Nanoparticles Targeting the Adenosine A\textsubscript{2A} Receptor as a Potential Immunotherapy in Renal Cell Carcinoma

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Introduction
Adenosine (Ado) accumulates within solid tumors and signals through the Ado A\textsubscript{2A} receptor (A\textsubscript{2A}R) to decrease T cell motility and cytokine release. However, the effect of Ado on the chemotaxis of CD8\textsuperscript{+} T cells from renal cell carcinoma (RCC) patients is unknown. Additionally, antagonism of the A\textsubscript{2A}R can rescue T cell migration, so a targeted therapy that decreases A\textsubscript{2A}R expression in CD8\textsuperscript{+} T cells may be of clinical utility.

Hypothesis
Ado inhibits the chemotaxis of peripheral blood (PB) CD8\textsuperscript{+} T cells of RCC patients. Lipid nanoparticles (NPs) can selectively deliver A\textsubscript{2A}R siRNAs to CD8\textsuperscript{+} T cells, downregulating the A\textsubscript{2A}R specifically in this cell type.

Methods
Cytotoxic T cells were isolated from RCC patients' peripheral blood (n=4) and the three-dimensional chemotaxis of these cells in the absence of Ado was compared to that of cells in a gradient of Ado. The Y center of mass (Y-COM) was determined for each condition, which reflects the collective endpoint to which the cells traveled. To determine the efficacy of the A\textsubscript{2A}R siRNAs, PB T cells from healthy donors were nucleofected with either A\textsubscript{2A}R or scramble siRNAs, and mRNA and protein levels assessed with RT-qPCR and flow cytometry, respectively. Fluorescent NPs were fabricated, labeled with anti-CD8 antibody, and added to PB T cells. Flow cytometry was used to assess the selectivity of the NPs for CD8\textsuperscript{+} T cells.

Results
Ado was responsible for a 66 \pm 3.8\% (mean \pm SE) reduction in the Y-COM in 3 RCC, while one patient did not respond to Ado. A\textsubscript{2A}R siRNAs reduced A\textsubscript{2A}R mRNA by 56\%, 61\%, 45\%, and 35\% at 12, 24, 48, and 72 hours, respectively. Decreases in A\textsubscript{2A}R protein expression of 22\%, 32\%, and 39\% were seen at 24, 48, and 72 hours, respectively. Flow cytometry analysis revealed that anti-CD8 labeled NPs are specific to CD8\textsuperscript{+} T cells.

Conclusions
RCC CD8\textsuperscript{+} T cells’ ability to infiltrate a tumor-like microenvironment is severely compromised by Ado. We now have the appropriate elements to fabricate CD8-specific NPs loaded with A\textsubscript{2A}R siRNAs, which should render CD8\textsuperscript{+} T cells less sensitive to Ado – providing a potential therapy in RCC.

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