Applying Multiomics to Basosquamous Carcinoma

Gavin R. Long[1], Arinnae I. Kurdian[1] and Scott X. Atwood[1,2,3]

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Basosquamous carcinoma (BSC) is a rare form of skin cancer defined by combined phenotypes of 2 common skin cancers: basal cell carcinoma (BCC) and cutaneous squamous cell carcinoma (cSCC). The heterogeneity of BSC provides tumors an intrinsic resistance to drugs that only target 1 phenotype, making standard therapy ineffective (Zelin et al, 2022). Jussila et al (2023[1]) utilize single-cell transcriptomics, spatial transcriptomics, and whole-exome sequencing to interrogate and spatially map phenotypic differences in a drug-resistant tumor from a patient with Gorlin syndrome. Defining the molecular mechanisms driving BSCs in this way provides insight into phenotypic switching within tumors as well as a framework to study other types of heterogeneous drug-resistant cancers.

BCCs are epithelial tumors arising from misactivation of the Hedgehog (HH) pathway and represent 60–80% of skin cancers. In the absence of the HH ligand, PTCH1 inhibits the G-protein coupled receptor Smoothened (SMO), preventing activation and nuclear localization of the GLI transcription factors. HH binding inhibits PTCH1, leading to SMO activation and accumulation of nuclear GLI with subsequent target gene expression. BCCs typically harbor either inactivating alterations in *PTCH1* (70–80%) or activating alterations in *SMO* (~20%) (Bonilla et al, 2016). Treatments for metastatic or advanced BCCs commonly use SMO inhibitors (SMOi). However, drug resistance is becoming a major roadblock to effective treatment, partly attributable to tumor cells transitioning to a more squamous phenotype (Haensel et al, 2022; Jussila et al, 2023[1]; Kuonen et al, 2021; Ransohoff et al, 2015).

cSCC is the second most common form of skin cancer (20–30%), arising primarily from a wide variety of genetic alterations. The stepwise progression from normal keratinocytes to cSCC involves cumulative alterations in genes to promote proliferation, lose cellular connections and cell–cell communication, reduce apoptosis, and alter interactions with the tumor microenvironment. Owing to the number of genes that drive cSCC development, such as activating alterations in *RAS* and phosphoinositide 3-kinase gene *PI3K* or inactivating alterations in *TP53*, *TGFBR1/2*, *CDKN2A*, and *NOTCH1/2*, monotherapies are usually ineffective in treating cSCC (Winge et al, 2023).

Even though BSCs only comprise 1.2–2.7% of skin cancers, untangling their heterogeneous drivers may provide insight into the innate resistance of many types of heterogeneous cancers. Genetically, BSCs likely originate as BCCs that partially transition to cSCC-like features through accumulation of additional alterations in genes such as *ARID1A* or alterations that activate the RAS/MAPK pathway (Chiang et al, 2019). The shift to high RAS/MAPK pathway activity results in SMOi resistance and accompanying squamatization of the tumor. In previous work, Kuonen et al (2021) utilized single-cell and bulk-level RNA sequencing of BCCs, cSCCs, and BSCs to identify cell-surface markers that tracked the BCC (GLI1) to BSC (TACSTD2, LYPD3, LY6D, MUC1) to cSCC (CD44) transition. LY6D emerged as an especially useful biomarker for the BSC transition state that is present in a subset of *PTCH1*-deficient sporadic BCCs, is selected for during SMOi therapy, and can drive SMOi resistance (Haensel et al, 2022).

IDENTIFICATION OF BSCs IN A MIXED POPULATION

Observation of squamatized regions within a patient's SMOi-resistant *PTCH1*-deficient Gorlin syndrome tumor led Jussila et al (2023[1]) to probe for LY6D expression. LY6D-positive cells accumulate in many epithelial tumors with poor clinical prognosis, including pancreatic carcinomas. The investigators found high levels of LY6D in the cSCC-like portions of the tumor and high levels of GLI1 in the BCC-like portions. Because the patient had been on tumor-suppressive doses of SMOi for over 10 years, the appearance of LY6D-positive tumor cells suggests that a subset of Gorlin tumors escape therapy through the LY6D-driven BSC transition pathway. To comprehensively profile this SMOi-resistant tumor, the researchers used single-cell RNA-sequencing (scRNA-seq) and spatial transcriptomics to interrogate and spatially map phenotypic differences. This combination of technologies allows for greater resolution of expressed transcripts in tumor cells and their microenvironment while maintaining spatial information (Figure 1). By superimposing cell state clusters from scRNA-seq data onto the cell state clusters from spatial transcriptomics analysis of Visium data, the investigators validated other BSC marker genes that coincided with *LY6D* expression, such as *LYPD3* and *TACSTD2*. These methods illustrate the ease of novel marker gene discovery and give insight into molecular mechanisms driving heterogeneous cellular states.

To answer lineage and driver alteration questions, the investigators incorporated whole-exome sequencing into their analysis. Guided by the scRNA-seq and spatial transcriptomics data, they extracted DNA from specific regions

[1]*Department of Developmental and Cell Biology, School of Biological Sciences, University of California, Irvine, Irvine, California, USA;* [2]*Department of Dermatology, School of Medicine, University of California, Irvine, Irvine, California, USA; and* [3]*Chao Family Comprehensive Cancer Center, University of California, Irvine, Irvine, California, USA*

Correspondence: Scott X. Atwood, Department of Developmental and Cell Biology, University of California, Irvine, 4238 McGaugh Hall, Irvine, California 92697, USA. E-mail: satwood@uci.edu

[1] Jussila AR, Haensel D, Gaddam S, Oro AE. Acquisition of drug resistance in basal cell nevus syndrome tumors through basal to squamous cell carcinoma transition. bioRxiv 2023.

Clinical Points

- Multiomics are useful to define heterogeneous cancers and identify novel biomarkers.
- LY6D is an effective biomarker for the basal-to-squamous cell carcinoma transition.
- Combination of PCYT2 and glycolysis inhibitors may be viable therapeutics for basosquamous carcinoma.

within the same biopsy that corresponded to unique tumor states. Common acquired somatic alterations were identified among sampled regions and indicated a shared lineage between the BCC- and cSCC-like regions of the tumor. Furthermore, whole-exome sequencing analysis revealed 35 genes that could contribute to basosquamous transition, with two altered genes, *PCYT2* and *ETNK1*, encoding sequential enzymes in the phosphatidylethanoalamine (PE) pathway. Both alterations cause premature stop codons and would likely result in reduced enzymatic activity and intracellular

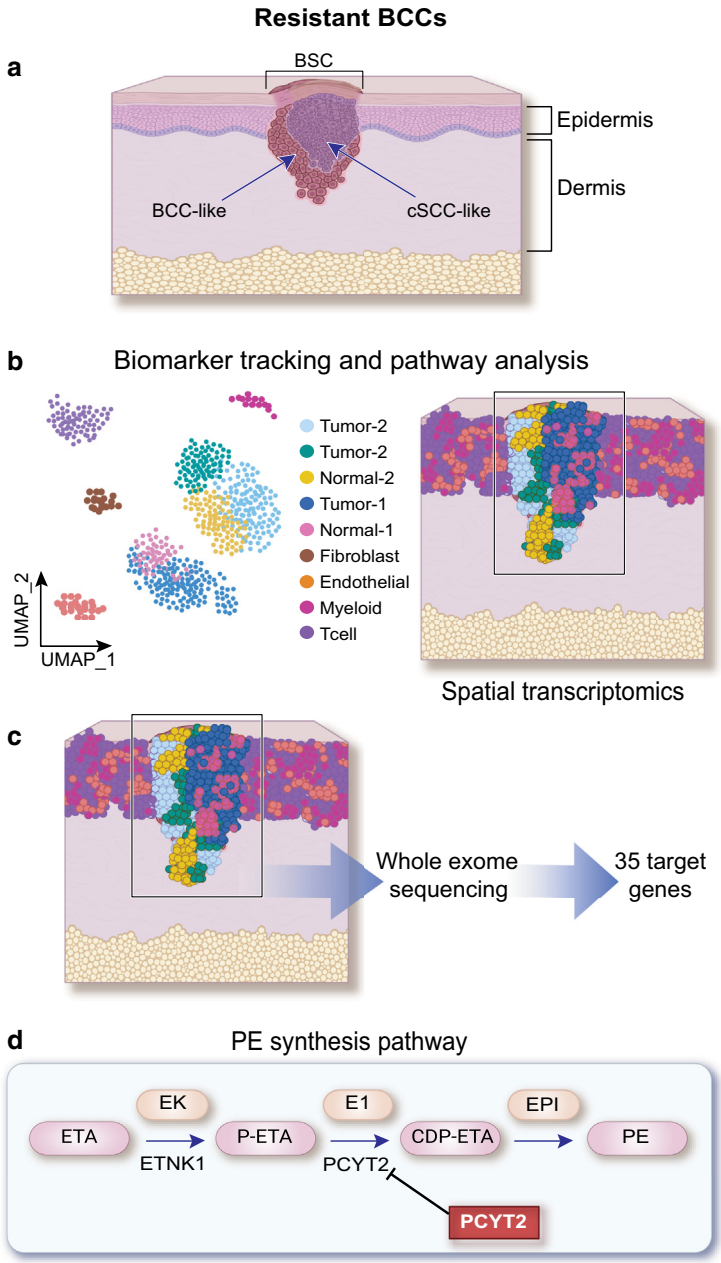

Figure 1. Application of multiomics in clinical samples. (**a**) Resistant BCC tumors taken from a patient with SMOi-resistant Gorlin syndrome show both BCC-like and cSCC-like morphologies. **(b)** Spatial transcriptomics combined with scRNA-seq separate tumor cells by gene expression, allowing for spatial analysis and lineage tracing of cells. **(c)** Whole-exome sequencing of tumors identifies 35 differentially expressed genes that may drive the cSCC-like phenotype of BSC. **(d)** Multiomic analysis identifies the PE pathway as a likely key driver of BSC and PCYT2 as a potential therapeutic target. Figure draft was created with BioRender.com; figure was revised by Jan Ruvido Stebbins, Ruvido Medical Illustration (Dexter, MI). BCC, basal cell carcinoma; BSC, basosquamous carcinoma; cSCC, cutaneous squamous cell carcinoma; ETA, ethanolamine; PE, phosphatidylethanolamine; scRNA-seq, single-cell RNA sequencing; SMOi, Smoothened inhibitor; UMAP, Uniform Manifold Approximation and Projection.

phosphoethanolamine, leading to mitochondria hyperactivation, ROS production, and DNA damage (Fontana et al, 2020). Treating mouse BCC cells with a PCYT2 inhibitor reduced *Gli1* and increased *Ly6d* expression, suggesting that the PE pathway may control the transition to BSC.

THERAPEUTIC TARGETS OF BSCs

Could targeting PCYT2 be used as a therapy to treat BSC? Possibly, some clues lie in how tumor cells regulate their metabolism. A classic view of cancer cell metabolism suggests a switch from glucose oxidation in normal tissues to glycolysis in cancer, resulting in a decrease in oxidative phosphorylation in mitochondria. Inhibition of PCYT2 alters energy metabolism by increasing phosphoethanolamine, resulting in lower mitochondrial respiration and a dependence on energy production from glycolysis. Treatment with PFKFB3 inhibitors, which target an enzyme that promotes glycolysis, can result in synthetic lethality in combination with PCYT2 inhibitors where cancer cells are no longer able to acquire enough energy through glycolysis or oxidative phosphorylation and has been shown to work in preclinical studies of acute myeloid leukemia and liver cancer cell lines and a xenotransplantation model (Guan et al, 2020). This treatment strategy may be useful in targeting the whole spectrum of BCC-to-cSCC transition, broadening their impact to include most skin cancers.

ORCIDs
Gavin R. Long: http://orcid.org/0009-0009-1817-2015
Arinnae I. Kurdian: http://orcid.org/0009-0009-2650-063X
Scott X. Atwood: http://orcid.org/0000-0001-7407-9792

CONFLICT OF INTEREST
The authors state no conflict of interest.

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