

Table of contents

Title	i
List of abbreviations	v
1 INTRODUCTION	1
1.1 CELL-CELL ADHESION	1
1.2 THE CADHERIN SUPERFAMILY	1
1.2.1 Classical cadherin	3
1.2.2 7D-cadherins	4
1.3 LI-CADHERIN	5
1.4 REGULATION OF THE EXPRESSION OF CLASSICAL CADHERINS AND 7D-CADHERINS	7
1.5 GENE SPECIFIC EXPRESSION IN INTESTINAL EPITHELIAL CELLS	8
1.6 AIM	11
2 MATERIAL AND METHODS	11
2.1 MATERIAL	11
2.1.1 Equipment	11
2.1.2 Consumables	11
2.1.3 Reagents	12
2.1.4 Buffers	13
2.1.5 Enzymes	14
2.1.6 Reaction kits	15
2.1.7 Antibodies	15
2.1.8 Oligonucleotides	15
2.1.9 Molecular size and weight standards	16
2.1.10 DNA plasmids	16
2.1.11 Bacteria	16
2.1.12 Cell lines	16
2.2 METHODS: MOLECULAR BIOLOGY	18
2.2.1 Purification of DNA	18
2.2.2 Isolation of DNA from agarose gel	18
2.2.3 Quantification of nucleic acids	19
2.2.4 PCR amplification	19
2.2.5 Nucleic acid gel electrophoresis	22
2.2.6 Digestion of DNA with restriction endonucleases	22
2.2.7 Dephosphorylation of DNA	23
2.2.8 Ligation of DNA	23
2.2.9 Transformation of <i>E.coli</i>	23
2.2.10 Plasmid-DNA preparation	24
2.2.11 DNA sequencing	24
2.2.12 RNA isolation	25
2.2.13 Rapid amplification of cDNA ends (RACE)	26
2.2.14 Primer extension	26
2.2.15 Generation of nested sets of deletions with Exonuclease III	26
2.2.16 Site-directed mutagenesis	27
2.2.17 Electrophoretic mobility shift assay	31
2.3 METHODS: CELL BIOLOGY	34
2.3.1 Cultivation of eukaryotic cells	34
2.3.2 Transfection of DNA into cultured eukaryotic cells	34
2.3.3 Fluorescence microscopy	36
2.3.4 Determination of promoter activity	36
2.3.5 Indirect immunofluorescence of murine colon carcinoma cells	37
2.3.6 Preparation of nuclear extracts from cell cultures	37
2.4 METHODS: PROTEIN CHEMISTRY	38
2.4.1 Preparation of protein lysates from cell cultures	38
2.4.2 Determination of protein concentration	38
2.4.3 SDS-polyacrylamide gel electrophoresis	38
2.4.4 Staining of protein gels	39
2.4.5 Western blot analysis	39
2.5 STATISTICS	40

3	<u>RESULTS</u>	41
3.1	<u>TRANSCRIPTION START SITE OF THE MURINE LI-CADHERIN GENE</u>	41
3.1.1	<u>5'-RACE-PCR</u>	41
3.1.2	<u>Primer extension</u>	42
3.1.3	<u>Sequence of the proximal 5'-flanking region of the LI-cadherin gene</u>	44
3.2	<u>REPORTER GENE SYSTEMS FOR ANALYZING THE LI-CADHERIN PROMOTER</u>	44
3.2.1	<u>Cloning of the 5'-flanking region of the murine LI-cadherin gene</u>	45
3.2.2	<u>Sequence analysis of the putative promoter and the 5'-flanking region</u>	46
3.2.3	<u>Characterization of an in vitro cell-system</u>	49
3.2.4	<u>Qualitative reporter gene assay: GFP-readout</u>	49
3.2.5	<u>Quantitative reporter gene assay: Luciferase readout</u>	51
3.2.6	<u>Functional analysis of the LI-cadherin promoter-region</u>	52
3.3	<u>SERIAL 5'-DELETION ANALYSIS OF THE LI-CADHERIN PROMOTER</u>	54
3.4	<u>BINDING OF NUCLEAR PROTEINS TO THE LI-CADHERIN PROMOTER</u>	59
3.5	<u>MUTATIONAL ANALYSIS</u>	63
3.5.1	<u>Effects of mutations on binding of nuclear extract to the LI-cadherin promoter</u>	63
3.5.2	<u>Effects of mutations on LI-cadherin promoter activity</u>	65
3.6	<u>EVALUATION OF TISSUE- AND SPECIES-DEPENDENT ACTIVITY OF THE LI-CADHERIN PROMOTER</u>	67
4	<u>DISCUSSION</u>	71
4.1	<u>MAPPING OF THE TRANSCRIPTION START SITE OF THE LI-CADHERIN GENE</u>	71
4.2	<u>VERIFICATION AND EXAMINATION OF LI-CADHERIN PROMOTER ACTIVITY</u>	72
4.3	<u>SEQUENCE ANALYSIS OF THE 5'-FLANKING REGION OF THE LI-CADHERIN GENE</u>	72
4.4	<u>DELETION ANALYSIS OF THE 5'-FLANKING REGION OF THE MURINE LI-CADHERIN GENE</u>	76
4.5	<u>DNA-PROTEIN INTERACTIONS AND INFLUENCE OF MUTATIONS ON THE PROMOTER ACTIVITY</u>	77
4.6	<u>TISSUE- AND SPECIES-DEPENDENT ACTIVITY OF THE LI-CADHERIN PROMOTER</u>	80
5	<u>SUMMARY AND FUTURE PERSPECTIVES</u>	83
6	<u>ZUSAMMENFASSUNG UND AUSBLICK</u>	85
7	<u>REFERENCES</u>	87
8	<u>ATTACHMENTS</u>	96
	Publications	99
	Curriculum vitae	100
	Acknowledgement	101