Drugs of the Future 2003, 28(9): 897-904

CCC: 0377-8282/2003

Review Article

Metastatic melanoma and matrix metalloproteinases

Gregory Riedlinger,[1] Stephen Kridel,[1] Ali M. Al-Housseini,[1] Jennifer M. Brow,[1] Thomas H. Bugge,[2] Stephen H. Leppla,[3] Nick Duesbery,[4] George Vande Woude[4] and Arthur Frankel[1]*

*[1]Department of Cancer Biology, Hanes 4046, Wake Forest University School of Medicine, Medical Center Blvd., Winston-Salem, North Carolina 27157; [2]Oral and Pharyngeal Cancer Branch, National Institute of Dental and Craniofacial Research, NIH, Bethesda, Maryland 20892; [3]Microbial Pathogenesis Section, National Institute of Allergy and Infectious Diseases, NIH, Bethesda, Maryland 20892; [4]Laboratories of Developmental Cell Biology and Molecular Oncology, Van Andel Research Institute, Grand Rapids, MI 49503, USA. *Correspondence*

CONTENTS

Abstract

Metastatic melanoma is a rapidly progressive, incurable malignancy for which novel therapies are needed. Analysis of the biology of melanoma cells reveals a dependency upon several critical pathways, with matrix metalloproteinases (MMPs) essential for tumor spread and dissemination. We describe a growing number of protein therapeutics which target melanoma cells by requiring activation by MMPs. These agents should have distinct side effects and may be useful in combinations with standard cytotoxic chemotherapy or immunotherapies.

Introduction

Over 55,000 people will develop malignant melanoma and close to 8,000 people will die in the U.S. in 2003 (1). Once malignant melanomas invade subcutaneous tissues and spread to other organs, cure is extremely rare. Cytotoxic chemotherapy has modest palliative benefit (2). Five year survival is less than 10% and median survival is 6-8 months. A minority of patients (approximately 5%) respond to interferon-α or interleukin-2 (3). Patients with metastases to skin, subcutaneous tissue or nodes do slightly better than patients with lung metastases who, in turn, do better than patients with metastases to other visceral organs (4).

Melanocyte transformation

Melanocytes are normally present in the basal layer of the epidermis. They have dendritic processes that contact keratinocytes and they contain melanosomes. Melanocytic transformation is observed histopathologically in distinct stage-nevi without dysplastic changes, dysplastic nevi, radial-growth phase melanoma, vertical growth phase melanoma and metastatic melanoma. The genetic changes associated with each of these stages are only partially understood. Information from cases of familial melanoma and mouse models implicate changes in at least 3 signaling pathways (5,6). The *RB pathway* includes the INK4a or p16 protein which inhibits CDK4/6-cyclin D1 phosphorylation of RB. Once RB is phosphorylated, it dissociates from E2F protein. The free E2F transcription factor activates genes necessary for progression into S phase. RB can be separately inactivated by the Myc protein. Thus, melanomas in human or mice have been associated with loss of INK4a, inhibition of INK4a binding to CDK4 by a mutation in CDK4 or inactivation of RB by Myc overexpression. The *p53 pathway* includes p14/p19 ARF protein which inhibits MDM2 protein which,

in turn, mediates ubiquitination and degradation of p53. p53 triggers apoptosis of abnormally proliferating cells. Melanomas in human are associated with mutation or loss of ARF. Melanomas in mice have been generated with p53 mutations. The *MAPK pathway* integrates signals from receptor tyrosine kinases and G-protein-coupled receptors. Downstream effectors include Ras protein, Raf proteins, MEKs, ERKs and specific nuclear substrates involved in regulating cell differentiation, proliferation and survival. Constitutive activation mutations of N-Ras, H-Ras and B-Raf occur in human and murine melanomas. Seventy percent of patients have evidence for MAPK pathway activation – most commonly B-Raf V599E (7,8).

The mutagenesis which leads to these pathway changes in melanocytes occurs more commonly in people with UV exposure and the RHC "red hair color" phenotype. This phenotype is controlled by the *MC1R pathway*. Components of this pathway include α-melanocyte-stimulating hormone, the melanocortin-1 receptor (a G-protein-coupled receptor), G_s proteins, adenylyl cyclase, cyclic AMP, protein kinase A, the CREB (cAMP-responsive-element binding protein) family, MITF basic helix-loop-helix transcription factor containing a CRE sequence in its promoter and proteins with E box promoters including tyrosinase and dopachrome tautomerase. Particular MC1R variants yield more pheomelanin than eumelanin and increase UV-induced mutagenesis and melanoma incidence (9).

Melanoma progression and spread

Metastatic melanoma involves the escape of melanoma cells from the epidermis, growth and development of a blood supply, dissemination of melanoma cells to other organs, and their growth at these locations. These steps require additional genetic and phenotypic changes by the transformed melanocytes. A number of genetic and epigenetic changes have been linked to the development of melanoma metastases including overexpression of the c-MET tyrosine-kinase receptor and HGF/SF hepatocyte growth factor (10, 11). The *HGF/SF-Met signaling pathway* stimulates proliferation, angiogenesis and motility, and disrupts adhesions between melanocytes and keratinocytes. Downstream effector molecules recruited to its multidocking sites include Grb2 and Gab1 and, secondarily, Src, PI3K, Ras and MAPK members. Cdc42 and Rac are activated and actin cytoskeleton reorganized; integrins are clustered; and matrix metalloproteinases (MMPs) are expressed and activated. E-cadherin-β-catenin adhesion junctions are dissembled. Met activation yields the vasculogenic mimicry phenotype in melanoma cells (M. Hendrix, personal communication). Other aspects of the highly invasive vasculogenic mimicry phenotype include release of laminin 5γ2 and MMP-2, MMP-9 and MMP-14 (12-16). Cleaved fragments of laminin are generated-laminin γ2' and laminin γ2x. These fragments associate with melanoma cell surface integrin α3β1 and signal expression of VE-cadherin (CD144), EphA2 epithelial cell kinase and further production of laminin 5γ2. MMP degradation of collagen IV to expose the HUIV26 cryptic epitope enhances tumor cell interaction with αVβ3 integrin and cell motility (17). Additional changes may further enhance metastatic potential including overexpression of osteopontin and chondroitin sulfate proteoglycan and decreased expression of PITSLRE protein kinase and tissue inhibitor of metalloproteinases (TIMP) 2 (6). The role of MMPs is central to the metastatic process. They modify the extracellular matrix to remove physical barriers, release growth factors, and expose cryptic protein sequences for integrin binding.

Matrix metalloproteinases (MMPs)

The MMPs are a family that includes 23 zinc-dependent endopeptidases (18). MMPs can be grouped into 4 main subcategories (Fig. 1). All members of the MMP family are regulated by a prodomain that maintains the proteinase in a latent zymogen state through a cysteine switch mechanism. The free cysteine in the prodomain ligates the catalytic zinc to render the proteinases inactive. Activation occurs when the prodomain is removed by either furin, other MMPs or plasmin. The first subgroup which includes MMP-7 and MMP-26 consists solely of the catalytic domain. The catalytic module which is common to all MMPs has a HEXGHXXGXXHS sequence within the active site. The 3 histidine residues coordinate the catalytic zinc. The second subgroup of which MMP-1 and MMP-3 are prototypic members, also have a hemopexin domain that is important for protein-protein interactions. The domain consists of 4 parts arranged symmetrically around a central axis and forming a 4-bladed, propeller-like structure. The third subgroup, the gelatinases represented solely by MMP-2 and MMP-9, have an additional 3 fibronectin type II repeats within the amino terminus of the catalytic domain that facilitates gelatin binding. MMP-9 also has a collagen V-like insert in the hinge region. The fourth subgroup, the membrane type MMPs, have a carboxyl-terminal transmembrane domain and cytoplasmic domain. Additionally, there are also MMPs that are membrane tethered by either a GPI anchor or an N-terminal signal anchor (19).

MMP activity is regulated by transcriptional control, zymogen activation and extracellular inhibitors. Expression of MMP mRNAs is increased by a variety of stimuli or growth factors including basic fibroblast growth factor signaling (20), osteopontin-NFκB signaling (21), AP-1 (22,23) and MAPK signaling (24-26) and inhibited by tenascin-X (27). Proteolysis by furin, MMPs or plasmin is needed to remove the prodomain to activate the MMP zymogen. Extracellular inhibitors include TIMPs, the membrane-anchored molecule, reversion-inducing cysteine-rich protein with Kazal motifs (RECK) and α2-macroglobulin. The cytoplasmic domains of the membrane-type MMPs (MMP-14, MMP-15, MMP-16) may route the protease to particular cell surface or

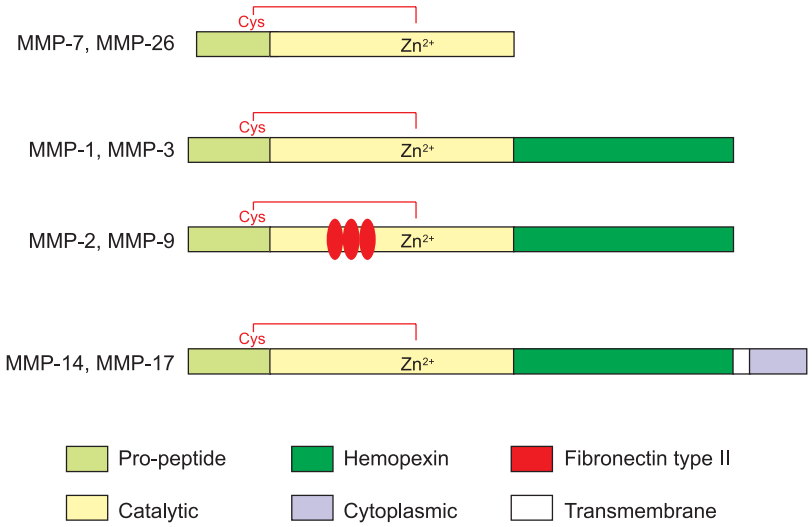

Fig. 1. Diagram of MMP structure. Dark green shows the propeptide domain; yellow shows the catalytic domain; light green shows the hemopexin domain; gray shows the cytoplasmic domain; red shows the fibronectin type II sequences; white shows the transmembrane domain. The blocking cysteine is shown in red (53).

intracellular compartments. Cell surface receptors such as thrombospondin-2 and integrins may bind and regulate the distribution of other MMPs such as MMP-2 and MMP-13.

The substrate preferences of MMPs have been partially identified based on the study of cleaved protein substrates and MMP activities on a series of synthetic peptide substrates. Because the structural features of the catalytic clefts of all MMPs are similar, the substrates show some similarities (Fig. 2). A deep $S_{1'}$ pocket in MMPs provides a docking point for a large hydrophobic residue at the $P_{1'}$ position. Similarly, the S_3 pocket is well fitted by a proline at position P_3. The consensus peptide motif for MMP cleavage is $PXXX_{Hy}$ (28-30). Further modifications can refine substrate specificity for particular MMPs: MMP-2 prefers a small residue at P_2 such as A or S; MMP-9 prefers R at P_1 and P_2 and a S/T at $P_{2'}$; and MMP-14 prefers R at P4 and L/I at $P_{1'}$.

Overexpression of MMPs in metastatic melanomas has been noted since 1980 (31). At that time, the metastatic potential of melanoma cells was correlated with enzymatic degradation of collagen. Various MMPs (MMP-1, MMP-2, MMP-3, MMP-8, MMP-9, MMP-13 and MMP-14) are overexpressed in both primary and metastatic melanoma cells (32-39). This is summarized in Table I. In most cases, a high level of MMP expression correlated with shorter disease-free survival. Furthermore, uveal melanoma metastases were associated with a decreased expression of TIMP3 (40).

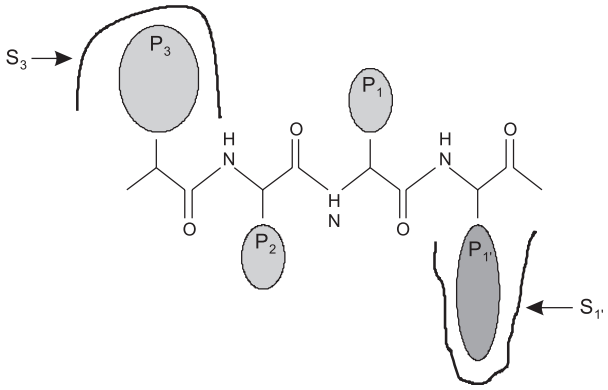

Fig. 2. Schematic diagram of an MMP docked to substrate. Proteinase-substrate interactions are indicated according to Schechter and Berger nomenclature. The deep $S_{1'}$ pocket accommodates binding of the $P_{1'}$ substrate residue that is generally a large hydrophobic amino acid. Similarly, the P_3 position in the substrate occupies a cavernous S_3 pocket in the enzyme. Differences in preference between various MMPs and substrate are determined by the residues that define the cognate S pockets of the enzyme. Specificity of proteinase-substrate binding is further determined by backbone interactions between substrate and enzyme across the active site cleft of the MMP (28).

Recombinant toxins targeting melanoma cell MMPs

Three different hybrid proteins have been prepared that target toxins to MMP-expressing tumor cells (41-43). In each case, potent selective toxicity was observed. Mansour and coworkers reacted the maleimideotriethylene glycol octapeptide derivative (Mal-GPLGIAGQ) with doxorubicin hydrochloride in dimethylformamide in the presence of 1-hydroxybenzotriazole hydrate, 4-methylmorpholine and *N,N'*-diisopropylcarbodiimide (41). The Mal-GPLGIAGQ-Doxo conjugate was mixed with human albumin and purified by size exclusion chromatography.

*Table I: MMP expression in high-grade human malignant melanomas.**

MMP	Assay	Percent strongly positive (%)	Ref.
MMP-1	IH, IS	6, 67, 68	36, 78, 83
MMP-2	IH	25, 60, 66, 78	32, 34, 77, 79
MMP-3	IH	25, 38	36, 77
MMP-8	IH	100	82
MMP-9	IH	10, 20	34, 81
MMP-11	IH	0	80
MMP-13	IS	48, 44	36, 78
MMP-14	IH	90	79

*IH, immunohistochemistry; IS, *in situ* cDNA hybridization. High grade includes Clark level IV or Breslow 2 mm or metastatic lesions.

The Dox-QGAIGLPG-albumin conjugate was cytotoxic to A375 melanoma cells with an IC_{50} of 10 μM. In nude mice, the Mal-GPLGIAGQ-Doxo molecule was used because it rapidly formed a stable bond to the free cysteine thiol of albumin. The prodrug had a maximal tolerated dose (MTD) that was 4-fold higher than doxorubicin alone. This permitted dose escalation *in vivo* with dramatic remissions of subcutaneous A375 melanomas lasting over 40 days. The conjugate releases doxorubicin adjacent to the tumor cells. Thus, the mechanism of cell intoxication should be similar to doxorubicin-inhibition of topoisomerase II. Multidrug resistant melanoma cells may show resistance to this conjugate as well, although the higher dose achievable locally may improve the response rate. Holle and colleagues biotinylated a MMP-2 cleavage sequence-melittin peptide (NH_2-KQGAIGQPQRKRKI-WSILAPLGTT-LVKLVAGIG-COOH) (42). The melittin is a bee venom amphipathic peptide which forms pores in cell membranes leading to cell lysis. The conjugate was mixed with avidin and purified by exposure to streptavidin matrix. The MMP-2 cleavage activated peptide-melittin conjugate was incubated with cell lines and showed some cytotoxicity at MMP-2-secreting DU145 and -SK-OV-3 cells with 10% cell lysis at 1.5 μg/ml. No cytotoxicity above control was seen with B16 melanoma cells which had low MMP-2 secretion. Intratumoral injections of the conjugate produced some tumor growth inhibition of B16 melanoma cells grown s.c. in C57Bl6 mice. There is insufficient evidence of activity of this agent in tissue culture or animal models for its further development for therapy of metastatic melanoma. Liu and colleagues genetically modified anthrax protective antigen (PrAg) to replace the furin cleavage sequence RKKR with a MMP-2 and MMP-9 cleavage sequence, GPLGMLSQ (43). The protein was expressed in *Bacillus anthracis* and purified by ammonium sulfate precipitation and anion exchange chromatography. The modified PrAg (PrAg-L1) was mixed with FP59 and the human melanoma A2058 cell line. FP59 is a recombinant fusion protein composed of the amino-terminal fragment of anthrax lethal factor (amino acids 1-254) fused to the ADP-ribosylation

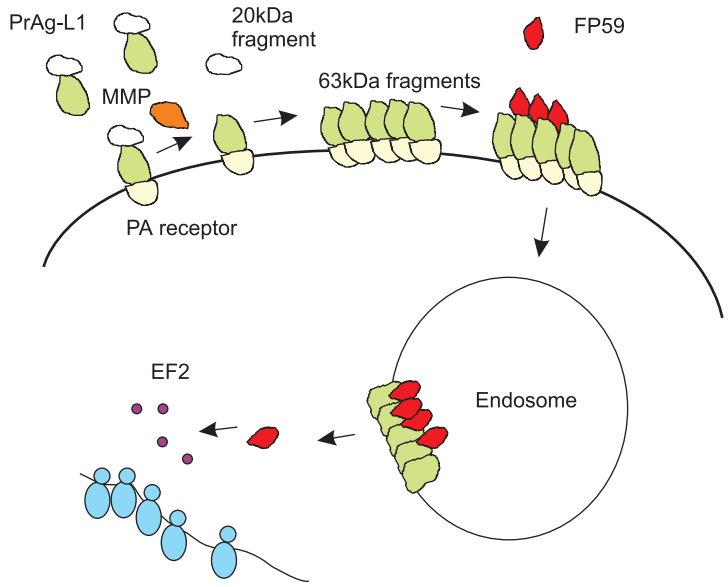

Fig. 3. Mechanism of action of anthrax PrAg-L1/FP59. (a) Binding of PrAg-L1 to the anthrax toxin receptor on the melanoma cell; (b) Proteolytic cleavage and release of PA_{20} by the MMP; (c) Oligomerization of PA_{63} to heptamers; (d) Binding of up to three FP59 molecules to the heptamer; (e) Receptor-mediated endocytosis; (f) Acidification in the endosome and membrane insertion of the PA_{63} heptamer to form a 14-strand β-barrel pore; (g) Translocation of FP59 to the cytosol; (h) FP59 ADP-ribosylation of elongation factor 2 (EF2) leading to inactivation of cytosolic protein synthesis and cell death.

Table II: Melanoma animal models.

Name	Transplantable cells	Metastases	Species	Ref.
B16-C57Bl/6J	Yes	Yes	Mouse	51
S91-DBA or C57Bl/6J	Yes	Yes	Mouse	54
Harding-Passey-C57Bl/6J or ICR	Yes	No	Mouse	55
Ma/Ab-Syrian golden hamster	Yes	Yes	Hamster	56
D12,R18,U25,A7-nude mice	Yes	Yes	Human/mouse	57
PSM-*Xiphophorus*	No	No	Fish	58
L2-weaning *Monodelphis*	Yes	Yes	Opossum	59
K1735-C3H	Yes	Yes	Mouse	60
JB/RH-C57Bl/6J	Yes	Yes	Mouse	61
Tyr-SV40E mice	No	Yes	Mouse	62
DMBA-AxC rats	No	Yes	Rat	63
M4Be,7GP,T1P26-newborn rats	Yes	Yes	Human/rat	64
GP1,GP5,GP6,GP8-guinea pigs	No	Yes	Guinea pig	65
UISO-SSCM433, UISO-SSCM438-newborn swine	No	No	Swine	66
IGR-37, IGR-39-nude mice	Yes	No	Human/mouse	67
RPMI1846-newborn hamster	Yes	No	Hamster	68
A2058-nude mice	Yes	No	Human/mouse	69
C32-nude mice	Yes	No	Human/mouse	70
A375-nude mice	Yes	No	Human/mouse	71
Hs695T, Hs294T-nude mice	Yes	No	Human/mouse	72
HT144,Malme-3M,MeWo,SK-MEL1-nude mice	Yes	No	Human/mouse	73
MT-HGF + perinatal UV transgenic mice	No	Yes	Mouse	46
Tyr-H-ras + Ink4a/Arf2/3 transgenic mice	No	No	Mouse	74
Transgene B transgenic mice	No	Yes	Mouse	75
CDK4R24C knockin mice	No	Yes	Mouse	76

domain of Pseudomonas exotoxin (44). A schema for the mechanism of cell intoxication by the PrAg-L1/FP59 proteins is shown in Figure 3. The PrAg-L1/FP59 proteins showed an IC_{50} of 4 ng/ml or 40 pM PrAg-L1 and 1,000 pM LF. Among the 3 conjugates, the modified anthrax toxins showed the greatest potency *in vitro*, and we await *in vivo* efficacy data. Exciting animal model data have been generated with a urokinase cleavage site modified protective antigen PrAg-U2/FP59 combination (45). We hope similar or better activity will be seen with the PrAg-L1/FP59 combination in melanoma models.

Melanoma animal models

Numerous animal models have been developed for melanoma that can be used for testing of novel agents such as the MMP-targeted proteins (46-51). A partial list of the animal models include Sinclair swine, Camargue horses, Angora goats, *Xiphorphorus* fish, the marsupial opossum, guinea pig strains, Syrian hamster strains, transgenic mice, human melanoma xenografts and the B16 murine melanoma-C57/Bl6 model. The B16 model has been the most extensively used for evaluation of melanoma metastases. A list of some of the melanoma animal models is shown in Table II with an emphasis on rapid, reproducible models with transplantable cells and metastases. These characteristics facilitate testing of therapeutic agents.

Conclusions

The limited clinical efficacy of MMP inhibitors in melanoma and other MMP-expressing tumors may be attributable in part both to the cytostatic nature of the agents and the multiple protease pathways that may bypass the inhibitor (52). In contrast, catalytic toxins activated by MMP-expressing cells will likely be more potent agents. Further, the peptide sequence may be modified to improve specificity for particular MMPs. By combining melanoma specificity for MMPs with the intracellular signaling pathway target of MAPKK proteins, further improvements in the therapeutic index may be achieved. This may be accomplished by combining an MMP-activated moiety (such as the modified anthrax protective antigen) with an enzyme selective for melanoma cells (such as anthrax lethal factor) (50). Efficacy of MMP activation can be further enhanced by modification of the MMP cleavage sites within the targeted toxins, as has been demonstrated by various techniques, to minimize killing of nontumor cells and to increase MMP sensitivity and selectivity. Both tissue culture and animal model testing will be needed. Nevertheless, exciting targeted therapeutic proteins most likely will have an impact on the treatment of patients with this currently incurable disease.

References

1. Jemal, A., Murray, T., Samuels, A., Chafoor, A., Ward, E., Thun, M.J. *Cancer statistics 2003*. CA Cancer J Clin 2003, 53: 5-26.

2. Brown, C.K., Kirkwood, J.M. *Medical management of melanoma.* Surg Clin N Am 2003, 83: 283-322.

3. Keilholz, U., Gore, M.E. *Biochemotherapy for advanced melanoma.* Semin Oncol 2002, 29: 456-61.

4. Masci, P., Borden, E.C. *Malignant melanoma: Treatments emerging, but early detection is still key.* Cleveland Clinic J Med 2002, 69: 529-45.

5. Chin, L. *The genetics of malignant melanoma: Lessons from mouse and man.* Nat Rev Cancer 2003, 3: 559-70.

6. Herlyn, M., Padarathsingh, M., Chin, L. et al. *New approaches to the biology of melanoma: A workshop of the National Institutes of Health Pathology B Study Section.* Am J Pathol 2002, 161: 1949-57.

7. Dong, J., Phelps, R.G., Qiao, R., Yao, S., Benard, O., Ronai, Z., Aaronson, S.A. *BRAF oncogenic mutations correlate with progression rather than initiation of human melanoma.* Cancer Res 2003, 63: 3883-5.

8. Gorden, A., Osman, I., Gai, W. et al. *Analysis of BRAF and N-RAS mutations in metastatic melanoma tissues.* Cancer Res 2003, 63: 3955-7.

9. Sturm, R.A. *Skin colour and skin cancer: MC1R, the genetic link.* Melanoma Res 2002, 12: 405-16.

10. Zhang, Y.W., Vande Woude, G.F. *HGF/SF-Met signaling in the control of branching morphogenesis and invasion.* J Cell Biochem 2003, 88: 408-17.

11. Cruz, J., Reis-Filho, J.S., Silva, P., Lopes, J.M. *Expression of c-met tyrosine kinase receptor is biologically and prognostically relevant for primary cutaneous malignant melanomas.* Oncology 2003, 65: 72-82.

12. Maniotis, A.J., Folberg, R., Hess, A. et al. *Vascular channel formation by human melanoma cells in vivo and in vitro: Vasculogenic mimicry.* Am J Pathol 1999, 155: 739-52.

13. Hendrix, M.J.C., Seftor, E.A., Meltzer, P.S. et al. *Expression and functional significance of VE-cadherin in aggressive human melanoma cells: Role in vasculogenic mimicry.* Proc Natl Acad Sci USA 2001, 98: 8018-23.

14. Hendrix, M.J.C., Seftor, E.A., Kirschmann, D.A., Quaranta, V., Seftor, R.E.B. *Remodeling of the microenvironment by aggressive melanoma tumor cells.* Ann NY Acad Sci 2003, 995: 151-61.

15. Tsuj, T., Kawada, Y., Kai-Murozono, M. et al. *Regulation of melanoma cell migration and invasion by laminin-5 and $\alpha_3\beta_1$ integrin (VLA-3).* Clin Exp Metastasis 2002, 19: 127-34.

16. Kuratomi, Y., Nomizu, M., Tanaka, K., Ponce, M.L., Komiyama, S., Kleinman, H.K., Yamada, Y. *Laminin gamma 1 chain peptide, C-16 (KAFDITYVRLKF), promotes migration, MMP-9 secretion, and pulmonary metastases of B16-F10 mouse melanoma cells.* Br J Cancer 2002, 86: 1169-73.

17. Xu, J., Rodriguez, D., Petitclerc, E. et al. *Proteolytic exposure of a cryptic site within collagen type IV is required for angiogenesis and tumor growth in vivo.* J Cell Biol 2001, 154: 1069-79.

18. Brinckerhoff, C.E., Matrisian, L.M. *Matrix metalloproteinases: A tail of a frog that became a prince.* Nat Rev Mol Cell Biol 2002, 3: 207-14.

19. Pei, D., Kang, T., Qi, H. *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 2000, 275: 33988-97.

20. Wandel, E., Raschke, A., Hildebrandt, G., Eberle, J., Dummer, R., Anderegg, U., Saalbach, A. *Fibroblasts enhance the invasive capacity of melanoma cells in vitro.* Arch Dermatol Res 2002, 293: 601-8.

21. Philip, S., Kundu, G.C. *Osteopontin induces nuclear factor kappa B-mediated promatrix metalloproteinase-2 activation through I κ B α/IKK signaling pathways, and curcumin (diferulolylmethane) downregulates these pathways.* J Biol Chem 2003, 278: 14487-97.

22. Ho, L.L., Chen, W.J., Liin-Shiau, S.Y., Lin, J.K. *Penta-O-galloyl-β-D-glucose inhibits the invasion of mouse melanoma by suppressing metalloproteinase-9 through down-regulation of activator protein-1.* Eur J Pharmacol 2002, 453: 149-58.

23. Ishii, Y., Ogura, T., Tatemichi, M., Fujisawa, H., Otsuka, F., Esumi, H. *Induction of matrix metalloproteinase gene transcription by nitric oxide and mechanisms of MMP-1 gene induction in human melanoma cell lines.* Int J Cancer 2003, 103: 161-8.

24. Tower, G.B., Coon, C.C., Benbow, U., Vincenti, M.P., Brinckerhoff, C.E. *Erk 1/2 differentially regulates the expression from the 1G/2G single nucleotide polymorphism in the MMP-1 promoter in melanoma cells.* Biochim Biophys Acta 2002, 1586: 265-74.

25. Ge, X., Fu, Y.M., Meadows, G.G. *U0126, a mitogen-activated protein kinase kinase inhibitor, inhibits the invasion of human A375 melanoma cells.* Cancer Lett 2002, 179: 133-40.

26. Govindarajan, B., Bai, X., Cohen, C. et al. *Malignant transformation of melanocytes to melanoma by constitutive activation of mitogen-activated protein kinase kinase (MAPKK) signaling.* J Biol Chem 2003, 278: 9790-5.

27. Matsumoto, K., Takayama, N., Ohnishi, J., Ohnishi, E., Shirayoshi, Y., Nakatsuji, N., Ariga, H. *Tumour invasion and metastasis are promoted in mice deficient in tenascin-X.* Genes Cells 2001, 6: 1101-11.

28. Chen, E.I., Kridel, S.J., Howard, E.W., Li, W., Godzik, A., Smith, J.W. *A unique substrate recognition profile for matrix metalloproteinase-2.* J Biol Chem 2002, 277: 4485-91.

29. Kridel, S.J., Chen, E., Kotra, L.P., Howard, E.W., Mobashery, S., Smith, J.W. *Substrate hydrolysis by matrix metalloproteinase-9.* J Biol Chem 2001, 276: 20572-8.

30. Kridel, S.J., Sawai, H., Ratnikov, B.I. et al. *A unique substrate binding mode discriminates membrane type-1 matrix metalloproteinase from other matrix metalloproteinases.* J Biol Chem 2002, 277: 23788-93.

31. Liotta, L.A., Tryggvason, K., Garbisa, S., Hart, I., Foltz, C.M., Shafie, S. *Metastatic potential correlates with enzymatic degradation of basement membrane collagen.* Nature 1980, 284: 67-8.

32. Vaisanen, A., Touminen, H., Kallioinen, M., Turpeeenniemihujanen, T. *Matrix metalloproteinase-2 (72 kD type IV collagenase) expression occurs in the early stage of human melanocytic tumour progression and may have prognostic value.* J Pathol 1996, 180: 283-9.

33. Kurschat, P., Wickenhauser, C., Groth, W., Krieg, T., Mauch, C. *Identification of activated matrix metalloproteinase-2 (MMP-2) the main gelantinolytic enzyme in malignant melanoma by in situ zymography.* J Pathol 2002, 197: 179-87.

34. Simonetti, O., Lucarini, G., Brancorsini, D., Nita, P., Bernardini, M.L., Biagini, G., Offidani, A. *Immunohistochemical expression of vascular endothelial growth factor, matrix metalloproteinase 2, and matrix metalloproteinase 9 in cutaneous melanocytic lesions.* Cancer 2002, 95: 1963-70.

35. Giambernardi, T.A., Sakaguchi, A.Y., Gluhak, J. et al. *Neutrophil collagenase (MMP-8) is expressed during early development in neural crest cells as well as in adult melanoma cells.* Matrix Biol 2001, 20: 577-87.

36. Nikkola, J., Vihinen, P., Vlaykova, T., Hahka-Kemppinen, M., Kahari, V.M., Pyrhonen, S. *High expression levels of collagenase-1 and stromelysin-1 correlate with shorter disease-free survival in human metastatic melanoma.* Int J Cancer 2002, 97: 432-8.

37. Sounni, N.E., Baramova, E.N., Munaut, C., Maquoi, E., Frankenne, F., Foidart, J.M., Noel, A. *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 2002, 98: 23-8.

38. Kurschat, P., Zigrino, P., Nischt, R. et al. *Tissue inhibitor of matrix metalloproteinase-2 regulates matrix metalloproteinase-2 activation by modulation of membrane-type 1 matrix metalloproteinase activity in high and low invasive melanoma cell lines.* J Biol Chem 1999, 274: 21056-62.

39. Fiebig, H.H., Kostermeyer, A., Schuler, J.B., Burger, A. *Characterization of matrix metalloproteinases in 47 human tumor xenografts show high expression of MMP-2 in melanomas and sarcomas.* Proc Am Assoc Cancer Res 1999, 40: 463.

40. Van der Velden, P.A., Zuidervaart, W., Hurks, M.H. et al. *Expression profiling reveals that methylation of TIMP3 is involved in uveal melanoma development.* Int J Cancer 2003, 106: 472-9.

41. Mansour, A.M., Drevs, J., Esser, N., Hamada, F.M., Badary, O.A., Unger, C., Fichtner, I., Kratz, F. *A new approach for the treatment of malignant melanoma: Enhanced antitumor efficacy of an albumin-binding doxorubicin prodrug that is cleaved by matrix metalloproteinase 2.* Cancer Res 2003, 63: 4062-6.

42. Holle, L., Song, W., Holle, E., Wei, Y., Wagner, T., Yu, X. *A matrix metalloproteinase 2 cleavable melittin/avidin conjugate specifically targets tumor cells in vitro and in vivo.* Int J Oncol 2003, 22: 93-8.

43. Liu, S., Netzel-Arnett, S., Birkedal-Hansen, H., Leppla, S.H. *Tumor cell-selective cytotoxicity of matrix metalloproteinase-activated anthrax toxin.* Cancer Res 2000, 60: 6061-7.

44. Arora, N., Leppla, S.H. *Residues 1-254 of anthrax toxin lethal factor are sufficient to cause cellular uptake of fused polypeptides.* J Biol Chem 1993, 268: 3334-41.

45. Liu, S., Aaronson, H., Mitola, D.J., Leppla, S.H., Bugge, T.H. *Potent antitumor activity of a urokinase-activated engineered anthrax toxin.* Proc Natl Acad Sci USA 2003, 100: 657-62.

46. Noonan, F.P., Dudek, J., Merlino, G., De Fabo, E.C. *Animal models of melanoma: An HGF/SF transgenic mouse model may facilitate experimental access to UV initiating events.* Pigment Cell Res 2003, 16: 16-25.

47. Bardeesy, N., Wong, K.K., DePinho, R.A., Chin, L. *Animal models of melanoma: Recent advances and future prospects.* Adv Cancer Res 2000, 79: 123-56.

48. Kusewitt, D.F., Ley, R.D. *Animals models in melanoma.* Cancer Surv 1996, 25: 35-70.

49. Ito, A., Watabe, K., Koma, Y., Kitamura, Y. *An attempt to isolate genes responsible for spontaneous and experimental metastasis in the mouse model.* Histol Histopathol 2002, 17: 951-9.

50. Koo, H.-M., VanBrocklin, M., McWilliams, M.J., Leppla, S.H., Duesbery, N.S., Vande Woude, G.F. *Apoptosis and melanogenesis in human melanoma cells induced by anthrax lethal factor inactivation of mitogen-activated protein kinase kinase.* Proc Natl Acad Sci USA 2002, 99: 3052-7.

51. Ortega, A., Ferrer, P., Carretero, J., Obrador, E., Asensi, M., Pellicer, J.A., Estrela, J.M. *Down-regulation of glutathione and Bcl-2 synthesis in mouse B16 melanoma cells avoids their survival during interaction with the vascular endothelium.* J Biol Chem, in press.

52. Nelson, A.R., Fingleton, B., Rothenberg, M.L., Matrisian, L.M. *Matrix metalloproteinases: Biological activity and clinical implications.* J Clin Oncol 2000, 18: 1135-49.

53. Elkins, P.A., Ho, Y.S., Smith, W.W. et al. *Structure of the C-terminally truncated human proMMP9, a gelatin-binding matrix metalloproteinase.* Acta Crystallographica 2002, D58: 1182-92.

54. Weinzweig, J., Tattini, C., Lynch, S., Zienowicz, R., Weinzweig, N., Spangenberger, A., Edstrom, L. *Investigation of the growth and metastasis of malignant melanoma in a murine model: The role of supplemental vitamin A.* Plast Reconstr Surg 2003, 112: 152-8.

55. Toda, M., Martuza, R.L., Rabkin, S.D. *Tumor growth inhibition by intratumoral inoculation of defective herpes simplex virus vectors expressing granulocyte-macrophage colony-stimulating factor.* Mol Ther 2000, 2: 324-9.

56. Kozlowska, K., Cichorek, M., Zarzeczna, M., Brozek, J., Witkowski, J.M. *Heterogenous susceptibility to spontaneous and induced apoptosis characterizes two related transplantable melanomas with different biological properties.* Pigment Cell Res 2002, 15: 233-8.

57. Rofstad, E.K., Henriksen, K., Galappathi, K., Mathiesen, B. *Antiangiogenic treatment with thrombospondin-1 enhances primary tumor radiation response and prevents growth of dormant pulmonary micrometastases after curative radiation therapy in human melanoma xenografts.* Cancer Res 2003, 63: 4055-61.

58. Wakamatsu, Y. *Establishment of a cell line form the platyfish-swordtail hybrid melanoma.* Cancer Res 1981, 41: 679-80.

59. Rohman, E.S., Dooley, T.P. *A new allogeneic model for metastatic melanoma.* Eur J Cancer 1995, 31A: 2302-8.

60. Hellstrom, K.E., Hellstrom, I. *Therapeutic vaccination with tumor cells that engage CD137.* J Mol Med 2003, 81: 71-86.

61. Berkelhammer, J., Luethans, T.N., Hook, R.R., Oxenhandler, R.W. *Phenotypic instability of mouse melanomas after propagation in vivo and in vitro.* Cancer Res 1986, 46: 2923-8.

62. Bradl, M., Klein-Szanto, A., Porter, S., Mintz, B. *Malignant melanoma in transgenic mice.* Proc Natl Acad Sci USA 1991, 88: 164-8.

63. Iglesias, R., Salinas, S. *Transplantable melanotic tumors induced in AxC rats by a single feeding of DMBA.* J Invest Dermatol 1970, 54: 89-92.

64. Boukerche, H., Benchaibi, M., Berthier-Vergnes, O., Lizard, G., Bailly, M., Bailly, M., McGregor, J.L. *Two human melanoma*

cell-line variants with enhanced in vivo tumor growth and metastatic capacity do not express the beta 3 integrin subunit. Eur J Biochem 1994, 220: 485-91.

65. Liao, S.K., Smith, J.W., Kwong, P.C., Natali, P.G., Kusama, M., Hamby, C.V., Ferrone, S. *Cross-reactivity of murine anti-human high molecular weight melanoma associated antigen monoclonal antibodies with guinea pig melanoma cells.* Cancer Res 1987, 47: 4835-41.

66. Green, A., Shilkaitis, A., Bratescu, L., Amoss, M.S., Beattie, C.W. *Establishment and characterization of four Sinclair swine cutaneous malignant melanoma cell liens.* Cancer Genet Cytogenet 1992, 61: 77-92.

67. Aubert, C., Rouge, F., Galindo, J.R. *Tumorigenicity of human malignant melanocytes in nude in relation to their differentiation in vitro.* JNCI 1980, 64: 1029-40.

68. Moore, G.E. *Culture of malignant tumors of the Syrian hamster.* J Natl Cancer Inst 1963, 31: 1217-37.

69. Stetler-Stevenson, W.G., Krutzsch, H.C., Liotta, L.A. *Tissue inhibitor of metalloproteinase (TIMP-2). A new member of the metalloproteinase inhibitor family.* J Biol Chem 1989, 264: 17374-8.

70. Chen, T.R. *Evolution in vitro of stemlines with minimal karyotypic deviation in a human heteroploid cell line.* J Natl Cancer Inst 1978, 61: 277-84.

71. Gershwin, M.E., Ikeda, R.M., Kawakami, T.G., Owens, R.B. *Immunobiology of heterotransplanted human tumors in nude mice.* J Natl Cancer Inst 1977, 58: 1455-63.

72. Creasey, A.A., Smith, H.S., Hackett, A.J., Fukuyama, K., Epstein, W.L., Madin, S.H. *Biological properties of human melanoma cells in culture.* In Vitro 1979, 15: 342-50.

73. Fogh, J., Fogh, J.M., Orfeo, T. *One hundred and twenty-seven cultured human tumor cell lines producing tumors in nude mice.* J Natl Cancer Inst 1977, 59: 221-6.

74. Chin, L., Pomerantz, J., Polsky, D., Jacobson, M., Cohen, C., Cordon-Cardo, C., Horner, J.W., DePinho, R.A. *Cooperative effects of INK4a and ras in melanoma susceptibility in vivo.* Genes Dev 1997, 11: 2822-34.

75. Zhu, H., Reuhl, K., Zhang, X., Botha, R., Ryan, K., Wei, J., Chen, S. *Development of heritable melanoma in transgenic mice.* J Invest Dermatol 1998, 110: 247-52.

76. Sotillo, R., Garcia, J.F., Ortega, S., Martin, J., Dubus, P., Barbacid, M., Malumbres, M. *Invasive melanoma in Cdk4-targeted mice.* Proc Natl Acad Sci USA 2001, 98: 13312-7.

77. Walker, R.A., Woolley, D.E. *Immunolocalisation studies of matrix metalloproteinases-1,-2, and -3 in human melanoma.* Virchows Arch 1999, 435: 574-9.

78. Airola, K., Karonen, T., Vaalamo, M. et al. *Expression of collagenase-1 and -3 and their inhibitors TIMP-1 and -3 correlates with the level of invasion in malignant melanoma.* Br J Cancer 1999, 80: 733-43.

79. Hofmann, U.B., Westphal, J.R., Zendman, A.J.W., Becker, J.C., Ruiter, D.J., van Muijen, G.N.P. *Expression and activation of matrix metalloproteinase-2 (MMP-2) and its co-localization with membrane-type I matrix metalloproteinase (MT1-MMP) correlate with melanoma progression.* J Pathol 2000, 191: 245-56.

80. Thewes, M., Worret, W.I., Engst, R., Ring, J. *Stromelysin-3 (ST-3) immunohistochemical characterization of the matrix metalloproteinase (MMP)-11 in benign and malignant skin tumours and other skin disorders.* Clin Exp Dermatol 1999, 24: 122-6.

81. van den Oord, J.J., Paemen, L., Opdenakker, G., de Wolf Peeters, C. *Expression of gelatinase B and the extracellular matrix metalloproteinase inducer EMMPRIN in benign and malignant pigment cell lesions of the skin.* Am J Pathol 1997, 151: 665-70.

82. Giambernardi, T.A., Sakaguchi, A.Y., Gluhak, J., Pavlin, D., Troyer, D.A., Das, G., Rodeck, U., Klebe, R.J. *Neutrophil collagenase (MMP-8) is expressed during early development in neural crest cells as well as in adult melanoma cells.* Matrix Biol 2001, 20: 577-87.

83. Woolley, D.E., Grafton, C.A. *Collagenase immunolocalization studies of cutaneous secondary melanomas.* Br J Cancer 1980, 42: 260-5.