Cyclin B1 and Other Cyclins as Tumor Antigens in Immunosurveillance and Immunotherapy of Cancer

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Abstract
Uncontrolled cell division is an indispensable event in tumor progression, and numerous molecules involved in this process have been the focus of intense investigation in tumor biology. Cyclins, molecules that orchestrate normal cell cycle progression, are abnormally overexpressed in various human cancers. We review evidence that the immune system recognizes some abnormally expressed cyclins as tumor antigens, such as cyclin B1, and we analyze the potential of cyclins D, E, and A to serve a similar function in cancer immunosurveillance. (Cancer Res 2006; 66(1): 6-9)

Introduction
The immune system has evolved to protect individual organisms from external pathogens as well as from internal changes that might threaten the integrity of the organism. One such threat is cancer, and immune responses to the altered self employ both the innate and the adaptive arms of the immune system. Examples of tumor-specific alterations include changes in protein sequence and/or protein quantity, as seen during activation of proto-oncogenes or overexpression of nonfunctional tumor suppressor genes (e.g., Her-2/neu and p53, respectively). Such changes are found in virtually all tumors and have been used increasingly by pathologists to assess tissue biopsies for cancer diagnosis and prognosis. Immunologists have also learned that many mutated or overexpressed proteins are processed and presented to the immune system as tumor antigens that can trigger both humoral and cellular immune responses (1). This review is focused on the tumor-associated changes in the expression of one family of cell cycle regulatory proteins, the cyclins, and the potential of some of these proteins to serve as stimulators of antitumor immunity and targets of immunotherapy.

Unlike normal cells that turn on transient expression of minute amounts of cyclin proteins at specific points in the cell cycle, many tumors have high constitutive levels of one or more cyclins. In normal and tumor cells, cyclins are ubiquitinated for immediate degradation by proteasomes into peptides. This same degradation pathway is used for processing and presentation of antigens to the immune system. It would thus be expected that immunosurveillance of cyclin peptides presented by normal cells, where they appear transiently and probably below the threshold of immune detection, would differ significantly from those presented by cancer cells, where the peptides are constitutively present at high levels, presumably reaching the threshold for immune recognition.

We and others have found that at least one member of the cyclin family, cyclin B1, plays an important role as a tumor antigen. Cyclin B1 is recognized by the immune system early in cancer development, and both antibodies and T cells are generated in response to its aberrant expression. In this review, we use our knowledge of cyclin B1 regulation and expression in cancer and its documented tumor antigen potential as a template to assess the potential of other aberrantly expressed cyclins to be recognized by the immune system. Because cyclins are expected to be deregulated early in disease, we propose that monitoring immune responses against cyclins that are recognized as tumor antigens can be an important approach to early cancer detection (2). Furthermore, because cyclin deregulation is associated with increased tumor malignancy (3), we suggest that eliciting or boosting immunity to tumor cyclins through vaccines or other immunotherapy approaches could lead to the elimination of the most malignant cancer cells and thus to a more favorable disease outcome.

Cyclin B1, the Prototype Cyclin Tumor Antigen
In 1997, it was reported that patients with hepatocellular carcinoma generate antibodies to cyclin B1, whereas such antibodies were not found in the healthy control subjects (4). Subsequently, in our efforts to identify new tumor antigens, we found that a peptide fraction eluted from breast adenocarcinoma MHC class I molecules was able to stimulate tumor-specific cytotoxic T cells from breast and head and neck cancer patients (5). Sequencing of the peptides in the immunostimulatory fraction identified the peptides as having been derived from cyclin B1 (5). Antibody responses against cyclin B1 have since been reported by us and others in patients with prostate, breast, colorectal, lung, and hepatocellular cancers (6, 7). In recursive partitioning analyses assessing antibody levels against a panel of tumor antigens as a method to differentiate subjects with cancer from those without, the presence of anti-cyclin B1 antibodies was an important discriminator for subjects with the aforementioned cancers (6). Importantly for early cancer detection and intervention, we also found that in lung cancer, early transformation events led to aberrant expression of cyclin B1 in premalignant lung lesions, and this correlated with the presence of T cell–dependent antibody responses (7). Under normal conditions, cyclin B1 is expressed at very low levels and accumulates appreciably only at the G2-M cell cycle transition, concurrent with its scheduled translocation to the nucleus (8). In cancer cells, cyclin B1 is found overexpressed throughout the cell cycle and resides primarily in the cytoplasm. This alteration in the regulation of cyclin B1 expression was found by us to be the result of inactivation of the tumor suppressor
protein p53 function (9). Our finding that cyclin B1–derived peptides can be eluted from MHC class I molecules on tumor cells shows that this constitutively overexpressed protein is naturally processed into peptides that bind MHC class I molecules and stimulate cytotoxic T cells. Furthermore, the finding of T helper cell–dependent anti-cyclin B1 antibodies (IgG and IgA) in cancer patients shows that the overexpressed protein can activate B cells and can also be taken up by antigen-presenting cells and processed and presented to helper T cells (see Fig. 1).

Other Cyclins as Tumor Antigens

In the ongoing effort to discover tumor antigens, the immunogenicity of overexpressed and aberrantly localized normal cellular components is emerging as a common theme. Some notable examples of overexpressed normal cellular proteins are the tumor antigens MUC1 (10), HER-2/neu (11), and p53 (12).

A, D, and E type cyclins all have been shown to be overexpressed in many cancers, where their overexpression has been associated frequently with poor clinical outcome. Like cyclin B1, these cyclins undergo ubiquitin-mediated proteolysis and could, therefore, be available as whole proteins as well as processed peptides for presentation to the immune system. However, whether or not these other peptides can effectively stimulate the immune response will depend on the precise molecular mechanisms that underlie the aberrant expression of each of the individual cyclins and on the extent of quantitative and qualitative differences in their expression between normal and cancer cells. Thus, some cyclins may be anticipated to be more immunogenic or better immunotherapy targets than others. Using the criteria we and others have determined to be important for cyclin B1 immunogenicity and tumor specificity, it is possible to examine alterations in the expression and localization of cyclins D, E, and A in cancer as a way to evaluate their potential to alter antigen loading at the cancer cell surface or provide increased levels of protein for cross-presentation by antigen-presenting cells.

**D type cyclins.** Cyclin D1 is the most commonly reported overexpressed cyclin in cancer and the best characterized of the

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**Figure 1.** Establishment of an immune response to cyclin B1. **Top,** the priming phase. At the tumor site, overexpressed cyclin B1 is released by cancer cells, endocytosed by immature dendritic cells (DC) and transported to the tumor draining lymph node. In the lymph node, the mature dendritic cell process and cross-present cyclin B1–derived peptides to naive T cells. This leads to the activation and expansion of helper (CD4⁺) and cytotoxic (CD8⁺) cyclin B1–specific T cells. Soluble cyclin B1 protein is also drained to the lymph node, where it stimulates naive B cells and primes them to receive help from cyclin B1–specific T cells in the production of anti-cyclin B1 antibody. **Bottom,** the effector phase. The lytic function of cyclin B1–specific cytotoxic (CD8⁺) T cells is restricted to cancer cells overexpressing cyclin B1. Activated cyclin B1–specific CD8⁺ T cells migrate from the lymph node to the tumor site, where they recognize and kill the cancer cells that present a high density of CB1 peptides. Normal cells that express transient, low levels of cyclin B1 do not provide sufficient peptide density to trigger the T-cell function.
D type cyclins (D1, D2, and D3). Cyclin D1 overexpression has often been found to correlate with worse disease prognosis (13). As in the case of cyclin B1, cytoplasmic localization of aberrantly expressed cyclin D1 protein has been noted in non–small cell lung cancer. However, in the majority of tumors, overexpressed cyclin D1 protein resides primarily in the nucleus (13). Processing and presentation of antigens overexpressed in the nucleus have not been studied extensively; thus, the effect of cyclin D1 nuclear overexpression on peptide loading into MHC class I and consequent increase in its tumor antigen potential will have to be specifically determined. Studies to date exploring the potential of cyclin D1 to stimulate immune responses include one report that 16% of patients with high-grade prostate cancer make T cell–dependent anti-cyclin D1 antibodies (14). Given the frequently documented cyclin D1 overexpression in cancer, additional studies of the immune response in cancer patients to this potential new tumor antigen are warranted.

E type cyclins. Along with cyclin D1, the cyclins E1 and E2 direct and amplify the transition of cells from G1 phase into S phase of the cell cycle. Cyclin E1 has been more extensively studied and has been frequently found to be deregulated in tumor cells. As in the case of cyclins B1 and D1, deregulation of cyclin E1 has been reported to be associated with worse prognosis in lung and breast cancers (reviewed in ref. 15). Multiple mechanisms underlying increased expression of cyclin E1 are possible, including gene amplification, disruption of Rb transcription regulatory pathways, and errors in ubiquitination and degradation processes (16). Furthermore, there are low molecular weight forms of cyclin E that are produced at high levels only in cancers and can serve as a source of antigenic peptides (17). Cyclin E appearance is normally tightly restricted to mid-G1 phase to S phase of the cell cycle. In contrast, in several bladder cancer cell lines, cyclin E has been found to be present throughout the cell cycle (18). This transition to unscheduled expression suggests the possibility of higher levels of cell surface presentation of cyclin E–derived peptides due to increased accessibility to antigen processing machinery. However, aberrantly expressed cyclin E is constitutively localized to the nucleoli rather than the cytoplasm; thus, it is postulated to be retained and not degraded (18). If the major mechanism for this aberrant expression is a lack of degradation, then cyclin E is unlikely to be seen by the immune system as a tumor antigen. The accumulated protein can still be released by tumor cells and taken up by antigen-presenting cells and B cells to stimulate helper T cells and production of anti-cyclin E antibodies. However, in the only published study to date, that has evaluated the presence of antibodies to cyclin E in sera from cancer patients, none of the patients tested had anti-cyclin E antibodies (4).

A type cyclins. Cyclins A1 and A2 activate cyclin-dependent kinase 2 (cdk2) and cdk1 and function to promote transit through S phase and into mitosis. Cyclin A1, the expression of which is confined mainly to hematopoietic progenitor cells and male germ cells, has been found to be highly overexpressed in acute myeloid leukemia (AML) and testicular cancer (15). AML patients whose tumors had high levels of cyclin A1 have significantly lower survival than those with low levels of cyclin A1 (19). In normal hematopoietic cells, cyclin A1 is predominantly nuclear; however, in leukemic cell lines and tumor cells from AML patients, cyclin A1 is predominantly cytoplasmic (20). Overexpression of cyclin A2, which is expressed in all dividing somatic cells, has also been associated with poor prognosis in several cancers (15). Thus far, nothing much is known about immune responses to A type cyclins in patients with AML or other tumors of hematopoietic origin. However, two recent reports describe antibodies to cyclin A2 in sera of patients with solid tumors (4, 14). Although there is relatively limited information on cyclin A, its alteration in cancers similar to cyclin B1 should prompt further attention concerning cyclin A as a potentially important tumor antigen.

Summary

The importance of altered cyclin function in malignant transformation has long been appreciated. More recently, the consequences of aberrantly expressed cyclins on tumor behavior are beginning to be understood and tumor cyclin status is increasingly recognized as an important cancer prognostic indicator. Immune responses that target specific tumor antigens can also be important determinants of cancer and premalignant disease course. We have summarized evidence to date regarding cyclin B1 as a tumor antigen and reviewed what is known about aberrant expression of cyclins D, E, and A that might affect their abilities to stimulate the immune system. We conclude that in addition to cyclin B1, which is increasingly being recognized as an important player in the immune control of tumor growth, cyclins A and D have the potential to be targets of immune surveillance and hold promise as candidate targets for cancer immunotherapy.

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References


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