(Olsen et al., 2015). Such correlation demonstrates the effectiveness of physical sun protection as a valuable preventative method against a higher tumor burden in patients with BCNS despite their strong genetic risks.

DNA repair mechanisms may be useful therapeutic targets for genomically instable BCCs

Chiang et al also examined the mutation spectra in sporadic tumors and found that the average baseline mutation rate was 254.6 mutations/tumor with a whopping 736 UV signature mutations/tumor. When looking for causes of this high mutation load, the authors found more mutations in genes involved in DNA checkpoint repair and genome stability with 5.9 mutations per sporadic BCC versus 1.9 mutations per BCNS BCC. Genes that were highly or differentially affected included *ATM*, *BRCA2*, *MSH2*, and *TP53*. Although these mutation numbers align with the overall mutation loads in the two types of BCC, the real consequence is likely a more unstable genome. Genome stability is critically important to cells, where DNA repair mechanisms often work redundantly to preserve the fidelity of the genome (Kelley et al., 2014). If an alternative repair pathway is disrupted through mutations, impairing critical steps in the main repair pathway can force cells to use inadequate backups that result in accumulation of additional mutations and cell death. Current therapies, such as PARP inhibition, are effective in *BRCA*-deficient breast cancers or *ATM*-deficient glioblastomas and use this principle of synthetic lethality. PARP inhibition forces the cell to abrogate the base excision repair pathway, a main DNA repair mechanism, causing accumulation of single- and double-stranded DNA breaks. BRCA and ATM are critical components of the homologous recombination repair pathway that would normally take over to fix double-stranded breaks; however, *BRCA*- or *ATM*-deficient cells are forced to use the error-prone nonhomologous end-joining repair pathway that cannot handle double-stranded breaks, resulting in cell death. As *ATM* and *BRCA2* are differentially mutated in sporadic versus BCNS BCC, inhibition of PARP, as well as other DNA repair pathways (Kelley et al., 2014), may be a viable therapeutic option (Figure 1b).

CONFLICT OF INTEREST

The authors state no conflict of interest.

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Sex Matters: Interfering with the Oxidative Stress Response in Pachyonychia Congenita

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Pachyonychia congenita is an incurable and often debilitating genodermatosis. Topical application of the antioxidative response inducer sulforaphane, however, alleviates disease symptoms in a murine pachyonychia congenita model, forecasting clinical benefits. The Coulombe laboratory now reports sex-dependent differences in sulforaphane responsiveness of pachyonychia congenita mice, thereby dampening treatment expectations but also unveiling novel aspects of sex-specific oxidative stress reactivity in the epidermis.

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Introduction

We report and comment on an article by Kerns et al. (2018) that is one of a series of publications from the laboratory of Pierre Coulombe dealing with the effect of sulforaphane, a small molecule activator of the antioxidant inducer NRF2, in keratinopathies as a potential treatment option. Kerns et al. report on sex-dependent differences in responsiveness using a murine model for pachyonychia congenita (PC).

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Clinical Implications

- Sex-specific differences should be considered in the pathogenesis of pachyonychia congenita.
- Local NRF2 activation has great potential for reducing hyperkeratosis in pachyonychia congenita.
- Hormone status should be considered as a strong modulator of therapeutic NRF2 stimulation.

PC: Mutation of structural proteins leading to alterations in the oxidative stress response

PC is a very rare skin disease that presents exorbitant nail thickening and painful palmoplantar keratoderma (PPK) (Figure 1a and b). It has been linked to dominant negative mutations in the genes encoding the type II keratins K6a and K6b and their keratin type I polymerization partners K16 and K17 (http://www.interfil.org). The K6/16 pair is selectively expressed in plantar glabrous skin, nail epithelia, and ectoderm-derived appendages and is absent from interfollicular epidermis. However, K6/16 are strongly induced in epidermis upon wounding, UV irradiation, and arsenite poisoning, as well as in inflammatory and hyperproliferative epidermal disorders such as psoriasis and in cancer (Moll et al., 2008). This unique distribution pattern reflects specialized K6/16 functions in a distinct set of epidermal responses and pathologies (McGowan and Coulombe, 1998). Correspondingly, the PC phenotype differs profoundly from that observed in epidermolysis bullosa simplex, a skin disease caused by mutations in the keratin type II/I pair K5/14. Although the latter is characterized by cytolysis, the former is typified by hyperproliferation and extreme cornification. Thus, a mere structural dysfunction is unlikely the sole cause of PC pathogenesis.

A promising lead in elucidating PC pathomechanisms has been the observation of increased oxidative stress and dysfunctional NRF2 in *Krt16*$^{-/-}$ mice, a well-established PC model developing copious PPK (Kerns et al., 2016). In the wild-type situation, NRF2 is activated by oxidative stress, that is, by a reduced glutathione (GSH)/glutathione disulfide (GSSG) ratio, and involves NRF2 release from Keap1 and PKCδ-mediated phosphorylation. After nuclear translocation of NRF2, genes with an antioxidant response element are switched on, resulting in increased expression of antioxidant proteins and an increased GSH/GSSG ratio. This pathway is linked to K16 in several ways:

- The *Krt16* gene contains a functional antioxidant response element in its promoter region (Endo et al., 2008).
- Unlike K5/14, K16 does not sequester RACK1-bound active PKC δ (Kerns et al., 2016).
- Absence of K16 is associated with a reduced GSH/GSSG ratio (Kerns et al., 2016).
- Absence of K16 leads to a reduction of the NRF2 upstream regulators PKC δ and RACK1 (Kerns et al., 2016).
- Absence of K16 increases NRF2 levels by increased oxidative stress despite an increase in Keap1 (Kerns et al., 2016).

The overall scenario results in the peculiar situation that loss of K16 is associated with increased levels of NRF2, which, however, is not activated because of a reduction of RACK1-activated PKCδ. Taken together, loss of K16 or K16 dysfunction appears to impede NRF2-mediated antioxidant activity (Figure 1c).

Based on these findings, it was hypothesized that NRF2 activation may correct for the absence or malfunction of K16. Using the small molecule NRF2 activator sulforaphane (SF), which is contained in cruciferous vegetables such as broccoli, Brussels sprouts, and cabbages, proof of principle was obtained in support of this idea. A significant reduction of epidermal paw

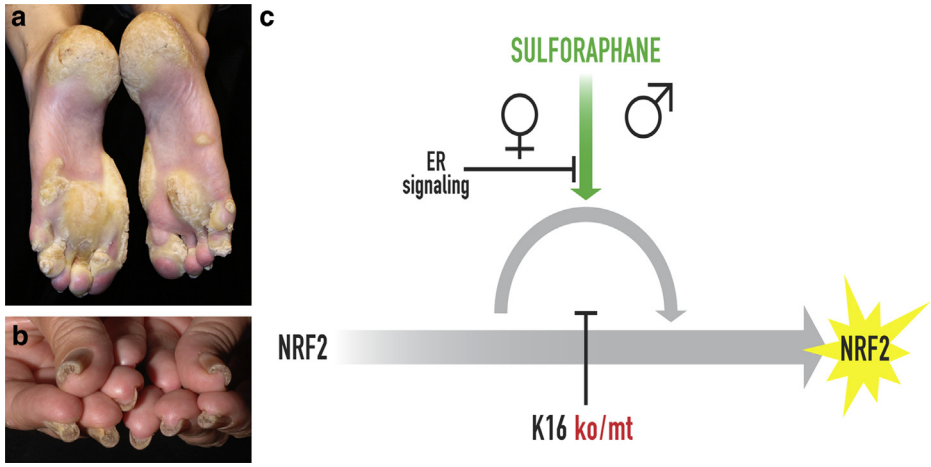

Figure 1. Images of typical PC symptoms and schematic representation of main results described by Kerns et al. (2018). (**a, b**) The images of (**a**) plantar hyperkeratosis and (**b**) nail thickening of two PC patients were kindly provided by the Pachyonychia Congenita Project (www.pachyonychia.org). (**c**) The scheme depicts the observed interference of K16 knockout (and presumably also PC-related K16 mutation) with NRF2 activation that is overcome by SF treatment in male *Krt16*$^{-/-}$ mice, resulting in reduced palmoplantar keratoderma. These beneficial effects were not observed in female *Krt16*$^{-/-}$ mice. Simultaneous treatment of SF and diarylpropionitrile, which is an estrogen receptor-α selective agonist and estrogen receptor-β antagonist, however, led to NRF2 activation and reduced palmoplantar keratoderma. ER, estrogen receptor; K, keratin; ko, knockout; mt, mutant; PC, pachyonychia congenita; SF, sulforaphane.

thickening was achieved upon local SF treatment coincident with increased ^{P}NRF2, ^{P}PKCδ, and GSH/GSSG ratio (Kerns et al., 2010).

Male and female: The challenge of unearthing the difference in ultra-rare diseases

Especially in situations of ultra-rare diseases such as PC, sex aspects are difficult to assess and are often impossible to examine systematically. The situation becomes even more complicated when considering varying hormonal status during the different life phases, especially in female individuals who experience prepuberty, puberty, adolescence with cycling hormone levels, menopause, and senescence. The use of murine models helps with investigation of the influence of these changes.

The side-by-side comparison of female and male *Krt16*$^{-/-}$ PC model mice was therefore instrumental in appreciating the relevance of sex-dependent pathogenesis and drug responsiveness. At first glance, the situation did not seem to differ between male and female PC mice. At 4 weeks, that is, at the time of symptomatic PC onset, decreased GSH and GSH-synthesizing enzymes and increased NRF2 were observed in paws of both male and female mice in the absence of elevated active ^{P}NRF2 and NRF2 target gene expression. Remarkably, males treated only with jojoba oil, which was used as vehicle for topical drug application later, developed significantly increased hyperkeratosis compared with females. Furthermore and quite unexpectedly, SF treatment increased ^{P}NRF2 levels in male but not female mice, coincident with a dramatic reduction of PPK in male mice and no reduction of PPK in female mice.

The availability of the well-characterized PC animal model subsequently allowed a very detailed temporal analysis of PPK lesion formation, showing a delay of approximately 1.5 weeks in female *Krt16*$^{-/-}$ mice. This correlated with delays in transient GSSG level increases observed in both wild-type and mutant females around the same time. How these observations relate to the onset of puberty and the first luteinizing hormone surge remains to be investigated, but they provided the impetus for experiments that uncovered a slightly premature rise in estrogen receptor-α mRNA in *Krt16*$^{-/-}$ females versus wild-type controls. Furthermore, sex-specific differences in NRF2 phosphorylation were observed in the same time interval between male and female mice.

Building on these findings, Kerns et al. (2018) hypothesized that stimulating estrogen receptor-β, which is known to positively affect NRF2 signaling and, conversely, inhibiting estrogen receptor-α activity, which has been shown to activate NRF2 signaling, might overcome the SF resistance of female mice. This hypothesis was tested using diarylpropionitrile (DPN), which is an estrogen receptor-β selective agonist and estrogen receptor-α antagonist. As predicted, the combination of DPN with SF induced a significant decrease of PPK and NRF2 activation and led to an increased GSH/GSSG ratio in female mice (Kerns et al., 2016).

Sex and the skin

Sex-dependent dimorphism often extends beyond the obvious, affecting all cell and tissue types in the body through complex modifications of multiple pathways. The skin is no exception. However, the affected processes remain poorly investigated. Elucidation of their mode of action, however, may lead to modification of strategies in disease management. The study by Kerns et al. (2018) adds another valuable piece to the multifaceted mosaic of sex-specific pathway regulation in epidermal cells. These insights will help alter treatment rationales in a devastating human disease and also lead to new research questions that require further investigation, as in the following examples.

How do estrogen levels modulate the oxidative response in epidermis? The observed beneficial effect of DPN on SF action in female mice remains to be further worked out. Kerns et al. (2018) suggest that DPN may exert this effect by (i) antagonizing estrogen receptor-α signaling, which may directly inhibit NRF2 activation; (ii) continuously stimulating estrogen receptor-β, which is contrary to the temporary estrogen receptor-β increase occurring physiologically in untreated *Krt16*$^{-/-}$ female paw skin; or (iii) acting in synergy with SF in an estrogen-independent fashion through anti-inflammatory or antioxidant effects. Beyond that, the presented data open up an entirely new area of research, which warrants a detailed analysis of estrogen level-dependent stress response regulation in the skin.

How does sexual dimorphism affect SF treatment in other keratinopathies? SF treatment has also proven advantageous in a *Krt14*$^{-/-}$ mouse, which mimics features of epidermolysis bullosa simplex (Kerns et al., 2007). As mentioned, however, the disease pathogenesis appears to be fundamentally different. K5/14 presumably sequesters RACK1 together with active PKCs and thereby hinders NRF2 activation (Kroger et al., 2013), whereas K16 supports PKCδ action and NRF2 target gene expression. Consistent with this, NRF2 activation is reduced in *Krt16*$^{-/-}$ paw epidermis (Kerns et al., 2016) and, conversely, NRF2 target gene expression is increased in keratinocytes lacking K5/14 (Kumar et al., 2015). Of note, however, SF-dependent K17 induction occurs in both situations (Kerns et al., 2007; 2010). Analyses of sulforaphane action in other epidermal keratinopathies have not been performed to date.

How are oxidative and other stress responses affected in the epidermis by sex- and keratin isotype-dependent differences in mitochondrial activity? A strong indication of fundamental differences in the stress response of male and female *Krt16*$^{-/-}$ epidermis was the jojoba oil-induced fulminant hyperkeratosis in male but not female mice. This may reflect multiple differences at several levels including cytoplasmic organization, organelle function, skin barrier formation, or even sensory nociception. More specifically, the importance of intermediate filaments for mitochondrial distribution, morphology, and function is generally assumed and has been shown to be relevant in the case of epidermal keratin intermediate filaments (for recent review, see Schwarz and Leube, 2016). The relevant molecular mechanisms, however, are still poorly understood and appear to be highly complex, considering the diversity in keratin expression and cell type-specific patterns of mitochondrial distribution and function. A link to sexual dimorphism has not yet been shown.

How do these findings relate to the situation in humans? A major caveat of the current findings is that they are mostly restricted to *Krt16*$^{-/-}$ mice. The murine model lacks K16, but PC patients harbor a

mutant allele together with a K16 wild-type allele. It is therefore unlikely that the human phenotype is solely determined by loss of K16 function but is further confounded by gain of toxic function through the mutant K16 polypeptides. Beyond these differences, murine skin is much thinner than human skin, indicating profound functional differences. Despite these caveats, the initial findings in human PC patients are highly encouraging: NRF2 is elevated but hypoactive in lesional plantar epidermis of male and female PC patients (Kerns et al., 2016, 2018), and a recent clinical trial of SF treatment in healthy individuals presented promising results with respect to NRF2 induction presenting a sex-dependent bias of NRF2 baseline expression and induction of K17 (Kerns et al., 2017).

Given the well-organized and dedicated group of patients and researchers through the International Pachyonychia Congenita Consortium (www.pachyonychia.org), there is well-founded hope that there will be symptomatic and curable treatments for PC available in the near future based on rational pharmacologic and genetic approaches with potential implications for other keratinopathies and skin diseases.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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A Microbiota-Dependent, STAT3-Driven Mouse Model of Cutaneous T-Cell Lymphoma

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In recent years, much has been learned about the molecular genetics of cutaneous T-cell lymphomas. Fanok et al. (2018) translate knowledge from systematic genomic and transcriptomic analyses to develop a mouse model that tests the hypothesis that activated STAT3 in CD4$^+$ T cells may be a driver of cutaneous T-cell lymphomas. The transgenic mouse that they developed exhibits clinical features of mycosis fungoides, as well as Sezary syndrome, two well-known entities in the cutaneous T-cell lymphoma spectrum. Furthermore, these authors show that TCR engagement and microbiota are required for development of the complete clinical phenotype. This mouse model, which develops progressive disease, provides a new tool to understand cutaneous T-cell lymphoma biology and to potentially test new therapies.

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Animal models of human diseases facilitate dissection of molecular pathogenesis, and also allow researchers to evaluate and optimize therapeutic strategies that are not mature enough to be tested in patients. The earliest studies using animal models of cutaneous T-cell lymphomas (CTCLs) were published more than 30 years ago, just 10 years after the term *cutaneous T cell lymphoma* was introduced in 1974 (Edelson, 2010; Piepkorn and Tigelaar, 1984). Prior to the early animal models, a large number of reports had examined the phenotype of the neoplastic T cells in mycosis fungoides (MF) and Sezary syndrome (SS) through the use of functional assays or monoclonal antibody–defined cell surface markers. Although spontaneous canine MF was proposed to be an ideal model for human CTCL based on clinical skin findings, dermal–epidermal infiltrate, immunophenotypes, TCR gene rearrangements, it is extremely low incidence and slow progression, as well as the high costs for animal care and husbandry prevented widespread application (Fivenson et al., 1994; Shadduck et al., 1978, Shadduck, 1979).

Many attempts were made to model CTCL in mice, which rarely develop a CTCL-like condition spontaneously, but are widely studied because they are relatively inexpensive to work with and

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