

EZH2 SENSES METABOLIC STRESS TO RESTORE MHC-I ANTIGEN PRESENTATION AND ICB RESPONSE IN METASTATIC MELANOMA

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Background Unleashing the immune anti-tumor response through immune checkpoint blockade (ICB) therapy has been successful in combating solid-tumor malignancies, including metastatic melanoma. When successful, the anti-tumor response is potent; however, half of melanoma patients fail to respond. ICB responsiveness is dictated by the immune milieu and antigenicity of the tumor microenvironment (TME), with therapy-resistant melanomas exhibiting reduced expression of major histocompatibility complex class I (MHC-I) and increased levels of endoplasmic reticulum (ER) stress. Though the TME is known to be immunosuppressive, the specific mechanisms governing metabolic stress and antigen presentation in the context of ICB response have not been well characterized.

Methods To further study the regulation of MHC-I, we cultured melanoma in conditions of prolonged metabolic stress, forcing cells to adapt to the absence of glucose. Proteomic profiling indicates this model establishes a reversible, adaptive phenotype reminiscent of the changes seen in published ICB-responder datasets. Quantification of tumor immunogenicity was achieved by RNA signatures, flow cytometry, and immunoblotting. Functional assessment of immune-mediated killing was achieved through co-culture killing assays with B16-OVA cells and activated OT-1 CD8⁺ T-cells. Chromatin immunoprecipitation (ChIP) was used to quantify chromatin modification, globally and at specific gene loci. To assess the role and functionality of specific epigenetic regulators on ICB response, mutant cell lines were established through lentiviral transduction of B16 melanoma cell lines.

Results Melanoma metabolically adapted to glucose-free media had increases in MHC-I antigen presentation. Remodeling the energy metabolism globally restored MHC-I genes and significantly increases tumor sensitivity to T-cells, independent of IFN- γ . Proteomic analysis exhibited significant dysregulation in histone modifiers, specifically the loss EZH2 (Enhancer of Zeste Homolog 2), a histone methyltransferase characterized by transcriptional repression. As confirmed by ChIP-Sequencing and ChIP-PCR, the catalytic mark of EZH2, H3K27me3, is reduced in metabolically reprogrammed melanoma at gene loci specific to MHC-I antigen presentation. Additionally, EZH2 mutant melanoma cell lines demonstrate differential sensitivity to activated CD8⁺ T-cells, further implicating EZH2 in the determination of ICB response.

Conclusions The melanoma response to ICB is influenced by the metabolic state of the tumor. EZH2, a potent regulator of gene expression, is critical to the interferon-independent mechanisms of antigen presentation and immune escape, which dictate the ICB response. Conditions of increased mitochondrial respiration and ER stress significantly decrease EZH2 levels and presence of H3K27me3 at the promoter regions of MHC-I antigen-presenting genes. This demonstrates the clear potential for metabolic and EZH2 status as a prognostic indicator for ICB-responsiveness in metastatic melanoma

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