RNA-seq and Tumor Infiltrating Lymphocytes as Markers of Tumor Microenvironment Changes after Cryoablation


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**Introduction**

Cryoablation is a minimally invasive procedure that induces target tissue necrosis through freeze/thaw cycles, reaching less than < -40° C, while preserving surrounding tissue architecture. While the tumor is killed, potential cancer antigens are preserved. Since the ablated tumor remains in the patient, this introduces the potential for augmentation of an immune response to tumor antigens still present which might target the cells that have already escaped for systemic disease (Apcosalp Effect).

**Objective**

Immunological microenvironment and tumor-infiltrating lymphocytes (TILs) in breast cancer is becoming increasingly relevant as new therapeutic targets. This study explored changes in TILs and RNA-seq in breast cancers after cryoablation of low-risk tumors.

**Materials and Methods**

- Women diagnosed with ER+, PR+, and HER2-infiltrating ductal carcinomas ≤ 1.5 cm were treated with cryoablation using a Visica® 2 Treatment System.
- Biopsy of tumor bed performed pre-cryoablation and at 6-month follow-up.
- TIL scores calculated using the International TIL Working Group guidelines.
- Whole transcriptome sequencing was performed on an Illumina NextSeq 500 instrument.
- Sequencing reads were mapped to the human reference genome Hg38.
- Differentially Expressed Genes (DEGs) were identified between pre and post cryoablation as ≥ two-fold changes and a correction p-value of ≤ 0.05.
- DEGs analyzed with Ingenuity Pathway Analysis Software (IPA®, QIAGEN).

**Results**

- 15 breast cancer patients treated with cryoablation.
- All tumors completely ablated with no residual disease seen on mammogram, ultrasound and MRI at 6 months.
- 5 Patients (33.3%) had pre and post cryoablation tissue samples analyzed.
- Mean ± (SD) tumor size at presentation was 9.34 ± (2.86) mm.
- Histopathology showed fat necrosis, collagen, foamy histiocytes and lymphocytes.
- Average TIL scores increased from 4.8% (range 1-10%) to 5.6% (range 1-15%) post cryoablation.
- RNA-seq analysis identified 1646 DEGs with 1096 genes up-regulated and 550 genes down-regulated.

**Conclusion**

Immune canonical pathways showed gene expression changes associated with tissue damage signaling, recruitment of leukocytes, phagocytic tissue damage clean up, increased antigen presentation, both TH1 and TH2 T cell immune responses and decreased adaptive immune resistance enhancing antitumor immunity. Larger sample size is needed to study clinical relevance of immune response to cryoablation.

**Future**

Clinical Study: Local Therapy Optimization by Grouping Immune-modulation with Cryoablation (LOGIC) for High-Risk Breast Cancers.

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