

EPIGENETIC REMODELING ENHANCES THERAPEUTIC RESPONSES TO STING AGONISM *IN VIVO*

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Background Despite evidence of therapeutic activity in preclinical models, STING activating agents have shown limited efficacy in early phase clinical trials. This discrepancy in outcomes suggests that in addition to antigen presenting cells, targeting STING pathway could be also functionally relevant in tumor cells with well documented epigenetically driven STING suppression.^{1, 2} In this study, we determined if reversal of tumor cell-intrinsic STING silencing using a DNA methyltransferase inhibitor can improve the efficacy of a STING agonist in mouse models of melanoma.

Methods Using genome-wide methylation profiling, we assessed methylation levels of STING in B16-F10 and Yumm1.7 mouse melanoma cell lines before and after treatment with 5-aza-2'-deoxycytidine (5AZADC). We also evaluated their activation of STING following stimulation with the STING agonist ADU-S100. Using B16-F10 and Yumm1.7 models in STING-deficient and STING-sufficient hosts, we next assessed the effect of 5AZADC treatment on the efficacy of ADU-S100. Additionally, we performed mechanistic studies using T-cell depletion experiments as well as phenotypic and gene expression profiling.

Results While we observed hypermethylation of STING in both B16-F10 and Yumm1.7 cell lines, their treatment with 5AZADC resulted in a decrease in STING methylation levels. We also found reconstitution of STING protein expression in 5AZADC-pretreated cell lines as well as up to a 46-fold increase in induction of IFN- β ($p < 0.001$) and a 4.5-fold increase in MHC class I surface expression ($p < 0.01$) compared to untreated controls following stimulation with ADU-S100. In tumor-bearing mice, while treatment with a combination of 5AZADC and ADU-S100 resulted in a marked increase in *Ifnb1* transcripts within tumors ($p < 0.001$), it significantly delayed tumor growth ($p < 0.05$). Antibody-mediated depletion studies in mice receiving the combination therapy further indicated that this antitumor activity depends on the generation of functional tumor antigen-specific CD8⁺ T cells ($p < 0.001$); however, tumor growth remained unaltered by the depletion of CD4⁺ T cells.

Conclusions We have shown that although epigenetic silencing of STING in melanoma cells can confer resistance to STING agonist therapy, a rational combination of a clinically available DNA methylation inhibitor with a STING agonist can reverse this silencing and lead to robust antitumor responses in the setting of two STING^{low} murine models of melanoma. Therefore, identification and pharmacologic restoration of tumor cell-intrinsic STING defects through epigenetic reprogramming might be critical for the successful use of STING agonists in the clinic.

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