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Review

Tumor immunogenicity and responsiveness to cancer vaccine therapy: The state of the art☆

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ABSTRACT

Despite enormous effort, promising pre-clinical data in animal studies and over 900 clinical trials in the United States, no cancer vaccine has ever been approved for clinical use. Over the past decade a great deal of progress has been in both laboratory and clinical studies defining the interactions between developing tumors and the immune system. The results of these studies provide a rationale that may help explain the failure of recent therapeutic cancer vaccines in terms of vaccine principles, in selecting which tumors are the most appropriate to target and instruct the design and implementation of state-of-the-art cancer vaccines.

1. Introduction

Cancer is a disease arising from a prolonged period of genetic instability that extends the lifespan of a normal cell. The triggering event that marks the beginning of this period is variable between cell types, but is commonly the acquisition of a mutation in a tumor suppressor gene (such as p53 or Rb), a mutation in a proto-oncogene (such as KRAS or myc) or infection of the cell with an oncogenic virus (such as HPV16 or EBV). Whatever the origin, cells that acquire mutations in genes that enable them to escape normal growth controls or cell death pathways then become more likely to acquire additional such mutations. At some point a cell has acquired enough mutations, typically thought to be at least six, that it is no longer responsive to intrinsic or extrinsic signals that would restrain its growth or trigger apoptosis. Although it may sometimes be the case that a very small number of mutations are sufficient to transform cells, recent analysis of the genetic makeup of human tumors by The Cancer Genome Atlas suggests that it is far more common that a tumor contain several dozens of mutations than just a handful [1–4].

Because tumors arise from our own cells, our bodies' immune systems are initially tolerant to those cells. The acquisition of tumorigenic mutations may or may not lead to the production of a mutated protein containing an epitope that is sufficiently non-self to become immunogenic. If a cell acquires an immunogenic mutation, then it may be sought out and destroyed by the host immune system, a process known as immunosurveillance [5]. A variety of murine studies lend support to the immune surveillance hypothesis [6–8] and also suggest that innate in addition to so-called adaptive immune responses may facilitate rejection of immunogenic tumors [9–11]. Such innate responses may be evoked through induced expression of NK activating signals such as NKG2D ligand expression or following DNA damage incurred as a result of mutagenic or viral processes. Some cells that acquire immunogenic mutations also gain the capacity to engage normal immune regulatory systems that dampen anti-self-immune responses [12]. The pathways driving the activation of host regulatory mechanisms are poorly understood. Still other cells may gain a number of oncogenic mutations without ever producing an immunogenic peptide that leads to the activation of the host immune system. Therefore, tumor cells that produce an immunogenic peptide during their transformation must continuously evade anti-tumor immune responses in order to survive, whereas tumors that become transformed without activating the immune system may not rely on such immune regulatory mechanisms for survival. This phenomenon of variable tumor immunogenicity has been largely ignored when designing and testing cancer immunotherapeutics.

Cancer vaccines fall under a category of therapeutics known as biological response modifiers (BRMs). Prophylactic cancer vaccines such as Gardasil (Merck & Co.) and Cervarix (GlaxoSmithKline) as well as a variety of therapeutic cancer vaccines, which have not yet received FDA approval, fall into this category. Also included are

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innovative approaches that employ viral vectors or that augment immune cell activation in an attempt to directly lyse tumor cells and/or invoke an effective anti-tumor immune response. These latter approaches do not necessarily introduce new tumor antigens, and therefore do not meet the definition of a vaccine, but much of their efficacy is considered to be due to immune activation through a process dubbed 'vaccination *in situ*'. Therefore, the primary focus of this review will be to review prophylactic and therapeutic cancer vaccines currently in clinical development, but a discussion of certain non-vaccine BRMs is also included where their use has instructed us as to the immunogenicity of certain tumors and the requirement for combinatorial therapeutics.

2. Tumor antigens and immunogenicity

For over a century there has been a struggle both within and outside the scientific community in an effort to provide unequivocal proof that the immune system is capable of identifying and eliminating spontaneous tumors [13]. This argument has been largely limited to spontaneous tumors, whereas there has been general agreement that the immune system should be capable of recognizing tumors of viral origin. The crux of this disparity in consensus is related to whether or not spontaneous tumors ever gain sufficient immunogenicity via the acquisition of genetic mutations to break immune self-tolerance. Breaking self-tolerance is not an obstacle for viral antigens implicated in virally induced cancers (because viral antigens are inherently non-self), however the loss of dependence of transformed cells upon those viral antigens for long-term survival [14–16] suggests that virally induced cancers should be thought of simply as highly immunogenic tumors, rather than as a separate category.

There are two basic categories of tumor antigens: abnormal self-antigens (ASAs) and tumor-specific antigens (TSAs). ASAs are antigens that may be generated in a variety of ways including; induction of embryonal and developmental genes not normally expressed in most adult tissues, expression of normal proteins with abnormal sugar moieties or expression of self-proteins at abnormally high levels. TSAs result from spontaneous somatic mutations or breaks in the germline DNA that lead to missense, frameshift errors in the open reading frame of normal mRNA transcripts or to fusion proteins, respectively [17]. Not all such mutations alter the immunogenicity of transformed cells however, because specific residues in mutated self-proteins must be flanked by anchor residues in order to facilitate loading onto the MHC. It remains unclear what percentage of TSAs satisfy the requirements for MHC binding. For breast and colorectal cancers however, epitope mapping based on the results of The Cancer Genome Atlas (TCGA) estimated that approximately 10 and 7, respectively, TSAs are generated on average in individual tumors with appropriate anchor residues for MHC loading [18].

Large numbers of both ASAs and TSAs have been described and a useful database of these antigens is maintained by the Academy of Cancer Immunology (http://www.cancerimmunity.org/peptidedatabase/Tcellepitopes.htm). In addition, TCGA has recently uncovered a multitude of potential antigens in pancreatic adenocarcinoma, glioblastoma multiforme, breast and colorectal cancers. The comprehensive cancer genome sequencing effort led by TCGA has provided enormous insight into both the heterogeneity and the potential number of TSAs both between and among particular cancers. As was predicted by Hanahan and Weinberg, the most commonly mutated somatic genes are those that are involved in the regulation of cell growth and death pathways (mutations in proteins thought to be the 'drivers' of oncogenesis), however in total there are far more so-called 'passenger' mutations scattered throughout the genome of transformed cells [1–4,19]. The

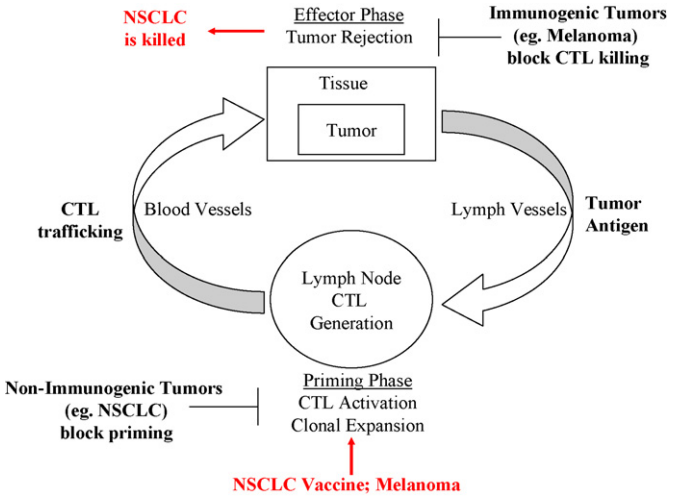

Fig. 1. Differential interactions between immunogenic versus non-immunogenic tumors and the immune system. Tumors develop within tissues and release tumor antigens into local lymphoid organs. For immunogenic tumors (such as melanoma), those antigens lead to cytotoxic T lymphocyte (CTL) activation and subsequent infiltration of the tumor by tumor-antigen specific CTL. The observation that many immunogenic tumors develop in spite of such a response is evidence that immunogenic tumors develop regulatory characteristics that lead to resistance to tumor cell killing by CTL. Non-immunogenic tumors on the other hand (such as NSCLC) release antigens that do not efficiently prime an anti-tumor immune response, and as a result such tumors need not develop regulatory mechanisms to counteract the killing activity of tumor-antigen primed CTL. Therapeutic vaccination aims to prime the immune system against tumor antigens, so it is anticipated that such a response will be more effective against tumors (non-immunogenic) that have not already acquired an immune regulatory phenotype.

relative frequency of ASAs and TSAs is poorly understood, as is the frequency of shared mutations between individual patients. Both of these questions are critical to the logical design of cancer vaccines intended to treat a large number of patients with a similar cancer, let alone patients with unrelated tumors.

Equally important to the availability of ASAs and TSAs for incorporation into vaccination strategies is a recognition of which of these antigens have already led to the activation of T cell immunity. Tumors that commonly induce spontaneous anti-tumor immune responses, engage immunosurveilling T cells and still develop in spite of these responses, are thought to express ASAs and TSAs and are considered immunogenic tumors. A surrogate marker for the overall immunogenicity of a tumor is the presence of tumor infiltrating lymphocytes (TILs). The presence of TILs indicates that the tumor microenvironment is permissive for leukocyte trafficking and extravasation. Importantly, *ex vivo* cytotoxicity assays utilizing purified TILs demonstrates that in many cases TILs are tumor-antigen specific and have no intrinsic deficits in cell-mediated cytotoxic functions [20,21]. Since the objective of a cancer vaccine is to induce tumor-antigen specific T cell responses that are capable of killing tumor cells, we must ask ourselves whether patients with immunogenic tumors bearing large numbers of TILs can benefit from vaccination, or whether the presence of TILs should be taken as evidence of *vaccination in situ*. Thus, the rationale design of a state-of-the-art vaccine must now take into account recent data characterizing the interplay between a developing tumor and the immune system, and in particular the predicted differences in immune interactions between immunogenic and non-immunogenic tumors (Fig. 1).

A number of recent reviews have unfortunately generalized the failure of a number of vaccinations strategies, citing overall response rates of only 3.3% in trials of over a thousand patients, without emphasizing that 96% of the patients treated on these trials had a single type of cancer; *melanoma* [22]. The scientific