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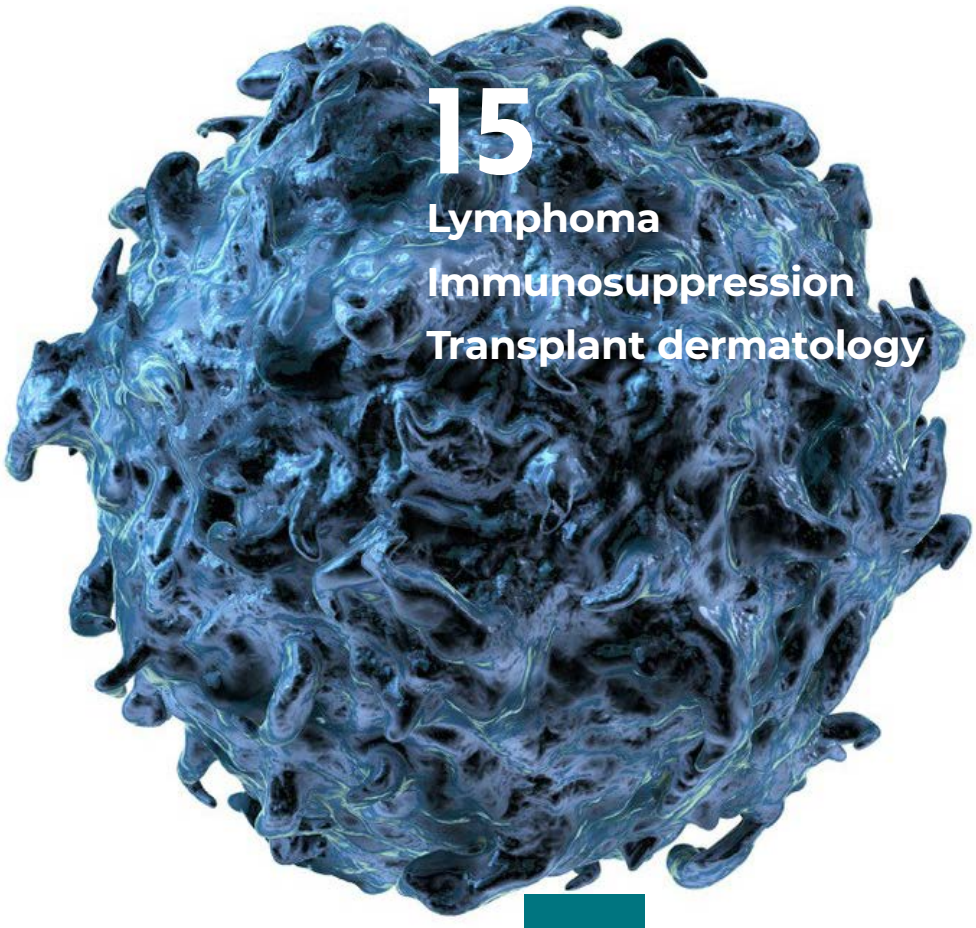

Société Suisse de Dermatologie et Vénéréologie
Società Svizzera di Dermatologia e Venereologia
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WHAT'S NEW

Live-Tissue Biobanking: A new method for viable tissue preservation

This section is a contribution from the SKINTEGRITY.CH interdisciplinary research consortium. The present work was performed by Dr. Restivo, Dr. Tastanova, Dr. Balázs and colleagues, in a collaboration that included SKINTEGRITY.CH principal investigators Prof. Hafner and Prof. Levesque at the University Hospital Zurich.

Maarten Schledorn
Scientific coordinator SKINTEGRITY.CH

Gaetana Restivo, Aizhan Tastanova.
In this study we showed that live tumor tissues, slow-frozen after surgery, could be used for several downstream applications common in cancer research and recapitulate the tissue composition of freshly processed samples.

This study, resulting from a collaboration of four groups from Zurich and Basel (Mitch Levesque and Michael Krauthammer in Zürich, and Salvatore Piscuoglio and Momo Bentires-Alj in Basel), demonstrated that solid tumors (i.e., melanoma, basal cell carcinoma, breast and colorectal) could be slow-frozen in FCS with 10 % DMSO shortly after collection. Viable tissues were then recovered after thawing and successfully used for different lab techniques including the establishment of cell and tissue cultures and single-cell RNA sequencing (scRNAseq).

Biobanking of surplus human samples from healthy or diseased tissue is essential for diagnostics and translational research. Formalin-fixed and paraffin-embedded (FFPE), Tissue-Tek OCT embedded, or snap-frozen tissues are preserved in many biobanks worldwide and have been the basis of translational studies. However, the use of those samples is limited to assays that do not require viable cells. The access to intact and viable human material is a prerequisite for translational validation of basic research results, for novel therapeutic target discovery, and functional hypothesis testing.

The project workflow is described in *Figure 1*. Briefly, tumor samples were either cut into small pieces or collected as shave biopsies. The small tumor pieces were either immediately digested into single-cell suspension (F) or slow-frozen (S) and subsequently used for 2D and 3D culture establishment and scRNAseq. Shave biopsies were either fresh (F) or slow-frozen (S) and used to establish ex vivo cultures. Moreover, melanoma FNAs were either slow-frozen or directly processed for scRNAseq. The results of the different applications on slow frozen samples were compared to fresh samples analyzed directly after surgery.

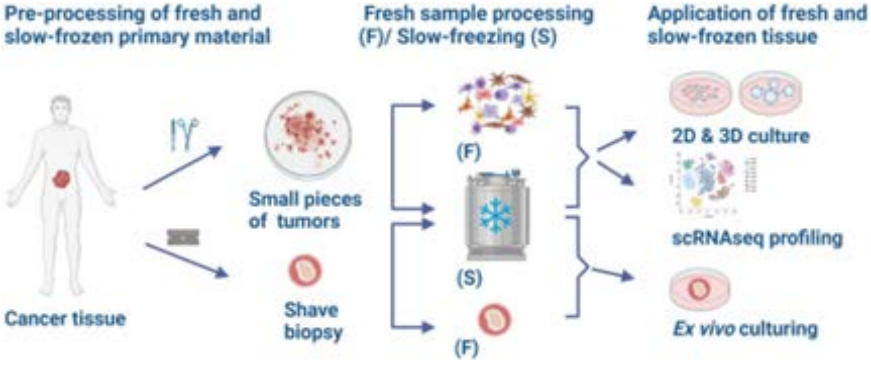

Figure 1: Schematic representation of the project workflow.

2D cell establishment from melanoma biopsies

We conducted a retrospective analysis on cell lines isolated from resected melanoma biopsies or from slow-frozen biopsies and found a comparable establishment rate. In particular, we analyzed 35 cell cultures obtained from fresh material and 36 derived from slow-frozen tissue (not paired). For both groups we generated 22 successful cell cultures (success rate fresh = 63 %, slow-frozen = 60 %).

Establishment of patient derived organoids (PDO) from colon rectal cancer (CRC)

We compared the success rate of PDO generation and histopathological features from fresh and cryopreserved matched tissues. First, we compared cell viability in fresh and matched slow-frozen tissue samples and observed no substantial difference. Then, we monitored organoid development and morphology for 4 weeks in 3D culture. We were able to generate organoids from fresh and matched slow-frozen tissues after 4 days and 8–10 days of culturing, respectively. After the organoids were established, we did not observe differences in growth and morphology. We also assessed the expression of common markers for CRC diagnosis: the homeobox protein CDX2, which is a transcription factor respon-

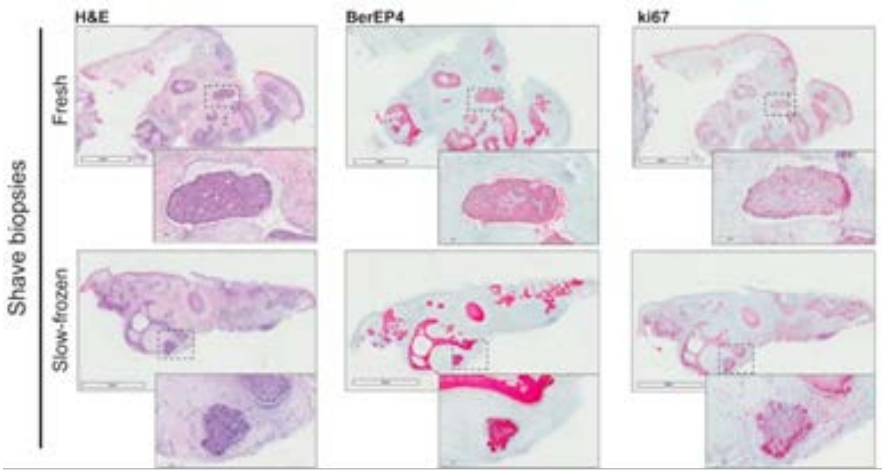

Figure 2: Ex vivo culture of BCC from fresh and live slow frozen shave biopsies.

sible for the differentiation and maintenance of the intestinal phenotype and keratin 20 (CK20). Both markers were expressed in PDO obtained from fresh and slow-frozen material.

Ex-vivo tissue cultures of BCC

We compared ex vivo tissue cultures of shave biopsies from BCC. The shave biopsies were cut in 2 pieces and either directly put in culture or slow-frozen. Five days after cultivation the tissues were analyzed by immunohistochemistry for proliferation (ki67) and the BCC marker BerEP4 *(Figure 2)*. The results from fresh or live, slow-frozen biopsies were comparable, thus the thawing and cultivation did not disturb tumor morphology and proliferation. We thus assume that slow-frozen tissue can be used subsequent to storage for drug testing.

scRNAseq from CRC, BCC, and melanoma FNA

We analyzed paired fresh and slow-frozen tissues from CRC and BCC, as well as fine needle aspirates (FNA) of a melanoma metastasis. Similar numbers of cells and comparable tissue heterogeneity was recovered from fresh and slow-frozen samples, with different relative proportions of cell types. Slow-frozen samples showed enrichment in fibroblasts and vascular endothelial cells in all tissue types. Epithelial cells were underrepresented in BCCs, but not CRC samples, suggesting that keratinocytes are sensitive to the slow-freezing procedure. Among immune cell populations cell lineage marker expression was equally captured for the majority of the immune cell types in both fresh and slow-frozen samples, except for granulocytes. Neutrophils and mast cells were barely detectable in slow-frozen samples, underlining the importance of using fresh tissue when studying these cells. Differential gene expression analysis between fresh and live-frozen samples identified response to heat stress in the majority of cell types, nonetheless the gene expression profile recovered from both fresh and slow-frozen samples allowed cell type identification. To further demonstrate the applicability of slow-frozen biobanking we analyzed five breast cancer samples. The resected breast cancer tissue was split into two parts, one was slow-frozen and the other part was preserved as an FFPE block. scRNAseq of slow-frozen breast cancer tissue identified a heterogeneous cancer microenvironment and tumor cells. We next quantified protein abundance from paired FFPE sections and correlated it to the corresponding mRNA levels in the scRNAseq data. Expression of mRNA and protein abundance in stromal and tumor cells showed a good correlation, especially in tumor cells.

In summary, we showed that 2D, 3D, and ex vivo cultures can be established from live, slow-frozen small tumors and shave biopsies and that the success rates and features are maintained when compared to fresh material. Moreover, we showed scRNAseq can be applied to slow-frozen small tumors and FNA and that the overall tumor heterogeneity and microenvironment are maintained with different proportions of certain cell types and minimal transcriptome differences between fresh and slow-frozen tissue pairs.

Authors

Dr. sc. nat. Gaetana Restivo, Dermatology Department, University of Zurich, University Hospital Zurich
Dr. sc. ETH Zurich Aizhan Tastanova, Dermatology Department, University of Zurich, University Hospital Zurich

Gaetana Restivo

Aizhan Tastanova

Reference

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**Equal contribution*