

Structural and functional characterization of the
membrane type 4 matrix metalloproteinase
(MT4-MMP/ MMP17)

**Dissertation zur Erlangung des Doktorgrades
der Naturwissenschaften (Dr. rer. nat.)**

**Fakultät Naturwissenschaften
Universität Hohenheim**

Institut für Biologische Chemie und Ernährungswissenschaften

Prof. Dr. Lutz Graeve

Vorgelegt von
Bettina Hieronimus

Aus Herrenberg

2016

Contents

1	Introduction	5
1.1	Metalloproteases	5
1.1.1	Matrix Metalloproteinases	6
1.1.2	Membrane-Type 4 Matrix Metalloproteinase	8
1.2	Migration and Invasion	10
1.2.1	Extracellular Matrix and Integrins in Cell Migration.....	11
1.2.2	Small GTPases	12
1.3	Melanoma	15
1.3.1	Melanocytes.....	15
1.3.2	Melanoma genesis	16
1.4	Objectives	16
2	Materials	19
2.1	Cell Lines	19
2.2	Antibodies	20
2.3	Reagents and Buffers	20
2.3.1	Cell Biology Methods	20
2.3.2	Molecular Biology Methods	22
2.3.3	Protein Biochemistry Methods	24
2.4	Laboratory Equipment	26
2.5	Software	26
3	Methods	27
3.1	Tissue Sample Collection	27
3.2	Cell Biology Methods	27
3.2.1	Cultivation of Cells	27
3.2.2	Trypsinizing and Subculturing of Cells.....	28
3.2.3	Freezing and Thawing of Cells.....	28
3.2.4	Mycoplasma Testing	28

- 3.2.5 Lysates for WB.....29
- 3.2.6 RNA Isolation.....29
- 3.2.7 Immunocytochemistry (ICC).....29
- 3.2.8 Transfection and Creation of Stable Cell Clones30
- 3.2.9 Proteinase K digest of surface proteins.....31
- 3.2.10 Biotin labeling of surface proteins32
- 3.2.11 Shedding of GPI-anchored proteins with PI-PLC32
- 3.2.12 MTT Assay32
- 3.2.13 *In vitro* scratch assay/ wound healing assay33
- 3.2.14 An agarose spot assay for chemotactic invasion.....33
- 3.2.15 Colony formation assay.....33
- 3.2.16 Stimulation of cells.....34
- 3.3 Molecular Biology Methods34
 - 3.3.1 Transformation of *Escherichia Coli*.....34
 - 3.3.2 DNA Preparation34
 - 3.3.3 Side directed mutagenesis35
 - 3.3.4 Quantitative PCR35
- 3.4 Protein Biochemistry Methods.....36
 - 3.4.1 Protein determination and sample preparation36
 - 3.4.2 SDS-PAGE36
 - 3.4.3 Western blotting36
 - 3.4.4 Membrane Stripping37
 - 3.4.5 Separation of detergent resistant membranes (DRM).....37
 - 3.4.6 Subcellular fractionation37
 - 3.4.7 Nucleus cytosol fractionation.....38
 - 3.4.8 PNGase F digest.....38
- 3.5 Data analyzes38
- 4 Results.....39
 - 4.1 Which model to choose?.....39
 - 4.1.1 MT4-MMP expression in cancer cell lines.....39
 - 4.1.2 MT4-MMP in melanoma and skin tissue samples39
 - 4.2 Subcellular localization of the MT4-MMP protein variants.....41

4.2.1 Digestion of membrane bound proteins by proteinase K	41
4.2.2 Labeling of extracellular proteins with biotin	42
4.2.3 Shedding of MT4-MMP by PI-PLC	43
4.2.4 The MT4-MMP variants locate to different membrane domains	43
4.2.5 The 69 kDa variants of MT4-MMP reside in the ER/Golgi compartment.....	44
4.2.6 MT4-MMP is not located in the nucleus	45
4.3 Understanding the nature of the MT4-MMP variants	46
4.3.1 Cleavage of the pro-domain by furin is needed for MT4-MMP processing into the 58 kDa forms.....	47
4.3.2 Half-life of the MT4-MMP forms	48
4.3.3 The MT4-MMP is N-glycosylated	49
4.3.4 The proliferation of MDCK clones is unaffected by MT4-MMP expression	51
4.3.5 Sorting of MT4-MMP in stably transfected MDCK cells	52
4.4 MT4-MMP knockdown in SK-Mel-28 cells with shRNA	53
4.4.1 Generation of MT4-MMP knockdown clones	54
4.4.2 MT4-MMP knockdown does not influence the proliferation of the SK-Mel-28 clones	55
4.4.3 Migration assays	56
4.4.4 Migration associated proteins	59
4.4.5-MT4-MMP increases the clonogenic capacity of SK-Mel-28 cells (Colony formation assay)	61
5 Discussion	63
5.1 Protein processing of MT4-MMP.....	63
5.2 MT4-MMP influences the migration of SK-Mel-28 cells	71
Summary	77
Zusammenfassung	79
Bibliography	81
Appendices.....	95
A Additional Data	96
A.1 Methods.....	96
A.2 Results.....	97
B List of Figures	99

C List of Tables.....	101
D Glossary and Acronyms	103
E Curriculum Vitae	105
F Eidesstattliche Versicherung	107