MIR-15A AND MIR-15B MODULATE NATURAL KILLER AND CD8+ T-CELL ACTIVATION AND ANTI-TUMOR IMMUNE RESPONSE BY TARGETING PD-L1 IN NEUROBLASTOMA

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Background Neuroblastoma (NB) is an enigmatic and deadliest pediatric cancer to treat. The major obstacles to effective immuno-therapy treatments in NB are defective immune cells and the immune evasion tactics deployed by the tumor cells and the stromal microenvironment. Nervous system development during embryonic and pediatric stages is critically mediated by non-coding RNAs such as micro RNAs (miR). However, how miRs influence T and NK cell function and anti-tumor immune response in NB remains poorly understood.1,3

Methods We explored the role of miRs in anti-tumor immune response via a range of data-driven workflows and in vitro & in vivo experiments. Using the TARGET, NB patient dataset (n=249), we applied the robust bioinformatic workflows incorporating differential expression, co-expression, survival, heatmaps, and box plots. We performed PD-L1 mRNA 3'-untranslated region sequence-specific luciferase activity and Ago2 RNA immunoprecipitation assays. NB cells expressing miR-15a/miR-15b were cocultured with CD8+T and NK cells and analyzed the activation and cytotoxicity against NB in vitro. Murine stable NB cells expressing miR-15a were subcutaneously injected into C57/BL6 mice and analyzed tumor size, tumor vasculature, and the activation and infiltration of tumor CD8+T and NK cells. Further, surface PD-L1 was blocked using an anti-PD-L1 antibody and CD8+T, and NK cell-mediated anti-tumor responses were studied.

Results We initially demonstrated the role of miR-15a-5p (miR-15a) and miR-15b-5p (miR-15b) as tumor suppressors, followed by their negative association with stromal cell percentages and a statistically significant negative regulation of T and natural killer (NK) cell signature genes, especially CD274 (PD-L1) in stromal-low patient subsets. The NB phase-specific expression of the miR-15a/miR-15b-PD-L1 axis was further corroborated using the PDX (n=24) dataset. We demonstrated miR-15a/ miR-15b mediated degradation of PD-L1 mRNA through its interaction with the 3'-untranslated region and the RNA-induced silencing complex using sequence-specific luciferase activity and Ago2 RNA immunoprecipitation assays. In addition, we established miR-15a/miR-15b induced CD8+T and NK cell activation and cytotoxicity against NB in vitro. Moreover, the injection of murine cells expressing miR-15a reduced tumor size and tumor vasculature and enhanced the activation and infiltration of CD8+T and NK cells into the tumors in vivo. We further established that blocking the surface PD-L1 using an anti-PD-L1 antibody rescued miR-15a/miR-15b induced CD8+T and NK cell mediated anti-tumor responses.

Conclusions These findings demonstrate that miR-15a and miR-15b induce an anti-tumor immune response by targeting PD-L1 in NB.

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Dataset: Therapeutically Applicable Research to Generate Effective Treatments (TARGET) (https://ocg.cancer.gov/programs/target) initiative, phs000218.

PDXs: Childhood Cancer Repository of the COG and Solid Tumor Network of St. Jude Children’s Research Hospital