Unraveling the Spatial Landscape of Cutaneous Squamous Cell Carcinoma

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Introduction

Cutaneous squamous cell carcinoma (cSCC) is the second most common non-melanoma skin cancer, with a rising global incidence. Derived from keratinocytes, cSCC frequently evolves from actinic keratoses (AK) driven by chronic UV-B exposure—its primary risk factor and typically presents clinically as solitary, erythematous, scaly plaques on sun-exposed areas. While the metastatic potential is relatively low (1.2%-5%), advanced stages carry a significant mortality risk. These tumors are therefore characterized by a high mutational burden. While surgical resection remains the standard of care, often supplemented by radiotherapy or systemic agents, therapeutic challenges persist. In particular, the complex spatial architecture and tumor ecosystem drive intratumoral heterogeneity and resistance. Elucidating these spatially determined mechanisms is essential for understanding disease progression and developing next-generation therapeutic strategies.

Methods

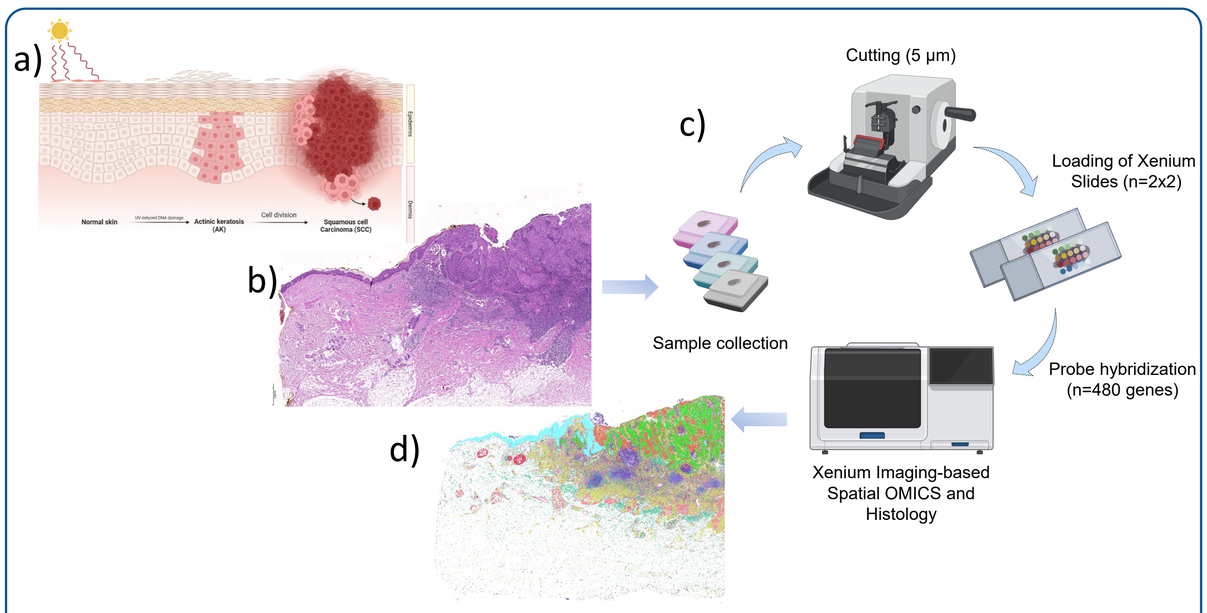

Figure 1: a) A schematic representation of cSCC initiation and progression, which arises from actinic keratosis. **b)** Representative histological section of cSCC tissue stained with H&E. **c)** An overview of the spatial transcriptomics workflow using the Xenium platform. **d)** Bioinformatic analysis and processing of spatial transcriptomic data in RStudio. Figure a) and c) were created with BioRender.com

Spatial transcriptomics was performed on whole-slide human skin samples (n=4) with histopathologically confirmed actinic keratosis (AK) or cutaneous squamous cell carcinoma (cSCC) using the Xenium platform (10x Genomics). A custom 100-gene panel was combined with a 360-gene immuno-oncology panel. Data were analyzed at single-cell resolution in RStudio using VoltRon and Seurat, followed by H&E staining of the scanned slides.

Results

We identified significant stage-specific changes in the cell type composition and gene expression patterns particularly in the transition zones (TZ) from normal skin (NS) to actinic keratosis (AK) and/or to tumor tissue (cSCC). Single-cell-resolved spatial transcriptomic profiling of patient skin samples with histopathologically validated AK or cSCC revealed a clear separation of keratinocyte stages and enabled deeper molecular analysis of functional cellular networks. We observed a loss of KRT1 and KRT10, along with a gain of MET, MMP1, FTH1 and ANXA1.
Spatial mapping of gene signatures associated with hypoxia activation, interferon-mediated processes, DNA repair, and immune cell enrichment clearly distinguished tumor cells and precancerous lesions from normal skin.

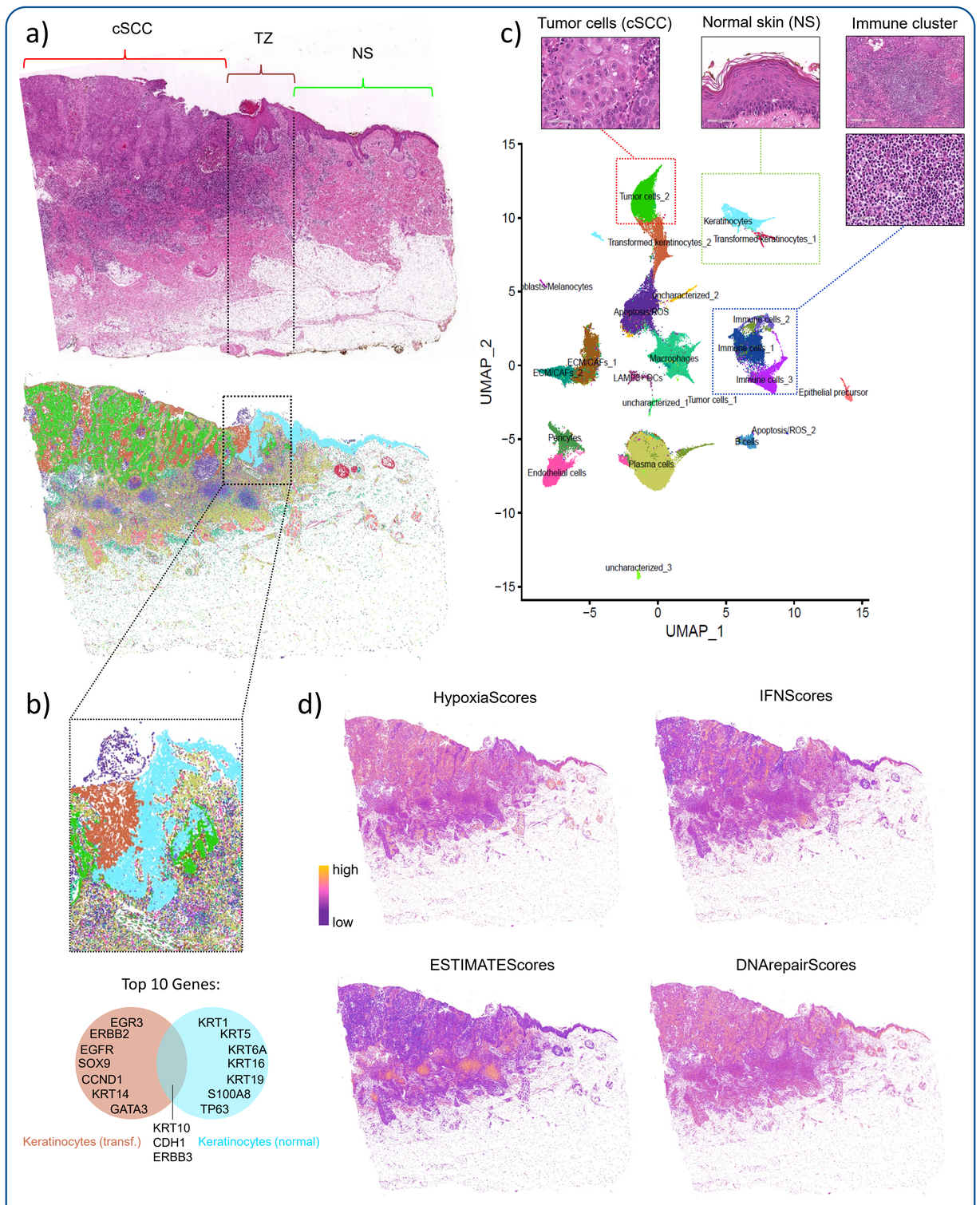

Figure 2: a) H&E scan and spatial distribution of different cell clusters of a human tumor tissue with the transition zone (TZ), normal skin (NS) and tumor tissue (cSCC). **b)** Magnified view of the transition zone. The top ten expressed genes of the two dominant cell clusters (transformed keratinocytes and keratinocytes) in the TZ. **c)** UMAP of different cell clusters, with an enlargement of the H&E scan for the present cell types. **d)** Spatial distribution of levels of hypoxia scores, IFN scores, ESTIMATE scores, and DNA repair scores.

Conclusion

Our study provides a detailed characterization of cutaneous squamous cell carcinoma (cSCC) progression, highlighting distinct molecular and cellular signatures across normal skin, actinic keratosis, transition states, and invasive tumor tissue. By incorporating spatial context, we identified key biomarkers and transcriptional changes associated with early tumorigenesis and disease progression.
Overall, our findings reveal spatially defined, stage-specific immune and tumor cell compositions, underscoring the critical role of the tumor microenvironment in cSCC development.

Outlook

Future work will focus on elucidating the key molecules driving cSCC progression in spatial transition zones. We will validate candidate pathways to identify new therapeutic targets and establish biomarkers for early detection, patient stratification, and treatment response.

References: doi: https://doi.org/10.1101/2025.08.29.25334740

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