

CD73: A Novel Target for Cancer Immunotherapy

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Abstract

The promise of cancer immunotherapy has not been translated into clinical successes, in large part because of tumor-associated immune suppression that blocks effective antitumor immunity. Recent findings show a tumor-induced immunosuppressive mechanism, whereby tumor-derived CD73 functions as an ecto-enzyme to produce extracellular adenosine, which promotes tumor growth by limiting antitumor T-cell immunity via adenosine receptor signaling. Results with small molecule inhibitors, or monoclonal antibodies targeting CD73 in murine tumor models, suggest that targeted CD73 therapy is an important alternative and realistic approach to effective control of tumor growth. In particular, it helps T-cell-based therapy by enhancing the adaptive immune response machinery, which may increase the function of tumor-infiltrating T lymphocytes, and subsequently lead to improved survival in cancer patients. *Cancer Res*; 70(16); 6407–11. ©2010 AACR.

Cancer immunotherapy by endogenous or adoptively transferred antitumor T cells is complementary to conventional therapies, including surgery, radiotherapy, or chemotherapy. However, a number of obstacles hinder the generation of effective antitumor T-cell immunity. During tumor progression, tumor cells foster a tolerant microenvironment and activate a plethora of immunosuppressive mechanisms, which may act in concert to counter effective immune responses (1). The focus of this review is to discuss the newly available experimental evidence showing that CD73 on tumor cells impairs antitumor T-cell responses, strongly supporting and extending the concept that extracellular adenosine and the A2A adenosine receptor (A2AR) comprise a pivotal axis in tumor immune escape (2–5). Importantly, these data point out a potential and realistic strategy of targeting CD73 for cancer treatment in addition to, or instead of, the A2AR.

Tumor CD73 Controls Cancer Progression

CD73, known as ecto-5'-nucleotidase (ecto-5'-NT, EC 3.1.3.5) is a glycosyl-phosphatidylinositol (GPI)-linked 70-kDa cell surface enzyme found in most tissues (6, 7). CD73, originally defined as a lymphocyte differentiation antigen, is thought to function as a cosignaling molecule on T lymphocytes, and as an adhesion molecule that is important for lymphocyte binding to endothelium. Recent studies have implicated CD73 in the control of a variety of physiologic

responses, including epithelial ion and fluid transport, ischemic preconditioning, tissue injury, platelet function, hypoxia, and vascular leak (6–8). However, the role of CD73 in cancer remains unclear. Previous studies reported that CD73 participates in cell-cell and cell-matrix interactions and implicated CD73 in drug resistance and tumor promotion (9). Using flow cytometry, we have shown that CD73 is widely expressed on many tumor cell lines and is upregulated in cancerous tissues (10). In agreement, genetic data indicate that CD73 is upregulated in various human carcinomas, including those of colon, lung, pancreas, and ovary. Importantly, higher expression levels of CD73 are associated with tumor neovascularization, invasiveness, and metastasis, and with shorter patient survival time in breast cancer (9). Moreover, recent studies confirm that CD73 promotes invasion, migration, and adhesion of human breast cancer cells (11). Upregulated expression of CD73 has been found in highly invasive human melanoma cell lines, but not in melanocytes or in primary tumor cells. The participation of CD73 *per se* as a proliferative factor involved in the control of glioma cell growth was described. Clinically, the strong expression of CD73 in papillary thyroid carcinomas has been suggested as a diagnostic aid in the differential diagnosis of thyroid tumors. Meanwhile, upregulated CD73 ecto-enzymatic activity was proposed to have prognostic value for colon cancer patients. Thus, the increase in CD73 activity during tumor development might be a physiologic attempt by cancer cells to provide more substrate to accelerate purine salvage pathway activity. These results suggest that CD73 is an important player in controlling tumor progression (9).

Extracellular Adenosine Generated by Tumor CD73 Accumulates in the Tumor Microenvironment

Elevated levels of extracellular adenosine within the mouse tumor microenvironment have recently been described

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(2, 12). Several sources of extracellular adenosine may be passively or actively released into the tumor microenvironment. Extracellular adenosine is likely produced either owing to passive diffusion or active transport of intracellular adenosine or because of enzymatic hydrolysis of extracellular ATP (4, 5). Local tissue hypoxia that follows damage to endothelial cells and microcirculation and the interruption of normal blood and oxygen supply is associated with an increase in intracellular AMP, accumulation of intracellular adenosine, and subsequent transport or diffusion of intracellular adenosine into the extracellular space. Indeed, passive efflux of adenosine is observed in tissue injury, necrosis, and ischemia, and, therefore, may be a significant source of this extracellular nucleoside in solid tumors (4, 9), although this specific mechanism has yet to be clearly described. Because the amount of released adenosine depends on the extent and severity of ischemia and/or necrosis in tumor tissues, this mechanism may not contribute greatly to extracellular adenosine generation.

Biological actions of CD73 (ecto-5'-NT) are mainly a consequence of the regulated enzymatic phosphohydrolytic activity on extracellular nucleotides. This ecto-enzymatic cascade in tandem with CD39 (ecto-ATPase) generates adenosine from ATP, which in turn activates adenosine receptors. In contrast to the intracellular generation of adenosine from cytosolic pools of adenine nucleotides catalyzed by cytosolic 5'-NT in the heart, production of extracellular adenosine by CD73 is likely the predominant means of adenosine generation in epithelial cells, despite depending tightly on the availability of extracellular AMP. We have shown that mouse and human epithelial tumor cells express CD39 and CD73.¹ Importantly, CD73 is significantly upregulated in cancerous tissues accompanied by high enzymatic activity, which can mediate the production of extracellular adenosine (10). Therefore, our results clearly support the concept that tumor cells themselves contribute to the elevated levels of adenosine in the tumor microenvironment through surface CD73 enzymatic activity. We assume that high levels of tumor CD73 expression are likely induced in the local tumor microenvironment. This concept is supported by our demonstration of CD73 upregulation on cultured ovarian cancer cells treated *in vitro* with malignant ascites, suggesting that soluble factors (presumably proinflammatory cytokines) in the tumor induce CD73 expression (10). However, reports in the literature about the regulation of CD73 expression by proinflammatory cytokines are conflicting. We do not know whether these inflammatory cytokines affect CD73 on cancer cells, but a hypoxia inducible factor-1 α (HIF-1 α)-dependent regulatory pathway for CD73 on epithelial cells has been recently recognized (13). Thus, the present evidence supports the concept that hypoxia, HIF-1 α , and CD73 in sequential order convert AMP to adenosine, leading to elevated levels of extracellular adenosine in the tumor (4, 10).

¹ B. Zhang, unpublished data.

Because the constant release of adenosine is counteracted by uptake followed by metabolism by either adenosine kinase (AK; phosphorylation to form AMP), or by adenosine deaminase (ADA; deamination to inosine), it is more relevant to examine the ratio of the adenosine-producing activity of CD73 to the combined adenosine-using activities of ADA and AK. In humans (but not in rodents), ADA can be found on the cell surface bound to CD26 in addition to its normal cytoplasmic location. Recently, a number of examples showed a significant correlation between increased expression of CD73 and/or decreased expression of ADA and cancer progression (9). Moreover, HIF-1 α can repress intracellular AK as well as upregulate CD73 (4). Therefore, it appears that these four enzymatic activities (CD39-CD73, ADA, and AK) collaborate to maintain high adenosine concentrations in solid tumors, but further work is needed for formal demonstration.

Extracellular Adenosine Generated by Tumor CD73 Impairs Antitumor T-Cell Responses

Adenosine potently inhibits a series of T-cell responses, including antigen-induced proliferation; secretion of interleukin-2 (IL-2) and proinflammatory cytokines such as interferon- γ and tumor necrosis factor- α (TNF- α); upregulation of CD25; induction of cytolytic effector molecules such as perforin and Fas ligand; adhesion of killer lymphocytes to tumor cells; and granule exocytosis by cytotoxic T lymphocytes (CTL; ref. 14). In addition, adenosine and adenosine analogues can suppress natural killer (NK) cell as well as lymphokine-activated killer (LAK) cell function (14). Thus, given the strong immunosuppressive properties of adenosine, and its presumed high concentration in solid tumors, it is reasonable to infer that adenosine may constitute an important part of the so-called "immunologic barrier," leading to a failure of mounting an effective antitumor immune response. Because many tumor cells express functional CD73, we examined the regulatory role of tumor CD73 in antitumor T-cell immunity. We found that extracellular adenosine generated by tumor CD73 *in vitro* and *in vivo* inhibited both the activation phase and effector phase of the antitumor T-cell response and promoted T-cell apoptosis. Moreover, knockdown of CD73 on tumor cells by small interfering RNA (siRNA) completely restored efficacy of adoptive T-cell therapy and led to long-term tumor-free survival of tumor-bearing mice, suggesting that tumor CD73-mediated immune suppression, through its enzymatic activity, significantly contributes to cancer immune evasion (10). Consistent with our finding, another group simultaneously showed that inhibition of CD73 by an anti-CD73 monoclonal antibody (mAb) triggered adaptive antitumor immunity and inhibited breast tumor growth and metastasis (15). We each concluded that CD73 can serve as a novel target for cancer treatment to improve antitumor immunity (10, 15).

Extensive mouse and human studies using adenosine receptor subtype-selective agonists and antagonists indicate

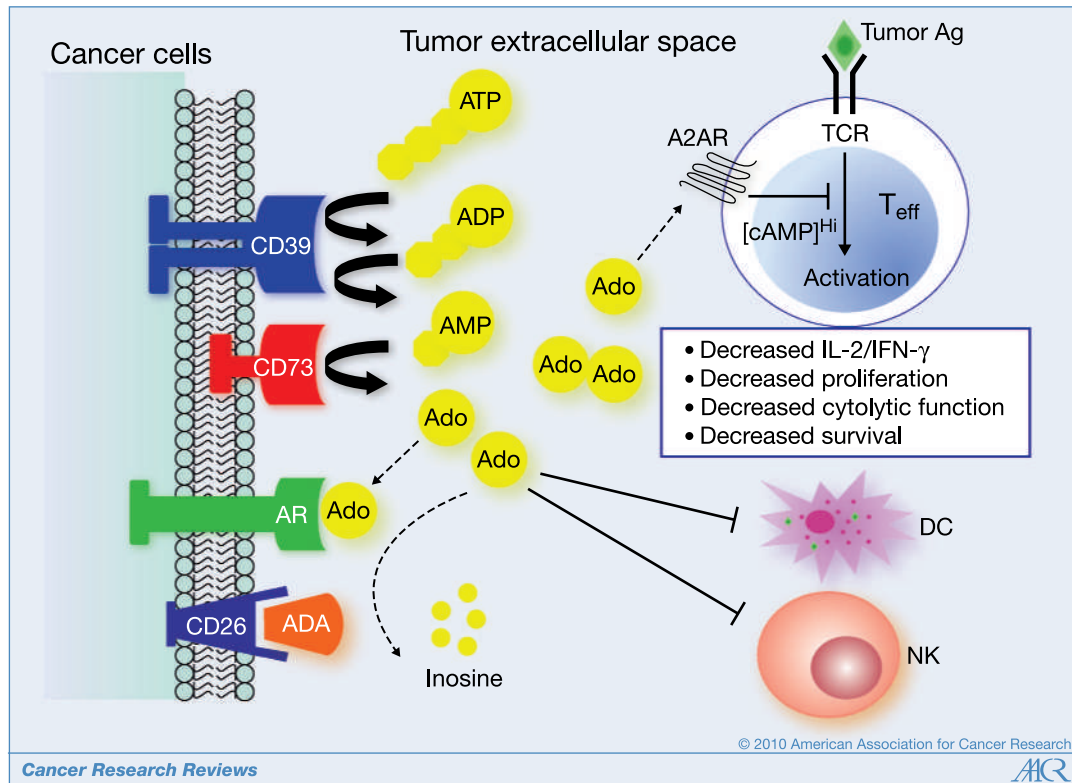


Figure 1. Schema of extracellular adenosine metabolism in tumor-induced immune suppression. The sequential action of CD39 and CD73, both of which can be induced by tumor hypoxia to degrade ATP, represents a primary pathway of extracellular adenosine generation in the tumor microenvironment. It is very likely that coordinated expression and activity of CD39–CD73 (increased) and ecto-adenosine deaminase ADA (decreased), which in human cells can be bound to the cell surface by CD26, contribute to the elevated levels of extracellular adenosine during tumor progression. The exact roles of CD39–CD73 on the nonmalignant host cells (e.g., Treg) in the tumor microenvironment remain unclear. High concentrations of adenosine suppress antitumor T-cell activation, survival, and effector function through A2AR engagement. Meanwhile, adenosine may trigger certain adenosine receptors (AR) on cancer cells to promote tumor cell chemotaxis and metastasis. In addition, adenosine can negatively modulate differentiation and function of dendritic cells (DC), and further inhibit NK cell proliferation and cytolytic function.

that adenosine inhibits T-cell activation and effector activity, primarily via the A2AR. We showed that blockade of the A2AR with a selective antagonist augmented the efficacy of adoptive T-cell anticancer therapy (10), which is compatible with a previous ground-breaking study showing that endogenous or adoptively transferred antitumor T cells were much more resistant to inhibition in the tumor microenvironment by either genetic ablation of the A2AR or A2AR antagonist treatment (2). It has been shown that A2AR engagement suppresses T-cell proliferation, inflammatory cytokine secretion, and reduces surface expression of cytokine receptors by elevating the intracellular levels of cyclic (c)AMP through adenylyl cyclase stimulation (5, 16). Because A2BR (2) and A3 (17) adenosine receptors are also involved in T-cell activity, further studies are needed to determine the exact contribution of A2BR and A3R to the function of antitumor T cells (2).

We and others found that blockade of CD73 had no impact on primary tumor growth in T-cell-deficient mice (10, 15), suggesting that CD73 may enhance tumor growth in a T-cell-dependent manner, but independently of any

effect on NK cells. This finding is further supported by the finding that depletion of NK cells using anti-asialo-GM1 antibody did not affect tumor growth in T-cell-deficient mice (15). However, we cannot exclude the possibility that extracellular adenosine in the tumor modulates NK cell function, because several studies have reported that adenosine and adenosine analogues suppress NK-cell proliferation and killing activity (14).

Implications and Future Directions

One of the most critical questions is to determine whether adenosine is a mere passive product of necrosis and ischemia in solid tumors, or if it is actively released during tumor progression as a result of the activity of adenosine-generating enzymes. It is now evident that extracellular adenosine produced through the activity of the ecto-enzymes (CD39 and CD73) on tumor cells can sufficiently downregulate antitumor immunity (Fig. 1). We have shown that adenosine generated by tumor CD73 impairs cellular antitumor immune responses at

multiple levels, including T-cell activation, clonal expansion of tumor-specific T cells with helper and cytolytic effector function, tumor cell killing by CTL, and survival of CTL. This result suggests a tumor-“autonomous” role of CD73 in tumor immune escape via generation of adenosine, which is sufficient to inhibit antitumor T cells, and mimics the paracrine secretion of adenosine previously described for regulatory T cells (Treg; ref. 18). Thus, targeting the enzymatic activity of tumor CD73 appears to be therapeutic for the tumor-bearing host. Indeed, it is impossible to target adenosine directly *in vivo*, because adenosine has a short half-life (<10 seconds) and is vital to preserve normal tissue functions. Importantly, we have not noticed any signs of autoimmunity in mice treated by tumor-antigen-specific T cells and tumor CD73 knockdown (10). Our data further support the recent view, stated by Ohta and colleagues (2), that targeting the adenosine-A2AR pathway is a cancer immunotherapy strategy to prevent inhibition of antitumor T cells in the tumor microenvironment. We expect that blocking A2AR signals on T cells and targeting CD73 on tumor cells could improve therapeutic efficacy beyond that achievable with either alone. Interestingly, we also found that cancer cells express both CD39 and CD73, indicating that extracellular adenosine derived from ADP and/or ATP is generated most likely through the combined action of CD39 and CD73. ATP has recently been reported as an important immunologic danger signal that recruits antigen-presenting cells and promotes the maturation of dendritic cells in the tumor (19). Consequently, antitumoral immune responses could be substantially improved by the inhibition of tumor CD39. Whether additional benefits would be obtained by inhibiting the expression of both CD39 and CD73 remains to be explored.

CD73-directed therapies have not been well developed. The CD73 inhibitor α,β -methylene ADP (APCP) has been successfully tested in our tumor models (10). Moreover, others have documented use of APCP in various murine models (7). The drug APCP functions as an inhibitor of CD73 because it is a nonhydrolyzable structural mimic of ADP. It has the attraction of being cheap, widely available, and well tolerated *in vivo*. However, at levels achievable *in vivo*, it might not fully inhibit the enzymatic activity of CD73 as its half-life *in vivo* and biodistribution are not well characterized. Thus, tumor regression with APCP could be incomplete compared with that achieved by genetic deletion of CD73, which accomplishes nearly 100% reduction of CD73 enzyme activity for 100% of the time. Furthermore, undesirable side effects could occur when APCP is administered intensively and frequently. In view of these considerations, it would be interesting to design and synthesize a new generation of CD73 enzyme inhibitors with the ability to inhibit enzyme activity irreversibly, and with increased stability *in vivo* by structural optimization to achieve stronger antitumor effects. Although the anti-CD73 mAb (TY/23) is less effective in inhibiting CD73 enzymatic activity than APCP, its effects in cancer treatment have been shown (15). Moreover, the effects of an anti-CD73 mAb may extend be-

yond inhibition of CD73 enzymatic activity. For instance, anti-CD73 mAb may directly inhibit the adhesion of tumor cells to endothelial cells, thereby inhibiting their invasiveness. It should be noted that the above observed antitumor effects of APCP or anti-CD73 mAb may or may not be attributed only to the inhibition of CD73 enzyme activity on tumor cells. It appears that the generation of extracellular adenosine by CD73 can protect tumors in both a tumor-autonomous way and by inhibiting incoming antitumor T cells by host Treg. Indeed, it was found that CD73 is overexpressed on Treg cells (20), and the CD39-CD73 tandem suppresses T-cell function (18). In this regard, we have evidence that host CD39-CD73 forms an important part of the immunosuppressive apparatus of Treg and the endothelial barrier to antitumor T-cell homing to tumors.² Moreover, it has been recently shown that human tumor-associated Treg cells, highly expressing CD39, suppressed Th17 cell development through the adenosinergic pathway (21), which could be a previously unappreciated mechanism by which tumors evade the immune system (22). Therefore, the actual *in vivo* effects of a therapeutic approach that uses CD39-CD73 as a molecular target for cancer treatment may far exceed the expectations raised by our published experimental data.

Of note, the success of the proposed strategy is extremely dependent on the existence of ongoing antitumor T-cell immunity. If antitumor T cells are not present, the ablation of CD73 likely will have a minimal effect on tumor regression, except in situations when it can interfere with CD73-mediated tumor cell adhesion and/or chemotaxis (15). Because endogenous antitumor immunity that can be induced is often insufficient and transient, targeted CD73 therapy may be most effective combined with other forms of immunotherapies, such as adoptive T-cell transfer, immune-stimulating mAbs, or dendritic cell vaccines. Indeed, we show that tumor-bearing mice can be cured by combinatory treatment of CD73 inhibition and T-cell therapy (10). In addition, targeting CD73 may have benefits other than enhancing adaptive antitumor immunity, and could be effective even for the treatment of nonimmunogenic or weakly immunogenic tumors. For instance, an important recent finding suggested that tumor CD73-generated adenosine promoted spontaneous lung metastasis of 4T1 breast tumors in the absence of T cells (15). Others have shown that knockdown of CD73 in MB-MDA-231 human breast cancer cells prevented their adhesion to extracellular matrix and inhibited their migration (11).

Finally, our identification of CD39-CD73-dependent generation of extracellular adenosine by tumor cells supports mutual dependence of CD39-CD73 and A2AR-mediated immunosuppression in the tumor microenvironment. These data raise the feasibility of potent strategies to overcome this tumor-induced immunosuppression by

² L. Wang, et al. Host CD73 mediates the immune barrier and favors tumor growth, submitted for publication.

targeting the important axis of CD39-CD73-A2AR in the tumor. This targeted CD39-CD73-A2AR therapy directs the development of focused pharmacologic strategies to reduce or ablate the impact of adenosinergic immune suppression in cancer patients, thereby increasing the effectiveness of therapeutic cancer vaccines and other T-cell-based cancer immune therapies.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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