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Defining the Genetics of Basosquamous Carcinoma

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Basosquamous carcinoma (BSC) is a rare form of skin cancer with both basaloid and squamous morphology. Chiang et al. (2019) genetically define BSCs and demonstrate that BSCs likely originate as basal cell carcinomas that partially squamatize through accumulation of ARID1A mutations and RAS/MAPK pathway activation.

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Basosquamous carcinoma (BSC) is a rare form of skin cancer, which displays phenotypes of both basal cell carcinoma (BCC) and squamous cell carcinoma (SCC). However, the molecular mechanisms behind this malignancy, as well as the genetic lineage, is highly debated. Potential clues to the origins of BSCs may be found by examining what drives the individual components of this form of cancer. BCCs are common locally invasive epithelial tumors characterized by inappropriate activation of the Hedgehog (HH) pathway and account for 60%-80% of skin cancers. In the absence of the Hedgehog ligand, the receptor Patched1 (PTCH1) inhibits Smoothened (SMO), which allows Suppressor of Fused (SUFU) to keep the GLI transcription factors within the cytoplasm. In the presence of HH-induced PTCH1 inhibition, SMO is able to suppress SUFU allowing the GLI transcription factors to enter the nucleus and regulate the transcription of downstream target genes associated with skin appendage development and homeostasis. Sporadic BCCs arise from progenitor cells found within the basal layer of the epidermis, predominantly from mutations in PTCH1 (73%) and SMO (20%) (Bonilla et al., 2016). SMO inhibitors are used to treat advanced or metastatic BCCs. However, tumors can gain resistance to these drugs partly by undergoing squamatization (Ransohoff et al., 2015; Saintes et al., 2015; Zhao et al., 2015; Kuonen et al., 2019).

SCC is the second most common form of skin cancer, comprising approximately 20% of cases, and typically arises from suprabasal keratinocytes. Unlike BCCs, SCCs tend to have a more complex genetic lineage because of the cellular heterogeneity of post-mitotic suprabasal cells at various stages of differentiation. Many genes are implicated in the progression of SCC, including TP53, NOTCH1/2, CDKN2A and members of the RAS/MAPK pathway (Dotto and Rustgi 2016). Monotherapies are not as effective in treating SCC, partly because of the large number of genetic alterations needed to promote proliferation, lose cellular connections and communication, reduce apoptosis, and stimulate a mutator phenotype to push keratinocytes to progress to SCC.

Although not as common as either BCC or SCC, BSCs comprise 1.2%-2.7% of skin cancer cases with a 5% incidence of metastasis (Garcia et al. 2009). These tumors have histological characteristics of both BCC and SCC but are routinely considered a type of BCC with a higher rate of reoccurrence and metastasis. Due to the low reported incidence rate of BSC, there is a lack of awareness of BSC pathology and molecular drivers of the disease. Chiang et al., 2019 provide a detailed genetic characterization of BSCs and find that HH pathway mutations are likely to initiate tumor formation with a surprising bifurcation event where ARID1A mutations may promote SCC driver mutations and squamatization that leads to the mixed nature of BSCs.

Genetic Lineage of BSC

Using targeted sequencing of 1641 cancer genes from 20 BSCs, whole exome sequencing from 16 BCCs, and a mixture of previously published whole exome and whole genome datasets from 52 SCCs, Chiang et al., 2019 found that BSC tumors more closely resembled BCCs than SCCs. Forty-five percent of BSC and 44% of BCC tumors contained deleterious mutations within PTCH1, whereas only 10% of SCCs showed PTCH1 mutations. Additionally, 15% of BSCs and 19% of BCCs carried mutations for MYCN, a downstream effector of HH signaling, while only 6% of SCCs showed MYCN mutations. Similar relationships were also seen with putative BCC drivers such as PTEN and PIK3CA. However, not all mutational drivers of BCC were found at comparable rates in BSC. For instance, the constitutively active SMO W535L mutation was only found in 5% of BSCs compared with 25% in BCCs. Other putative BCC drivers, such as PPP6C, GRIN2A, and PREX2, showed similar mutational frequencies between BSC and SCC.

When comparing the mutational frequencies of SCC driver genes, BSCs were more similar to BCC than SCC. For instance, major SCC driver genes, such as CDKN2A, KRAS, NRAS, and HRAS, were not mutated in either BSC or BCC. The NOTCH genes were also mutated less frequently in BSC (~18%) than SCC, where approximately 42% of SCC and 28% of BCC tumors showed NOTCH1 and NOTCH2 mutations. However, some oncogenic drivers showed more similarity between BSC and SCC (e.g., TP53, TP63, and RAC1).

Interestingly, 45% of BSCs displayed mutations in ARID1A, a component of the SWI/SNF chromatin remodeling complex. This was a significantly

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Clinical Implications

- ARID1A mutations and RAS/MAPK activation may serve as biomarkers for BSC and likely promote resistance to SMO inhibitors.
- PARP inhibition may be a viable therapeutic for BSC and unstable sporadic BCC.

greater amount than the 19% of BCCs and SCCs that harbored ARID1A mutations. ARID1A disruption promotes mammalian cell proliferation and regeneration, imparting a plasticity that enhances cell survival in part by reducing the restrictive nature of chromatin remodeling in terminally differentiated cells (Sun et al. 2016). ARID1A mutations may allow keratinocytes to sample different fates and undergo squamatization under selective pressure, an event seen in the clinic where SMO inhibitor treatment can promote de novo SCC development from BCC tumors (Ransohoff et al., 2015; Saintes et al., 2015; Zhao et al., 2015).

ERK1/2 activation in BSC

Pathway switching from HH to RAS/MAPK is one route that HH-dependent tumors can take to evade SMO inhibition (Zhao et al., 2015). In medulloblastoma, pathway switching circumvents HH pathway dependency and enhances metastatic behavior, whereas pathway switching in BCC can lead to squamatization. Furthermore, loss of primary cilia, a microtubule-based signaling organelle that is essential for highly sustained HH signaling, can promote RAS/MAPK pathway activation to push the BCC-to-SCC transition (Kuonen et al., 2019). Here, primary cilia may serve as a biomarker for SMO inhibitor efficacy where BCCs with primary cilia display high GLI1 signal and are responsive to SMO inhibition, whereas BCCs without primary cilia can display high RAS/MAPK signal, are non-responsive to SMO inhibition, and may squamatize. Together, this data suggests a balance between HH and RAS/MAPK signaling that dictates tumor fates (Figure 1). The authors showed a similar relationship between the two pathways, where basaloid cells within BSC tumors displayed high levels of nuclear GLI1 and low levels of p-MEK, and squamatized areas showed greater levels of p-MEK and the loss of nuclear GLI1. Basaloid cells adjacent to the squamatized areas had an intermediate state with higher levels of p-ERK staining, while maintaining GLI1 levels, suggesting that RAS/MAPK pathway activation through p-ERK and p-MEK may serve as biomarkers for the efficacy of SMO inhibitors in HH-dependent tumors.

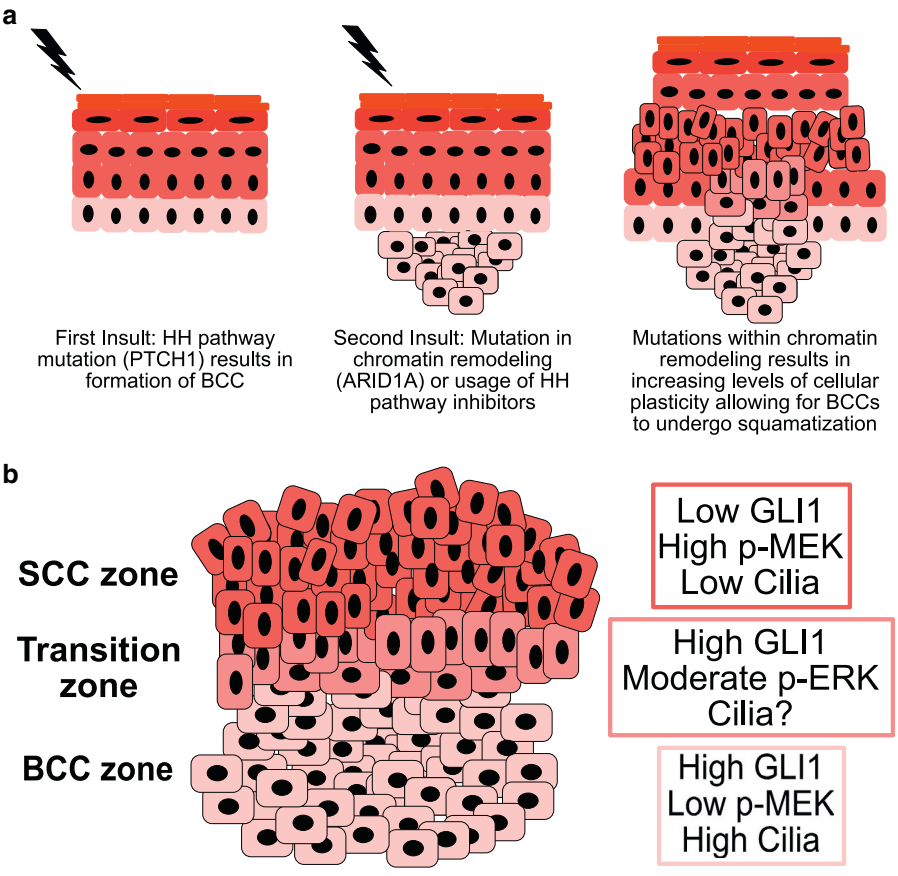

Figure 1. Genetic lineage of BSC. (**a**) Activating HH pathway mutations initially drive formation of BCCs. Acquisition of de novo ARID1A mutations or other chromatin remodeling mutations under pharmacological SMO inhibition drive cellular plasticity, pushing basal cells to undergo squamatization and leading to BSC formation.(**b**) Within the BCC zone, high levels of HH signaling, low RAS/MAPK pathway activity, and high levels of ciliation drive tumor growth. Within the SCC zone, RAS/MAPK signaling increases with a concomitant reduction in HH pathway activity and ciliation. Within the Transition zone, cells begin to show higher levels of RAS/MAPK pathway activity while maintaining HH signaling. The levels of ciliation are unknown.

Therapeutic targets for BSCs

The balance between the HH and RAS/MAPK pathways illustrates a potential problem when treating BCCs, where SMO inhibitors may push HH-dependent cancers to adopt additional pathways to maintain tumor growth. The absence of RAS/MAPK pathway activation or ARID1A mutations in BCC-like tumors may serve as a "go" signal to use SMO inhibitors with a low probability of drug resistance, whereas the presence of either biomarker should instill caution toward the efficacy of SMO inhibitors. For those tumors with ARID1A mutations, the recent success of PARP inhibitors on ARID1A-deficient cancer cells may serve as an alternative therapeutic strategy to treat BSCs (Shen et al. 2015). In fact, targeting DNA repair mechanisms may be therapeutically useful to treat genomically unstable BCCs, broadening their impact to

include the majority of skin cancers (Nguyen and Atwood, 2018).

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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Langerhans Cells Spy on Keratinocytes

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Tunneling nanotubes (TNTs) have been described as a novel mechanism for intercellular communication. However, the ability of epidermal cells to utilize TNTs remains a mystery. In this issue, Su and Igyártó (2019) showed that Langerhans cells (LCs) obtain mRNA from keratinocytes (KC) in vivo presumably via TNTs. The demonstration of exchange of genetic material from KC to LC in vivo is an an unexpected method of antigen acquisition by LC and also an important consideration when analyzing transcriptomic data.

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Cell-to-cell communication is fundamental for the maintenance of tissue homeostasis and the execution of efficient physiological responses to an array of stimuli. Hemopoietic and non-hemopoietic cells accomplish this task by secreting and responding to soluble ligands (e.g., cytokines and hormones), through gap junctions, extracellular vesicles (exosomes and microvesicles), and tunneling nanotubes (TNTs) (Mittal et al., 2019; van Niel et al., 2018). The latter two mechanisms transfer a large diversity of molecular cargo (e.g., DNA, protein, lipids, and mRNA) that is not secreted normally. Since their recent discovery, TNTs have been reported to be utilized by epithelial cells and myeloid cells (e.g., monocytes, macrophages, and dendritic cells) (Dupont et al., 2018; Rustom et al., 2004). However, the ability of epidermal Langerhans cells (LCs) to use TNTs to communicate with keratinocytes (KCs) or dendritic epidermal T cells (DETCs) had yet to be explored. In a report in the Journal of Investigative Dermatology, Su et al. provide evidence that LCs have the ability to exchange mRNA to other epidermal cells, presumably through TNTs (Su and Igyarto, 2019).

Su and colleagues began by carefully analyzing transcriptomic datasets provided by the IMMGEN Genome project, a scientific collaboration that collects and curates transcriptomic data from flow cytometry–purified mouse immune cells. They noted the presence of several KC-specific transcripts such as keratins (e.g., K14, K10, and K5) in epidermal LCs. This phenomenon had been noted in unpublished work by several groups, but it was attributed to potential cross-contamination of KCs with LCs during sorting experiments. Su and colleagues (2019) dug deeper and confirmed that epidermal LCs contained KC-specific mRNA transcripts. LCs contained easily detectable levels of keratin mRNA, but chromatin analysis of LCs clearly indicated that *K14* and other keratin gene loci were unavailable, indicating that LCs were not actively transcribing *K14*. Despite not actively transcribing keratins, keratin protein was evident in LCs. These data suggest that LCs can acquire mRNA from KCs, but a more formal demonstration required a clever but complex experimental approach. To accomplish this, the authors created K14-YFP mice in which the *K14* promoter drives the expression of Cre recombinase in a ROSA26.YFP fate reporter mouse, resulting in YFP expression in KCs. To ensure that Cre and YFP expression are absolutely excluded from LCs, K14-YFP mice were bred to huLangerin-DTA mice that lack LCs. These mice then were transplanted with bone marrow from wild-type (WT) mice to replenish the mice with WT LCs. This resulted in mice where Cre and YFP are robustly expressed by KCs, and there is no possibility that they are expressed by LCs. Despite the absence of *YFP*

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