with green fluorescent Vybrant® CFDA SE Cell Tracer were seeded upon LEC or HDBEC monolayers on a gelatin coated semipermeable membrane of Falcon cell culture inserts in endothelial growth medium containing 1% serum. Medium containing 10% fetal calf serum was used in the lower chamber as an attractant. After 12 h the cells were fixed, and the cells from upper chamber were removed by wiping. The membranes were mounted on the objective glasses and photographed with upright fluorescent microscope Axioplan (Zeiss). The areas of transmigrated cells/microscopic field were quantified using ImageJ software.

20. Statistical analysis (II, III)

All numerical values represent mean ± SD. Statistical significance was determined using the Mann-Whitney test and PASW software.

RESULTS

1. Large latent TGF-β complex (LLC) is released from the ECM upon endothelial cell activation (I)

Most non-malignant cells secrete TGF-β as a large latent TGF-β complex (LLC). LTBP-1 regulates the secretion, availability and activation of TGF-β by facilitating its secretion and by targeting LLC to the ECM. The release of LLC from ECM is considered to be among the first steps in TGF-β activation. Culturing HUVEC endothelial cells for 4 d resulted in massive deposition of LTBP-1 into the subendothelial matrix. Activation of endothelial cells with PMA (phorbol 12-myristate 13-acetate) caused morphological transformation from cobblestone-like to fibroblastoid spindle cell appearance. Similar morphological changes can be induced by inflammatory cytokines, such as IL-1β, INF-γ, and TNF-α (Bujan et al., 1999; Koziol et al., 2012; Montesano et al., 1985). During this process, LTBP-1 was almost completely depleted from the ECM in conjunction with the release of truncated LTBP-1 and LLC, as well as traces of LTBP-1 -free small latent TGF-β complex (SLC), and translocated into the conditioned medium. The depletion of LTBP-1 and LLC from the ECM was almost complete after 48 h. As analyzed by immunofluorescence, collagen type IV was also depleted from the ECM, and the amount of laminin was reduced. In addition, fibronectin and LTBP-2 staining patterns became fragmented. PMA is a direct protein kinase C agonist, which induces also Erk1/2 phosphorylation and PI-3 kinase pathway (Taylor et al., 2006). The morphological change and processing of LTBP-1 induced by PMA were abrogated by PKC and MEK inhibitors, but only slightly by PI-3 and CK-II inhibitors.

2. Silencing of MT1-MMP abrogates the release of TGF-β from the subendothelial matrix (I)

Since proteases such as MMP-2, MMP-9, and serine proteases have been implicated in TGF-β release from the ECM or TGF-β activation (Dallas et al., 2002; Taipale et al., 1992; Taipale et al., 1995; Yu and Stamenkovic, 2000), we defined by protease inhibitors which endogenous proteases participate in TGF-β release from endothelial cell ECM. LTBP-1 and LLC release from the ECM was inhibited by synthetic wide spectrum MMP and ADAM inhibitors TAPI-1 and GM6001, but not by serine protease inhibitors. Further, morphological activation of endothelial cells by PMA was associated with induction of MMP-2 activation, reflecting increased MT-MMP activity. MT1-MMP was the only notably expressed MT-MMP in HUVECs, and its expression was further enhanced by PMA. Both ADAM10 and -17 were also expressed in these cells, but their expression was not affected by PMA. To assess which MMPs or ADAMs contributed to LLC release from the ECM, we assessed the effect of tissue inhibitors of metalloproteinases, TIMP-1, -2 and -3, which have different specifities towards MMPs. TIMP-1 inhibits mainly secreted MMPs and ADAM10, while TIMP-2 inhibits secreted and membrane-type MMPs, and TIMP-3 inhibits all MMPs and ADAM10 and -17, among others (Baker et al., 2002). Since TIMP-2 and TIMP-3, but not TIMP-1, abrogated

LTBP-1 and LLC release from the ECM, we concluded that MT-MMPs mediated LTBP-1 cleavage in activated endothelial cells. Indeed, specific silencing of MT1-MMP by lentiviral short hairpin RNA reversed LTBP-1 depletion from the ECM, indicating that MT1-MMP mediated LLC release from the subendothelial matrix.

3. MT3-MMP is expressed in lymph node metastases of melanoma and in metastatic melanoma cell lines (II)

In addition to promoting angiogenesis MT1-MMP has important functions in cancer cell invasion and metastasis. In melanomas MT1-MMP is often overexpressed in the invading edges of primary tumors, and its expression is associated with melanoma metastatic progression (Hofmann et al., 2000; Riker et al., 2008). Its close homologue, MT3-MMP, is specifically expressed in the most aggressive melanoma subtype, nodular melanoma (Jaeger et al., 2007; Ohnishi et al., 2001), but its functions in cancer progression are largely unknown. To begin to characterize MT3-MMP functions in melanoma progression, we assessed MT1-MMP and MT3-MMP mRNA expression in human biopsy samples of normal skin, benign nevi, and metastases to lymph nodes and different organs. MT1-MMP and MT3-MMP were not upregulated in benign nevi compared to normal skin. Remarkably, MT3-MMP was upregulated >8-fold in biopsies of lymph node metastases compared to normal skin. In contrast, MT1-MMP expression was high in normal skin and benign nevi, but was not significantly increased in biopsies of metastases.

In parallel, we assessed the expression of MT1-MMP and MT3-MMP in cultured melanoma cell lines derived from primary tumors and metastases. MT1-MMP was expressed in four metastatic cell lines (WM852, WM164, WM165 and WM239) and in three cell lines derived from primary tumors (Bowes, G361, WM793). MT3-MMP was expressed in three metastatic cell lines (WM852, WM165 and WM239) and WM793 cell line derived from vertical growth phase primary melanoma, suggesting that MT3-MMP might be associated with melanoma progression.

4. MT3-MMP promotes the adhesive phenotype of melanoma cells in conjunction with increased fibrin invasion and restricted collagen invasion (II, III)

MT1-MMP drives tumor cell invasion through collagen type I and the basement membranes. It localizes often to invasive structures of the cells, where it cleaves native collagen type I and other ECM proteins to permit tumor cell movement (Ota et al., 2009; Sabeh et al., 2004). Although MT3-MMP is a close homologue of MT1-MMP, it cannot cleave collagen type I, but it cleaves collagen type III, fibrin, and laminin (Li et al., 2008; Sabeh et al., 2004; Shimada et al., 1999). To investigate the growth and invasion of melanoma cells in 3D conditions, we implanted them as single cells in cross-linked acid-extracted collagen type I. Remarkably, we found that MT3-MMP expression was associated with expansive growth of melanoma colonies or growth as rounded single cells, while cells with undetectable MT3-

MMP expression grew in collagen as invasive sprouting colonies or elongated single cells. To further analyze the invasive phenotypes of melanoma cells, we plated WM852 and Bowes cells atop 3D collagen type I and fibrin, which is a component of provisional matrix found in melanoma tumor/host interface. WM852 cells, derived from nodular melanoma metastases and expressing MT3-MMP, displayed marked fibrin-invasive activity and limited collagen invasion. Bowes cells, derived from radial growth phase melanoma and not expressing MT3-MMP, invaded efficiently into both matrices. Consistently with previous reports on the functions of MT-MMPs in cell invasion (Hotary et al., 2000; Hotary et al., 2002; Sabeh et al., 2004), both the collagen and fibrin invasion were abrogated by the MMP inhibitor. MT1-MMP silencing inhibited collagen and fibrin invasion of WM852 cells. Remarkably, MT3-MMP silencing promoted collagen invasion but inhibited fibrin invasion in these cells. Furthermore, stable overexpression of MT3-MMP in Bowes cells resulted in marked increase in fibrin invasion and significant attenuation of collagen invasion.

To assess the interrelated functions of MT-MMPs in cell invasion, we expressed MT1-MMP and MT3-MMP alone or in combination in COS cells, which lack endogenous MT-MMPs. COS cells expressing MT1-MMP invaded efficiently both 3D collagen and fibrin, whereas cells expressing MT3-MMP invaded only into fibrin. Interestingly, co-expression of MT1-MMP and MT3-MMP further induced fibrin invasion of COS cells, but abrogated collagen invasion, suggesting that MT3-MMP reduced collagenolytic activity of MT1-MMP.

In 2D cell culture, WM852 cells overlaid with 3D collagen grew as cohesive colonies. MT3-MMP silencing resulted in morphological shift from cohesive growth pattern to single cell growth. Furthermore, MT3-MMP silencing induced reduction of ZO-1 in cell-cell contacts. To further elucidate the functions of MT3-MMP in melanoma cell invasion, we stably silenced MT3-MMP in WM852 and WM165 melanoma cells using lentiviral shRNA. Stable MT3-MMP silencing resulted in profound induction of sprouting growth pattern and invasion in 3D collagen. In contrast, MT3-MMP silencing markedly reduced the invasive growth of melanoma cells in 3D fibrin, indicating that endogenous MT3-MMP supports cohesive growth of 2D cultures and promotes fibrin invasion of melanoma cells, whereas in collagen it shifts their growth from invasive to expanding type.

5. MT3-MMP restricts the collagenolytic activity of MT1-MMP by cleaving it and reducing its cell surface localization (II, III)

Both MT1-MMP and MT3-MMP undergo autocatalytic inactivation, which generates 44 kDa and 37 kDa cell-bound fragments, respectively (Lehti et al., 1998). When MT3-MMP was stably expressed in Bowes cell, it was detected in cell lysates mainly as a processed 37 kDa form, and as a ~65 kDa form on the cell surface. Full-length ~65 kDa protein was markedly increased in cell lysates after inhibition of both MMP and lysosomal activities, indicating that autocatalytic processing and subsequent internalization and lysosomal degradation resulted in rapid MT3-MMP turnover. MT3-MMP reduced both the endogenous and ectopically expressed full-length MT1-MMP in Bowes cells lysates and induced the processing of ectopically expressed MT1-MMP to 37-44 kDa fragments in Bowes and COS cells. Since MT3-MMP induced the cleavage of catalytically inactive MT1-MMP E/A mutant, we

concluded that MT3-MMP cleaved MT1-MMP directly rather than induced its autocatalytic cleavage.

Oligomerization of MT1-MMP promotes the autocatalytic cleavage of MT1-MMP and proMMP2 activation (Lehti et al., 2002). We found that MT1-MMP was co-immunoprecipitated with MT3-MMP in Bowes and COS-1 cells, suggesting that they existed in the same complexes on the cell surface. Further, when MT1-MMP was expressed in COS cells, which lack endogenous MT-MMPs, it was detected mainly on the cell surface by surface biotinylation and immunofluorescence. In contrast, co-expression of MT1-MMP with catalytically active MT3-MMP resulted in the shift of MT1-MMP localization from cell surface to mainly intracellular. Consistently, stable silencing of MT3-MMP by lentiviral shRNA in WM852 cells resulted in induction of MT1-MMP on the cell surface both in cell culture *in vitro* and in melanoma xenografts *in vivo*.

6. Silencing of MT3-MMP increases the growth rate of melanoma xenografts and induces a switch from lymphatic to blood vessel invasion (III)

To investigate MT3-MMP functions in melanoma progression *in vivo*, tumor growth and invasion were analyzed after subcutaneous injection of WM852 cells expressing control shRNA (Ctrl) and shRNA targeting MT3-MMP (shMT3) into SCID mice. The control tumors grew slowly, reaching the average weight of 0.24 ± 0.1 g in ~8 weeks. MT3-MMP silencing induced the growth rate of tumors, which reached the average weight of 0.55 ± 0.2 g during the same period of time. Similar results were obtained from two independent experiments.

To analyze tumor blood and lymphatic vessel density, tumor sections were stained by CD31 and LYVE-1, a panendothelial marker and a marker for lymphatic vessels, respectively. Intratumoral blood and lymphatic density in shMT3 tumors was slightly reduced, while peritumoral lymphatic densities remained comparable. Notably, we observed marked lymphatic vessel invasion (LVI) by tumor cells in the control group, which was abrogated by MT3-MMP silencing. LVI was more prominent in peritumoral than intratumoral vessels. By contrast, negligible blood vessel invasion (BVI) of control tumors was increased by ~10-fold after MT3-MMP silencing.

7. Silencing of MT3-MMP affects the ECM composition of melanoma xenografts (III)

Since MT-MMPs are strong modulators of ECM, we analyzed ECM composition of control and shMT3 tumors. Collagen type IV thickness at vascular basement membrane was comparable in both groups. In contrast, prominent collagen type I fibers in control tumors were markedly decreased after MT3-MMP silencing as detected by both immunofluorescence and immunohistochemistry. The mRNA expression of human collagen type I and mouse collagen type I and III in mouse xenografts remained comparable, suggesting that collagen degradation was enhanced. By contrast, control tumors displayed only minor amounts of

extracellular fibrin, and MT3-MMP silencing resulted in prominent fibrin extracellular network detected by immunohistochemistry. Consistently with the observed increase of collagen degradation in shMT3 tumors, MT1-MMP localization shifted from mainly intracellular in control tumors to cell surface after MT3-MMP silencing.

8. Three-dimensional matrix facilitates melanoma cell transmigration across LECs (III)

To define the contribution of ECM composition and melanoma/endothelial cell interactions in intravasation, we cultured melanoma cells atop blood endothelial cell (BEC) and lymphatic endothelial cell (LEC) monolayers. WM852 melanoma cells adhered efficiently to human BECs but poorly to LECs. Silencing of MT3-MMP further increased adhesion to BECs, whereas adhesion to LECs was less affected. Furthermore, only few melanoma cells transmigrated across the LEC monolayer within 12 h, whereas transmigration across the BEC monolayer was efficient. The transmigration of the cultured cells across BECs was further enhanced after MT3-MMP silencing. To better mimic tumor cell-endothelial cell interactions and intravasation in the ECM microenvironment, we cultured melanoma cells together with BEC and LEC spheroids in 3D fibrin gels. Opposite to the weak transmigration across LEC monolayer, control WM852 melanoma cells efficiently invaded into the bodies of LEC spheroids grown in fibrin, suggesting that 3D matrix may facilitate transmigration. The cell invasion into both LEC and BEC spheroids was decreased after MT3-MMP silencing, which is in line with limited ability of these cells to invade in the fibrin matrix. Therefore, MT3-MMP expression supports intravasation of WM852 cells into 3D LEC spheroids.

9. Low MT1-MMP and/or high MT3-MMP expression correlates with cohesive growth phenotype of human melanoma tumors (III)

To investigate the functions of MT3-MMP in human melanoma tumors, we assessed MT1-MMP and MT3-MMP expression and collagen content in eight melanoma biopsies. For mRNA expression benign nevus biopsies were used as a control. MT1-MMP expression was markedly elevated in three melanoma biopsies, and MT3-MMP expression was increased in two melanoma biopsies compared to benign nevi. In the eight Herovici-stained melanoma biopsy specimens, five tumors exhibited cohesive morphology with expansive melanoma cell nests surrounded by collagen fibers. All of these tumors had either low MT1-MMP or high MT3-MMP expression. Three melanomas exhibited infiltrating growth pattern. Two of these melanomas had high MT1-MMP and low MT3-MMP expression, and in one melanoma both MT1-MMP and MT3-MMP were low. In addition, we stained two whole tumor specimens with highest MT1-MMP expression for podoplanin to detect lymphatic vessels. M4 tumor expressed high MT3-MMP mRNA, and M7 tumor expressed low MT3-MMP, resembling the expression pattern of control and shMT3 WM852 cells. Notably, M4 tumor exhibited prominent lymphatic invasion, whereas no lymphatic invasion was observed in M7 tumor. Similar to cohesive growth in 2D culture and low collagenolytic ability of WM852 cells,

melanoma cells of M4 tumor grew as expansive cell nests surrounded by collagen fibers. In contrast, melanoma cells of M7 tumor infiltrated the underlying dermis diffusely, suggestive of high collagenolytic activity. Consistently with prominent lymphatic invasion, M4 patient had macrometastases in all lymph nodes analyzed, as well as distant metastases, whereas no lymph node metastases were observed in M7 patient. These results suggest that MT3-MMP may affect collagen degradation ability and lymphatic invasion of melanoma cells also in human tumors.

DISCUSSION

1. The release of ECM-bound large latent TGF-β complex by MT1-MMP (I)

TGF-β regulates a wide array of cellular functions, including angiogenesis. It is essential for both the initial phases of endothelial sprouting, and the resolution and maturation phase of the angiogenesis. The activation of TGF-β is tightly regulated by various mechanisms. TGF-β is targeted to ECM via large latent TGF-β complex (LLC), which release from the ECM promotes TGF-β activation. We explored the endogenous mechanisms of LLC release from the ECM in endothelial cells. We found that following morphological activation of endothelial cells by PMA, MT1-MMP expression and activity is induced, with concomitant release of ECM-bound LTPB-1 and LLC. Silencing of MT1-MMP with lentiviral shRNA abrogated this release. The release of LLC was cell type specific, since treatment of human lung fibroblasts with PMA failed to induce LTBP-1 and LLC depletion from the ECM. In addition, LTBP-2 cleavage was not observed, indicating that morphological activation of endothelial cells was associated specifically with LTBP-1 cleavage.

Both MT1-MMP and TGF-β have been implicated in angiogenesis. MT1-MMP is specifically expressed in sprouting tip during neovessel formation, where it acts as a collagenase. In contrast, vascular smooth muscle cells suppress MT1-MMP activity in mature and stabilized vessels (Yana et al., 2007). Recently, some of the MT1-MMP functions in angiogenesis have been reported to be mediated by TGF-β activation. For example, prostaglandin E2 induces MT1-MMP clustering on the endothelial cell surface, which leads to TGF-β activation and induction of angiogenesis (Alfranca et al., 2008). Further, MT1-MMP knockout mice exhibit decreased levels of total TGF-β and defects in angiogenesis. Consistently, mice with mutated cleavage resistant Col1α1(I) and upregulated MT1-MMP activity display increased active TGF-β, which results in enhanced vascular leakage (Sounni et al., 2010). We could not confirm TGF-β activation in our experimental conditions, probably due to the low levels of active TGF-β. The phosphorylation of Smad2/3, the downstream effector of TGF-β signalling, was not observed in activated endothelial cells. However, since the release of latent TGF-β is the first step in its activation, our results demonstrate that MT1-MMP increases the bioavailability of latent TGF-β, which could be activated in the physiological microenvironment. Consistently, the activation of TGF-β secreted by endothelial cells may require the presence of smooth muscle cells (Sato et al., 1993).

According to previous reports, serine proteases and secreted MMPs can activate TGF-β (Dallas et al., 2002; Yu and Stamenkovic, 2000). However, neither of them was involved in LTPB-1 cleavage in our results, since serine protease inhibitors or TIMP-1, which targets secreted MMPs, did not affect LTBP-1 depletion from the ECM. PKC pathway is essential for mediating angiogenic signals. VEGF induces Erk1/2 phosphorylation via PKC kinase leading to increased endothelial cell proliferation (Wong and Jin, 2005). We observed marked induction in Erk1/2 phosphorylation downstream of PKC. Both PKC and MEK inhibitors abrogated endothelial cell morphology change and LTBP-1 release from the ECM. Therefore,

LLC release from the ECM can be general feature of endothelial cells activated to proliferate or engage in sprouting morphogenesis.

2. Interrelated functions of MT1-MMP and MT3-MMP in melanoma cell invasion (II, III)

MT-MMPs are well-recognized players in cancer progression. In addition to cleaving ECM proteins, they can activate secreted MMPs by cleaving their prodomains. We observed previously uncharacterized type of interaction between two MT-MMPs. MT3-MMP reduced cell surface localization and collagenolytic activity of MT1-MMP. Despite MT1-MMP and MT3-MMP being close homologues (66% homology of amino acid sequence of catalytical domain, (Takino et al., 1995)), their different substrate specificity for collagen type I, the most abundant protein in human body, results in strikingly different biological functions when expressed in tumor cells. Since MT3-MMP is not merely unable to cleave collagen type I, but also reduces collagenolytic activity of MT1-MMP, its expression has profound effects on the growth and invasion phenotype of tumor cells. On the other hand, MT3-MMP can promote melanoma cell invasion in fibrin even more efficiently than MT1-MMP, indicating that the net effect of MT3-MMP on melanoma cell invasion depends on the surrounding tissue.

We found that MT3-MMP reduces MT1-MMP cell surface levels both *in vitro* (II) and *in vivo* (III), providing another mechanism for posttranslational regulation of MT1-MMP protein expression and subcellular localization. When co-expressed in COS or Bowes cells, MT3-MMP induced MT1-MMP cleavage to 44 kDa fragment, which corresponds to the fragment generated by autocatalytic cleavage, and 37 kDa fragment. The smaller fragment has been reported before to be generated by recombinant MMP-2, MMP-3, MMP-13, and soluble MT1-MMP (Stanton et al., 1998), which may represent another cleavage site accessed by other MMPs but not by cell surface MT1-MMP itself. Cleavage of endogenous MT1-MMP was not detected in WM852 cells, probably due to high levels of TIMP-2 and TIMP-3 in these cells (Airola et al., 1999), or rapid internalization of 44 kDa cleaved fragment. However, MT3-MMP reduced full-length cell surface MT1-MMP also in WM852 cells. Co-expression of catalytically inactive MT3-MMP mutant did not induce MT1-MMP cleavage, affect cell surface localization of MT1-MMP, or reduce MT1-MMP-driven collagen invasion. Therefore, MT3-MMP catalytical activity is required for the regulation of MT1-MMP cell surface localization either directly by inducing its cleavage and internalization, or indirectly by affecting MT1-MMP trafficking.

Reduced cell surface MT1-MMP correlated with decreased invasiveness in collagen *in vitro* (II) and collagen accumulation in tumors *in vivo* (III). MT3-MMP expressing melanoma xenografts displayed slow restricted growth (III). This suggests that limited ability of MT3-MMP expressing cells to cleave collagen type I resulted in collagen accumulation and tumor growth restriction. In contrast, the opposite pattern of fibrin accumulation was observed. Although both MT1-MMP and MT3-MMP cleave fibrin, extracellular fibrin network was observed only in tumors with silenced MT3-MMP, suggesting that MT3-MMP is more potent enzyme to cleave fibrin in these cells than MT1-MMP. Since fibrin is a component of the provisional matrix often observed at tumor/host interface and perivascular spaces, MT3-MMP may promote tumor dissemination by driving cell invasion through fibrin-rich tissues.

In human melanoma tumors, low MT1-MMP and high MT3-MMP expression correlated with cohesive growth of melanoma cell nests, which grew expansively pushing collagen fibers outwards (III). In two human melanoma tumors with high MT1-MMP and low MT3-MMP expression, melanoma cells infiltrated underlying dermis diffusely, and did not form cell nests, suggesting that the expression of both MT1-MMP and MT3-MMP may determine the net collagenolytic activity, and growth phenotype. Since MT1-MMP affects many cell-cell and cell-ECM adhesive proteins, it is possible that cohesive vs. infiltrating phenotype of melanoma cells was affected, in addition to collagenolytic ability, by cell-cell contacts.

Interestingly, MT3-MMP is specifically upregulated in the most aggressive subtype of melanoma, namely nodular melanoma (Jaeger et al., 2007; Ohnishi et al., 2001). This may seem unexpected since melanoma grows in collagen-rich skin and may thus depend on unperturbed MT1-MMP activity. Nodular melanomas are typically small nodular tumors, which lack radial growth phase, and grow rapidly in thickness instead, having metastasizing capacity already at the early stages. In 3D collagen culture of seven melanoma cell lines, MT3-MMP expression was associated with expansive spherical growth, contrasted by the invasive sprouting growth of melanoma cell lines without MT3-MMP (II). This phenotype was found to be MT3-MMP-dependent, since silencing MT3-MMP in two melanoma cell lines resulted in the shift from expansive to invasive growth. Thus, MT3-MMP does not inhibit colony growth in 3D collagen, but rather affects the growth pattern, indicating that low MT1-MMP activity is still present at the cell surface. Consistently, cell surface levels of MT1-MMP determine the invasive vs. expansive growth phenotype of ovarian carcinoma cells (Moss et al., 2009), suggesting that MT3-MMP regulates melanoma growth phenotype by affecting MT1-MMP cell surface activity.

In addition, MT3-MMP expressing WM852 cells grew as adherent monolayers in 2D culture, while MT3-MMP silencing rendered them to elongated single cells (III). MT1-MMP expression and induced collagenolytic activity and invasion are linked to epithelial-to-mesenchymal transition-like phenotype observed at the initial stages of many carcinomas (Ota et al., 2009). Mesenchymal-like single cell invasion may facilitate local spreading and intravasation, but it impedes metastatic colony formation at the distant sites. Instead, carcinoma cells must return to the epithelioid phenotype at the late stages of metastatic process to be able to form adhesive colonies in the distant organs (Polyak and Weinberg, 2009). Furthermore, cells which engage in collective invasion and grow as cohesive colonies colonize distant organs more efficiently than those moving in a single cell manner (Chui, 2012; Friedl and Gilmour, 2009; Giampieri et al., 2009). Therefore, MT3-MMP expression may give advantage to nodular melanomas by suppressing excessive MT1-MMP activity and promoting adhesive phenotype of melanoma cells. We observed upregulation of MT3-MMP expression in two out of eight human melanoma biopsies (III), in biopsies of human melanoma lymph node metastases (II), and in three out of four metastatic melanoma cell lines (II). Thus, MT3-MMP may be associated more widely with metastatic dissemination in melanoma.

3. MT3-MMP in vascular invasion of melanoma cells (III)

Solid tumors disseminate to distant organs either via lymphatic or blood vessels. Some tumors, such as melanoma and breast cancer, spread mainly via lymphatics, first to tumor draining sentinel lymph nodes, and further to the other lymph nodes, circulation, and distant organs. In our results, MT3-MMP expression was associated with prominent lymphatic invasion (LVI), while MT3-MMP silencing abrogated LVI and shifted melanoma cell invasion towards blood vessel invasion (BVI). Enhanced BVI correlated with increased cell surface MT1-MMP and collagen degradation.

The mechanisms of tumor cell intravasation and its contribution to metastatic progression are widely debated issues. Since the amount of circulating tumor cells does not correlate with the metastatic colony formation, it has been proposed that tumor cell intravasation is not a rate-liming step for metastatic dissemination (Butler and Gullino, 1975; Fidler, 2003). Furthermore, discontinuous basement membrane and defects in endothelial cell lining in tumor vessels could allow the passive passage into the blood vessels without need of active invasion or proteolytic activity (Bockhorn et al., 2007; Hashizume et al., 2000). However, others report that blood vessel intravasation requires active permeabilization of the vessel wall by tumor cells, and that MT1-MMP activity is involved in this process (Perentes et al., 2011; Shen et al., 2010; Stoletov et al., 2007). We find that in melanoma xenografts blood vessel invasion is restricted when collagen type I is accumulated around the blood vessels in tumors with low MT1-MMP activity, suggesting that active proteolytic activity is required for BVI, at least in collagen-rich environment. Taken together, reduced BVI in MT3-MMP expressing tumors may reflect both the reduced invasion towards the blood vessels, and the limited ability to induce blood vessel permeabilization due to low MT1-MMP activity.

According to The Human Protein Atlas, MT3-MMP protein is expressed moderately in normal human keratinocytes, and weakly in fibroblasts and melanocytes in the skin (www.proteinatlas.org). Out of twenty cancer tissues examined in the Atlas, the highest protein expression was observed in melanoma biopsies (in 50% of which the staining was strong, in 42% - moderate and in 8% - weak), suggesting that MT3-MMP is involved in melanoma pathogenesis. We observed high MT3-MMP expression in two out of eight human melanoma biopsies. While the nodular vs. superficially spreading origin of melanoma biopsies was not defined, the expression frequency of MT3-MMP resembled the occurrence of nodular melanoma (~15% of melanoma cases). Prominent lymphatic vessel invasion was observed in the human tumor with high MT1-MMP and MT3-MMP expression, as compared to no lymphatic invasion in the tumor with high MT1-MMP and low MT3-MMP expression.

Clear correlation between lymphatic invasion with poor outcome in breast cancer and melanoma patients suggests that lymphatic vessel invasion may be essential for metastatic progression of certain cancers (Egger et al., 2011; Niakosari et al., 2008; Petersson et al., 2009; Ran et al., 2010; Rose et al., 2011). Since lymphatic vessels lack basement membrane, pericyte lining, and exhibit intraendothelial channels, lymphatic vessel invasion could occur passively. The preference to invade into blood vs. lymphatic vessels depends also on the adhesive phenotype of the cells. Thus, tumor cells invading in single cell manner intravasate into blood vessels, while cells engaging in collective invasion enter lymphatic vessels (Giampieri et al., 2009). We found that MT3-MMP induced the adhesive phenotype of melanoma cells, which may provide an advantage for LVI, while MT3-MMP silencing resulted in elongated single cell growth pattern. We did not observe any macrometastases in

our xenograft models, probably due to the short follow up period and low metastatic ability of the cell line used. However, the observed shift in the initial stage of metastatic dissemination from LVI to BVI in MT3-MMP silenced cells provides new insights to the factors which modulate intravasation process.

Metastatic melanoma is a disease which is highly resistant to current treatments. Recently, B-raf inhibitors have been accepted to treat metastatic melanoma with *BRAF* mutations. However, ~50-60% of melanomas do not harbour this mutation (Lee et al., 2010), emphasizing a need to explore the biology of *BRAF*-wild type tumors. WM852 cells used in this study are wild type for *BRAF*, but have mutated *NRAS*, consistently with the observation that *BRAF* mutation frequently occurs in slowly progressing superficially spreading melanoma, while mutated *NRAS* is seen more often in aggressive nodular melanomas (Lee et al., 2010), from which WM852 cells are originating.

Sentinel lymph node (SLN) biopsy, a standard procedure for ≥1 mm thick melanomas, is associated with increased morbidity without improving overall survival (Morton et al., 2006). In addition, the fraction of thin (<1 mm) melanomas will eventually form distant metastases. Therefore, new markers which can be assessed already from the primary tumor helping to predict SLN and distant metastasis, and to find patients who would benefit from lymphadenectomy are needed (Shields et al., 2004). Shields et al proposed the use of Shields index, which combines lymphatic density, Breslow thickness and LVI, and predicts metastatic status even more accurately than Breslow thickness alone (Emmett et al., 2010; Shields et al., 2004). We found here that MT3-MMP expression is associated with LVI, suggesting that MT3-MMP might also predict melanoma metastatic dissemination.

LVI is observed in 16-63% of melanoma samples and correlates with lymph node metastasis and poor survival of melanoma patients (Egger et al., 2011; Niakosari et al., 2008; Petersson et al., 2009; Rose et al., 2011). By contrast, BVI, detected in 3-43% of melanoma samples, does not correlate with melanoma progression or prognosis (Storr et al., 2012). LVI correlates also with the aggressive nodular melanoma subtype, where MT3-MMP is specifically expressed (Jaeger et al., 2007; Storr et al., 2012). Taken together, since MT3-MMP is associated with nodular melanoma subtype (Jaeger et al., 2007; Ohnishi et al., 2001), lymph node metastasis (II), and LVI (III), its expression may predict poor prognosis of melanoma patients and the need of early aggressive treatment.
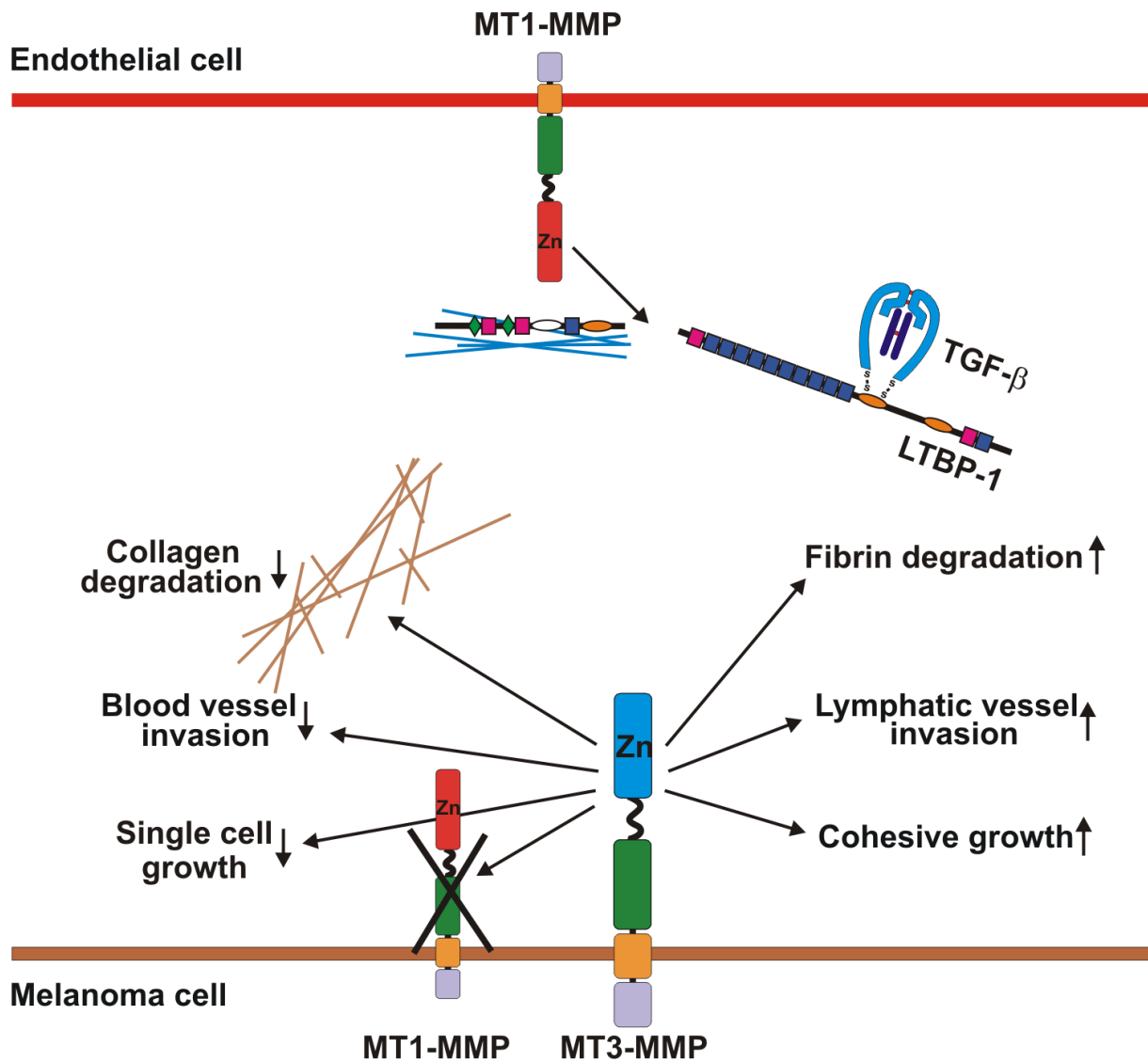
Figure 7. The main results of the thesis. MT1-MMP expressed by endothelial cells releases ECM-bound large latent TGF-β complex by inducing the cleavage of LTBP-1, which N-terminal region is bound to ECM proteins. MT3-MMP cleaves MT1-MMP leading to reduced collagen degradation and blood vessel invasion of melanoma cells, as well as motility shift from single cell to collective migration. Collagen accumulation and collective motility impair blood vessel invasion, but facilitate lymphatic vessel invasion.

CONCLUSIONS AND PERSPECTIVES

MT1-MMP and TGF-β are involved in neovessel formation. MT1-MMP is specifically expressed at the sprouting tips of growing vessels. TGF-β is secreted from endothelial cells as a large latent TGF-β complex, which targets it to the ECM. The data presented here identifies MT1-MMP as an enzyme which has the ability to release large latent TGF-β complex from the ECM of endothelial cells (Fig. 7). MT1-MMP cleaved LTBP-1 from the ECM after endothelial cell activation by a protein kinase C agonist, PMA. Since protein kinase C mediates angiogenic signals of VEGF and FGF, MT1-MMP-mediated TGF-β release may be a general mechanism to increase bioavailability of TGF-β during angiogenesis.

MT1-MMP is a potent collagenase which drives tumor cell invasion through basement membranes and collagen-rich interstitial tissues. MT3-MMP is a close homologue to MT1-MMP, but it cannot cleave collagen type I. We found here that MT3-MMP restricts the collagenolytic activity of MT1-MMP by reducing its cell surface levels both *in vitro* and *in vivo*. This results in decreased collagen invasion, but induces growth of cohesive colonies *in vitro*. Limited collagenolytic ability and cohesive growth impair blood vessel invasion of melanoma cells, but permit their lymphatic invasion. Taken together, MT3-MMP expression correlates with lymphatic vessel invasion, a process associated with poor prognosis of melanoma patients (Fig. 7). The elucidation of the mechanisms of lymphatic invasion by tumor cells can help to discover new prognostic markers for metastatic dissemination, and facilitate the development of specific intravasation inhibitors. The correlation of MT3-MMP expression of melanoma tumors with metastatic progression or patient survival should bring valuable information of MT3-MMP functions in *in vivo* melanoma progression. In the coming era of personalized medicine, when gene sequencing of individual tumors will be widely performed, the information about key modifiers of tumor progression will accumulate and will guide the choice of patient treatment.

ACKNOWLEDGEMENTS

I thank the leaders of three groups I visited during my rotation summers: Docent Anu Jalanko, Professor Markku Heikinheimo, and Professor Erkki Hölttä, and their group members for introducing to me the world of science from different perspectives.

I am exceedingly grateful to my friends from Ressu Anastasia Shulga, Olga Koiverianine, and Leena Sommarberg for long time friendship, common sport activities, movie nights, and giving me an escape from the world of medicine from time to time. Marjo Mattila, Sirpa Kilpijärvi, and Marika Suontaka are warmly thanked for walking with me the path of medical studies, and for staying my friends after graduating.

I would like to thank my dear parents for their love, support, and creating the study-friendly environment starting from my first grade. I owe you everything I am. I thank my aunt Lilja, cousin Svetlana, and all the relatives for supporting me and believing in me, and my cousins Jana and Anton for all the fun we have together.

My special gratitude belongs to Alexander for his understanding, care, and love. Your support, endless energy, and excitement about everything in life have widened my horizons, and have given me courage and trust in myself.

Helsinki, April 2013

REFERENCES

Abraham, R., Schafer, J., Rothe, M., Bange, J., Knyazev, P., and Ullrich, A. (2005). Identification of MMP-15 as an anti-apoptotic factor in cancer cells. J. Biol. Chem. *280,* 34123-34132.

Agarwal, D., Goodison, S., Nicholson, B., Tarin, D., and Urquidi, V. (2003). Expression of matrix metalloproteinase 8 (MMP-8) and tyrosinase-related protein-1 (TYRP-1) correlates with the absence of metastasis in an isogenic human breast cancer model. Differentiation *71,* 114-125.

Ahmad, M., Takino, T., Miyamori, H., Yoshizaki, T., Furukawa, M., and Sato, H. (2006). Cleavage of amyloid-beta precursor protein (APP) by membrane-type matrix metalloproteinases. J. Biochem. *139,* 517-526.

Airola, K., Karonen, T., Vaalamo, M., Lehti, K., Lohi, J., Kariniemi, A.L., Keski-Oja, J., and Saarialho-Kere, U.K. (1999). Expression of collagenases-1 and -3 and their inhibitors TIMP-1 and -3 correlates with the level of invasion in malignant melanomas. Br. J. Cancer *80,* 733-743.

Alexander, S., Koehl, G.E., Hirschberg, M., Geissler, E.K., and Friedl, P. (2008). Dynamic imaging of cancer growth and invasion: a modified skin-fold chamber model. Histochem. Cell Biol. *130,* 1147-1154.

Alfranca, A., Lopez-Oliva, J.M., Genis, L., Lopez-Maderuelo, D., Mirones, I., Salvado, D., Quesada, A.J., Arroyo, A.G., and Redondo, J.M. (2008). PGE2 induces angiogenesis via MT1-MMP-mediated activation of the TGFbeta/Alk5 signaling pathway. Blood *112,* 1120-1128.

Alitalo, A., and Detmar, M. (2012). Interaction of tumor cells and lymphatic vessels in cancer progression. Oncogene *31,* 4499-4508.

Amour, A., Knight, C.G., Webster, A., Slocombe, P.M., Stephens, P.E., Knäuper, V., Docherty, A.J., and Murphy, G. (2000). The in vitro activity of ADAM-10 is inhibited by TIMP-1 and TIMP-3. FEBS Lett. *473,* 275-279.

Annabi, B., Lachambre, M., Bousquet-Gagnon, N., Page, M., Gingras, D., and Beliveau, R. (2001). Localization of membrane-type 1 matrix metalloproteinase in caveolae membrane domains. Biochem. J. *353,* 547-553.

Antonelli-Orlidge, A., Saunders, K.B., Smith, S.R., and D'Amore, P.A. (1989). An activated form of transforming growth factor beta is produced by cocultures of endothelial cells and pericytes. Proc. Natl. Acad. Sci. U. S. A. *86,* 4544-4548.

Apte, S.S., Fukai, N., Beier, D.R., and Olsen, B.R. (1997). The matrix metalloproteinase-14 (MMP-14) gene is structurally distinct from other MMP genes and is co-expressed with the TIMP-2 gene during mouse embryogenesis. J. Biol. Chem. *272,* 25511-25517.

Arai, I., Nagano, H., Kondo, M., Yamamoto, H., Hiraoka, N., Sugita, Y., Ota, H., Yoshioka, S., Nakamura, M., Wada, H.*, et al.* (2007). Overexpression of MT3-MMP in hepatocellular carcinoma correlates with capsular invasion. Hepatogastroenterology *54,* 167-171.

Arozarena, I., Bischof, H., Gilby, D., Belloni, B., Dummer, R., and Wellbrock, C. (2011). In melanoma, beta-catenin is a suppressor of invasion. Oncogene *30,* 4531-4543.

Artym, V.V., Zhang, Y., Seillier-Moiseiwitsch, F., Yamada, K.M., and Mueller, S.C. (2006). Dynamic interactions of cortactin and membrane type 1 matrix metalloproteinase at invadopodia: defining the stages of invadopodia formation and function. Cancer Res. *66,* 3034-3043.

Astarci, E., Erson-Bensan, A.E., and Banerjee, S. (2012). Matrix metalloprotease 16 expression is downregulated by microRNA-146a in spontaneously differentiating Caco-2 cells. Dev. Growth Differ. *54,* 216-226.

Atkinson, S.J., Crabbe, T., Cowell, S., Ward, R.V., Butler, M.J., Sato, H., Seiki, M., Reynolds, J.J., and Murphy, G. (1995). Intermolecular autolytic cleavage can contribute to the activation of progelatinase A by cell membranes. J. Biol. Chem. *270,* 30479-30485.

Attisano, L., and Wrana, J.L. (2002). Signal transduction by the TGF-beta superfamily. Science *296,* 1646-1647.

Azzali, G. (2007). Tumor cell transendothelial passage in the absorbing lymphatic vessel of transgenic adenocarcinoma mouse prostate. Am. J. Pathol. *170,* 334-346.

Azzam, H.S., and Thompson, E.W. (1992). Collagen-induced activation of the M(r) 72,000 type IV collagenase in normal and malignant human fibroblastoid cells. Cancer Res. *52,* 4540-4544.

Baker, A.H., Edwards, D.R., and Murphy, G. (2002). Metalloproteinase inhibitors: biological actions and therapeutic opportunities. J. Cell. Sci. *115,* 3719-3727.

Balbin, M., Fueyo, A., Tester, A.M., Pendas, A.M., Pitiot, A.S., Astudillo, A., Overall, C.M., Shapiro, S.D., and Lopez-Otin, C. (2003). Loss of collagenase-2 confers increased skin tumor susceptibility to male mice. Nat. Genet. *35,* 252-257.

Balch, C.M., Gershenwald, J.E., Soong, S.J., Thompson, J.F., Atkins, M.B., Byrd, D.R., Buzaid, A.C., Cochran, A.J., Coit, D.G., Ding, S.*, et al.* (2009). Final version of 2009 AJCC melanoma staging and classification. J. Clin. Oncol. *27,* 6199-6206.

Balkwill, F. (2004). Cancer and the chemokine network. Nat. Rev. Cancer. *4,* 540-550.

Balzer, E.M., Tong, Z., Paul, C.D., Hung, W.C., Stroka, K.M., Boggs, A.E., Martin, S.S., and Konstantopoulos, K. (2012). Physical confinement alters tumor cell adhesion and migration phenotypes. Faseb j. *26,* 4045-4056.

Barbolina, M.V., Adley, B.P., Ariztia, E.V., Liu, Y., and Stack, M.S. (2007). Microenvironmental regulation of membrane type 1 matrix metalloproteinase activity in ovarian carcinoma cells via collagen-induced EGR1 expression. J. Biol. Chem. *282,* 4924-4931.

Bar-Eli, M. (2001). Gene regulation in melanoma progression by the AP-2 transcription factor. Pigment Cell Res. *14,* 78-85.

Bashkin, P., Doctrow, S., Klagsbrun, M., Svahn, C.M., Folkman, J., and Vlodavsky, I. (1989). Basic fibroblast growth factor binds to subendothelial extracellular matrix and is released by heparitinase and heparin-like molecules. Biochemistry *28,* 1737-1743.

Bass, M.D., Morgan, M.R., Roach, K.A., Settleman, J., Goryachev, A.B., and Humphries, M.J. (2008). p190RhoGAP is the convergence point of adhesion signals from alpha 5 beta 1 integrin and syndecan-4. J. Cell Biol. *181,* 1013-1026.

Beadle, C., Assanah, M.C., Monzo, P., Vallee, R., Rosenfeld, S.S., and Canoll, P. (2008). The role of myosin II in glioma invasion of the brain. Mol. Biol. Cell *19,* 3357-3368.

Beasley, N.J., Prevo, R., Banerji, S., Leek, R.D., Moore, J., van Trappen, P., Cox, G., Harris, A.L., and Jackson, D.G. (2002). Intratumoral lymphangiogenesis and lymph node metastasis in head and neck cancer. Cancer Res. *62,* 1315-1320.

Beauvais, D.M., and Rapraeger, A.C. (2003). Syndecan-1-mediated cell spreading requires signaling by alphavbeta3 integrins in human breast carcinoma cells. Exp. Cell Res. *286,* 219-232.

Bedogni, B., Welford, S.M., Cassarino, D.S., Nickoloff, B.J., Giaccia, A.J., and Powell, M.B. (2005). The hypoxic microenvironment of the skin contributes to Akt-mediated melanocyte transformation. Cancer. Cell. *8,* 443-454.

Belkin, A.M., Akimov, S.S., Zaritskaya, L.S., Ratnikov, B.I., Deryugina, E.I., and Strongin, A.Y. (2001). Matrix-dependent proteolysis of surface transglutaminase by membrane-type metalloproteinase regulates cancer cell adhesion and locomotion. J. Biol. Chem. *276,* 18415-18422.

Bergers, G., and Hanahan, D. (2008). Modes of resistance to anti-angiogenic therapy. Nat. Rev. Cancer. *8,* 592-603.

Berking, C., Takemoto, R., Schaider, H., Showe, L., Satyamoorthy, K., Robbins, P., and Herlyn, M. (2001). Transforming growth factor-beta1 increases survival of human melanoma through stroma remodeling. Cancer Res. *61,* 8306-8316.

Bharadwaj, A.G., Kovar, J.L., Loughman, E., Elowsky, C., Oakley, G.G., and Simpson, M.A. (2009). Spontaneous metastasis of prostate cancer is promoted by excess hyaluronan synthesis and processing. Am. J. Pathol. *174,* 1027-1036.

Bingle, L., Brown, N.J., and Lewis, C.E. (2002). The role of tumour-associated macrophages in tumour progression: implications for new anticancer therapies. J. Pathol. *196,* 254-265.

Blackburn, J.S., Liu, I., Coon, C.I., and Brinckerhoff, C.E. (2009). A matrix metalloproteinase-1/protease activated receptor-1 signaling axis promotes melanoma invasion and metastasis. Oncogene *28,* 4237-4248.

Bockhorn, M., Jain, R.K., and Munn, L.L. (2007). Active versus passive mechanisms in metastasis: do cancer cells crawl into vessels, or are they pushed? Lancet Oncol. *8,* 444-448.

Borg, I., Squire, M., Menzel, C., Stout, K., Morgan, D., Willatt, L., O'Brien, P.C., Ferguson-Smith, M.A., Ropers, H.H., Tommerup, N., Kalscheuer, V.M., and Sargan, D.R. (2002). A cryptic deletion of 2q35 including part of the PAX3 gene detected by breakpoint mapping in a child with autism and a de novo 2;8 translocation. J. Med. Genet. *39,* 391-399.

Boyd, P.J., Doyle, J., Gee, E., Pallan, S., and Haas, T.L. (2005). MAPK signaling regulates endothelial cell assembly into networks and expression of MT1-MMP and MMP-2. Am. J. Physiol. Cell. Physiol. *288,* C659-68.

Brabek, J., Mierke, C.T., Rosel, D., Vesely, P., and Fabry, B. (2010). The role of the tissue microenvironment in the regulation of cancer cell motility and invasion. Cell. Commun. Signal. *8,* 22-811X-8-22.

Bradford, P.T., Goldstein, A.M., McMaster, M.L., and Tucker, M.A. (2009). Acral lentiginous melanoma: incidence and survival patterns in the United States, 1986-2005. Arch. Dermatol. *145,* 427-434.

Brassart-Pasco, S., Senechal, K., Thevenard, J., Ramont, L., Devy, J., Di Stefano, L., Dupont-Deshorgue, A., Brezillon, S., Feru, J., Jazeron, J.F.*, et al.* (2012). Tetrastatin, the NC1 domain of the alpha4(IV) collagen chain: a novel potent anti-tumor matrikine. PLoS One *7,* e29587.

Bravo-Cordero, J.J., Marrero-Diaz, R., Megias, D., Genis, L., Garcia-Grande, A., Garcia, M.A., Arroyo, A.G., and Montoya, M.C. (2007). MT1-MMP proinvasive activity is regulated by a novel Rab8-dependent exocytic pathway. Embo j. *26,* 1499-1510.

Breslow, A. (1970). Thickness, cross-sectional areas and depth of invasion in the prognosis of cutaneous melanoma. Ann. Surg. *172,* 902-908.

Brew, K., Dinakarpandian, D., and Nagase, H. (2000). Tissue inhibitors of metalloproteinases: evolution, structure and function. Biochim. Biophys. Acta *1477,* 267-283.

Brinckerhoff, C.E., and Matrisian, L.M. (2002). Matrix metalloproteinases: a tail of a frog that became a prince. Nat. Rev. Mol. Cell Biol. *3,* 207-214.

Bujan, J., Gimeno, M.J., Prieto, A., Pascual, G., Bellon, J.M., and Alvarez-Mon, M. (1999). Modulation of PECAM-1 (CD31) expression in human endothelial cells: effect of IFNgamma and IL-10. J. Vasc. Res. *36,* 106-113.

Buommino, E., De Filippis, A., Gaudiello, F., Balato, A., Balato, N., Tufano, M.A., and Ayala, F. (2012). Modification of osteopontin and MMP-9 levels in patients with psoriasis on anti-TNF-alpha therapy. Arch. Dermatol. Res. *304,* 481-485.

Burton, J.B., Priceman, S.J., Sung, J.L., Brakenhielm, E., An, D.S., Pytowski, B., Alitalo, K., and Wu, L. (2008). Suppression of prostate cancer nodal and systemic metastasis by blockade of the lymphangiogenic axis. Cancer Res. *68,* 7828-7837.

Busam, K.J., Berwick, M., Blessing, K., Fandrey, K., Kang, S., Karaoli, T., Fine, J., Cochran, A.J., White, W.L., and Rivers, J. (1995). Tumor vascularity is not a prognostic factor for malignant melanoma of the skin. Am. J. Pathol. *147,* 1049-1056.

Butler, G.S., Butler, M.J., Atkinson, S.J., Will, H., Tamura, T., Schade van Westrum, S., Crabbe, T., Clements, J., d'Ortho, M.P., and Murphy, G. (1998). The TIMP2 membrane type 1 metalloproteinase "receptor" regulates the concentration and efficient activation of progelatinase A. A kinetic study. J. Biol. Chem. *273,* 871-880.

Butler, T.P., and Gullino, P.M. (1975). Quantitation of cell shedding into efferent blood of mammary adenocarcinoma. Cancer Res. *35,* 512-516.

Carmeliet, P. (2005). Angiogenesis in life, disease and medicine. Nature *438,* 932-936.

Carmeliet, P., and Jain, R.K. (2000). Angiogenesis in cancer and other diseases. Nature *407,* 249-257.

Carnochan, P., Briggs, J.C., Westbury, G., and Davies, A.J. (1991). The vascularity of cutaneous melanoma: a quantitative histological study of lesions 0.85-1.25 mm in thickness. Br. J. Cancer *64,* 102-107.

Casanovas, O., Hicklin, D.J., Bergers, G., and Hanahan, D. (2005). Drug resistance by evasion of antiangiogenic targeting of VEGF signaling in late-stage pancreatic islet tumors. Cancer. Cell. *8,* 299-309.

Caterina, J.J., Yamada, S., Caterina, N.C., Longenecker, G., Holmback, K., Shi, J., Yermovsky, A.E., Engler, J.A., and Birkedal-Hansen, H. (2000). Inactivating mutation of the mouse tissue inhibitor of metalloproteinases-2(Timp-2) gene alters proMMP-2 activation. J. Biol. Chem. *275,* 26416-26422.

Chabottaux, V., Sounni, N.E., Pennington, C.J., English, W.R., van den Brule, F., Blacher, S., Gilles, C., Munaut, C., Maquoi, E., Lopez-Otin, C.*, et al.* (2006). Membrane-type 4 matrix metalloproteinase promotes breast cancer growth and metastases. Cancer Res. *66,* 5165-5172.

Chamberlain, A.J., Fritschi, L., Giles, G.G., Dowling, J.P., and Kelly, J.W. (2002). Nodular type and older age as the most significant associations of thick melanoma in Victoria, Australia. Arch. Dermatol. *138,* 609-614.

Chan, K.M., Wong, H.L., Jin, G., Liu, B., Cao, R., Cao, Y., Lehti, K., Tryggvason, K., and Zhou, Z. (2012). MT1-MMP inactivates ADAM9 to regulate FGFR2 signaling and calvarial osteogenesis. Dev. Cell. *22,* 1176-1190.

Chen, P., Huang, Y., Bong, R., Ding, Y., Song, N., Wang, X., Song, X., and Luo, Y. (2011). Tumor-associated macrophages promote angiogenesis and melanoma growth via adrenomedullin in a paracrine and autocrine manner. Clin. Cancer Res. *17,* 7230-7239.

Chernov, A.V., and Strongin, A.Y. (2011). Epigenetic regulation of matrix metalloproteinases and their collagen substrates in cancer. Biomol. Concepts *2,* 135-147.

Choi, W.W., Lewis, M.M., Lawson, D., Yin-Goen, Q., Birdsong, G.G., Cotsonis, G.A., Cohen, C., and Young, A.N. (2005). Angiogenic and lymphangiogenic microvessel density in breast carcinoma: correlation with clinicopathologic parameters and VEGF-family gene expression. Mod. Pathol. *18,* 143-152.

Chua, R., Setzer, S., Govindarajan, B., Sexton, D., Cohen, C., and Arbiser, J.L. (2009). Maspin expression, angiogenesis, prognostic parameters, and outcome in malignant melanoma. J. Am. Acad. Dermatol. *60,* 758-766.

Chui, M.H. (2012). Insights into cancer metastasis from a clinicopathologic perspective: Epithelial-Mesenchymal Transition is not a necessary step. Int. J. Cancer

Chun, T.H., Sabeh, F., Ota, I., Murphy, H., McDonagh, K.T., Holmbeck, K., Birkedal-Hansen, H., Allen, E.D., and Weiss, S.J. (2004). MT1-MMP-dependent neovessel formation within the confines of the three-dimensional extracellular matrix. J. Cell Biol. *167,* 757-767.

Claffey, K.P., Brown, L.F., del Aguila, L.F., Tognazzi, K., Yeo, K.T., Manseau, E.J., and Dvorak, H.F. (1996). Expression of vascular permeability factor/vascular endothelial growth factor by melanoma cells increases tumor growth, angiogenesis, and experimental metastasis. Cancer Res. *56,* 172-181.

Clark, W.H.,Jr, From, L., Bernardino, E.A., and Mihm, M.C. (1969). The histogenesis and biologic behavior of primary human malignant melanomas of the skin. Cancer Res. *29,* 705-727.

Colorado, P.C., Torre, A., Kamphaus, G., Maeshima, Y., Hopfer, H., Takahashi, K., Volk, R., Zamborsky, E.D., Herman, S., Sarkar, P.K.*, et al.* (2000). Anti-angiogenic cues from vascular basement membrane collagen. Cancer Res. *60,* 2520-2526.

Costantini, V., and Zacharski, L.R. (1992). The role of fibrin in tumor metastasis. Cancer Metastasis Rev. *11,* 283-290.

Cukierman, E., Pankov, R., and Yamada, K.M. (2002). Cell interactions with three-dimensional matrices. Curr. Opin. Cell Biol. *14,* 633-639.

Cullen, P.J. (2001). Ras effectors: buying shares in Ras plc. Curr. Biol. *11,* R342-4.

Dabovic, B., Chen, Y., Colarossi, C., Obata, H., Zambuto, L., Perle, M.A., and Rifkin, D.B. (2002). Bone abnormalities in latent TGF-[beta] binding protein (Ltbp)-3-null mice indicate a role for Ltbp-3 in modulating TGF-[beta] bioavailability. J. Cell Biol. *156,* 227-232.

Dadras, S.S., Lange-Asschenfeldt, B., Velasco, P., Nguyen, L., Vora, A., Muzikansky, A., Jahnke, K., Hauschild, A., Hirakawa, S., Mihm, M.C., and Detmar, M. (2005). Tumor lymphangiogenesis predicts melanoma metastasis to sentinel lymph nodes. Mod. Pathol. *18,* 1232-1242.

Dadras, S.S., Paul, T., Bertoncini, J., Brown, L.F., Muzikansky, A., Jackson, D.G., Ellwanger, U., Garbe, C., Mihm, M.C., and Detmar, M. (2003). Tumor lymphangiogenesis: a novel prognostic indicator for cutaneous melanoma metastasis and survival. Am. J. Pathol. *162,* 1951-1960.

Dallas, S.L., Keene, D.R., Bruder, S.P., Saharinen, J., Sakai, L.Y., Mundy, G.R., and Bonewald, L.F. (2000). Role of the latent transforming growth factor beta binding protein 1 in fibrillin-containing microfibrils in bone cells in vitro and in vivo. J. Bone Miner. Res. *15,* 68-81.

Dallas, S.L., Park-Snyder, S., Miyazono, K., Twardzik, D., Mundy, G.R., and Bonewald, L.F. (1994). Characterization and autoregulation of latent transforming growth factor beta (TGF beta) complexes in osteoblast-like cell lines. Production of a latent complex lacking the latent TGF beta-binding protein. J. Biol. Chem. *269,* 6815-6821.

Dallas, S.L., Rosser, J.L., Mundy, G.R., and Bonewald, L.F. (2002). Proteolysis of latent transforming growth factor-beta (TGF-beta)-binding protein-1 by osteoclasts. A cellular mechanism for release of TGF-beta from bone matrix. J. Biol. Chem. *277,* 21352-21360.

Danielson, K.G., Fazzio, A., Cohen, I., Cannizzaro, L.A., Eichstetter, I., and Iozzo, R.V. (1993). The human decorin gene: intron-exon organization, discovery of two alternatively spliced exons in the 5' untranslated region, and mapping of the gene to chromosome 12q23. Genomics *15,* 146-160.

Davidson, B., Konstantinovsky, S., Nielsen, S., Dong, H.P., Berner, A., Vyberg, M., and Reich, R. (2004). Altered expression of metastasis-associated and regulatory molecules in effusions from breast cancer patients: a novel model for tumor progression. Clin. Cancer Res. *10,* 7335-7346.

Davies, H., Bignell, G.R., Cox, C., Stephens, P., Edkins, S., Clegg, S., Teague, J., Woffendin, H., Garnett, M.J., Bottomley, W.*, et al.* (2002). Mutations of the BRAF gene in human cancer. Nature *417,* 949-954.

de Larco, J.E., and Todaro, G.J. (1978). Growth factors from murine sarcoma virus-transformed cells. Proc. Natl. Acad. Sci. U. S. A. *75,* 4001-4005.

de Snoo, F.A., and Hayward, N.K. (2005). Cutaneous melanoma susceptibility and progression genes. Cancer Lett. *230,* 153-186.

Demou, Z.N., Awad, M., McKee, T., Perentes, J.Y., Wang, X., Munn, L.L., Jain, R.K., and Boucher, Y. (2005). Lack of telopeptides in fibrillar collagen I promotes the invasion of a metastatic breast tumor cell line. Cancer Res. *65,* 5674-5682.

Dennis, P.A., and Rifkin, D.B. (1991). Cellular activation of latent transforming growth factor beta requires binding to the cation-independent mannose 6-phosphate/insulin-like growth factor type II receptor. Proc. Natl. Acad. Sci. U. S. A. *88,* 580-584.

Derynck, R., Jarrett, J.A., Chen, E.Y., Eaton, D.H., Bell, J.R., Assoian, R.K., Roberts, A.B., Sporn, M.B., and Goeddel, D.V. (1985). Human transforming growth factor-beta complementary DNA sequence and expression in normal and transformed cells. Nature *316,* 701-705.

Deryugina, E.I., and Quigley, J.P. (2006). Matrix metalloproteinases and tumor metastasis. Cancer Metastasis Rev. *25,* 9-34.

Deryugina, E.I., Soroceanu, L., and Strongin, A.Y. (2002). Up-regulation of vascular endothelial growth factor by membrane-type 1 matrix metalloproteinase stimulates human glioma xenograft growth and angiogenesis. Cancer Res. *62,* 580-588.

Deryugina, E.I., Zijlstra, A., Partridge, J.J., Kupriyanova, T.A., Madsen, M.A., Papagiannakopoulos, T., and Quigley, J.P. (2005). Unexpected effect of matrix metalloproteinase down-regulation on vascular intravasation and metastasis of human fibrosarcoma cells selected in vivo for high rates of dissemination. Cancer Res. *65,* 10959-10969.

Drews, F., Knobel, S., Moser, M., Muhlack, K.G., Mohren, S., Stoll, C., Bosio, A., Gressner, A.M., and Weiskirchen, R. (2008). Disruption of the latent transforming growth factor-beta binding protein-1 gene causes alteration in facial structure and influences TGF-beta bioavailability. Biochim. Biophys. Acta *1783,* 34-48.

Dubois, C.M., Laprise, M.H., Blanchette, F., Gentry, L.E., and Leduc, R. (1995). Processing of transforming growth factor beta 1 precursor by human furin convertase. J. Biol. Chem. *270,* 10618-10624.

Dudas, B., and Semeniken, K. (2012). Glycosaminoglycans and neuroprotection. Handb. Exp. Pharmacol. *(207):325-43. doi,* 325-343.

Egawa, N., Koshikawa, N., Tomari, T., Nabeshima, K., Isobe, T., and Seiki, M. (2006). Membrane type 1 matrix metalloproteinase (MT1-MMP/MMP-14) cleaves and releases a 22-kDa extracellular matrix metalloproteinase inducer (EMMPRIN) fragment from tumor cells. J. Biol. Chem. *281,* 37576-37585.

Egger, M.E., Gilbert, J.E., Burton, A.L., McMasters, K.M., Callender, G.G., Quillo, A.R., Brown, R.E., St Hill, C.R., Hagendoorn, L., Martin, R.C.,2nd, Stromberg, A.J., and Scoggins, C.R. (2011). Lymphovascular invasion as a prognostic factor in melanoma. Am. Surg. *77,* 992-997.

Ellerbroek, S.M., Wu, Y.I., Overall, C.M., and Stack, M.S. (2001). Functional interplay between type I collagen and cell surface matrix metalloproteinase activity. J. Biol. Chem. *276,* 24833-24842.

Emmett, M.S., Symonds, K.E., Rigby, H., Cook, M.G., Price, R., Metcalfe, C., Orlando, A., and Bates, D.O. (2010). Prediction of melanoma metastasis by the Shields index based on lymphatic vessel density. BMC Cancer *10,* 208-2407-10-208.

Endo, K., Takino, T., Miyamori, H., Kinsen, H., Yoshizaki, T., Furukawa, M., and Sato, H. (2003). Cleavage of syndecan-1 by membrane type matrix metalloproteinase-1 stimulates cell migration. J. Biol. Chem. *278,* 40764-40770.

English, W.R., Puente, X.S., Freije, J.M., Knäuper, V., Amour, A., Merryweather, A., Lopez-Otin, C., and Murphy, G. (2000). Membrane type 4 matrix metalloproteinase (MMP17) has tumor necrosis factor-alpha convertase activity but does not activate pro-MMP2. J. Biol. Chem. *275,* 14046-14055.

Engvall, E., Ruoslahti, E., and Miller, E.J. (1978). Affinity of fibronectin to collagens of different genetic types and to fibrinogen. J. Exp. Med. *147,* 1584-1595.

Ferraro, G.B., Morrison, C.J., Overall, C.M., Strittmatter, S.M., and Fournier, A.E. (2011b). Membrane-type matrix metalloproteinase-3 regulates neuronal responsiveness to myelin through Nogo-66 receptor 1 cleavage. J. Biol. Chem. *286,* 31418-31424.

Fidler, I.J. (2003). The pathogenesis of cancer metastasis: the 'seed and soil' hypothesis revisited. Nat. Rev. Cancer. *3,* 453-458.

Fish, R.J., and Neerman-Arbez, M. (2012). Fibrinogen gene regulation. Thromb. Haemost. *108,* 419-426.

Flaumenhaft, R., Abe, M., Sato, Y., Miyazono, K., Harpel, J., Heldin, C.H., and Rifkin, D.B. (1993). Role of the latent TGF-beta binding protein in the activation of latent TGF-beta by co-cultures of endothelial and smooth muscle cells. J. Cell Biol. *120,* 995-1002.

Friedl, P. (2004). Prespecification and plasticity: shifting mechanisms of cell migration. Curr. Opin. Cell Biol. *16,* 14-23.

Friedl, P., and Gilmour, D. (2009). Collective cell migration in morphogenesis, regeneration and cancer. Nat. Rev. Mol. Cell Biol. *10,* 445-457.

Friedl, P., Locker, J., Sahai, E., and Segall, J.E. (2012). Classifying collective cancer cell invasion. Nat. Cell Biol. *14,* 777-783.

Friedl, P., and Wolf, K. (2003). Tumour-cell invasion and migration: diversity and escape mechanisms. Nat. Rev. Cancer. *3,* 362-374.

Friedl, P., Wolf, K., and Lammerding, J. (2011). Nuclear mechanics during cell migration. Curr. Opin. Cell Biol. *23,* 55-64.

Frisch, S.M., and Morisaki, J.H. (1990). Positive and negative transcriptional elements of the human type IV collagenase gene. Mol. Cell. Biol. *10,* 6524-6532.

Fuchs, E. (2008). Skin stem cells: rising to the surface. J. Cell Biol. *180,* 273-284.

Fukunaga-Kalabis, M., Martinez, G., Nguyen, T.K., Kim, D., Santiago-Walker, A., Roesch, A., and Herlyn, M. (2010). Tenascin-C promotes melanoma progression by maintaining the ABCB5-positive side population. Oncogene *29,* 6115-6124.

Galvez, B.G., Matias-Roman, S., Yanez-Mo, M., Sanchez-Madrid, F., and Arroyo, A.G. (2002). ECM regulates MT1-MMP localization with beta1 or alphavbeta3 integrins at distinct cell compartments modulating its internalization and activity on human endothelial cells. J. Cell Biol. *159,* 509-521.

Gartner L.P., and Hiatt J.L. (2001), Color Textbook of Histology, 2nd edition, ISBN-10: 0721688063, 2001, Philadelphia, USA.

Ge, G., and Greenspan, D.S. (2006). BMP1 controls TGFbeta1 activation via cleavage of latent TGFbeta-binding protein. J. Cell Biol. *175,* 111-120.

Gengrinovitch, S., Berman, B., David, G., Witte, L., Neufeld, G., and Ron, D. (1999). Glypican-1 is a VEGF165 binding proteoglycan that acts as an extracellular chaperone for VEGF165. J. Biol. Chem. *274,* 10816-10822.

Gentry, L.E., Lioubin, M.N., Purchio, A.F., and Marquardt, H. (1988). Molecular events in the processing of recombinant type 1 pre-pro-transforming growth factor beta to the mature polypeptide. Mol. Cell. Biol. *8,* 4162-4168.

Gerhardt, H. (2008). VEGF and endothelial guidance in angiogenic sprouting. Organogenesis *4,* 241-246.

Gerli, R., Ibba, L., and Fruschelli, C. (1990). A fibrillar elastic apparatus around human lymph capillaries. Anat. Embryol. (Berl) *181,* 281-286.

Giampieri, S., Manning, C., Hooper, S., Jones, L., Hill, C.S., and Sahai, E. (2009). Localized and reversible TGFbeta signalling switches breast cancer cells from cohesive to single cell motility. Nat. Cell Biol. *11,* 1287-1296.

Giltay, R., Kostka, G., and Timpl, R. (1997). Sequence and expression of a novel member (LTBP-4) of the family of latent transforming growth factor-beta binding proteins. FEBS Lett. *411,* 164-168.

Gingras, D., and Beliveau, R. (2010). Emerging concepts in the regulation of membrane-type 1 matrix metalloproteinase activity. Biochim. Biophys. Acta *1803,* 142-150.

Gingras, D., Bousquet-Gagnon, N., Langlois, S., Lachambre, M.P., Annabi, B., and Beliveau, R. (2001). Activation of the extracellular signal-regulated protein kinase (ERK) cascade by membrane-type-1 matrix metalloproteinase (MT1-MMP). FEBS Lett. *507,* 231-236.

Gioia, M., Fasciglione, G.F., Marini, S., D'Alessio, S., De Sanctis, G., Diekmann, O., Pieper, M., Politi, V., Tschesche, H., and Coletta, M. (2002). Modulation of the catalytic activity of neutrophil collagenase MMP-8 on bovine collagen I. Role of the activation cleavage and of the hemopexin-like domain. J. Biol. Chem. *277,* 23123-23130.

Golubkov, V.S., Chekanov, A.V., Doxsey, S.J., and Strongin, A.Y. (2005). Centrosomal pericentrin is a direct cleavage target of membrane type-1 matrix metalloproteinase in humans but not in mice: potential implications for tumorigenesis. J. Biol. Chem. *280,* 42237-42241.

Golubkov, V.S., and Strongin, A.Y. (2012). Insights into Ectodomain Shedding and Processing of Protein-tyrosine Pseudokinase 7 (PTK7). J. Biol. Chem. *287,* 42009-42018.

Gomis-Ruth, F.X. (2003). Structural aspects of the metzincin clan of metalloendopeptidases. Mol. Biotechnol. *24,* 157-202.

Graham, C.H., Rivers, J., Kerbel, R.S., Stankiewicz, K.S., and White, W.L. (1994). Extent of vascularization as a prognostic indicator in thin (< 0.76 mm) malignant melanomas. Am. J. Pathol. *145,* 510-514.

Grass, G.D., Bratoeva, M., and Toole, B.P. (2012). Regulation of invadopodia formation and activity by CD147. J. Cell. Sci. *125,* 777-788.

Gremel, G., Rafferty, M., Lau, T.Y., and Gallagher, W.M. (2009). Identification and functional validation of therapeutic targets for malignant melanoma. Crit. Rev. Oncol. Hematol. *72,* 194-214.

Gross, J., and Lapiere, C.M. (1962). Collagenolytic activity in amphibian tissues: a tissue culture assay. Proc. Natl. Acad. Sci. U. S. A. *48,* 1014-1022.

Ha, H.Y., Moon, H.B., Nam, M.S., Lee, J.W., Ryoo, Z.Y., Lee, T.H., Lee, K.K., So, B.J., Sato, H., Seiki, M., and Yu, D.Y. (2001). Overexpression of membrane-type matrix metalloproteinase-1 gene induces mammary gland abnormalities and adenocarcinoma in transgenic mice. Cancer Res. *61,* 984-990.

Haas, T.L., Davis, S.J., and Madri, J.A. (1998). Three-dimensional type I collagen lattices induce coordinate expression of matrix metalloproteinases MT1-MMP and MMP-2 in microvascular endothelial cells. J. Biol. Chem. *273,* 3604-3610.

Haas, T.L., and Madri, J.A. (1999). Extracellular matrix-driven matrix metalloproteinase production in endothelial cells: implications for angiogenesis. Trends Cardiovasc. Med. *9,* 70-77.

Halata, Z., Grim, M., and Bauman, K.I. (2003). Friedrich Sigmund Merkel and his "Merkel cell", morphology, development, and physiology: review and new results. Anat. Rec. A. Discov. Mol. Cell. Evol. Biol. *271,* 225-239.

Hanahan, D., and Weinberg, R.A. (2011). Hallmarks of cancer: the next generation. Cell *144,* 646-674.

Haqq, C., Nosrati, M., Sudilovsky, D., Crothers, J., Khodabakhsh, D., Pulliam, B.L., Federman, S., Miller, J.R.,3rd, Allen, R.E., Singer, M.I.*, et al.* (2005). The gene expression signatures of melanoma progression. Proc. Natl. Acad. Sci. U. S. A. *102,* 6092-6097.

Harper, J.I., Godwin, H., Green, A., Wilkes, L.E., Holden, N.J., Moffatt, M., Cookson, W.O., Layton, G., and Chandler, S. (2010). A study of matrix metalloproteinase expression and activity in atopic dermatitis using a novel skin wash sampling assay for functional biomarker analysis. Br. J. Dermatol. *162,* 397-403.

Harris, A.L. (2002). Hypoxia--a key regulatory factor in tumour growth. Nat. Rev. Cancer. *2,* 38-47.

Hashizume, H., Baluk, P., Morikawa, S., McLean, J.W., Thurston, G., Roberge, S., Jain, R.K., and McDonald, D.M. (2000). Openings between defective endothelial cells explain tumor vessel leakiness. Am. J. Pathol. *156,* 1363-1380.

He, Y., Rajantie, I., Pajusola, K., Jeltsch, M., Holopainen, T., Yla-Herttuala, S., Harding, T., Jooss, K., Takahashi, T., and Alitalo, K. (2005). Vascular endothelial cell growth factor receptor 3-mediated activation of lymphatic endothelium is crucial for tumor cell entry and spread via lymphatic vessels. Cancer Res. *65,* 4739-4746.

Heino, J. (2007). The collagen family members as cell adhesion proteins. Bioessays *29,* 1001-1010.

Hendrix, M.J., Seftor, E.A., Hess, A.R., and Seftor, R.E. (2003). Vasculogenic mimicry and tumour-cell plasticity: lessons from melanoma. Nat. Rev. Cancer. *3,* 411-421.

Henriet, P., Blavier, L., and Declerck, Y.A. (1999). Tissue inhibitors of metalloproteinases (TIMP) in invasion and proliferation. Apmis *107,* 111-119.

Hirakawa, S., Kodama, S., Kunstfeld, R., Kajiya, K., Brown, L.F., and Detmar, M. (2005). VEGF-A induces tumor and sentinel lymph node lymphangiogenesis and promotes lymphatic metastasis. J. Exp. Med. *201,* 1089-1099.

Hiraoka, N., Allen, E., Apel, I.J., Gyetko, M.R., and Weiss, S.J. (1998). Matrix metalloproteinases regulate neovascularization by acting as pericellular fibrinolysins. Cell *95,* 365-377.

Hlubek, F., Spaderna, S., Jung, A., Kirchner, T., and Brabletz, T. (2004). Beta-catenin activates a coordinated expression of the proinvasive factors laminin-5 gamma2 chain and MT1-MMP in colorectal carcinomas. Int. J. Cancer *108,* 321-326.

Hofmann, U.B., Houben, R., Brocker, E.B., and Becker, J.C. (2005). Role of matrix metalloproteinases in melanoma cell invasion. Biochimie *87,* 307-314.

Hofmann, U.B., Westphal, J.R., Zendman, A.J., Becker, J.C., Ruiter, D.J., and van Muijen, G.N. (2000). Expression and activation of matrix metalloproteinase-2 (MMP-2) and its co-localization with membrane-type 1 matrix metalloproteinase (MT1-MMP) correlate with melanoma progression. J. Pathol. *191,* 245-256.

Holmbeck, K., Bianco, P., Caterina, J., Yamada, S., Kromer, M., Kuznetsov, S.A., Mankani, M., Robey, P.G., Poole, A.R., Pidoux, I., Ward, J.M., and Birkedal-Hansen, H. (1999). MT1-MMP-deficient mice develop dwarfism, osteopenia, arthritis, and connective tissue disease due to inadequate collagen turnover. Cell *99,* 81-92.

Hotary, K., Allen, E., Punturieri, A., Yana, I., and Weiss, S.J. (2000). Regulation of cell invasion and morphogenesis in a three-dimensional type I collagen matrix by membrane-type matrix metalloproteinases 1, 2, and 3. J. Cell Biol. *149,* 1309-1323.

Hotary, K., Li, X.Y., Allen, E., Stevens, S.L., and Weiss, S.J. (2006). A cancer cell metalloprotease triad regulates the basement membrane transmigration program. Genes Dev. *20,* 2673-2686.

Hotary, K.B., Allen, E.D., Brooks, P.C., Datta, N.S., Long, M.W., and Weiss, S.J. (2003). Membrane type I matrix metalloproteinase usurps tumor growth control imposed by the three-dimensional extracellular matrix. Cell *114,* 33-45.

Hotary, K.B., Yana, I., Sabeh, F., Li, X.Y., Holmbeck, K., Birkedal-Hansen, H., Allen, E.D., Hiraoka, N., and Weiss, S.J. (2002). Matrix metalloproteinases (MMPs) regulate fibrin-invasive activity via MT1-MMP-dependent and -independent processes. J. Exp. Med. *195,* 295-308.

Hudson, B.G., Reeders, S.T., and Tryggvason, K. (1993). Type IV collagen: structure, gene organization, and role in human diseases. Molecular basis of Goodpasture and Alport syndromes and diffuse leiomyomatosis. J. Biol. Chem. *268,* 26033-26036.

Huh, M.I., Lee, Y.M., Seo, S.K., Kang, B.S., Chang, Y., Lee, Y.S., Fini, M.E., Kang, S.S., and Jung, J.C. (2007). Roles of MMP/TIMP in regulating matrix swelling and cell migration during chick corneal development. J. Cell. Biochem. *101,* 1222-1237.

Huhtala, P., Chow, L.T., and Tryggvason, K. (1990). Structure of the human type IV collagenase gene. J. Biol. Chem. *265,* 11077-11082.

Huovila, A.P., Turner, A.J., Pelto-Huikko, M., Karkkainen, I., and Ortiz, R.M. (2005). Shedding light on ADAM metalloproteinases. Trends Biochem. Sci. *30,* 413-422.

Hussein, M.R. (2005). Transforming growth factor-beta and malignant melanoma: molecular mechanisms. J. Cutan. Pathol. *32,* 389-395.

Hyytiäinen, M., Penttinen, C., and Keski-Oja, J. (2004). Latent TGF-beta binding proteins: extracellular matrix association and roles in TGF-beta activation. Crit. Rev. Clin. Lab. Sci. *41,* 233-264.

Hyytiäinen, M., Taipale, J., Heldin, C.H., and Keski-Oja, J. (1998). Recombinant latent transforming growth factor beta-binding protein 2 assembles to fibroblast extracellular matrix and is susceptible to proteolytic processing and release. J. Biol. Chem. *273,* 20669-20676.

Iacobuzio-Donahue, C.A., Ryu, B., Hruban, R.H., and Kern, S.E. (2002). Exploring the host desmoplastic response to pancreatic carcinoma: gene expression of stromal and neoplastic cells at the site of primary invasion. Am. J. Pathol. *160,* 91-99.

Illman, S.A., Lehti, K., Keski-Oja, J., and Lohi, J. (2006). Epilysin (MMP-28) induces TGF-beta mediated epithelial to mesenchymal transition in lung carcinoma cells. J. Cell. Sci. *119,* 3856-3865.

Ilmonen, S., Kariniemi, A.L., Vlaykova, T., Muhonen, T., Pyrhonen, S., and Asko-Seljavaara, S. (1999). Prognostic value of tumour vascularity in primary melanoma. Melanoma Res. *9,* 273-278.

Isogai, Z., Ono, R.N., Ushiro, S., Keene, D.R., Chen, Y., Mazzieri, R., Charbonneau, N.L., Reinhardt, D.P., Rifkin, D.B., and Sakai, L.Y. (2003). Latent transforming growth factor beta-binding protein 1 interacts with fibrillin and is a microfibril-associated protein. J. Biol. Chem. *278,* 2750-2757.

Itoh, T., Ikeda, T., Gomi, H., Nakao, S., Suzuki, T., and Itohara, S. (1997). Unaltered secretion of beta-amyloid precursor protein in gelatinase A (matrix metalloproteinase 2)-deficient mice. J. Biol. Chem. *272,* 22389-22392.

Itoh, Y., and Seiki, M. (2006). MT1-MMP: a potent modifier of pericellular microenvironment. J. Cell. Physiol. *206,* 1-8.

Itoh, Y., Takamura, A., Ito, N., Maru, Y., Sato, H., Suenaga, N., Aoki, T., and Seiki, M. (2001). Homophilic complex formation of MT1-MMP facilitates proMMP-2 activation on the cell surface and promotes tumor cell invasion. Embo j. *20,* 4782-4793.

Jaeger, J., Koczan, D., Thiesen, H.J., Ibrahim, S.M., Gross, G., Spang, R., and Kunz, M. (2007). Gene expression signatures for tumor progression, tumor subtype, and tumor thickness in laser-microdissected melanoma tissues. Clin. Cancer Res. *13,* 806-815.

Janji, B., Melchior, C., Gouon, V., Vallar, L., and Kieffer, N. (1999). Autocrine TGF-beta-regulated expression of adhesion receptors and integrin-linked kinase in HT-144 melanoma cells correlates with their metastatic phenotype. Int. J. Cancer *83,* 255-262.

Jenei, V., Nystrom, M.L., and Thomas, G.J. (2011). Measuring invasion in an organotypic model. Methods Mol. Biol. *769,* 223-232.

Jiang, A., Lehti, K., Wang, X., Weiss, S.J., Keski-Oja, J., and Pei, D. (2001). Regulation of membrane-type matrix metalloproteinase 1 activity by dynamin-mediated endocytosis. Proc. Natl. Acad. Sci. U. S. A. *98,* 13693-13698.

Jiang, A., and Pei, D. (2003). Distinct roles of catalytic and pexin-like domains in membrane-type matrix metalloproteinase (MMP)-mediated pro-MMP-2 activation and collagenolysis. J. Biol. Chem. *278,* 38765-38771.

Jiang, W.G., Davies, G., Martin, T.A., Parr, C., Watkins, G., Mason, M.D., and Mansel, R.E. (2006). Expression of membrane type-1 matrix metalloproteinase, MT1-MMP in human breast cancer and its impact on invasiveness of breast cancer cells. Int. J. Mol. Med. *17,* 583-590.

Joyce, J.A., and Pollard, J.W. (2009). Microenvironmental regulation of metastasis. Nat. Rev. Cancer. *9,* 239-252.

Kaartinen, V., Voncken, J.W., Shuler, C., Warburton, D., Bu, D., Heisterkamp, N., and Groffen, J. (1995). Abnormal lung development and cleft palate in mice lacking TGF-beta 3 indicates defects of epithelial-mesenchymal interaction. Nat. Genet. *11,* 415-421.

Kadono, Y., Okada, Y., Namiki, M., Seiki, M., and Sato, H. (1998). Transformation of epithelial Madin-Darby canine kidney cells with p60(v-src) induces expression of membrane-type 1 matrix metalloproteinase and invasiveness. Cancer Res. *58,* 2240-2244.

Kahn, H.J., Bailey, D., and Marks, A. (2002). Monoclonal antibody D2-40, a new marker of lymphatic endothelium, reacts with Kaposi's sarcoma and a subset of angiosarcomas. Mod. Pathol. *15,* 434-440.

Kaipainen, A., Korhonen, J., Mustonen, T., van Hinsbergh, V.W., Fang, G.H., Dumont, D., Breitman, M., and Alitalo, K. (1995). Expression of the fms-like tyrosine kinase 4 gene becomes restricted to lymphatic endothelium during development. Proc. Natl. Acad. Sci. U. S. A. *92,* 3566-3570.

Kajita, M., Itoh, Y., Chiba, T., Mori, H., Okada, A., Kinoh, H., and Seiki, M. (2001). Membrane-type 1 matrix metalloproteinase cleaves CD44 and promotes cell migration. J. Cell Biol. *153,* 893-904.

Kalluri, R. (2003). Basement membranes: structure, assembly and role in tumour angiogenesis. Nat. Rev. Cancer. *3,* 422-433.

Kang, T., Yi, J., Yang, W., Wang, X., Jiang, A., and Pei, D. (2000). Functional characterization of MT3-MMP in transfected MDCK cells: progelatinase A activation and tubulogenesis in 3-D collagen lattice. Faseb j. *14,* 2559-2568.

Kantola, A.K., Keski-Oja, J., and Koli, K. (2008). Fibronectin and heparin binding domains of latent TGF-beta binding protein (LTBP)-4 mediate matrix targeting and cell adhesion. Exp. Cell Res. *314,* 2488-2500.

Kantola, A.K., Ryynänen, M.J., Lhota, F., Keski-Oja, J., and Koli, K. (2010). Independent regulation of short and long forms of latent TGF-beta binding protein (LTBP)-4 in cultured fibroblasts and human tissues. J. Cell. Physiol. *223,* 727-736.

Kanzaki, T., Olofsson, A., Moren, A., Wernstedt, C., Hellman, U., Miyazono, K., Claesson-Welsh, L., and Heldin, C.H. (1990). TGF-beta 1 binding protein: a component of the large latent complex of TGF-beta 1 with multiple repeat sequences. Cell *61,* 1051-1061.

Kashani-Sabet, M., Sagebiel, R.W., Ferreira, C.M., Nosrati, M., and Miller, J.R.,3rd. (2002). Tumor vascularity in the prognostic assessment of primary cutaneous melanoma. J. Clin. Oncol. *20,* 1826-1831.

Katz, M.E., and McCormick, F. (1997). Signal transduction from multiple Ras effectors. Curr. Opin. Genet. Dev. *7,* 75-79.

Kelly, J.W., Chamberlain, A.J., Staples, M.P., and McAvoy, B. (2003). Nodular melanoma. No longer as simple as ABC. Aust. Fam. Physician *32,* 706-709.

Kerjaschki, D., Bago-Horvath, Z., Rudas, M., Sexl, V., Schneckenleithner, C., Wolbank, S., Bartel, G., Krieger, S., Kalt, R., Hantusch, B.*, et al.* (2011). Lipoxygenase mediates invasion of intrametastatic lymphatic vessels and propagates lymph node metastasis of human mammary carcinoma xenografts in mouse. J. Clin. Invest. *121,* 2000-2012.

Kessenbrock, K., Plaks, V., and Werb, Z. (2010). Matrix metalloproteinases: regulators of the tumor microenvironment. Cell *141,* 52-67.

Kielty, C.M., Sherratt, M.J., and Shuttleworth, C.A. (2002). Elastic fibres. J. Cell. Sci. *115,* 2817-2828.

Kilpinen, S., Autio, R., Ojala, K., Iljin, K., Bucher, E., Sara, H., Pisto, T., Saarela, M., Skotheim, R.I., Björkman, M.*, et al.* (2008). Systematic bioinformatic analysis of expression levels of 17,330 human genes across 9,783 samples from 175 types of healthy and pathological tissues. Genome Biol. *9,* R139.

Knäuper, V., Cowell, S., Smith, B., Lopez-Otin, C., O'Shea, M., Morris, H., Zardi, L., and Murphy, G. (1997). The role of the C-terminal domain of human collagenase-3 (MMP-13) in the activation of procollagenase-3, substrate specificity, and tissue inhibitor of metalloproteinase interaction. J. Biol. Chem. *272,* 7608-7616.

Koli, K., and Keski-Oja, J. (1993). Vitamin D3 and calcipotriol enhance the secretion of transforming growth factor-beta 1 and -beta 2 in cultured murine keratinocytes. Growth Factors *8,* 153-163.

Koli, K., Saharinen, J., Karkkainen, M., and Keski-Oja, J. (2001). Novel non-TGF-beta-binding splice variant of LTBP-4 in human cells and tissues provides means to decrease TGF-beta deposition. J. Cell. Sci. *114,* 2869-2878.

Koski, C., Saharinen, J., and Keski-Oja, J. (1999). Independent promoters regulate the expression of two amino terminally distinct forms of latent transforming growth factor-beta binding protein-1 (LTBP-1) in a cell type-specific manner. J. Biol. Chem. *274,* 32619-32630.

Koziol, A., Gonzalo, P., Mota, A., Pollan, A., Lorenzo, C., Colome, N., Montaner, D., Dopazo, J., Arribas, J., Canals, F., and Arroyo, A.G. (2012). The protease MT1-MMP drives a combinatorial proteolytic program in activated endothelial cells. Faseb j. *26,* 4481-4494.

Krantz, S.B., Shields, M.A., Dangi-Garimella, S., Cheon, E.C., Barron, M.R., Hwang, R.F., Rao, M.S., Grippo, P.J., Bentrem, D.J., and Munshi, H.G. (2011). MT1-MMP cooperates with Kras(G12D) to promote pancreatic fibrosis through increased TGF-beta signaling. Mol. Cancer. Res. *9,* 1294-1304.

Krasagakis, K., Kruger-Krasagakes, S., Fimmel, S., Eberle, J., Tholke, D., von der Ohe, M., Mansmann, U., and Orfanos, C.E. (1999). Desensitization of melanoma cells to autocrine TGF-beta isoforms. J. Cell. Physiol. *178,* 179-187.

Kriehuber, E., Breiteneder-Geleff, S., Groeger, M., Soleiman, A., Schoppmann, S.F., Stingl, G., Kerjaschki, D., and Maurer, D. (2001). Isolation and characterization of dermal lymphatic and blood endothelial cells reveal stable and functionally specialized cell lineages. J. Exp. Med. *194,* 797-808.

Krubasik, D., Eisenach, P.A., Kunz-Schughart, L.A., Murphy, G., and English, W.R. (2008). Granulocyte-macrophage colony stimulating factor induces endothelial capillary formation through induction of membrane-type 1 matrix metalloproteinase expression in vitro. Int. J. Cancer *122,* 1261-1272.

Kulesa, P.M., Kasemeier-Kulesa, J.C., Teddy, J.M., Margaryan, N.V., Seftor, E.A., Seftor, R.E., and Hendrix, M.J. (2006). Reprogramming metastatic melanoma cells to assume a neural crest cell-like phenotype in an embryonic microenvironment. Proc. Natl. Acad. Sci. U. S. A. *103,* 3752-3757.

Kulkarni, A.B., Huh, C.G., Becker, D., Geiser, A., Lyght, M., Flanders, K.C., Roberts, A.B., Sporn, M.B., Ward, J.M., and Karlsson, S. (1993). Transforming growth factor beta 1 null mutation in mice causes excessive inflammatory response and early death. Proc. Natl. Acad. Sci. U. S. A. *90,* 770-774.

Kwak, J.H., Woo, J.S., Shin, K., Kim, H.J., Jeong, H.S., Han, D.C., Kim, S.I., and Park, C.S. (2005). Expression and regulation of latent TGF-beta binding protein-1 transcripts and their splice variants in human glomerular endothelial cells. J. Korean Med. Sci. *20,* 628-635.

Laakkonen, P., Waltari, M., Holopainen, T., Takahashi, T., Pytowski, B., Steiner, P., Hicklin, D., Persaud, K., Tonra, J.R., Witte, L., and Alitalo, K. (2007). Vascular endothelial growth factor receptor 3 is involved in tumor angiogenesis and growth. Cancer Res. *67,* 593-599.

Laakkonen, P., Zhang, L., and Ruoslahti, E. (2008). Peptide targeting of tumor lymph vessels. Ann. N. Y. Acad. Sci. *1131,* 37-43.

Lafleur, M.A., Handsley, M.M., Knäuper, V., Murphy, G., and Edwards, D.R. (2002). Endothelial tubulogenesis within fibrin gels specifically requires the activity of membrane-type-matrix metalloproteinases (MT-MMPs). J. Cell. Sci. *115,* 3427-3438.

Lallas, A., Apalla, Z., and Chaidemenos, G. (2012). New trends in dermoscopy to minimize the risk of missing melanoma. J. Skin Cancer. *2012,* 820474.

Lapiere, J.C., Woodley, D.T., Parente, M.G., Iwasaki, T., Wynn, K.C., Christiano, A.M., and Uitto, J. (1993). Epitope mapping of type VII collagen. Identification of discrete peptide sequences recognized by sera from patients with acquired epidermolysis bullosa. J. Clin. Invest. *92*, 1831-1839.

Lauffenburger, D.A., and Horwitz, A.F. (1996). Cell migration: a physically integrated molecular process. Cell *84*, 359-369.

Leco, K.J., Waterhouse, P., Sanchez, O.H., Gowing, K.L., Poole, A.R., Wakeham, A., Mak, T.W., and Khokha, R. (2001). Spontaneous air space enlargement in the lungs of mice lacking tissue inhibitor of metalloproteinases-3 (TIMP-3). J. Clin. Invest. *108*, 817-829.

Lee, J.H., Choi, J.W., and Kim, Y.S. (2010). Frequencies of BRAF and NRAS mutations are different in histologic types and sites of origin of cutaneous melanoma: a meta-analysis. Br. J. Dermatol.

Lehti, K., Allen, E., Birkedal-Hansen, H., Holmbeck, K., Miyake, Y., Chun, T.H., and Weiss, S.J. (2005). An MT1-MMP-PDGF receptor-beta axis regulates mural cell investment of the microvasculature. Genes Dev. *19*, 979-991.

Lehti, K., Lohi, J., Juntunen, M.M., Pei, D., and Keski-Oja, J. (2002). Oligomerization through hemopexin and cytoplasmic domains regulates the activity and turnover of membrane-type 1 matrix metalloproteinase. J. Biol. Chem. *277*, 8440-8448.

Lehti, K., Lohi, J., Valtanen, H., and Keski-Oja, J. (1998). Proteolytic processing of membrane-type-1 matrix metalloproteinase is associated with gelatinase A activation at the cell surface. Biochem. J. *334 (Pt 2)*, 345-353.

Lehti, K., Valtanen, H., Wickstrom, S.A., Lohi, J., and Keski-Oja, J. (2000). Regulation of membrane-type-1 matrix metalloproteinase activity by its cytoplasmic domain. J. Biol. Chem. *275*, 15006-15013.

Lens, M.B., Dawes, M., Newton-Bishop, J.A., and Goodacre, T. (2002). Tumour thickness as a predictor of occult lymph node metastases in patients with stage I and II melanoma undergoing sentinel lymph node biopsy. Br. J. Surg. *89*, 1223-1227.

Leong, S.P., Nakakura, E.K., Pollock, R., Choti, M.A., Morton, D.L., Henner, W.D., Lal, A., Pillai, R., Clark, O.H., and Cady, B. (2011). Unique patterns of metastases in common and rare types of malignancy. J. Surg. Oncol. *103*, 607-614.

Levental, K.R., Yu, H., Kass, L., Lakins, J.N., Egeblad, M., Erler, J.T., Fong, S.F., Csiszar, K., Giaccia, A., Weninger, W.*, et al.* (2009). Matrix crosslinking forces tumor progression by enhancing integrin signaling. Cell *139*, 891-906.

Lewalle, J.M., Munaut, C., Pichot, B., Cataldo, D., Baramova, E., and Foidart, J.M. (1995). Plasma membrane-dependent activation of gelatinase A in human vascular endothelial cells. J. Cell. Physiol. *165*, 475-483.

Li, X.Y., Ota, I., Yana, I., Sabeh, F., and Weiss, S.J. (2008). Molecular dissection of the structural machinery underlying the tissue-invasive activity of membrane type-1 matrix metalloproteinase. Mol. Biol. Cell *19*, 3221-3233.

Lin, E.Y., Nguyen, A.V., Russell, R.G., and Pollard, J.W. (2001). Colony-stimulating factor 1 promotes progression of mammary tumors to malignancy. J. Exp. Med. *193*, 727-740.

Lin, F., Wang, X., Jie, Z., Hong, X., Li, X., Wang, M., and Yu, Y. (2011). Inhibitory effects of miR-146b-5p on cell migration and invasion of pancreatic cancer by targeting MMP16. J. Huazhong Univ. Sci. Technolog Med. Sci. *31*, 509-514.

Llano, E., Pendas, A.M., Freije, J.P., Nakano, A., Knäuper, V., Murphy, G., and Lopez-Otin, C. (1999). Identification and characterization of human MT5-MMP, a new membrane-bound activator of progelatinase a overexpressed in brain tumors. Cancer Res. *59*, 2570-2576.

Löffek, S., Zigrino, P., Angel, P., Anwald, B., Krieg, T., and Mauch, C. (2005). High invasive melanoma cells induce matrix metalloproteinase-1 synthesis in fibroblasts by interleukin-1alpha and basic fibroblast growth factor-mediated mechanisms. J. Invest. Dermatol. *124*, 638-643.

Löffek, S., Zigrino, P., Steiger, J., Kurschat, P., Smola, H., and Mauch, C. (2006). Melanoma cell-derived vascular endothelial growth factor induces endothelial tubulogenesis within fibrin gels by a metalloproteinase-mediated mechanism. Eur. J. Cell Biol. *85*, 1167-1177.

Lohi, J., Lehti, K., Valtanen, H., Parks, W.C., and Keski-Oja, J. (2000). Structural analysis and promoter characterization of the human membrane-type matrix metalloproteinase-1 (MT1-MMP) gene. Gene *242*, 75-86.

Lohi, J., Lehti, K., Westermarck, J., Kähäri, V.M., and Keski-Oja, J. (1996). Regulation of membrane-type matrix metalloproteinase-1 expression by growth factors and phorbol 12-myristate 13-acetate. Eur. J. Biochem. *239*, 239-247.

Lopez, J.I., Kang, I., You, W.K., McDonald, D.M., and Weaver, V.M. (2011). In situ force mapping of mammary gland transformation. Integr. Biol. (Camb) *3*, 910-921.

Lowy, A.M., Clements, W.M., Bishop, J., Kong, L., Bonney, T., Sisco, K., Aronow, B., Fenoglio-Preiser, C., and Groden, J. (2006). beta-Catenin/Wnt signaling regulates expression of the membrane type 3 matrix metalloproteinase in gastric cancer. Cancer Res. *66*, 4734-4741.

Lu, P., Weaver, V.M., and Werb, Z. (2012). The extracellular matrix: a dynamic niche in cancer progression. J. Cell Biol. *196,* 395-406.

Mäkitie, T., Summanen, P., Tarkkanen, A., and Kivelä, T. (2001). Tumor-infiltrating macrophages (CD68(+) cells) and prognosis in malignant uveal melanoma. Invest. Ophthalmol. Vis. Sci. *42,* 1414-1421.

Makris, G., Wright, J.D., Ingham, E., and Holland, K.T. (2004). The hyaluronate lyase of Staphylococcus aureus - a virulence factor? Microbiology *150,* 2005-2013.

Malik, G., Knowles, L.M., Dhir, R., Xu, S., Yang, S., Ruoslahti, E., and Pilch, J. (2010). Plasma fibronectin promotes lung metastasis by contributions to fibrin clots and tumor cell invasion. Cancer Res. *70,* 4327-4334.

Manka, S.W., Carafoli, F., Visse, R., Bihan, D., Raynal, N., Farndale, R.W., Murphy, G., Enghild, J.J., Hohenester, E., and Nagase, H. (2012). Structural insights into triple-helical collagen cleavage by matrix metalloproteinase 1. Proc. Natl. Acad. Sci. U. S. A. *109,* 12461-12466.

Marks, M.S., and Seabra, M.C. (2001). The melanosome: membrane dynamics in black and white. Nat. Rev. Mol. Cell Biol. *2,* 738-748.

Massague, J. (2012). TGFbeta signalling in context. Nat. Rev. Mol. Cell Biol. *13,* 616-630.

Massague, J., Czech, M.P., Iwata, K., DeLarco, J.E., and Todaro, G.J. (1982). Affinity labeling of a transforming growth factor receptor that does not interact with epidermal growth factor. Proc. Natl. Acad. Sci. U. S. A. *79,* 6822-6826.

Massi, D., Puig, S., Franchi, A., Malvehy, J., Vidal-Sicart, S., Gonzalez-Cao, M., Baroni, G., Ketabchi, S., Palou, J., and Santucci, M. (2006). Tumour lymphangiogenesis is a possible predictor of sentinel lymph node status in cutaneous melanoma: a case-control study. J. Clin. Pathol. *59,* 166-173.

Matsumoto, S., Katoh, M., Saito, S., Watanabe, T., and Masuho, Y. (1997). Identification of soluble type of membrane-type matrix metalloproteinase-3 formed by alternatively spliced mRNA. Biochim. Biophys. Acta *1354,* 159-170.

Mazzone, M., Baldassarre, M., Beznoussenko, G., Giacchetti, G., Cao, J., Zucker, S., Luini, A., and Buccione, R. (2004). Intracellular processing and activation of membrane type 1 matrix metalloprotease depends on its partitioning into lipid domains. J. Cell. Sci. *117,* 6275-6287.

McAllister, K.A., Grogg, K.M., Johnson, D.W., Gallione, C.J., Baldwin, M.A., Jackson, C.E., Helmbold, E.A., Markel, D.S., McKinnon, W.C., and Murrell, J. (1994). Endoglin, a TGF-beta binding protein of endothelial cells, is the gene for hereditary haemorrhagic telangiectasia type 1. Nat. Genet. *8,* 345-351.

McCawley, L.J., Crawford, H.C., King, L.E.,Jr, Mudgett, J., and Matrisian, L.M. (2004). A protective role for matrix metalloproteinase-3 in squamous cell carcinoma. Cancer Res. *64,* 6965-6972.

McCawley, L.J., and Matrisian, L.M. (2001). Matrix metalloproteinases: they're not just for matrix anymore! Curr. Opin. Cell Biol. *13,* 534-540.

McCormack, V.A., and dos Santos Silva, I. (2006). Breast density and parenchymal patterns as markers of breast cancer risk: a meta-analysis. Cancer Epidemiol. Biomarkers Prev. *15,* 1159-1169.

Mehnert, J.M., and Kluger, H.M. (2012). Driver mutations in melanoma: lessons learned from bench-to-bedside studies. Curr. Oncol. Rep. *14,* 449-457.

Michaloglou, C., Vredeveld, L.C., Soengas, M.S., Denoyelle, C., Kuilman, T., van der Horst, C.M., Majoor, D.M., Shay, J.W., Mooi, W.J., and Peeper, D.S. (2005). BRAFE600-associated senescence-like cell cycle arrest of human naevi. Nature *436,* 720-724.

Migita, K., Eguchi, K., Kawabe, Y., Ichinose, Y., Tsukada, T., Aoyagi, T., Nakamura, H., and Nagataki, S. (1996). TNF-alpha-mediated expression of membrane-type matrix metalloproteinase in rheumatoid synovial fibroblasts. Immunology *89,* 553-557.

Mimori, K., Fukagawa, T., Kosaka, Y., Ishikawa, K., Iwatsuki, M., Yokobori, T., Hirasaki, S., Takatsuno, Y., Sakashita, H., Ishii, H., Sasako, M., and Mori, M. (2008). A Large-Scale Study of MT1-MMP as a Marker for Isolated Tumor Cells in Peripheral Blood and Bone Marrow in Gastric Cancer Cases. Ann. Surg. Oncol.

Miner, J.H. (2008). Laminins and their roles in mammals. Microsc. Res. Tech. *71,* 349-356.

Miyazono, K., Hellman, U., Wernstedt, C., and Heldin, C.H. (1988). Latent high molecular weight complex of transforming growth factor beta 1. Purification from human platelets and structural characterization. J. Biol. Chem. *263,* 6407-6415.

Miyazono, K., Olofsson, A., Colosetti, P., and Heldin, C.H. (1991). A role of the latent TGF-beta 1-binding protein in the assembly and secretion of TGF-beta 1. Embo j. *10,* 1091-1101.

Mohammed, R.A., Ellis, I.O., Elsheikh, S., Paish, E.C., and Martin, S.G. (2009). Lymphatic and angiogenic characteristics in breast cancer: morphometric analysis and prognostic implications. Breast Cancer Res. Treat. *113,* 261-273.

Monaco, S., Gioia, M., Rodriguez, J., Fasciglione, G.F., Di Pierro, D., Lupidi, G., Krippahl, L., Marini, S., and Coletta, M. (2007). Modulation of the proteolytic activity of matrix metalloproteinase-2 (gelatinase A) on fibrinogen. Biochem. J. *402,* 503-513.

Monea, S., Lehti, K., Keski-Oja, J., and Mignatti, P. (2002). Plasmin activates pro-matrix metalloproteinase-2 with a membrane-type 1 matrix metalloproteinase-dependent mechanism. J. Cell. Physiol. *192,* 160-170.

Montesano, R., Orci, L., and Vassalli, P. (1985). Human endothelial cell cultures: phenotypic modulation by leukocyte interleukins. J. Cell. Physiol. *122,* 424-434.

Montesano, R., Soriano, J.V., Hosseini, G., Pepper, M.S., and Schramek, H. (1999). Constitutively active mitogen-activated protein kinase kinase MEK1 disrupts morphogenesis and induces an invasive phenotype in Madin-Darby canine kidney epithelial cells. Cell Growth Differ. *10,* 317-332.

Mori, H., Tomari, T., Koshikawa, N., Kajita, M., Itoh, Y., Sato, H., Tojo, H., Yana, I., and Seiki, M. (2002). CD44 directs membrane-type 1 matrix metalloproteinase to lamellipodia by associating with its hemopexin-like domain. Embo j. *21,* 3949-3959.

Morris, M.J., and Basu, S. (2009). An unusually stable G-quadruplex within the 5'-UTR of the MT3 matrix metalloproteinase mRNA represses translation in eukaryotic cells. Biochemistry *48,* 5313-5319.

Morrison, C.J., Butler, G.S., Rodriguez, D., and Overall, C.M. (2009). Matrix metalloproteinase proteomics: substrates, targets, and therapy. Curr. Opin. Cell Biol. *21,* 645-653.

Morton, D.L., Thompson, J.F., Cochran, A.J., Mozzillo, N., Elashoff, R., Essner, R., Nieweg, O.E., Roses, D.F., Hoekstra, H.J., Karakousis, C.P.*, et al.* (2006). Sentinel-node biopsy or nodal observation in melanoma. N. Engl. J. Med. *355,* 1307-1317.

Morton, D.L., Wen, D.R., Wong, J.H., Economou, J.S., Cagle, L.A., Storm, F.K., Foshag, L.J., and Cochran, A.J. (1992). Technical details of intraoperative lymphatic mapping for early stage melanoma. Arch. Surg. *127,* 392-399.

Moss, N.M., Liu, Y., Johnson, J.J., Debiase, P., Jones, J., Hudson, L.G., Munshi, H.G., and Stack, M.S. (2009). Epidermal growth factor receptor-mediated membrane type 1 matrix metalloproteinase endocytosis regulates the transition between invasive versus expansive growth of ovarian carcinoma cells in three-dimensional collagen. Mol. Cancer. Res. *7,* 809-820.

Moussai, D., Mitsui, H., Pettersen, J.S., Pierson, K.C., Shah, K.R., Suarez-Farinas, M., Cardinale, I.R., Bluth, M.J., Krueger, J.G., and Carucci, J.A. (2011). The human cutaneous squamous cell carcinoma microenvironment is characterized by increased lymphatic density and enhanced expression of macrophage-derived VEGF-C. J. Invest. Dermatol. *131,* 229-236.

Müller, A., Homey, B., Soto, H., Ge, N., Catron, D., Buchanan, M.E., McClanahan, T., Murphy, E., Yuan, W., Wagner, S.N.*, et al.* (2001). Involvement of chemokine receptors in breast cancer metastasis. Nature *410,* 50-56.

Munshi, H.G., and Stack, M.S. (2006). Reciprocal interactions between adhesion receptor signaling and MMP regulation. Cancer Metastasis Rev. *25,* 45-56.

Myers, C., Charboneau, A., Cheung, I., Hanks, D., and Boudreau, N. (2002). Sustained expression of homeobox D10 inhibits angiogenesis. Am. J. Pathol. *161,* 2099-2109.

Myllyharju, J., and Kivirikko, K.I. (2004). Collagens, modifying enzymes and their mutations in humans, flies and worms. Trends Genet. *20,* 33-43.

Nagase, H. (1997). Activation mechanisms of matrix metalloproteinases. Biol. Chem. *378,* 151-160.

Nakada, M., Nakamura, H., Ikeda, E., Fujimoto, N., Yamashita, J., Sato, H., Seiki, M., and Okada, Y. (1999). Expression and tissue localization of membrane-type 1, 2, and 3 matrix metalloproteinases in human astrocytic tumors. Am. J. Pathol. *154,* 417-428.

Nakahara, H., Howard, L., Thompson, E.W., Sato, H., Seiki, M., Yeh, Y., and Chen, W.T. (1997). Transmembrane/cytoplasmic domain-mediated membrane type 1-matrix metalloprotease docking to invadopodia is required for cell invasion. Proc. Natl. Acad. Sci. U. S. A. *94,* 7959-7964.

Nakamura, Y., Yasuoka, H., Tsujimoto, M., Imabun, S., Nakahara, M., Nakao, K., Nakamura, M., Mori, I., and Kakudo, K. (2005). Lymph vessel density correlates with nodal status, VEGF-C expression, and prognosis in breast cancer. Breast Cancer Res. Treat. *91,* 125-132.

Neptune, E.R., Frischmeyer, P.A., Arking, D.E., Myers, L., Bunton, T.E., Gayraud, B., Ramirez, F., Sakai, L.Y., and Dietz, H.C. (2003). Dysregulation of TGF-beta activation contributes to pathogenesis in Marfan syndrome. Nat. Genet. *33,* 407-411.

Niakosari, F., Kahn, H.J., McCready, D., Ghazarian, D., Rotstein, L.E., Marks, A., Kiss, A., and From, L. (2008). Lymphatic invasion identified by monoclonal antibody D2-40, younger age, and ulceration: predictors of sentinel lymph node involvement in primary cutaneous melanoma. Arch. Dermatol. *144,* 462-467.

Nie, J., and Pei, D. (2003). Direct activation of pro-matrix metalloproteinase-2 by leukolysin/membrane-type 6 matrix metalloproteinase/matrix metalloproteinase 25 at the asn(109)-Tyr bond. Cancer Res. *63,* 6758-6762.

Nomura, H., Sato, H., Seiki, M., Mai, M., and Okada, Y. (1995). Expression of membrane-type matrix metalloproteinase in human gastric carcinomas. Cancer Res. *55,* 3263-3266.

Nothnick, W.B. (2001). Reduction in reproductive lifespan of tissue inhibitor of metalloproteinase 1 (TIMP-1)-deficient female mice. Reproduction *122,* 923-927.

Nurmenniemi, S., Sinikumpu, T., Alahuhta, I., Salo, S., Sutinen, M., Santala, M., Risteli, J., Nyberg, P., and Salo, T. (2009). A novel organotypic model mimics the tumor microenvironment. Am. J. Pathol. *175,* 1281-1291.

Nyalendo, C., Beaulieu, E., Sartelet, H., Michaud, M., Fontaine, N., Gingras, D., and Beliveau, R. (2008). Impaired tyrosine phosphorylation of membrane type 1-matrix metalloproteinase reduces tumor cell proliferation in three-dimensional matrices and abrogates tumor growth in mice. Carcinogenesis *29,* 1655-1664.

Nyalendo, C., Michaud, M., Beaulieu, E., Roghi, C., Murphy, G., Gingras, D., and Beliveau, R. (2007). Src-dependent phosphorylation of membrane type I matrix metalloproteinase on cytoplasmic tyrosine 573: role in endothelial and tumor cell migration. J. Biol. Chem. *282,* 15690-15699.

Nyberg, P., Xie, L., Sugimoto, H., Colorado, P., Sund, M., Holthaus, K., Sudhakar, A., Salo, T., and Kalluri, R. (2008). Characterization of the anti-angiogenic properties of arresten, an alpha1beta1 integrin-dependent collagen-derived tumor suppressor. Exp. Cell Res. *314,* 3292-3305.

Ohnishi, Y., Tajima, S., and Ishibashi, A. (2001). Coordinate expression of membrane type-matrix metalloproteinases-2 and 3 (MT2-MMP and MT3-MMP) and matrix metalloproteinase-2 (MMP-2) in primary and metastatic melanoma cells. Eur. J. Dermatol. *11,* 420-423.

Okada, A., Bellocq, J.P., Rouyer, N., Chenard, M.P., Rio, M.C., Chambon, P., and Basset, P. (1995). Membrane-type matrix metalloproteinase (MT-MMP) gene is expressed in stromal cells of human colon, breast, and head and neck carcinomas. Proc. Natl. Acad. Sci. U. S. A. *92,* 2730-2734.

Okada, A., Tomasetto, C., Lutz, Y., Bellocq, J.P., Rio, M.C., and Basset, P. (1997). Expression of matrix metalloproteinases during rat skin wound healing: evidence that membrane type-1 matrix metalloproteinase is a stromal activator of pro-gelatinase A. J. Cell Biol. *137,* 67-77.

Okumura, Y., Sato, H., Seiki, M., and Kido, H. (1997). Proteolytic activation of the precursor of membrane type 1 matrix metalloproteinase by human plasmin. A possible cell surface activator. FEBS Lett. *402,* 181-184.

Olofsson, A., Ichijo, H., Moren, A., ten Dijke, P., Miyazono, K., and Heldin, C.H. (1995). Efficient association of an amino-terminally extended form of human latent transforming growth factor-beta binding protein with the extracellular matrix. J. Biol. Chem. *270,* 31294-31297.

Olofsson, A., Miyazono, K., Kanzaki, T., Colosetti, P., Engstrom, U., and Heldin, C.H. (1992). Transforming growth factor-beta 1, -beta 2, and -beta 3 secreted by a human glioblastoma cell line. Identification of small and different forms of large latent complexes. J. Biol. Chem. *267,* 19482-19488.

O'Reilly, M.S., Boehm, T., Shing, Y., Fukai, N., Vasios, G., Lane, W.S., Flynn, E., Birkhead, J.R., Olsen, B.R., and Folkman, J. (1997). Endostatin: an endogenous inhibitor of angiogenesis and tumor growth. Cell *88,* 277-285.

Ortega, N., and Werb, Z. (2002). New functional roles for non-collagenous domains of basement membrane collagens. J. Cell. Sci. *115,* 4201-4214.

Ota, I., Li, X.Y., Hu, Y., and Weiss, S.J. (2009). Induction of a MT1-MMP and MT2-MMP-dependent basement membrane transmigration program in cancer cells by Snail1. Proc. Natl. Acad. Sci. U. S. A. *106,* 20318-20323.

Overall, C.M., and Kleifeld, O. (2006). Tumour microenvironment - opinion: validating matrix metalloproteinases as drug targets and anti-targets for cancer therapy. Nat. Rev. Cancer. *6,* 227-239.

Padera, T.P., Kadambi, A., di Tomaso, E., Carreira, C.M., Brown, E.B., Boucher, Y., Choi, N.C., Mathisen, D., Wain, J., Mark, E.J., Munn, L.L., and Jain, R.K. (2002). Lymphatic metastasis in the absence of functional intratumor lymphatics. Science *296,* 1883-1886.

Palumbo, J.S., Potter, J.M., Kaplan, L.S., Talmage, K., Jackson, D.G., and Degen, J.L. (2002). Spontaneous hematogenous and lymphatic metastasis, but not primary tumor growth or angiogenesis, is diminished in fibrinogen-deficient mice. Cancer Res. *62,* 6966-6972.

Pasonen-Seppänen, S., Takabe, P., Edward, M., Rauhala, L., Rilla, K., Tammi, M., and Tammi, R. (2012). Melanoma cell-derived factors stimulate hyaluronan synthesis in dermal fibroblasts by upregulating HAS2 through PDGFR-PI3K-AKT and p38 signaling. Histochem. Cell Biol. *138,* 895-911.

Patwari, P., Gao, G., Lee, J.H., Grodzinsky, A.J., and Sandy, J.D. (2005). Analysis of ADAMTS4 and MT4-MMP indicates that both are involved in aggrecanolysis in interleukin-1-treated bovine cartilage. Osteoarthritis Cartilage *13,* 269-277.

Pearson, G., Robinson, F., Beers Gibson, T., Xu, B.E., Karandikar, M., Berman, K., and Cobb, M.H. (2001). Mitogen-activated protein (MAP) kinase pathways: regulation and physiological functions. Endocr. Rev. *22,* 153-183.

Pei, D. (1999). Leukolysin/MMP25/MT6-MMP: a novel matrix metalloproteinase specifically expressed in the leukocyte lineage. Cell Res. *9,* 291-303.

Pei, D., Kang, T., and Qi, H. (2000). Cysteine array matrix metalloproteinase (CA-MMP)/MMP-23 is a type II transmembrane matrix metalloproteinase regulated by a single cleavage for both secretion and activation. J. Biol. Chem. *275,* 33988-33997.

Pencheva, N., Tran, H., Buss, C., Huh, D., Drobnjak, M., Busam, K., and Tavazoie, S.F. (2012). Convergent Multi-miRNA Targeting of ApoE Drives LRP1/LRP8-Dependent Melanoma Metastasis and Angiogenesis. Cell *151,* 1068-1082.

Penttinen, C., Saharinen, J., Weikkolainen, K., Hyytiäinen, M., and Keski-Oja, J. (2002). Secretion of human latent TGF-beta-binding protein-3 (LTBP-3) is dependent on co-expression of TGF-beta. J. Cell. Sci. *115,* 3457-3468.

Perentes, J.Y., Kirkpatrick, N.D., Nagano, S., Smith, E.Y., Shaver, C.M., Sgroi, D., Garkavtsev, I., Munn, L.L., Jain, R.K., and Boucher, Y. (2011). Cancer cell-associated MT1-MMP promotes blood vessel invasion and distant metastasis in triple-negative mammary tumors. Cancer Res. *71,* 4527-4538.

Petersen, O.W., Nielsen, H.L., Gudjonsson, T., Villadsen, R., Rank, F., Niebuhr, E., Bissell, M.J., and Ronnov-Jessen, L. (2003). Epithelial to mesenchymal transition in human breast cancer can provide a nonmalignant stroma. Am. J. Pathol. *162,* 391-402.

Petersson, F., Diwan, A.H., Ivan, D., Gershenwald, J.E., Johnson, M.M., Harrell, R., and Prieto, V.G. (2009). Immunohistochemical detection of lymphovascular invasion with D2-40 in melanoma correlates with sentinel lymph node status, metastasis and survival. J. Cutan. Pathol. *36,* 1157-1163.

Petitclerc, E., Boutaud, A., Prestayko, A., Xu, J., Sado, Y., Ninomiya, Y., Sarras, M.P.,Jr, Hudson, B.G., and Brooks, P.C. (2000). New functions for non-collagenous domains of human collagen type IV. Novel integrin ligands inhibiting angiogenesis and tumor growth in vivo. J. Biol. Chem. *275,* 8051-8061.

Petrie, R.J., Gavara, N., Chadwick, R.S., and Yamada, K.M. (2012). Nonpolarized signaling reveals two distinct modes of 3D cell migration. J. Cell Biol. *197,* 439-455.

Plaisier, M., Kapiteijn, K., Koolwijk, P., Fijten, C., Hanemaaijer, R., Grimbergen, J.M., Mulder-Stapel, A., Quax, P.H., Helmerhorst, F.M., and van Hinsbergh, V.W. (2004). Involvement of membrane-type matrix metalloproteinases (MT-MMPs) in capillary tube formation by human endometrial microvascular endothelial cells: role of MT3-MMP. J. Clin. Endocrinol. Metab. *89,* 5828-5836.

Platz, A., Egyhazi, S., Ringborg, U., and Hansson, J. (2008). Human cutaneous melanoma; a review of NRAS and BRAF mutation frequencies in relation to histogenetic subclass and body site. Mol. Oncol. *1,* 395-405.

Poincloux, R., Lizarraga, F., and Chavrier, P. (2009). Matrix invasion by tumour cells: a focus on MT1-MMP trafficking to invadopodia. J. Cell. Sci. *122,* 3015-3024.

Polyak, K., and Weinberg, R.A. (2009). Transitions between epithelial and mesenchymal states: acquisition of malignant and stem cell traits. Nat. Rev. Cancer. *9,* 265-273.

Postovit, L.M., Seftor, E.A., Seftor, R.E., and Hendrix, M.J. (2006). Influence of the microenvironment on melanoma cell fate determination and phenotype. Cancer Res. *66,* 7833-7836.

Prevo, R., Banerji, S., Ferguson, D.J., Clasper, S., and Jackson, D.G. (2001). Mouse LYVE-1 is an endocytic receptor for hyaluronan in lymphatic endothelium. J. Biol. Chem. *276,* 19420-19430.

Provenzano, P.P., Inman, D.R., Eliceiri, K.W., Knittel, J.G., Yan, L., Rueden, C.T., White, J.G., and Keely, P.J. (2008). Collagen density promotes mammary tumor initiation and progression. BMC Med. *6,* 11-7015-6-11.

Prudova, A., auf dem Keller, U., Butler, G.S., and Overall, C.M. (2010). Multiplex N-terminome analysis of MMP-2 and MMP-9 substrate degradomes by iTRAQ-TAILS quantitative proteomics. Mol. Cell. Proteomics *9,* 894-911.

Quan, T., Wang, F., Shao, Y., Rittie, L., Xia, W., Orringer, J.S., Voorhees, J.J., and Fisher, G.J. (2012). Enhancing Structural Support of the Dermal Microenvironment Activates Fibroblasts, Endothelial Cells, and Keratinocytes in Aged Human Skin In Vivo. J. Invest. Dermatol.

Radisky, E.S., and Radisky, D.C. (2010). Matrix metalloproteinase-induced epithelial-mesenchymal transition in breast cancer. J. Mammary Gland Biol. Neoplasia *15,* 201-212.

Rager, E.L., Bridgeford, E.P., and Ollila, D.W. (2005). Cutaneous melanoma: update on prevention, screening, diagnosis, and treatment. Am. Fam. Physician *72,* 269-276.

Ramchandran, R., Dhanabal, M., Volk, R., Waterman, M.J., Segal, M., Lu, H., Knebelmann, B., and Sukhatme, V.P. (1999). Antiangiogenic activity of restin, NC10 domain of human collagen XV: comparison to endostatin. Biochem. Biophys. Res. Commun. *255,* 735-739.

Ran, S., Volk, L., Hall, K., and Flister, M.J. (2010). Lymphangiogenesis and lymphatic metastasis in breast cancer. Pathophysiology *17,* 229-251.

Ratnikov, B.I., Rozanov, D.V., Postnova, T.I., Baciu, P.G., Zhang, H., DiScipio, R.G., Chestukhina, G.G., Smith, J.W., Deryugina, E.I., and Strongin, A.Y. (2002). An alternative processing of integrin alpha(v) subunit in tumor cells by membrane type-1 matrix metalloproteinase. J. Biol. Chem. *277,* 7377-7385.

Raub, C.B., Suresh, V., Krasieva, T., Lyubovitsky, J., Mih, J.D., Putnam, A.J., Tromberg, B.J., and George, S.C. (2007). Noninvasive assessment of collagen gel microstructure and mechanics using multiphoton microscopy. Biophys. J. *92,* 2212-2222.

Raub, C.B., Unruh, J., Suresh, V., Krasieva, T., Lindmo, T., Gratton, E., Tromberg, B.J., and George, S.C. (2008). Image correlation spectroscopy of multiphoton images correlates with collagen mechanical properties. Biophys. J. *94,* 2361-2373.

Reed, J.A., McNutt, N.S., Prieto, V.G., and Albino, A.P. (1994). Expression of transforming growth factor-beta 2 in malignant melanoma correlates with the depth of tumor invasion. Implications for tumor progression. Am. J. Pathol. *145,* 97-104.

Reinke, J.M., and Sorg, H. (2012). Wound repair and regeneration. Eur. Surg. Res. *49,* 35-43.

Remacle, A., Murphy, G., and Roghi, C. (2003). Membrane type I-matrix metalloproteinase (MT1-MMP) is internalised by two different pathways and is recycled to the cell surface. J. Cell. Sci. *116,* 3905-3916.

Ribatti, D., Nico, B., Cimpean, A.M., Raica, M., Crivellato, E., Ruggieri, S., and Vacca, A. (2012). B16-F10 melanoma cells contribute to the new formation of blood vessels in the chick embryo chorioallantoic membrane through vasculogenic mimicry. Clin. Exp. Med.

Ribeiro, S.M., Poczatek, M., Schultz-Cherry, S., Villain, M., and Murphy-Ullrich, J.E. (1999). The activation sequence of thrombospondin-1 interacts with the latency-associated peptide to regulate activation of latent transforming growth factor-beta. J. Biol. Chem. *274,* 13586-13593.

Riker, A.I., Enkemann, S.A., Fodstad, O., Liu, S., Ren, S., Morris, C., Xi, Y., Howell, P., Metge, B., Samant, R.S.*, et al.* (2008). The gene expression profiles of primary and metastatic melanoma yields a transition point of tumor progression and metastasis. BMC Med. Genomics *1,* 13.

Robinson, P.N., and Godfrey, M. (2000). The molecular genetics of Marfan syndrome and related microfibrillopathies. J. Med. Genet. *37,* 9-25.

Rodeck, U., Nishiyama, T., and Mauviel, A. (1999). Independent regulation of growth and SMAD-mediated transcription by transforming growth factor beta in human melanoma cells. Cancer Res. *59,* 547-550.

Roeb, E., Schleinkofer, K., Kernebeck, T., Potsch, S., Jansen, B., Behrmann, I., Matern, S., and Grotzinger, J. (2002). The matrix metalloproteinase 9 (mmp-9) hemopexin domain is a novel gelatin binding domain and acts as an antagonist. J. Biol. Chem. *277,* 50326-50332.

Rose, A.E., Christos, P.J., Lackaye, D., Shapiro, R.L., Berman, R., Mazumdar, M., Kamino, H., Osman, I., and Darvishian, F. (2011). Clinical relevance of detection of lymphovascular invasion in primary melanoma using endothelial markers D2-40 and CD34. Am. J. Surg. Pathol. *35,* 1441-1449.

Rozanov, D.V., Hahn-Dantona, E., Strickland, D.K., and Strongin, A.Y. (2004). The low density lipoprotein receptor-related protein LRP is regulated by membrane type-1 matrix metalloproteinase (MT1-MMP) proteolysis in malignant cells. J. Biol. Chem. *279,* 4260-4268.

Ruoslahti, E., and Vaheri, A. (1974). Novel human serum protein from fibroblast plasma membrane. Nature *248,* 789-791.

Ryynanen, M., Knowlton, R.G., Parente, M.G., Chung, L.C., Chu, M.L., and Uitto, J. (1991). Human type VII collagen: genetic linkage of the gene (COL7A1) on chromosome 3 to dominant dystrophic epidermolysis bullosa. Am. J. Hum. Genet. *49,* 797-803.

Sabeh, F., Ota, I., Holmbeck, K., Birkedal-Hansen, H., Soloway, P., Balbin, M., Lopez-Otin, C., Shapiro, S., Inada, M., Krane, S.*, et al.* (2004). Tumor cell traffic through the extracellular matrix is controlled by the membrane-anchored collagenase MT1-MMP. J. Cell Biol. *167,* 769-781.

Sabeh, F., Shimizu-Hirota, R., and Weiss, S.J. (2009). Protease-dependent versus -independent cancer cell invasion programs: three-dimensional amoeboid movement revisited. J. Cell Biol. *185,* 11-19.

Saharinen, J., Hyytiäinen, M., Taipale, J., and Keski-Oja, J. (1999). Latent transforming growth factor-beta binding proteins (LTBPs)--structural extracellular matrix proteins for targeting TGF-beta action. Cytokine Growth Factor Rev. *10,* 99-117.

Saharinen, J., Taipale, J., Monni, O., and Keski-Oja, J. (1998). Identification and characterization of a new latent transforming growth factor-beta-binding protein, LTBP-4. J. Biol. Chem. *273,* 18459-18469.

Sanford, L.P., Ormsby, I., Gittenberger-de Groot, A.C., Sariola, H., Friedman, R., Boivin, G.P., Cardell, E.L., and Doetschman, T. (1997). TGFbeta2 knockout mice have multiple developmental defects that are non-overlapping with other TGFbeta knockout phenotypes. Development *124,* 2659-2670.

Sato, H., Kinoshita, T., Takino, T., Nakayama, K., and Seiki, M. (1996). Activation of a recombinant membrane type 1-matrix metalloproteinase (MT1-MMP) by furin and its interaction with tissue inhibitor of metalloproteinases (TIMP)-2. FEBS Lett. *393,* 101-104.

Sato, H., Takino, T., Okada, Y., Cao, J., Shinagawa, A., Yamamoto, E., and Seiki, M. (1994). A matrix metalloproteinase expressed on the surface of invasive tumour cells. Nature *370,* 61-65.

Sato, Y., Okada, F., Abe, M., Seguchi, T., Kuwano, M., Sato, S., Furuya, A., Hanai, N., and Tamaoki, T. (1993). The mechanism for the activation of latent TGF-beta during co-culture of endothelial cells and smooth

muscle cells: cell-type specific targeting of latent TGF-beta to smooth muscle cells. J. Cell Biol. *123,* 1249-1254.

Sato, Y., and Rifkin, D.B. (1989). Inhibition of endothelial cell movement by pericytes and smooth muscle cells: activation of a latent transforming growth factor-beta 1-like molecule by plasmin during co-culture. J. Cell Biol. *109,* 309-315.

Seftor, E.A., Brown, K.M., Chin, L., Kirschmann, D.A., Wheaton, W.W., Protopopov, A., Feng, B., Balagurunathan, Y., Trent, J.M., Nickoloff, B.J., Seftor, R.E., and Hendrix, M.J. (2005). Epigenetic transdifferentiation of normal melanocytes by a metastatic melanoma microenvironment. Cancer Res. *65,* 10164-10169.

Seftor, R.E. (1998). Role of the beta3 integrin subunit in human primary melanoma progression: multifunctional activities associated with alpha(v)beta3 integrin expression. Am. J. Pathol. *153,* 1347-1351.

Seppinen, L., and Pihlajaniemi, T. (2011). The multiple functions of collagen XVIII in development and disease. Matrix Biol. *30,* 83-92.

Shayan, R., Karnezis, T., Murali, R., Wilmott, J.S., Ashton, M.W., Taylor, G.I., Thompson, J.F., Hersey, P., Achen, M.G., Scolyer, R.A., and Stacker, S.A. (2012). Lymphatic vessel density in primary melanomas predicts sentinel lymph node status and risk of metastasis. Histopathology

Shen, Q., Lee, E.S., Pitts, R.L., Wu, M.H., and Yuan, S.Y. (2010). Tissue inhibitor of metalloproteinase-2 regulates matrix metalloproteinase-2-mediated endothelial barrier dysfunction and breast cancer cell transmigration through lung microvascular endothelial cells. Mol. Cancer. Res. *8,* 939-951.

Sheppard, D. (2005). Integrin-mediated activation of latent transforming growth factor beta. Cancer Metastasis Rev. *24,* 395-402.

Shi, J., Son, M.Y., Yamada, S., Szabova, L., Kahan, S., Chrysovergis, K., Wolf, L., Surmak, A., and Holmbeck, K. (2008). Membrane-type MMPs enable extracellular matrix permissiveness and mesenchymal cell proliferation during embryogenesis. Dev. Biol. *313,* 196-209.

Shields, J.D., Borsetti, M., Rigby, H., Harper, S.J., Mortimer, P.S., Levick, J.R., Orlando, A., and Bates, D.O. (2004). Lymphatic density and metastatic spread in human malignant melanoma. Br. J. Cancer *90,* 693-700.

Shimada, T., Nakamura, H., Ohuchi, E., Fujii, Y., Murakami, Y., Sato, H., Seiki, M., and Okada, Y. (1999). Characterization of a truncated recombinant form of human membrane type 3 matrix metalloproteinase. Eur. J. Biochem. *262,* 907-914.

Shipley, J.M., Mecham, R.P., Maus, E., Bonadio, J., Rosenbloom, J., McCarthy, R.T., Baumann, M.L., Frankfater, C., Segade, F., and Shapiro, S.D. (2000). Developmental expression of latent transforming growth factor beta binding protein 2 and its requirement early in mouse development. Mol. Cell. Biol. *20,* 4879-4887.

Shiryaev, S.A., Savinov, A.Y., Cieplak, P., Ratnikov, B.I., Motamedchaboki, K., Smith, J.W., and Strongin, A.Y. (2009). Matrix metalloproteinase proteolysis of the myelin basic protein isoforms is a source of immunogenic peptides in autoimmune multiple sclerosis. PLoS One *4,* e4952.

Shofuda, K., Yasumitsu, H., Nishihashi, A., Miki, K., and Miyazaki, K. (1997). Expression of three membrane-type matrix metalloproteinases (MT-MMPs) in rat vascular smooth muscle cells and characterization of MT3-MMPs with and without transmembrane domain. J. Biol. Chem. *272,* 9749-9754.

Shull, M.M., Ormsby, I., Kier, A.B., Pawlowski, S., Diebold, R.J., Yin, M., Allen, R., Sidman, C., Proetzel, G., and Calvin, D. (1992). Targeted disruption of the mouse transforming growth factor-beta 1 gene results in multifocal inflammatory disease. Nature *359,* 693-699.

Silye, R., Karayiannakis, A.J., Syrigos, K.N., Poole, S., van Noorden, S., Batchelor, W., Regele, H., Sega, W., Boesmueller, H., Krausz, T., and Pignatelli, M. (1998). E-cadherin/catenin complex in benign and malignant melanocytic lesions. J. Pathol. *186,* 350-355.

Sironen, R.K., Tammi, M., Tammi, R., Auvinen, P.K., Anttila, M., and Kosma, V.M. (2011). Hyaluronan in human malignancies. Exp. Cell Res. *317,* 383-391.

Skobe, M., Hawighorst, T., Jackson, D.G., Prevo, R., Janes, L., Velasco, P., Riccardi, L., Alitalo, K., Claffey, K., and Detmar, M. (2001). Induction of tumor lymphangiogenesis by VEGF-C promotes breast cancer metastasis. Nat. Med. *7,* 192-198.

Smoller, B.R. (2006). Histologic criteria for diagnosing primary cutaneous malignant melanoma. Mod. Pathol. *19 Suppl 2,* S34-40.

Sodek, K.L., Brown, T.J., and Ringuette, M.J. (2008). Collagen I but not Matrigel matrices provide an MMP-dependent barrier to ovarian cancer cell penetration. BMC Cancer *8,* 223.

Sohail, A., Sun, Q., Zhao, H., Bernardo, M.M., Cho, J.A., and Fridman, R. (2008). MT4-(MMP17) and MT6-MMP (MMP25), A unique set of membrane-anchored matrix metalloproteinases: properties and expression in cancer. Cancer Metastasis Rev. *27,* 289-302.

Soulie, P., Carrozzino, F., Pepper, M.S., Strongin, A.Y., Poupon, M.F., and Montesano, R. (2005). Membrane-type-1 matrix metalloproteinase confers tumorigenicity on nonmalignant epithelial cells. Oncogene *24,* 1689-1697.

Sounni, N.E., Baramova, E.N., Munaut, C., Maquoi, E., Frankenne, F., Foidart, J.M., and Nöel, A. (2002b). Expression of membrane type 1 matrix metalloproteinase (MT1-MMP) in A2058 melanoma cells is associated with MMP-2 activation and increased tumor growth and vascularization. Int. J. Cancer *98,* 23-28.

Sounni, N.E., Dehne, K., van Kempen, L., Egeblad, M., Affara, N.I., Cuevas, I., Wiesen, J., Junankar, S., Korets, L., Lee, J.*, et al.* (2010). Stromal regulation of vessel stability by MMP14 and TGFbeta. Dis. Model. Mech. *3,* 317-332.

Sounni, N.E., Devy, L., Hajitou, A., Frankenne, F., Munaut, C., Gilles, C., Deroanne, C., Thompson, E.W., Foidart, J.M., and Nöel, A. (2002a). MT1-MMP expression promotes tumor growth and angiogenesis through an up-regulation of vascular endothelial growth factor expression. Faseb j. *16,* 555-564.

Sounni, N.E., Roghi, C., Chabottaux, V., Janssen, M., Munaut, C., Maquoi, E., Galvez, B.G., Gilles, C., Frankenne, F., Murphy, G., Foidart, J.M., and Nöel, A. (2004). Up-regulation of vascular endothelial growth factor-A by active membrane-type 1 matrix metalloproteinase through activation of Src-tyrosine kinases. J. Biol. Chem. *279,* 13564-13574.

Srichai, M.B., Colleta, H., Gewin, L., Matrisian, L., Abel, T.W., Koshikawa, N., Seiki, M., Pozzi, A., Harris, R.C., and Zent, R. (2011). Membrane-type 4 matrix metalloproteinase (MT4-MMP) modulates water homeostasis in mice. PLoS One *6,* e17099.

Stacker, S.A., Achen, M.G., Jussila, L., Baldwin, M.E., and Alitalo, K. (2002). Lymphangiogenesis and cancer metastasis. Nat. Rev. Cancer. *2,* 573-583.

Standeven, K.F., Ariens, R.A., and Grant, P.J. (2005). The molecular physiology and pathology of fibrin structure/function. Blood Rev. *19,* 275-288.

Stang, A., Pukkala, E., Sankila, R., Soderman, B., and Hakulinen, T. (2006). Time trend analysis of the skin melanoma incidence of Finland from 1953 through 2003 including 16,414 cases. Int. J. Cancer *119,* 380-384.

Stanton, H., Gavrilovic, J., Atkinson, S.J., d'Ortho, M.P., Yamada, K.M., Zardi, L., and Murphy, G. (1998). The activation of ProMMP-2 (gelatinase A) by HT1080 fibrosarcoma cells is promoted by culture on a fibronectin substrate and is concomitant with an increase in processing of MT1-MMP (MMP-14) to a 45 kDa form. J. Cell. Sci. *111 (Pt 18),* 2789-2798.

Steffen, A., Le Dez, G., Poincloux, R., Recchi, C., Nassoy, P., Rottner, K., Galli, T., and Chavrier, P. (2008). MT1-MMP-dependent invasion is regulated by TI-VAMP/VAMP7. Curr. Biol. *18,* 926-931.

Steffensen, B., Wallon, U.M., and Overall, C.M. (1995). Extracellular matrix binding properties of recombinant fibronectin type II-like modules of human 72-kDa gelatinase/type IV collagenase. High affinity binding to native type I collagen but not native type IV collagen. J. Biol. Chem. *270,* 11555-11566.

Sterner-Kock, A., Thorey, I.S., Koli, K., Wempe, F., Otte, J., Bangsow, T., Kuhlmeier, K., Kirchner, T., Jin, S., Keski-Oja, J., and von Melchner, H. (2002). Disruption of the gene encoding the latent transforming growth factor-beta binding protein 4 (LTBP-4) causes abnormal lung development, cardiomyopathy, and colorectal cancer. Genes Dev. *16,* 2264-2273.

Sternlicht, M.D., and Werb, Z. (2001). How matrix metalloproteinases regulate cell behavior. Annu. Rev. Cell Dev. Biol. *17,* 463-516.

Stockmann, C., Doedens, A., Weidemann, A., Zhang, N., Takeda, N., Greenberg, J.I., Cheresh, D.A., and Johnson, R.S. (2008). Deletion of vascular endothelial growth factor in myeloid cells accelerates tumorigenesis. Nature *456,* 814-818.

Stoletov, K., Montel, V., Lester, R.D., Gonias, S.L., and Klemke, R. (2007). High-resolution imaging of the dynamic tumor cell vascular interface in transparent zebrafish. Proc. Natl. Acad. Sci. U. S. A. *104,* 17406-17411.

Storr, S.J., Safuan, S., Mitra, A., Elliott, F., Walker, C., Vasko, M.J., Ho, B., Cook, M., Mohammed, R.A., Patel, P.M.*, et al.* (2012). Objective assessment of blood and lymphatic vessel invasion and association with macrophage infiltration in cutaneous melanoma. Mod. Pathol. *25,* 493-504.

Straume, O., Sviland, L., and Akslen, L.A. (2000). Loss of nuclear p16 protein expression correlates with increased tumor cell proliferation (Ki-67) and poor prognosis in patients with vertical growth phase melanoma. Clin. Cancer Res. *6,* 1845-1853.

Streit, M., and Detmar, M. (2003). Angiogenesis, lymphangiogenesis, and melanoma metastasis. Oncogene *22,* 3172-3179.

Strongin, A.Y., Collier, I., Bannikov, G., Marmer, B.L., Grant, G.A., and Goldberg, G.I. (1995). Mechanism of cell surface activation of 72-kDa type IV collagenase. Isolation of the activated form of the membrane metalloprotease. J. Biol. Chem. *270,* 5331-5338.

Su, G., Blaine, S.A., Qiao, D., and Friedl, A. (2008). Membrane type 1 matrix metalloproteinase-mediated stromal syndecan-1 shedding stimulates breast carcinoma cell proliferation. Cancer Res. *68,* 9558-9565.

Sugiyama, N., Varjosalo, M., Meller, P., Lohi, J., Chan, K.M., Zhou, Z., Alitalo, K., Taipale, J., Keski-Oja, J., and Lehti, K. (2010a). FGF receptor-4 (FGFR4) polymorphism acts as an activity switch of a membrane type 1 matrix metalloproteinase-FGFR4 complex. Proc. Natl. Acad. Sci. U. S. A. *107,* 15786-15791.

Sugiyama, N., Varjosalo, M., Meller, P., Lohi, J., Hyytiäinen, M., Kilpinen, S., Kallioniemi, O., Ingvarsen, S., Engelholm, L.H., Taipale, J.*, et al.* (2010b). Fibroblast growth factor receptor 4 regulates tumor invasion by coupling fibroblast growth factor signaling to extracellular matrix degradation. Cancer Res. *70,* 7851-7861.

Sun, L., Yan, W., Wang, Y., Sun, G., Luo, H., Zhang, J., Wang, X., You, Y., Yang, Z., and Liu, N. (2011). MicroRNA-10b induces glioma cell invasion by modulating MMP-14 and uPAR expression via HOXD10. Brain Res. *1389,* 9-18.

Sun, Q., Weber, C.R., Sohail, A., Bernardo, M.M., Toth, M., Zhao, H., Turner, J.R., and Fridman, R. (2007). MMP25 (MT6-MMP) is highly expressed in human colon cancer, promotes tumor growth, and exhibits unique biochemical properties. J. Biol. Chem. *282,* 21998-22010.

Taipale, J., and Keski-Oja, J. (1997). Growth factors in the extracellular matrix. Faseb j. *11,* 51-59.

Taipale, J., Koli, K., and Keski-Oja, J. (1992). Release of transforming growth factor-beta 1 from the pericellular matrix of cultured fibroblasts and fibrosarcoma cells by plasmin and thrombin. J. Biol. Chem. *267,* 25378-25384.

Taipale, J., Lohi, J., Saarinen, J., Kovanen, P.T., and Keski-Oja, J. (1995). Human mast cell chymase and leukocyte elastase release latent transforming growth factor-beta 1 from the extracellular matrix of cultured human epithelial and endothelial cells. J. Biol. Chem. *270,* 4689-4696.

Taipale, J., Miyazono, K., Heldin, C.H., and Keski-Oja, J. (1994). Latent transforming growth factor-beta 1 associates to fibroblast extracellular matrix via latent TGF-beta binding protein. J. Cell Biol. *124,* 171-181.

Takino, T., Sato, H., Shinagawa, A., and Seiki, M. (1995). Identification of the second membrane-type matrix metalloproteinase (MT-MMP-2) gene from a human placenta cDNA library. MT-MMPs form a unique membrane-type subclass in the MMP family. J. Biol. Chem. *270,* 23013-23020.

Talve, L., Sauroja, I., Collan, Y., Punnonen, K., and Ekfors, T. (1997). Loss of expression of the p16INK4/CDKN2 gene in cutaneous malignant melanoma correlates with tumor cell proliferation and invasive stage. Int. J. Cancer *74,* 255-259.

Tam, E.M., Wu, Y.I., Butler, G.S., Stack, M.S., and Overall, C.M. (2002). Collagen binding properties of the membrane type-1 matrix metalloproteinase (MT1-MMP) hemopexin C domain. The ectodomain of the 44-kDa autocatalytic product of MT1-MMP inhibits cell invasion by disrupting native type I collagen cleavage. J. Biol. Chem. *277,* 39005-39014.

Taylor, C.J., Motamed, K., and Lilly, B. (2006). Protein kinase C and downstream signaling pathways in a three-dimensional model of phorbol ester-induced angiogenesis. Angiogenesis *9,* 39-51.

Tellez, C., and Bar-Eli, M. (2003). Role and regulation of the thrombin receptor (PAR-1) in human melanoma. Oncogene *22,* 3130-3137.

Teti, A., De Giorgi, A., Spinella, M.T., Migliaccio, S., Canipari, R., Onetti Muda, A., and Faraggiana, T. (1997). Transforming growth factor-beta enhances adhesion of melanoma cells to the endothelium in vitro. Int. J. Cancer *72,* 1013-1020.

Tomari, T., Koshikawa, N., Uematsu, T., Shinkawa, T., Hoshino, D., Egawa, N., Isobe, T., and Seiki, M. (2009). High throughput analysis of proteins associating with a proinvasive MT1-MMP in human malignant melanoma A375 cells. Cancer. Sci. *100,* 1284-1290.

Toth, M., Osenkowski, P., Hesek, D., Brown, S., Meroueh, S., Sakr, W., Mobashery, S., and Fridman, R. (2005). Cleavage at the stem region releases an active ectodomain of the membrane type 1 matrix metalloproteinase. Biochem. J. *387,* 497-506.

Tsuji, T., Okada, F., Yamaguchi, K., and Nakamura, T. (1990). Molecular cloning of the large subunit of transforming growth factor type beta masking protein and expression of the mRNA in various rat tissues. Proc. Natl. Acad. Sci. U. S. A. *87,* 8835-8839.

Tuominen, H., and Kallioinen, M. (1994). Increased tenascin expression in melanocytic tumors. J. Cutan. Pathol. *21,* 424-429.

Uchibori, M., Nishida, Y., Nagasaka, T., Yamada, Y., Nakanishi, K., and Ishiguro, N. (2006). Increased expression of membrane-type matrix metalloproteinase-1 is correlated with poor prognosis in patients with osteosarcoma. Int. J. Oncol. *28,* 33-42.

Uekita, T., Itoh, Y., Yana, I., Ohno, H., and Seiki, M. (2001). Cytoplasmic tail-dependent internalization of membrane-type 1 matrix metalloproteinase is important for its invasion-promoting activity. J. Cell Biol. *155,* 1345-1356.

Ugurel, S., Rappl, G., Tilgen, W., and Reinhold, U. (2001). Increased serum concentration of angiogenic factors in malignant melanoma patients correlates with tumor progression and survival. J. Clin. Oncol. *19,* 577-583.

Unsöld, C., Hyytiäinen, M., Bruckner-Tuderman, L., and Keski-Oja, J. (2001). Latent TGF-beta binding protein LTBP-1 contains three potential extracellular matrix interacting domains. J. Cell. Sci. *114,* 187-197.

Ushiki, T. (2002). Collagen fibers, reticular fibers and elastic fibers. A comprehensive understanding from a morphological viewpoint. Arch. Histol. Cytol. *65,* 109-126.

Van der Auwera, I., Van den Eynden, G.G., Colpaert, C.G., Van Laere, S.J., van Dam, P., Van Marck, E.A., Dirix, L.Y., and Vermeulen, P.B. (2005). Tumor lymphangiogenesis in inflammatory breast carcinoma: a histomorphometric study. Clin. Cancer Res. *11,* 7637-7642.

van der Schaft, D.W., Pauwels, P., Hulsmans, S., Zimmermann, M., van de Poll-Franse, L.V., and Griffioen, A.W. (2007). Absence of lymphangiogenesis in ductal breast cancer at the primary tumor site. Cancer Lett. *254,* 128-136.

Van Meter, T.E., Broaddus, W.C., Rooprai, H.K., Pilkington, G.J., and Fillmore, H.L. (2004). Induction of membrane-type-1 matrix metalloproteinase by epidermal growth factor-mediated signaling in gliomas. Neuro Oncol. *6,* 188-199.

Vehviläinen, P., Hyytiäinen, M., and Keski-Oja, J. (2009). Matrix association of latent TGF-beta binding protein-2 (LTBP-2) is dependent on fibrillin-1. J. Cell. Physiol. *221,* 586-593.

Vehviläinen, P., Hyytiäinen, M., and Keski-Oja, J. (2003). Latent transforming growth factor-beta-binding protein 2 is an adhesion protein for melanoma cells. J. Biol. Chem. *278,* 24705-24713.

Velasco, G., Cal, S., Merlos-Suarez, A., Ferrando, A.A., Alvarez, S., Nakano, A., Arribas, J., and Lopez-Otin, C. (2000). Human MT6-matrix metalloproteinase: identification, progelatinase A activation, and expression in brain tumors. Cancer Res. *60,* 877-882.

Viljoen, D. (1994). Congenital contractural arachnodactyly (Beals syndrome). J. Med. Genet. *31,* 640-643.

Vitellaro-Zuccarello, L., Garbelli, R., and Rossi, V.D. (1992). Immunocytochemical localization of collagen types I, III, IV, and fibronectin in the human dermis. Modifications with ageing. Cell Tissue Res. *268,* 505-511.

Vlaykova, T., Laurila, P., Muhonen, T., Hahka-Kemppinen, M., Jekunen, A., Alitalo, K., and Pyrhönen, S. (1999). Prognostic value of tumour vascularity in metastatic melanoma and association of blood vessel density with vascular endothelial growth factor expression. Melanoma Res. *9,* 59-68.

Vuoriluoto, K., Högnäs, G., Meller, P., Lehti, K., and Ivaska, J. (2011). Syndecan-1 and -4 differentially regulate oncogenic K-ras dependent cell invasion into collagen through alpha2beta1 integrin and MT1-MMP. Matrix Biol. *30,* 207-217.

Vuylsteke, R.J., van Leeuwen, P.A., Statius Muller, M.G., Gietema, H.A., Kragt, D.R., and Meijer, S. (2003). Clinical outcome of stage I/II melanoma patients after selective sentinel lymph node dissection: long-term follow-up results. J. Clin. Oncol. *21,* 1057-1065.

Walker, G.J., Flores, J.F., Glendening, J.M., Lin, A.H., Markl, I.D., and Fountain, J.W. (1998). Virtually 100% of melanoma cell lines harbor alterations at the DNA level within CDKN2A, CDKN2B, or one of their downstream targets. Genes Chromosomes Cancer *22,* 157-163.

Wang, P., Nie, J., and Pei, D. (2004). The hemopexin domain of membrane-type matrix metalloproteinase-1 (MT1-MMP) Is not required for its activation of proMMP2 on cell surface but is essential for MT1-MMP-mediated invasion in three-dimensional type I collagen. J. Biol. Chem. *279,* 51148-51155.

Wang, Y., Johnson, A.R., Ye, Q.Z., and Dyer, R.D. (1999). Catalytic activities and substrate specificity of the human membrane type 4 matrix metalloproteinase catalytic domain. J. Biol. Chem. *274,* 33043-33049.

Watt, F.M., and Fujiwara, H. (2011). Cell-extracellular matrix interactions in normal and diseased skin. Cold Spring Harb Perspect. Biol. *3,* 10.1101/cshperspect.a005124.

Wei, Y., Lukashev, M., Simon, D.I., Bodary, S.C., Rosenberg, S., Doyle, M.V., and Chapman, H.A. (1996). Regulation of integrin function by the urokinase receptor. Science *273,* 1551-1555.

Weninger, W., Partanen, T.A., Breiteneder-Geleff, S., Mayer, C., Kowalski, H., Mildner, M., Pammer, J., Sturzl, M., Kerjaschki, D., Alitalo, K., and Tschachler, E. (1999). Expression of vascular endothelial growth factor receptor-3 and podoplanin suggests a lymphatic endothelial cell origin of Kaposi's sarcoma tumor cells. Lab. Invest. *79,* 243-251.

White, R.R., Stanley, W.E., Johnson, J.L., Tyler, D.S., and Seigler, H.F. (2002). Long-term survival in 2,505 patients with melanoma with regional lymph node metastasis. Ann. Surg. *235,* 879-887.

Wickström, S.A., Alitalo, K., and Keski-Oja, J. (2002). Endostatin associates with integrin alpha5beta1 and caveolin-1, and activates Src via a tyrosyl phosphatase-dependent pathway in human endothelial cells. Cancer Res. *62,* 5580-5589.

Widera, D., Hauser, S., Kaltschmidt, C., and Kaltschmidt, B. (2012). Origin and regenerative potential of vertebrate mechanoreceptor-associated stem cells. Anat. Res. Int. *2012,* 837626.

Wigle, J.T., and Oliver, G. (1999). Prox1 function is required for the development of the murine lymphatic system. Cell *98,* 769-778.

Wiley, H.E., Gonzalez, E.B., Maki, W., Wu, M.T., and Hwang, S.T. (2001). Expression of CC chemokine receptor-7 and regional lymph node metastasis of B16 murine melanoma. J. Natl. Cancer Inst. *93,* 1638-1643.

Will, H., Atkinson, S.J., Butler, G.S., Smith, B., and Murphy, G. (1996). The soluble catalytic domain of membrane type 1 matrix metalloproteinase cleaves the propeptide of progelatinase A and initiates autoproteolytic activation. Regulation by TIMP-2 and TIMP-3. J. Biol. Chem. *271,* 17119-17123.

Wolf, K., Alexander, S., Schacht, V., Coussens, L.M., von Andrian, U.H., van Rheenen, J., Deryugina, E., and Friedl, P. (2009). Collagen-based cell migration models in vitro and in vivo. Semin. Cell Dev. Biol. *20,* 931-941.

Wolf, K., and Friedl, P. (2011). Extracellular matrix determinants of proteolytic and non-proteolytic cell migration. Trends Cell Biol. *21,* 736-744.

Wolf, K., and Friedl, P. (2009). Mapping proteolytic cancer cell-extracellular matrix interfaces. Clin. Exp. Metastasis *26,* 289-298.

Wolf, K., Mazo, I., Leung, H., Engelke, K., von Andrian, U.H., Deryugina, E.I., Strongin, A.Y., Brocker, E.B., and Friedl, P. (2003). Compensation mechanism in tumor cell migration: mesenchymal-amoeboid transition after blocking of pericellular proteolysis. J. Cell Biol. *160,* 267-277.

Wolf, K., Wu, Y.I., Liu, Y., Geiger, J., Tam, E., Overall, C., Stack, M.S., and Friedl, P. (2007). Multi-step pericellular proteolysis controls the transition from individual to collective cancer cell invasion. Nat. Cell Biol. *9,* 893-904.

Wong, C., and Jin, Z.G. (2005). Protein kinase C-dependent protein kinase D activation modulates ERK signal pathway and endothelial cell proliferation by vascular endothelial growth factor. J. Biol. Chem. *280,* 33262-33269.

Woods, A., Longley, R.L., Tumova, S., and Couchman, J.R. (2000). Syndecan-4 binding to the high affinity heparin-binding domain of fibronectin drives focal adhesion formation in fibroblasts. Arch. Biochem. Biophys. *374,* 66-72.

Wu, J., Rosenbaum, E., Begum, S., and Westra, W.H. (2007). Distribution of BRAF T1799A(V600E) mutations across various types of benign nevi: implications for melanocytic tumorigenesis. Am. J. Dermatopathol. *29,* 534-537.

Wyckoff, J.B., Jones, J.G., Condeelis, J.S., and Segall, J.E. (2000). A critical step in metastasis: in vivo analysis of intravasation at the primary tumor. Cancer Res. *60,* 2504-2511.

Xia, H., Qi, Y., Ng, S.S., Chen, X., Li, D., Chen, S., Ge, R., Jiang, S., Li, G., Chen, Y*., et al.* (2009). microRNA-146b inhibits glioma cell migration and invasion by targeting MMPs. Brain Res. *1269,* 158-165.

Xing, F., Saidou, J., and Watabe, K. (2010). Cancer associated fibroblasts (CAFs) in tumor microenvironment. Front. Biosci. *15,* 166-179.

Xu, J., Rodriguez, D., Petitclerc, E., Kim, J.J., Hangai, M., Moon, Y.S., Davis, G.E., and Brooks, P.C. (2001). Proteolytic exposure of a cryptic site within collagen type IV is required for angiogenesis and tumor growth in vivo. J. Cell Biol. *154,* 1069-1079.

Xu, X., Yamamoto, H., Liu, G., Ito, Y., Ngan, C.Y., Kondo, M., Nagano, H., Dono, K., Sekimoto, M., and Monden, M. (2008). CDC25A inhibition suppresses the growth and invasion of human hepatocellular carcinoma cells. Int. J. Mol. Med. *21,* 145-152.

Yan, C., and Boyd, D.D. (2007). Regulation of matrix metalloproteinase gene expression. J. Cell. Physiol. *211,* 19-26.

Yana, I., Sagara, H., Takaki, S., Takatsu, K., Nakamura, K., Nakao, K., Katsuki, M., Taniguchi, S., Aoki, T., Sato, H., Weiss, S.J., and Seiki, M. (2007). Crosstalk between neovessels and mural cells directs the site-specific expression of MT1-MMP to endothelial tip cells. J. Cell. Sci. *120,* 1607-1614.

Yana, I., and Weiss, S.J. (2000). Regulation of membrane type-1 matrix metalloproteinase activation by proprotein convertases. Mol. Biol. Cell *11,* 2387-2401.

Yee, V.S., Thompson, J.F., McKinnon, J.G., Scolyer, R.A., Li, L.X., McCarthy, W.H., O'Brien, C.J., Quinn, M.J., Saw, R.P., Shannon, K.F., Stretch, J.R., and Uren, R.F. (2005). Outcome in 846 cutaneous melanoma patients from a single center after a negative sentinel node biopsy. Ann. Surg. Oncol. *12,* 429-439.

Yoshiyama, Y., Sato, H., Seiki, M., Shinagawa, A., Takahashi, M., and Yamada, T. (1998). Expression of the membrane-type 3 matrix metalloproteinase (MT3-MMP) in human brain tissues. Acta Neuropathol. *96,* 347-350.

Yu, J.L., Rak, J.W., Carmeliet, P., Nagy, A., Kerbel, R.S., and Coomber, B.L. (2001). Heterogeneous vascular dependence of tumor cell populations. Am. J. Pathol. *158,* 1325-1334.

Yu, J.L., Rak, J.W., Klement, G., and Kerbel, R.S. (2002). Vascular endothelial growth factor isoform expression as a determinant of blood vessel patterning in human melanoma xenografts. Cancer Res. *62,* 1838-1846.

Yu, Q., and Stamenkovic, I. (2000). Cell surface-localized matrix metalloproteinase-9 proteolytically activates TGF-beta and promotes tumor invasion and angiogenesis. Genes Dev. *14,* 163-176.

Zhai, Y., Hotary, K.B., Nan, B., Bosch, F.X., Munoz, N., Weiss, S.J., and Cho, K.R. (2005). Expression of membrane type 1 matrix metalloproteinase is associated with cervical carcinoma progression and invasion. Cancer Res. *65,* 6543-6550.

Zhang, H., Wong, C.C., Wei, H., Gilkes, D.M., Korangath, P., Chaturvedi, P., Schito, L., Chen, J., Krishnamachary, B., Winnard, P.T.,Jr, *et al.* (2012a). HIF-1-dependent expression of angiopoietin-like 4 and L1CAM mediates vascular metastasis of hypoxic breast cancer cells to the lungs. Oncogene *31,* 1757-1770.

Zhang, Q.W., Liu, L., Gong, C.Y., Shi, H.S., Zeng, Y.H., Wang, X.Z., Zhao, Y.W., and Wei, Y.Q. (2012b). Prognostic significance of tumor-associated macrophages in solid tumor: a meta-analysis of the literature. PLoS One *7,* e50946.

Zhou, Z., Apte, S.S., Soininen, R., Cao, R., Baaklini, G.Y., Rauser, R.W., Wang, J., Cao, Y., and Tryggvason, K. (2000). Impaired endochondral ossification and angiogenesis in mice deficient in membrane-type matrix metalloproteinase I. Proc. Natl. Acad. Sci. U. S. A. *97,* 4052-4057.

Zhu, S., Zhou, Y., Wang, L., Zhang, J., Wu, H., Xiong, J., Zhang, J., Tian, Y., Wang, C., and Wu, H. (2011). Transcriptional upregulation of MT2-MMP in response to hypoxia is promoted by HIF-1alpha in cancer cells. Mol. Carcinog. *50,* 770-780.

Zhuge, Y., and Xu, J. (2001). Rac1 mediates type I collagen-dependent MMP-2 activation. role in cell invasion across collagen barrier. J. Biol. Chem. *276,* 16248-16256.

Zigrino, P., Kuhn, I., Bäuerle, T., Zamek, J., Fox, J.W., Neumann, S., Licht, A., Schorpp-Kistner, M., Angel, P., and Mauch, C. (2009). Stromal expression of MMP-13 is required for melanoma invasion and metastasis. J. Invest. Dermatol. *129,* 2686-2693.

Zigrino, P., Nischt, R., and Mauch, C. (2011). The disintegrin-like and cysteine-rich domains of ADAM-9 mediate interactions between melanoma cells and fibroblasts. J. Biol. Chem. *286,* 6801-6807.

Ziyad, S., and Iruela-Arispe, M.L. (2011). Molecular mechanisms of tumor angiogenesis. Genes Cancer. *2,* 1085-1096.