

Table of Content

1. Summary	1
1. Zusammenfassung	3
2. Introduction	5
2.1. The liver and its metabolic activity	5
2.1.1. Epidemiology and management of liver malignancies	7
2.1.2. Liver cancer in mouse models	9
2.1.3. The Tumor Microenvironment	10
2.2. The metabolic process of fasting	12
2.2.1. Using fasting as a therapeutic approach	15
2.2.2. Effects of Fasting on Cancer/Stress resistance	15
2.3. Nanomedicine based tumor-targeted therapy	17
2.3.1. Liposomes and their properties	18
2.3.2. Liposomal uptake through endocytosis	19
2.3.3. Tumor-directed delivery of liposomes	21
2.4. Aim of the study	21
3. Material and Methods	23
3.1. Chemicals	23
3.2. Solutions	24
3.3. Buffer recipes	24
3.4. Devices and Software	24
3.5. Consumables	26
3.6. Liposomes	26
3.7. Cell lines	27
3.7.1. Hepa1.6 cell line	27
3.7.2. J774.A1	28
3.7.2.1. Polarizing J774.A1 to Macrophages	28
3.7.3. Hep55.1C cell line	28
3.8. Liposome uptake	28
3.8.1. Uptake under standard cell culture conditions	29
3.8.2. Uptake under the influence of forskolin	29
3.8.3. Inhibition of Liposome-uptake	29

Table of content

3.8.4.	Inhibition of uptake under the influence of Forskolin	29
3.9.	Proliferation and Apoptosis <i>in vitro</i>	29
3.10.	Mouse models	30
3.10.1.	Preparation of cells for injection	30
3.10.2.	Allogenic ectopic tumor	30
3.10.3.	Allogenic orthotopic tumor	31
3.11.	Animal experiments	31
3.11.1.	Fasting/refeeding experiments	31
3.11.2.	Weekly μ CT scans	32
3.11.3.	Weekly MRI scans	32
3.11.4.	Hybrid μ CT-FMT imaging experiments	33
3.12.	Imaging analysis	33
3.12.1.	μ CT	33
3.12.2.	MRI	34
3.12.3.	μ CT-FMT	34
3.13.	Tissue and biopsies	34
3.14.	Fluorescence-Activated Cell Sorting (FACS)	34
3.15.	Immunofluorescence staining and microscopy	34
3.15.1.	Immunofluorescence staining analysis of cell lines	35
3.15.2.	Immunofluorescence staining analysis of tissue	35
3.16.	Sirius Red staining	36
3.17.	TUNEL Assay	36
3.18.	Two-photon laser scanning microscopy	36
3.19.	Western Blot	37
3.20.	Statistical analysis	38
4.	Results	39
4.1.	Liposome uptake on the cellular level	39
4.1.1.	Proof of concept for liposomal uptake <i>in vitro</i>	39
4.1.2.	The influence of fasting conditions on liposome uptake <i>in vitro</i>	40
4.1.3.	Liposome uptake inhibition	40
4.2.	The influence of fasting on tumor drug delivery	43
4.2.1.	The ectopic tumor model	43
4.2.1.1.	The impact of fasting on ectopic tumor-directed liposome delivery	45

Table of content

4.2.1.2.	The influence of fasting on tumor microenvironment	47
4.2.2.	The orthotopic tumor model	51
4.2.2.1.	The impact of fasting on orthotopic tumor-directed liposome delivery	52
4.2.2.2.	The influence of fasting on the intrahepatic tumor microenvironment	54
5.	Discussion	57
6.	Literature	64
7.	Appendix	A
7.1.	Supplementary figures	A
7.2.	Abbreviations	C
7.3.	Table index	F
7.4.	Figure index	G
7.5.	Appendix figure index	H
7.6.	Eidesstattliche Erklärung	I