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Mini Review

Actions of Adenosine on Cullin Neddylation: Implications for Inflammatory Responses

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ABSTRACT

There is intense interest in understanding how the purine nucleoside adenosine functions in health and during disease. In this review, we outline some of the evidence that implicates adenosine signaling as an important metabolic signature to promote inflammatory resolution. Studies derived from cultured cell systems, animal models and human patients have revealed that nucleotide metabolism is significant component of the overall inflammatory microenvironment. These studies have revealed a prominent role for the transcription factors NF-κB and hypoxia-inducible factor (HIF) and that these molecules are post-translationally regulated through similar components, namely the neddylation of cullins within the E3 ligase that are controlled through adenosine receptor signaling. Studies defining differences and similarities between these responses have taught us a number of important lessons about the complexity of the inflammatory response. A clearer definition of these pathways has provided new insight into disease pathogenesis and importantly, the potential for new therapeutic targets.

Contents

1. Introduction

Adenosine is a purine nucleoside that has long been implicated in normal cell growth and metabolism and more recently has been demonstrated to possess potent anti-inflammatory properties. Surprisingly little is known about the actual mechanisms of adenosine-mediated anti-inflammation. While signal transduction through the various adenosine receptors is reasonably well characterized, less is known about post-receptor signaling events and consequences of such signaling. One recently appreciated mechanism suggests that adenosine acts on the proteasomal degradation machinery that controls inflammation-associated transcription factors. This short review focuses on specific molecular pathways that have been identified downstream of adenosine signaling, including NF-κB and hypoxia inducible factor (HIF) pathways, and explores their role in inflammation, resolution and as potential new targets for therapy.

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2. Adenosine and Inflammation

At sites of injury and inflammation, adenosine is released and plays an important role in regulating inflammatory responses and limiting inflammatory tissue destruction [1]. Increased levels of adenosine have been detected during both inflammation and hypoxia and in these conditions, adenosine is both anti-inflammatory and cytoprotective [2,3]. The adenosine pathway has multiple points of regulation including metabolism of purine nucleotides via CD39 (ectonucleoside triphosphate diphosphohydrolase 1, E-NTPDase1) and CD73 (ecto-5′-nucleotidase, Ecto5′NTase), signaling via adenosine A2A and A2B receptors, and transport via ENT1/2 (equilibrative nucleoside transporters 1/2) [4,5]. Therefore, multiple potential therapeutic targets of the adenosine pathway have been evaluated for various inflammatory diseases and ischemia reperfusion disorders [6,7].

Recent work has defined the relationship between adenosine, adenosine receptor (AR) signaling, and anti-inflammation [4]. The signal transduction through the various ARs has been well characterized, however, less is known about the post-receptor events. Work in various cell types has shown that adenosine inhibits NF-κB activation through a number of distinct mechanisms, including elevation of intracellular cyclic adenosine monophosphate (cAMP) and activation of protein kinase A (PKA) which blocks IκB phosphorylation thus inhibiting NF-κB activation [8], inhibition of tumor necrosis factor (TNF)-α-induced NF-κB activity and subsequent gene expression by inhibition of nuclear translocation of active NF-κB without influencing IκB phosphorylation or degradation [9,10], and increased SUMO-1 modifications of IκBα by adenosine inhibition of phosphorylation and degradation of IκBα, which attenuates NF-κB activation [11]. Our work in recent years has found that adenosine inhibits NF-κB through actions on proteasomal degradation of IκB proteins via an alternative adenosine-mediated mechanism. Studies using an NF-κB luciferase reporter assay confirmed that adenosine, generated under the adaptive pathway of hypoxic preconditioning, significantly suppressed NF-κB activation [12]. As previous studies had demonstrated a connection between IκB degradation, NF-κB inhibition and Cullin-1 (Cul-1) protein deneddylation [13,14], the neddylation status of Cul-1 after AR stimulation revealed that adenosine indeed modulated Cul-1 neddylation and further influenced IκB protein stabilization and downstream targets. Regulated protein degradation is an essential part of cell signaling for many adaptive processes. The proteasomal degradation of IκB (Fig. 1), which inhibits NF-κB, is but one example of a rapid response by the cell to signal for growth, differentiation, apoptosis, or inflammation. The E3 SCF ubiquitin ligase is specific to the IκB family and is comprised of SKP1, Cul-1 and the F-box domain of β-TrCP and is responsible for the polyubiquitination of IκB [15]. The COP9 signalsome (CSN) must conjugate the small protein Nedd8 to Cul-1 in order for the E3 SCF to be active, and deneddylated Cul-1 therefore inhibits the ubiquitination of IκB, inactivating NF-κB [16]. It is currently unclear which of these adenosine-mediated pathways predominately regulates NF-κB activity.

One mechanism of deneddylation occurs through the deneddylase-1 (DEN-1, also called SENP8) protein. DEN-1 is a Nedd8-specific protease that has isopeptidase activity capable of directly deneddylating cullin targets [17,18]. The influence of these other cullin targets on pathways that can mediate inflammation, such as Cullin-2 (Cul-2) and the HIF pathway, and the potential influence of adenosine on DEN-1 activity are areas currently being studied.

3. Adenosine and Ischemia and Reperfusion Injury

Ischemia-reperfusion injury is a pathologic condition characterized by an initial restriction of blood supply, followed by the subsequent restoration of perfusion and concomitant re-oxygenation. In its classic manifestation, occlusion of a coronary artery is caused by a coronary thrombus and results in a severe imbalance of metabolic supply and demand causing tissue hypoxia [19]. In the second stage of the disease, blood flow is rapidly restored. Somewhat surprisingly, the restoration of blood flow along with re-oxygenation is in many instances associated with an exacerbation of tissue injury and a profound inflammatory response (so called “reperfusion injury”) [20].

A recent study provided new insights into potential mechanisms of how adenosine and HIF-1 could function in mediating cardioprotection from ischemia and reperfusion injury [21,22]. It has been known for some time that one of the critical functions of HIF is the induction of glycolytic enzymes, which are considered critical components of enhancing the capacity of hypoxic or ischemic tissues to increase

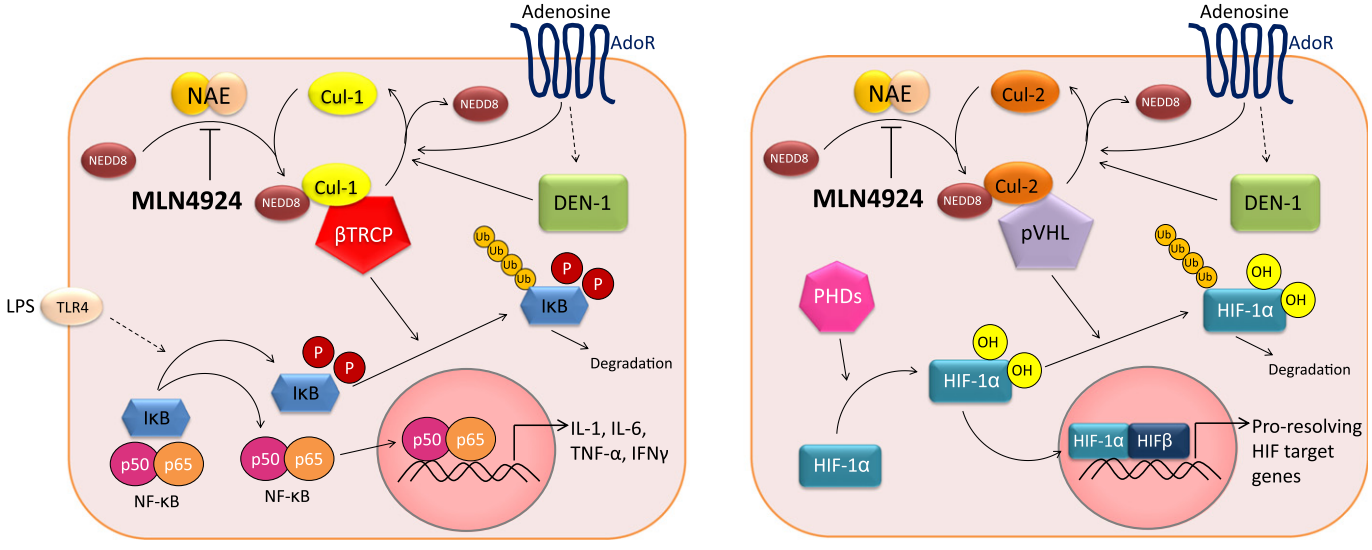

Fig. 1. Neddylation pathways influencing NF-κB and HIF-1α. Left, NF-κB pathway: Pro-inflammatory stimuli, such as LPS, facilitate the phosphorylation of IκB, leading to the recognition of p-IκB by the Cul-1–Nedd8–βTRCP complex, culminating in its polyubiquitination and proteasomal degradation. The conjugation of Nedd8 to Cul-1 is required for polyubiquitination and is achieved through a multi-enzyme process wherein DEN-1 cleaves the Nedd8 precursor to its mature form, allowing for conjugation to cullin proteins. Loss of DEN-1, or pharmacological inhibition of Nedd8 conjugation by MLN4924 through inhibition of the Nedd8-activating enzyme (NAE), prevents the activation of Cul-1, preventing the liberation of NF-κB from IκB and quenching pro-inflammatory signaling. The binding of adenosine to adenosine receptors (AdoR) also results in deneddylation of Cul-1. Studies are ongoing regarding a potential regulation of DEN-1 activity by adenosine and AdoR. Right, HIF-1α pathway: In contrast to NF-κB, HIF-1α in its hydroxylated form is degraded by the proteasome after ubiquitination via the Cul-2–Nedd8–pVHL complex. Pharmacological inhibition of Cul-2 neddylation using MLN4924 stabilizes cellular HIF-1α levels, leading to increased transcription of pro-resolving HIF target genes. Loss of DEN-1 also positively influences barrier function of intestinal epithelial cells.