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See related article on pg 123

Deregulation of Adenosine Receptors in Psoriatic Epidermis: An Option for Therapeutic Treatment

Stefania Merighi[1], Pier Andrea Borea[1], Katia Varani[1] and Stefania Gessi[1]

Purinergic signaling is involved in psoriasis, a chronic skin disease characterized by increased epidermis cell growth. In particular, Andrés et al. focus on the keratinocyte biology modulated by adenosine receptors providing evidence that the A_{2B} subtype plays a prominent role in the reduction of keratinocyte proliferation whereas A_{2A} and A_{2B} agonists have antiinflammatory effects independent of adenosine receptors. The authors report that psoriatic epidermis presents a deregulated adenosine receptor expression profile with reduced A_{2B} and increased A_{2A}.

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Psoriasis and adenosine

Psoriasis is a common relapsing and remitting autoimmune disease, affecting the skin and joints, now thought to be caused by the dysregulation of cytokines controlling inflammatory pathways, a mechanism that likely contributes to the various comorbidities observed in patients affected by it. Biologic treatments specifically target the altered inflammatory milieu, and they have been shown to be effective for moderate to severe psoriasis, commonly after other systemic treatments have failed. However, the first-line inexpensive systemic treatment of psoriasis is represented by methotrexate, an inhibitor of dihydrofolate reductase that blocks DNA synthesis and cell mitosis of rapidly dividing cells. Importantly, it has a well-established safety profile. It is well known that many of its effects are mediated by the activation of an adenosine receptor. In fact, methotrexate induces adenosine release both "in vitro" and "in vivo," in both animal models of inflammation and patients with rheumatoid arthritis (Cronstein, 2010). Adenosine concentrations in normal physiological conditions are low, in the nanomolar range, due to the activity of enzymes and transporters, but they increase in different pathologic settings, up to micromolar levels. Interestingly, high levels of adenosine have been reported to be found in the blood of patients with psoriasis (Burnstock et al., 2012). Indeed, adenosine is a ubiquitous autacoid, one that exerts a wide variety of physiological effects through interaction with four G protein-coupled receptors, named A_1, A_{2A}, A_{2B}, and A_3. They are able to modulate adenylyl cyclase activity, being A_1 and A_3 inhibitors, whereas A_{2A} and A_{2B} are stimulators of this enzyme through interactions with G_i and G_s proteins, respectively. In addition, A_{2B} and A_3 are also coupled to phospholipase C, via G_q proteins, thereby increasing calcium levels (Borea et al., 2015). Finally, all of these molecules modulate mitogen-activated protein kinases, with important consequences in the modulation of cell proliferation (Borea et al., 2016).

Andrés et al. (2017) investigated the role of adenosine receptors in keratinocyte proliferation, and they reported that the A_{2B} receptor inhibits proliferation whereas the A_{2A} subtype stimulates it. In addition, the authors focused on the antiinflammatory effects mediated by A_{2A} and A_{2B} agonists and concluded that these are not related to adenosine receptor binding.

Effects of adenosine receptors on keratinocyte proliferation

The literature includes few studies concerned with the therapeutic application of adenosinergic signaling in the skin, and the effects of adenosine regulation of epidermal cells are not well studied (Merighi et al., 2002). Adenosine receptor expression has been shown in keratinocytes, and the A_{2B} receptor is the major receptor subtype (Andrés et al., 2017; Braun

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Clinical Implications

- Agonists at A_{2A} receptors, upregulated in psoriasis, may counteract inflammation.
- Deregulated A_{2B}/A_{2A} receptors in psoriasis may be monitored during conventional therapy to evaluate remitting or relapsing pathology.
- A_{2B} agonists/A_{2A} antagonists could be developed to decrease keratinocyte proliferation.

et al., 2006; Brown et al., 2000). With respect to the other adenosine receptors, conflicting results have been reported, probably due to low levels of expression (Andrés et al., 2017). The effects of adenosine on keratinocyte cell growth have been investigated, also with conflicting findings. They may be stimulatory (Braun et al., 2006) or inhibitory (Brown et al., 2000), possibly depending on the ligands employed (adenosine vs. synthetic agonists), as well as their concentrations. The work of Andrés et al. (2017) now sheds light on the role of adenosine receptors in keratinocyte proliferation, addressing this long-lasting controversy. Indeed, the authors, using appropriately selected pharmacological tools, found that both increases and decreases in the rates of proliferation were obtained after adenosine receptor modulation, with inhibitory effects attributed to the A_{2B} receptor and stimulatory effects attributed to the A_{2A} subtype. The antiproliferative effect of the A_{2B} receptor was induced by an intracellular calcium increase, in a fashion similar to that of several antipsoriatic drugs, whereas the stimulatory effect of A_{2A} was due to p38 mitogen-activated protein kinase stimulation. Both these effects were mediated without affecting cAMP.

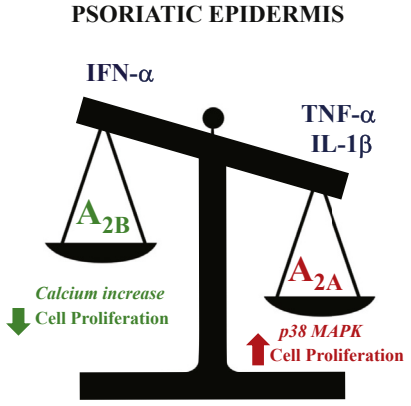

Figure 1. Schematic representation of adenosine receptor subtypes differentially expressed in psoriatic epidermis. A_{2B} and A_{2A} receptor subtypes are down- and upregulated, respectively, in psoriatic epidermis and play the opposite role in keratinocyte proliferation. IFN-α, TNF-α, and IL-1β play key roles in adenosine receptor deregulation. MAPK, mitogen-activated protein kinase; TNF-α, tumor necrosis factor-alpha.

Deregulation of adenosine receptors in psoriatic epidermis

Adenosine, acting at its receptors, is a potent inhibitor of inflammation through the modulation of the function of neutrophils, macrophage/monocytes, dendritic cells, and lymphocytes in diseases characterized by overactivated states of immune cells (Antonioli et al., 2013). Andrés et al. (2017) found that adenosine, as well as selective A_{2A} and A_{2B} agonists, inhibited tumor necrosis factor-alpha and IL-8 production, showing antiinflammatory profiles, without the involvement of adenosine receptors, as antagonists did not revert these effects. In particular, the mechanism was attributed to the activation of membrane phosphatases. Furthermore, a reciprocal interaction was found in normal human epidermal keratinocytes between a number of proinflammatory cytokines and adenosine receptors, which were decreased in the case of A_{2B} and increased in the case of the A_{2A} subtype. Accordingly, a similar deregulation was observed in psoriatic epidermis, which included reduced A_{2B} and increased A_{2A} profile expression (Figure 1). These data are in agreement with the upregulation of A_{2A} and A_3 receptors found in inflammatory cells of different autoimmune disorders (Ochaion et al., 2009; Varani et al., 2011), pointing to a role for adenosine receptors as immunomodulators, with the function of offering protection as opposed to inflammation, like a "guardian angel" (Borea et al., 2016). Interestingly, an A_3 receptor agonist is under evaluation in phase II/III clinical trials for patients with moderate to severe plaque psoriasis (David et al., 2012).

Conclusions and perspectives

On the basis of the new knowledge reported in the article by Andrés et al. (2017), we are now beginning to understand the molecular adenosinergic machinery that underlies fundamental events in keratinocyte biology. This study opens new avenues for the investigation and development of new therapies for hyperproliferative skin diseases, such as psoriasis, based on the intriguing dual roles of the A_{2A} and A_{2B} adenosine receptors in modulating keratinocyte proliferation and on altered adenosine receptor expression in psoriatic epidermis. In particular, future studies will assess whether the best therapeutic approach is related to A_{2B} agonists/A_{2A} antagonists to decrease keratinocyte proliferation or to A_{2A} agonists to reduce inflammation. Furthermore, the modulation of adenosine receptor expression by inflammatory cytokines in psoriasis could be monitored under the influence of existing therapeutic approaches in order to clarify their roles in remissions and relapses of psoriatic pathology.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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See related article on pg 179

sm"FISH"ing for Hedgehog

Michael L. Drummond[1] and Scott X. Atwood[1,2]

Patched (Ptch) receptors are critical negative regulators of Hedgehog signaling, where *Ptch1* loss causes basal cell carcinoma and *Ptch1;Ptch2* loss disrupts skin and hair follicle development. Adolphe et al. use single molecule fluorescent in situ hybridization to show quantitatively that Ptch receptors create a Hedgehog signaling gradient that may specify hair follicle development.

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The Hedgehog (Hh) pathway is an evolutionarily conserved signaling mechanism that allows graded responses from cells and tissues to control fundamental biological processes such as cell fate specification, tissue patterning, regulation of proliferation, and maintenance of tissue homeostasis for developing and adult organisms. Hh pathway activation is also essential for proper development of the skin and its appendages, where overactivation of the pathway can lead to hyperproliferation of the epidermis, defects in differentiation, and cancer (Adolphe et al., 2014; Atwood et al., 2014). However, the relationship between levels of Hh pathway activation and tissue development and disease is unclear, because precise quantitative measurements are lacking.

Patched1 (Ptch1) and, to a lesser extent, patched2 (Ptch2) are transmembrane receptors that act to inhibit Hh pathway activation in vertebrates. Ptch1 acts by keeping the G-protein coupled receptor Smoothened out of the primary cilium, which prevents the Gli transcription factors (Gli1 and Gli2) from entering the nucleus. On Hh ligand binding to Ptch1, Smoothened relocalizes to the primary cilium, allowing the Gli proteins to enter the nucleus and activate Hh target genes that include *Ptch1* and *Gli1*. As the *Ptch1* transcript is upregulated, newly formed Ptch1 protein binds and internalizes available Hh ligand, thus attenuating Hh signaling events. Mutations that inactivate Ptch1, or activate Smoothened, lead to constitutive Hh pathway activation that is able to initiate and drive the growth of select cancers, including basal cell carcinoma (BCC) (Atwood et al., 2014). Ptch2 may act redundantly with Ptch1, but its role in Hh pathway regulation is yet to be fully elucidated.

Although both *Ptch1* and *Ptch2* are expressed in the developing hair follicle, *Ptch1* seems to be the prominent regulator of Hh signaling, as the authors have shown previously that its removal is sufficient to drive hyperplastic growth, whereas loss of *Ptch2* does not (Adolphe et al., 2014). Concomitant loss of *Ptch1* and *Ptch2* leads to more severe neoplasias, with features resembling human BCCs. Although multiple investigative groups suggest that the severity of phenotypes observed in skin with constitutively active Hh signaling depends on Hh activation levels, precise spatiotemporal quantitative measurements are lacking (Epstein, 2011; Grachtchouk et al., 2003). Adolphe et al. (2017) extend their own work by precisely quantifying Hh transcript levels of wild-type, *Ptch1*-deficient, and *Ptch1;Ptch2*-deficient skin using single molecule RNA fluorescent in situ hybridization (smFISH), and they have found a gradient of Hh pathway activation that may specify hair follicle progenitor cells and BCC growth.

smFISH quantifies Hh signaling levels at precise locations within mouse skin

smFISH uses fluorescently labeled oligonucleotide probes, combined with fluorescent microscopy, to visualize individual mRNA molecules (Femino et al., 1998). This technique allows for precise counts of transcripts within single cells, an important issue as appreciation of cellular heterogeneity within a tissue grows. Another advantage of smFISH is the quantification of transcripts at their precise cellular localization, which gives information on when and where a protein is translated to potentially create signaling gradients and restrict protein function to exact cellular locations (Crosetto et al., 2015).

Adolphe et al. (2017) use smFISH in the developing mouse skin to quantify *Gli1* and *Ptch1* transcripts, an accepted proxy for Hh signaling levels. They observed an increase in Hh signaling within the interfollicular epidermis and hair follicle in *K5Cre:Ptch1*$^{lox/lox}$ mice. Although the same increase was not

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