Targetable immunogenic tumor specific antigens can be identified in non-coding regions of the genome

Franceschetti T, Zhao Q, Vincent K, Perreault C, Milosevic S and Sommermeyer D

1Medigene Immunotherapies GmbH, a subsidiary of Medigene AG, Planegg, Germany; 2Institute for Research in Immunology and Cancer, Université de Montréal, Montréal, Quebec, Canada

For further information and questions please contact Daniel Sommermeyer: d.sommermeyer@medigene.com

Abstract

CD8+ cytotoxic T cells are the main mediators of immune responses during cancer immunotherapy. Effective T cell functionality depends on the specific interaction with major histocompatibility (MHC)-class I-bound peptide antigen. Significant efforts are being dedicated to the identification of novel tumor specific antigens (TSA), investigating not only the known proteins, but also non-coding regions of the genome, that would allow for improved discrimination between cancer cells and healthy tissues. Through extensive comparisons of tumor and healthy tissues at the transcriptional and MHC-presented peptide levels, TSAs were identified that derive from the translation in canonical and non-coding reading frames of non-mutated non-coding genomic regions, including 5’ and 3’ untranslated regions (UTRs), introns and intergenic regions. A remarkable feature of these TSAs is that they are shared among patients and solid tumor types, thus representing ideal targets for cancer immunotherapies, including vaccines and adoptive T cell therapies. The non-coding region screening procedure was used to investigate the immunogenicity of 47 TSAs in the context of five common HLA types. Constructs harboring the TSA sequences were expressed and transfected into HLA-matched monocyte-derived dendritic cells (mDCs) that were used to stimulate autologous CD8+ T cells. TSA-reactive T cells were enriched upon stimulation with antigen-positive and negative cells using the T cell activation marker CD150 and sorted as single cells. Reactivity of individual T cell clones towards specific TSAs was confirmed by measuring cytokine release upon co-culture with HLA-matched TSA-positive and negative cells. The identified TSAs were found to be processed and presented, including at least one immunogenic TSA for each of the five analyzed HLA. For some of these antigens, specific T cells were found in multiple healthy donors. The identified immunogenic TSAs derive from a variety of non-coding regions, such as introns, 5’UTRs and non-coding RNAs. The T cell receptor (TCR) and epitope sequences of TSA-reactive T cell clones were identified by NGS, engineered into a retroviral expression construct and transduced into Tet-On inducible ATLR-matched T cells against TSA-positive cells. Following selection of the TSA-reactive cell lines, TSA-positive peptides were isolated from introns and intergenic regions of cancer genomes. A key tool developed in this study for the identification of TSA-reactive TCRs was the isolation of TSA-positive T cells using a multiplex tetramer assay that exploits the variable TCR binding specificity of TSA-presenting cells.

Figure 1. Schematic representation of TSA identification in tumor samples. Tumor samples were processed for RNA extraction and MHC class I-associated peptide isolation. a) MHC class I-associated peptides were immunoprecipitated (IP) and identified by liquid chromatography-tandem mass spectrometry (LC-MS-MS). b) Tumor RNA samples were analyzed by RNA-seq and used to identify a custom TSA database. This database contains two modules: the canonical protein derived from in-frame translation of the personalized exome containing single-nucleotide variants (SNVs) and the cancer-specific proteome consisting of the 3-frame translation of cancer RNA sequences not detected in normal tissue. Proteomics data were integrated with the custom cancer database to identify novel TSAs. 47 TSAs, resulting from in- and out-of-frame translation of non-mutated genomic regions were selected for further analysis. The TCR repertoire was expressed in normal tissues and were shared among different samples and tumor types (Zhao et al. at JMMJ). TSA candidates derive from non-mutated non-coding genomic regions and are shared across tumor types.

Figure 2. TSA candidates identified by proteogenomics. 47 potential TSAs were identified in ovarian (OC), breast (BC), and lung (LC) cancer tissue samples in the context of five HLA types: HLA-A*01:01, A*02:01, A*11:01 and B*07:01. Most of these antigens were expressed in multiple cancer types and are transcribed from non-coding genomic regions.

Figure 3. Immunochemistry screening workflow. Minigene constructs containing the TSA sequences were developed and electroporated into healthy donor T cells. These were used as antigen-presenting cells for autologous CD8+ T cells. TSA-reactive T cells were enriched and subsequently sorted as single cells by fluorescence activated cell sorting (FACS). CD150 was used as a marker of T cell activation after stimulation with antigen-negative and positive cells. Individual T cell clones were tested for reactivity by IFN-γ ELISA after co-culture with peptide-loaded and unloaded HLA-matched K562 cells. Subsequently, the HLA restriction and peptide-reactivity of TSA-reactive clones as well as their TCR sequences were identified.

Figure 4. Identification of HLA-peptide specificities of TSA-reactive clones. To identify the specificity of reacting T cell clones, these were co-cultured with HLA-matched K562 cell lines loaded with tryptophan of TSA peptides or B) individual peptides. TSA recognition was analyzed by IFN-γ release. Representative clones recognizing two HLA-A2 and two HLA-A3-associated TSAs are shown. This procedure was carried out for all reactive clones across the five different HLA types.

Figure 5. TSA-reactive TCRs can recognize endogenously processed antigens. The TCR coding sequences of TSA-reactive clones were identified by NGS and cloned into retroviral expression vectors. CD8+ T cells isolated from a healthy donor were transduced with five of these constructs, each expressing a different HLA-peptide tetramer. The ability of TCR-transgenic or untransduced T cells to react against TSA-pulsed target cells was assessed after co-culture with K562 cells expressing the corresponding peptides. A TSA-reactive TCR also recognized a T cell line in the context of HLA-A1 isolated from the same donor.

Figure 6. Identification of HLA-peptide specificities of TSA-reactive clones. To identify the specificity of reacting T cell clones, these were co-cultured with HLA-matched K562 cell lines loaded with tryptophan of TSA peptides or B) individual peptides. TSA recognition was analyzed by IFN-γ release. Representative clones recognizing two HLA-A2 and two HLA-A3-associated TSAs are shown. This procedure was carried out for all reactive clones across the five different HLA types.

Summary

The high throughput screening procedure described here was able to successfully detected immunogenic non-mutated tumor-specific antigens (TSAs) among a list of 47 candidates. Ten immunogenic TSAs were identified across the different HLA types. At the same time, this approach also uncovered the sequences of functional TSA-reactive TCRs, which represents a critical step in the development of TCR-based immunotherapies targeting TSAs. The reactivity of these transgenic TCRs against indication relevant cancer cell lines endogenously expressing the TCR of interest is ongoing.