P09.08 THE TUMOR IMMUNE MICROENVIRONMENT OF HEAD AND NECK CANCER IN RELATION TO ANATOMICAL SITE CLASSIFICATION

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Background Head and neck squamous cell carcinomas (HNSCCs) arise in the mucosal linings of the upper aerodigestive tract. Risk factors are smoking, excessive alcohol consumption and infection with human papillomavirus (HPV). Tumors are classified according to stage, HPV status, and to anatomical site: oral cavity, hypopharynx, larynx and oropharynx. Immune checkpoint inhibitors belong to the treatment arsenal for HNSCC but are effective in only a minority of patients. Treatment response and prognosis are influenced by the tumor immune microenvironment (TiME). Generally, HPV-related HNSCCs are associated with increased immune infiltration, but for different HNSCC anatomical sites such data are lacking.

Materials and Methods Using flow cytometry, we investigated the TiME of 58 fresh HNSCC samples. We further examined the spatial distribution of CD8+ subsets using multiplex immunohistochemistry (mIHC) on 20 samples. Additionally, single cell RNA-sequencing (scRNA-seq) was performed on five samples to obtain a comprehensive understanding of individual cells in the TiME of HNSCC.

Results Increased T cell frequencies were observed with flow cytometry in oral cavity squamous cell carcinoma (OCSCC) in comparison to HPV-unrelated HNSCC at other anatomical sites. Using mIHC, no significant differences were found in tissue-resident or proliferating CD8+ densities between sites. However, a trend towards increased proliferating CD8+ densities was observed in OCSCC. Moreover, the number of infiltrating CD8+ cells within 30µm from tumor cells was highest in OCSCC. Using scRNA-seq, we were able to annotate cell types and perform subclustering. This enabled us to distinguish among others CD8+ exhausted T cells.

Conclusions Our data indicate that, compared to other anatomical sites, oral cavity SCCs are more often populated by T cells. This suggests that particularly OCSCC may more easily respond to immunotherapy strategies aimed at activating T cells.

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P09.09 A CAR-T CELL-BASED APPROACH FOR THE TREATMENT OF MALIGNANT T CELL DISEASES

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Background The T cell receptor (TCR) is uniformly expressed in monoclonal T cell lymphoma (TCL) populations and would thus represent an ideal target antigen for a CAR-based immunotherapy.[1] However, the TCR is present on all T cells including malignant, healthy and CAR-T cells. Strategies to increase tumor specificity of TCR-specific CAR-T cells are therefore required. To address this issue, CARs targeting one of the two TCR-β constant chains and CARs targeting the CDR3 region of a malignant clone have been designed. [2,3] However, in the first case the off-tumor activity would affect about 50% of T cells, which could lead to impaired T cell immunity in the patient. The latter approach is highly specific to the malignant T cell clone but would require creation of individual CDR3-specific CARs for every patient which is currently impracticable. The TCR V-segment chains can be grouped into families and targeting TCR malignancies via V-family-specific CARs would spare the majority of healthy T cells. [4–6] Targeting the TCR V-segments would unite high tumor specificity with the limited effort of creating a panel of CAR molecules that could be used for all patients.

Materials and Methods The TCR sequence of a malignant clone from a patient with γδ-TCL was obtained and cloned into a lentiviral expression plasmid. This γδ-TCR was expressed in Jurkat E.6.1 TCL cells together with a panel of other γδ-τCRs to create target cells with different variable chain usage. A Vδ1-specific CAR molecule was designed and the CAR was expressed in a previously described Jurkat E.6.1 based T cell reporter cell line, that allows to measure T cell activation by flow cytometry.[7]

Results A panel of γδ-TCR expressing target cells with different variable chain usage was created. This panel included a lymphoma-patient derived Vγ2Vδ1 TCR and several other TCRs consisting of Vδ1 chains with different CDR3 regions paired to various Vγ chains. Vγ9Vδ2- and αβ-TCR expressing target cells served as control cells. The Vδ1-specific CAR was highly expressed in Jurkat E.6.1 reporter cells. Co-culture experiments revealed strong activation of reporter cells by all Vδ1 chain expressing target cells whereas no reactivity towards Vδ1 negative TCRs was detected.

Conclusion We show highly specific activation of Vδ1-specific CAR T cells in a reporter cell based in-vitro model of γδ-TCL. Provided that a panel of V-segment specific CARs would be created, these CAR-T cells would be able to specifically kill malignant T cells with very limited collateral damage to the non-malignant T cell repertoire. Unlike CDR3 regions the V-segments are largely unaffected by V(D)J-recombination and the panel of CARs could be reused for multiple patients as part of a personalized, precision immunotherapeutic treatment approach. We therefore propose the TCR V-segments as ideal targets for immunotherapies against TCLs.

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P09.10 DYSREGULATED EXPRESSIONS OF INHIBITORY CHECKPOINT MOLECULES AND THEIR LIGANDS ON T-CELLS AND BLASTS IN AML RELAPSES AFTER STEM CELL TRANSPLANTATION (SCT)

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Background Upregulation of inhibitory checkpoint molecules (ICM) on T-Cells and their ligands on AML blasts may be a mechanism of AML relapse after allogeneic stem cell transplantation (SCT). Better understanding of relapse biology may improve treatment efficacy.
Materials and Methods We examined peripheral blood (PB) and bone marrow (BM) samples of 5 AML patients (PTs) relapsing after SCT and PB from 5 healthy individuals, including 2 stem cells donors. ICM (PD-1, CTLA-4)/ligand (CD86, PD-L1, PD-L2) expression on T-cells and blasts was assessed by flow cytometry. PTs' PB was cultivated with 'KitM' (GM-CSF+PGE-1) and without (Ctr) to generate leukemia-derived dendritic cells (DC_{leu}), followed by MLC enriched with PTs'/donors' T-Cells. After MLC, immune activation and functionality (degranulation, intracellular cytokine production, blast lysis) was assessed.
Results All PTs showed high expression of PD-1 on T-Cells, additional overexpression of CTLA-4 correlated negatively with responses to relapse treatment. Expression of ICM was low on T-Cells of 4/5 healthy individuals.

Influence of KitM on ICM/ligand expression:

a. ICM/ligand expression on uncultured T-Cells/blasts: Contrary to H, PTs presented high expressions of CTLA-4 and PD-1 on PB T-Cells. PTs also showed high frequencies of PB/BM blasts expressing CD86.

b. DC/DC_{leu} in PB: Generation of DC_{leu} in AML and generation of DC in H was successful with KitM pretreated PB vs. Ctr.

c. ICM expression on T-Cells after MLC: MLC of KitM treated PB enriched with unstimulated PTs' T-Cells resulted in reduced frequencies of ICM-positive T-Cells in 3/5 PTs and increased frequencies of activated (leukemia specific) T-Cells in 3/5 PTs. Blast lysis was improved in 4/5 samples treated with KitM vs. Ctr.

Possible impact of ICM on clinical outcome (case study): PT1suffered early relapses after 2 SCTs from her healthy father. A role of ICM in AML relapse was suggested by CTLA-4/PD-1 expression on her T-Cells and CD86 expression on her blasts. Also, >90% of the father's T-Cells expressed CTLA-4/PD-1, which might have contributed to treatment failure. In contrast, T-Cells from PT1's mother presented with low ICM levels, suggesting that she may have been a better donor. Stimulation of PT1's PB cells with KitM generated DC_{leu}, decreased ICM expression and increased T-Cell activity. KitM pretreated samples showed improved blast lysis after MLC.
Conclusions Concisely, T-cells and blasts of AML PTs relapsing after SCT uniformly expressed ICM and their ligands, possibly leading to inferior immune responses.High aberrant ICM expression on donor T-Cells (particularly CTLA-4) may be a reason for relapse after SCT by inhibiting antileukemic immune reactions. Further, generation of DC_{leu} through KitM triggers immune responses in MLC along reduced ICM expression on T-Cells, possibly reducing their inhibitory effects and thereby improving antileukemic responses.

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P09.11 ANTI-PROLIFERATIVE EFFECTS OF GUT MICROBIAL METABOLITES ON HUMAN GASTRIC ADENOCARCINOMA CELLS AND THEIR POTENTIAL SYNERGY WITH DEXAMETHASONE

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Background The relevance of gut microbiota in the development, treatment and clinical outcome of cancers is an emerging area of translational research that can pave new avenues in cancer therapeutics. Our recently published reviews have underlined the immunomodulatory and anti-tumoural effects of gut microbiota in cancers through the production of different metabolites [1, 2]. Studies have also reported the anti-cancer activity of gut microbial metabolites such as short-chain fatty acids (SCFAs; including butyrate, propionate, and acetate) against different cancers. However, the detailed molecular mechanisms of action of these SCFAs and their potential synergistic interactions with standard chemotherapy and immunotherapy have not been investigated thoroughly, especially against gastric cancer.
Materials and Methods This study was designed to evaluate the anti-proliferative effects of three SCFAs against the AGS gastric adenocarcinoma cells using the Alamar Blue assay and to decipher the molecular mechanisms of action of the most active metabolite using label-free quantification proteomics and flow cytometric (apoptotic and cell cycle assays) analyses as per our previously validated methods [3]. The potential synergy between the most active gut metabolite and the anti-inflammatory drug dexamethasone against the AGS cells was also quantified using the combination index (CI) model [4].
Results All three gut metabolites sodium butyrate (NaB), sodium propionate and magnesium acetate exhibited strong anti-proliferative activity with NaB displaying the greatest inhibitory effect ($p < 0.05$) against the AGS cells. In addition, NaB induced cell cycle arrest and apoptosis in the AGS cells as evident in the flow cytometric and proteomics analyses and potentially synergised the activity of dexamethasone (CI value < 1) against the AGS cells.
Conclusions The findings of this study demonstrated the potential implementation of gut microbial metabolites against gastric adenocarcinoma as a combination therapy with dexamethasone. However, further in vivo studies are warranted to validate these findings.

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