Mutation-Derived Tumor Antigens: Novel Targets in Cancer Immunotherapy

December 15, 2015 | Oncology Journal [1]
By John P. Finnigan, Jr, BS [2], Alex Rubinsteyn, PhD [3], Jeffrey Hammerbacher, BA [4], and Nina Bhardwaj, MD, PhD [5]

Because of the abundance of promising preclinical and early-phase clinical data, mutation-derived tumor antigens an exciting new class of targets in cancer immunotherapy.

Introduction

The ability to leverage the adaptive immune system to treat human cancers has been a goal of immunologists and oncologists dating back to the pioneering experiments of William Coley. The ideal immunotherapy should mobilize the immune system to clear an existing population of malignant cells, without damaging adjacent healthy cells, and remain active in the event of recurrence. In practice, these goals have yet to be fully realized, in part because of the difficulties associated with identifying targets of the antitumor immune response. However, the introduction of highly effective immunotherapeutics—such as immune checkpoint blockade—coupled with advances in gene sequencing, has changed the face of the field entirely. Recent evidence indicates that mutations unique to a given individual’s tumor may be the long-sought-after targets of effective antitumor immunity and may hold the key to the design of the next generation of immune-based therapeutics.

Background

Descriptions of tumor antigens first appeared in the literature in the middle of the 20th century following observations of immune-mediated clearance of carcinogen-induced tumors in murine experimental models.[1-4] In the subsequent decades many tumor antigens have been identified, including developmental (carcinoembryonic antigen, WT1 [Wilms tumor 1]), lineage-specific (Melan-A/MART-1 [melanoma antigen recognized by T cells 1], prostatic acid phosphatase), and cancer/testis (MAGE-A [melanoma-associated antigens], NY-ESO-1, PRAME [preferentially expressed antigen in melanoma]) antigens, all of which are produced by genes overexpressed by malignant cells.[5] These antigens are easily identifiable and often shared among a cohort of subjects.[6,7] However, they are, by definition, “self” antigens and, like other “self” antigens, subject to immune tolerance.[8-11] Thus, the qualities that make these tumor antigens amenable to study may in turn limit their therapeutic utility.[12-15]

Recent advances in genetic sequencing technologies have facilitated the discovery of a new class of tumor antigen derived from somatic mutations, which we have termed mutation-derived tumor antigens (MTAs) but which are also known as neoantigens, or tumor-specific antigens.[16,17] MTAs are the product of nonsynonymous somatic variations randomly acquired by malignant cells as a result of deregulated progression through the cell cycle. Nonsynonymous somatic variations change protein sequence and function, facilitating the acquisition of traits associated with replicative advantage and tissue invasiveness.[18,19] Yet, when a nonsynonymous somatic variant is expressed and the variant sequence translated, the immune system may perceive the variant protein as “foreign” or “nonself” and respond to the cell as though it were infected with a pathogen.[20] Notably, the likelihood of generating MTAs appears to be roughly proportional to the number of somatic mutations present within a given tumor.[21,22] However, the production of MTAs does not depend on the type of somatic variation. MTAs have arisen from substitutions, insertions, and deletions, as well as from larger structural rearrangements, such as duplications, inversions, and translocations. Nor does the production of MTAs depend on the oncologic significance of the instigating mutation. MTAs have arisen from both classic tumor suppressors and oncogenes, as well as from somatic mutations of unknown significance. Finally, the production of MTAs does not appear to depend on clinical characteristics of age, sex, and disease histology. MTAs have been identified in
patients with multiple types of solid and hematologic malignancies.[23-37] Evidence from both murine and human studies suggests that MTAs play a significant role in the endogenous adaptive immune response to tumors, as well as the induced adaptive immune response to tumors treated with cytotoxic T-lymphocyte–associated antigen 4 (CTLA-4) or programmed death 1 (PD-1) antagonists. Indeed, patients with melanoma or squamous-cell non–small-cell lung cancer who are treated with immune checkpoint blockade appear to stand a greater chance of clinical benefit as the burden of somatic mutations increases.[38-40] Furthermore, patients with tumors deficient in DNA mismatch repair, in which the number of somatic mutations can be multiple orders of magnitude greater than in tumors of comparable origin, also appear to fare significantly better when treated with immune checkpoint blockade.[41] However, prospective trials will be required to establish the utility of MTAs as predictive biomarkers. Given the considerable excitement brought about by these findings, as well as the ongoing development of therapeutic approaches that directly target MTAs, we will consider how these tumor antigens are identified, highlight deficiencies in current processes, and speculate as to how existing methods may be improved through further research.

Identification of MTAs

The identification of somatic variations in tumors and, by extension, MTAs relies on the use of high-throughput sequencing (Figure 1).[42-44] Typically, material from both the tumor and a matched normal sample are sequenced in tandem.[45,46] Due to economic and technical considerations, sequencing efforts are restricted to the known protein-coding regions of the genome (exome). However, the use of targeted sequencing panels is not advised because this severely limits the ability to identify novel MTAs. Sequencing experiments designed to identify MTAs are performed at higher coverage depths than are typically encountered in the literature. This is true with regard to the sequencing of tumor material, as well as material derived from matched normal samples. Sequence coverage depth is described in terms of the mean number of reads successfully realigned to targeted loci. However, the process by which the exome is enriched prior to sequencing can result in significant nonuniformity in coverage depth. To achieve acceptable mean coverage across all target loci, a mean sequence coverage depth of 150× may be required for normal samples.[47] When tumor samples are sequenced, coverage depths are typically doubled (300×) to account for additional confounders, including intrinsic tumor heterogeneity and contamination of tumor samples with material from stromal and/or other infiltrating cell types. These and other sources of bias may be reduced even further through random sampling of multiple, distinct regions of the tumor or through sequencing of circulating tumor material (cells, DNA).

MTAs are produced when a nonsynonymous somatic variant is expressed and the variant sequence is translated into protein. The targeting of MTAs derived from expressed variants can lead to therapeutic responses in vivo in animal models, but the utility of MTAs derived from nonexpressed variants has not been established.[42-44,48] DNA sequencing can be used to measure sequence variation; however, it does not provide a measure of gene expression. Quantifying expression eliminates nonexpressed variants that can confound downstream analysis. In addition, expression analysis through RNA sequencing, as opposed to array-based methods, directly determines transcript sequence and structure, which mitigates the requirement for accurate annotation of the transcriptome. Unfortunately, because of both biologic and technical variables, it is difficult to provide a general recommendation with regard to target depth for RNA sequencing experiments. However, depths in excess of 50 to 100 million reads per sample can be required to unambiguously resolve the sequence of variant-containing transcripts.

The technical details of DNA and RNA sequence analysis are beyond the scope of this article. However, we would like to emphasize that the choice of parameters used in sequence analysis can introduce systematic error into the results of these analyses. To overcome these issues, it is advisable to perform all analyses with extensively vetted combinations of bioinformatics tools.[49] It may also be advisable to use many such vetted combinations and to consider only those results arrived at by consensus.[50,51]

The final step in identifying MTAs further refines the list of expressed nonsynonymous variants and eliminates those that are unlikely to be immunologically relevant. This procedure has been the subject of a number of recent reviews and will not be exhaustively detailed here. Fritsch et al, van Buuren et al, Rajasagi et al, and Gubin et al provide comprehensive overviews of the methodology.[52-55] Briefly, computational tools are used to assess the variant protein sequence
and to determine whether it will be presented by host major histocompatibility complex (MHC) class I or II molecules—surface receptors required for the initiation of a cellular adaptive immune response. It has been demonstrated that putative MTAs that are predicted to bind to MHC molecules by this method can lead to therapeutic responses in vivo.[42-44] However, the criteria used to make this distinction were developed for the infectious disease setting, and additional studies will be required to formally establish criteria for use in identifying MTAs. Finally, there is an urgent need for new animal models that can be used to evaluate MTA-specific therapeutic approaches in a manner that can be translated into studies performed in humans.

**Therapeutic Targeting of MTAs**

Much remains to be determined regarding the specific dose, schedule, and route of delivery of MTA-targeting immune-based therapeutics and the relative role of CD4- vs CD8-mediated immunity (Figure 2).[48] At present, a number of early-phase clinical trials are assessing MTA-targeting vaccines, and the preliminary results are encouraging.[56] Tumor-lysate–, peptide–, RNA–, and dendritic cell–based vaccine substrates are being studied, but the optimal choice of vaccine adjuvant remains to be determined. Trials currently underway have largely employed certain synthetic toll-like receptor ligands, which have been studied in other vaccination projects. However, adjuvants that target other innate immune pattern recognition receptors, including C-type lectin (CLR), nucleotide-binding oligomerization domain (NOD), RIG-I-like (RLR), and stimulator of interferon genes (STING) receptor pathways, remain viable, unstudied alternatives. The use of stimulatory hematopoietins, such as FMS-like tyrosine kinase 3 ligand and granulocyte-macrophage colony-stimulating factor (sargramostim), should also be considered.[57-59]

Evidence suggests that indirect targeting of MTAs through the use of vaccine adjuvants or oncolytic viruses injected or infused in situ or directly into the body of existing tumors may represent yet another approach to the stimulation of an MTA-specific immune response.[60,61] Preclinical work suggests that in situ vaccination may be used as a bridge to formal MTA-targeted vaccination for patients with advanced primary or recurrent disease, thus broadening the range of patient groups who are eligible to receive precision immune-based therapeutics. Finally, it will be important to consider the use of MTA-targeted agents in conjunction with CTLA-4 and PD-1/programmed death ligand 1 antagonists (see Figure 2). Although combination approaches have not been formally tested in humans, preliminary evidence suggests that MTA-targeted approaches can boost existing MTA-specific responses and also provoke MTA-specific responses de novo.[56] Thus, combination therapy may serve as a safe and effective means of overcoming resistance to immune checkpoint blockade.[61]

**Conclusion**

At present much remains to be determined regarding the efficacy of MTA-specific immune-based therapeutics, used individually or in combination with other therapeutics. Generating these data will require innovative prospective trial designs compatible with the degree of subject-to-subject variability inherent to this approach. However, because of the abundance of promising preclinical and early-phase clinical data, many in the field are initiating such trials, making the MTAs an exciting new class of targets in cancer immunotherapy.

**Financial Disclosure:** Dr. Bhardwaj is a cofounder of Check Point Sciences. The other authors have no significant financial interest in or other relationship with the manufacturer of any product or provider of any service mentioned in this article.

Figure 2. Challenges Associated With the Implementation of Personalize...

References:


Source URL: http://www.cancernetwork.com/oncology-journal/mutation-derived-tumor-antigens-novel-targets-cancer-immunotherapy

Links:
[1] http://www.cancernetwork.com/oncology-journal
[2] http://www.cancernetwork.com/authors/john-p-finnigan-jr-bs
[3] http://www.cancernetwork.com/authors/alex-rubinsteyn-phd
[4] http://www.cancernetwork.com/authors/jeffrey-hammerbacher-ba
[5] http://www.cancernetwork.com/authors/nina-bhardwaj-md-phd