Discovery of immunogenic ERV-derived antigens as targets for melanoma immunotherapy

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Abstract

Background

Recent advances in immunotherapy have confirmed that adaptive immune responses can recognize and eliminate cancer cells. Past approaches using tumor-associated antigens have elicited poor immune responses and the cancer vaccine field has shifted to single nucleotide variant-derived neoantigens to avoid T-cell depletion by central tolerance. These highly personalized vaccines are beginning to show promise. We hypothesized that highly immunogenic target antigens exist within the cancer genome and become aberrantly expressed due to the epigenetic changes that accompany neoplasia. We utilized markers of endogenous retroviruses (ERVs), which are particularly abundant in repressed regions of DNA, to search for novel, high-value melanoma antigens, to circumvent the need for personalization.

Methods

We created a *de novo* pan-cancer transcriptome assembly with RNA-seq reads from 31 cancer types obtained from TCGA. Transcript sequences were subsequently filtered to identify those containing ERV elements. Next, they were subject to differential expression analysis selecting those expressed in the melanoma patients compared to normal tissues (utilizing GTEx data). To discover *bona fide* antigens encoded by our melanoma-specific transcripts, we interrogated the ORFs from these transcripts using public and independently-generated mass spectrometry-based immunopeptidomics data from melanoma samples. HLA-bound peptides from these analyses matching the known proteome were removed, and those peptides solely mapping to our predicted ORF sequences were used to identify novel transcript-derived antigens specifically expressed and presented in primary melanoma tissue. Confirmation of tumor cell-specific expression of antigen-encoding transcripts was carried out using RNAScope®. The ability of detected epitopes from our discovered antigens to elicit an adaptive immune response was assessed by characterization of antigen-specific T-cell responses from naïve donors.

Results

Our *de novo* assembly revealed the presence of approximately 100 melanoma-specific transcripts encoding over 2,000 potential antigens (ORFs). Interrogation of these ORFs against MS immunopeptidomic datasets mapped HLA-bound peptides to dozens of ORFs, demonstrating presentation of our novel antigens in multiple melanoma patient tissues. RNAScope® revealed melanoma-specificity at the transcript level, with little to no transcript expression identified across normal tissues. Assessment of immunogenicity in naïve subject T-cells revealed strongly reactive T-cells that were able to kill peptide-pulsed APCs, indicating a lack of central-tolerance deletion of T-cells specific for these ERV-derived peptides.

Conclusion

We have identified a number of novel melanoma-specific antigens that are shared among patients. T-cells reactive for these antigens can be detected in naïve subjects, and thus these antigens show promise as candidates for development of off-the-shelf cancer vaccine-based immunotherapies.

Introduction

- Ervaxx has identified proprietary, endogenous retrovirus (ERV)-derived Dark Antigens™ that map to melanoma and generate robust, antigen-specific T-cell responses. We are leveraging these insights to advance a pipeline of off-the-shelf cancer vaccines and TCR-based immunotherapies.
- Our Dark Antigens derive from vast untapped expanses of genomic "dark matter" beyond the normal coding regions of the genome, which are silent in normal tissue but can become epigenetically activated in cancer.
- ERVs are a component of genomic dark matter and make up ~8% of the human genome. Thousands of novel ERV sequences have been identified by Ervaxx and our collaborators with enriched expression in > 30 tumor types.

The EDAPT™ platform for antigen discovery and validation

- EDAPT (Ervaxx Dark Antigen Platform Technology) is designed to explore the new and expanding Dark Antigen repertoire, and to identify and assess its tumor specificity and immunogenic potential to combat cancers.
- EDAPT is a powerful and proprietary approach combining bioinformatics, immunopeptidomics and state-of-the-art T-cell immunology. The platform systematically examines the entire cancer genome to understand changes in transcription patterns and discover and validate previously hidden Dark Antigens for use in immunotherapy.

Results - Discovery

ERV-derived antigen transcript discovery

- In order to scour regions of the genomic dark matter, a *de novo* pan-cancer transcript assembly was generated[1] using raw RNAseq reads from 768 patient samples selected from The Cancer Genome Atlas public database.
- The filtering approach outlined in Table 1 led to the *in silico* discovery of 97 melanoma-specific, ERV-derived transcripts, encoding 2,269 potential open reading frames (ORFs) for immunopeptidomic interrogation.

Table 1. Filtering for melanoma-specific, ERV-derived transcripts

Step	Transcripts
Pan-cancer assembly generation	1,001,931
Filter for cancer-specific transcripts • Expressed in > 25% samples for a given cancer • Expressed at < 10 TPM in > 90% normal samples • Expressed in any given cancer > 3x median expression of any control	32,264
Filter for ERV element containing transcripts	5,923
Filter for transcripts present in primary skin melanoma	403
Manual QC for transcript structure and expression levels	97

MS-immunopeptidomics validates presentation of ERV-derived antigens

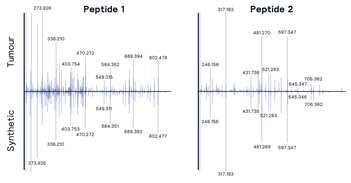
- Mass spectrometry-based immunopeptidomics allows the identity of HLA-bound peptides to be defined and searched against prospective antigens.
- 2,269 ORFs from 97 melanoma-specific, ERV-derived transcripts were interrogated against public[2] and independently-generated immunopeptidomic data from melanoma tumor samples.

Table 2. Filtering for melanoma-specific, ERV-derived transcripts

MS dataset	Sample number	Unique peptides	Unique ERV peptides	ERV-derived ORFs discovered
Public[2] dataset	25	164,884	46	32
Ervaxx-generated	16	71,368	24	17*

* Overlaps with public dataset

Figure 1. Example spectral validation with synthetic peptides confirms identity of peptides derived from an ERV-derived antigen detected in tumor samples



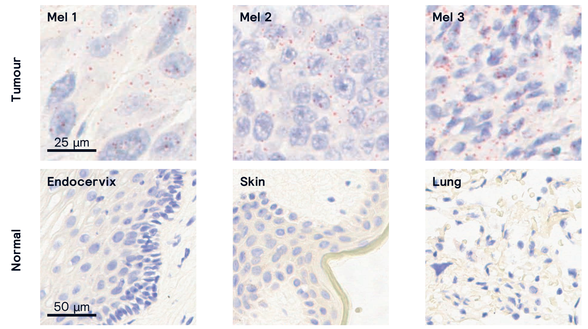
- Synthetic peptides were generated corresponding to each of the tumor-derived peptide sequences mapping to ERV-derived antigens.
- Spectral comparisons confirm the identity of the tumor-derived peptides.

Results - Validation

RNAScope® validates tumor-specificity and patient breadth of ERV-derived transcripts encoding MS discovered antigens

- RNAScope® is a method of *in situ* hybridization allowing for specific, sensitive detection of transcripts present in tissue sections with probes designed against large stretches of the target transcript.
- Tissue arrays of tumor and normal tissue cores were tested for expression of the transcripts encoding ERV-derived antigens discovered through immunopeptidomics.

Figure 2. Example images of RNAScope® staining for transcript encoding ERV Antigen-1 across melanoma and normal tissue cores



Normal donor CD8+ T-cell response to ERV-derived antigens confirms immunogenicity and lack of central tolerance

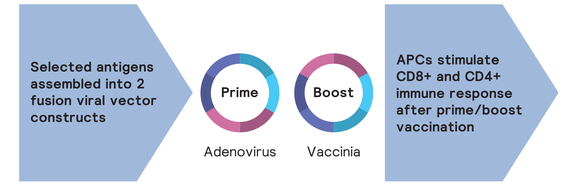
- ELISpot assay utilized to test CD8+ T-cell response to peptides derived from ERV antigens.
- Normal donor CD8+ cells were incubated with autologous monocytes loaded with selected peptides and assayed after a 2w expansion.

Figure 3. Example of naïve CD8+ T-cell priming and expansion by a peptide derived from ERV Antigen-1

- Reactive CD8+ T-cells from healthy bonors after incubation with ERV-derived antigen peptides suggests lack of central and peripheral tolerance against peptide sequences.
- Responses are present in the naïve CD8+ (CD45ROlow) T-cell population, as opposed to the memory CD8+ (CD45ROhigh) population, consistent with a lack of prior visibility of these antigens to the immune system.

Vaccine development

Multiple candidate antigens are combined into a fusion protein construct for prime/boost viral vectors



- Clinically proven viral vectors being utilized for delivery of fusion constructs containing multiple ERV-derived antigens displaying breadth of expression and potential epitopes across the patient cohort and ability to generate a robust immune response.
- Fusion constructs designed with linker sequences between antigens to avoid the generation of aberrant HLA restricted epitopes or sequences homologous to the known human proteome.
- Distinct antigen order and linker sequences used between prime/boost constructs to minimize the risk of amplifying unintended immune responses.
- Lead prime/boost vectors currently in preGMP development.

Conclusions

- **Creation and mining of a *de novo* pan-cancer transcriptome generated a library of candidate ERV-derived antigens uniquely expressed in melanoma.**
- **Mass spectrometry-based immunopeptidomics on primary melanoma patient tumor samples confirms the *bona fide* presentation of candidate antigens on the surface of tumor cells.**
- **Melanoma-specific expression and prevalence across patient samples of candidate ERV-derived antigens validated by RNAScope® *in situ* hybridization assay.**
- **Identification of response in naïve CD8+ T-cells from healthy donors confirms that ERV antigen-specific T-cells are not deleted during thymic or peripheral selection.**
- **Generation of fusion vaccine vectors with multiple ERV-derived antigens ensures broad patient coverage in an off-the-shelf melanoma vaccine currently being developed.**

References

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