09. Cell Therapy in Solid Tumors

P09.01 ADAPTING IMMUNE CELLS TO THE HYPOGLYCEMIC TUMOR MICROENVIRONMENT BY SOLUTE CARRIER 2A1 (*SLC2A1*/GLUT1) OVEREXPRESSION

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Background In recent years, T cell-based immunotherapies have shown promising results in hematologic malignancies. However, these strategies seem to be limited in solid cancers, posing more complex challenges including a hostile TME with nutrient deprivation and tissue hypoxia [1]. Additionally, metabolic reprogramming has been identified as a crucial factor for proper cytotoxic T-cell functions upon their activation. Such energy demands are answered by the upregulation of glycolysis, oxidative phosphorylation, and upregulation of nutrient transporters represented by SLCs [2,3]. Within the TME, tumor and immune cells compete for nutrients and shape a distinct metabolic milieu, resulting in an ineffective effector function [4]. Herein, we aim to metabolically engineer T cells to improve their fitness in the glucose-deprived TME and optimize ACT.

Materials and Methods We retrovirally overexpressed the glucose transporter Slc2a1/GLUT1 in murine CD8$^+$ T cells (CD8$+^{Slc2a1}$). To assess T-cell fitness we conducted experiments in physiologic (5mM) and hypoglycemic (0.5mM) media conditions. CellTrace^{TM}-based proliferation experiments and killing assays in the OT1-OVA model are used to examine differences to MOCK in functionality and were analyzed via flow cytometry and microscopy, respectively. Furthermore, Seahorse analyses, bulk RNA-Seq, and metabolomic analyses were performed to examine the mechanical background. Murine *in vivo* studies are performed to approach the translatability of this system into living organisms.

Results CD8$+^{Slc2a1}$ cells possessed a higher proliferative capacity in all conditions tested but most prominently in hypoglycemic (0.5mM) media. This better functional activity of CD8$+^{Slc2a1}$ was also translated to higher killing rates in coculture assays with tumor cells, especially in low-glucose environments. Metabolic flux analyses and multi-omics suggested greater metabolic activity of CD8$+^{Slc2a1}$ and revealed higher ROS production and upregulation of correlating antioxidative pathways, especially the pentose-phosphate pathway. Preliminary *in vivo* studies support the *in vitro* killing in a syngeneic tumor model. Furthermore, signs of altered memory formation were visible, expressed in a higher proportion of effector memory cells.

Conclusions Our data point to the role of GLUT1 overexpression in T cells for improved cytotoxic activity, proliferation, and long-term persistence. Therefore, combinatorial approaches with GLUT1 overexpression could serve as a potential approach to increase efficacy in ACT against solid cancer. We also identified GLUT1-dependent reprogramming in CD8$+^{Slc2a1}$ cells which is further investigated in ongoing studies. Additionally, we are evaluating the potential risk of this approach to neoplastic formation.

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P09.02 LOADING OF CAR-T CELLS WITH SUPERPARAMAGNETIC IRON OXIDE NANOPARTICLES ALLOWS THEIR MAGNETIC TARGETING FOR LOCAL INDUCTION OF ANTIGEN-SPECIFIC ANTI-TUMOR RESPONSES

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Background Different hematological cancer types have shown promising responses to autologous chimeric antigen receptor (CAR)-T cell therapy. However, the efficacy of this treatment in solid tumors is hindered by challenges such as poor tumor infiltration, long-term retention of CAR-T cells, and systemic side effects. To address these limitations, we developed citrate-coated superparamagnetic iron oxide nanoparticles (SPIONs), which have the ability to adhere to and be taken up by T cells, thereby enabling the control of CAR-T cells through an external magnetic field (1-3). In future, magnetic guidability should help to enrich CAR-T cells in the tumor microenvironment, leading to site-specific anti-tumor responses. This study aims to investigate the influence of SPION-loading of CAR-T cells on their efficacy in anti-tumor cell responses *in vitro*.

Materials and Methods T cells were isolated from peripheral blood of healthy donors and received mRNA encoding a chondroitin sulfate proteoglycan 4 (CSGP4)-specific CAR via electroporation (4, 5). The cells were then incubated with SPIONs for 4h to magnetically functionalize them. Subsequently, T cells were co-incubated with melanoma tumor cells expressing CSGP4 on their surface. Afterwards, the cells were analyzed for their antigen-specific anti-tumor responses and

compared to non-loaded CAR-T cells or CSGP4-negative tumor cells by flow cytometry. Additionally, tumor cell lysis was investigated via impedance-based monitoring of cell viability and microscopic analysis of the dissolution of three-dimensional tumor spheroids.

Results We observed that SPION-loading did not affect the expression of activation markers, differentiation, or proliferation of CAR-T cells. Furthermore, SPION-loaded CAR-T cells retained their capability for antigen-specific tumor cell lysis over multiple days. Additionally, these CAR-T cells demonstrated the ability to be controlled by an external magnetic field, as well as infiltrating and dissolving tumor spheroids.

Conclusions In summary, we demonstrated that SPION-loading did not compromise the functionality of CAR-T cells, as they were still able to perform the investigated effector functions with similar efficacy as the non-loaded control CAR-T cells. These findings underscore the potential of SPIONs in enhancing site-specific anti-tumor responses of CAR-T cells in the therapy of solid cancers in the future.

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P09.03 LOADING OF T CELLS WITH SUPERPARAMAGNETIC IRON OXIDE NANOPARTICLES GIVES THEM MAGNETIC CONTROLLABILITY WHILE RETAINING ANTIGEN-SPECIFIC EFFECTOR FUNCTIONS

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Background The composition of the tumor microenvironment in solid tumors is of crucial importance for the prognosis and clinical outcome of patients with solid cancers (1). Infiltration of CD8+ T cells into the tumor can improve the prognosis and treatment options of patients. Adoptive T cell therapy is intended to increase the number of CD8+ T cells in the tumor. However, only a fraction of cancer patients benefit from this option, partially because the T cells do not effectively reach the tumor (2). We developed citrate-coated superparamagnetic iron oxide nanoparticles (SPIONs) for the loading of T cells to make them magnetically controllable (3,4). After intra-arterial application and magnetic enrichment in the tumor region, SPION-loaded T cells must pass through the vessel wall to reach the tumor and they must retain antigen-specific effector functions to fight the tumor. This study investigated the effects of SPION loading on primary human T cells, particularly on antigen-specific effector functions and their cellular migration capacity (5).

Materials and Methods T cells were freshly isolated from human whole blood and subsequently loaded with SPIONs for 4 h. Unloaded T cells served as controls. Using a Boyden-Chamber-based assay, we acquired information about the ability of T cell to migrate towards a CXCL12-gradient. Furthermore, the tethering and attachment of T cells on an endothelial cell monolayer was investigated by fluorescence microscopy. The deformability upon SPION-loading was investigated using Real-Time Deformability Cytometry (RT-DC). Antigen-specific effector functions were examined after stimulation via an introduced exogenous T cell receptor (TCR) specific for the melanoma antigen MelanA or the endogenous TCR specific for the cytomegalovirus antigen pp65.

Results SPION-loading had no effect on the attachment of T cells to an endothelial monolayer, however, the chemotactic migration was reduced by SPIONs, which was cancelled out by magnetic attraction. RT-DC ruled out stiffening of the cells due to nanoparticle loading, which is important for squeezing through the vessel walls during transmigration. Lastly, we observed no alterations in antigen-specific effector functions regarding proliferation, expression of activation markers, cytokine secretion, or tumor cell killing after antigen-specific activation mediated by endo- or exogenous TCRs.

Conclusions In sum, we showed that SPION loading did not impair cellular mechanics or antigen-specific effector functions. With regard to cell transmigration, possible negative effects of SPION-loading on the T cells were compensated by magnetic attraction. These results underline the potential of SPIONs for the enrichment of T cells in the tissue of solid tumors through magnetic attraction.

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P09.04 ISOLATION OF A HIGH AVIDITY TCR TARGETING A NEWLY IDENTIFIED EPITOPE OF A COMMON CANCER TESTIS ANTIGEN EXPRESSED BY SOLID TUMORS

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Background T cell receptor (TCR)-based immunotherapy is a promising cancer treatment particularly as the TCR antigen repertoire detects both intracellular and extracellular tumor antigens. Our newly identified cancer-testis antigen (CTA) is an intracellular antigen exclusively expressed by cancer and reproductive tissue making it an ideal target for TCR-T cell therapy. Here, we aim to isolate a TCR against an HLA-A*02:01 restricted CTA epitope from the human HLA-A*02:01 negative repertoire to evaluate its safety and efficacy *in vitro* and *in vivo*.