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A_{2A} blockade enhances anti-metastatic immune responses

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The specific targeting of tumor-elicited immunosuppression is a promising strategy for the treatment of cancer. We have recently demonstrated that targeting the immunosuppressive pathway mediated by CD73-derived adenosine through the blockade of A_{2A}/A_{2B} adenosine receptors significantly reduced the metastatic potential of CD73$^+$ breast carcinomas and melanomas via both immunological and non-immunological mechanisms.

Targeting tumor elicited-immunosuppressive pathways has proven to be an efficient means of boosting antitumor immunity. The potential of this approach is underlined by the clinical success of monoclonal antibodies targeting cytotoxic T lymphocyte-associated protein 4 (CTLA4), programmed cell death 1 (PDCD1, best known as PD-1) and CD274 (best known as PD-L1).[1] One of the immunosuppressive pathways that has largely been overlooked relies on the generation of adenosine by 5'-nucleotidase, ecto (NT5E, best known as CD73). The expression of this ectoenzyme enhances tumor progression and metastatic dissemination. In line with this notion, the expression of CD73 is a poor prognostic marker among triple negative breast cancer patients.[2–4] The activation of both A_{2A} and A_{2B} adenosine receptors has been shown to suppress antitumor T-cell responses within the tumor microenvironment.[3,5,6] However, the mechanisms by which CD73 promotes metastasis were less clear. Therefore, we recently set out to investigate the molecular and cellular cascades that underlie the pro-metastatic activity of CD73.[7]

We first investigated whether ectopic CD73 expression is sufficient to increase the metastatic potential of CD73$^-$ tumors. We chose AT-3 breast carcinoma and B16F10 melanoma cells since they constitute a weakly metastatic and a highly metastatic tumor model, respectively. In both settings, CD73 expression significantly enhanced tumor metastasis. Although the major biological function of CD73 is the generation of adenosine, this ectoenzyme has been attributed with additional roles.[8] Therefore, we investigated whether exogenous adenosine could mimic the pro-metastatic effects of CD73. The pre-treatment of mice with 5'(*N*-ethylcarboxamido) adenosine (NECA), a stable analog of adenosine that operates as a pan-agonist for A_1, A_{2A}, A_{2B}, and A_3 receptors, significantly enhanced the metastatic potential of B16F10 tumor cells. To investigate which adenosine receptors would be involved in the effects of NECA, we simultaneously treated mice with NECA and selective A_{2A} (SCH58261) or A_{2B} (PSB-1115) antagonists. Both SCH58261 and PSB-1115 partially reversed the pro-metastatic effects of NECA. Accordingly, a selective A_{2A} (CGS21680) or A_{2B} (BAY60–6583) agonist was sufficient to exacerbate tumor metastasis. Although these results indicated that the activation of A_{2A} or A_{2B} receptors could promote the metastatic dissemination of malignant cells, they did not formally demonstrate that this pathway would underpin the increased metastatic potential of CD73$^+$ tumors. Therefore, we investigated the ability of these antagonists to reduce the dissemination of B16F10-CD73$^+$ and 4T1.2 cells, breast carcinoma cells that endogenously express CD73. A_{2A} or A_{2B} blockade significantly reduced the metastatic potential of these cancer cell lines. To confirm that the effects of SCH58261 were mediated by the A_{2A} receptor we investigated the metastatic dissemination of B16F10-CD73$^+$ tumor cells in A_{2A}-deficient (*Adora2a*$^{-/-}$) mice. These mice were significantly protected against metastases as compared with their wild-type (WT) counterparts, confirming the pro-metastatic role of host A_{2A} receptors.

To investigate whether the blockade of A_{2A} or A_{2B} receptors would reduce metastasis via an immunological mechanism, we next examined whether A_{2A}/A_{2B} targeting was effective in immunocompromised mice lacking natural killer (NK) and T cells. We observed that NECA promotes metastasis in these mice, albeit to a lesser extent than in WT animals. Notably, the blockade of A_{2A} receptors was no longer effective in immunocompromised mice, indicating that A_{2A} stimulation enhances the metastatic dissemination of cancer

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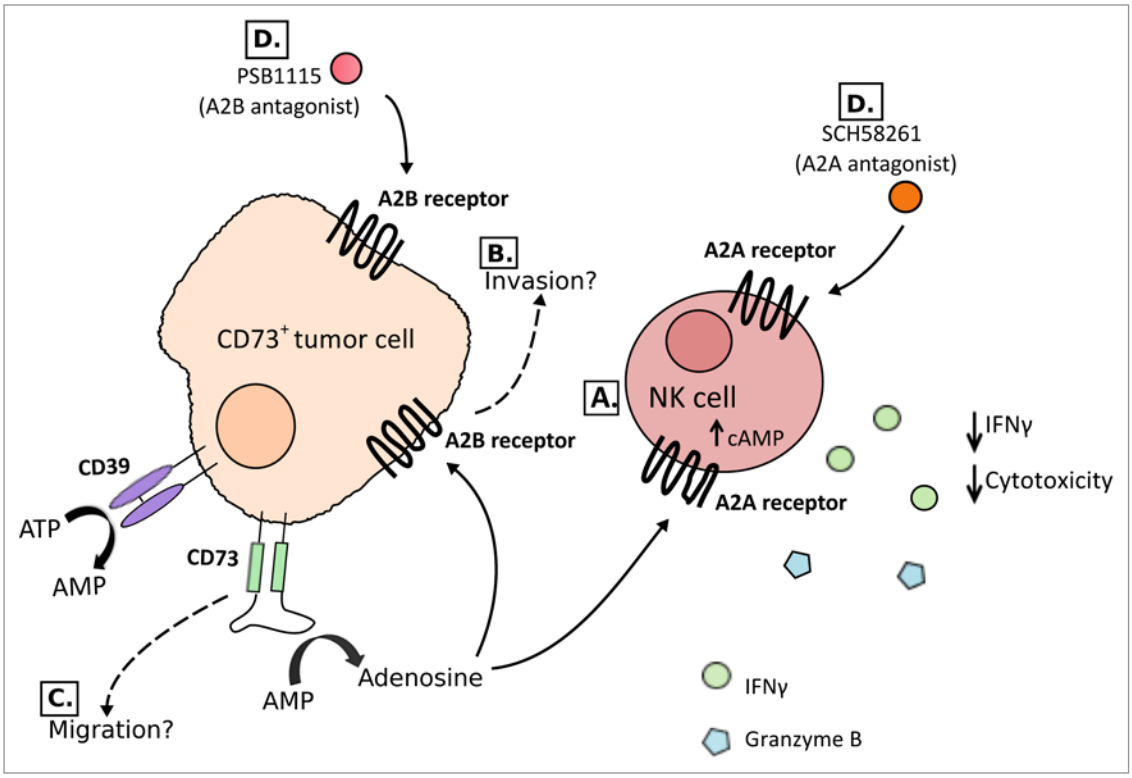

Figure 1. The expression of CD73 by malignant cells enhances metastasis through the activation of A_{2A} and A_{2B} adenosine receptors. (**A**) The expression of CD73 on malignant cells converts AMP into adenosine, which activates A_{2A} receptors on natural killer (NK) cells. This inhibits the cytotoxic functions of NK cells as well as their ability to produce pro-inflammatory cytokines including interferon γ (IFNγ). (**B**) The activation of A_{2B} receptors also stimulates metastatic dissemination through an alternative, hitherto unclear, pathway. This may potentially coincide with the activation of A_{2B} receptors on tumor cells. (**C**) CD73 may also promote metastasis in an adenosine-independent manner. (**D**) The blockade of A_{2A} or A_{2B} receptors with the A_{2A} antagonist SCH58261 or the A_{2B} antagonist PSB-1115, respectively, significantly reduces the metastatic dissemination of $CD73^+$ tumors.

cells as it suppress host lymphocytes. By contrast, the blockade of A_{2B} maintained its ability to limit metastatic dissemination in the absence of NK and T cells, indicating that A_{2B} stimulation enhances metastasis through a distinct mechanism. Since the metastatic dissemination of B16F10 cells is known to be controlled by NK cells and that the anti-metastatic effects of A_{2A} blockade relied on host lymphocytes, we hypothesized that such anti-metastatic effects might be due to enhanced NK-cell effector functions. Indeed, we observed that the stimulation of A_{2A} receptors suppresses the ability of NK cells to kill B16F10 or 4T1.2 cancer cells in vitro and that the activity of the A_{2A} antagonist SCH58261 is attenuated in perforin-deficient ($Prf^{-/-}$) mice. Since the phenotype of $Prf1^{-/-}$ mice could potentially be explained by enhanced cytotoxic functions of either NK cells or $CD8^+$ T lymphocytes, we analyzed the phenotype of tumor-infiltrating NK cells in mice treated with A_{2A} or A_{2B} antagonist. Intratumoral NK cells isolated from mice treated with SCH58261, but not PSB-1115, exhibited increased expression levels of granzyme B. Taken together, these results indicate that the blockade of A_{2A} receptors exacerbates the cytotoxic activity of NK cells in vivo.

Although our data show an unequivocal role for A_{2A}-mediated immunosuppression in the pro-metastatic effect of CD73, this is not the only mechanism whereby CD73 stimulates tumor metastasis. Indeed, we found that CD73 expression promotes the metastatic dissemination of tumor cells in immunocompromised mice, indicating that CD73 also exerts tumor-promoting effects via non-immunological mechanisms. Notably, the autoactivation of A_{2B} receptors on neoplastic cells has previously been shown to enhance their invasive potential by stimulating the formation of filopodia.[3,9] Intriguingly, it has recently been shown that CD73 can promote the metastatic dissemination of tumor cells in vivo independently of its catalytic activity, indicating that the activation of adenosine receptors may not fully account for the pro-metastatic effects of CD73.[10]

In summary, our data indicate that CD73 favors tumor metastasis by multiple mechanisms including the generation of adenosine, and activation of A_{2A} receptors of the host (**Fig. 1**). Our data clearly suggest that the blockade of A_{2A} or A_{2B} represents a potential therapeutic strategy to limit metastasis. As antagonists of these receptors are already under clinical development for other indications and show good safety profiles, the translation of our findings to cancer patients appears feasible.

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Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.