Discovery and characterization of actionable tumor antigens

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Abstract

The nature of tumor antigens detectable by T cells remains unclear. In several patients with melanoma, T cells were shown to react against major histocompatibility complex (MHC)-associated peptides (MAPs) derived from exonic mutations. However, a recent multi-omic study revealed that in hepatocellular carcinomas, mutated exonic MAPs were exceedingly rare. This study questions the accuracy of methods currently used for antigen identification and demonstrates the importance of broadening the scope of tumor antigen discovery efforts.

Keywords: Antigen processing, Bioinformatics, Cancer immunology, Genomics, Major histocompatibility complex, Mass spectrometry, Transcriptomics, Tumor antigens.

Melanoma immunotherapy: from solid breakthroughs to data misinterpretation

Melanoma immunotherapy has fueled the current enthusiasm for cancer immunotherapy by leading to two seminal breakthroughs. Indeed, melanoma regression can now be achieved via injection of in vitro expanded tumor-infiltrating lymphocytes or immune checkpoint therapy targeting regulatory pathways in T cells. What is the nature of melanoma antigens capable of triggering therapeutic T-cell responses? A series of seminal observations suddenly brought exon-derived mutated MAPs (mMAPs) into the limelight. Exonic mMAPs can be detected by mass spectrometry (MS) on melanoma cells, these MAPs can elicit potent T-cell responses, and favorable response to immune checkpoint blockade correlates with the mutational load (1). The frenzy over discovery of exonic mMAPs led to a large acceptance of a speculative concept and the introduction a semantic bias. The unproven concept is that the repertoire of exonic mMAPs can be predicted (without MS validation) by combining exome sequencing and MHC binding prediction algorithms. The semantic reductionist bias was introduced when exonic mMAPs were named tumor-specific antigens (TSAs) (AKA neoantigens) and these terms were used synonymously. Formally, the term TSA must encompass not only exonic mMAPs but rather all MAPs present only on cancer cells, irrespective of their genomic origin (exonic or not) and mutational status. This is not a trivial issue because exons represent only 2% of the genome, while 75% of the genome can be transcribed and potentially translated.

The MAP repertoire of cancer cells: insights from MS analyses of primary tumors

In this issue of *Genome Medicine*, Löffler and colleagues report on a seminal study of 16 primary human hepatocellular carcinomas where they performed exome and transcriptome sequencing, high-throughput shotgun MS analyses of the proteome and MAP repertoire complemented with highly-sensitive targeted MS analyses of selected MAPs (2). The results were striking. Based on exome and transcriptome sequencing, MHC binding algorithms predicted that individual tumors would present an average of 118 exonic mMAPs. Remarkably, none of these exonic mMAPs were detected by MS analyses. Two tentative conclusions can be drawn from these comprehensive analyses. First, consistent with recent reports (3), they cast serious doubts on the validity of predictions based solely on next-generation sequencing and MHC binding algorithms. This is because current algorithms fail to take into account the numerous translational and posttranslational events that regulate MAP biogenesis (4). Second, exonic mMAPs are so rare in non-melanoma tumors that, for most patients, they do not represent realistic therapeutic targets. Over the last few months, similar findings were reported in a large cohort of chronic myelogenous leukemia patients (5) and a few other non-melanoma tumors (6).

How can we reconcile the rarity of exonic mMAPs with compelling evidence that many non-melanoma tumors display immunogenic MAPs? Arguably, the most parsimonious explanation is that the MAP repertoire of cancer cells contains a substantial amount of dark matter (antigens not detected with current approaches). In line with this, a recent study found that most TSAs present in human acute lymphoblastic leukemias and lung cancers derived from unmutated non-exonic sequences located in introns, intergenic

regions and other noncanonical reading frames (6). These aberrantly expressed TSAs (aeTSAs) were coded by RNAs that are not expressed in adult somatic cells, including medullary thymic cells (mTECs). mTECs deserve special attention here in view of their key role in establishing central tolerance and their ability to promiscuously express more transcripts than other types of somatic cells (7). The presence of aeTSAs on cancer cells results from epigenetic changes causing expression of genomic sequences normally repressed in somatic cells (e.g., endogenous retroelements).

Proposed guidelines for global analyses of the tumor antigen landscape

The study by Löffler and colleagues confirms that MS is the best and most robust method for high-throughput analyses of the MAP repertoire of tumor cells (2). Notably, the breadth and sensitivity of MS analyses can be adjusted according to user preferences and sample size (8). We therefore suggest that MS analyses should be included at the discovery and/or validation stage in tumor antigen discovery pipelines. Furthermore, we strongly encourage the sharing of MS datasets via the SysteMHC Atlas (9). In the short term, sharing of immunopeptidomic data will accelerate further analyses of tumor antigens' features: sharing among different tumors, abundance in tumor cells (at the RNA and peptide level), immunogenicity, etc. In the long term, it will then be possible to use large tumor antigen datasets as learning material for artificial neural networks. Until large tumor antigen datasets are available, it will remain impossible to use the most powerful bioinformatic techniques, such as deep neural networks, for development of predictive tools.

Rapid progress in the field and lack of a standardized nomenclature commonly lead to some confusion in the classification of tumor antigens. We therefore offer a simple classification of tumor antigens based on three criteria: tissue expression profile, genomic origin and mutational status (Table 1). Tumor-associated antigens (TAAs) are MAPs that show superior abundance on tumor cells but are nonetheless present on normal cells and therefore may induce central immune tolerance (10). TSAs are segregated into two main groups: mTSAs and aeTSAs (6). mTSAs derive from mutated DNA sequences that can be either exonic or non-exonic. aeTSAs result from aberrant expression of transcripts that are not expressed in any normal somatic cell, including mTECs. Finally, a peculiar antigen family, cancer-germline antigens (CGAs), is astride the TAA and aeTSA categories. CGAs are coded by canonical exons normally expressed only by germinal cells, and their aberrant expression in cancer cells is mostly driven by epigenetic alterations. However, some CGAs are expressed by adult mTECs (7). We propose to classify CGAs expressed in mTECs as TAAs and those not expressed by mTECs as genuine aeTSAs. One advantage of this simple classification is that antigen classes are linked to functional features. Thus, in contrast to TAAs, mTSAs and aeTSAs should not induce central immune tolerance and are expected to display superior immunogenicity. Also, TAAs and aeTSAs may present two advantages over mTSAs: they are more numerous and evidences suggest that some are shared by many tumors (6, 10-12). In principle, aeTSAs may offer the best of both worlds but much more work is needed in order to cogently evaluate the value of various classes of tumor antigens. A lot of immunopeptidomic dark matter has yet to be explored before conclusions can be reached.

Table 1. Classes of Tumor Antigens

	TAAs	**mTSAs**	**aeTSAs**
Genomic Origin	Exonic ++++	Exonic++ Non-exonic ++	Exonic + (some CGAs) Non-exonic ++++
Expression in mTECs	Yes	No	No
Mutation	No	Yes	No
Shared	Often	No/rarely	Often
Presence due to	Overexpression due to genetic and epigenetic changes	Somatic mutations	Epigenetics Aberrant splicing

Abbreviations

aeTSA: aberrantly-expressed TSA; CGA, cancer-germline antigen; MAP: MHC-associated peptide; MS, mass spectrometry; MHC: Major histocompatibility complex; mTEC: medullary thymic epithelial cell; mTSA: mutated TSA; TAA: Tumor-associated antigen; TSA: Tumor-specific antigen

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Authors' contributions

GE and CP wrote the manuscript. Both authors read and approved the final manuscript.

Competing interests

CP is a named inventor on a patent application entitled “Proteogenomic-based method for identifying tumor-specific antigens” filed by Université de Montréal. GE has no competing interests.

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