ABSTRACT

Cancer stands as the most significant challenge in terms of clinical, societal, and economic impact among all human diseases. It arises from the disruption of the orderly process of cell senescence. Genomic instability is a prominent hallmark of cancer, it facilitates for mutations that can contribute to the disorderliness in the senescence process and aids in tumors survival, progression, as well as immune evasion. Evading immune response is another hallmark of cancer. The immune system, which is ordained to protect self from non-self or altered self is either suppressed or manipulated to support cancer cells in a process termed as immune editing. In immune editing, initial immune surveillance is followed by an equilibrium phase, where immune resistant and immune susceptible clone coexist in equilibrium. The equilibrium is tilted by accumulation of mutations favouring immune escape leading to expansion of immune resistant clones that manipulate the tumor immune microenvironment to facilitate secretion of immune-suppressive molecules and infiltration of immune suppressive cells fuelling tumor growth. It can therefore be hypothesized that mutations in immune response related genes have the potential to influence tumor growth, prognosis and survival. In this doctoral research we focus on exploring immune related genes with genomic instability in order to better understand how changes in these genes affect the disease. For the study, immune-related genes exhibiting genomic instability in cancer were selected to evaluate their influence on disease outcome, altered gene expression, enriched pathways and immune cell infiltration to the tumor immune microenvironment.

Datasets for 24 types of cancer and a list of immune-related genes were downloaded from cBioPortal and ImmPort, respectively. Mutation data for genes in the immune-related gene list was obtained and analysed for mutational summaries and screening frequently mutated genes using maftools. CNA data from the 24 different cancer dataset was analysed using GISTIC 2.0. Given the potential of secondary nucleic acid structures, such as G-quadruplexes, to induce genomic instability and their association with the immune system, we investigated their relationship with mutations in immune-related genes in

cancer. FASTA sequences for the human genome were downloaded from NCBI, and G4Hunter was used to examine the occurrence of G-quadruplexes. Mutations from 24 datasets were mapped onto G-quadruplex locations in the human genome using bedtools intersect. We identified a list of 19 frequently mutated genes, with PIK3CA and TG being common to 14 and 15 cancer types, respectively. PIK3CA and TG were amplified in 8 and 7 different cancer types, respectively, marking them as immune-related genes with a high density of nucleotide changes. Kaplan-Meier survival curves for PIK3CA, TG, and four antigen-presenting genes (HLA-A, HLA-B, HLA-DRB1, CIITA) from list of frequently mutated immune-related genes generated using maftools. Cancers having significant impact on survival due to mutations were split into two datasets: mutated and non-mutated. EdgeR was used to detect differential gene expression in the mutated versus non-mutated datasets. The genes with differences in expression levels were subsequently utilized to identify overrepresented pathways. Differences in immune infiltration between mutated and non-mutated datasets were examined using CIBERSORT. The effect of variations in the expression of four antigen-presenting genes (HLA-A, HLA-B, HLA-DRB1, and CIITA) on survival was investigated using GEPIA, as the genes did not achieve significant values for survival analysis when mutations were taken into account.

Our study suggests that mutational summary of immune related genes in different cancer type was distinct. G-quadruplexes were more prevalent in immune-related genes than proto-oncogenes, particularly in genes involved in the TCR and BCR signalling pathways. Although G-quadruplexes are widespread in the immune system, they were not responsible for cancer mutations as the number of mutations overlapping the G-quadruplex sites was remarkably low. This suggests, G-quadruplexes must have a significant function in the regulation of BCR and TCR pathways and mutations of immune related genes in cancer were not introduced through influence of G-quadruplexes.

PIK3CA and TG were highlighted with most frequent alterations. Our findings indicate that the mutant PIK3CA is related with higher 5-year survival in UCEC but is linked to poor survival in LGG. The complement and coagulation pathways was seen as an overrepresented pathway in both cancer types. Higher infiltration of M1 macrophages in the TIME suggest an anti-tumor microenvironment in PIK3CA mutated UCEC while, in

LGG, an elevated abundance of follicular helper T cells, plasma cells and M1 macrophages indicate a pro-tumor microenvironment. The data offer valuable insights into the role of PIK3CA in immune response within cancer, emphasizing its potential role in combination therapy and providing a foundation for increasing the efficacy of PIK3CA-targeting drugs in specific cancers.

TG mutations were associated with poor survival in BRCA. TG mutated dataset had a higher infiltration of M1 macrophages, but the macrophages were associated with immune-suppressive genes like ACE2 and IDO2 hinting us that the M1 macrophages might act as immunosuppressive in TIME of TG mutated BRCA.

HLA-A, HLA-B, HLA-DRB1 and CIITA were upregulated in LGG. High expression levels of HLA-A, HLA-B, HLA-DRB1 and CIITA correlated with poor survival in LGG. CIITA, together with RFX, NFY, and CREB, is responsible for regulating MHC expression. Elevated levels of RFX, CREB, and HLA-E have been associated with poor survival outcomes. In LGG, upregulated HLA-E showed a strong positive correlation to the 4 genes in our study. The upregulation of HLA-E is associated with evasion from both NK cells and cytotoxic T cells. Additionally, expression levels of HLA-A, HLA-B, HLA-DRB1, and CIITA correlate negatively to CD4+ T cell while the correlation was positive for M2 infiltration validating their immune suppressive nature in LGG TIME. This suggests that the combined high expression of HLA-A, HLA-B, HLA-DRB1, and CIITA might facilitate immune suppression in the tumor microenvironment of LGG. The data obtained should help to better appreciate the mechanisms behind immune evasion in tumors and might prove to be useful in considering suitable therapeutics for better outcomes in LGG patients.

SCIENTIFIC PUBLICATIONS FROM THE THESIS

1. Bora, M., Sarmah, N., **Das, B**., Baruah, M. N., Deka, G., Hazarika, S. G., & Baruah, S. (2022). A comparative study on regulation of HLA-G expression in bad obstetric history and in head and neck squamous cell carcinoma from Northeast India. *Human Immunology*, *83*(5), 453-457.

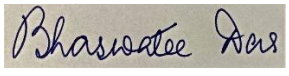
SCIENTIFIC PRESENTATIONS FROM THE THESIS

1. **Das,B** and Baruah,S, G-quadruplexes, HLA-G and Cancer: What is the connection ?, *National seminar on Advances in Basic and Translational Research in Biology (ABTRiB),* Department of Molecular Biology and Biotechnology, Tezpur University. 11th and 12th March, 2022
2. **Das,B** and Baruah,S, Mutations of immune response related genes in Head and Neck Squamous Cell Carcinoma (HNSCC), *48th Annual Conference of the Indian Immunology Society (Immunocon 2022),* Department of Molecular and Human Genetics, Banaras Hindu University, Varanasi, India, 8th – 9^{h} July , 2022 (Virtual).
3. **Das,B** and Baruah,S, Mutation and expression of PIK3CA favored survival in UCEC but not KIRC, *Golden Jubilee Conference of the Indian Immunology Society (Immunocon 2023),* JL Auditorium, AIIMS, India, 5th-8th, October, 2023.

OTHER SCIENTIFIC PUBLICATIONS

2. **Das, B**., Choudhury, B., Kumar, A., & Baruah, V. J. (2021). Genomic instability and DNA repair in cancer. In *DNA-Damages and Repair Mechanisms*. IntechOpen.

Signature of the Student



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