Supplementary Materials for

Comprehensive analysis of spatial architecture in primary liver cancer

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The PDF file includes:

Figs. S1 to S15
Legends for tables S1 to S5

Other Supplementary Material for this manuscript includes the following:

Tables S1 to S5
Figure S1. Sampling process and ST sequencing. (A) Samples were taken from different positions of the tumor according to the experiment design. Photo Credit: Rui
Wu, International Cooperation Laboratory on Signal Transduction, Eastern Hepatobiliary Surgery Hospital, Shanghai 200438, China. (B) Spatial transcriptomics technology can detect ~5,000 spatially barcoded spots of 55 μm diameter and 100 μm center-to-center distance in a capture area (6.5*6.5 mm²). (C) Spatial feature plots of the number of expressed transcripts (nUMIs) and genes (nGene).
Figure S2, related to Figure 1

Figure S2. H&E staining and expression of marker genes. (A) H&E staining and the spatial feature plots of six marker genes of L-sections.
Figure S3, related to Figure 1

Figure S3. H&E staining and expression of marker genes. (A) H&E staining and the spatial feature plots of six marker genes of N/T/P-sections.
Figure S4, Cluster comparison and the cell type scoring. (A) Diffusion map of the clusters across different patients, showing the first two diffusion components. The clusters (dots) were colored by patient sources (left) and tissue regions (right) information. HCC-1.1 represented cluster-1 of HCC-1. The clusters’ normal, stromal...
and tumor region labels were annotated according to the expression profile of marker genes and the corresponding tissue types in H&E images. (B) Violin plots of the six stromal and immune cell type scores in each cluster. (C) The MIA maps of the ST-defined clusters and the single-cell identified cell types from a published HCC scRNA-seq dataset. Each element in the heatmap indicated the enrichment degree (-log10(p-value) of hypergeometric test) of cell types in ST clusters, which were measured by testing on the overlap of their differential expression genes. (D) Comparison between the mean of ST signature-based cell type scores and the enrichment degree by MIA. Each dot indicated one ST cluster.
Figure S5, related to Figure 3

(A) Density plots showed the distribution of six stromal and immune cell type scores in normal and tumor regions. The dashed lines marked the median of the scores in the two types of regions, respectively. (B) Comparison of the median of stromal and
immune cell subtype scores between the normal (x-axis) and tumor (y-axis) regions in each L-section.
Figure S6. Mapping the changes of hallmark pathway activities on both sides of the transition region. (A) Gradient area division results on both sides of the transition region with the interval of 5 spots in L-sections. (B) The changes of hallmark pathways’ activities along with the gradient divisions on both of the tumor and normal sides. Each dot indicated the median of the pathway activities in the corresponding area.
Figure S7. Comparison between tumor clusters. (A) Clustered heatmap of the metabolic pathway averaged activities of tumor clusters in Module-1. (B) The survival curves of two groups of patients in TCGA and LCI cohorts to compare the relative malignancy of ST tumor cluster pairs (cluster-2 vs 6, and cluster-5 vs 6 in HCC-1T).
These two groups were divided according to which ST tumor cluster the bulk samples were more similar to at expression level. Log-rank test was used to measure the statistical significance of their relative malignancy degrees.
Figure S8. CNV Profiles of HCC-1 and HCC-3. (A-B) CNV profiles of the bulk samples of HCC-1 and HCC-3 patients. The three columns indicated the samples from different tissue regions: the normal region of the leading-edge sections (left), the tumor region of the leading-edge sections (middle), and the tumor sections (right). (C)
Heatmap of the inferred CNV profiles for tumor cluster spots (row). Red: amplifications; blue: deletions. The CNVs of normal references from N-sections were also presented at the top. (D) The averaged CNV profiles for each tumor cluster in HCC-3, inferred from spatial transcriptomes.
Figure S9. The correlation between hallmark pathway activities and CSC marker expression levels. (A-B) Scatter plots showing the correlation between hallmark pathway activities and the expression levels of the stemness markers (CD47, PROM1, and EPCAM) on the TCGA and LCI cohort datasets, respectively. Linear regression and Pearson correlation were used to measure their relation.
Figure S10. Characteristics of the clusters in HCC-5. (A) Heatmap showing the differentially expressed genes of the 6 clusters in HCC-5. (B) Clustered heatmap showing the relative activities (average of the z-scores) of oncogenic pathways of tumor clusters in HCC-5.
Figure S11. The accuracy of TLS-50 gene signature for TLS identification. (A-B) Comparing of identification accuracy between TLS-50 and other signatures in all L-sections and HCC-5. White number: the TLS region number. (C) Scatter plots showing the correlation between TLS-50 and 12-Chemokine signature scores on bulk
sequencing data of 239 HCC cases.
Figure S12, related to Figure 7

Figure S12. Immunohistochemistry and CODEX of TLSs on HCC tissue microarrays. (A) Immunohistochemical staining on HCC tissue microarrays showed the high expression of CD52 and CD53 in TLSs. (B) CODEX on HCC tissue microarrays. High expression of CD4&CD8 (T cell markers), CD20 (B cell marker),
and podoplanin (lymphatic endothelial cell marker) confirmed the TLSs, while high expression of HLA-DR in these TLSs confirmed the accuracy of TLS-50 signature (HLA-DQB1 and HLA-DQA1 are ranked the 42nd and 50th in the TLS-50 signature, respectively).
Figure S13. The H&E staining and TME characteristics of TLSs. (A) TLS identified by TLS-50 signature and their corresponding H&E staining images in the L-sections. (B) Violin plots showing the difference of immune cell type scores in the TLSs and their surrounding stromal backgrounds. Statistical significances were determined
with two-sided Wilcoxon rank-sum tests. * p<0.05; ** p<0.01; *** p<0.001; **** p<0.0001.
Figure S14. Diverse TLSs composition shaped by their distance to tumor cells. (A) The distance from each TLS spot to tumor regions on H&E staining images of L-sections. (B) Scatter plots showing the correlation between the T cell subtype enrichment levels of TLS spots with their distances to tumor regions. Linear regression and Pearson correlation were used to measure the relation. (C) Scatter plots showing the correlation between the gene expression levels of TLS spots with their distances to
tumor regions. Genes that significantly changed in 3 or more cases were displayed. Linear regression and Pearson correlation were used to measure the relation.
Figure S15. The spatial changes of signature genes in TLSs. (A) Spatial plots showing the surrounding regions (5 spots wide) of TLS in L-sections and HCC-5, which were colored by the distances to the TLS spots. (B) The spatial changes of signature
genes around TLSs. For each gene, top: spatial plots showing genes’ expression distribution on the surrounding regions (5 spots wide) of TLS, which were colored by the genes’ expression levels; bottom: scatter plots showing the relation between the expression levels and the distances to TLS spots. Linear regression and Pearson correlation were used to measure the relation.

SUPPLEMENTARY TABLES

Table S1. Clinical and Pathological Data of Each Patient.

Table S2. Sample Spatial Transcriptomics Sequencing Data Summary.

Table S3. Signature Genes of Indicated Cell Types.

Table S4. Differentially Expressed Genes of Cluster-2, 5 and 6 in HCC-1T.

Table S5. Differentially Expressed Genes of Cluster-6 in HCC-5 and the TLS-50 Signature Genes.