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Requirement of NK cells for selective A_{2A} receptor blockade to suppress $CD73^+$ tumor metastasis

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Abstract

CD73 is becoming an emerging therapeutic target for the prevention of tumor growth and metastasis. However, the mechanism by which CD73 promotes tumor metastasis is unclear. Beavis *et al.* evaluated the efficacy of A_{2A} and A_{2B} adenosine receptor antagonists in inhibiting the metastasis of tumors expressing CD73, either endogenously or ectopically. They demonstrate distinct mechanisms whereby A_{2A} versus A_{2B} adenosine receptors could contribute to $CD73^+$ tumor metastasis. As A_{2A}receptor (R)/A_{2B}R antagonists have been tested in clinical trials in other disease settings, this study highlights the potential therapeutic application of an A_{2A}R/A_{2B}R blockade strategy for treatment of $CD73^+$ metastatic tumors.

Keywords

adenosine receptor; CD73; ecto-5′-nucleotidase; metastasis; NK cell

Tumor-induced immunosuppression is one of the main causes of tumor escape and failure of immunotherapy. Thus, understanding immunosuppressive mechanisms is critical for optimization of cancer immunotherapeutic benefit. CD73-mediated adenosinergic effects can now be viewed as among the most important immunosuppressive regulatory pathways in the tumor microenvironment [1,2]. CD73 is a cell surface ectoenzyme (ecto-5′-nucleotidase) that catalyzes the dephosphorylation of extracellular AMP into adenosine [3,4]. Importantly, this ectoenzymatic cascade, in tandem with CD39 (ecto-ATPase), generates adenosine from ATP that in turn signals through four known cell surface G protein-coupled receptors (A_1, A_{2A}, A_{2B}, A_3), among which the A_{2A} receptor (R) and A_{2B}R particularly exert potent anti-inflammatory activity [5]. They share a common signaling pathway, both leading to the activation of G proteins and the accumulation of intracellular

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cyclic AMP. Extensive studies demonstrate that $A_{2A}R/A_{2B}R$ activation contributes to tumor growth and metastasis by the immunosuppressive effects of adenosine [6,7].

Upregulation of CD73 expression has been observed in a broad spectrum of solid cancers and leukemias [1]. Moreover, a number of studies suggest that CD73 expression is associated with more aggressive, metastatic behaviors, and could serve as a diagnostic/prognostic marker and/or therapeutic target in certain cancer types [8,9]. In support, the feasibility of potent strategies to harness antitumor immune responses by targeting the important CD39/CD73-adenosine receptor axis in tumors has been recently documented [1,2,10,11]. Several preclinical studies demonstrate a pivotal role of tumor and host CD73-mediated adenosinergic effects on tumor growth and metastasis using an anti-CD73-blocking monoclonal antibody and a small-molecular inhibitor of CD73 activity [12–16]. The tumor-inhibiting advantage of CD73 blockade was due to improved antitumor T-cell immune responses, likely as a result of reduced $A_{2A}R$-mediated immunosuppression [6]. However, there is also considerable evidence to support the idea that CD73 promotes tumor growth and metastasis through multiple mechanisms [1,2] independently of antitumor T-cell regulation, that need further investigation.

Summary of methods & results

In their recent paper, Beavis and colleagues investigated the mechanism by which CD73 promotes metastasis. They transduced $CD73^-$ murine tumor cells with retrovirus-encoding murine CD73 cDNA to generate $CD73^+$ tumor cell lines, and then compared the metastatic capacity of the $CD73^-$ versus $CD73^+$ cells. They found that $CD73^-$ and $CD73^+$ tumor cells grew at equal rates *in vivo*. By contrast, ectopic CD73 expression promoted lung metastasis when tumor cells were injected either subcutaneously (spontaneous metastasis), or intravenously (experimental metastasis).

To investigate whether the prometastatic effect of tumor CD73 is dependent on adenosine receptor activation, a pan adenosine receptor agonist (adenosine analog), 5′-(*N*-ethylcarboxamido) adenosine (NECA), was used. In this setting, wild-type mice were treated with NECA before receiving intravenously injected $CD73^-$ tumor cells. NECA pretreatment significantly increased pulmonary metastasis. To further test which specific adenosine receptor was involved in this process, $A_{2A}R$ or $A_{2B}R$ antagonists were given before NECA. The prometastatic effect of NECA was partially reversed by either $A_{2A}R$ or $A_{2B}R$ blockade, suggesting that both $A_{2A}R$ and $A_{2B}R$ contributed to the prometastatic effect of adenosine signaling. This conclusion was further confirmed by the direct use of $A_{2A}R/A_{2B}R$ agonists that increased metastasis. The authors investigated whether $A_{2A}R/A_{2B}R$ activation also affected metastasis in immunocompromised $RAG^{-/-}c\gamma^{-/-}$ mice (lacking T, B and NK cells). They found that NECA pretreatment in $RAG^{-/-}c\gamma^{-/-}$ mice did increase metastasis, albeit to a significantly lesser extent than in wild-type mice. The increased metastasis could be partially reversed by $A_{2B}R$ blockade, but not by $A_{2A}R$ blockade. The results suggested that adenosine receptor activation promoted metastasis in both immune-dependent and immune-independent manners. It appears that the antimetastatic effect of $A_{2A}R$ blockade was dependent on immune cells that are deficient in $RAG^{-/-}c\gamma^{-/-}$ mice, while blocking A_{2B} receptors reduced metastasis through a distinct mechanism.

Notably, CD73 expression on tumor cells was required for the metastasis-promoting effects of adenosine receptor activation because blockade of either $A_{2A}R$ or $A_{2B}R$ did not inhibit the metastasis of $CD73^-$ tumors. In addition, $A_{2A}R$ blockade failed to affect metastasis of $CD73^+$ tumors in $A_{2A}R^{-/-}$ mice, indicating that $A_{2A}R$ activation on host immune cells, rather than tumor cells, contributed to CD73-mediated metastasis.

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To investigate which specific host immune cells were affected by $A_{2A}R$ activation, the authors focused on the NK cell subset, which is deficient in $RAG^{-/-}c\gamma^{-/-}$ mice. As expected, adding NECA significantly inhibited cytokine production, activation and cytolytic activity of NK cells *in vitro*. This effect was reversed by $A_{2A}R$ antagonists, indicating a role for $A_{2A}R$ activation in suppressing NK cell functions. These results could be also recapitulated *in vivo* because the activity of NK cells from $A_{2A}R^{-/-}$ mice was not modulated by NECA. Moreover, $A_{2A}R$ blockade failed to reduce both spontaneous and experimental metastasis of $CD73^+$ tumors from perforindeficient mice, in which the cytolytic function of NK cells is impaired. Interestingly, further new evidence showed that the proportion of tumorinfiltrating NK cells expressing granzyme B was significantly increased by $A_{2A}R$ blockade, but not $A_{2B}R$ blockade. Thus, the authors concluded that $A_{2A}R$, rather than $A_{2B}R$, activation promoted $CD73^+$ tumor metastasis through inhibition of perforin-dependent NK cytotoxicity.

While exploring which immune subsets participate in the $A_{2B}R$-mediated prometastatic effect, the authors found that the frequencies of myeloidderived suppressor cells, macrophages and dendritic cells in metastatic lungs were unchanged following $A_{2B}R$ blockade, suggesting that $A_{2B}R$ stimulation may enhance metastasis through a mechanism independent of these immune cells. Indeed, $A_{2B}R$ activation on tumor cells was reported to reduce $CD73^+$ tumor metastasis and invasiveness [14,17]. Together, these data suggested CD73 promotes metastasis by $A^{2A}R$ and $A^{2B}R$ activation, invoking both immune-dependent and immune-independent mechanisms.

Discussion & significance

CD73 expression on tumor cells has been shown to promote tumor growth and metastasis. The mechanism of CD73-mediated tumor growth has been well studied, and an inhibition of antitumor T-cell responses by CD73 has been revealed [12–16]. However, the underlying mechanism by which CD73 influences tumor metasstasis remains largely unknown. The current study demonstrates that tumor CD73 enhances metastasis through both immune-dependent and immune-independent mechanisms. The immune-dependent mechanism relies on $A_{2A}R$-mediated suppression of perforin-dependent NK cell cytotoxicity. Conversely, the contribution of the $A_{2B}R$ to CD73-promoted tumor metatastasis likely occurs through its activation on tumor cells. Although the majority of current CD73-focused cancer therapy studies have been concentrated on CD73-mediated adenosinergic effects, it appears there may also be an adenosine- independent role of CD73. This derives from a more recent study [18], which showed that an antihuman CD73 monoclonal antibody inhibited development of metastasis in a spontaneous animal model of human metastatic breast cancer by causing the clustering and internalization of CD73. This appears to limit the ability of circulating tumor cells to extravasate and colonize, suggesting a nonenzymatic role for CD73 in cell adhesion. Therefore, CD73 promotes tumor growth and metastasis in a multifactorial manner, highlighting that CD73 is a promising novel target for effective cancer therapy. As there are several adenosine receptor antagonists already in clinical trials for other disease settings, a more rapid transition in anti-CD73 cancer therapy from bench to bedside is warranted.

Future perspective

The most significant result reported here is the demonstration that the metastasis of $CD73^+$ tumors can be suppressed by restoring NK cell activity through $A_{2A}R$ blockade. In contrast to $A_{2A}R$ blockade, Beavis *et al.* also showed that $A_{2B}R$ blockade alone was sufficient to inhibit metastasis in a NK cell-independent manner. Although $A_{2B}R$ blockade did not change the percentage of myeloid-derived suppressor cells, macrophage and dendritic cells in metastatic tissue, we cannot formally exclude the possibility that $A_{2B}R$ antagonists

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reduced $CD73^+$ tumor metastasis through these immune cell activities. Indeed, $A^{2B}R$ blockade has previously been shown to modulate the differentiation and function of these myeloid cells [19,20]. Further investigations with $A_{2B}R$-deficient mice are certainly needed. Moreover, it would be interesting to dissect the contribution of tumor $A_{2B}R$ versus host $A_{2B}R$ to CD73-mediated tumor metastasis. Finally, exploring the possible correlative analysis of $A_{2A}R/A_{2B}R$ expression between the phenotypic characterization of varied immune infiltrates and clinical outcome in a large cohort of different cancer specimens would be very informative to validate the clinical implication for the $A_{2A}R/A_{2B}R$ antagonists.

References

1. Beavis PA, Stagg J, Darcy PK, Smyth MJ. CD73: a potent suppressor of antitumor immune responses. Trends Immunol. 2012; 33:231–237. [PubMed: 22487321]
2. Zhang B. Opportunities and challenges for anti-CD73 cancer therapy. Immunotherapy. 2012; 4:861–865. [PubMed: 23046227]
3. Resta R, Yamashita Y, Thompson LF. Ectoenzyme and signaling functions of lymphocyte CD73. Immunol Rev. 1998; 161:95–109. [PubMed: 9553767]
4. Colgan SP, Eltzschig HK, Eckle T, Thompson LF. Physiological roles for ecto-5′-nucleotidase (CD73). Purinergic Signal. 2006; 2:351–360. [PubMed: 18404475]
5. Hasko G, Linden J, Cronstein B, Pacher P. Adenosine receptors: therapeutic aspects for inflammatory and immune diseases. Nat Rev Drug Discov. 2008; 7:759–770. [PubMed: 18758473]
6. Ohta A, Gorelik E, Prasad SJ, et al. A_{2A} adenosine receptor protects tumors from antitumor T cells. Proc Natl Acad Sci USA. 2006; 103:13132–13137. [PubMed: 16916931]
7. Cekic C, Sag D, Li Y, Theodorescu D, Strieter RM, Linden J. Adenosine A_{2B} receptor blockade slows growth of bladder and breast tumors. J Immunol. 2012; 188:198–205. [PubMed: 22116822]
8. Stagg J, Smyth MJ. Extracellular adenosine triphosphate and adenosine in cancer. Oncogene. 2010; 29:5346–5358. [PubMed: 20661219]
9. Zhang B. CD73: a novel target for cancer immunotherapy. Cancer Res. 2010; 70:6407–6411. [PubMed: 20682793]
10. Bastid J, Cottalorda-Regairaz A, Alberici G, Bonnefoy N, Eliaou JF, Bensussan A. ENTPD1/CD39 is a promising therapeutic target in oncology. Oncogene. 2013; 32:1743–1751. [PubMed: 22751118]
11. Longhi MS, Robson SC, Bernstein SH, Serra S, Deaglio S. Biological functions of ectoenzymes in regulating extracellular adenosine levels in neoplastic and inflammatory disease states. J Mol Med (Berl). 2013; 91:165–172. [PubMed: 23292173]
12. Stagg J, Beavis PA, Divisekera U, et al. CD73- deficient mice are resistant to carcinogenesis. Cancer Res. 2012; 72:2190–2196. [PubMed: 22396496]
13. Stagg J, Divisekera U, Duret H, et al. CD73- deficient mice have increased antitumor immunity and are resistant to experimental metastasis. Cancer Res. 2011; 71:2892–2900. [PubMed: 21292811]
14. Stagg J, Divisekera U, McLaughlin N, et al. Anti-CD73 antibody therapy inhibits breast tumor growth and metastasis. Proc Natl Acad Sci USA. 2010; 107:1547–1552. [PubMed: 20080644]
15. Jin D, Fan J, Wang L, et al. CD73 on tumor cells impairs antitumor T-cell responses: a novel mechanism of tumor-induced immune suppression. Cancer Res. 2010; 70:2245–2255. [PubMed: 20179192]
16. Wang L, Fan J, Thompson LF, et al. CD73 has distinct roles in nonhematopoietic and hematopoietic cells to promote tumor growth in mice. J Clin Invest. 2011; 121:2371–2382. [PubMed: 21537079]
17. Desmet CJ, Gallenne T, Prieur A, et al. Identification of a pharmacologically tractable Fra-1/ADORA2B axis promoting breast cancer metastasis. Proc Natl Acad Sci USA. 2013; 110:5139–5144. [PubMed: 23483055]

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18. Terp MG, Olesen KA, Arnspang EC, et al. Anti-human CD73 monoclonal antibody inhibits metastasis formation in human breast cancer by inducing clustering and internalization of CD73 expressed on the surface of cancer cells. J Immunol. 2013; 191(8):4165–4173. [PubMed: 24043904]

19. Ryzhov S, Novitskiy SV, Goldstein AE, et al. Adenosinergic regulation of the expansion and immunosuppressive activity of $CD11b^{+}Gr1^{+}$ cells. J Immunol. 2011; 187:6120–6129. [PubMed: 22039302]

20. Novitskiy SV, Ryzhov S, Zaynagetdinov R, et al. Adenosine receptors in regulation of dendritic cell differentiation and function. Blood. 2008; 112:1822–1831. [PubMed: 18559975]

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Executive summary

- CD73 expression on tumor cells is required for A_{2A}receptor (R)/A_{2B}R-mediated tumor metastasis.
- A_{2A}R blockade inhibits $CD73^+$ tumor metastasis by reversing NK cell functions.
- A_{2B}R blockade inhibits $CD73^+$ tumor metastasis by a distinct mechanism which may be independent of immune regulation.
- Targeting the CD73 A_{2A}R/A_{2B}R axis represents a new way to effectively treat cancer metastasis.