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Enhancing anti-tumor immune response by modulating splicing to generate neoantigens

Anurag Prabhu, Adi Mogilevsky, Ariel Feiglin, Zahava Kluger, Michael Berger, Erez Levanon, Rotem Karni

Hebrew University of Jerusalem, Israel

Background: The formation of neoantigens is governed by the tumor mutational burden and many of the common cancers possess low mutation burden. Revolutionary check-point inhibitor immunotherapies are inefficient in the absence of neoantigens, expressed by the tumor cells. To overcome these limitations, we present a novel approach to induce production of neoantigens in tumor cells by mRNA splicing modulation.

Methods: As a part of a collaboration, a computational pipeline was created to predict target exons which can be skipped to induce a frame-shift in the mRNA forming unnatural aberrant isoforms. These are predicted to be presented by the tumor cells on their MHC-I molecules at the cell membrane. The predicted events were filtered to focus on over-expressed transcripts in cancer compared to normal tissues. We employed CRISPR/Cas9 to disrupt the splice sites of the predicted exons to induce target exon skipping in selected murine cancer cell lines. Next, we immunized immune-competent mice with predicted peptides and injected the CRISPR modified cells to assess immune-mediated tumor inhibition.

Results: The immunogenicity of the predicted neoantigens was assessed by injecting these peptides into immune-competent mice. $CD8^+$ T cells were activated upon restimulation with the resulting neoantigen *in vitro*. Furthermore, we observed reduced tumor growth in immune-competent mice that were injected with cancer cells expressing target exon skipping compared to control tumors. We also observed significant activation of $CD8^+$ T cells and tumor infiltration in the mice that were injected with CRISPR modified cells. Injections in immune-deficient mice showed no significant difference in tumor growths between the groups.

Conclusion: The final goal of our research is to determine if splicing induced neoantigens can improve the anti-tumor response of existing immune checkpoint blockade (ICB) therapies.This proposal demonstrates a novel approach to generate neoantigens with high therapeutic potential for treating various cancers and offers possible solutions to augment current immunotherapies.