

TABLE OF CONTENTS

Table of contents	i
List of Figures	iv
List of Abbreviations	vi
1. INTRODUCTION	1
1.1 Epidemiology of pancreatic cancer.....	1
1.2 Pancreatic ductal adenocarcinoma	1
1.3 The therapy of pancreatic cancer	2
1.3.1 Surgical treatment	2
1.3.2 Targeted radiotherapy	3
1.3.3 Chemotherapy	3
1.3.4 Immunotherapy	4
1.4 Exosomes	20
1.4.1 Introduction of Exosomes	20
1.4.2 The role of pancreatic cancer-derived exosomes in pre-metastatic niche formation and cancer metastasis	23
1.4.3 TEX (tumor-exosomes) and immune responses	24
1.5 Aim of the thesis	28
2. MATERIALS AND METHODS	30
2.1 Materials.....	30
2.1.1 Instruments	30
2.1.2 Miscellaneous material	31
2.1.3 Chemicals and reagents.....	31
2.1.4 Cell culture medium and supplements.....	32
2.1.5 Buffers and solutions.....	33
2.1.6 Kits	34
2.1.7 Antibodies	34
2.1.8 Computer software	40
2.1.9 Tumor cell lines.....	40
2.1.10 Animals	40
2.2 Methods.....	40
2.2.1 Cell culture	40
2.2.2 Tissue preparation and cell isolation	41

2.2.3	In vitro dendritic cell generation and DC-TEX vaccination preparation	41
2.2.4	In vitro myeloid-derived suppressor cells (MDSC) generation	42
2.2.5	Cell-lysate preparation.....	42
2.2.6	TEX preparation.....	42
2.2.7	Carboxyfluorescein succinimidyl ester-labelling and Dio18 labelling (Exo)	43
2.2.8	Flow-cytometry	44
2.2.9	Immunohistochemistry.....	45
2.2.10	Tcell proliferation.....	46
2.2.11	Cytotoxicity assay	46
2.2.12	Apoptosis assay	46
2.2.13	Trogocytosis (TRAP assay)	47
2.2.14	In vivo assays	47
2.2.15	Statistics.....	48
3.	RESULTS	49
3.1	DC loading with tumor exosomes and the distribution of DC in the tumor-bearing host.....	49
3.2	DC-TEX vaccination prolongs the survival time and affects UNKC dissemination	52
3.3	The immunosuppressive microenvironment produced by orthotropic tumor growth	57
3.4	Managing the expansion, activation and recruitment of MDSC.....	61
3.4.1	ATRA combined with DC-TEX.....	61
3.4.2	Combined Sunitinib treatment and DC-TEX vaccination	65
3.4.3	Gemcitabine combined with DC-TEX.....	71
3.5	Efficacy of DC-TEX vaccination was intensified by attacking maturation and activation of MDSC	76
4.	DISCUSSION	82
4.1	Appropriateness of the murine model for controlling the efficacy of DC vaccination in PaCa.....	82
4.2	Appropriateness of UNKC TEX loaded DC as vaccine and the likelihood of DC-TEX to reach their target.....	84
4.3	Dealing with expansion and activity of MDSC in PaCa during active vaccination	85
4.3.1	ATRA.....	85
4.3.2	Sunitinib.....	86
4.3.3	Gemcitabine	87
4.3.4	Combination of ATRA, Sunitinib and Gemcitabine (AGS).....	88

5. CONCLUSION AND OUTLOOK.....	90
6. SUMMARY AND ZUSAMMENFASSUNG.....	92
7. REFERENCES	96
8. LIST OF PUBLICATIONS	109
9. CURRICULUM VITAE	110
10. ACKNOWLEDGEMENTS.....	112