Title: A potential oncogenic role and immunoregulatory mechanisms of MZB1 in melanoma

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Scientific Abstract:

While immune checkpoint blockade (ICB) therapies have drastically improved melanoma treatment, a majority of patients fail to achieve a durable response. Thus, there is a critical need to advance our understanding of melanoma immune evasion mechanisms and identify novel targets capable of enhancing ICB responses. MZB1 (Marginal Zone B and B1 Cell Specific Protein), an immune regulator gene, is differentially expressed in metastatic melanoma compared to primary melanoma or normal skin. In a limited number of cancers, MZB1 has been shown to function as either an oncogene or a tumor suppressor. However, its role and significance in melanoma is not known. In this study, we determined the functional significance and immunoregulatory mechanisms of MZB1 in human melanoma cell lines using MZB1 overexpression (OE) (in SK-MEL-2, and SK-MEL-28) and CRISPR knockout (KO) (in A375 and Hs294T) lines. MZB1 OE significantly increased melanoma cell proliferation and expression of the proliferation markers Ki67 and PCNA, while MZB1 KO yielded the opposite effect. To identify immunoregulatory mechanisms associated with MZB1, we performed differential gene expression analysis of 770 immune-related genes using NanoString nCounter technology. nSolver analysis identified 153 differentially expressed genes (DEGs) as a result of MZB1 knockout in Hs294T cells (adjusted $p < 0.05$, 1.5-fold change cutoff). MZB1 KO strongly reduced the expression of the HLA class-II family genes and altered the expression of various cytokines, suggesting an immunoregulatory role of MZB1. Further, MZB1 KO significantly downregulated genes related to cancer progression and metastasis promotion. Additionally, MZB1 KO reduced expression of common “cancer stem cell” genes, such as RXRG, ALDH1A1, AQP1, NFATC2, LPAR1, indicating MZB1 expression in melanoma may promote a dedifferentiated or neural crest-like state. In addition, an Ingenuity Pathway Analysis (IPA) of the DEGs revealed many cancer-related functions altered upon MZB1 KO (p adjusted < 0.05) and displayed a negative enrichment for cancer-promoting pathways including tumor growth, metastasis, and proliferation. IPA regulatory analysis predicted MZB1 KO to decrease the activation of oncogenic signaling components including PI3K, MEK, and ERK1/2. Further, IPA predicted that MZB1 inhibition could positively sensitize melanoma cells towards higher immune responses by activating mechanisms associated with T cell proliferation and activation, leukocyte migration, and macrophage activation. Overall, our study suggests MZB1 promotes oncogenic signaling in melanoma to drive proliferation and dedifferentiation, as well as regulates a wide range of immune-related genes that may be responsible for immune evasion in melanoma cells. Thus, MZB1 merits further investigation to determine if the immune consequences yielded by targeting MZB1 can complement ICB approaches in the management of melanoma.

Written Lay Abstract:

One type of treatment for melanoma (skin cancer) is immune checkpoint blockade (ICB) therapy. However, ICB therapy does not work for most patients with melanoma. Scientists are trying to better understand melanoma to find more effective treatments.

In normal skin cells, the gene MZB1 controls cell growth and immune responses, such as increasing inflammation and antibodies. In metastatic melanoma (skin cancer that has spread), MZB1 does not act normally**.** In this study, the researchers studied how MZB1 works in human melanoma cells. In one test, they changed the DNA in cells so they would have more MZB1 activity. In another test, they changed cell DNA to remove MZB1.

In melanoma cells with more MZB1 activity, there was more cancer cell growth and more immune responses. In melanoma cells without MZB1, there was less cancer cell growth. The scientists found that MZB1 affected 153 of 770 genes that control immune response. MZB1 also affected other genes that control cancer cell growth and spread.

This research tells us that MZB1 affects cancer cell growth and immunity, which makes it difficult for ICB therapy to work on these cancer cells. Future studies can look at how to target MZB1 so that ICB therapy can better treat melanoma.

Visual Lay Abstract: