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 (Abscopal Effect).

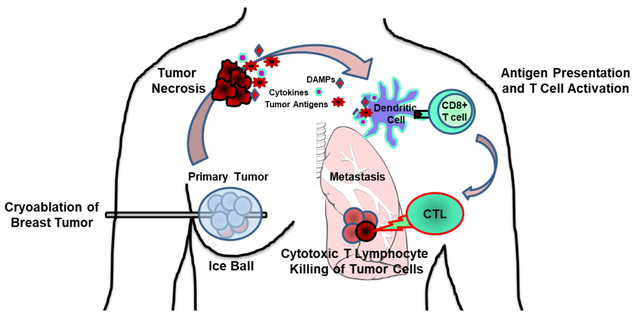


Figure 1. Cryoablation and Immune Response

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).

Figure 2. Experimental Approach

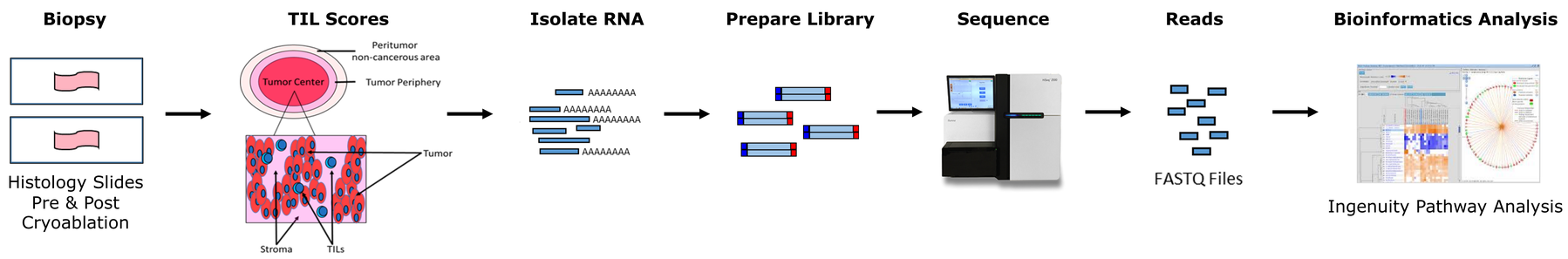
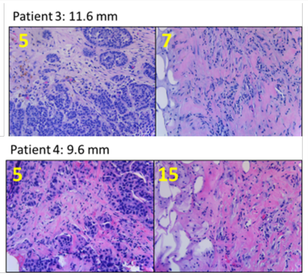


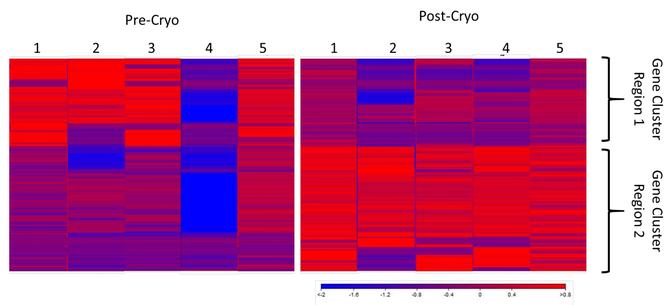
Figure 3. Tumor Characteristics and TIL Scores



ID	Age	Stage	Molecular Subtype	Tumor Size (mm)	Histopathology (6 month)	TIL Score Pre	TIL Score Post
1	75	1A	LumA	**7.5**	FN, Stromal fibrosis	**1**	**3**
2	81	1B	LumA	**13**	Lymphocytes near blood vessels	**3**	**2**
3	76	1A	LumA	**11.6**	FN, Foamy Histocytes	**5**	**7**
4	61	1A	LumB	**9.6**	FN w/ multinucleated Giant Cell Rxn.	**5**	**15**
5	68	1A	LumA	**5**	Collagen, FN, MicroCalc	**10**	**1**
Ave	72.2			**9.34**	Average	**4.80**	**5.60**
					Student T-test	**p = 0.81**	

FN = Fat Necrosis

Figure 4. Heat Map of Differentially Expressed Genes (DEGs)



Post Cryo vs. Pre Cryo DEGS (≥2 fold Change)	
Total	1646
Up-regulated	1096
Down Regulated	550

Figure 5. IPA for Immune Canonical Pathway Analysis.

Individual Patient Comparison for DEGS ≥ 2-Fold Change

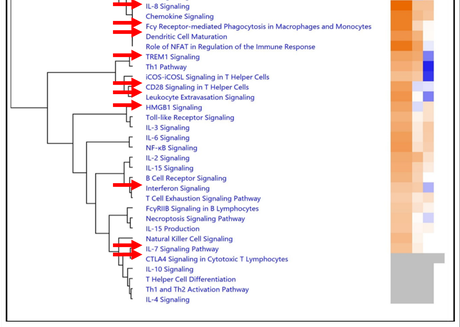
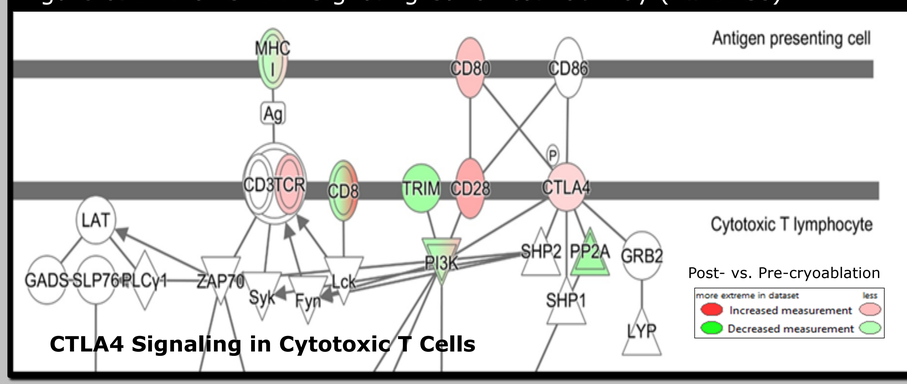


Table 1. Gene query - CTLA4 Checkpoint Pathway

T cell Activation / Co-stimulatory		
Gene	**log2FC**	**p value**
CTLA4	1.050	0.567
CD28	2.178	0.046
CD80	1.624	0.303
CD86	0.357	0.728
PD1	3.299	0.172
PDL1	-0.827	0.443
CD276	0.669	0.169
CD8	#N/A	#N/A
CD3	#N/A	#N/A
CD4	1.630	0.021
ICOS	-0.377	0.803
CD27	-1.768	0.289
PDL2	2.207	0.044
TNFRSF4	-2.742	0.135
TNFRSF9	1.010	0.485
TNFRSF18	-0.996	0.568
CSK	0.723	0.484
LCK	-0.608	0.724
PTCRA	-1.522	0.419

Red = up-regulated, Green = down-regulated

Figure 6. IPA for CTLA4 Signaling Canonical Pathway (All DEGs)



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 tissues 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.
- Overall, gene expression associated with immune cell functions were increased.
- IPA gene analysis showed increased gene expression for immune canonical pathways: HMGB1 Signaling, IL-8 Signaling, CXCR4 Pathway, Leukocyte Extravasation, FCγ Receptor Mediated Phagocytosis in Macrophages and Monocytes, Dendritic Cell Maturation, Natural Killer Cell Signaling, CD28 Signaling in T Helper Cells, IL-15 Production, and decreases in genes associated with the PD-1, PD-L1 Cancer Immunotherapy Pathway.
- Up-regulation of T cell costimulatory CD28 (> 2-fold) and inhibitory CTLA-4 (< 2-fold) receptors.

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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