

## Table of contents

Contents		Page No.
Abstract		i-iii
Declaration by Candidate		iv
Certificate of the Supervisor		v
Acknowledgement		vi-vii
Table of Contents		viii-xi
List of Figures		xii-xiii
List of Tables		xiv
List of Abbreviations		xv-xviii
<b>Chapter I</b>	<b>Introduction</b>	<b>1-33</b>
1.1.	Cancer: A preview	2-4
1.2.	Types of Cancer	4-5
1.3.	Hallmarks of cancer	5
	1.3.1 Sustained proliferative signalling	5-6
	1.3.2 Evading growth suppressors	6
	1.3.3 Enabling replicative immortality	7
	1.3.4 Tissue invasion and metastasis	7
	1.3.5 Inducing angiogenesis	7-8
	1.3.6 Resisting cell death	8
	1.3.7 Avoiding immune destruction	8-9
	1.3.8 Reprogramming energy metabolism in cancer cells	9
1.4	Tumor microenvironment (TME)	9-10
	1.4.1 Inflammation in tumor microenvironment	10-12
	1.4.2 Components of Tumor microenvironment	12-15
	1.4.3 Tumor Associated Macrophages (TAM)	15
	1.4.4 Pro-tumor and Anti-tumor state of Tumor Associated Macrophages (TAM)	15-16
	1.4.5 Targeting TAMs to combat tumor	17-19
1.5	Breast cancer	19-20
1.6	High salt and cancer	20-22

1.7	References	23-33
<b>Chapter II</b>	<b>Review of literature</b>	<b>34-61</b>
2.1	Salt regulation and cellular homeostasis	35-37
2.2	Regulation of sodium homeostasis by Immune cells	38-39
2.3	Sodium accumulation in tumor microenvironment	39-40
2.4	Role of high salt in influencing immune cell's function	41-42
2.5	Role of salt in influencing macrophage cell's function	42-43
2.6	Role of high salt in influencing T and dendritic cell's function	44-45
2.7	Mediators of Immunosuppressive state in TME	46
2.8	The contradictory role of high salt in cancer progression	47-51
2.9	Objectives of the study	51
2.10	References	52-61
<b>Chapter III</b>	<b>Materials and Method</b>	<b>62-75</b>
3.1	Reagents	63
3.2	Cell lines and cell culture	63
3.3	Cytotoxicity assay	64
3.4	Apoptosis assay using flow cytometer	64
3.5	Cell cycle analysis	64-65
3.6	Cell proliferation assay	65
3.7	Measurement of reactive oxygen species (ROS) level	65
3.8	Wound healing assay	65-66
3.9	Adhesion assay	66
3.10	Matrigel invasion assay	66
3.11	Total RNA isolation	66-67
3.12	First strand cDNA synthesis	70
3.13	Western blot analysis	70
3.14	Harvesting tumor-conditioned media	71
3.15	Polarization of THP-1 monocytes to macrophage	71
3.16	<i>In vitro</i> chemotaxis assay	71-72
3.17	Generation of tumor associated macrophage (TAM)	72
3.18	Co-culture of TAM and cancer cells	72

3.19	RNA sequencing analysis	72-73
3.20	Statistical analysis	73
3.21	References	74-75
<b>Chapter IV</b>	<b>A study on the effect of high dietary salt on tumorigenic properties of breast cancer cell lines</b>	<b>76-109</b>
4.1	Introduction	77
4.2	Results	78
	4.2.1 To study the effect of NaCl on cell viability in MDA MB-231 and MCF-7 cell lines	78
	4.2.2 High salt induces apoptosis in MDA MB-231 and MCF-7 cell lines	79-81
	4.2.3 Expression of apoptotic markers and ROS generation in response to high salt	81-83
	4.2.4 High salt inhibits cell proliferation in MDA MB-231 and MCF-7 cell lines	83-85
	4.2.5 High salt inhibits clonogenic property of breast cancer cell lines	85-86
	4.2.6 High salt arrests cell cycle of breast cancer cells in S phase	86-89
	4.2.7 High salt suppresses migration property in breast cancer cell lines	89-91
	4.2.8 High salt suppresses adhesion to extracellular matrix (ECM) in breast cancer cell lines	91
	4.2.9 High salt induces global transcriptomic alterations in high salt treated MDA MB-231 cells	91-95
	4.2.10 Validation of transcriptomic data	95-96
4.3	Discussion	100-103
4.4	References	104-109
<b>Chapter V</b>	<b>A study on the effect of high dietary salt on the crosstalk between cancer cells and macrophages / TAMs in vitro</b>	<b>110-148</b>
5.1	Introduction	111
5.2	Results	112

	5.2.1	Standardizing conversion of THP-1 monocytes to macrophage-like cells	112-113
	5.2.2	Standardization of THP-1 to TAM conversion and study the impact of high salt on TAM polarization	113-115
	5.2.3	High salt induces pro-inflammatory state in THP-1 macrophage cells	115-116
	5.2.4	High salt induces pro-inflammatory genes involved in anti-tumor function in macrophages and TAM cells	116-117
	5.2.5	High salt treated breast cancer cells induces chemotaxis of THP-1 monocytes	117-119
	5.2.6	Macrophage and tumor associated macrophage (TAM) suppresses MDA MB-231 cell proliferation under high salt condition	119-120
	5.2.7	High salt treated macrophage and TAMs suppressed MDA MB-231 migration under co-culture condition	120-121
	5.2.8	High salt treated macrophage and TAMs suppressed MDA MB-231 invasion	121-123
	5.2.9	High salt treated macrophage and TAMs suppressed MDA MB-231 adhesion to extracellular matrix under co-culture condition	123
	5.2.10	Effect of high salt on global transcriptome profile of MDA MB-231 cells co-cultured with TAM	124-127
	5.2.11	Validation of genes differentially regulated in RNA sequencing analysis	127-128
5.3	Discussion		133-139
5.4	References		140-148
<b>Chapter VI</b>	<b>Conclusions and Future Prospects</b>		<b>149-155</b>
6.1	Conclusion		150-153
6.2	Future prospects		153
6.3	References		154-155
<b>Appendix I</b>			<b>156-157</b>