Illuminating Alternative Strategies to Treat Targeted Chemotherapy-Resistant Sporadic Basal Cell Carcinoma

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Sporadic and basal cell nevus syndrome basal cell carcinomas show differential response rates to Smoothened inhibitors. Chiang et al. demonstrate notable decreases in UV-induced mutagenesis, total mutation load, genomic instability, and drug-resistant mutations among basal cell nevus syndrome basal cell carcinomas using whole exome sequencing, which may explain the differences in drug response rates.

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Basal cell carcinomas (BCCs) are locally invasive epithelial tumors characterized by inappropriate activation of the Hedgehog pathway, an evolutionarily conserved signaling mechanism that controls cell proliferation, cell fate specification, tissue patterning, and tissue homeostasis in developing and adult organisms. Recently, Smoothened inhibitors (SMOis) such as vismodegib and sonidegib have been used to treat patients with advanced or multiple tumors with varying levels of success, and previous work has identified several SMOi resistance mechanisms in advanced sporadic tumors (Atwood et al., 2015; Sharpe et al., 2015). The two main types of BCC, sporadic and inherited, are indistinguishable histologically but have widely divergent SMOi response rates (Sekulic et al., 2015; Tang et al., 2016). Although both BCC types are derived from mutations that overactivate the Hedgehog pathway, it is unclear why inherited BCCs have a higher response rate to SMOi.

In the absence of Hedgehog ligand, a 12-pass transmembrane receptor Patched1 inhibits the G-protein coupled receptor Smoothened (SMO), allowing Suppressor of Fused to sequester Glioma-associated Oncogene transcription factors in the cytoplasm. Binding of Hedgehog ligand to Patched1 enables SMO to suppress Suppressor of Fused, leading to activation of Glioma-associated Oncogene and subsequent transcription of downstream target genes that are essential for the development of the skin and its appendages. Sporadic BCCs are driven predominantly by mutations that inhibit Patched1 or activate SMO in the basal layer of sun-exposed epidermis (Bonilla et al., 2016). On the other hand, inherited or basal cell nevus syndrome (BCNS) BCCs mainly originate from individuals carrying germline mutations of *Patched1*. Unlike sporadic BCC tumors that generally appear much fewer in number, BCNS BCCs may be present in tens to several hundred throughout the patient's body.

Standard surgical excision and chemotherapy are common effective methods to treat small nodular and superficial BCCs of both types (Atwood et al., 2014). However, patients with large advanced tumors or with an overwhelming number of tumors are strong candidates for SMOis. Interestingly, more than 99% of BCNS BCCs showed complete response, whereas only approximately 47% of locally advanced and 33% of metastatic BCCs from sporadic cases showed an objective response to drug (Sekulic et al., 2015; Tang et al., 2016). Chiang et al. (2017) provide a detailed genetic evaluation of sporadic and BCNS BCCs and find a surprising mutator phenotype that leads to higher genomic instability in sporadic BCCs that is largely absent in BCNS BCCs and may explain the wide variance in SMOi response rates.

SMOi remains effective for BCNS BCCs due to lack of drug-resistant mutations

Whole-exome sequencing of SMOi-sensitive and SMOi-resistant BCCs has revealed that the majority of SMOi resistance in advanced tumors is driven by *SMO* mutations that disrupt SMOi binding or suspend SMO autoinhibitory activity (Atwood et al., 2015; Sharpe et al., 2015). Only 9 of the 27 SMOi-resistant tumors were from patients with BCNS, suggesting that BCNS tumors have a lower chance of developing drug resistance. In support of this, 12 months of follow-up analysis from the ERIVANCE BCC study showed objective response rates to SMOi of 33.3% in 33 metastatic BCCs and 47.6% in 63 locally advanced BCCs (Sekulic et al., 2015), whereas an extended multicenter phase 2 36-month trial showed response rates of more than 99.9% to SMOi in more than 2,775 BCNS tumors (Tang et al., 2016). Chiang et al. took a closer look at this disparity using targeted and whole-exome sequencing of the *SMO* gene in 80 sporadic and 51 BCNS drug-naïve BCCs (Figure 1a). They found that 10% of sporadic drug-naïve tumors harbored SMOi-resistant mutations that would suspend SMO autoinhibitory activity, in agreement with other studies (Atwood et al., 2015; Bonilla et al., 2016), whereas none of the BCNS drug-naïve tumors harbored SMOi-resistant mutations. The presence of these inherent SMOi-resistant mutations in sporadic but not BCNS BCCs suggests that SMOi therapy

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Clinical Implications

- As ATM and BRCA2 are differentially mutated in sporadic versus basal cell nevus syndrome basal cell carcinoma, inhibition of PARP, as well as other DNA repair pathways, may be a viable therapeutic option.

continues to be an overwhelmingly effective strategy to treat patients with BCNS.

UV damage prevention likely impedes progression toward higher BCC burden

Prior clinical studies have associated UV exposure to increased incidence of skin cancer. Specifically, UVB signature mutations (C→T transition) were found in approximately 50% of sporadic BCCs (Rass and Reichrath, 2008). In general, UV-induced mutations are often caused by failure in nucleotide excision repair mechanisms involving genes such as DNA ligase 1, endonuclease ERCC1, or the MSH mismatch repair family. Chiang et al. explored differences in UV-induced mutations by performing whole-exome sequencing of 20 drug-naïve BCNS tumors from 16 patients with BCNS that ranged in number of BCCs at baseline. This group of patients was representative of a larger cohort of patients that showed a bimodal distribution of BCC burden, where low-burden patients had less than 51 tumors and high-burden patients had more than 51 tumors. Surprisingly, tumors from low-burden and high-burden patients displayed a nearly identical baseline number of non-UV mutations at 145.7 versus 144.5 mutations/tumor, respectively. The real difference came from the UV signature mutations, where low-burden patients had 104.5 mutations/tumor, whereas high-burden patients had 306.9 mutations/tumor. The relative stability of baseline mutations between low and high tumor burden populations suggests that UV-induced mutations are likely the root cause of tumor number expansion in high-burden patients. Interestingly, the lower UV-induced mutation frequency was inversely correlated with a survey response of more frequent sunscreen usage, which is consistent with other clinical findings

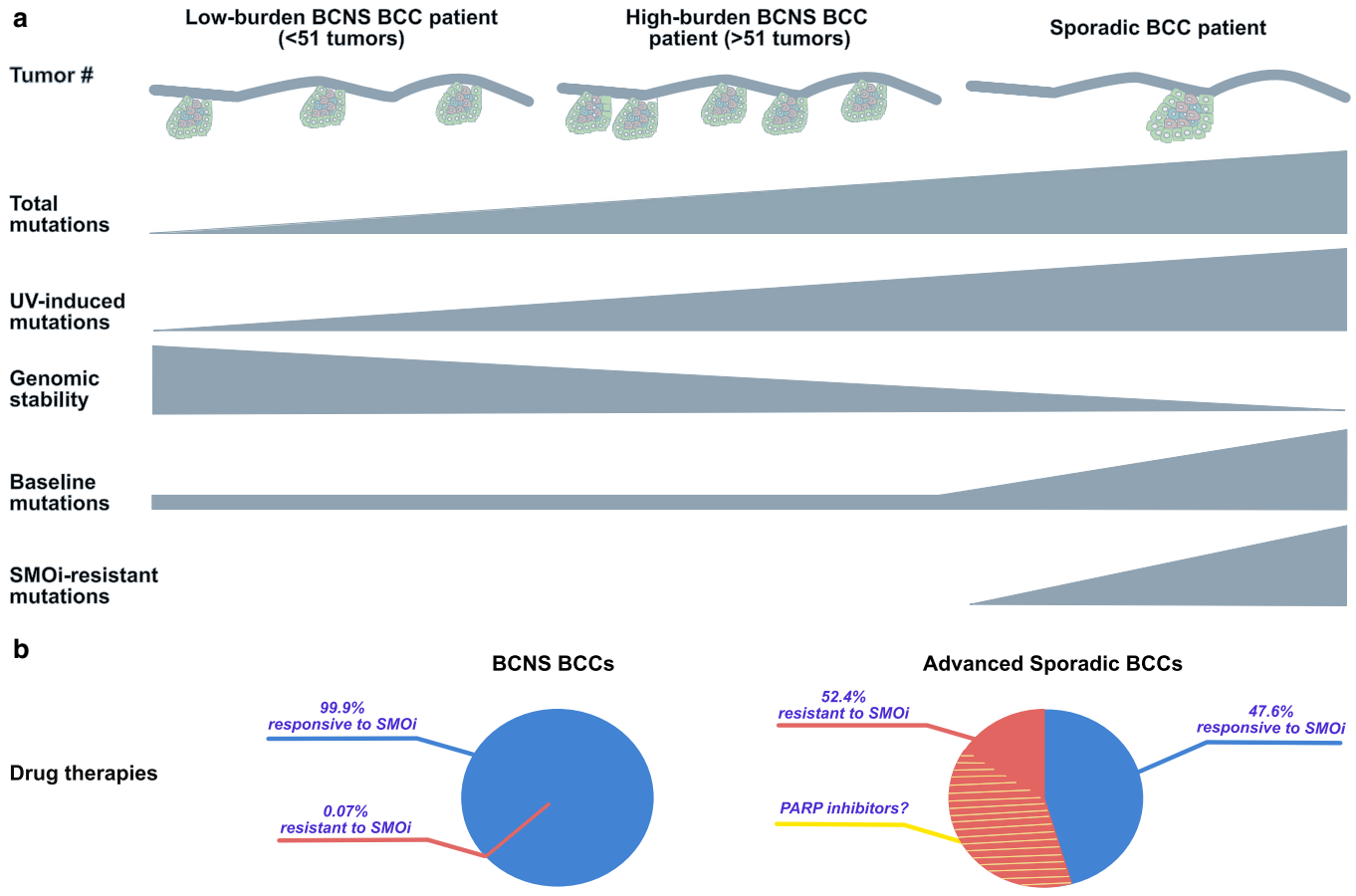

Figure 1. Genomic differences between BCNS and sporadic BCCs likely contribute to differential drug response. (**a**) BCNS and sporadic BCC tumors are histologically similar, but differ in the number of tumors. Low-burden patients with BCNS display less than 51 tumors, high-burden patients with BCNS display more than 51 tumors, and patients with sporadic BCC typically display few tumors. Total mutation load, UV-induced mutations, and genomic instability increase from low-burden BCNS, to high-burden BCNS, to sporadic BCCs, whereas baseline non-UV signature mutations remain the same between low- and high-burden BCNS tumors but rises in sporadic tumors. SMOi-resistant mutations are present in sporadic but not BCNS BCCs. (**b**) SMOi is effective in more than 99.9% of BCNS BCCs and in 47.6% advanced sporadic BCCs. Higher number of DNA repair mutations in sporadic BCCs compared with BCNS BCCs via whole-exome sequencing data from Chiang et al. suggest PARP inhibitors, or other DNA repair pathway inhibitors, as potential alternative therapy for SMOi-resistant BCCs. BCC, basal cell carcinoma; BCNS, basal cell nevus syndrome; SMOi, Smoothened inhibitor.

(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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