Studies on immune related genes with genomic instability in cancer

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Doctor of Philosophy

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Chapter V:

Summary & Conclusion

Summary:

Cancer is a multifaceted disease characterized by the uncontrolled growth and spread of abnormal cells. It arises from mutations that can alter the normal regulatory processes of cell division and death, resulting in unregulated cellular proliferation. These mutations can be triggered by various factors, including environmental exposures, lifestyle choices, infections, and inherited genetic predispositions. Cancer cells possess distinct capabilities, known as the hallmarks of cancer, which enable their growth and survival. There are 14 such hallmarks of cancer, genomic instability and evasion of immune destruction are among them. Genomic instability is a key hallmark of cancer, promoting mutation accumulation and tumor evolution. The role of our immune system is often compromised, allowing tumors to evade immune detection. Tumors can manipulate the tumor microenvironment with upregulation of immune checkpoint molecules, secretion of immunosuppressive cytokines and altering immune infiltration to TME creating an immunosuppressive environment aiding in immune evasion. The process of immune editing, where the immune system shapes tumor immunogenicity, further allows cancer cells to evade destruction. The alterations in immune functions are primarily achieved through mutations. Therefore, immune related genes with frequent mutations could be considered crucial to immune function in cancer.

We investigated the influence of immune related genes with frequent mutations on survival and immune function. To understand the probable mechanism, we examine the differentially expressed genes between mutated and non-mutated datasets, as well as the overrepresented pathways and immune infiltrations in cancers where mutations in the frequently mutated genes could have a significant impact on survival outcomes. The variations in proportions of immune cell recruitment to TME in the mutant compared to non-mutated datasets, as well as the association of these cells to differently expressed genes, adds to our understanding of the impact of immune-related gene alterations. However, due to limitation of both mutation and clinical data we could not attain

significant values for certain genes. Therefore, we investigated the influence of their altered expression on survival and immune response.

Mutations in the genome may be influenced by secondary nucleic acid structures such as G-quadruplexes. G-quadruples are associated to the immune system in various ways. They are abundant in promoters of cytokines. They play crucial role in class switching. Therefore, we investigated the abundance of G-quadruplex in immune related genes and the frequency of mutations in G-quadruplex locations.

Our investigation on influence of mutations in frequently mutated immune response related genes yielded the following results:

1. The mutational profiles of immune response related genes vary across different types of cancer.
2. Mutations in immune related genes in cancers are not influenced by G-quadruplex structures in the human genome. G4 are abundant in genes of B-cell receptor and T-cell receptor pathways, suggesting they play a significant role in B-cell and T-cell development. It is established that proto-oncogenes have a higher frequency of G-quadruplexes. The density of G-quadruplexes in immune-related genes is even higher than in proto-oncogenes. This suggests that G-quadruplexes in immune-related genes, may have unique regulatory features and potentially significant roles in the immune response.
3. PIK3CA and TG were found to be the most frequently mutated immune-related genes. Including the two genes a group of 19 genes were identified that could be categorized into three major groups namely cellular regulation, cellular migration, and antigen presentation.
4. Mutated PIK3CA showed positive association to survival in UCEC but the association was negative in case of LGG. Complement and coagulation pathway was highlighted as an over-represented pathway in mutant dataset of both the cancers; in LGG complement regulatory proteins were upregulated but in UCEC

the regulatory proteins were downregulated. Elevated expression of complement regulatory proteins should facilitate formation of MAC at sub-lytic concentrations that protect the cell from complement mediated lysis and induce cell proliferation. Anaphylatoxins that were released due to complement activation can help cancer cells in anti-apoptotic responses, cell migration and facilitating epithelial-mesenchymal transition. In LGG, infiltration levels of M1macrophages and Tfh were elevated but both of them were associated to pro-tumor genes. In case of UCEC M1 macrophage levels were elevated and associated positively to anti-tumor genes.

5. The poor survival outcome in TG-mutated BRCA may be attributed to the presence of immunosuppressive M1 macrophages influenced by TG mutations.
6. HLA-A, HLA-B, HLA-DRB1 and CIITA were the frequently mutated immune related genes involved in antigen processing and presentation. Higher expression of all the 4 genes were associated to poor survival in LGG. All the genes correlate to HLA-E, a checkpoint molecule that can supress NK cells. These 4 genes showed a positive correlation to M2 macrophage whereas they were negatively correlated to CD4+ T cells infiltration. The data hint us induction of an immune-suppressive microenvironment in LGG by the genes together.

Conclusion:

Cancer hijacks our immune system in large part due to mutations in immune-related genes. The mutational profiles of these genes vary across different types of cancer. Including PIK3CA and TG, a total of 19 genes were frequently mutated and could be categorized into three broad groups: cellular regulation, cellular migration and antigen presentation. We investigated the impact of mutations in PIK3CA and TG on survival, gene expression, pathways, and immune infiltration. Although mutations in four antigen-presenting genes (HLA-A, HLA-B, HLA-DRB1, CIITA) could not show a significant result in survival analysis due to limitations in sample size with both mutation and clinical data, we analysed the effect of their altered expression on survival and the tumor microenvironment.

The survival outcomes of PIK3CA-mutated datasets in cancers may be influenced by modulation of the tumor immune microenvironment with implication of the complement and coagulation cascades. In UCEC, the positive association between PIK3CA mutations and survival may be influenced by the anti-tumor nature of the TIME. Conversely, in LGG, the negative association between PIK3CA mutations and survival could be attributed to the pro-tumor characteristics of the TIME. These divergent behaviours of mutated PIK3CA in the two cancers may be driven by alterations in the complement and coagulation cascades (Figure 5.1, Figure 5.2). This information is pivotal for contemplating the potential inclusion of PIK3CA inhibitors in combinational therapy or considering PIK3CA as a targeted therapy, particularly in the case of LGG. Anti-complement therapies, already available for diseases like paroxysmal nocturnal hemoglobinuria and atypical hemolytic uremic syndrome, validate their potential as cancer therapeutics as well. However, research in this area is still in the preliminary stages.

The poor survival outcome in TG mutated BRCA may be due to immunosuppressive M1 macrophages influenced by TG mutations. Further research will be required to understand

the mechanism underlying how thyroglobulin mutations may alter survival outcomes and immune infiltration in BRCA.

In LGG, higher expression of HLA-A, HLA-B, HLA-DRB1, and CIITA correlated with poor survival outcomes, potentially due to the induction of a pro-tumor immune microenvironment. This environment may be characterized by the inhibition of CD4+ T cells infiltration, promotion of M2 macrophage infiltration and the induction of HLA-E, which can facilitate escape from NK cells. In LGG, these genes together contribute to an immune landscape that supports tumor growth and progression, underscoring the complex role of these genes in immune evasion and cancer prognosis. This information could be instrumental in determining appropriate treatments for patients with LGG (Figure 5.3).

G-quadruplexes (G4), secondary nucleic acid structures known to have the potential to induce mutations, were found to be abundant in immune-related genes. However, these structures did not coincide with mutations in cancer, indicating that mutations in cancers are not influenced by the presence of G4 structures. G-quadruplexes (G4) are plenty in the B-cell receptor (BCR) and T-cell receptor (TCR) pathway genes, suggesting a significant role they might play in B-cell and T-cell development. A more detailed analysis can shed light on the importance of G4 structures in these genes and their potential impact on immune function and development. G4 inhibitors are currently gaining traction in cancer treatment, yet they encounter challenges related to their efficacy. A deeper understanding of how these inhibitors function within the immune system could potentially help address these challenges. This avenue of research may offer insights into optimizing their effectiveness and minimizing unintended impacts on immune function.

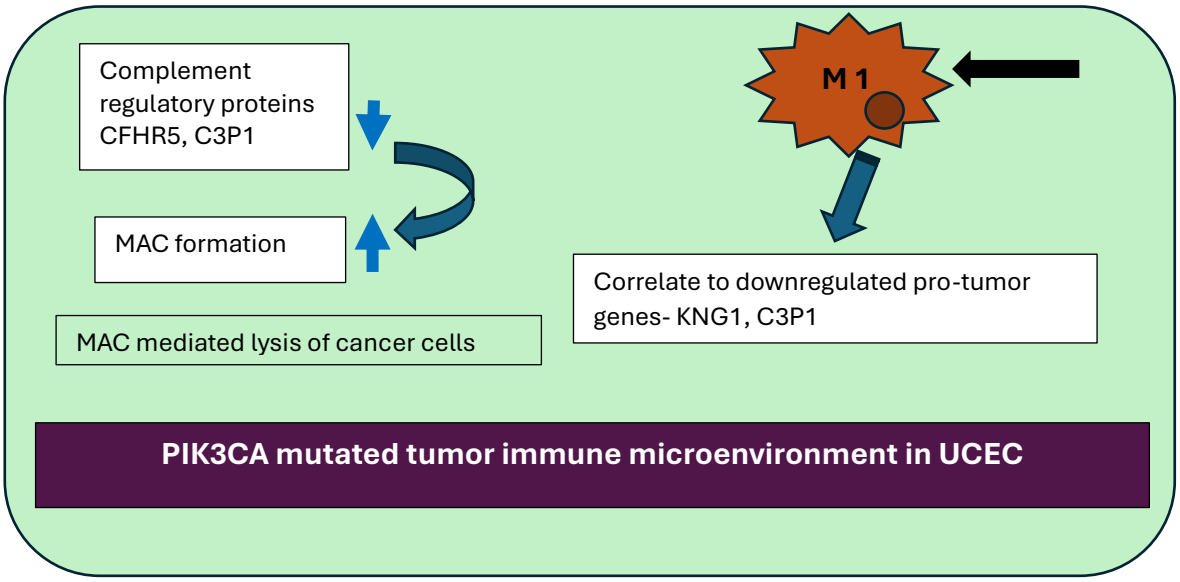

Figure 5.1: A diagram representing changes in immune microenvironment with PIK3CA mutations in UCEC

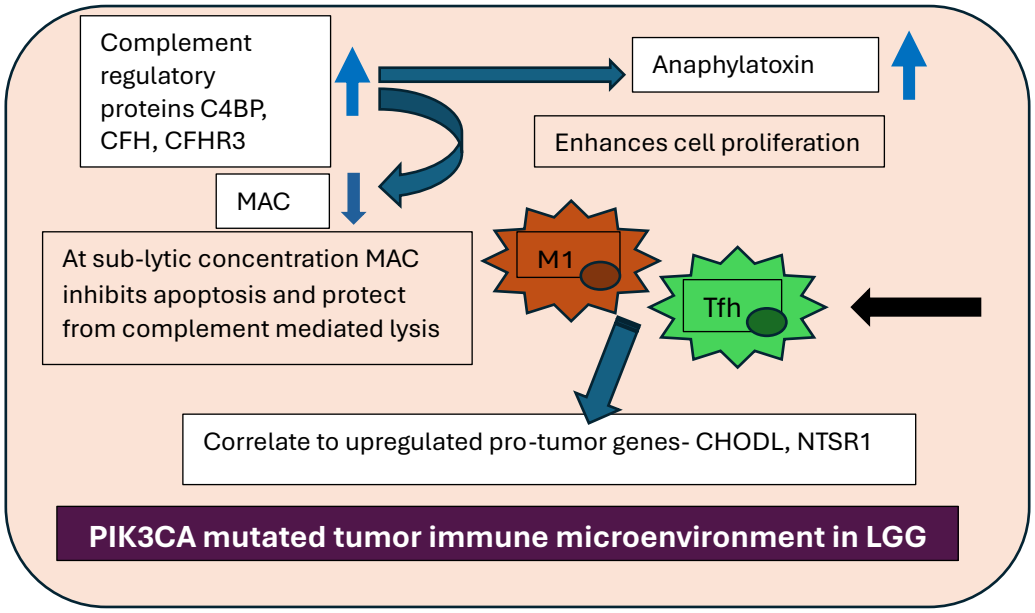

Figure 5.2: A diagram representing changes in immune microenvironment with PIK3CA mutations LGG

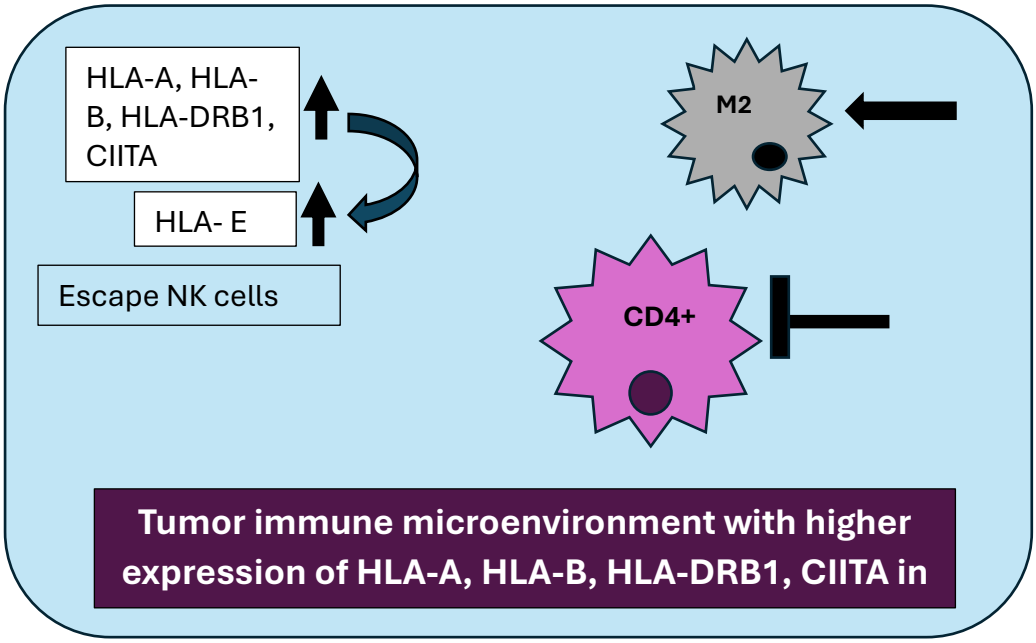

Figure 5.3: A diagram representing changes in immune microenvironment with alterations in expression levels of HLA-A, HLA-B, HLA-DRB1, CIITA in LGG