Abstract

The incidence of skin cancer (squamous cell carcinoma, basal cell carcinoma and melanoma) has been increasing over the past several years. It is expected that there will be a parallel demand for cutaneous tumor samples for biomedical research studies. Tissue availability, however, is limited due to the cost of establishing a biorepository and the lack of protocols available for obtaining clinical samples that do not interfere with clinical operations. A protocol was established to collect and process cutaneous tumor and associated blood and saliva samples that have minimal impact on routine clinical procedures on the date of Mohs surgery. Tumor samples are collected and processed from patients undergoing their first layer of Mohs surgery for biopsy-proven cutaneous malignancies by the Mohs histotechnologist. Adjunct normal tissue (ANT) is collected at the time of surgical closure. Additional samples that may be collected are whole blood and buccal swabs. Tumor and blood tissue may also be collected from melanoma patients. By utilizing tissue samples that are normally discarded, a biorepository was generated that offers several key advantages for being based in the clinic versus the laboratory setting. These include a wide range of samples collected, access to de-identified patient records including pathology reports, and, for the typical donor, access to additional samples during follow-up visits. The samples have been stored in downstream applications (expant culture, RNA isolation, western blot analysis, cell cultures, and histological evaluation) and have been validated.

Outline of Sample Collection & Processing

Consented Mohs Microsurgery Patient

Initial Patient Sample

Processing Shop

Stroed Sample

Downstream Applications

Analysis of Proteins extracted from ANT and SCC

SCC Markers are Differentially Expressed in Tumor Tissue

M biomarkers recapitulate the expected differential expression

Explant Culture and RNA Isolation

Immunocytochemistry Analysis of SCC Sample

Immunoanalysis of SCC Sample

Figure 3: (A) Phase contrast image of explant cultures containing mixed keratinocytes and fibroblasts generated from SCC sample (B) Immunostaining of SCC nuclei garnished as part of Mohs micrographic surgery excision, an immunostaining of Nuclei stained with DAPI (Blue) and in yellow with anti-cytokeratin (Cy5) showing positive staining for the expression of transthyretin and S100P in SCC.

Figure 4: RNA integrity

Figure 5: (A) Malignant melanomas are seen under light microscopy using H&E from patient donor tissue sample. (B) Preserved CTCs isolated from patient donor blood sample

Circulating Tumor Cells (CTCs) Isolated from Culture

Discussion

To the author’s knowledge, this protocol is the first of its kind that focuses on the clinical procurement of cutaneous tissue samples in both a cost-effective and quick approach on the date of Mohs surgery. Each sample type has been tested in downstream applications to validate collection procedures:

- Tumor and adjunct normal tissue have been successfully used in protein and RNA isolation, and can potentially be used for DNA isolation.
- Viable explants established from the tissue sections have been evaluated by microscopy while stored histological slides have been used for immunohistochemistry and immunofluorescence.
- Circulating tumor cells have been isolated from patient donor blood samples.

We hope that the clinic-based biorepository model may be utilized to help alleviate the shortage of patient samples in biomedical research.

- Melanoma samples may also be obtained and stored in the biorepository on the date of excision.

Future Directions

- By following the protocol described here, it is possible to extend this model to other dermatology clinics, other tumor types (such as melanoma), and other surgical specialties and practices to provide human tumor samples for multi-faceted research into human cancers.

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