

Table of contents

Table of contents	I
List of abbreviations	IV
1. Introduction.....	1
1.1 Obesity, NAFLD and NASH.....	1
1.2 NASH-HCC	4
1.3 Rodent NASH-HCC models.....	7
1.4 mTOR protein pathway and mTOR inhibition	10
2. Aim of the study.....	15
3. Materials and Methods	17
3.1 Chemicals and devices	17
3.2 Antibodies and primers	19
3.3 Cell culture	21
3.3.1 Isolation of N-HCC25 cells	21
3.3.2 Cell culture conditions	21
3.3.3 Treatment regiments	23
3.3.4 Real-time cell analysis (RTCA)	24
3.3.5 2D-Flow cytometry	25
3.4 Nucleic acid analysis	26
3.4.1 RNA isolation and quality control	26
3.4.2 cDNA synthesis.....	26
3.4.3 Polymerase chain reaction (PCR).....	27
3.4.4 Agarose gel electrophoresis.....	27
3.5 Proteomics.....	28
3.5.1 Protein isolation from cells	28
3.5.2 Western blot analysis	29
3.6 Animal experiments	32
3.6.1 Strains	32
3.6.2 Animal model	33
3.6.3 Clinical status	35
3.6.4 Application of DMBA or acetone	37
3.6.5 Sample preparation for genotyping	37
3.6.6 Intraperitoneal glucose tolerance test (IP-GTT).....	38

3.6.7	Blood samples.....	39
3.6.8	<i>In vivo</i> micro-computed tomography (μ CT).....	40
3.6.9	Explantation of organs and tissue preparation.....	42
3.7	Histology	43
3.7.1	Sample fixation and paraffin embedding.....	43
3.7.2	Hematoxylin-eosin staining	43
3.7.3	Reticulin staining	44
3.7.4	PAS staining.....	46
3.7.5	Sirius red staining.....	46
3.7.6	Immunohistochemical stainings: pS6, CD45, F4/80	47
3.7.7	NAS and SAF Score	49
3.8	Statistics	50
4.	Results	52
4.1	The role of mTOR inhibition in an <i>in vitro</i> model of wild type NASH-HCC.....	52
4.1.1	Characterization of N-HCC25: A fast proliferating NASH-HCC cell line that depends on the supply of D-glucose and FBS	52
4.1.2	mTORC1/2 inhibition has a higher inhibitory effect on N-HCC25 than mTORC1 inhibition in a concentration dependent manner by inducing G ₁ /G ₀ cell cycle arrest.....	56
4.2	The role of mTOR inhibition <i>in vivo</i> : A two hit NASH-HCC model with conditional liver-specific mTOR knock-out mice	61
4.2.1	KO ^{DMBA+WD} showed a significant higher tumor burden than WT ^{DMBA+WD}	61
4.2.2	Investigation of characteristic metabolic and histopathological changes supporting tumorigenesis in NASH-HCC.....	63
4.2.2.1	Weight gain.....	64
4.2.2.2	Dyslipidemia and impaired glucose tolerance	66
4.2.2.3	Histological investigation of NASH	68
4.2.2.4	Additional inflammatory targets: AST/ALT, CD45, F4/80 ..	70
4.2.3	Clinical status	72

5. Discussion.....	73
5.1 mTOR inhibitors restrict proliferation of model-specific murine wild type NASH-HCC cells with a higher magnitude for mTORC1/2 inhibition <i>in vitro</i>	73
5.2 Mice with postnatal conditional liver-specific mTOR-KO (KO ^{DMBA+WD}) showed a significant higher tumor burden than WT control (WT ^{DMBA+WD}) in the two hit NASH-HCC model	76
5.3 Limitations of the study	82
6. Summary	84
7. References	86
8. Supplements	98
Acknowledgement/ Danksagung	107
Declaration on data retention/ Erklärung zur Datenaufbewahrung	109
Affidativ/ Eidesstattliche Erklärung	110
gemäß § 5 Abs. (1) und § 11 Abs. (3) 12. der Promotionsordnung	110