Mutations In BRAF Are Associated With Higher Levels of Immune Infiltrates in Microsatellite-Stable Colon Cancer

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Introduction

While BRAF is among the most well-established oncogenes in human cancers, more recently it has garnered attention for its role in suppressing antitumor immunity, especially in melanoma. Because tumor-infiltrating lymphocyte (TIL) density is strongly prognostic of colorectal cancer (CRC)\(^1\), we decided to investigate the connection between TIL density and the BRAF-activating V600E mutation in CRC.

Methods

We downloaded clinical and mRNA expression data for the colon adenocarcinoma (COAD) cohort of The Cancer Genome Atlas (TCGA) via the UCSC Cancer Portal. We used ESTIMATE to infer the presence of immune cells in the TCGA COAD dataset (n = 216); this is an algorithm that uses a gene-expression signature of 141 immune-related genes to infer the presence of immune cells in the tumor infiltrate\(^2\). Using R, we ran Wilcoxon tests in comparing mean ESTIMATE scores between BRAF-V600E and BRAF-WT groups, as well as an ANOVA on a linear model for ESTIMATE that included terms for microsatellite phenotype, BRAF mutation status and an interaction term. We obtained our data for the BROAD CRC cohort (n=445) directly from the authors of a previous study\(^3\), including immune scores obtained by immunostaining. We performed two kinds of tests: Wilcoxon tests comparing the mean raw immune scores of the BRAF-V600E and BRAF-WT groups, and Fisher’s tests comparing the number of positive (score > 0) and negative (score = 0) binarized immune scores of each group.

Results

COAD samples with the BRAF-V600E mutation have higher levels of immune cells than those without the mutation (p < 0.05, Figure 1). The MSI-H subtype of CRC is characterized by epigenetic silencing of DNA mismatch-repair genes and higher mutational load, neoantigen expression and TIL density. Since it has been previously established that BRAF mutation is highly correlated with MSI-H phenotype\(^4\), we performed an ANOVA to assess if BRAF mutation status predicts ESTIMATE score independently of MSI-H phenotype. While the BRAF term was not significant in this multivariate linear model and TIL density. Since it has been previously established that BRAF mutation is associated with higher immune-cell infiltration\(^5\), we included an interaction term that was significant (p < 0.005), suggesting that both variables contribute to ESTIMATE score.

To determine the effect of the interaction, we grouped samples into MSI-H and MSS groups and compared the wild-type and BRAF V600E subgroups within them. In the MSS group only, immune scores were higher in the BRAF-V600E subgroup (p = 0.05, Figure 2). Next, we sought to reproduce our results in an independent cohort of CRC tumor samples from the BROAD institute that have been assigned immune scores based on immunostaining\(^6\). We grouped samples into TIL-positive and TIL-negative groups and compared the numbers of BRAF-V600E and BRAF-WT samples within each group. We observed a significant correlation between BRAF mutation and TIL+ immune score within the MSS group (p = 0.01352, odds ratio 5.76, Figure 3). Notably, the MSS group is the same group from the TCGA cohort in which we observed a significant correlation between BRAF mutation and ESTIMATE score.

Discussion

The analysis we present here reveals a statistically significant correlation between the BRAF-V600E mutation and increased immune infiltrate in MSS tumors across two independent colon cancer datasets. Because V600E is an activating mutation, this finding suggests an immunity-stimulating role for BRAF in CRC, in contrast to BRAF’s role in melanoma, which appears largely immunosuppressive.

References

6. Marco-Doria, Paolo Fagone, Fernando Nicotelli, Nicola D’Angelo, Omid Mahdavi, For the Shoklo Malaria Research Unit and Molecular and Nuclear Medicine Group (2012) BRAF inhibition improves tumor recognition by the immune system, Oncogene, 31(13), 1747-1753. DOI: 10.1038/onc.2011.237