Hypothesis/Commentary

The Use of FoxP3 as a Biomarker and Prognostic Factor for Malignant Human Tumors

Taylor H. Schreiber
Sheila and David Fuente Program in Cancer Biology, University of Miami Miller School of Medicine, Miami, Florida

Abstract

Only since the early 21st century has it been proven that the immune system can actively defend the body against the development of malignant tumors. Escape from this process, termed immunosurveillance, has been shown to be required for the development of many tumors in both mice and humans, and may be a necessary prerequisite for the establishment of many malignancies. Serendipitously, an evolution in the understanding and characterization of immunosuppressor cells, regulatory T cells, has coincided with the establishment of tumor immunosurveillance. These two fields merged when it was found that the recruitment of regulatory T cells within tumors was a dominant mechanism tumors used to escape immunosurveillance. Regulatory T cells are specifically identified with antibodies which recognize the transcription factor, FoxP3. The presence of FoxP3+ cells within tumors has been shown to predict the prognosis, invasiveness, and metastatic ability of some tumors by modulating the ability of the immune system to target tumor cells. Furthermore, depletion of regulatory T cells from tumors could lead to the rejection of both early- and late-stage tumors by the host immune system. These findings suggest that the widespread use of FoxP3 as a biomarker should be explored for human tumors to enable physicians to make better decisions in oncologic care and to prepare the field for novel therapeutic agents directed at the elimination of regulatory T cells within tumors. (Cancer Epidemiol Biomarkers Prev 2007;16(10):1931–4)

Introduction

The field of tumor immunology traces its origins back to the early 20th century New York City surgeon, William B. Coley. Coley is credited with making the observation that some of his patients suffering from sarcomas would undergo tumor regression if they became bacteremic during the course of treatment. Later, he created a killed bacterial cocktail, named Coley’s toxin, which was capable of stimulating tumor rejection in a small number of patients. This evidence lay dormant until the 1950s, when Burnet and Thomas first proposed a theory of tumor immunosurveillance. This hypothesis suggested that the immune system contained natural mechanisms to recognize, contain, and destroy developing tumor cells throughout the life of an organism. However, Burnet and Thomas were unable to provide experimental evidence supporting this model. This evidence did not begin to appear until the late 1970s, when it was shown by Robert North and others that not only were T cells important for the rejection of established tumors but that other populations of suppressor T cells existed and were capable of inhibiting antitumor immunity (1-5). In the late 1980s and early 1990s, the laboratories of Hans Hengartner, Eckhard Podack, Mark Smyth, and others began to identify specific elements of the antitumor immune response involved in tumor cell killing (6-9). Finally in the late 1990s and early 21st century, Robert Schreiber and Lloyd Old were the first to provide direct evidence that not only does the immune system actively control developing malignancies, but that the development of a life-threatening tumor may be dependent on the immune escape of those tumor cells from immune recognition (10, 11).

To gain evidence in support of the hypothesis of tumor immunosurveillance, Schreiber’s laboratory generated tumors by chemical induction (3-methylycholanthrene) in both wild-type and RAG2−/− mice. RAG2−/− mice are deficient in all B and T cell responses but have a largely preserved innate immune system (neutrophils, macrophages, and natural killer cells). It was proposed that if immunosurveillance occurred in mice, the tumors that developed in wild-type mice (immunocompetent) would have developed mechanisms to either evade or suppress immune detection. The tumors that developed in RAG2−/− mice (immunocompromised) would by comparison be highly susceptible to immune surveillance in an immunocompetent mouse. The first critical finding was that 58% of RAG2−/− mice developed tumors compared with only 19% of wild-type mice following chemical induction. Once the immunocompetent and immunodeficient tumors were established in wild-type or RAG2−/− mice, respectively, cells from the
immunocompetent tumors were injected into immunocompromised mice and cells from the immunodeficient tumors were injected into immunocompetent mice. The immunocompetent tumor cells formed tumors equally in immunocompetent and immunocompromised mice. The immunodeficient tumor cells, however, failed to form tumors in 40% of immunocompetent mice despite forming tumors in 100% of immunocompromised mice (10). This set of experiments decisively showed that immunosurveillance occurred in immunocompetent mice and that it was capable of rejecting tumors that had developed in the absence of an intact immune system.

At around the same time period, a controversy that had been brewing for >20 years regarding the existence of immunosuppressive T cells drew to a close. In 1995, Shimon Sakaguchi reported that depletion of CD25-expressing CD4+ cells in mice leads to the development of various autoimmune diseases (12). This report gave new life to the notion that a population of circulating lymphocytes was involved in immunosuppression. It was not until the late 1990s that this idea was fully accepted by the scientific community (13), but the fund of knowledge about these regulatory T cells (Tregs) has since exploded. It is now well demonstrated that Tregs exist in various forms throughout the life cycle of mammals to control autoimmune disease by preventing the expansion of self-reactive T cell clones that escape negative selection in the thymus (14). Early detection of Tregs depended on the coexpression of the surface markers CD4 and CD25. In a noninflamed state, ~10% of the circulating CD4+ cells are CD25+ and ~1% of the CD8+ cells are CD25+. Of the CD4/CD25 double-positive cells, a variable majority exhibit regulatory activity (12). Thus, it was necessary to find a better marker for Tregs. This marker came in 2003 when the laboratories of Shimon Sakaguchi and Alexander Rudensky discovered that the forkhead transcription factor FoxP3 is required for regulatory T cell function and that it is found almost exclusively within Tregs following intracellular staining (15, 16).

Shortly after proving the existence of tumor immunosurveillance, Schreiber’s lab and others (17) began to study the mechanisms involved in both immune-mediated tumor clearance and in tumor-mediated immune escape. There are several potential mechanisms that tumors could use to escape from immune recognition. Initially, it was thought that among a heterogeneous population of tumor cells, some exist that express molecules which are immunogenic and will thus be recognized by the immune system. Among this heterogeneous population, there would also be some clones that are not recognized by the immune system and could eventually be selected for following immune-mediated clearance of the immunogenic cells. A second hypothesis could be that tumors develop a program of cytokine and chemokine expression that prohibits the entry of the immunosurveilling cells into the tumor site. A third hypothesis suggests that tumors may develop the ability to actively suppress immune surveillance and tumor cell killing by hijacking circulating immunosuppressor cells. Serendipitously, support for tumor immunosurveillance and the discovery of Tregs occurred simultaneously. Thus, Schreiber’s lab looked into the existing tumor models with immunocompetent and immunocompro-mised tumors to determine whether they harbored cells with suppressive activity. In these studies, it was shown that in the chemically induced sarcomas, a higher proportion of Tregs to total CD4+ cells were found in the immunocompetent tumors (18). Causality was later shown in experiments in which immunocompetent tumors were rejected in mice that had been depleted of Tregs by pretreatment with a CD25-specific antibody.

The studies mentioned above suggest that the presence of Tregs within tumors might be a necessary precedent to the formation of a morbid or metastatic malignancy. Since the work of Bui et al. demonstrating that accumulation of Tregs within tumors confers a dominant mechanism for immune escape (18), a multitude of human tumors have been found to exhibit similar characteristics. In cervical cancer, higher proportions of Tregs are found within metastatic lymph nodes compared with nonmetastatic lymph nodes (19), and are also implicated in the progression from cervical intraepithelial neoplasia (20). In colorectal cancer, higher proportions of FoxP3+ cells are found in the peripheral blood and within the tumor than in surrounding tissues (21). In hepatocellular carcinoma, high proportions of Tregs within the liver are thought to precede the development of tumorigenesis and are associated with an unfavorable prognosis (22-24). Aggressive forms of adult T cell leukemia/lymphoma have been shown to up-regulate FoxP3 within the tumor cells themselves, conferring the ability of the tumor to actively suppress other lymphocytes (25). Bladder carcinomas have been found to be dominated by immunosuppressive regulatory T cells (26). High-risk breast cancers, especially breast cancers at risk for recurrence, recruit high numbers of Tregs, suggesting a correlation with disease prognosis (27). A recent study showed that, in a wide variety of atypical nevi and melanoma lesions in humans, there is an unusually high proportion of Tregs, suggesting that Treg-mediated immunosuppression may be occurring during the progression of many cutaneous malignancies (28). In addition to these tumors, high proportions of regulatory T cells within non–small cell lung cancer including lung adenocarcinoma, mesothelioma, pancreatic carcinoma, and prostate cancer have all been found to correlate with tumor invasiveness and metastatic potential (29-34). The fact that an imbalance between immunosuppressive cells and immune effector cells has been observed in so many different tumors within <4 years since the FoxP3 marker was first identified suggests that high proportions of Tregs will continue to be found in other tumors, and may be found to correlate with prognosis and metastatic potential. Importantly, in follicular lymphoma, it is has been shown that the presence of Tregs is associated with the control of tumor growth (35-37).

Due to the prevalence of Tregs within different tumors, combined with evidence suggesting that the presence of Tregs correlates with metastasis and overall disease prognosis, efforts must be made to investigate the utility of FoxP3 staining of tumor biopsies in the clinic. Oncologists rely on evidence-based medicine for proper clinical decision-making. These practices are based on what the likely clinical outcome may be for any individual cancer patient. Currently, prognosis is determined from demographic and clinical variables, which
guide the choice of treatment option. For many human tumors, including hepatocellular carcinoma, the absence of large-scale randomized trials leaves treatment strategies at the discretion of treatment centers based on local physician experience and retrospective studies (38). The lack of large-scale randomized trials is particularly true for the use of newer immunotherapeutic agents. The growing knowledge of FoxP3 correlating it with cancer recurrence, metastasis, and prognosis suggests that knowledge of this marker within tumors may better guide oncologic decision-making in primary, neoadjuvant, and adjuvant therapy.

The most intriguing conclusions that can be drawn from the observations that Treg are prevalent within such a wide variety of tumors are that these tumors may require the presence of suppressor cells for both development and maintenance. Because FoxP3 is a transcription factor, it is not amenable to targeting by antibodies in vivo. Thus, several investigators have studied the effects of depleting Tregs with the use of anti-CD25 monoclonal antibodies. This approach has been used in murine tumor models with some success both as an adjuvant (39, 40) and a primary therapy (18). Although these therapies have shown success in eliminating residual disease and prolonging disease-free intervals, therapy with anti-CD25 is not optimal for clinical use for several reasons. First, CD25 is expressed on immune effector cells, which have a significant role in the proposed mechanism of tumor clearance. Therefore, by depleting Tregs with CD25, there is also the unwanted side effect of also depleting the host of the cells that are essential for the development of a strong antitumor immune response. Secondly, because CD25+ effector cells are critical for the normal function of the immune system, there is the potential for opportunistic infections to thrive during this kind of treatment.

In a recent study by Nair et al., a new strategy for Treg depletion was accomplished by vaccination against FoxP3. This approach is counterintuitive for several reasons. First, FoxP3 is a “self” protein that is normally expressed throughout the life cycle of an organism and therefore should not be immunogenic. The authors proposed however that because FoxP3 resides within the nucleus, the presence of high levels of FoxP3 presented by dendritic cells may be capable of stimulating an immune response. The second reason that this approach is unorthodox is because of the necessity for Treg to prevent lethal autoimmune disease independent of the presence of a tumor. Vaccination with FoxP3 carries the potential of completely depleting Tregs and certainly leading to autoimmune disease. Surprisingly, these authors showed that vaccination with FoxP3 led to a robust FoxP3-specific CTL response that was specific to the tumor microenvironment and that did not affect circulating CD25+ helper cells (41). These data provide exciting new insights into tumor-specific vaccination and Treg-specific depletion strategies.

The widespread utility of most disease biomarkers is based on a combination of the sensitivity and specificity of the marker and the prevalence of the disease being tested. Biomarkers that are highly disease-specific are often not used as diagnostic tools if the prevalence of the disease they identify is very low. Over the past decade, a new class of biomarkers has been identified that aid physicians both by identifying specific disease and by guiding treatment. The prototype for this kind of biomarker is ErbB-2 (HER2/neu). Despite the low frequency of breast masses that are malignant and despite the fact that only 25% to 30% of breast malignancies express ErbB-2, it is commonly used by pathologists in breast tissue biopsies. The reason that this marker is commonly used is because it’s identification within a breast tumor guides the course of treatment for the patient. Identification of FoxP3 within tumors could have a similar effect on both prognosis and treatment, and it is likely that pathologic levels of FoxP3 expression will be found on a larger percentage of malignancies than ErbB-2.

Mounting evidence suggests that the recruitment of regulatory T cells into both the primary tumor and to sites of micrometastasis is a critical event in the development of many malignancies. Immunohistochemical staining of the transcription factor FoxP3 can be used to specifically identify Tregs that have infiltrated a tumor specimen. Because the presence of Tregs within many murine and human tumors has been shown to correlate with the prognosis and/or invasiveness of a tumor, it is imperative that clinical protocols are developed to establish guidelines for the use of FoxP3 as a biomarker of malignant potential. Furthermore, the presence of Tregs in both primary and metastatic tumors suggest that active immunosuppression is required by many tumors for establishment and progression. This suggests that the elimination of Treg by targeting FoxP3, from tumors could be enough to allow the immune system to generate an effective antitumor cytotoxic response. The generation of compounds that specifically eliminate Tregs, or inhibit their function will continue to occur and become better defined in mouse models. If clinical best practices are established that identify clinically meaningful levels of FoxP3 expression that predict the invasive potential of certain tumors now, it will expedite the approval of these compounds as a new class of anticancer agents in the near future.

References


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