NKG2DL AS A PROMISING TARGETS FOR COLORECTAL CANCER IMMUNOTHERAPY

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Background: Colorectal cancer is the third most commonly diagnosed cancer and the second leading cause of cancer-related death. Immunotherapy for colorectal cancer is a relatively new area of effective treatment. A special place in immunotherapy is occupied by CAR-T, the targets for which can be NKG2D ligands. These molecules are widely found on various types of tumors and stressed cells. These include MICA, MICB, and ULBPs, which we tested for expression in human colorectal cell lines HT-29, RKO, LOVO, HCT116. These lines were also tested for resistance to FOLFOX, cytokines, and NKG2D-CAR-T itself.

Materials and methods: Ligand expression was tested by flow cytometry and qPCR. MTT assay was used to test FOLFOX sensitivity. Cytokine and CAR-T cell death assay was tested by RTCA (real-time cytotoxicity assay).

Results: The colorectal cancer cell lines tested displayed a heterogeneous pattern of NKG2D ligand expression, with distinct differences in their sensitivity to treatment. Among the panel, the RKO line emerged as the most resistant to the chemotherapeutic regimen FOLFOX, showing only limited loss of viability under drug exposure. In contrast, HT-29 and LOVO cells responded markedly to cytokine stimulation, indicating a higher degree of vulnerability to immune-mediated cytotoxic signals. RKO cells, however, exhibited relative insensitivity to cytokines, reinforcing their resistant phenotype. Importantly, when challenged with NKG2D-based CAR-T cells, all colorectal cancer lines tested—including RKO—were efficiently recognized and lysed. None of the lines displayed measurable resistance to NKG2D-CAR-T activity, underscoring the broad potential of this approach to overcome intrinsic heterogeneity in cytokine and chemotherapy responses.

Conclusion: Our NKG2D-CAR-Ts demonstrate versatility against human colorectal cell lines and lack of resistance to them even in cancer types that are potentially resistant to chemotherapy or cytokine therapy.

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References: Schmiedel, D., Mandelboim, O. NKG2D ligands–critical targets for cancer immune escape and therapy. Front. Immunol. 9, 2040 (2018).

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