ID: 03375

Topic: INNOVATIVE THERAPIES

Enhancing NK Cell-Mediated Cytotoxicity in Neuroblastoma through MYC Inhibition with Omomyc: A Promising Strategy for High-Risk Pediatric Tumors

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Background: Allogeneic natural killer (NK) cell therapy has shown promising results in hematological malignancies but remains less effective in solid tumors such as neuroblastoma (NB). NB is a pediatric cancer of the sympathetic nervous system, with more than 50% of children with high-risk NB experiencing relapse and a 5-year overall survival rate of less than 10%. The MYCN oncogene is amplified in around 20% of NB cases, particularly in high-risk patients, and is associated with tumor progression, immune evasion, metastasis, and poor prognosis. Recent studies have demonstrated the potential of Omomyc, a dominant-negative MYC mutant, to reduce tumor cell proliferation and induce tumor regression through microenvironmental changes. Suppressing MYC via Omomyc may also enhance NK cell-mediated immunosurveillance, potentially restoring tumor cell sensitivity to NK cell killing. **Aim**: This study aims to assess whether MYC inhibition through Omomyc enhances the sensitivity of neuroblastoma cell lines to NK cell-mediated cytotoxicity. **Methods**: Doxycycline-inducible neuroblastoma cell sublines were created from SH-EP, LA-N-1, and LA-N-5 cell lines by transducing them with pTRIZ-RFP or pTRIZ-RFP-Omomyc plasmids using third-generation lentiviruses. Transduced cells were sorted using flow cytometry after doxycycline induction. NK cells were activated and expanded from peripheral blood mononuclear cells (PBMCs) of healthy donors using IL-2 and IL-15. Proliferation assays and NK cell-mediated cytotoxicity assays were conducted at different effector-to-target cell ratios (1:10, 1:2, 1:1), with continuous monitoring using the IncuCyte® S5X live-cell imaging system. The expression of NK cell ligands (MICA/B, ULBP 2/5/6, ULBP 3) on neuroblastoma cells was assessed before and after Omomyc induction by flow cytometry. **Results**: MYC inhibition via Omomyc reduced cell proliferation in all neuroblastoma cell lines. Furthermore, Omomyc expression enhanced NK cell-mediated cytotoxicity, restoring neuroblastoma cell sensitivity to NK cell killing. This was associated with increased expression of NK cell-activating ligands such as MICA/B, ULBP 2/5/6, and ULBP 3. These effects were more pronounced in MYCN-amplified cell lines (LA-N-1, LA-N-5) compared to non-amplified SH-EP cells. **Conclusions**: MYC inhibition by Omomyc sensitizes neuroblastoma cells to NK cell-mediated cytotoxicity. These findings suggest that combining MYC inhibition with Omomyc and adoptive NK cell therapy may offer a promising new treatment strategy for neuroblastoma.