Chapter II:

Review of literature

2. Review of literature

2.1.1. Hallmarks of cancer

Hallmarks of cancer is a concept that serve as an analytical tool to filter the infinite complexities of cancer. Therefore, a provisional set of underlying principles with phenotypes and genotypes into account was considered to establish the hallmarks of cancer. This conceptualization was guided by the realization that human cancers develop as outcome of multistep processes. With advancement of knowledge on mechanisms of cancer development, other dimensions of the disease have emerged as potential factors. Initially six hallmarks were defined namely- sustaining proliferative signalling, evading growth suppressors, resisting cell death, enabling replicative immortality, inducing/accessing vasculature, activating invasion and metastasis(44). These six was later expanded to eight by addition of deregulating cellular metabolism and avoiding immune destruction into the list. In 2011, 2 more hallmarks namely- tumor-promoting inflammation and genome instability and mutation were added making the number to 10(45). Further “unlocking phenotypic plasticity,” “nonmutational epigenetic reprogramming,” “polymorphic microbiomes,” and “senescent cells” were added to the list in 2022, marking the number of hallmarks to a total of 14 presently (Figure 2.1) (46).

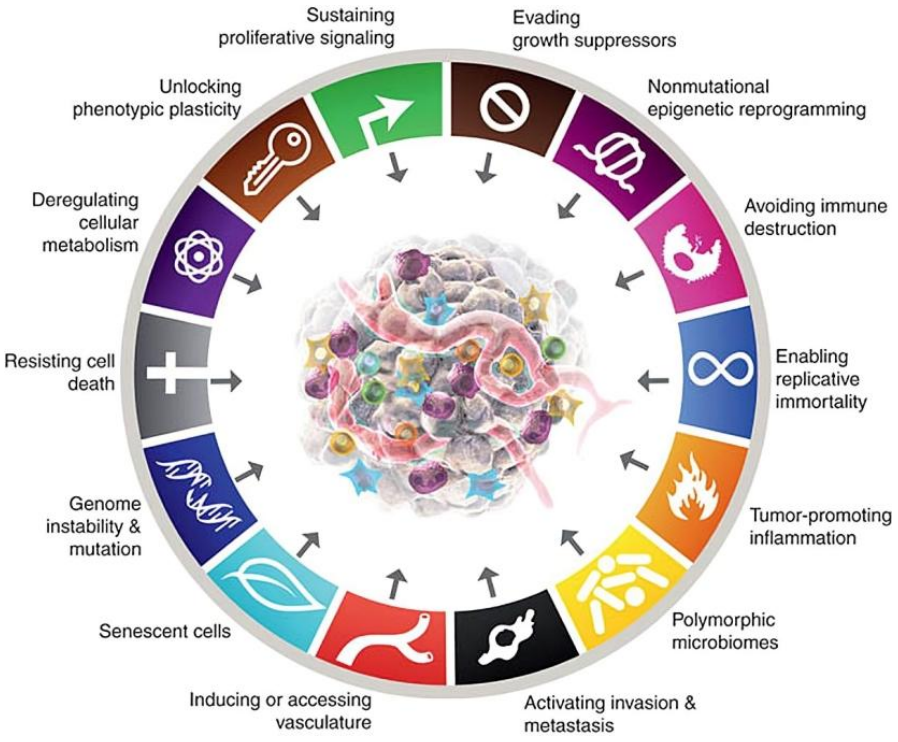

Figure 2.1: A diagram representing all 14 hallmarks of cancer

Image courtesy: Hanahan, D. (2022). Hallmarks of cancer: new dimensions. *Cancer discovery*, *12*(1), 31-46.

Cancer cell formation is fundamentally rooted in the alteration of DNA sequences that might give rise to genome instability(47). These changes can disrupt the cellular homeostasis, supporting various other hallmark characteristic of cancer. One such hallmark is avoiding immune destruction, the process by which the cancer cell attains the state to avoid immune destruction is termed as cancer immune editing (48). Therefore, generation of genome instability is a prime event in cancer development and advancement.

2.1.2. Genomic instability

The purpose of cell division in healthy somatic cells is to precisely duplicate the whole genome and distribute it evenly between the two daughter cells. This procedure guarantees that the daughter cells contain identical genetic material as the parent cell. However, if the procedure fails or there is an abnormally high frequency of mistakes, the offspring cells may experience a variety of genetic changes. These changes include chromosome segment mutations, amplifications, deletions, or rearrangements, as well as the addition or loss of entire chromosomes. The aggregation of these genetic changes can lead to disturbances in proper cell division and, eventually, cancer.(49). Genomic instability is described as an increased susceptibility to genetic changes. During cell division, genomic instability is caused by parental cells' failure to accurately copy the genome and distribute genomic material evenly across daughter cells. Cell divisions in normal tissues are strictly controlled to prevent neoplastic transformation or tumorigenesis. The molecular process of carcinogenesis can be described as the accumulation of genomic changes throughout several cell divisions. Changes to essential genes in a progenitor cell can change a normal cell into a pre-cancerous cell. Though pre-cancerous cells cannot be clinically diagnosed as cancer, subsequent genomic modifications can confer growth advantages to some of these cells. As a result, this newly formed group of neoplastic cells has the potential to develop into a clinically detectable cancer.

Further genetic changes within cancer cells can result in distinct populations with more aggressive features. Several types of genetic changes can occur and accumulate in discrete subsets of cell populations during neoplastic transformation and progression, which is an important aspect of this paradigm. This explains the variety in genetic backgrounds of cancer cells, which contribute to the heterogeneity found in cancer (47).

These changes can be caused by a variety of reasons, including carcinogen exposure, mistakes during DNA replication, or defects in DNA repair mechanisms(50). When the

genome undergoes instability, the normal regulatory mechanisms that control cell growth and division may be disrupted, resulting in uncontrolled cell proliferation—a defining feature of cancer. The accumulation of genetic changes over time contributes to the acquisition of other hallmark cancer traits, including avoiding immune destruction and the ability to invade surrounding tissues(51). Developments in genomic technologies have allowed experts to analyze the mutational landscape of several malignancies, providing important information for development of targeted medicines.

2.1.3. Evading immune destruction

Cancerous cells have a remarkable capacity to adapt tactics that allow them to avoid identification and attack by the immune system.(52). Two key mechanisms through which they achieve this evasion are alterations in antigen presentation and modulation of immune checkpoints. Cancer cells typically present antigens on their surfaces. However, cancer cells can undergo changes in the presentation of these antigens, making it difficult for the immune system to identify them as threats. This alteration may involve downregulating the expression of certain antigens or modifying the normal antigen presentation process. As an outcome, the immune system may not develop an effective defense against cancer cells.(15, 17).

Immune checkpoints molecules regulate the immune response to avoid damage to normal tissues(53). The cancer cells use the checkpoints molecules to suppress the immune response. By upregulating certain checkpoint proteins, like PD-L1, cancer cells can interact with corresponding receptors on immune cells, sending inhibitory signals that suppress the immune response. This interaction essentially put a brake on the immune response, allowing cancer to evade destruction. Interplay between alterations in antigen presentation and immune checkpoint modulation creates a sophisticated defence mechanism for cancer cells(16).

Understanding these manipulated mechanisms and targets has led to the development of immunotherapies that aim to reinvigorate the ability of the immune system to identify and

eradicate cancer cells. We can consider immune checkpoint inhibitors as an example, they prevent the interactions between checkpoint proteins and their receptors, allowing the immune response to identify and eliminate tumor cells. This method has demonstrated encouraging outcomes in several tumors, highlighting the potential of immunotherapy in overcoming immune evasion strategies employed by cancer cells(54). Ongoing research continues to refine these therapeutic strategies and explore new ways to enhance the immune system's effectiveness against cancer.

2.2.1. Genomic instability in immune related genes

Alterations in the nucleotide sequence of immune related genes is the main source to generate a compromised immune system in cancer(48). A large repertoire of literature is available on genomic instability of cell cycle genes and DNA repair genes in cancer but compared to the same such study on immune related genes are scarce.

2.2.2. Avoiding immune recognition

Cancer cells achieve this by tempering the usual antigen presentation process either by alteration or mutation of human leukocyte antigen on cancer cells or manipulating molecules in antigen processing and presentation pathway or by downregulating their NK activators. In initial case, cancer cells may cease expressing tumor antigens with MHC on their surfaces, evading detection by cytotoxic T cells(55). This MHC-low phenotype has been found in a variety of human malignancies, including breast, melanoma, colorectal, head and neck squamous cell carcinoma, prostate, hepatocellular carcinoma and NSCLC (56). HLA mutations and deletions can reduce antigen presentation and increase resistance to T-cell effector molecules like IFN-γ and TNF-α (57). Furthermore, to overcome the onslaught of NK cells, breast and lung cancer cells downregulate cell surface NK activators, making them undetectable to NK cells (58). Upregulation of checkpoint molecules such as HLA-G and HLA-E is another such mechanism used by cancer cells to suppress both NK cell and cytotoxic T cell activity(59, 60).

2.2.3. Inducing immune suppressive microenvironment

The process includes production of immunosuppressive molecules such as TGF-β, IL-10 or expression of inhibitory checkpoint molecules like CTLA-4, PD-L1, or initiation of Tregs, TAMs, and MDSCs recruitment by tumor-associated chemokines like as CSF1, CCL2, CCL22, CCL5, CXCL8, CXCL5, as they should attain immune escape for survival.(61).

2.3.1. Mutations in immune related genes

Mutations in immune-related genes can have profound impacts on the immune system's ability to recognize and fight cancer. Mutations in IDH1**(**Isocitrate Dehydrogenase 1) or IDH2**(**Isocitrate Dehydrogenase 2) can produce an oncometabolite (2-hydroxyglutarate) that affects immune cell function and promotes an immunosuppressive tumor microenvironment(62). Mutations or alterations in the STING (Stimulator of Interferon Genes) pathway can impair the production of type I interferons, weakening immune response against cancer(63). Mutations or loss of HLA class 1 alleles can lead to reduced antigen presentation and immune evasion by cancer cells(55, 64).

2.3.2. CNA in immune related genes

Copy Number Alterations in immune-related genes can significantly influence the immune landscape in tumors and impact the efficiency of immunotherapy. Amplification of the PD-L1 gene can lead to its overexpression on tumor cells, promoting immune evasion by inhibiting T cell activity(65). Deletions in the B2M gene can impair the process of presenting antigen and enable immunological escape.(65).

2.4.1. Frequently mutated genes in cancer

Cancer is characterized by genetic mutations that drive proliferation of cells in unregulated manner. Understanding the role of these frequently mutated genes in cancer can provide insights into the mechanisms of tumorigenesis and help in the development

of targeted therapies. Many of these mutations are key biomarkers for diagnosis, prognosis and treatment selection in oncology.

2.4.2. PIK3CA (Phosphatidylinositol-4,5-Bisphosphate 3-Kinase Catalytic Subunit Alpha)

The PIK3CA gene encodes the p110α subunit of PI3K, which is an enzyme from PI3K/AKT signalling pathway. This pathway plays a crucial role in regulation of various cellular processes, including growth, proliferation, survival and immune response. Mutations in PIK3CA are among the most common genetic alterations in cancer and play a significant role in oncogenesis. PIK3CA mutations frequently occur at specific "hotspots," primarily in the helical (exon 9)(E542K and E545K) and kinase (exon 20) (H1047R) domains of the gene (66). PIK3CA mutations are found in about 30-40% of breast cancers, particularly in hormone receptor-positive (HR+) subtypes(67). PIK3CA mutations are also observed in colorectal cancers, glioblastoma, gastric cancer, lung cancer, endometrial cancers and ovarian cancer at varying frequencies(66). Drugs targeting PI3K, such as alpelisib (specifically targets PIK3CA-mutant cancers), have been developed and approved for use in certain cancers, particularly breast cancer(68). Combining PI3K inhibitors with other treatments, such as hormonal therapies or other targeted agents, can enhance efficacy and overcome resistance(69). Ongoing research aims to refine these therapeutic strategies and overcome challenges such as drug resistance and adverse effects. PIK3CA has evident role in immune function like, migration and chemotaxis of immune cells(33), T cell activation (34), regulation of macrophage function (35).

PIK3CA mutations can alter the profile of chemokines and cytokines released by cancer cells, influencing infiltration of different immune cells.(70). Mutations in PIK3CA may support infiltration of immunosuppressive cells like MDSCs, and these might limit efficient anti-tumor responses.(71). PIK3CA mutations can activate checkpoint molecules like PD-L1, that binds to PD-1 expressed by T cells and hinders their

activity.(72). Combining PI3K inhibitors with immune checkpoint inhibitors (e.g., anti-PD-1/PD-L1 therapies) has shown promise in preclinical models(73), suggesting that targeting both the PI3K pathway and immune checkpoints can produce synergistic anti-tumor effects. PIK3CA mutations can significantly impact the immune response in tumors, influencing the tumor-immune microenvironment, facilitating immune evasion, and affecting patient outcomes. Understanding these interactions can guide the development of more effective therapies, including combination strategies that target both the PI3K pathway and the immune system.

2.4.3. TG (Thyroglobulin)

TG is a large glycoprotein produced by the thyroid gland, playing a crucial role in the synthesis of thyroid hormones (T3 and T4)(39). While TG mutations are more commonly associated with congenital hypothyroidism and other thyroid dysfunctions, they can also be involved in thyroid cancer (74). Elevated levels of thyroglobulin in the blood can indicate the presence of remaining cancer cell(75). The thyroid hormone can alter various components of the immune system by increasing cytokine expression, B cell differentiation, T memory cell generation, natural cell cytotoxicity, and dendritic cell antitumor immunity(40). Thyroglobulin is a tumor marker used to monitor differentiated thyroid malignancies such as papillary and follicular thyroid cancer(41).

2.4.4. HLA-A and HLA-B

Human Leukocyte Antigen A (HLA-A) and Human Leukocyte Antigen B (HLA-B) are major histocompatibility complex class I entities that play a vital role in the immune system by displaying peptide antigens to cytotoxic T lymphocytes.(76). Mutations and alterations in HLA-A and HLA-B can significantly impact immune surveillance and the body's ability to recognize and attack cancer cells. Tumors can evade immune detection through various mechanisms, including mutations in HLA-A and HLA-B, which can impair antigen presentation and reduce T cell recognition(56). Patients with colon or rectal cancer might reap advantages from approaches that promote HLA-B/C

expresion and stimulate T cell mediated immunity (77). HLA-B regulates integrin beta-1 expression and pancreatic cancer cell migration(78).

2.4.5. HLA-DRB1

HLA-DRB1 gene encodes a protein of the major histocompatibility complex (MHC) class II. It is essential in displaying extracellular antigens to CD4+ helper T lymphocytes.(76). While most of the cancer research has concentrated on MHC class I molecules (such as HLA-A and HLA-B) because of their involvement in presentation of antigens to cytotoxic T lymphocytes, MHC class II molecules, such as HLA-DRB1, play vital functions in the immune response to cancer. Downregulation of HLA-DRB1 is linked to poor prognosis and metastasis in cutaneous melanoma. It has the potential to accelerate the shift in the tumor microenvironment from inhibitory to beneficial.(79).

2.4.6. CIITA

CIITA (Class II Major Histocompatibility Complex Transactivator) is a transcriptional coactivator that controls the expression of MHC class II, which includes HLA-DR, HLA-DP, and HLA-DQ. It regulates transcription of Major Histocompatibility Complex (MHC) class I and II genes activated by γ-interferon. (80)Mutations in CIITA can disrupt this process, significantly impacting cancer immune surveillance and progression. CIITA is a gene that's altered in 1.42% of all cancers, including cancers of the central nervous system, intestinal cancer, and skin cancer(81). CIITA mutations are usual in Large B Cell Lymphoma and usually result in low expression of CIITA along with reduction of MHC class II surface expression creating an immune suppressive environment (82). In Hepatocellular carcinoma CIITA is downregulated with downregulation of MHC class II but it correlates to upregulation of MHC class I(83). CIITA correlates to high levels of HLA molecules in uveal melanoma that attributes to promotion of T cells infiltration to TME. Therefore, uveal melanoma could serve as an ideal target for T-cell based therapies(84).

2.5.1. G-quadruplex(G4)

G-quadruplexes are nucleic acid structures with four-strands formed in guanine-rich regions of DNA and RNA. These structures are stabilized by Hoogsteen hydrogen bonding between guanines, creating stacked G-tetrads and potassium ions (Figure 2.2). G4 are found all along the human genome, inclusive of telomeres, promoters and untranslated regions in mRNA(13). G4 structures are involved in several functions like regulating genomic stability, transcription, translation and replication. G4 structures can lead to genomic instability if not properly resolved, contributing to cancer progression. Specialized helicases, such as WRN (Werner Syndrome ATP-Dependent Helicase) and BLM (Bloom Syndrome RecQ Like Helicase), are required to resolve G4 to ensure proper replication. Mutations or deficiencies in helicases that resolve G4 are associated with increased cancer risk(85). Small molecules that stabilize G4 structures are being investigated as potential anticancer agents. These molecules can selectively bind to G4, inhibiting the replication of cancer cells and the expression of oncogenes as G4s are abundant in oncogenes. The ligands to G4 are still in the development process as high specificity is required to avoid off-target effects and toxicity(86). More research is needed to fully understand the diverse roles of G4 in various biological processes to develop a potent and efficient G4 ligand to use for therapeutic purpose in cancers.

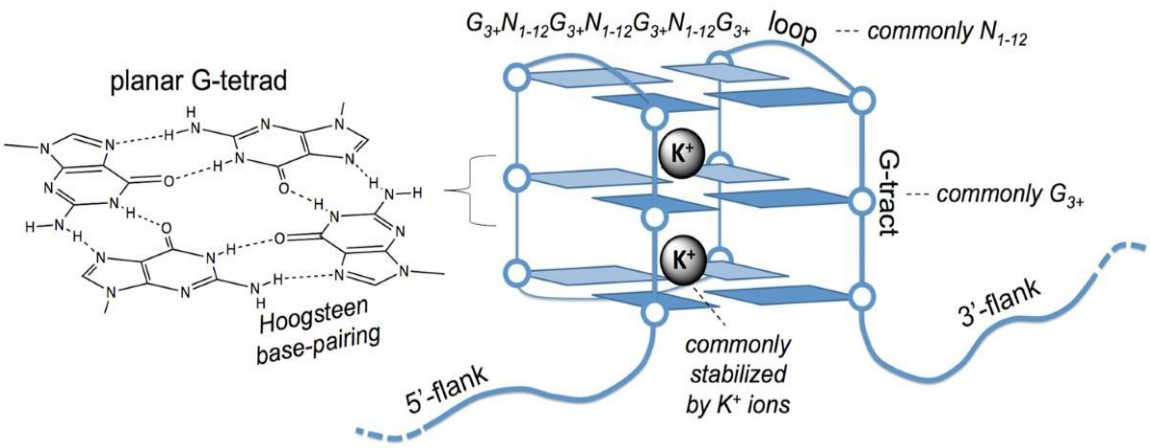

Figure 2.2: A diagrammatic illustration of the characteristics of G-quadruplex (G4) in DNA, including four guanine-rich tracts (G-tracts) that create planar G-tetrads via Hoogsteen base-pairing.

Image courtesy: Sahakyan, A. B., Chambers, V. S., Marsico, G., Santner, T., Di Antonio, M., & Balasubramanian, S. (2017). Machine learning model for sequence-driven DNA G-quadruplex formation. *Scientific reports*, *7*(1), 14535.

2.5.2. G-quadruplex in immune system

G4 are related to immune system in various ways. G4 ligands could be used as effective immunomodulators in combination immunotherapies against unresponsive malignancies(87). Promoters of cytokines like as IL-6, IL-12, IL-17, TNF, TGF-β and β-chain, as well as chemokines like TAFA and XC, have higher G4 frequency than proto-oncogenes.(12). The matured B cell diversifies antibody (Ig) production by class switching recombination, which allows distant "switch" (S) regions to connect. In mammals, S regions is G-rich, generating G4 that promote class switch recombination. G4 ligands were shown to influence class switch recombination in B cells(88).

2.5.3. G-quadruplex in cancer can generate genomic instability

G4 structure development can cause genomic instability by inducing mutations, deletions, and recombination events(11). A higher number of G4 structures can operate as a predictive marker for local single nucleotide variation frequency in cancer samples, this emphasized the necessity of addressing G4 structures in cancer research.(89).

Objectives of the study:

- Characterization of immune related genes with genomic instability in the human genome with respect to cancer
- Functional in silico analysis of immune related genes with genomic instability
- Investigating the crucial role of immune related genes with genomic instability in different types of cancer