CROSSTALK BETWEEN ADENOSINE AND NOTCH PATHWAYS IN CD8+ T-CELLS

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Immunosuppression in the tumor microenvironment is induced by elevated concentration of growth factors, inflammatory mediators and metabolites, including adenosine. Adenosine is an ATP-derived nucleoside which is released in response to pathophysiological conditions, such as hypoxia, ischemia, inflammation or trauma and acts as an "alarm" signal (Hasko et al., 2008). Extracellular adenosine acts through the interaction with G protein-coupled cell-surface receptors (GPCRs) that can either stimulate (A2AR and A2BR) or inhibit (A1R and A3R) adenylate cyclase (Antonioli et al., 2008). In the tumor microenvironment, immunosuppressive effects are mainly mediated by cAMP-elevating adenosine receptor A2AR. However, emerging evidence suggests that A2BR may also play a pivotal role in adenosine-mediated tumor progression (Feoktistov et al., 2002). Stromal accumulation of adenosine contributes to the recruitment of type II macrophages (TAM), regulatory T cells (Tregs) and myeloid-derived suppressor cells (MDSCs) which are involved in immune suppression, tumor growth and angiogenesis. T-cells are essential effectors of tumor immunity, and one of the crucial signaling pathways involved in T-cell development, lineage selection and activation is Notch. It is well documented that Notch signaling is activated after T-cell receptor (TCR) engagement, and is required for T-cell activation and for the cytotoxic activity of CD8 T-cells (Dongre et al., 2014). Notch1 is critical for the differentiation of CD8 effector cells, and constitutive expression of active Notch1 renders CD8 cells resistant to the immune-suppressive activity of MDSCs (Sierra et al., 2014). A number of pharmacological strategies have been developed to target Notch signaling, including antibodies to receptors or ligands and γ-secretase inhibitors (GSIs) that can prevent the generation and release of Notch intracellular domain (NICD) (Olsauskas-Kuprys et al., 2013).

The aim of this study was to investigate the mechanisms of Adenosine-Notch crosstalk pathway in CD8+T cells. We hypothesized that Adenosine suppresses CD8+T-cell activity by downregulating the expression or activity of Notch1 via A2AR or A2BR. Isolated and activated CD8+T-cells from spleens of naïve mice, treated with an A2A agonist (CGS-21680, 1 μM for 72 hours) showed reduced proliferation rate and IFNγ and Granzyme β production, and decreased expression of Notch1. Conversely, A2B agonist treatment (Bay-606583, 1 μM for 72 hours) had no effects on CD8+T-cells, suggesting that A2A is the only adenosine receptor involved in CD8+Tcell suppression through Notch1 modulation. Administration of ZM-241385 (A2A agonist, 1 μM for 72 hours) completely reversed the effects induced by CGS-21680 and restored Notch1 expression. These results suggest that activation of A2A receptor, and not A2BR, significantly inhibit CD8+T cells function and this effect is associated with reduced expression of Notch1. Accordingly, stimulation of A2A with CGS-21680 in combination with a GSI (PF-03084014, 1 μM for 48 hours) significantly decreased proliferation rate and impaired IFN-γ and Granzyme β production compared to the cells treated with either agent alone. These data indicate that A2AR activation and inhibition of Notch synergistically reduce the effector functions of activated CD8+T-cells. To determine whether the constitutive activation of Notch1 in CD8+T-cells protects these cells from the suppressive activity

of adenosine we used transgenic CD8 T-cells expressing Notch1 NICD under the Granzyme promoter compared with Flox CD8+T-cells. Treatment of activated CD8+T-cells expressing Notch1 NICD with CGS-21680 did not influence the expression of GZMB, or the secretion of IFN-γ or Granzyme β, in contrast to Flox CD8+T-cells. These effect were blocked by the A2A antagonist ZM241385, confirming that the suppressive activity of CGS21680 via A2A adenosine receptor is at least in part dependent on Notch1 inhibition. Accordingly, in CD8+T-cells expressing Notch1 NICD treated with the Notch1 inhibitor PF-03084014, the suppressive effects of CGS-21680 on GZMB expression were abrogated. Together, our data reveal a novel role for A2AR which, by repressing the expression of Notch in CD8+T-cells, regulates the T cells effector response. Our findings will have significant implications for the development of strategies to counteract adenosine-dependent tolerogenic immunosuppressive effects in cancer.

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