Archival FFPE blocks: The gift that keeps giving

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In a recent issue of *Cell*, Kaile and colleagues reported a robust high-throughput single-cell DNA sequencing (scDNA-seq) technique, named Arc-Well, that enables the researchers to overcome huge challenges while studying cancer genomic evolution in formalin-fixed and paraffin-embedded (FFPE) samples from clinical cancer patients. By the application of this technique, the team revealed a clearer view of the clonal diversity of breast ductal carcinoma *in situ* (DCIS) and genomic evolution of DCIS to recurrent disease.

Single-cell genomics have dramatically advanced our understanding of cancer evolution. A major hurdle to study cancer genomic evolution model(s) in patients is that archival clinical tissues with long-term patient outcome data have been routinely preserved as FFPE blocks for diagnostic purposes, while single-cell genomics are largely limited to the analysis of fresh or frozen tissues. In a recent issue of *Cell*, Wang et al. reported a powerful method, called Arc-Well, compatible with analyzing archival FFPE tissues by using a high-throughput scDNA-seq approach.[1] Utilizing Arc-Well enabled the researchers to fully leverage archival FFPE tissues for exploring the genomic lineages of breast DCIS progression to invasive ductal carcinoma (IDC) (Figure 1).

Three evolutionary models have been proposed for DCIS progression to IDC: independent lineages, evolutionary bottlenecks, and multi-clonal invasion (Figure 1). Yet, the genomic and evolutionary basis of progression from DCIS to IDC remains poorly understood. The rapid development of scDNA-seq technology has accelerated the process of resolving the sub-clonal heterogeneity and

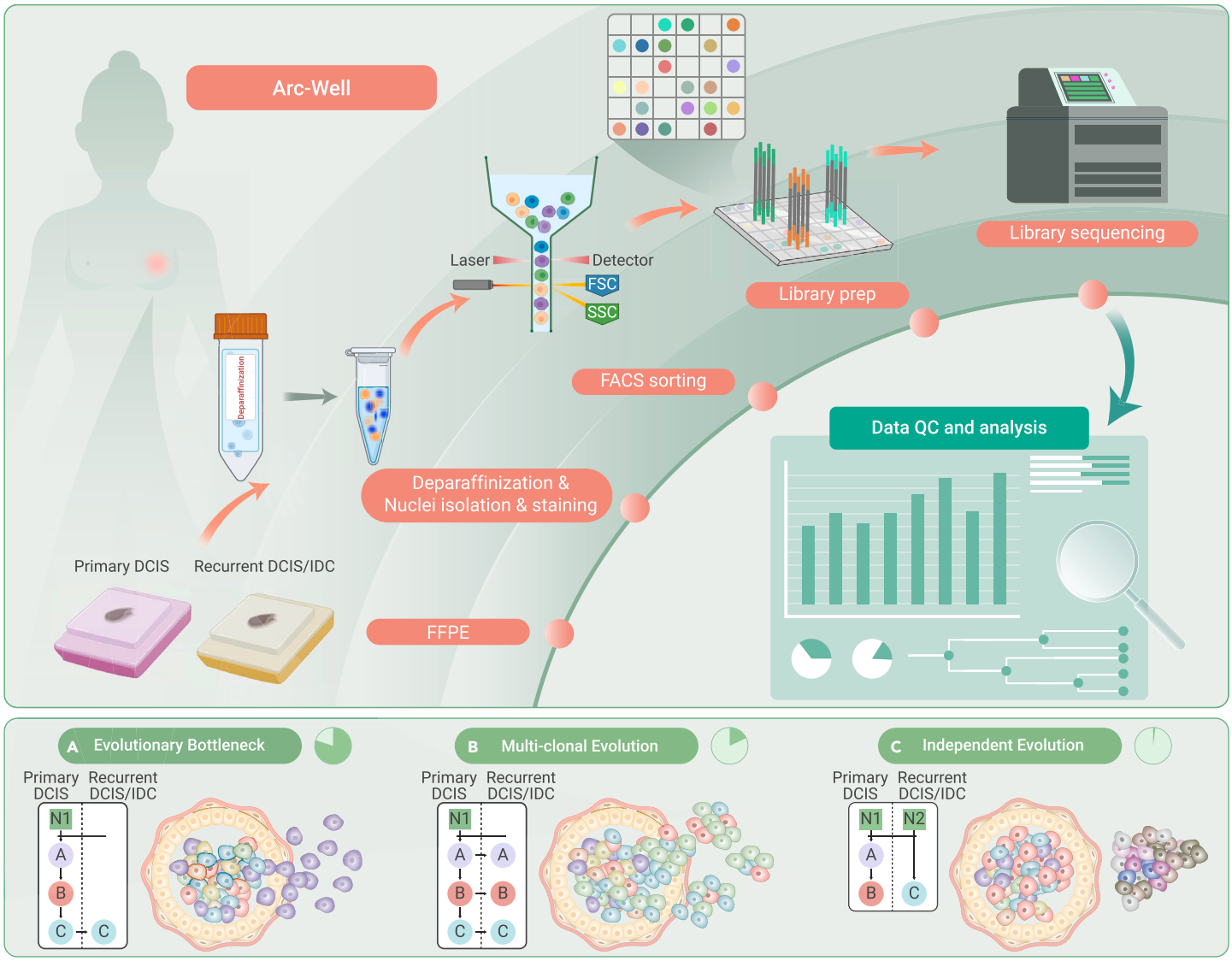

Figure 1. Overview of Arc-Well workflow and cancer genomic evolutionary models (A) Evolutionary bottleneck model shows that three clonal subpopulations are evolved from a single ancestral cell (N1), and a single clone is selected during invasion and expands to form the invasive carcinoma. (B) Multi-clonal evolution model shows that three clonal subpopulations are evolved from a single normal cell (N1) in the breast. In this model, all three clones escape the extinction and invade into the adjacent tissues to develop the invasive carcinoma. (C) Independent evolution model shows that the DCIS and IDC subpopulations are evolved from independent lineages that originated from two different normal cells (N1, N2) in the breast.

evolutionary lineage in synchronous DCIS and IDC by computational simulation upon quantification of copy number alterations (CNAs). Despite the high similarities in the genomic profiles and gene expression signatures of synchronous DCIS and IDC,[2] there are still clear differences in genome-wide copy number between them. As reported in this recent *Cell* paper, most DCIS lesions underwent an evolutionary bottleneck, in which persistent sub-clones became a common ancestor to form the recurrent invasive lesions.[1] Here, we highlight this powerful new technology (Arc-Well) applied to archival FFPE tissues and the emergent findings on the genomic evolution of DCIS (Figure 1).

Notably, FFPE preservation causes formalin-induced DNA-protein crosslinks and ultimately fragmentation that impedes the application of archival FFPE tissues for genomic amplification with existing whole-genome-amplification (WGA) chemistries. To overcome this challenge, Martelotto et al. firstly developed a method for whole-genome-sequencing-based copy number analysis of single cells derived from FFPE samples.[3] The newly developed Arc-Well method shows a number of improvements compared to previous methods including acoustic cell tagmentation,[4] for example: (1) improved cell yields, (2) decreased costs, (3) reduced technical variabilities, (4) optimized WGA chemistries, and (5) improved data quality. To further validate its performance, Arc-Well was tested in formalin-fixed breast cancer cell lines to confirm similar sequencing results in comparison to those without formalin fixation. Indeed, Arc-Well was found to provide comparable quality of sequencing results in over-dispersion and breadth of coverage metrics compared with other scDNA-seq platforms such as 10X Genomics Chromium Single-Cell Copy-Number Variation microdroplet system (10XCNV), direct library preparation (DLP), and degenerate-oligonucleotide-priming PCR-based method (DOP-PCR).[1] Moreover, the authors validated Arc-Well in lung and prostate FFPE tumors, and the sequencing results showed a better detection of copy number variation in those FFPE tumors, even in samples that were decades old.[1]

Using Arc-Well, the team studied 10 pairs of pure DCIS with matched recurrent IDC and revealed that most DCIS lesions (8/10 patients) shared highly similar DNA ploidy with matched recurrent IDC. In most of the patients, all sub-clones in primary DCIS were clustered together within a clade, whereas all the sub-clones in recurrent IDC were clustered in another clade, indicating a monoclonal expansion from a single ancestral sub-clone. This observation is consistent with the evolutionary bottleneck model, in which a common ancestor of persistent sub-clones was selected and expanded to form the recurrent cancers (Figure 1A). Additionally, two patients (2/10) had two or more sub-clones that expanded from the DCIS to form recurrent sub-clones in the recurrent IDC, which is consistent with the multi-clonal invasion model (Figure 1B). This model has been proposed in another *Cell* paper by the group led by N. Navin. It would be interesting to see cases with independent genomic lineages if they increase the patient numbers in this study by using Arc-Well technique, as previously Lips and Kurmar et al. reported that 18% of IDCs are actually unrelated to the DCIS, representing new independent lineages (Figure 1C).[5]

In conclusion, this recent *Cell* paper provides direct genomic evidence supporting that most DCISs undergo an evolutionary bottleneck at the time of progression.[1] Not so surprisingly, tumor recurrence is still the main cause of breast cancer death. The bottleneck and multi-clonal evolution models demonstrate that treatment with initial surgery for primary DCIS tumor isn't always sufficient to eliminate all cancer cells, as it allows persistent sub-clones to permanently exist and then expand as recurrent tumors. This posits two questions with regard to surgical resection and recurrence: what factors contribute to the leaving behind of cancer cells after surgery, and what mechanisms lead to tumor extinction even when some population of cells is left behind from surgery? In this recent *Cell* paper, Wang et al. showed that recurrent sub-clones acquired many clonal CNA events including gains of MYC, AKT3, FGFR4, ERBB2, and PIK3CA and loss of tumor suppressor genes such as PTEN, RB1, p53, and BRCA1/2.[1] Tumor clones harboring these genetic alterations exhibit great survival advantages, resisting extinction and resulting in the tumor recurrence. Thus, patients with such evolutional clones require additional therapy to reduce the recurrence, and such therapy might conceivably incorporate the genetic alterations to inform the choice of therapeutic targets. Importantly, archival FFPE tissues continue to prove to be valuable specimens, and their utility will increase as technologies to extract their biological insights continue to improve.

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DECLARATION OF INTERESTS

The authors declare no competing interests.