Establishing and Validating Patient-Derived Head and Neck SCC Cell Lines

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Background

Head and neck cancer is one of the most common cancers worldwide, accounting for around 900,000 cases and 400,000 deaths annually. Of all head and neck cancers, squamous cell carcinomas (HNSCC) account for roughly 90% of all cases. Cell lines are important *in vitro* models for disease, and their use in cancer biology research has led to significant advances in our understanding and treatment of numerous cancers in the last few decades. Currently, there are few head and neck cancer cell lines available for purchase on the market or to obtain from other laboratories.

Methods

Fresh HNSCC tumors were harvested in the operating room and brought back to the laboratory. Samples were mechanically diced into sub-millimeter pieces and then enzymatically digested using 1 mg/ml collagenase for 6 to 12 hours (duration dependent on the texture of the tumor). Digestion was stopped by adding fetal bovine serum (FBS). Cells were isolated via centrifugation, resuspended in Dulbecco's Modified Eagle Medium with 10% FBS, and plated on CellBind plates. Plates were monitored for cancer cell attachment and growth. Contaminant fibroblasts were removed using selectively trypsinization. Culture media was changed to PneumaCult-Ex Plus Medium to select for the growth of HNSCC cells over fibroblasts. Cell lines were validated using immunocytochemistry (ICC) and fluorescence microscopy.

Conclusion

- Primary, patient-derived cell lines are important *in vitro* models of HNSCC
- We describe a successful protocol to isolate, culture, and maintain primary HNSCC cells from fresh patient-derived tumor samples

Results

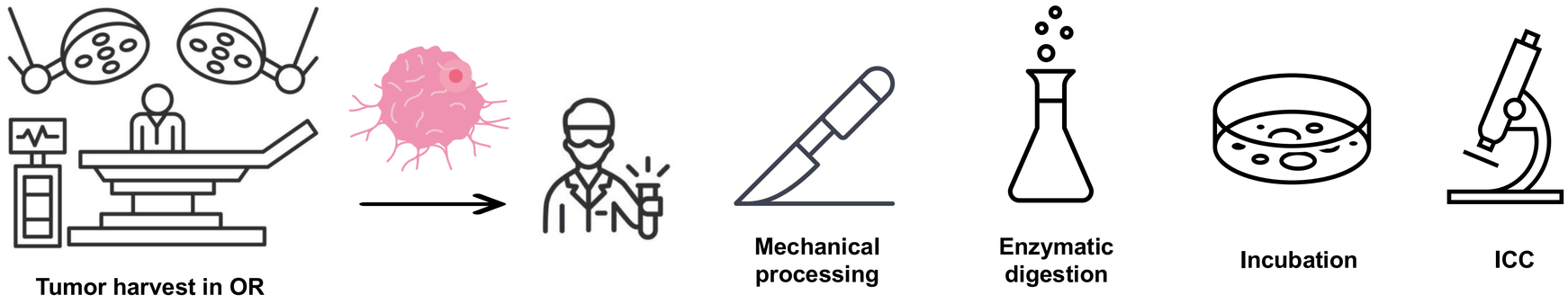

Figure 1. **Workflow of Tumor Harvesting and Processing**. After HNSCC is resected in the operating room, the sample is brought to the pathology lab where a chunk is harvested and brought back to the laboratory. There, the tumor chunk is mechanically processed using microscopic surgical scissors into sub-millimeter pieces and then enzymatically digested using collagenase. Cells are then isolated, plated, and incubated. After the first passage, culture media was changed to PneumaCult-Ex Plus Medium to select for growth of HNSCC cells over contaminant fibroblasts. Cell lines were validated using immunocytochemistry (ICC) and fluorescence microscopy.

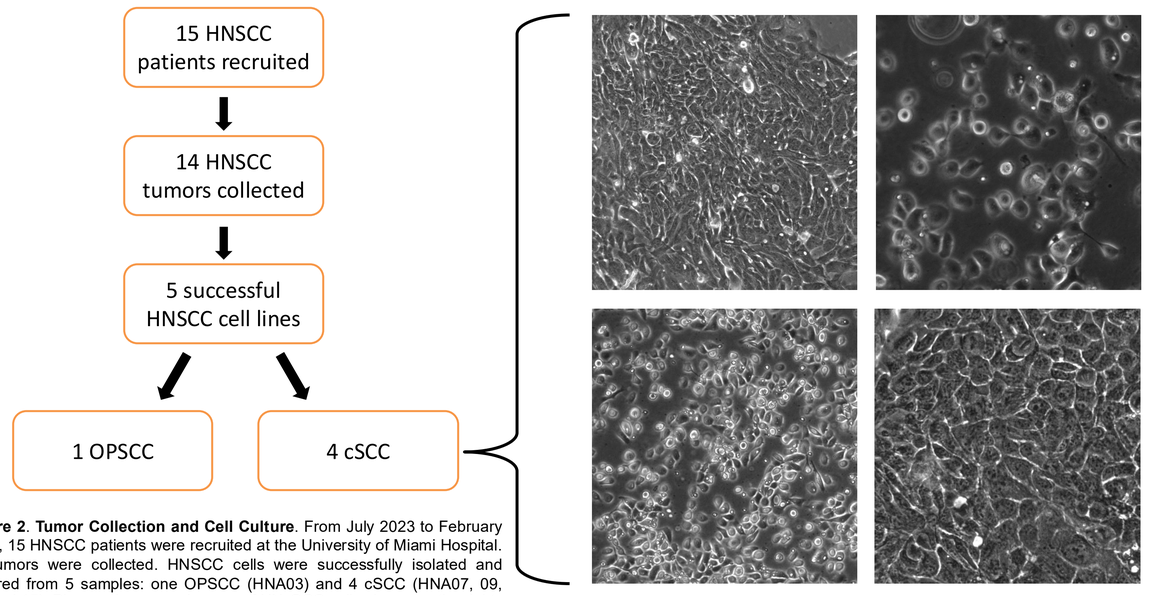

Figure 2. **Tumor Collection and Cell Culture**. From July 2023 to February 2025, 15 HNSCC patients were recruited at the University of Miami Hospital. 14 tumors were collected. HNSCC cells were successfully isolated and cultured from 5 samples: one OPSCC (HNA03) and 4 cSCC (HNA07, 09, 12, and 15). Phase microscopy images of HNcSCC cells shown on the right.

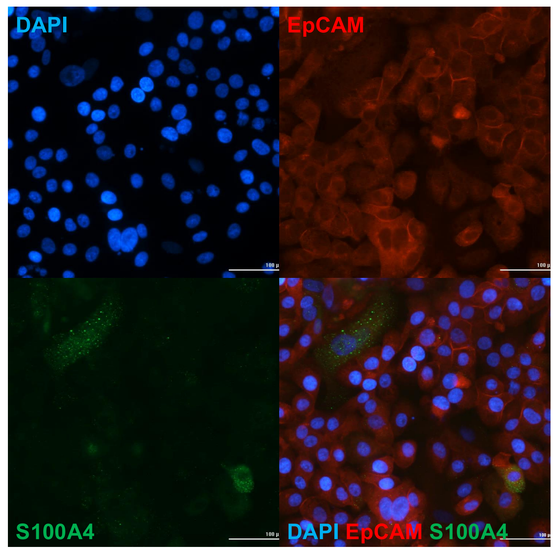

Figure 3. **cSCC Cell Type Validation using ICC**. cSCC cell lines were validated using ICC, staining positively for SCC marker epCAM (red) and negatively for fibroblast marker S100A4 (green).

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