Chapter I:

Introduction

Cancer poses the highest clinical, social, and economic burden among all human diseases. Cancer is the second most significant cause of death worldwide, responsible for approximately 8.97 million deaths each year, following ischemic heart disease. By 2060, cancer is projected to become the leading cause of death, with an estimated 18.63 million fatalities annually. The most fatal types of cancer in population are lung, stomach, and liver cancers, with breast and lung cancers accounting for majority of cancer-related deaths both in men and women. Prostate and thyroid cancers have the best prognosis, with approximately 100% 5-year survival. Cancers of the esophagus, liver, and pancreas have the worse prognosis, with a 5-year survival rate of less than 20%(1). Another concern is both short term and long-term effects of cancer and its treatment on employment and productivity of cancer survivors. Physical disability, distress and reduced quality of life are common after cancer and vary depending on the cancer (2).

The process of carcinogenesis is a dynamic complex multi-step process. As cancer progress through evolution, it adapts itself well into the microenvironment, accumulating significant number of genetic changes and generating heterogeneity. Several new disease characteristics have become apparent as we gained a better understanding of the mechanisms behind emergence of cancer. Therefore, to refine the vast complexity of the carcinogenesis process into an array of functional qualities that are gained as cells transform from normal to neoplastic developmental states, certain factors were determined (3). These factors were termed as “hallmarks of cancer”. There are 14 established hallmarks in cancer – evading growth suppressors, avoiding immune destruction, activating invasion and metastasis, sustaining proliferative signalling, tumor-promoting inflammation, enabling replicative immortality, inducing angiogenesis, avoiding immune destruction, genome instability and mutation, resisting cell death, Non-mutational epigenetic reprogramming, deregulating energetics, Polymorphic microbiomes, Senescent cells and Unlocking phenotypic plasticity (4).

Genomic instability is one such hallmark in cancer. It indicates an upsurge in the frequency of nucleotide sequence alterations over the course of a cell's life. The ultimate

purpose of replication in non-cancerous somatic cells is precise duplication of the whole genome and division into two cells. Inability to accomplish this, or cell division with an elevated rate of errors, can lead to cell cycle dysregulation and the genesis of cancer.(5, 6). Damage to DNA can be caused by exposure to radiation, chemicals, and other environmental mutagens. Within a tumor, genomic instability creates genetic variety, which offers a reservoir of mutations that can propel the tumor's development and adaptability. It exacerbates intratumoral heterogeneity which makes diagnosis and treatment more difficult(7). Tumors with significant genomic instability are more likely to develop resistance to therapy because they can quickly acquire mutations conferring resistance (8). A poor prognosis is frequently linked to high levels of genomic instability in a variety of malignancies(9, 10). It may be induced by generation of mutations and stimulating recombination events through non-canonical nucleic acid structures like G-quadruplexes (G4)(11). The significance of G4 in the immune system has been established as their abundance in inflammatory mediators has been confirmed(12) and studies have establish that they play a critical role in class switching of antigen-activated B cells (13).

Evading immune destruction is another hallmark in cancer (14). Tumor cells employ two main strategies to counter host defence, one includes avoiding immune recognition and the other is inducing immune-suppressive tumor microenvironment. Former one is achieved by deletion or mutation of human leukocyte antigen (HLA) or manipulating molecules in antigen processing and presentation pathway. Another strategy employed by cancer cells to undermine immune detection is to downregulate their NK (Natural killer cell) activators, rendering them undetectable to NK cells. The later strategy of inducing tumor favourable immune-suppressive microenvironment is achieved by release of immune suppressive molecules such as TGF-β and IL-10; upregulation of inhibitory checkpoint molecules like HLA-G, HLA-E, CTLA-4, PD-L1 and inducing recruitment of immune-suppressive cells that include Tregs (Regulatory T cells), TAMs (Tumor associated Macrophages) and MDSCs (Myeloid derived suppressor cells) by secretion of

tumor-associated chemokines such as CSF1, CCL2, CCL22, CCL5, CXCL5, CXCL8 (Figure 1.1) (15). In general, immune suppressive cells and molecules assist to maintain self-tolerance and homeostasis throughout an immune response, but cancer cells exploit them to maintain an immune suppressive milieu for their survival.(16). Blending together the strategies indicated above cancer cells build up an efficient and complex system for immune evasion(17). Targeting immune evasion systems for therapeutics is termed as immunotherapy. It is a form of treatment for cancer that uses the immune system to combat cancer.

Immunotherapy can be categorized into several types, including checkpoint inhibitors, CAR-T cell therapy, monoclonal antibodies and cancer vaccines. Checkpoint inhibitors work by inhibiting the immune checkpoints molecules, such as CTLA-4 and PD-1/PD-L1 which blocks the inhibitory signals and restores T cell activation (18). CAR-T therapy includes altering a patient's T cells to express cancer-specific receptors. These modified T cells are reinstated into the patient, where they attack and eliminate cancer cells(19). Monoclonal antibodies may attach to particular antigens on cancerous cells, allowing the immune system to destroy them, inhibit growth signals, or transfer poisonous compounds directly to the cells (20). Cancer vaccines are designed to treat existing cancer or prevent it from returning (21). The effectiveness of many immunotherapies highlights the important role played by immune evasion for cancer survival. Therefore, restoring immunological function alone may be sufficient to eliminate cancer.

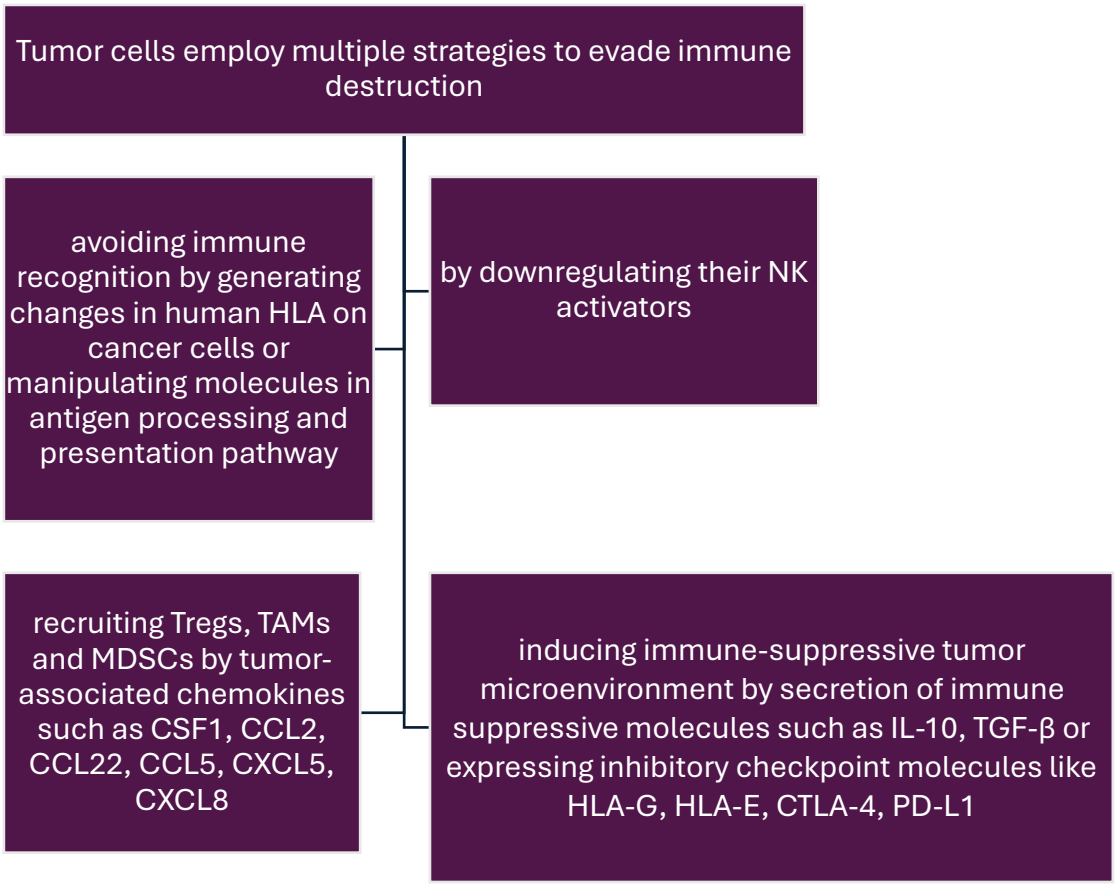

Figure 1.1: A diagram showing strategies used by tumor for immune suppression

The process of transitioning the immune response fighting the tumors to enslaving the immune system by tumors for their advancement is described as immunoediting. It is a dynamic scenario wherein the immune system engages with tumor and shapes their evolution. It involves three main phases: elimination, equilibrium, and escape, often referred to as "Three E's" of immunoediting. During first phase, immune system detects and eliminates cancer cells. The process includes both the innate and adaptive immune responses. Cells that are a part of innate immune response, like natural killer (NK) cells, identify and eradicate aberrant cells. The adaptive immune system, particularly T cells, targets and eliminates cancer cells using specific antigen recognition technique. Under circumstances if the immune response becomes unsuccessful in eliminating all tumor cells and certain cells survive the elimination phase, they enter a period of dormancy.

Throughout the time of dormancy, the immune system is in a state of equilibrium and controls their growth but does not completely eradicate them. This phase can persist for years, with tumor cells potentially accumulating mutations that will enable them to elude immune detection and destruction. This ongoing immune selection pressure drives the evolution of the tumor selecting only non-immunogenic tumor cells. Finally, the tumor cell population may lose its dormancy, leading to outgrowth and transitioning into the third phase known as escape phase. During this period, tumors with compromised immunogenicity begin to grow progressively in an immunologically unrestrained manner, establishing an immunosuppressive tumor microenvironment, and eventually become clinically detectable (Figure 1.2) (22). Therefore, immunoediting could accumulate and select favourable mutations, manipulating the tumor immune milieu and paving the way for immune escape and tumor development (23).

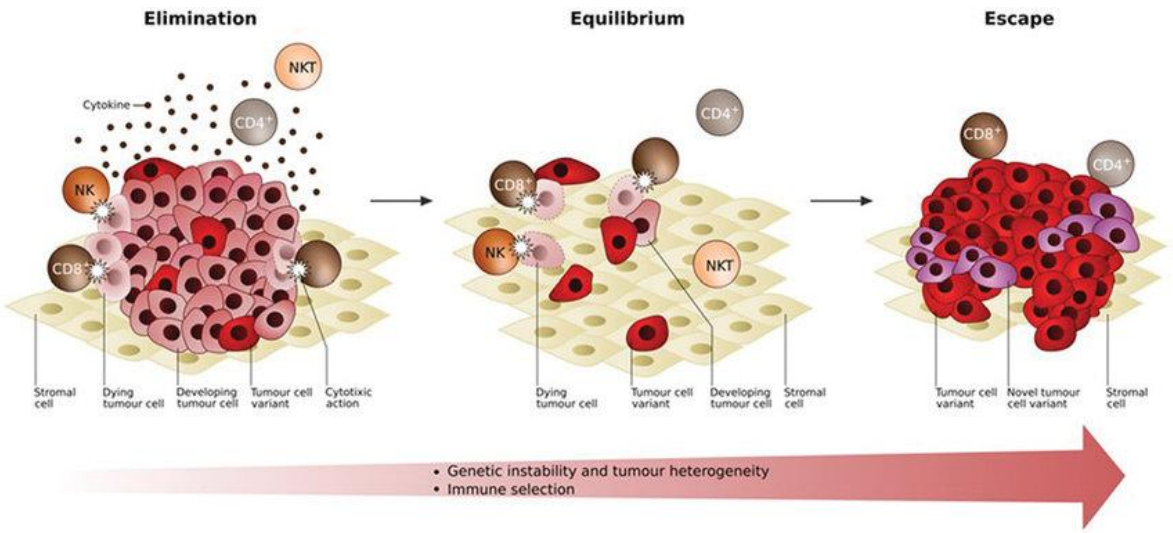

Figure 1.2: A schematic illustration depicting three phase of cancer immunoediting

Image courtesy: Pradeu, T. (2020). *Philosophy of immunology*. Cambridge University Press.

Mutations are pivotal to tumor survival and immune escape Prevalence of somatic mutations differs among different tumors with lung carcinomas at the top with 4.21 per Mb, followed by gastric cancers (2.10 per Mb), ovarian cancers (1.85 per Mb), colorectal cancers (1.21 per Mb) whereas renal cancers (0.74 per Mb), testis cancers (0.12 per Mb) have comparatively low number of mutations(24). Several detailed reviews are available on mutations and their subsequent effect on genes involved in cell cycle, cell migration, and cell growth but mutations in immune response related genes are less explored (25, 26). There is an intimate relationship between cancer and immune response; hence it is very tenable to conceive that mutations in immune response genes would affect tumor immune response with significant bearing on disease prognosis. Polymorphisms and mutations of immune-related genes have been demonstrated to impact gene function in a variety of cancers. Mutations of KRAS and BRAF promote decreased transcription of MHC class I molecules and modify the expression levels of genes producing molecules required for peptide loading (27). Mutation enriched CASP8 prevents cytolytic killing of tumors via FasL-Fas interactions by immune cells in various type of cancers (28). Copy number alterations (CNA) are another type of alteration that can occur within the nucleotide sequence. A substantial body of literature exists regarding the influence of CNA on cancer-immune interplay(29). In gastrointestinal cancer, diminished CNA can reduce cell cycle indicators while increasing cytotoxic marker expression, as well as increased immune cell infiltration(30). Conversely, increased CNA burden is related with lower immune cell recruitment in breast cancer.(31). Therefore, immune related genes that undergoes frequent alterations in cancer must have a significant contribution to cancer development and survival.

We intended to explore influence of frequently mutated immune related genes in different cancers. The list of frequently mutated immune genes obtained in the study could be classified into three principal groups according to their roles in cellular processes: cell regulation, cell migration, and antigen presentation. For our comprehensive investigation, we focused on TG (Thyroglobulin) in the cell regulation category, PIK3CA

(Phosphatidylinositol-4,5-Bisphosphate 3-Kinase Catalytic Subunit Alpha) in the cell migration category, and all the antigen presentation genes. PIK3CA is the gene encoding alpha catalytic subunit of phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K), an intracellular enzyme acting as a secondary messenger. It is an important component of the PIK3/AKT pathway and has a well described role in tumor growth and various immune functions. However, PIK3CA inhibitors for cancer therapy have not been very satisfying (32). PIK3CA has evident role in immune function like, migration and chemotaxis of immune cells(33), T cell activation (34), regulation of macrophage function (35) and regulation of immune cell survival (36). It is a commonly mutated gene in various cancers such as colorectal cancer, breast cancer, head and neck squamous cell carcinoma (37) and non-small lung cell carcinoma (38). Influence of PIK3CA mutation on factors such as cell homeostasis and survival are well studied but the information on its effect in TIME (tumor immune microenvironment) is limited.

TG is a glycoprotein homodimer produced predominantly by the thyroid gland; TG undergoes proteolytic degradation releasing T_4. T_4 is the dominant form of biologically active thyroid hormone. (39). Thyroid hormone can affect several components of the immune system by inducing expression of cytokines, B cell differentiation, inducing T memory cell production, enhancing natural cell cytotoxicity, dendritic cell antitumor immunity(40). Thyroglobulin is used as a marker in the monitoring differentiated thyroid cancers such as papillary and follicular thyroid cancer(41).

The Major Histocompatibility Complex (MHC) locus, which encodes the human leukocyte antigen (HLA), is the most variable gene cluster in the human genome. It is situated on the short arm of chromosome 6 (6p21.3). The primary function of HLA class I gene products such as HLA-A, -B, and -C is to present endogenous peptides to CD8+ T lymphocytes, whereas the class II coding molecules HLA-DR, -DP, and -DQ are restricted to antigen presenting cells. It assembles exogenous peptides for display to CD4+ helper T cells. Class III molecules encode proteins from the complement system and TNF family, such as tumor necrosis factor (TNF), heat shock proteins, factors C3 and

C5 of complement (42). In our study, HLA-A and HLA-B, the class I MHC subtype were found to be frequently mutated along with HLA-DRB1 of class II subtype and CIITA. CIITA is Class II transactivator, it acts as a transcriptional co-activator that regulates γ-interferon activated transcription for MHC class I and II genes. CIITA exerts its regulatory function by forming a complex with other transcription factors, including Regulatory Factor X (RFX), Nuclear Transcription Factor Y (NFY) and CAMP Responsive Element Binding Protein (CREB) (Figure 1.3). This complex is essential for initiating the transcription process of MHC class II molecules. RFX is a complex of 3 proteins mainly RFXANK, RFXAP, RFX5. NFY has three subunits namely NFYA, NFYB, NFYC (43). Their influence on regulation of gene expression, critical pathways and immune cell infiltration underscores their importance in cancer biology.

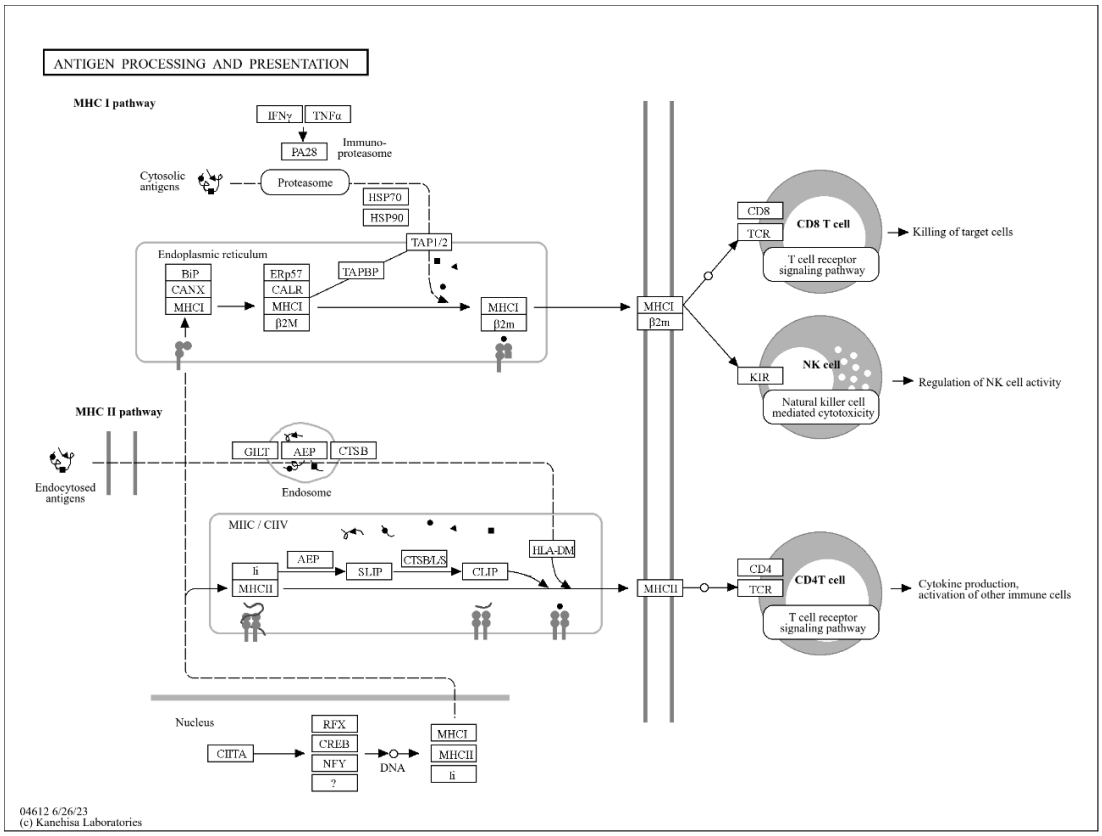

Figure 1.3: Antigen processing and presentation pathway downloaded from KEGG pathways database

Our study was focused on exploring immune related genes with frequent mutations. The objective was to understand their significance in disease prognosis and establish their associations with the functioning of the immune system in various cancers. By identifying changes in the tumor immune microenvironment of mutated tumors compared to non-mutated counterparts, we expect to identify key targets and gain insight into how these mutations facilitate the hijack of our immune system to promote cancer progression. We examined the impact of mutations in immune related genes on tumor immune microenvironment and explored their potential association with patient prognosis to identify new therapeutic targets, suggest appropriate combination therapy, or improve the efficacy of currently available therapeutic options.