GPR56 a Novel Immune Checkpoint on Tumor-Infiltrating Lymphocytes

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Despite the clinical efficacy of so-called immune checkpoint inhibitors (ICIs) in various cancers, some cancer types, including epithelial ovarian cancer (EOC), do not effectively respond to current therapeutics. Thus, the identification of new immune checkpoints that regulate T cell immunity remains of great interest. One as yet largely uninvestigated checkpoint of potential interest is the G protein-coupled receptor 56 (GPR56), which belongs to the adhesion GPCR family. Here in this study, it was identified that GPR56 is expressed on tumor infiltrating lymphocytes (TILs) and investigated its role as a potential immune checkpoint within the context of cancer. Based on the investigated data, GPR56 indeed appears to function as an immune checkpoint in TILs and may thus provide a novel immunotherapeutic target for the reactivation of tumor-infiltrating and tumor-reactive lymphocytes.

Cancer Immunotherapy | Tumor-Infiltrated Lymphocytes (TIL) | GPR56 | Immune Checkpoint

1. Introduction

In the past decades, diverse therapeutic approaches have been developed to specifically counteract the immune-suppressive mechanisms within the tumor microenvironment (TME), with the development of immune checkpoint inhibitors (ICIs) reaching a revolutionary milestone in the field of immuno-oncology. Most notably, ICIs targeting CTLA-4 or PD-L1/PD-1 have yielded remarkable clinical responses for a variety of cancer types, with a range of drugs currently approved targeting these axes available as cancer treatments [1][2][3][4][5][6][7][8][9][10][11][12][13][14][15][16][1].

However, despite the introduction of these promising ICIs some cancer types do not effectively respond. For instance, the treatment of PD-1 antibody Nivolumab, PD-L1 antibody Avelumab, PD-1 antibody Pembrolizumab, and CTLA-4 blocking antibody MDX-CTLA4 only yielded objective response rates (ORR) up to 15% in epithelial ovarian cancer (EOC). Similarly, the treatment of breast cancer and pancreatic cancer still face challenges, despite the introduction of ICIs [2][3][4][5]. Therefore, identifying novel immune-checkpoints are urgently needed for ovarian cancer. Besides from the recently identified immune-checkpoints LAG-3, TIM-3, TIGIT, VISTA and many others [6][7][8][9]. One particular, less established immune-checkpoint is the protein GPR56. GPR56 is an adhesion G-protein-coupled receptor with an array of functions, ranging from cortical development [10][11], anti-depressant response [12], hematopoietic development [13], to tumor cell adhesion and progression [14][15].

GPR56 expression and function have not been evaluated in the context of tumor infiltrating lymphocytes (TILs) yet. As the magnitude, composition, quality, and phenotypic features of the TIL population have been linked to

treatment response-rates and outcome [16], here it was identified that GPR56 is expressed on TILs and investigated its role as a potential immune checkpoint within the context of cancer.

2. GPR56 might be a potential novel immunotherapeutic target for reactivation of tumor-infiltrating and tumor-reactive lymphocytes

In this study (10.3390/cancers14133164), GPR56 expression was identified on TIL fractions from various tumor types and to subsequently phenotypically define these populations by consulting RNA sequencing datasets.

Within a single-cell tumor immune transcriptomic (scRNAseq) dataset covering different tumor types, GPR56 mRNA expression was detected in various tumor-infiltrating immune cell sub-types, especially the expression was high within the cytotoxic and terminally exhausted CD8 T cells subsets. Analysis of the differentially expressed genes (DEGs) of the GPR56-positive and GPR56- negative cytotoxic CD8 T cells and terminally exhausted CD8 T cells revealed that GPR56-positive population presented significantly higher expression of genes associated with cytotoxic/(pre-)exhausted/tumor-reactive gene signatures. Further, GPR56 expression levels, as well as expression of several effector/co-stimulatory molecules identified in the sequencing analysis, were confirmed on TILs from epithelial ovarian cancer (EOC) patients.

GPR56 was subsequently evaluated on healthy primary human T cells and was compared with TILs isolated from EOC patients. This revealed higher expression levels of GPR56 on the total CD3 population (4% vs. 15%) as well as on the CD4 (1% vs. 15%) and CD8 (8% vs. 11%) fractions of TILs compared to healthy primary human T cells, although clear patient variability was observed. Further, Upon MHC-independent activation of TCR signaling by anti-CD3 single-chain antibody fragment-expressing ES-2 cells (ES-2scFvCD3), GPR56-expressing TILs upregulated GPR56 expression by approximately three-fold (CD3, 5% to 15%; CD4, 6% to 16%; CD8, 3% to 12%). Such an increase was not detected on TCR-stimulated healthy primary human T cells.

MHC-independent activation of TCR signaling was also subsequently induced by ES-2scFvCD3 or FADUscFvCD3 cells in T cell line Jurkat ectopically expressing GPR56 (Jurkat.GPR56). This revealed a significantly stronger upregulation of activation markers CD25 and CD69 on Jurkat. GPR56 compared to wild type Jurkat (Jurkat.WT) or control Jurkat cells (Jurkat.EV).

Furthermore, after ectopically expressing GPR56 on primary healthy T cells and assessing the migration potential of those cells in response to chemoattractants. T cells. A reduced migratory potential towards chemoattractant SDF-1 (CXCL12) was subsequently observed for primary healthy T cells ectopically expressing GPR56 compared to empty vector-transduced primary healthy T cells. Thus, GPR56 expression on primary healthy T cells affects in vitro migration in response to chemoattractant SDF-1.

Interestingly, Upon the subsequent co-culture of GPR56-expressing Jurkat.NFAT-luc reporter cells with ES2-anti-CD3 cells, both GPR56-expressing and control Jurkat.NFAT-luc cells increased luminescence levels compared to

the Jurkat.NFAT-luc cells alone. However, a significant decrease in luminescence was detected for Jurkat.NFAT-luc cells expressing GPR56 compared to control cells. Thus, GPR56 inhibited T cell activation in the Jurkat.NFAT-luc model system.

Highlights of the study (10.3390/cancers14133164)

1. GPR56 Is Expressed in Cytotoxic and Terminally Exhausted CD8 TILs That Display a Tumor-Reactive Phenotype across Multiple Tumor Types
2. GPR56 Expression Is Elevated on Ovarian Cancer TILs Compared to Healthy Peripheral Blood Lymphocytes and Associates with an Effector Memory Phenotype
3. TCR Stimulation Upregulates GPR56 Expression on TILs and Enhances the Activation Status of T Cells
4. Ectopic GPR56 Expression Inhibits Migration of Primary Healthy T Cells

3. Conclusion

GPR56 mRNA expression was characterized in the context of TILs, with GPR56 expression being detected predominantly in tumor infiltrating CD8 T cells with a cytotoxic and (pre-)exhausted phenotype. In accordance with this mRNA profile, TILs from ovarian cancer patients expressed GPR56 primarily within the effector memory and central memory T cell subsets. GPR56 expression further increased on TILs upon T cell receptor (TCR)-mediated stimulation in co-cultures with cancer cells, whereas GPR56 expression on healthy primary human T cells did not. Further, the ectopic expression of GPR56 significantly reduced the migration of GPR56-positive T cells. Taken together, GPR56 is a potential immune-checkpoint in EOC found on (pre-)exhausted CD8 TILs that may regulate migratory behavior.

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