RESEARCH HIGHLIGHT

Unveiling the hidden battlefield: dissecting the invasive zone in liver cancer

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Understanding the tumor ecosystem, particularly the tumor invasive margins, is crucial for studying tumor progression and metastasis. Using nanoscale-resolution SpaTial Enhanced REsolution Omics-sequencing (Stereo-seq), Wu et al. identified an invasive zone around the tumor border in liver cancer patients characterized by the overexpression of serum amyloid A1 and A2, which is associated with immunosuppression and poor prognosis.

Tumor invasive zone at the margin areas, has been recognized as important zone where tumor infiltration or invasion occurs.[1,2] It is intimately linked to cancer progression, particularly metastasis, which remains one of the greatest challenges in cancer research and clinical management. The intricate interplay between tumor cells, surrounding normal cells, and the immune system plays a crucial role in dictating the fate of cancer progression and metastasis.[3] Consequently, in-depth investigation of the tumor ecosystem on this invasive zone surrounding the tumor border, is paramount for gaining insights into the underlying processes driving cancer progression and developing effective therapeutic strategies. However, until recently, the molecular and cellular mechanisms underlying tumor invasion within this zone remain largely elusive.

Recent developments in spatial transcriptomics have expanded the field exponentially and introduced new approaches to investigate the spatial organization of gene expression within tissues. Technologies such as Stereo-seq, Sequential Fluorescence In Situ Hybridization (seqFISH), Multiplexed Error-RobustFISH (MERFISH) and Slide-seq have emerged, enabling higher-resolution and multiplexed detection of RNA molecules with spatial information in intact tissue sections.[4–6] These methods utilize unique barcoding and imaging strategies, coupled with high-throughput sequencing, to capture and analyze gene expression patterns at single-cell resolution. In addition, advancements in computational algorithms and data analysis tools have enhanced the ability to decipher the complex spatial relationships between cells and understand the functional implications of their spatial organization.[7] These developments in spatial transcriptomics have opened up new avenues for studying tissue heterogeneity, cellular interactions, and the spatial dynamics of gene expression, ultimately deepening our understanding of biological processes and disease mechanisms within their native tissue context.

The recent groundbreaking study by Wu et al. utilizing nanoscale-resolution Stereo-seq has dissected and provided valuable insights into the invasive zone in liver cancer.[8] Stereo-seq is a cutting-edge spatial transcriptomics technology that combines DNA nanoball-patterned arrays and in situ RNA hybridization with nanoscale resolution at ~220 nm diameter and a center-to-center distance of 500–715 nm.[9] This technology, as developed by the same group, enables a detailed understanding of gene expression patterns and in-depth characterization of complex tumor ecosystem within tissues at sub-cellular level (Fig. 1a). In the current study published in *Cell Research*,[8] Wu et al. combined Stereo-seq and single-cell RNA sequencing (scRNA-seq), using the scRNA-seq data as a reference for cell type annotation on the Stereo-seq data, to analyze the tumor landscape in 21 patients with liver cancer (Fig. 1a). They identified the invasive zone, a 500 μm-wide region centered around the tumor border, using a novel tumor border scanning and digitization model. Remarkable immunosuppression, metabolic reprogramming and damage to hepatocytes were uncovered within this zone. In particular, they discovered a subpopulation of damaged hepatocytes, located in proximity to the tumor border on the paratumor (adjacent non-tumor liver) side, which exhibited elevated level of serum amyloid A1 and A2 (collectively referred to as SAAs) (Fig. 1b). Notably, the overexpression of CXCL6 in the neighboring malignant cells triggered activation of the JAK-STAT3 pathway in nearby hepatocytes, subsequently leading to the increased expression of SAAs (Fig. 1b). Furthermore, the overexpressed SAAs by the hepatocytes in the invasive zone bind to its receptors such as FPR1 for macrophage recruitment, and TLR2 for M2 polarization, thereby promoting local immunosuppression and potentially facilitating tumor progression (Fig. 1b).

Although the serum level of SAAs has been linked to poor prognosis in various solid tumors including HCC,[10,11] the impact of SAAs expressed within tumor marginal zone is not known. To validate the clinical relevance of the above findings, Wu et al. further demonstrated that overexpression of SAAs in the invasive zone is linked to a worse prognosis across an additional four independent cohorts comprising patients with primary and secondary liver cancer ($n = 423$) (Fig. 1c).[8] These findings highlight the prognostic significance of SAAs and provide insights into their contribution to tumor progression. In vivo experiments utilizing orthotopic implanted mouse liver tumor models further confirmed the pivotal role of SAAs. Knockdown of the genes encoding *SAA1* and *SAA2* in hepatocytes using pAAV-Saas-Sh resulted in significant inhibition of primary and secondary liver tumor growth in these mice (Fig. 1c). Moreover, reduced macrophage accumulation and M2 polarization were also observed in the invasive zone

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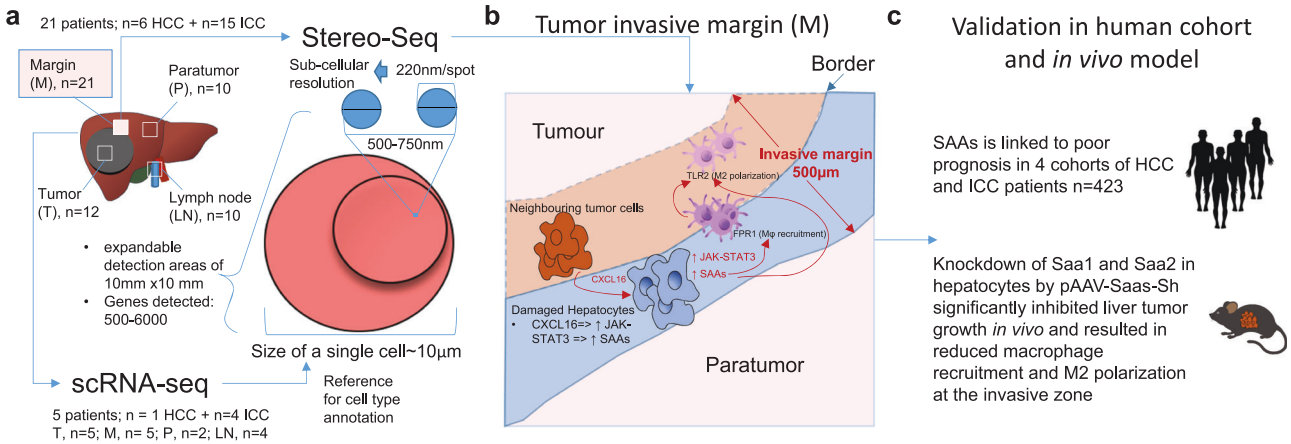

Fig. 1 Schematic summary of findings from Wu et al. on invasive margin in liver cancer. a Study design with combination of Stereo-seq and scRNA-seq to examine tumor (T), margin (M), paratumor (P) and lymph node (LN) tissues from 21 patients with liver cancer. Notably, Stereo-seq has sub-cellular resolution with detection spot at 220 nm diameter (45× smaller than the diameter of a typical single cell, 25 μm) and the spot-to-spot distance of 500–750 nm. **b** Main findings on the tumor invasive margin, a 500 μm zone centered around tumor border. **c** Validation of the clinical impact of SAAs in 4 cohorts of patients with primary or secondary liver cancers ($n = 423$). Additional validation of the role of SAAs in mouse liver tumor models orthotopically implanted with SAAs-knockdown cell lines.

(Fig. 1c). These findings not only shed light on the mechanisms of tumor invasion and metastasis but also have significant implications for the development of novel therapeutic strategies targeting advanced liver cancer and other solid tumors.

The comprehensive investigation of the invasive zone in liver cancer patients with high-resolution spatial information enabled by Stereo-seq has greatly enhanced our understanding of tumor biology, specifically on the intricate mechanisms underlying tumor invasion and metastasis. This novel zone provides valuable insights into the dynamic interactions between tumor cells, hepatocytes, and immune cells at the tumor margins. Most importantly, such spatial investigation was one of the first that offers high-dimension spatially-resolved data on cell–cell interaction as well as valuable insights into the hepatocytes, which are often too fragile to be captured in sufficient numbers using scRNA-seq. Furthermore, the detrimental effects of SAAs and their detailed mechanisms associated to macrophage recruitment and polarization were comprehensively characterized in an unprecedented resolution. The clinical association across multiple patient cohorts has further strengthened the prognostic significance of SAAs in the invasive zone. This not only emphasizes their potential as biomarkers for disease prognosis but also lays the foundation for the development of innovative therapeutic strategies targeting the invasive zone. The in vivo experiments in mouse liver tumor models have further confirmed the importance of SAAs in macrophage accumulation and tumor growth, providing additional support for their potential as therapeutic targets.

Overall, this study represents a significant advancement in our understanding of the invasive zone and its impact on tumor progression. The comprehensive insights gained from this research have the potential to revolutionize the field of cancer treatment and pave the way for future research aimed at developing innovative and personalized therapeutic approaches in the fight against cancer.

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ADDITIONAL INFORMATION

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