Clinical Implications

- Aspirin protected against UV-induced damage and inflammation in vitro and in vivo.
- Aspirin attenuated UV-induced keratinocyte carcinogenesis but did not impact UV-induced melanoma formation in mice.
- Through its anti-inflammatory properties, aspirin may be useful as a UV-protective agent.

could perhaps be incorporated into sunscreens?

Scientifically, the findings of Rahman et al. (2020) raise many questions. Does aspirin somehow prevent UV-mediated photoprotecton development or does it also impact their repair? There are indications from the data in this paper that it might do both. Also, why didn’t aspirin protect against melanoma when it reduced photoprotecton levels in Melan-A melanocytes? Was the lack of melanoma prevention because of the particular animal model that was used? In fact, one might expect that especially in melanocytes aspirin would be expected to lower CPDs caused by free radical–derived triplet state melanin (so-called dark photoproducts) because of its effects on reducing UV-induced ROS (Premi et al., 2015). I look forward to future work to clarify these issues and to possibly develop the use of anti-inflammatory agents in UV protection and skin cancer prevention.

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CONFLICT OF INTEREST

The author states no conflict of interest.

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See related article on pg 152

Phenformin: AMP(K)ed for Potential Repurposing

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The results in the article by Zhou et al. (2020) demonstrate that the antidiabetic drug phenformin inhibits skin tumor growth and promotes keratinocyte differentiation, and an underlying mechanism is also defined. In this commentary, additional potential mechanisms through which phenformin may exert its antimutogenic effect are described. Thus, the proposed repurposing of phenformin to treat skin cancer has merit.


Skin cancer is the most common cancer in the United States, with about 3 million Americans affected by nonmelanoma skin cancer (NMSC) each year (https://www.aad.org/media/stats-skin-cancer). The two most common forms of NMSC are basal cell carcinoma and squamous cell carcinoma (SCC). With early detection and proper treatment, NMSCs have good prognoses and survival rates. However, this cancer can have a significant impact on QOL, and aggregate effects on mortality can be significant. Therefore, it is important to discover and implement new therapies to treat these skin cancers.

The article by Zhou et al. (2020) proposes the use of phenformin, a biguanide drug originally developed to lower blood glucose levels in patients with diabetes, for the treatment of NMSCs. These authors show that oral administration of phenformin abrogates the growth of developed tumors in the 7,12-dimethylbenzanthracene (DMBA)-12-O-tetradecanoylphorbol-13-acetate (TPA) initiation–promotion two-stage mouse model of tumorigenesis. Further investigation into the possible mechanisms by which this inhibition occurs demonstrated that phenformin promotes the suspension-induced...
differentiation of human keratinocytes (KCs) through AMP-activated protein kinase (AMPK)–mediated and calcineurin-mediated activation of the transcription factor nuclear factor of activated T cell (NFAT)-c1 (NFATc1) pathway (Zhou et al., 2020). Knockdown of AMPK or calcineurin B1 prevents the differentiating effect of phenformin, as do inhibitors targeting NFAT (VIVIT) or calcineurin (cyclosporin A). Phenformin also induces KC differentiation marker protein expression in cyst formation assays with human KCs grafted onto nude mice (Zhou et al., 2020). These data support the development of phenformin for the treatment of skin diseases characterized by hyperproliferation and abnormal differentiation.

Zhou et al. (2020) have suggested that phenformin, by triggering KC differentiation through the AMPK-NFATc1 pathway, might be used to treat NMSC. However, whether or not this is the only mechanism by which phenformin functions to induce KC differentiation and inhibit skin tumor growth is as yet unknown. Phenformin is an analog of the commonly used antidiabetic drug metformin, and numerous studies indicate several potential mechanisms by which metformin may protect against skin and other cancers (reviewed in Cao and Wan [2009]). Like phenformin, metformin activates AMPK, and

**Clinical Implications**

- Phenformin inhibits skin tumorigenesis by activating AMP kinase to promote keratinocyte differentiation.
- Phenformin likely works through multiple pathways to inhibit skin cancer formation and/or growth.
- Topical phenformin might be developed clinically as a treatment for skin cancers.

**Figure 1.** Mechanisms of phenformin and another biguanide antidiabetic drug, metformin, to promote KC differentiation and inhibit skin cancer growth. (a) Illustrated are the known mechanisms by which phenformin triggers KC differentiation and reduces skin cancer growth. Blue color coding represents stimulated pathways, and red represents inhibited pathways. (b) Shown are the pathways that are activated (blue color coding) or inhibited (red color coding) by metformin and would be predicted to induce KC differentiation and/or inhibit skin cancer formation and progression; these mechanisms are likely also utilized by phenformin. AKT, protein kinase B; AMPK, AMP-activated protein kinase; ERK, extracellular signal–regulated kinase; KC, keratinocyte; MDSC, myeloid-derived suppressor cell; NFAT, nuclear factor of activated T cells.
AMPK activation is reduced in mouse and human SCC (Wu et al., 2013), suggesting that AMPK may be a tumor suppressor in KCs. Indeed, AMPK can activate multiple downstream pathways to promote DNA repair, inhibit skin cell growth, and reprogram tumor microenvironments, all of which would be predicted to be beneficial in inhibiting skin tumorigenesis.

Metformin promotes DNA repair through AMPK. AMPK is activated in response to UVB radiation, and activators of AMPK, including metformin, accelerate the repair of UVB-generated cyclobutane pyrimidine dimers (Wu et al., 2013). Consistent with a role for AMPK in DNA repair, AMPK deletion impairs DNA repair after UVB exposure likely because the deletion of AMPK is associated with a decrease in the XPC protein (Wu et al., 2013) that is important for nucleotide excision repair. Indeed, metformin was found to increase XPC expression and reduce UVB-induced skin tumorigenesis in mice (Wu et al., 2013). These results suggest that DNA repair is dependent on the AMPK pathway (through XPC) to protect against skin cancers. Because UV exposure leading to DNA mutation is a key risk factor for the development of NMSCs, these results suggest that AMPK activation should protect against these skin cancers. This same study found that metformin decreased cell proliferation through an AMPK-independent reduction of extracellular signal–regulated kinase (ERK) pathway activation (Wu et al., 2013), indicating that there may also be a separate AMPK-independent mechanism (or mechanisms) by which metformin acts to inhibit skin tumorigenesis.

Metformin also reduces the growth of SCC tumors through the mammalian target of rapamycin (mTOR)/protein kinase B (AKT) signaling pathway. A recent study showed that the reductions in tumor growth of human cutaneous SCC xenografts in mice treated with metformin are associated with increased AMPK expression and decreased mTOR/AKT signaling pathway activity (Chaudhary et al., 2012). These changes are consistent with the finding that AMPK activation is increased and mTOR complex (mTORC) activation is reduced upon UV exposure, thereby inhibiting cellular survival (and promoting growth arrest or apoptosis) (reviewed in Cao and Wan [2009]). In addition, a study on the effects of metformin and rapamycin on epidermal hyperplasia found that a combination of metformin and rapamycin produced greater inhibition of mTORC than either agent alone. The net result was a reduction in the growth of premalignant papillomas and SCC in mice in the DMBA-TPA model of tumorigenesis (Checkley et al., 2014). This finding suggests that the AMPK-mediated inhibition of mTORC is also important for protecting against NMSCs. Other studies have found that metformin can reduce the growth of SCC tumors by inhibiting NF-κB transcription factor to induce cell death and by decreasing ERK/p38 MAPK and AKT signaling to inhibit cell proliferation and survival (Chaudhary et al., 2012; Wu et al., 2013). Thus, there are multiple pathways through which metformin-activated AMPK can inhibit cell proliferation and tumor growth.

Metformin can also reprogram tumor microenvironments by reducing the number of myeloid-derived suppressor cells (MDSCs) in tumors. MDSCs are immature myeloid cells that inhibit T-cell responses, thereby protecting the tumor from the immune system. Metformin was found to block the migration of MDSCs into tumors in patients with esophageal cancer by activating AMPK1 (Qin et al., 2018). Moreover, activation of AMPK1 inhibits the secretion of the inflammatory chemokine CXCL1, which was found to increase MDSC recruitment into tumors in patients with esophageal cancer (Qin et al., 2018). Indeed, patients with advanced-stage cancer with lymph node metastasis have higher levels of CXCL1 expression, and these higher levels of CXCL1 expression are associated with poor prognosis (Qin et al., 2018). By extension, it seems likely that reducing the levels of CXCL1 would inhibit MDSC recruitment into NMSC tumors as well. The ability of metformin to inhibit CXCL1 production in human epidermal KCs treated to model psoriasis (Tsujii et al., 2020) also suggests that metformin might be protective in skin cancer. In KCs stimulated with TNF-α and IL-17a to mimic psoriasis, metformin decreases the levels of CXCL1 and other proinflammatory chemokines by modifying the secretion of mature IL-1β generated by the NLRP3 inflammasome (Tsuji et al., 2020). In particular, metformin inhibits caspase-1 activity through AMPK to prevent the cleavage of pro–IL-1β to mature IL-1β (Tsuji et al., 2020). Taken together, these results indicate that metformin-activated AMPK reduces the number of MDSCs in tumors and inhibits cancer-promoting inflammation and imply that metformin and/or phenformin may be useful for the treatment of patients with NMSC.

On the basis of these studies, we conclude that metformin has various mechanisms that allow it to act as a chemoprotective agent (Figure 1). There have been several previous studies that demonstrate that metformin protects against cancer development. A study of 16,237 patients with type II diabetes characterized as metformin ever-users or never-users showed that metformin users had a lower skin cancer risk (Tseng, 2018). The fact that phenformin is an analog of metformin and also inhibits KC proliferation and induces differentiation through AMPK (Zhou et al., 2020) suggests that phenformin and metformin may act through similar pathways to protect against NMSCs. However, phenformin may be more effective in improving treatment and prognosis in patients with cancer than metformin because it produces a greater decrease in the tumor-infiltrating MDSCs that are correlated with a worse prognosis (Qin et al., 2018). Phenformin is more lipophilic than metformin, and therefore, it acts independently of organic cation transporter-2 (OCT2), also known as solute carrier family 22 member 2, which is necessary for the uptake of metformin into MDSCs (Kim et al., 2017). OCT2 is important for the ability of metformin to inhibit cell proliferation in melanoma cancer cells as well (Kim et al., 2017), with relatively low expression in melanoma cell lines contributing to their resistance to metformin (Yuan et al., 2013). This result suggests possible better efficacy of phenformin than of metformin.

All of this evidence supports the proposed use of phenformin to treat NMSC.
However, it is important to acknowledge the clinical history of phenformin. Similar to metformin, phenformin was previously used for antidiabetic therapy. Currently, phenformin is rarely used because it has been associated with more severe lactic acidosis than metformin. Therefore, phenformin may be more useful as a topical agent than as a systemic agent. Phenformin also has specific physical characteristics that suggest that it might be successfully administered through a topical route. Phenformin is more hydrophobic than metformin, and it would, therefore, be predicted to better penetrate through the stratum corneum, especially with its low molecular weight (205 g/mole). In addition, as mentioned previously, whereas metformin is thought to require a specific transporter to cross membranes, and the expression of this transporter may be low or absent in certain cell types (Yuan et al., 2013), phenformin likely enters the cell in a transporter-independent manner. This suggests that phenformin may be more easily absorbed and taken up by skin cells when topically applied than metformin would be.

Although there are many available treatment options for NMSC, phenformin has the potential to enhance these therapeutic regimens. Zhou et al. (2020) have shown that phenformin can enhance the differentiation of KCs through the activation of AMPK; AMPK plays an important role in various chemoprotective pathways induced by metformin. Although the mechanisms by which phenformin acts are incompletely understood, some studies have shown that it acts like metformin and that it may be even more effective. Thus, along with induced KC differentiation and inhibited skin tumor growth, phenformin may increase DNA repair and reprogram the tumor microenvironment by decreasing the accumulation of MDSCs. Therefore, phenformin may act through multiple mechanisms and could be used in addition to current therapies to enhance the treatment of skin cancers.

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