



William Christopher Lamanna (Autor)

Functional characterization of the novel heparan sulfate 6O-endosulfatases Sulf1 and Sulf2

Functional characterization of the novel heparan sulfate 6O-endosulfatases Sulf1 and Sulf2

Zur Erlangung des Doktorgrades der Mathematisch -
Naturwissenschaftlichen Fakultäten der Georg-August-
Universität zu Göttingen

vorgelegt von
William Christopher Lamanna
aus Chapel Hill, North Carolina, USA

Göttingen 2008

<https://cuvillier.de/de/shop/publications/1424>

Copyright:

Cuvillier Verlag, Inhaberin Annette Jentzsch-Cuvillier, Nonnenstieg 8, 37075 Göttingen,
Germany

Telefon: +49 (0)551 54724-0, E-Mail: info@cuvillier.de, Website: <https://cuvillier.de>

Contents

Contents	I
1 Summary.....	1
2 Introduction.....	3
2.1 The sulfatase family.....	3
2.2 Heparan sulfate proteoglycans.....	5
2.2.1 Heparan sulfate proteoglycan biosynthesis.....	6
2.2.2 HSPG regulation of cell signaling	8
2.2.3 HSPG modulation of morphogen gradients.....	10
2.2.4 HSPG mediation of cellular adhesion.....	11
2.2.5 HSPG mediation of endocytosis and membrane trafficking	12
2.2.6 Knock out models for the HSPG biosynthetic machinery	13
2.3 Sulf1 and Sulf2	15
2.3.1 Sulf domain structure and function.....	16
2.3.2 Sulf regulation of cell signaling.....	17
2.3.3 The Sulf s and carcinogenesis.....	19
2.3.4 The Sulf s and mammalian development.....	20
2.4 Specific aims of this study	21
3 Materials	22
3.1 Laboratory equipment.....	22
3.2 Laboratory materials	23
3.3 Chemicals and reagents	24
3.4 Radioisotopes.....	27
3.5 Kits.....	27
3.6 Buffers and solutions	27
3.7 Mammalian cell culture medium	29
3.8 Bacterial cell culture medium	30
3.9 Mouse lines	31
3.10 Mammalian and bacterial cell lines	31

3.11	Enzymes	31
3.12	Antibodies	32
3.13	Primers for real time PCR analysis of MEFs	32
3.14	Primers for generating human Sulf mutants.....	32
3.15	Plasmids	32
3.16	DNA and protein markers	32
3.17	Software	32
4	Methods	32
4.1	Molecular biological methods.....	32
4.1.1	Determination of DNA and RNA concentrations	32
4.1.2	Generation of Sulf1-C87A/C88A and Sulf2-C88A/C89A mutants	32
4.1.3	Agarose gel electrophoresis	32
4.1.4	Purification of plasmid DNA from <i>Escherichia coli</i>	32
4.1.5	Restriction enzyme digests.....	32
4.1.6	Isolation of total RNA from MEF cell lines.....	32
4.1.6.1	Preparation of MEFs for RNA isolation	32
4.1.6.2	Isolation of total RNA from MEFs	32
4.1.6.3	DNaseI treatment of RNA samples.....	32
4.1.7	Microarray analysis	32
4.1.7.1	First strand sscDNA synthesis from total RNA	32
4.1.7.2	Generation of dscDNA from sscDNA	32
4.1.7.3	Generation of amino-modified cDNA oligonucleotide probes	32
4.1.7.4	Fluorescent dye coupling to amino-modified cDNA oligos	32
4.1.7.5	Microarray hybridization and analysis.....	32
4.1.8	Real time PCR expression analysis.....	32
4.1.8.1	Real time PCR primer design and specificity	32
4.1.8.2	Real time PCR primer efficiency analysis	32
4.1.8.3	Real time PCR analysis	32
4.2	Bacterial and mammalian cell culture methods	32
4.2.1	Generation of chemically competent <i>Escherichia coli</i>	32
4.2.2	Transformation of chemically competent <i>Escherichia coli</i>	32

4.2.3	Glycerol stocks of <i>Escherichia coli</i>	32
4.2.4	Mammalian cells and culture conditions	32
4.2.5	Cryo-conservation and thawing of mammalian cell lines	32
4.2.6	Transfection of mammalian cell lines.....	32
4.3	Biochemical and cell biological methods	32
4.3.1	Bradford determination of protein concentration	32
4.3.2	Scintillation counting of radiolabeled samples	32
4.3.3	SDS polyacrylamide electrophoresis (SDS-PAGE)	32
4.3.4	Western blotting.....	32
4.3.5	Coating of glass plates or coverslips with poly-L-lysine and laminin....	32
4.3.6	Immunofluorescence microscopy	32
4.3.7	Growth factor mitogenesis and cell signaling.....	32
4.3.8	FACS analysis of cell surface HSPGs	32
4.3.9	Membrane association and detergent solubility of the Sulf.....	32
4.3.10	Sulf secretion analysis in HT1080 cells.....	32
4.3.11	Sulf <i>in vitro</i> activity analysis	32
4.4	Glycobiological methods	32
4.4.1	Preparation of bacterial GAG lyases	32
4.4.2	Heparan sulfate proteoglycan profiling	32
4.4.2.1	Cell surface heparan sulfate proteoglycan profiling	32
4.4.2.2	Glypican heparan sulfate proteoglycan profiling.....	32
4.4.2.3	Shed heparan sulfate proteoglycan profiling	32
4.4.2.4	Extracellular matrix heparan sulfate proteoglycan profiling	32
4.4.3	Heparan sulfate purification.....	32
4.4.3.1	Metabolic $^3\text{H}/^{35}\text{S}$ radiolabeling of GAG chains	32
4.4.3.2	Extraction of radiolabeled total cell and shed HSPGs	32
4.4.3.3	Extraction of radiolabeled cell surface HSPGs.....	32
4.4.3.4	Extraction of radiolabeled GPI-anchored HSPGs	32
4.4.3.5	Extraction of radiolabeled ECM associated HSPGs.....	32
4.4.3.6	Removal of free label from GAG fractions	32
4.4.3.7	Elution of radiolabeled GAGs from DEAE columns	32

4.4.3.8	Re-concentration of GAGs following DEAE elution	32
4.4.3.9	Purification of full length HS from CS/DS GAGs.....	32
4.4.4	Heparan sulfate analysis.....	32
4.4.4.1	Heparan sulfate disaccharide composition analysis	32
4.4.4.2	Heparan sulfate heparinase III domain analysis.....	32
4.4.4.3	Heparan sulfate low pH nitrous acid domain analysis	32
5	Results	32
5.1	Sub-cellular localization of the Sulf	32
5.1.1	Immunofluorescence microscopy of Sulf expressing cell lines	32
5.1.2	Membrane association and detergent solubility of the Sulf.....	32
5.1.3	Secretion and proteolytic processing of Sulf1 and Sulf2	32
5.2	<i>In vitro</i> Sulf activity	32
5.2.1	Sulf <i>in vitro</i> activity from cell lysates	32
5.2.2	Secreted Sulf <i>in vitro</i> activity.....	32
5.3	The molecular phenotype of the Sulf1 and Sulf2 knock out.....	32
5.3.1	HS disaccharide analysis of primary Sulf knock out MEFs.....	32
5.3.2	The effect of Sulf loss on N-sulfate distribution	32
5.3.3	The effect of Sulf loss on HS transition zones	32
5.3.4	The effect of Sulf loss on S-domain composition	32
5.3.5	The effect of Sulf loss on mitogenic response	32
5.4	The effect of Sulf loss on topologically and functionally distinct heparan sulfate proteoglycans.....	32
5.4.1	Heparan sulfate proteoglycan profiling.....	32
5.4.2	The effect of Sulf loss on cell surface, shed, GPI-anchored and ECM heparan sulfate sulfation.....	32
5.4.3	The effect of Sulf loss on non-substrate <i>N</i> -, 2 <i>O</i> - and 6 <i>O</i> -sulfation.....	32
5.4.4	The effect of Sulf loss on FGF signaling	32
5.5	Genome wide microarray analysis of Sulf knock out MEFs	32
6	Discussion.....	32
6.1	The Sulfs are cell surface bound enzymes which can be secreted and proteolytically processed.....	32

6.2	Sulf1 and Sulf2 exhibit restricted 6 <i>O</i> -endosulfatase activity towards heparan sulfate S-domains <i>in vitro</i>	32
6.3	Discrete <i>in vivo</i> effects of Sulf1 and Sulf2 loss in primary MEFs	32
6.4	The Suls act cooperatively to modulate HS sulfation patterns of multiple functionally distinct HSPG substrates	32
6.5	Microarray analysis of Sulf knock out MEFs indicates extensive effects of Sulf loss on gene expression.....	32
6.6	Outlook	32
7	References.....	32
8	Appendix.....	xxxii
8.1	Sequences and alignments	xxxii
8.1.1	Protein sequence alignment of human Sulf1, Sulf2 and GlcNAc6S ..	xxxii
8.1.2	Protein and cDNA sequence of human Sulf1	xxxii
8.1.3	Protein and cDNA sequence of human Sulf1-C87A/C88A.....	xxxii
8.1.4	Protein and cDNA sequence of human Sulf2	xxxii
8.1.5	Protein and cDNA sequence of human Sulf2-C88A/C89A.....	xxxii
8.2	Heparan sulfate composition raw data.....	xxxii
8.2.1	<i>In vitro</i> heparan sulfate Sulf activity analysis raw data	xxxii
8.2.2	Primary wild-type, Sulf1 and Sulf2 knock out heparan sulfate disaccharide composition raw data	xxxii
8.2.3	Primary wild-type, Sulf1 and Sulf2 knock out heparinase III disaccharide fraction raw data	xxxii
8.2.4	Immortalized wild-type, Sulf1, Sulf2 and Sulf1/2 knock out HSPG disaccharide composition raw data	xxxii
8.3	Microarray commonality lists.....	xxxii
8.3.1	Gene targets common among Sulf1, Sulf2 and Sulf1/2 knock out microarray short list data sets	xxxii
8.4	List of figures.....	xxxii
8.5	List of tables.....	xxxii
8.6	Abbreviations.....	xxxii
9	Acknowledgements	xxxii
<i>Curriculum vitae</i>	xxxii