

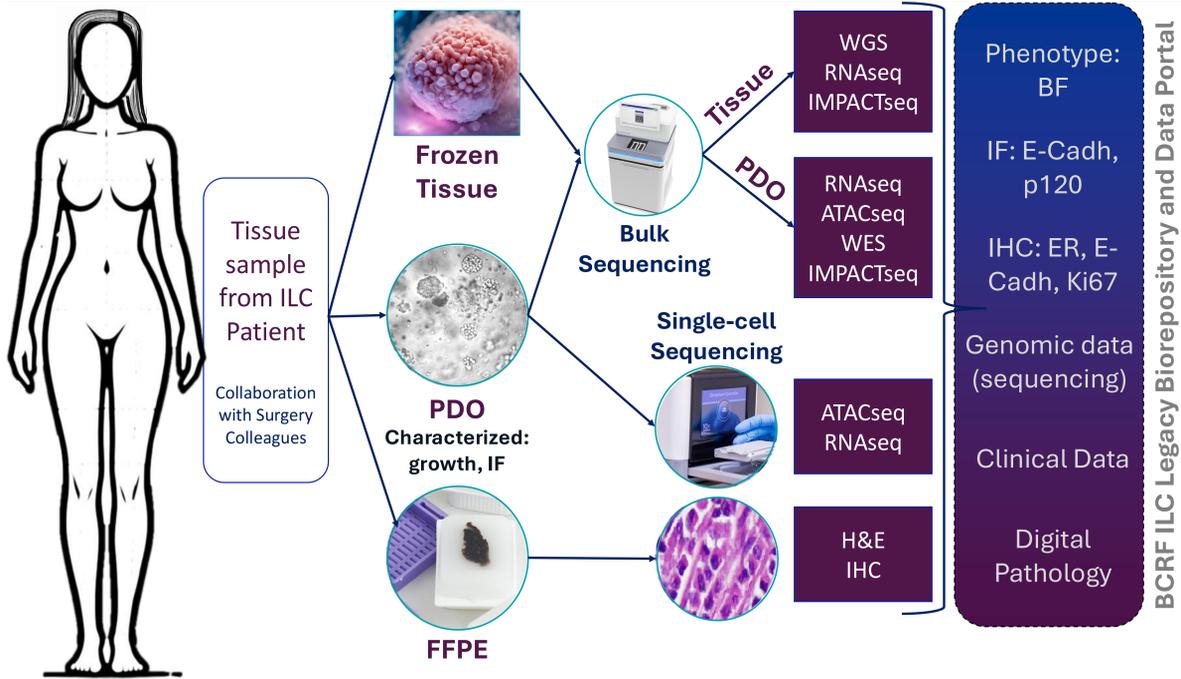
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Abstract

Background: Invasive lobular carcinoma (ILC) is a distinct histologic type of breast cancer with unique features. The lack of well-characterized ILC organoid models has limited research and therapy development. We aim to establish a well-characterized ILC organoid biobank under the BCRF Legacy Project, creating a valuable resource for ILC research. **Methods:** We performed whole-genome (WGS) and RNA-sequencing (seq) on patient FFPE tumor tissues. ILC organoids are subjected to whole-exome (WES), bulk RNA-seq, single nuclei (sn)RNA-seq, snATAC-seq, and MSK-IMPACT targeted sequencing. STR profiling confirmed specimen relatedness. Data were analyzed using validated bioinformatics tools. Immunofluorescence (IF) analysis of E-cadherin and p120 was performed. **Results:** After establishing a total of 9 ILC organoids, WES was performed on all 9 organoids while WGS was performed on 2 patient samples corresponding to 2 of these organoids. WGS analysis of bilateral ILCs (TP19-M179), corresponding to LIO-046 organoid, revealed that both tumors shared a *CDH1* p.L333Wfs23 frameshift mutation coupled with loss-of-heterozygosity (LOH), concurrent 1q gain and 16q loss, and dominant aging/clock mutational signatures. The left ILC was found to have fewer copy number alterations and somatic mutations than the right ILC. LIO-046 organoid was generated from right breast tumor tissue. Organoid IPM-BO-084 was generated from left ovarian metastasis of a mixed ILC (TP20-M130). Based on WGS analysis, the primary invasive carcinoma and ovarian metastases were found to be characterized by a somatic *BRCA2* p.Q2157fs18 mutation associated with LOH, and displayed genomic features of homologous recombination deficiency (HRD). The levels of chromosomal instability were higher in ovarian metastases compared to the primary lesion. WES analysis of ILC organoids identified genetic alterations affecting *CDH1*, which correlated with IF data where frameshift or nonsense mutations were associated with loss of E-cadherin and cytoplasmic p120 expression. **Conclusions:** We successfully established long-term ILC organoids. Our comprehensive genomic analysis of ILC organoids and patient samples underscores the genomic heterogeneity of ILC. Advanced sequencing techniques provide a framework for understanding ILC's molecular landscape. As we expand our biobank, these organoids and their data will be invaluable for developing targeted therapies and advancing ILC research. We are committed to making the genomic data and organoid models available to the research community. Our future goal is to foster collaboration and accelerate advancements in ILC research through this shared resource.

Project Goals: Development of the ILC Organoids Biorepository



Immunofluorescence

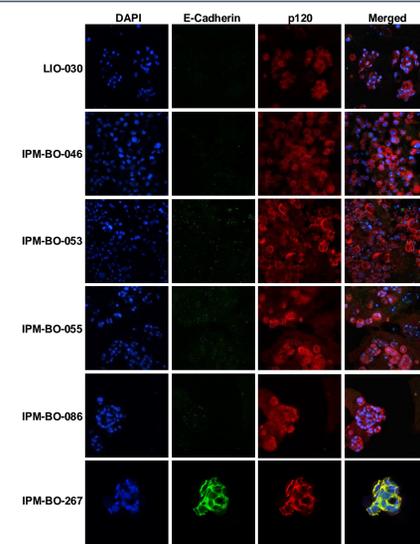


Figure 1: Immunofluorescence analysis of E-cadherin/p120 expression and localization in PDOs. IF confirms loss of E-Cadherin and cytoplasmic p120 in all ILC PDOs and membranous E-Cad/p120 in IPM-BO-267.

Results

Bulk whole-genome sequencing – tumor tissue

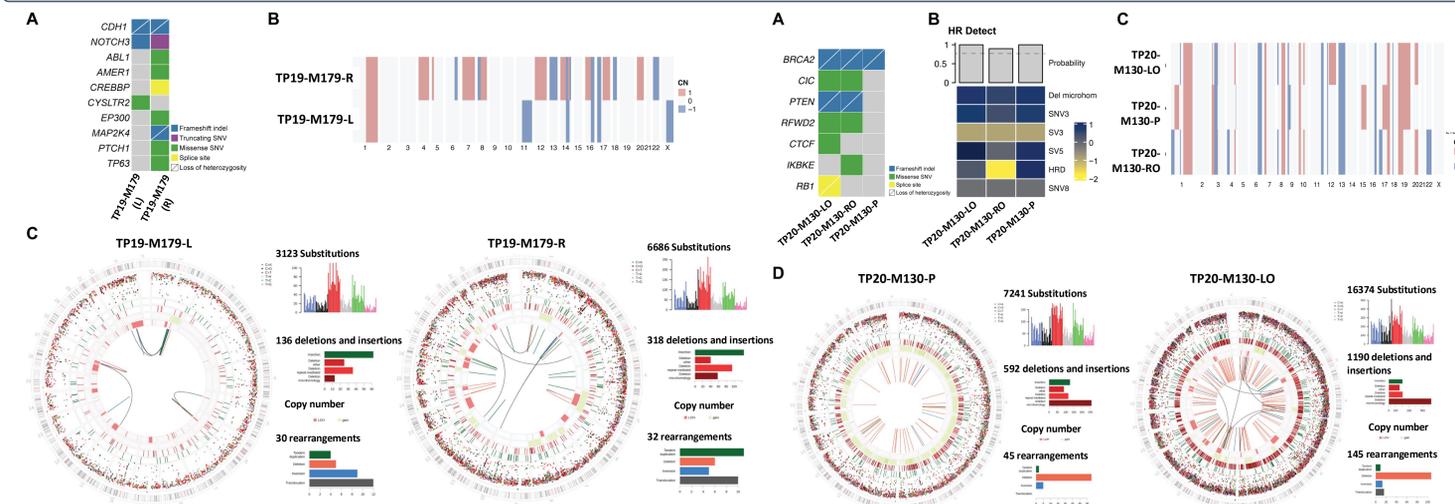


Figure 2. Genomic landscape of patient tissue sample TP19-M179. A, Somatic mutations affecting cancer-related genes. B, Copy number (CN) alterations with gains in red and losses in blue. C, Circos plots depicting, outside to inside, chromosome ideograms, single base substitutions, insertions, deletions, CN, loss of heterozygosity and structural variants. TP19-M179-L; left breast mastectomy, TP19-M179-R; right breast mastectomy. LIO-046 developed from TP19-M179-R.

Figure 3. Genomic landscape of patient tissue sample TP20-M130. A, Somatic mutations affecting cancer-related genes. B, HRDetect analysis. The primary tumor, TP20-M130-P, and ovarian metastases (TP20-M130-RO; right; TP20-M130-LO; left ovary) were HR-deficient. C, CN alterations with gains and losses. C, Circos plots depicting, single base substitutions, insertions, deletions, CN, regions of loss of heterozygosity and structural variants.

Honoring Leigh Pate's Legacy



Bulk targeted sequencing - organoids

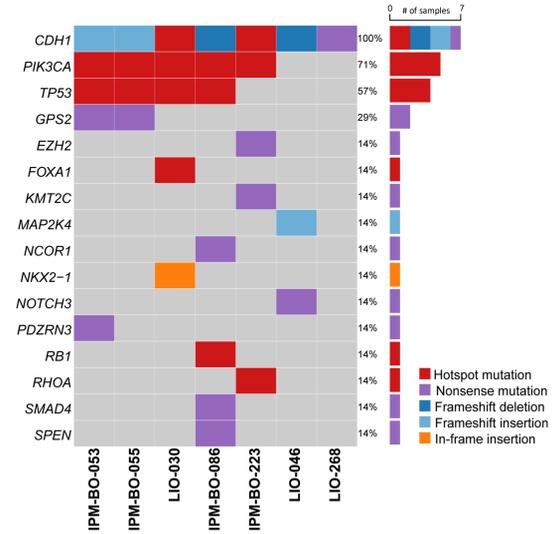


Figure 4: Landscape of cancer-related gene alterations in established ILC organoids. Recurrently altered genes in established ILC organoids, by targeted MSK-IMPACT panel sequencing. Only pathogenic alterations displayed.

Single-nuclei RNA and ATAC sequencing - organoids

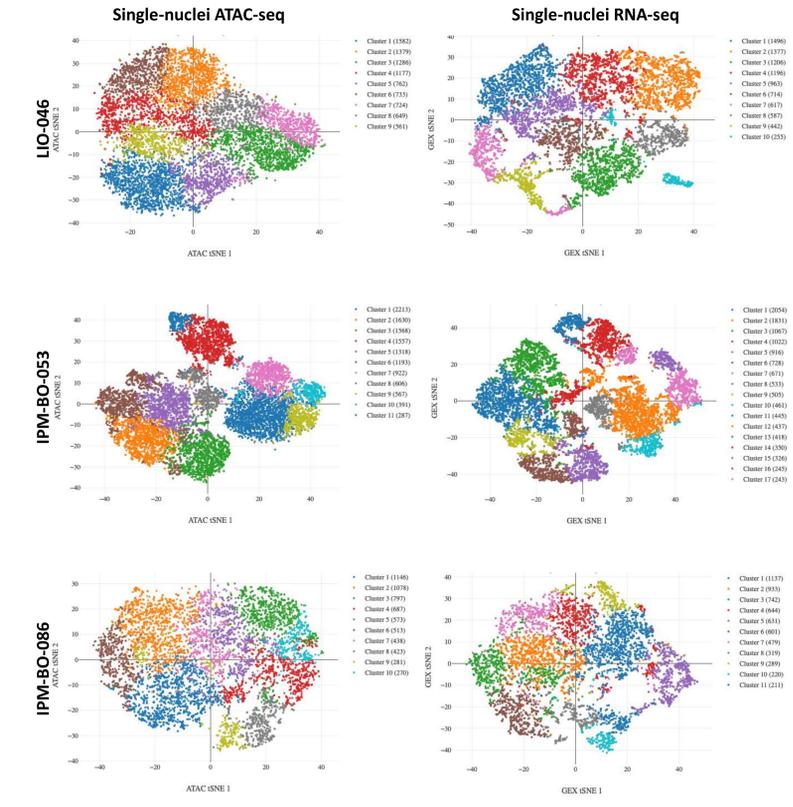
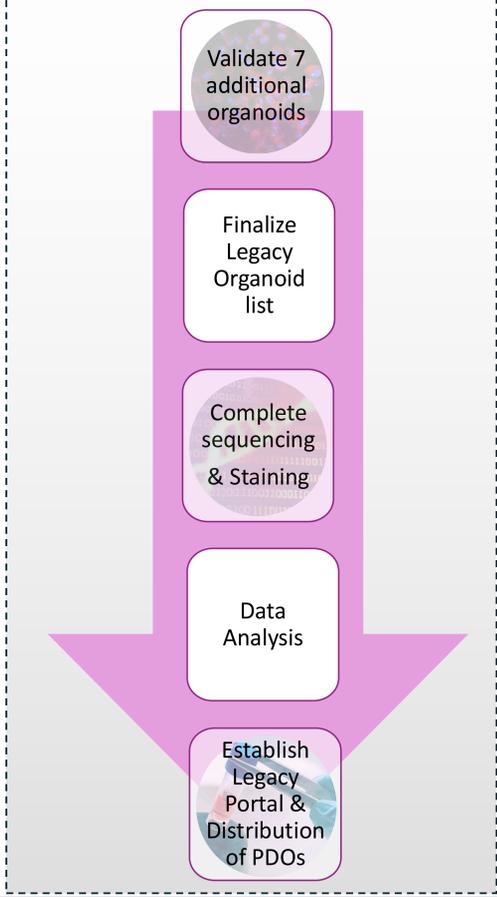


Figure 5: Single-nuclei Chromatin Accessibility and Gene Expression Profiles of ILC Organoids. Single-nuclei ATAC-seq (chromatin accessibility; left) and single-nuclei RNA-seq (gene expression; right) analysis of three ILC organoids. Each panel displays t-SNE plots where each point represents an individual cell, with clusters indicating groups of cells with similarities in chromatin accessibility (ATAC-seq) or similarities in gene expression profiles (RNA-seq) within each organoid.

Path Forward



Key Milestones in Development and Characterization of ILC Organoids

	Prospective vs IPM	Source Pathology	Prim vs Met	ER/PR/HER2	IF E-Cad/p120	WES	RNAseq (Bulk)	sc RNAseq	Sc ATACseq	IMPACT seq	Patient Tissue Samples				
											IMPACT	WGS	RNAseq (Bulk)	Digital Pathology	
LIO-030	IPM	ILC	Primary	+/-	✓	✓	✓	0	0	✓					✓
IPM-BO-041	IPM	ILC	Primary	+/-	✓	✓	✓	0	0	0					✓
LIO-046	IPM	ILC	Primary	+/-	✓	✓	✓	✓	✓	✓	0	✓	✓	✓	✓
IPM-BO-053	IPM	ILC	Met (bone)	+/-	✓	✓	✓	✓	✓	✓	0	0	✓	✓	✓
IPM-BO-055	IPM	ILC	Met (bone)	+/-	✓	✓	✓	0	0	✓					✓
LIO-084	IPM	mDLC	Met (ovary)	+/+	✓	✓	✓	0	0	✓	✓	✓	✓	✓	✓
IPM-BO-086	IPM	ILC	Met (bone)	+/-	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
IPM-BO-223	IPM	ILC	Met (LN)	+/-	✓	✓	✓	0	0	✓					✓
LIO-268	Prospective	ILC	Met (LN)	+/+	✓	✓	✓	0	0	✓					✓

0: In-progress, ✓: Completed