Poster # 1520

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

Franceschetti T[1], Zhao Q[2], Vincent K[2], Perreault C[2], Milosevic S[1] and Sommermeyer D[1]

[1]Medigene Immunotherapies GmbH, a subsidiary of Medigene AG, Planegg, Germany; [2]Institute 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 antigens. Significant efforts are being dedicated to the identification of novel tumor specific antigens (TSAs), investigating not only the known proteome, 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 peptidome levels, TSAs were identified that derived from the translation in canonical and non-canonical 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 cell therapies. To identify TSAs that can elicit T cell responses, a high throughput 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 developed 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 CD137 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 cell lines. Ten immunogenic TSAs were identified with this procedure, including at least one immunogenic TSA for each of the five analyzed HLAs. 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) α and β chain sequences of TSA-reactive T cell clones were identified by NGS, engineered into a retroviral expression construct and transduced into CD8+ T cells. The reactivity of TCR-transgenic T cells against TSA-positive target cells was confirmed by recognition of TSA-peptide-loaded cell lines and target cells internally processing and presenting the TSAs. In conclusion, our high throughput screening approach successfully detected immunogenic TSAs. Furthermore, it can be used for the identification of TSA-reactive TCRs, thus representing a key tool in the development of novel TCR-based cancer immunotherapies targeting this novel class of TSAs.

TSA identification by proteogenomics

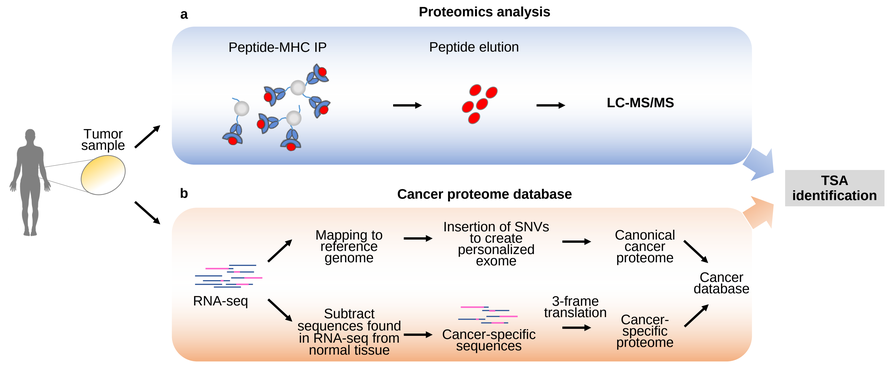

Figure 1. Schematic representation of TSA identification in tumor samples. Tumor samples were processed for RNA extraction and MHC class I-associated peptide isolation. **a)** MHC class I-associated peptides were immunoprecipitated (IP) and identified by liquid chromatography-tandem MS (LC-MS/MS). **b)** Tumor RNA samples were analyzed by RNA-seq and used to develop a custom proteome database. This database contains two modules: the canonical proteome 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. These antigens were not expressed in normal tissues and were shared among different samples and tumor types (*Zhao et al. 2020 Cancer Immunol Res; 8:544–55*).

TSA candidates derive from non-mutated non-coding genomic regions and are shared across tumor types

TSA #	HLA	Cancer types	Genomic origin
1	A*01:01	OC	Intronic
2	A*01:01	OC, BC, LC	Intronic
3	A*01:01	OC, BC, LC	Frameshift
4	A*01:01	OC, BC, LC	Intergenic
5	A*01:01	OC, BC, LC	5'UTR
6	A*01:01	OC	ncRNA
7	A*02:01	OC	Intronic
8	A*02:01	OC, BC, LC	Intergenic
9	A*02:01	OC, LC	Intergenic
10	A*02:01	OC	Intergenic
11	A*02:01	OC, BC, LC	Intronic
12	A*02:01	OC, LC	Annotated ORF
13	A*02:01	OC, BC, LC	Intronic
14	A*02:01	OC, LC	Antisense
15	A*02:01	OC	Intronic
16	A*03:01	OC, LC	Intergenic
17	A*03:01	OC	Intergenic
18	A*03:01	OC, BC, LC	5'UTR
19	A*03:01	OC	5'UTR
20	A*03:01	OC	ncRNA
21	A*03:01	OC	ncRNA
22	A*03:01	OC, BC	5'UTR
23	A*03:01	OC, BC, LC	Frameshift
24	A*03:01	OC	Intronic

TSA #	HLA	Cancer types	Genomic origin
25	A*03:01	OC, BC, LC	ncRNA
26	A*03:01	OC, LC	5'UTR
27	A*11:01	OC	Intergenic
28	A*11:01	OC, LC	ncRNA
29	A*11:01	OC, BC, LC	Intergenic
30	A*11:01	OC	ncRNA
31	A*11:01	OC	Intronic
32	A*11:01	OC	Intronic
33	A*11:01	OC, BC, LC	Annotated ORF
34	A*11:01	OC, BC, LC	Frameshift
35	A*11:01	OC, BC	Intergenic
36	B*08:01	OC	Intergenic
37	B*08:01	OC	Intronic
38	B*08:01	OC, BC, LC	Intergenic
39	B*08:01	OC	Intronic
40	B*08:01	OC, LC	Intergenic
41	B*08:01	OC, BC, LC	Intronic
42	B*08:01	OC	Intergenic
43	B*08:01	OC, BC	Intronic
44	B*08:01	OC, LC	Annotated ORF
45	B*08:01	OC, BC, LC	5'UTR
46	B*08:01	OC, LC	Intronic
47	B*08:01	OC	Frameshift

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*03:01, A*11:01 and B*08:01. Most of these antigens were expressed in multiple cancer types and are translated from non-coding genomic regions.

Procedure of immunogenicity screening

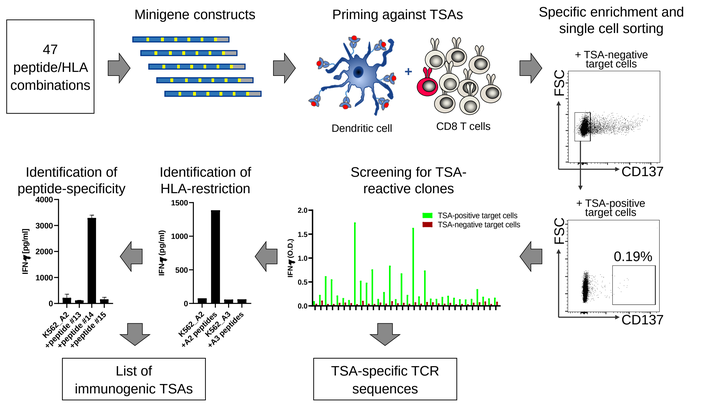

Figure 3. Immunogenicity screening workflow. Minigene constructs containing the TSA sequences were developed and electroporated into mature HLA-matched dendritic cells (mDCs). These were used as antigen-presenting cells for autologous CD8+ T cells. TSA-reactive T cells were enriched and subsequently sorted as single cells by fluorescence activated cell sorting (FACS). CD137 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-transgenic K562 cells. Subsequently, the HLA-restriction and peptide-specificity of TSA-reactive clones as well as their TCR sequences were identified.

Immunogenic TSAs can be identified

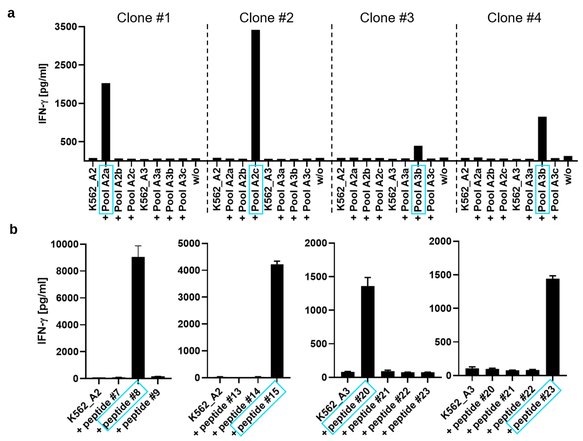

Figure 4. Identification of HLA-peptide specificities of TSA-reactive clones. To identify the specificity of reactive T cell clones, these were co-cultured with HLA-matched K562 cell lines loaded with **a)** pools of TSA peptides or **b)** individual peptides. TSA recognition was analyzed by IFN-γ release. 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.

TSA-specific TCR-T cells recognize TSA-expressing target cells

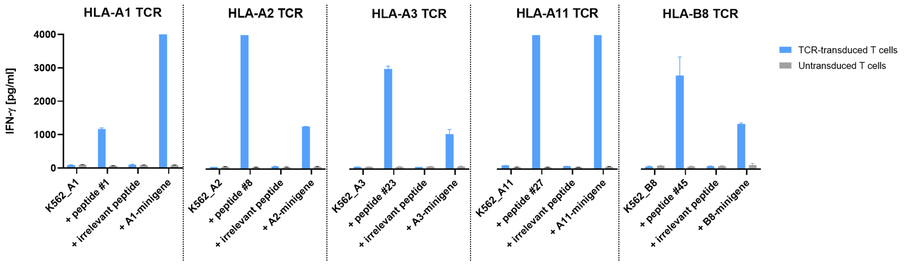

Figure 5. TSA-reactive TCRs can recognize endogenously processed antigens. The TCR coding sequences of TSA-reactive clones were identified by NGS and cloned into retroviral expression vectors. CD8+ T cells isolated from a healthy donor were transduced with five of these constructs, each recognizing a different HLA-peptide complex. The ability of TCR-transgenic or untransduced T cells to react against TSA-positive target cells was investigated by co-culture with HLA-matched K562 cell lines loaded with specific TSA peptides or irrelevant peptides at a concentration of 10^{-6} M. T cells were also co-cultured with HLA-matched K562 cells transduced with TSA-minigene constructs to confirm the recognition of internally processed and presented antigens. IFN-γ release was used as a readout of target cell recognition.

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 immunotherapeutics targeting TSAs. The reactivity of these transgenic TCRs against indication-relevant cancer cell lines endogenously expressing the TSA of interest is ongoing.