Nanoparticles Targeting the Adenosine A_{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_{2A} receptor ($A_{2A}R$) to decrease T cell motility and cytokine release. However, the effect of Ado on the chemotaxis of $CD8^+$ T cells from renal cell carcinoma (RCC) patients is unknown. Additionally, antagonism of the $A_{2A}R$ can rescue T cell migration, so a targeted therapy that decreases $A_{2A}R$ expression in $CD8^+$ T cells may be of clinical utility.

Hypothesis

Ado inhibits the chemotaxis of peripheral blood (PB) $CD8^+$ T cells of RCC patients. Lipid nanoparticles (NPs) can selectively deliver $A_{2A}R$ siRNAs to $CD8^+$ T cells, downregulating the $A_{2A}R$ 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 $A_{2A}R$ siRNAs, PB T cells from healthy donors were nucleofected with either $A_{2A}R$ 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 $CD8^+$ T cells.

Results

Ado was responsible for a 66 ± 3.8% (mean ± SE) reduction in the Y-COM in 3 RCC, while one patient did not respond to Ado. $A_{2A}R$ siRNAs reduced $A_{2A}R$ mRNA by 56%, 61%, 45%, and 35% at 12, 24, 48, and 72 hours, respectively. Decreases in $A_{2A}R$ 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 $CD8^+$ T cells.

Conclusions

RCC $CD8^+$ 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 $A_{2A}R$ siRNAs, which should render $CD8^+$ T cells less sensitive to Ado – providing a potential therapy in RCC.

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