Abstract

Transgenic T cell receptor (TCR)-based T cell therapies are a powerful treatment for cancer. However, one of the greatest remaining challenges is the successful identification of tumor-specific antigens (TSAs) that are shared between patients and tumor entities and which strong T cell responses. The non-coding region of the genome has become a promising source of such novel TSAs. Previously, we have identified eleven immunogenic shared TSAs derived from the translation in canonical and non-canonical reading frames of non-mutated non-coding genomic regions like introns, intergenic regions and 3′untranslated regions (Figure 1A). In the following process, we identified several TSAs specific for these TCRs (Figure 1B). Here we aimed at the validation of two TSA-specific TSAs in a model of ovarian cancer-derived organoids.

For that purpose, fresh ovarian tumor and normal ovarian tissue expressing the HLA of interest were obtained. The TSA of interest was detected both at the transcriptomic and protein level in the primary ovarian tumor tissue (Figure 2). Using frozen single cell suspensions of this tumor and corresponding normal tissue, tumor organoids and 2D-growing normal cell lines were successfully established and their integrity was confirmed (Figure 3). Both TSA-specific TCRs were efficiently expressed on CDT T cells from donors. TCR-transgenic T cells showed activation upon co-culture with tumor organoids but without recognition of normal ovarian cell lines. Normal cells were only recognized after loading with the specific target peptide (Figure 4).

In conclusion, the high relevance of two TSAs identified to be specific for a novel ovarian tumor-specific antigen was confirmed in a model of ovarian cancer organoids. The findings support further development of these TSAs for cancer immunotherapy and implementation of tumor organoids as a relevant tool for the characterization of TSA-specific TCRs.

TSA identification and TCR discovery

Figure 1. (A) Schematic representation of TSA identification in tumor samples. Tumor samples were processed in parallel for HLA class I-associated peptide isolation and RNA extraction. A total of 150 μg of RNA was loaded onto a 2D gel and subsequently stained. Using LC-MS/MS, tumor RNA samples were analyzed by mass spectrometry and used to develop a custom proteome database. Peptides were matched against the custom proteome database to identify novel TAs. (B) Immunogenicity screening workflow. Immunogenic TSAs were identified by enrichment assay. Candidate TSA sequences were electrophoretically into mature HLA-matched dendritic cells and used as antigen presenting cells for autologous CBDR T cells. TSA-reactive T cells were enriched based on CD107 expression upon stimulation with antigen-negative and -positive cells and subsequently used in a RMA assay. T cells fluorescence-activated cell sorting (FACS) T cell clones were tested for reactivity by IFN-γ ELISA after co-culture with peptide-loaded and unloaded HLA-expressing K562 cells. Subsequently, the HLA restriction and peptide-specificity of TCR-reactive clones as well as their TSA sequences were identified.

TSA is expressed in the primary ovarian cancer tissue

Figure 2. Confirmation of the TCR candidate expression in the selected primary ovarian cancer tissue by proteomics. From a glandular papillary carcinoma patient, freshly collected ovarian tumor tissue expressing the HLA of interest was mechanically disrupted enzymatically digested and cryopreserved. Cryopreserved material was used for the immunoprecipitation of peptide-HLA complexes. Subsequently, eluted peptides were quantified with tandem mass tagging (TMT). TMT labeling was parallel, and cryopreserved samples were used for eluted peptide and RNA-seq analysis. The resulting sequence reads were mapped to the genome containing a mapping coding sequence. The threshold for the expression of the coding sequence was set to 1.0 fmol.

Establishment and characterization of primary tissue derived ovarian cell lines

Figure 3. Ovarian cell line development from primary tissues (A) Schematic illustration of cell line generation workflow. Fresh ovarian tumor and normal ovarian tissue were dissociated and cryopreserved. Thawed single cell suspensions were used to generate tumor organoids growing in extracellular matrix or 2D-growing normal ovarian cell lines. (B) Representative bright-field images of tumor organoids and normal cell line at different days after seeding and different magnifications. (C) Generated cell lines resembled morphological pattern of primary tissue. To characterize generated cell lines, the presence or absence of at least one of two previously identified tumor-specific mutations was investigated by targeted Sanger sequencing in the genome of the tumor organoids, normal cell lines and primary tissues. Sequencing results detecting one mutation are shown.

TSA specific TCR-T cells recognize ovarian cancer organoids

Figure 4. (A) Overview of TCR reactivity assessment. Two TSA-specific TCRs (1606 and 1607) were retrovirally transduced into CD8 T cells of healthy donors. Expanded TSA-specific or mock CD8 T cells were co-cultured with single cell suspensions of tumor organoids or the 2D-growing normal ovarian cell line. T cells were also co-cultured with normal ovarian cell suspensions loaded with correspondingly TSA peptide. Mock-T cells were loaded with MIPCK Lysates ELISA as a positive control for mock TCR-T cells upon co-culture. (B) Both TSA-specific TCRs can represent TSA enzymatically processed and presented on the ovarian cancer organoids. Normal cells were only recognized after loading with the specific target peptide. Representative results for one donor out of three are shown.

Summary

Medigene’s well-established high-throughput TCR discovery process enabled identification of several TSA-specific TCRs.

The reactivity of two selected transgenic TSA-specific TCRs was confirmed here in the ovarian cancer organoid model.

Tumor organoids represent reliable and more physiologically relevant model to characterize TCRs in comparison to 2D established tumor cell lines.

Poster # 189
Targeting a novel shared tumor-specific antigen with T cell receptor transduced T cells for the treatment of ovarian cancer

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