Published in final edited form as:
Ann N Y Acad Sci. 2013 May ; 1284(1): 1–5. doi:10.1111/nyas.12105.

Cancer Immunoediting: antigens, mechanisms and implications to cancer immunotherapy

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Abstract

Accumulated data from animal models and human cancer patients strongly support the concept that the immune system can identify and control nascent tumor cells in a process called cancer immunosurveillance. Additionally, the immune system can also promote tumor progression through chronic inflammation, immunoselection of poorly immunogenic variants and suppressing anti-tumor immunity. Together, the dual host-protective and tumor-promoting actions of immunity are referred to as cancer immunoediting. The current framework of cancer immunoediting is a dynamic process comprised of three distinct phases: elimination, equilibrium, and escape. Recently, we demonstrated that immunoselection by $CD8^+$ T cells of tumor variants lacking strong tumor-specific antigens represents one mechanism by which cancer cells escape tumor immunity and points toward the future of personalized cancer therapy.

Keywords

cancer immunoediting; immunosurveillance; tumor antigens; immunotherapy; tumor escape; cancer genome

Introduction

Cancer immunoediting

A plethora of evidence now provides strong support for cancer immunoediting, a process wherein immunity functions not only as an extrinsic tumor suppressor but also to shape tumor immunogenicity[1]. In its most complex form, cancer immunoediting occurs in three sequential phases: elimination, equilibrium, and escape. Elimination is a modern view of the older notion of cancer immunosurveillance, in which innate and adaptive immunity work together to detect and destroy transformed cells long before they become clinically apparent. However, sometimes, tumor cell variants may not be completely eliminated but instead enter into an equilibrium phase in which the immune system controls net tumor cell outgrowth; in this phase, adaptive immunity constrains growth of clinically undetectable occult tumor cells and edits tumor cell immunogenicity.[2] Finally, the functional dormancy of the tumor cell population may be broken, leading to progression of the cells into the escape phase, during which edited tumors of reduced immunogenicity begin to grow progressively in an immunologically unrestrained manner, establish an immunosuppressive tumor microenvironment, and eventually become clinically apparent[3]. Importantly, escape from immune control is now acknowledged to be one of the hallmarks of cancer.[4]

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The antigens of unedited tumors

A central tenet of tumor immunology in general, and the cancer immunoediting process in particular, is that tumor cells express antigens that distinguish them from their non-transformed counterparts, thus permitting their recognition by T cells and their eventual destruction by immunological mechanisms. Although a deep understanding of human and mouse tumor antigens currently exists, it comes nearly entirely from analyses of tumor cells derived from immunocompetent hosts, which were likely subjected to the sculpting forces of cancer immunoediting during their development. Little is known about the antigens expressed in nascent tumor cells, for example, whether they are sufficient to induce host-protective, anti-tumor immune responses, or whether their expression is modulated by the immune system.

We realized that such questions might be answered by defining the antigens expressed in unedited sarcoma cell lines derived from 3′-methylcholanthrene (MCA)–treated, immunodeficient *Rag2*$^{-/-}$ mice because such induced tumor cells phenotypically resemble highly immunogenic, nascent, primary tumor cells.[5] However, current methods to identify tumor antigens using expression-cloning approaches are time and effort intensive, and are not well suited to establishing a tumor's antigenic landscape. Recent advances in genome sequencing have made possible rapid and cost effective methods to define cancer genomes, and have established that, whereas they acquire some mutations involved in the transformation process (*driver* mutations), cancer cells also develop many *passenger* mutations, in part, as a consequence of the genomic instability that is a characteristic of transformed cells. It has been proposed that some of these mutations result in the expression of tumor-specific proteins that are, in turn, tumor-specific antigens for T cells.[6] However, until recently, this had not been experimentally demonstrated.

Recent work from our laboratory[7] used a novel from of exome sequencing (cDNA capture sequencing or cDNA CapSeq) to define the mutational profile of two independent, unedited MCA sarcomas (d42m1 and H31m1). By pipelining the sequencing data for one of these tumors (d42m1) into major histocompatibility complex (MHC) class I epitope prediction algorithms, we identified a potential mutational antigen of the unedited d42m1cells, validated its identity as the major rejection antigen using expressing cloning techniques, and showed that antigen loss via a T cell–dependent immunoselection process represents the mechanism underlying cancer immunoediting of this tumor. This study[7] thus provides mechanistic insights into the process of cancer immunoediting and points to the future potential that cancer genome analysis may have on the fields of tumor immunology and cancer immunotherapy.

Cancer genome sequencing and antigen discovery

Using cDNA CapSeq we identified 3,737 non-synonymous mutations in d42m1 cells and 2,677 non-synonymous mutations in H31m1 cells. However, only 5% of the mutations were shared between d42m1 and H31m1, which explains the unique immunogenicity that each cell line displays. Interestingly, both d42m1 and H31m1 had activating mutations in codon 12 of the *Kras* proto-oncogene and inactivating mutations in the tumor suppressor gene *Trp53*. When comparing the sequence data of d42m1 and H31m1 sarcoma cells to those of human cancer genomes we found that the former most closely resemble genomes of carcinogen-induced lung cancers from smokers in both the number and type of mutations (e.g., C/A or G/T transversions).

Thus, cancer exome sequencing of d42m1 and H31m1 cells demonstrated a carcinogen signature similar to that found in lung cancer cells from smokers, but distinct from other

human cancers. This raises the possibility that a similar discovery approach may be used to find tumor- specific antigens in human lung cancer.

d42m1 tumor variants

The d42m1 sarcoma cell line displays a sporadic tendency to produce escape tumors following transplantation into naive, syngeneic wild-type mice. Furthermore, cell lines derived from escape tumors of parental d42m1 (d42m1-es1, d42m1-es2, and d42m1-es3) consistently formed progressively growing tumors when transplanted into naive syngeneic recipients. Thus, unedited d42m1 tumor cells can undergo immunoediting when transplanted into wild-type mice. The basis for the heterogeneous behavior of d42m1 tumor cells in naive immunocompetent mice is due the parental d42m1 cell line consisting of a disproportionate (80:20) mixture of regressor and progressor tumor cell clones.

Identifying potential d42m1 tumor antigens from genomic data

To identify tumor-specific antigens for $CD8^+$ T cells, we used the data from the cancer exome mutational analysis to identify the antigenic targets of d42m1-specific $CD8^+$ cytotoxic T lymphocytes (CTLs). First, we assessed the theoretical capacity of peptides containing each missense mutation to bind to MHC class I proteins (i.e., to function as neoantigens) by *in silico* analysis. Second, we used a d42m1-specific $CD8^+$ CTL clone derived from a wild-type mouse that had rejected parental d42m1 tumor cells to assess tumor reactivity *in vitro*; the readout of CTL activity against the tumor cells was IFN-γ production by the $CD8^+$ T cells. The d42m1- specific C3 CTL clone was stimulated by parental d42m1 tumor cells and with all regressor d42m1 tumor cell variants; but it was not stimulated by progressor d42m1 tumor cell variants or unrelated MCA sarcoma cells. These results revealed that regressor d42m1 tumor cells share a common rejection antigen. We therefore focused on the limited number of epitopes common to all d42m1 regressor variants. And together with the fact that recognition of all d42m1 regressor variants by the CTL clone was restricted by $H\text{-}2D^b$, we predicted that an R913L mutation in spectrin-β2 represented the most likely rejection antigen candidate because of its high affinity for $H\text{-}2D^b$.

Next, we established that C3 CTL cells could discriminate between the mutant and wild-type spectrin-β2 peptide sequence 905–913 when presented on $H\text{-}2D^b$. To document that the anti-R913L spectrin-β2 response occurred under physiologic conditions, we used labeled $H\text{-}2D^b$ tetramers carrying the mutant spectrin-β2 905–913 peptide to identify tumor antigen specific $CD8^+$ T cells in d42m1 tumors. Mutant spectrin-β2–specific $CD8^+$ T cells were detected in parental d42m1 tumors and draining lymph nodes and increased in numbers to peak values just prior to tumor rejection. In contrast, no mutant spectrin-β2–specific $CD8^+$ T cells were detected in d42m1-es3 tumors. These data demonstrate that a mutated gene expressed selectively in unedited d42m1 tumor cells gives rise to a mutant protein that evokes a naturally occurring T cell response in naive wild-type mice. Thus, mutant spectrin-β2 is a genuine tumor-specific antigen of d42m1 sarcoma cells.

Mutant spectrin-β2 is the major rejection antigen of d42m1 tumor cells

In order to explore whether mutant spectrin-β2 represented the major rejection antigen of parental d42m1 tumor cells, we enforced expression of either the mutant or wild type forms of spectrin-β2 into cells of one of the d42m1 escape variants, d42m1-es3. When injected into wild-type mice, d42m1-es3 tumor cell clones expressing wild-type spectrin-β2 grew progressively and displayed similar growth kinetics to the parental d42m1-es3 cell line. In contrast, d42m1-es3 tumor cell clones expressing mutant spectrin-β2 were rejected in wild-type but not $Rag2^{-/-}$ mice. Furthermore, $CD8^+$ T cells specific for mutant spectrin-β2 were detected by tetramer staining of d42m1-es3 tumors that had been reconstituted with mutant

spectrin-β2. These results demonstrate that expression of mutant spectrin-β2 is both necessary and sufficient for the rejection of d42m1 tumors, and thus validate it as a major rejection antigen of d42m1 sarcoma cells.

Immunoselection is the immunoediting mechanism for d42m1 tumor cells

Next, we formulated the hypothesis that T cell dependent immunoselection was a likely mechanism favoring outgrowth of tumor variants that lack strong rejection antigens. This possibility is consistent with our finding that every d42m1 clone that expresses mutant spectrin- β2 was rejected, while every clone or variant that lacks mutant spectrin-β2 formed progressively growing tumors. To formally test this hypothesis, we assessed the *in vivo* behavior of a disproportionate mixture of cells consisting of a majority of highly immunogenic d42m1 tumor cells expressing mutant spectrin-β2 (i.e., d42m1-T2) and a minority a d42m1 tumor cell clone lacking mutant spectrin-β2 (i.e., d42m1-T3). When this mixture was transplanted into wild-type mice, 5/20 (25%) developed escape tumors, a result that closely resembles what was observed with parental d42m1 tumor cells in wild-type recipients. Furthermore, tumors that grew out in wild-type mice consisted of 98% d42m1-T3 tumor cells and lacked mutant spectrin-β2. Thus, escape variants of parental d42m1 tumor cells develop as a consequence of a T cell–dependent immunoselection process that favors the outgrowth of tumor cell clones lacking the major rejection antigen.

For d42m1 tumor cells, we show that an immunoselection process acting on an oligoclonal parental tumor cell population leads to the outgrowth of tumor cell variants that lack the major tumor rejection antigen, in this case mutant spectrin-β2. The immunoselection that occurs upon exposure to an intact immune system is dependent on adaptive immunity since neither parental d42m1 tumor cells nor the mixture of regressor and progressor d42m1 tumor cell clones undergoes editing when passed through *Rag2*$^{-/-}$ mice; yet they are edited following transplantation into immunocompetent wild-type mice. Thus, in the case of d42m1, the target of the immunoselection process has been clearly identified as a major rejection antigen. However, this finding does not rule out the possibility that similar immunoediting mechanisms might select for mutations in critical components of the MHC class I antigen processing and presentation pathway, such as the class I heavy chain,[3] β2 microglobulin, or components of IFN-γ receptor signaling,[1] all of which are known to regulate tumor cell recognition by tumor-specific CD8^{+} T cells.

Personalized immunotherapy and genomics: the antigen landscape

Recent advances in genome sequencing have resulted in unprecedented opportunities to assess genetic influences on disease development. For cancer, most genome sequencing studies have focused on identifying new driver mutations that promote neoplastic development and metastasis, in the hope of obtaining insights that lead to novel cancer-targeted therapeutics or that provide prognostic value.[8] However, we have shown that this same technology, when combined with an *in silico* epitope prediction algorithm, can be used to identify expressed mutations in a cancer cells that result in expression of tumor-specific antigens that can be targets for immune-mediated elimination. We predict that this approach may not only provide new insights into basic mechanisms underlying cancer immunoediting but also new opportunities for individualized cancer immunotherapy.

The large datasets of information from the many cancer genome initiatives could be of value to tumor immunologists for defining the antigen landscape—as opposed to the mutational landscape—of human cancers[9]. One application of this approach is that it could be used to identify the subset of cancer patients whose tumors express antigens that may be better targeted by immunotherapy. For example, only about 25% of patients treated with anti-CTLA-4 respond positively, and the reasons for this limited response remain unknown. We

suggest that patients who respond to checkpoint blockade immunotherapy may have more immunogenic, tumor- specific mutations, and that these antigens can be identified using cancer exome sequencing and high-throughput bioinformatics; in other words, the genomics approach may provide a mechanism to stratify those patients who would benefit most from this type of therapy. A second application of this approach may provide a mechanism to longitudinally evaluate changes in a tumor's antigenic profile as a consequence of ongoing immunotherapy. A third application is the rapid identification of the most immunogenic epitopes within a tumor, with the goal of developing personalized cancer vaccines for patients.

It is difficult to predict whether this type of analysis will yield prognostic value in the clinic, as genome analysis can be costly and requires streamlined computational analysis. Nevertheless, third-generation sequencing technologies are already commercially available, and costs for cancer genome sequencing have already started to fall sharply and are likely to continue dropping over the next decade. It should thus be feasible to routinely perform this type of genomic analysis on individual patient's cancer cells in the not-to-distant future.

Remaining questions

Our recent study[7] demonstrates that immunoediting of a tumor results from T cell–dependent immunoselection for tumor cell variants that fail to express highly antigenic mutations. These results thus not only provide definitive evidence for at least one mechanism underlying the cancer immunoediting process, but also demonstrate the key role that tumor-specific mutations play in the development of a tumor's immunogenic phenotype and subsequent fate.

It is interesting that a single mutant protein manifests such immunodominance as d42m1 tumor cells, an immunodominance that in some ways resembles the immunodominance of certain viral antigens. Many factors may contribute to the immunodominance of mutant spectrin-β2. On the basis of *in silico* analysis, the mutant 905–913 sequence is predicted to interact with H-2D^{b} with very high affinity in contrast to the corresponding wild-type sequence, which is predicted to bind only weakly. However, several other factors may also contribute to the immunodominance of mutant spectrin-β2, including antigen abundance, antigen cross presentability, T cell repertoire, or presence of epitopes recognized by regulatory T cells. Clearly, more work is needed in order to refine the capacity to accurately predict the antigenicity of a mutated protein.

It is unclear from our work whether the mechanism of cellular transformation influences the types of antigens that are eventually expressed by cancer cells. In our model, sarcomas generated from chemical carcinogens are most similar, in the number and type of mutations, to carcinogen-induced human cancers, such as lung cancers from smokers. For the past six decades, the MCA sarcoma system has been an experimental workhorse for tumor immunologists. This may be due to the carcinogen's ability to generate a large number of passenger mutations, which allows for a greater number of potential neoantigens to form that may be recognized by the immune system. We speculate that oncogene-driven models of cancer, which harbor fewer passenger mutations than spontaneous cancers, may not be as readily eradicated or controlled by anti-tumor immunity. However, in a companion study to ours, Michel DuPage and Tyler Jacks et al. demonstrated a key role of dominant antigens in the cancer immunoediting process by using a sarcoma model driven by *Kras* activation and *Trp53* inactivation.[10]

Summary and conclusions

The recent revolution in genomics represents a significant transition in the evolution of the cancer immunoediting concept. In the past, efforts were mostly centered on demonstrating that the process occurs, identifying the key players in it, and attempting to define the positions that they play. Work in this area now enters a new phase in which researchers can begin to elucidate the molecular mechanisms that drive the process, and determine the quality and quantity of tumor antigens expressed in nascently transformed cells that drive immune-mediated elimination and/or sculpting.

The approach of exome sequencing, *in silico* analysis, and $CD8^+$ T cell cloning are beneficial to both basic and clinical scientists. By defining the specific antigenic targets in cancer cells, new levels of understanding of host responses to tumors during ongoing therapy can be obtained. This, in turn, should facilitate the development of new therapeutic opportunities that direct the power and specificity of the immune system to control, and/or destroy, cancer. It may also be useful for identifying subsets of cancer patients whose tumors express antigens that can be most effectively targeted by checkpoint blockade immunotherapy, and may provide a mechanism to longitudinally evaluate changes in a tumor's antigenic profile as a consequence of ongoing immunotherapy. Therefore, we predict that a genomic approach to tumor antigen identification may, in the future, facilitate the development of individualized cancer immunotherapies directed at tumor-specific—rather than simply cancer-associated—antigens.

Acknowledgments

This work was supported by grants to R.D.S from the National Cancer Institute, the Ludwig Institute for Cancer Research, the Cancer Research Institute, and the WWWW Foundation. M.D.V. is supported by a pre-doctoral fellowship from the Cancer Research Institute.

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