Double sword-edge action of adenosine

by Isabelle Plo and Iléana Antony-Debré

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The double-edged sword of adenosine

Isabelle Plo\textsuperscript{1,2,3} and Iléana Antony-Debré\textsuperscript{1,2,3}

\textsuperscript{1}INSERM, UMR 1287, Gustave Roussy, 114 rue Edouard Vaillant, Villejuif, France; \textsuperscript{2}Gustave Roussy, 114 rue Edouard Vaillant, Villejuif, France Villejuif and \textsuperscript{3}Université Paris-Saclay, Gif-sur-Yvette, France

Correspondence: Isabelle Plo
isabelle.plo@gustaveroussy.fr

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In this issue of \textit{Haematologica}, Midkar et al. show the dual role of adenosine signaling in erythroid and myeloid differentiation.\textsuperscript{1} Besides their involvement in DNA and RNA synthesis, nucleosides such as adenosine are also signaling molecules that regulate tissue homeostasis through various biological functions: cell growth, migration, neurotransmission, immune regulation, and vascular tone. Adenosine levels are highly up-regulated during hypoxia, ischemia, physical activity, epilepsy, pain, and cancers, and deal with oxygen demand and inflammation. This regulation implicates a tightly controlled network of catabolic enzymes, nucleoside transporters, and receptors. In particular, the extra-/intracellular levels of adenosine are controlled by the equilibrative nucleoside transporters (ENT) and the concentrative nucleoside transporters (CNT). Extracellular adenosine can be generated from dephosphorylation of extracellular ATP, ADP, and AMP through the action of the cell-surface-associated enzymes CD39 (ectonucleoside triphosphate diphosphohydrolase 1) and
CD73 (ecto-5'-nucleotidase). Adenosine can be rapidly phosphorylated to AMP by adenosine kinase or deaminated to inosine by adenosine deaminase. As a signaling molecule, adenosine can bind to four adenosine G protein-coupled receptor (AR) subtypes: A₁AR, A₂AAR, A₂BAR, and A₃AR. Transcriptomic databases of human hematopoiesis showed the predominant expression of \textit{ADORA3} and \textit{ADORA2B}, encoding A₃AR and A₂BAR, in CD34⁺ progenitors, and erythroid and myeloid cells. Following adenosine binding, activation of the receptors modulates the AMPc/PKA axis and related transcription factors and kinases, and the Phospholipase C and MAPK pathways (ERK, P38, JNK).²

The involvement of adenosine in the regulation of hematopoiesis is well known. However, very few studies, including those on the role of nucleosides in general, have reported its role in erythropoiesis, the process of differentiating hematopoietic stem cells into red blood cells (RBC).³ Defective erythropoiesis was observed both in \textit{ENT1}-null patients harboring the extremely rare Augustine blood group system and in \textit{Ent1} knock-out (KO) mice. Both models displayed increased extracellular nucleosides, including adenosine.⁴ In the present study, Midkar \textit{et al.} have focused on the impact of extracellular adenosine during early and late erythropoiesis. They used an \textit{in vitro} differentiation system derived from purified CD34⁺ progenitors with the addition of stem cell factor (SCF), interleukin-3 (IL-3), and erythropoietin. They showed that, in contrast to other nucleosides, a high concentration of adenosine is able to negatively impact erythropoiesis by inhibiting proliferation and differentiation. The combination of ENT1 potent bidirectional inhibitor (NTBI) and adenosine further potentiated the inhibitory effect on proliferation, leading to altered erythropoiesis, and suggesting the involvement of extracellular adenosine signaling. This hypothesis was elegantly confirmed by the use of the A₃AR agonist (CI-IB-MECA), which is in line with a previous study in primary human cells.⁵ Since A₂BAR was also expressed in myeloid and erythroid cells, the authors tested the BAY60-6583 agonist. This delayed erythroid maturation but did not impact proliferation. Taken together, this study provides evidence that extracellular adenosine is a signaling molecule that regulates erythropoiesis at different stages. It would be interesting to investigate the expression of these receptors in order to clarify their role.

Interestingly, the authors found that the negative effect of adenosine on erythropoiesis was through P53-mediated apoptosis. This was confirmed by a proteomic analysis of erythroblasts which showed numerous over-expressed proteins in both the p53-related apoptotic and the
DNA repair pathways. Of note, P53-induced apoptosis upon extracellular adenosine accumulation has already been described in breast and ovarian cancer cell lines. In particular, it was shown that P53 can promote transcription of ADORA2B, which in turn activates caspase- and Puma-dependent apoptotic pathways through P53. Alternatively, one could hypothesize that the activation of P53 by AR could be triggered through its phosphorylation by P38. P53 is known to be a critical molecule for effective erythropoiesis. In early stages, P53 should be tightly regulated to maintain a high rate of erythroblast proliferation. In later stages, when decreased ribosome biogenesis occurs in basophilic erythroblasts, P53 is required to respond to ribosome stress and ensure terminal maturation. A premature or abnormal activation of P53 during erythropoiesis, such as that observed upon extracellular adenosine treatment or in ribosomopathies, may lead to differentiation blockade and anemia.

Midkar et al. report that extracellular adenosine and A3AR agonist decreased erythroid differentiation in favor of myeloid differentiation, while the A2bAR agonist had no effect (Figure 1). Similarly, Ent1 KO mice displayed reduced numbers of erythroid cells while showing increased granulocytic cell counts, at both the mature and progenitor stages. A3AR activation has already been shown to promote mouse granulo-monocytic progenitor growth by increasing the effects of growth factors such as IL-3, SCF, and granulocyte macrophage colony-stimulating factor (GM-CSF) in vitro, and by promoting granulocyte (G)-CSF production in vivo in mice. In contrast, in a sickle cell disease mouse model harboring elevated levels of extracellular adenosine, treatment with polyethylene glycol-modified adenosine deaminase, which lowers adenosine concentrations, leads to a decrease in leukocyte counts, and increased RBC counts. Deletion of A3AR in mice leads to several abnormalities in the peripheral blood, such as decreased neutrophil and monocyte counts, increased erythrocyte and hemoglobin levels, and a decrease in platelets counts. However, the erythroid compartment still needs to be studied in more detail. While studies so far agree on the role of adenosine/A3AR signaling in erythroid/myeloid commitment and differentiation, other lineages, such as the megakaryocytic lineage, could also be affected, and there is still no clear indication as to how adenosine signaling can influence erythroid versus myeloid commitment. Could it strengthen the cytokine effect? Does it have an impact on transcription factor expression? Does it generate metabolic changes that will dictate differentiation?

The work of Midkar et al. adds adenosine to the plethora of factors that can modulate normal erythropoiesis. Different types of AR agonists are already in use or are being developed in
clinical trials in several diseases: auto-immune diseases, inflammation, neurological diseases, and diabetes. Therefore, one could consider their use in diseases with erythroid hyperplasia (hereditary erythrocytosis or myeloproliferative diseases such as polycythemia vera). In contrast, since adenosine plays a broad immunosuppressive role that regulates both innate and adaptive immune responses, inhibitors of extracellular adenosine have also been widely developed to promote antitumor activities in cancers. It would be interesting to determine the levels of extracellular adenosine in hematologic malignancies or diseases with defective erythropoiesis, and explore how these levels could contribute to disease development using adenosine inhibitors or agonists.
REFERENCES


Figure legend. Extracellular adenosine induces decreased erythropoiesis via adenosine receptors A_{2B}AR and A_{3}AR. While A_{3}AR inhibits erythroid proliferation and differentiation, A_{2B}AR inhibits erythroid maturation with no effect on proliferation. The defective erythropoiesis is due to activation of P53- and caspase 3-dependent apoptosis. Moreover, activation of A_{3}AR decreases erythropoiesis, which favors myelopoiesis.
Adenosine

A2BAR

A3AR

ENT1

P21/PUMA/BAX

p53

Nucleus

Proerythroblast

Reticulocyte

Erythroblast

Erythrocyte

Decreased erythropoiesis
↓ Proliferation
↓ Differentiation

Granulopoiesis

Myeloblast

Myelocyte

Promyelocyte

Metamyelocyte

Granulocyte

Increased granulopoiesis