The RATIOnal Role of Polyamines in Epidermal Differentiation

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Polyamines have been implicated in skin tumorigenesis; however, their role in epidermal homeostasis remains obscure. In a new article in the *Journal of Investigative Dermatology*, Rahim et al. (2021) report that keratinocyte differentiation requires a shift in polyamine ratios that is mediated by AMD1. Results suggest that targeting polyamine availability might be useful in the treatment of hyperproliferative skin disorders.


Polyamines regulate gene expression in epidermal differentiation

Polyamines are polycations that are essential for cell growth, regulating various stages of gene expression (Pegg, 2016). First, polyamines bind to negatively charged molecules, such as DNA and RNA, influencing their structure and stability. Second, polyamines are stably associated with ribosomes. Third, the polyamine spermine is required to post-translationally modify the translation initiation factor eIF5A. Finally, polyamines influence the epigenetic regulation of gene expression through the modulation of histone methylation and acetylation. Polyamine homeostasis is critical for cell survival and is achieved by the regulation of their synthesis, catabolism, and transport.

In a new article in the *Journal of Investigative Dermatology*, Rahim et al. (2021) demonstrate that keratinocyte (KC) differentiation depends on controlled changes in polyamine levels to modulate gene expression and drive cellular behavior changes.

Previously, changes in polyamine levels have been implicated in skin conditions such as psoriasis and different types of skin cancer (Broshtilova et al., 2012). Rahim et al. (2021) investigate the role of polyamines in epidermal homeostasis and identify AMD1 as a crucial regulator of KC differentiation. The authors show that AMD1 protein is abundant in differentiating KCs of the human epidermis and then confirm that AMD1 is upregulated during differentiation in vitro. This occurs without increased mRNA expression but rather through elevated translation of the AMD1 mRNA. The authors demonstrate that AMD1 is required for proper differentiation, using genetic or pharmacological inhibition of AMD1. Of note, this can be rescued by a supplementation with spermidine and spermine. To better understand underlying regulatory mechanisms during differentiation, the transcriptome was analyzed after AMD1 inhibition and rescue with spermidine and spermine. Intriguingly, a large proportion of genes that change with differentiation are AMD1 dependent, and it is shown that AMD1 and thus the polyamines control key transcription factors and signaling molecules that drive KC differentiation, including KLF4 and ZNF750. However, how the shift in polyamine ratios controls these gene expression changes remains unknown.

The results of Rahim et al. (2021) emphasize that different polyamine species have distinct roles in epidermal homeostasis. Whereas high putrescine levels correlate with high proliferation in basal cells of the epidermis, reduced putrescine abundance and increased spermine levels, achieved by elevated AMD1 expression, are required for KC differentiation. These data also demonstrate the context-dependent regulation of ODC and AMD1 expression (Figure 1). Because high spermidine and spermine levels induce the expression of SSAT, the acetylated polyamines N1-acetyl spermidine and N1-acetyl spermine are likely to accumulate when AMD1 is elevated. Although acetylated polyamines are mostly targeted for elimination from the cell, they might also play a role in epidermal homeostasis, which would be an interesting point for future investigation.

Increased putrescine availability favors hyperproliferation

Polyamine homeostasis is critical for cellular function and is achieved by the regulation of their synthesis, catabolism, and transportation. If control goes awry, the resulting changes in polyamine levels can have detrimental effects. For example, increased polyamine catabolism results in the depletion of spermidine and spermine and a total arrest in growth (Mandal et al., 2013). Consistently, elevated polyamine synthesis leads to increased proliferation as has been documented in tumors decades ago (Russell and Snyder, 1968).

High polyamine levels have also been implicated in skin conditions such as psoriasis and skin cancer (Broshtilova et al., 2012). In fact, elevated ODC expression in the outer root sheath of the hair follicle of transgenic mice affects epidermal tumor growth (O’Brien et al., 1997). This was investigated using carcinogen-mediated tumor initiation followed by repeated treatments with compounds driving hyperplasia and tumor promotion in initiated cells (Abel et al., 2009). ODC overexpression was sufficient to elevate proliferation after initial carcinogen treatment. The new results by Rahim et al. (2021) suggest that the three-fold...
Clinical Implications

- Different polyamine species have distinct cellular functions.
- High putrescine levels are detrimental in hyperproliferative disorders such as psoriasis or cancer.
- Manipulation of polyamine ratios by AMD1 is a potential therapeutic intervention.

increase in putrescine in the epidermis of ODC-overexpressing mice (O’Brien et al., 1997) was responsible for the increased proliferation. Consistent with this hypothesis, Weeks et al. (1982) showed that intraperitoneal injections of putrescine in combination with tumor-promoting agents increase tumor burden in mice. In summary, these studies suggest that the accumulation of putrescine correlates with hyperproliferation in skin carcinogenesis.

Modulation of polyamine availability

Because high polyamine levels are associated with cancer, lowering polyamine availability is a potential cancer therapy. Targeted development of polyamine metabolism inhibitors initiated after methylglyoxal-bisguanaylhydrazone, which was utilized in leukemia, was shown to inhibit AMD1 (Williams-Ashman and Schenone, 1972). Subsequently, 2-difluoromethylornithine (DFMO), which is an irreversible ODC inhibitor, was developed. Although DFMO suppresses skin carcinogenesis in mice by blocking proliferation in the tumor promotion phase (Weeks et al., 1982), it was ineffective in patients with leukemia or brain tumors (Gerner and Meyskens, 2004). An alternative to the inhibition of polyamine biosynthesis is the activation of polyamine catabolism to reduce intracellular polyamine pools. Polyamine analogs activate SSAT and consequently reduce the levels of natural polyamines, whereas the acetylated forms accumulate. Unfortunately, polyamine analogs showed no therapeutic benefit in clinical trials (Gerner and Meyskens, 2004).

AMD1 is an interesting rate-limiting enzyme in the polyamine biosynthesis pathway (Figure 1). Whereas ODC limits the production of putrescine, the AMD1 product decarboxylated S-adenosyl methionine (dcSAM) is required for the synthesis of spermidine and spermine from putrescine. Therefore, targeting AMD1 is a plausible approach to modulate polyamine ratios. Inhibition of AMD1 has been pursued as a potential strategy in cancer therapy and psoriasis treatment. Given that these diseases correlate with high polyamine concentrations, inhibition of AMD1 was a reasonable approach. However, the results of Rahim et al. (2021) indicate that AMD1 function might counter hyperproliferative skin diseases such as psoriasis and skin cancer by promoting differentiation. The authors suggest that high putrescine levels are required for proliferation, whereas decreased putrescine and increased spermine levels, as a result of elevated AMD1 expression, drive differentiation (Figure 1). Consistent with this notion, AMD1 activity is only minimally changed in papillomas, whereas ODC activity is considerably increased (Weeks et al., 1982). The consequent change in polyamine ratios toward increased putrescine levels correlated with papilloma size and growth (Weeks et al., 1982).

AMD1 regulation in the treatment of hyperproliferative skin disorders

To date, only the inhibition of polyamine biosynthesis has been investigated as a therapy in the context of hyperproliferative diseases. However, this approach ignores the fact that different polyamine species play distinct roles in cellular function. With their study, Rahim et al. (2021) demonstrate that a shift in polyamine ratios is required for KC differentiation. The authors shed new light on AMD1, which reduces putrescine levels to drive epidermal differentiation. Therefore, AMD1 activation rather than its inhibition might be beneficial in hyperproliferative diseases. AMD1 activation might be achieved by small molecules, such as putrescine analogs, or through the rewiring of AMD1 gene expression. Because AMD1 connects methionine metabolism to polyamine biosynthesis, close monitoring of methionine and SAM levels will be required to avoid toxic side effects. Overall, the study by Rahim et al. (2021) suggests that the rationale behind the treatment of hyperproliferative skin disorders has to be reconsidered. It is not the accumulation of polyamines per se but the ratio between the different polyamine species that matters in epidermal homeostasis and disease.

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CONFlict of INTEREST

The authors state no conflict of interest.
The skin’s major function is to act as a physical barrier between the human body and the external environment. It prevents invasion by foreign pathogens. Whereas diverse commensal microorganisms reside on the skin and contribute to protection, other species such as the fungus *C. albicans* may cause infections (Kashem et al., 2015). *C. albicans* is an opportunistic commensal largely found in the skin and mucous surfaces. It may become pathogenic when host defenses are compromised. Recent studies indicate that metabolites released into the extracellular environment, such as eATP and its main metabolite ADO, may actively modulate adaptive immune responses (Proietti et al., 2014). In their new article in the *Journal of Investigative Dermatology*, Zhang et al. (2021) assess the role of eATP release by *C. albicans* in the host immune response, in light of their previous work on T helper (Th)17 differentiation at mucosal sites during *C. albicans* infection (Igártó et al., 2011; Kashem et al., 2015; Zhang et al., 2021). The authors show that the release of eATP is variable among various strains of *C. albicans*, suggesting that there is a genetic control and that low levels of adenosine triphosphate (ATP) correlate with high infectivity.

### Innate response and innate recognition of *C. albicans*

Cutaneous and mucocutaneous immunity to *C. albicans* requires Th17 differentiation initiated by the recognition of filamentous *C. albicans* by innate immune receptors. The pattern recognition receptors, toll-like receptor (TLR) 2 and TLR4, and the C-type lectin receptors, dectin-1/2/3, detect the presence of phosphopilomannan, mannosyl proteins. α-Mannan induces the production of proinflammatory cytokines IL-1, IL-6, and IL-23 through Myd88 and TRIF adaptor molecules, NLRP3-dependent inflammasome, and NF-κB signaling that is required in the development of the inflammatory Th17 adaptive immune response. In the absence of dectin, filamentous forms may induce Th1 response without Th17 (Kashem et al., 2015; Wang et al., 2019).

Kashem et al. (2015) have shown that Th17 induction by *C. albicans* yeast through a dectin-1/IL-6-dependent mechanism is mediated by an interaction with Langerhans cells (LCs), whereas CD11b+ dermal dendritic cells failed to induce Th17 mainly owing to a lack of the hyphae forms found in the dermis. Although there are many redundant sources of IL-1β and TGFβ in

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