Editorial

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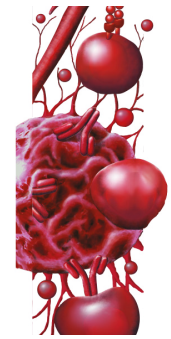
Immunotherapy



Potential of CD73 as a target for cancer immunotherapy

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"combination therapy by anti-CD73 and immune checkpoint inhibitors may lead to a robust synergistic outcome in cancer. This combination not only reduces immunosuppressive adenosine levels in the tumor site, but also enhances T-cell susceptibility to priming by antigen-presenting cells."

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Several escape mechanisms help cancer progression through overwhelming anticancer immune responses. These mechanisms include various cellular and molecular components that provide a complex network in tumor microenvironment leading to the expansion of cancer cells and resistance against anticancer immune responses. Interestingly, targeting these escape mechanisms has emerged as a potent anticancer immunotherapeutic approach in various cancers. Among the several immune escape mechanisms, generation of adenosine has been identified as one of the most important strategies used by cancer cells to overwhelm immune responses. Accordingly, overexpression of adenosine has been observed in several cancer types which were associated with disease progression and poor prognosis [1]. Adenosine is generated from adenosine triphosphate (ATP) by the function of two cell surface-expressed molecules, including CD39 and CD73. While CD39 degrades ATP to produce adenosine monophosphate (AMP), CD73 converts AMP to immunosuppressive adenosine. ATP degradation leads to depletion of immune danger signal (ATP) and generation of immunosuppressive factor (Adenosine) in tumor region [2]. The sequence of CD73 in mouse and rat exhibit about 90% homology with human, implying a highly conserved sequence. It is a homodimer molecule with 548 amino acids with the AMP binding site in carboxyl terminal [3]. In addition to the GPI-anchored surface-expressed form, CD73 is also detected as a soluble form (sCD73) in the plasma of cancer patients. However, its function, prevalence and prognostic value are a matter of debate [4].

Several factors can modulate the expression of CD73. Hypoxia in tumor microenvironment induces expression of CD39 and CD73, leading to overexpression of adenosine [5]. In addition to hypoxia, several factors including growth factor independent 1 (Gfi1), signal transducer and activator of transcription 3 (STAT3), Sp1, IL-6, interferon (IFN) type I, TGF-β1, IL-1β, TNF-α, prostaglandin E2, Wnt signaling and protein kinase C (PKC) can induce expression of CD73. In contrast, some factors such as IL-12, IL-21, IL-4 and IFN-γ suppress CD73 expression [6].

Adenosine is a potent immunosuppressive molecule which can robustly suppress proliferation and function of various immune cells, particularly T cells [7]. Interestingly, it can induce immune inhibitory cells, such as regulatory T cells (Tregs) and myeloid-derived cells (MDSCs). In addition to these immunosuppressive functions, adenosine can promote cancer cell proliferation, angiogenesis and metastasis [8]. All of these actions are mediated by adenosine receptors expressed on various cells, including A1, A2a, A2b and A3 adenosine receptors [9,10]. These extensive immunosuppressive and tumor-promoting functions introduced CD73 as a potential immunotherapeutic target for cancer therapy. Accordingly, several studies have targeted CD73 in various cancer models, which was associated with promising results. However, little is known regarding the efficacy of this strategy in human cancers [11–13]. Successful application of this therapeutic approach in human cancers needs clarification due to various unaddressed issues on this topic.

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One hesitating point regarding the CD73 targeting is the high expression rate of this molecule on both malignant and healthy cells. Besides cancer cells, several immune cells, including T cells (both $CD4^+$ and $CD8^+$ T cells), Tregs and B cells, can express CD73 [14]. Moreover, several nonimmune cells and organs can also express CD73 [15]. It should be noted that not only the number of CD73-expressing cells is essential, but also enzymatic function and intensity of this molecule are critical points [11]. It is assumed that targeting of CD73 may lead to capturing healthy cells expressing CD73, which is evident. I believe that blockade of CD73 in healthy (immune or nonimmune) cells not only is not deleterious, but also helps to maximize the impact of immunotherapy; since both host normal and cancerous cells in tumor microenvironment promote generation of adenosine and thereby enhance cancer progression.

It should be noted that although blockade of CD73 on healthy cells in the tumor microenvironment will be associated with ameliorative effects, it is unknown whether a similar outcome will be reported for other nonmalignant organs. Accordingly, CD73 is involved in platelet aggregation [15], and its mutations result in arterial and hand and foot joint calcification, as well as cardiovascular diseases [16]. I think that to avoid blockade of CD73 in nontumor sites, it is critical to focus and suppress this molecule only in the tumor microenvironment by using novel drug delivery systems, which can accurately deliver anticancer therapeutics to tumor sites through enhanced permeability and retention effect or active targeting [17,18]. We have successfully and consistently used this strategy to decline expression of CD73 in tumor site in preclinical studies [11,19]. However, this strategy also needs further investigation to identify and develop safe and effective drug delivery systems in humans.

Surprising ameliorative effects of CD73 targeting in preclinical investigations rigorously implied translation of this therapeutic strategy to clinical cancer patients. However, little is known regarding the efficacy of this method in cancer patients. Accordingly, several research groups are now trying to clarify the safety and efficacy of various CD73-directed inhibitors in the initial phases of clinical trials (www.clinicaltrials.gov). Various monoclonal antibodies against CD73 (such as CPI-006, CPI-444, oleclumab, TJ004309, NZV930 and BMS-986179) undergo primary evaluation in these studies. Furthermore, accumulating attempts are performed to develop other novel anti-CD73 drugs to achieve better clinical outcome [20]. Some clinical trials are investigating the possible synergism effects following combination therapy of cancer patients by using monoclonal antibodies against CD73 and immune checkpoints such as PD-1/PD-L1 (NCT03454451, NCT03835949 and NCT02503774). The results of these trials will address several unknown issues regarding the safety and efficacy of anti-CD73 therapy in cancer patients shortly. I believe that combination therapy by anti-CD73 and immune checkpoint inhibitors may lead to a robust synergistic outcome in cancer. This combination not only reduces immunosuppressive adenosine levels in the tumor site, but also enhances T-cell susceptibility to priming by antigen-presenting cells. Although these strategies seem to be interesting therapeutic protocols, their translation into the clinic should be associated with precise timing and dose scaling related to cancer grade.

Although tumor-promoting effects of CD73 is undeniable in preclinical and the majority of clinical studies, correlation of CD73 expression with good prognosis in some cancers such as ovarian and breast has made it challenging to make a precise decision regarding the efficacy of CD73 targeting as a general cancer biomarker in all cancers stages [21,22]. I therefore believe that the efficacy of CD73 targeting strictly relies on the type of cancer and disease stage, and evaluation of its expression and prognosis is a prerequirement to the administration of this therapeutic strategy. However, expression of CD73 on tumor-resident immune cells also persuades blockade of CD73, even in CD73-negative tumors. Consequently, considering CD73 as the universal tumor target or a personalized medicine needs to be further investigated in future studies. The results of clinical trials in this area currently underway should address this issue.

As previously mentioned, in addition to CD73, both CD39 and adenosine receptors are involved in the generation and signaling of adenosine; however, further research in to the blockade of these two is required to determine which would result in greater antitumor effects. It has been demonstrated that blockade of CD73 can potently suppress tumor growth in preclinical studies [11]. On the other hand, our group has shown that silencing of A2aR robustly restores anticancer responses of T cells in tumor bearing mice [23]. Based on current knowledge, it therefore seems that blockade of CD73 and A2aR is the most efficient strategy to neutralize tumor-driving adenosine effects. Since adenosine can also be generated in the CD73-independent pathway, I believe that blockade of A2aR, in combination with CD73, can maximize anticancer effects. Initial results of our current project (not yet published) also imply the efficacy of combined suppression of CD73 and A2aR in tumor models.

To conclude, before the potential of targeting these escape mechanisms can be realized, attention should be paid to various avenues of study in the future, including: evaluation of CD73 expression levels in different stages of

various cancers; investigation of the safety, efficacy and action mechanism of anti-CD73 monoclonal antibodies; clarification of adverse effects of CD73 blockade; identification of the impact of CD73 inhibition in CD73 positive and negative tumors; targeted blockade of CD73 in tumor region; prognostic value of CD73 in different cancers; and investigation of the efficacy of various combination therapies beside the CD73 inhibition.

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