

Table of Contents

Abbreviations	IV
I Introduction	1
1.1 Liver injury and fibrosis	1
1.2 Alpha-1-antitrypsin deficiency (AATD)	1
1.2.1 Alpha-1-antitrypsin (AAT)	1
1.2.2 AAT mutations and AAT deficiency	2
1.2.3 AATD-associated pulmonary and hepatic disease	3
1.2.4 PiZ mouse as model for AATD	4
1.3 Protein accumulation causes cell stress	5
1.3.1 Protein folding in the ER and the unfolded protein response (UPR) ...	5
1.3.2 Protein degradation	6
1.3.3 Apoptosis as an outcome of maladaptive ER stress	7
1.4 Interleukin 10	8
1.4.1 IL10 affects immunoregulation and inflammation	8
1.4.2 IL10 KO mouse model	8
1.4.3 IL10 in hepatic pathogenesis	9
1.5 Aim of the study	11
2 Materials and Methods	12
2.1 MATERIALS	12
2.1.1 Chemicals and reagents	12
2.1.2 Instruments and equipments	13
2.1.3 Consumables	14
2.1.4 Kits and enzymes	15
2.1.5 Antibodies	16
2.1.6 Oligonucleotides	17
2.1.7 Animal lines	19
2.1.8 Buffers and solutions	19
2.1.9 Softwares	22
2.2 Methods	23
2.2.1 Animal experiments	23
2.2.2 DNA analysis	24

2.2.3 RNA analysis	27
2.2.4 Protein analysis	30
2.2.5 Histological staining	34
2.2.6 Crypt length measurement	39
2.2.7 Statistics	39
3 Results	41
3.1 Characterization of PiZ-IL10 KO mice	41
3.1.1 Genotyping of mouse lines used in this project	41
3.1.2 Verification of AAT expression in the liver	41
3.1.3 PiZ-IL10 KO mice display an elevated accumulation of PASD positive aggregates in hepatocytes	43
3.1.4 PiZ-IL10 KO mice harbor similar AAT serum levels as PiZ transgenic mice	44
3.2 IL10 deficiency promotes liver damage in AATD mice	45
3.2.1 PiZ-IL10 KO mice display an increased liver-to-body weight ratio	45
3.2.2 PiZ-IL10 KO mice display significantly greater liver injury compared to PiZ transgenic mice	46
3.2.3 PiZ-IL10 KO mice display similar liver inflammation compared to PiZ transgenic mice	47
3.3 Hepatic changes	48
3.3.1 AAT protein accumulation in PiZ-IL10 KO mice is due to accumulation of insoluble AAT	48
3.3.2 IL10 deficiency loads the burden of PiZ folded protein in liver cells ..	50
3.3.3 PiZ-IL10 KO mice display signs of an autophagic impairment	51
3.3.4 PiZ-IL10 KO mice exhibit hepatic cell-death	52
3.3.5 3-month-old PiZ-IL10 KO mice didn't show signs of liver fibrosis	54
3.3.6 3-month-old PiZ-IL10 KO mice display clusters of CD45 positive cells in the liver	56
3.4 Evaluation of colitis in IL10-deficient animals	58
3.4.1 PiZ-IL10 KO mice show similar intestinal inflammation as IL10 KO mice	58
3.4.2 IL 10 deficiency results in increased expression of inflammatory cytokines in the intestine	59

3.4.3 PiZ-IL10 KO mice exhibited no histological abnormalities in colon tissue.....	60
4 Discussion	62
4.1. IL10 KO promotes liver injury in PiZ mice	62
4.2. IL10 leads to an altered AAT proteostasis in PiZ mice	62
4.3 IL10 KO may lead to enhanced UPR activation in PiZ mice	65
4.4. IL10 deficiency led to more cell death in the liver of PiZ mice	65
4.5. IL10 does not lead to marked inflammation in the liver of PiZ mice	66
4.6. The colitis phenotype of IL10 KO mice is not worsened by PiZ expression	66
5 Summary	68
6 Zusammenfassung	69
7 Reference	70
8 Appendix	79
8.1 List of tables	79
8.2 List of figures	79
Acknowledgements	81
Affidavit according to § 5 (1) for Data Retention	82
Affidavit according to § 5 (1) § 11 (3) 12 of the doctoral studies regulations	83
Curriculum vitae	83