Skin-Derived Vitamin D₃ Protects against Basal Cell Carcinoma

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UV in sunlight causes mutations that drive basal cell carcinomas. However, the incidence of these tumors plateaus with prolonged exposure, but the incidence of other skin cancers increases. Makarova et al. now show that vitamin D₃ produced in the skin by UVR protects against its oncogenic effects by inhibiting Hedgehog signaling, whereas dietary vitamin D₃ does not.


Introduction

It is well recognized that the sun’s UVR is mutagenic and causes various skin cancers. Exposure to sunlight and the risk of skin cancer are strongly correlated, and cancer prevention programs urge the importance of protection from UVR. Skin cancers that arise after exposure to sunlight differ in malignant potential and medical gravity. For instance, squamous cell carcinomas (SCCs) metastasize from the primary tumor site, and late-stage disease is associated with poor outcome. On the other hand, basal cell carcinomas (BCCs) are relatively indolent but have a very high incidence. Moreover, BCCs often occur in the face and, if left untreated, can severely disfigure patients.

An interesting incongruity has become apparent between the cumulative exposure to sunlight and the incidence of SCCs and BCCs: In contrast to SCCs, the incidence of BCCs appears to plateau: at a certain point, more exposure does not result in more cases of BCC (Rosso et al., 1996). It has been suggested that the UV-driven production of vitamin D₃ in the skin contributes to the protective effect of sunlight, explaining why the relative incidence of BCCs, as opposed to SCCs, does not increase with prolonged sunlight exposure. The Epstein group describes the use of a mouse model for UVR-driven BCCs to understand and explain this seemingly incongruous association (Makarova et al., 2017).

Hedgehog signaling in development and cancer

The developing embryo is shaped by gradients of molecules known as morphogens. The concentration, time of exposure, and combinations of the morphogens to which cells are exposed in a morphogenetic field will determine their fate and identity and sculpt tissues. One of the most prominent morphogen families to shape developing organisms is that of the Hedgehog proteins. Gradients of Hedgehog pattern the digits, face, gastrointestinal organs, central nervous system, and many other tissues and organs. This early role of Hedgehog signaling is matched by its role in the maintenance of various adult stem cell niches, including those in the skin and hair follicles. The widespread contribution of Hedgehog signaling to the developing embryo and stem cell niches is also reflected in the number of cancer types suspected or known to be driven by aberrant activation of the Hedgehog response. These include tumors of the skin and medulla and those forming in the derivatives of the foregut.

The Hedgehog signaling pathway is complex, and large gaps exist in our understanding of its regulation. In the absence of Hedgehog ligand, the putative transporter Patched inhibits the G-protein coupled receptor Smoothened, thus keeping the Hedgehog pathway inactive by a still poorly understood mechanism. Soon after its identification as the main receptor for Hedgehog ligand, Patched was found not to bind directly to Smo but rather to inhibit Smoothened via a catalytic mechanism (Taipale et al., 2002). Patched is a member of a family of transport proteins present in both prokaryotes and eukaryotes. As for most members of this family, the cargo of Patch is not known. Later work from us and others showed that Patched can act non-cell autonomously to inhibit Smoothened by the secretion of endogenous sterol-like molecules, most notably (non-hydroxylated) vitamin D₃ (Bijlsma et al., 2006; Linder et al., 2015; Roberts et al., 2016).

Vitamin D₃ is synthesized in the skin from 7-dehydrocholesterol by UVR. It is then converted in the kidneys and liver to yield hydroxylated vitamin D₃. In contrast to the classical activities of vitamin D₃ like bone calcification, this hydroxylation is not required for the inhibition of Smoothened. The biological precursor to 7-dehydrocholesterol is lathosterol, the conversion of which is mediated by sterol-C5-desaturase (Sc5d). In turn, 7-dehydrocholesterol is the precursor to cholesterol, a reaction catalyzed by 7-dehydrocholesterol reductase (Dhcr7). Targeting these enzymes allows indirect manipulation of vitamin D₃ levels: ablation or inhibition of Sc5d will lead to a depletion of 7-dehydrocholesterol and thus vitamin D₃, whereas inhibition of Dhcr7 leads to an accumulation of 7-dehydrocholesterol and increased levels of vitamin D₃. Humans lacking Dhcr7 display congenital abnormalities characteristic of decreased levels of Hedgehog signaling, an observation supporting the notion that (a derivative of) 7-dehydrocholesterol inhibits Smoothened.

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Endogenous inhibitors of Hedgehog signaling are effective against BCC

BCCs are prime examples of a tumor type in which the pathway components that regulate Hedgehog signaling act as proto-oncogenes and tumor suppressors. The oncogenic mutations responsible for BCC growth are well characterized: mutations in Patched that impede its catalytic inhibition of Smoothened and activating mutations in Smoothened that desensitize it to inhibition by Patched are two main causes of BCC (Aszterbaum et al., 1999; Hahn et al., 1996; Xie et al., 1998). The critical role for Hedgehog pathway activation in BCC formation and the known Hedgehog-suppressive effects of vitamin D3 imply that vitamin D3 could be effective against BCC.

The authors had previously observed that topically applied vitamin D3 is effective against BCC and showed that at least part of this effect was mediated by the inhibitory effect of vitamin D3 on the Hedgehog pathway (Tang et al., 2011). In the current article the authors take this observation further and prove that the same UVR exposure that drives BCC formation is also the UVR that generates tumor-suppressive vitamin D3 (Makarova et al., 2017). They do so by making use of the fact that the female heterozygous Patched mutant mice make vitamin D3 in UVR-exposed skin, whereas their male counterparts do not. It was observed that the mice that are able to synthesize vitamin D3 are protected from BCCs that typically arise in response to UVR. This was further supported by the use of conditional Sc5d^oe/oe mice, which cannot produce vitamin D3 in their skin and had much higher incidence of BCCs after UVR exposure. In addition, the authors found that UVR-generated or topically applied (non-hydroxylated) vitamin D3 was effective against BCCs but that dietary vitamin D3 was not. This is presumably due to the rapid hydroxylation of orally ingested vitamin D3, yielding a form of vitamin D3 that cannot inhibit Smoothened.

In summary, the authors provide evidence supporting the notion that the nonlinear relationship between high cumulative sunlight exposure and the incidence of BCC is caused by the increased local availability of vitamin D3 (see Figure 1). However, the intriguing idea that the increased vitamin D3 concentration directly affects Smoothened activity remains to be answered and differentiated from signaling by hydroxylated vitamin D3 through its canonical receptor (VDR), which also drives potent anti-tumor signaling. It is nevertheless tempting to speculate that local availability of vitamin D3 indeed inhibits BCCs through the same mechanism as does topical application of Smoothened inhibitors such as vismodegib and cyclopamine. Another question pertains to clinical applicability; if dietary vitamin D3 does not yield high systemic levels of non-hydroxylated vitamin D3, how can we achieve concentrations that are sufficiently high to inhibit Smoothened in populations at risk of developing BCC without exposing them to the very same sunlight that is oncogenic? Topical use of pharmacological inhibitors of, for instance, Dhcr7 could be considered, but this of course risks perturbing synthesis of important downstream sterols.

Clinical Relevance

- The authors show that vitamin D3 generated in the skin by UVR suppresses basal cell carcinoma growth.
- Basal cell carcinomas are commonly driven by an aberrantly activated Hedgehog pathway, and the tumor-suppressive effects of vitamin D3 are—at least in part—through inhibition of this pathway.
- These findings may explain why the incidence of basal cell carcinoma does not show a linear correlation with exposure to sunlight.

Figure 1. Schematic summary of the authors' findings. The sun's UVR is responsible for the synthesis of vitamin D3 in the skin and the mutations that drive skin cancer. For basal cell carcinoma, these have counteracting effects on tumor progression.
(cholesterol). If indeed vitamin D₃ synthesized in the skin because of UVR exposure is a significant tumor-suppressive mechanism, it can be hypothesized that inhibition of HMG CoA reductase by statins, thereby lowering levels of 7-dehydrocholesterol, will in fact exacerbate tumor incidence, but to our knowledge this has not been shown.

It is apparent that our current understanding of the relationship between developmental signaling, cancer, and the non-canonical actions of sterol-like molecules is insufficient to translate to clinical practice. However, articles such as those discussed here provide the small steps required to get there, hopefully enabling better prevention and treatment of the most common skin cancer in the near future.

**CONFLICT OF INTEREST**  
MFB has received research funding from Celgene. This party was not involved in drafting of the manuscript.

**REFERENCES**  


Reconstructing skin defects is a challenge for physicians and scientists. An aging population and a rise in comorbidities, such as diabetes, peripheral vascular disease, and obesity, have resulted in an increased prevalence of chronic wounds. Skin defects, from trauma and surgical resections for malignant and benign lesions as well as disorders such as epidermolysis bullosa, add to the need for skin regeneration. In addition, the health care costs related to such diseases are huge. In the United States, $25 billion are spent each year on the treatment of chronic wounds alone (Sen et al., 2009). Research described by Yamauchi et al. (2017) demonstrates potential for treating such conditions by reconstituting skin with differentiated multilineage-differentiating stress-enduring (MUSE) cells.

**MUSE cells demonstrate the potential to circumvent certain limitations of ESCs and iPSCs for skin regeneration, and the translational implications of this work are exciting.**

Recent advances in stem cell biology, genome editing capabilities, and skin grafting techniques hold promise for treating soft tissue defects. Cells from different origins can be harvested and either genetically engineered or induced to become various cell types to reconstitute skin (Sun et al., 2014). Guenou et al. (2009) documented formation of stratified epidermis using keratinocyte progeny of human embryonic stem cells. Subsequent research by Itoh et al. (2013) reconstituted skin from fibroblasts and keratinocytes that were derived from induced pluripotent stem cells (iPSCs). Now work by Yamauchi et al. (2017) has recapitulated this skin reconstitution with MUSE cells differentiated into melanocytes, fibroblasts, and keratinocytes.

MUSE cells were first reported by Kuroda et al. as stress-tolerant adult human stem cells capable of differentiating into the three germ layers. They

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**A MUSE for Skin Regeneration**

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With a rise in the prevalence of chronic wounds and other soft tissue defects, there is an urgent need to regenerate skin. Multilineage-differentiating stress-enduring cells were identified as distinct pluripotent stem cells in mesenchymal cell populations in humans. New research demonstrates the ability to effectively differentiate multilineage-differentiating stress-enduring cells into fibroblasts and keratinocytes for skin reconstitution.


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