Abstract:

Breast cancer is one of the most common cancers globally, comprising 12.5% of annual cases. In 2022, it affected 2.3 million women and caused 685,000 deaths worldwide. Dietary salt (NaCl) plays an important role in maintaining cellular homeostasis, fluid balance, blood pressure regulation, muscle function, nutrient transport, and nerve cell excitability. Although the daily recommended amount required for an average adult according to WHO is 4-5 g/day, recent data on salt intake shows that populations around the world are consuming much more (3-4 times) salt than physiologically required. High salt diet (HSD) has been reported to stimulate local tissue-specific accumulation of sodium in mice and humans in skin, thymus, liver, and spleen. Recent reports using ^{23}Na-MRI has also showed that sodium content in breast tumor cells is significantly higher compared to the surrounding normal breast tissue in tumor microenvironment. However, the factors leading to such local sodium accumulation remain unclear. Such accumulation of sodium in tumor microenvironment can trigger inflammation and affect extracellular matrix remodelling.

The tumor microenvironment (TME) consists of a complex network of cancer cells, stromal cells, immune cells, extracellular matrix components, and signalling molecules. These elements of the TME are crucial in tumor development, progression, and metastasis, and the environment is typically characterized by hypoxia, acidic pH, and abnormal blood vessels that support tumor cells while inhibiting immune responses. Tumor cells recruit and reprogram immune cells in the TME using cytokines such as IL-10, TGF-β, and M-CSF. The bidirectional interactions between cancer cells and immune cells promote tumor growth and immune suppression, creating a cycle that facilitates tumor progression. Immune cells in tumor microenvironment (TME) predominantly exists in immunosuppressive state and their phenotype is heavily influenced by their microenvironment. Recent research has highlighted the impact of ionic imbalance and immune cell metabolism on reshaping the TME. Tumor-associated macrophages (TAMs) are the most prevalent cells in the TME, typically displaying an M2-like, pro-tumor phenotype. However, high salt levels in the TME can induce a pro-inflammatory M1 state in macrophages, which usually exist in the pro-tumor M2 form under normal conditions. High salt also activates T-cells into an anti-

tumor state. This pro-inflammatory condition helps restore the anti-tumor function of immune cells in the TME. However, the role of high salt on cancer progression is controversial, the direct impact on breast cancer cells remains unexplored. Considering the gap in the field, the thesis work aimed to study the direct effect of high NaCl treatment on breast cancer cells and on TAMs.

The studies presented in this thesis have been grouped into six different chapters:

Chapter I outlines the introduction to the current study. It presents a brief history, types, and components of cancer. It addresses in details about the tumor microenvironment and components of tumor microenvironment. This chapter also discusses elaborately about immune cells and their impact on cancer progression in tumor microenvironment.

Chapter II presents the review of literature and justifies how the current topic is essential for the study. This chapter discusses the about the impact of high salt regulation on cellular homeostasis in general and specifically its role in tumor microenvironment. The effect of high salt in inducing inflammation in tumor microenvironment especially its effect on macrophage and tumor associated macrophage cells is also discussed. This chapter also delineates how high salt affects the cancer progression. Based on the current literature and the gaps in our understanding of the area, two objectives for the current study are presented.

Chapter III provides a detailed discussion about the specifications of biological reagents, cell lines, and instruments utilized in the study. Additionally, it thoroughly describes the experimental protocols, bioinformatics tools and statistical methodologies employed throughout the research. Each aspect of the procedures is covered in depth, ensuring a comprehensive understanding of the techniques and tools used.

Chapter IV presents experimental evidence of the direct effect of high salt on two breast cancer cell lines: triple negative MDA MB-231 and ER+ MCF-7. The chapter describes experiments to understand the effect of high salt on apoptosis, proliferation, cell-cycle, migration and adhesion property of breast cancer cells using various in vitro techniques like flow cytometry, real time PCR, microscopy and western blotting. Subsequently, to delve deeper into the effect of high salt on global transcriptomic changes, the RNA sequencing analysis of highly aggressive metastatic MDA

MB-231 cells are carried out. RNA-Sequencing analysis of reveals expression of tumor suppressor genes and genes associated with anti-tumor activity.

Chapter V describes the effect of high salt on THP-1 derived macrophage and tumor associated macrophages (TAM). The effect of high salt on inducing pro-inflammatory state in macrophage and tumor associated macrophage (TAM) cells are discussed in this chapter. It describes in detail the effect of high salt on the polarization of TAM from macrophage cells which is beneficial as anti-tumor strategy. Further it shows the effect of salt on the cross talk between macrophage and tumor associated macrophage cells with MDA MB-231 cells using co-culture system. To understand the genes and pathways of cancer cells involved in this cross-talk, RNA-Sequencing analysis of high salt treated macrophages and TAM co-cultured MDA MB-231 cells are presented. The analysis of DEG's involved in tumor suppression and anti-cancer function reveals how high salt impacts the cross-talk between TAM and cancer cells and results in inducing anti-tumorigenic effect.

Chapter VI presents the conclusion of the thesis work. The chapter also highlights some future prospects of the present study.