Supplementary materials

Figure S1

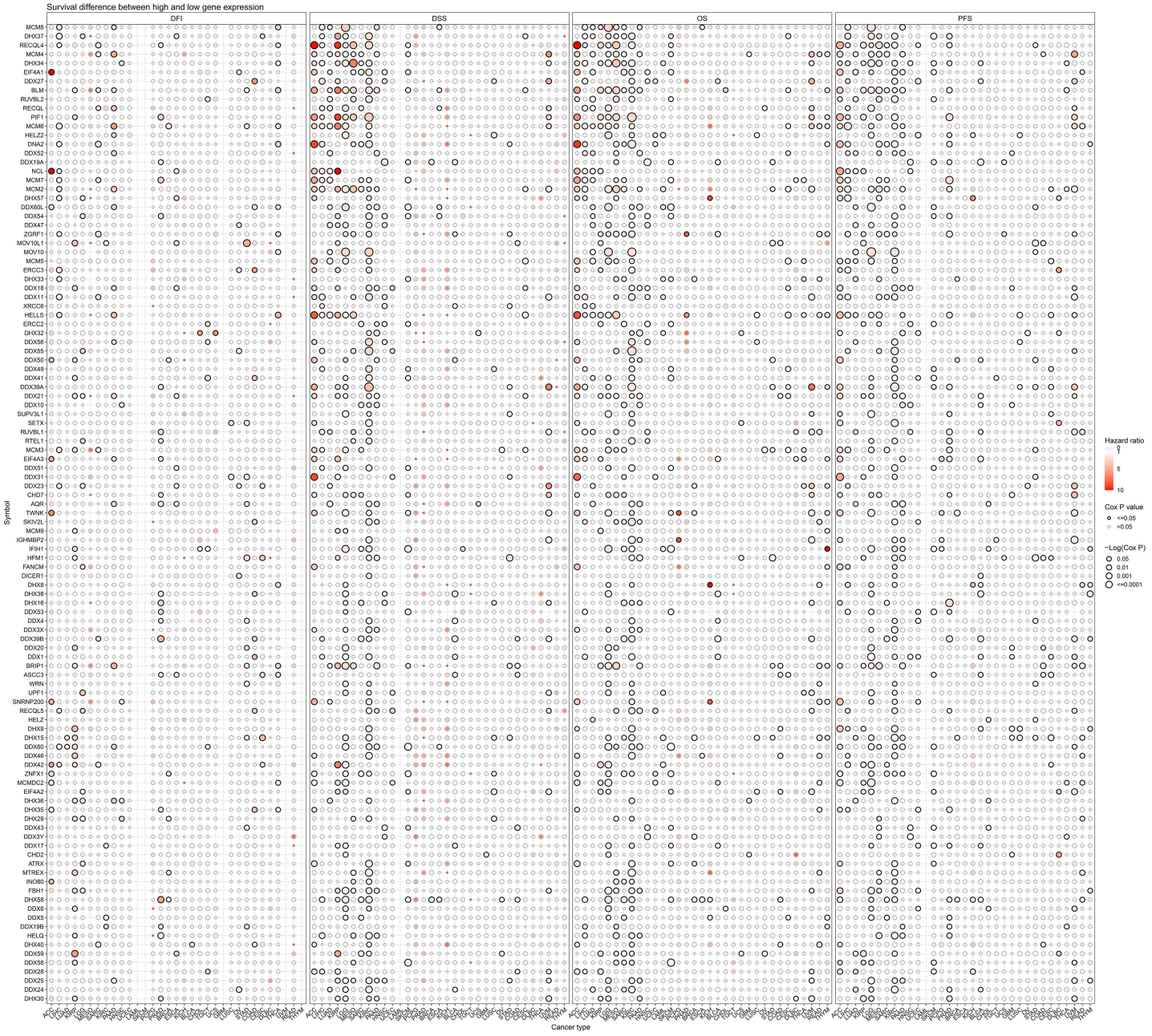

Figure S1 The correlation between disease-free survival (DFI)/disease-specific survival (DSS)/overall survival (OS)/progression-free survival (PFS) and expression data of helicases coding-genes (high or low) using the integrated TCGA Pan-cancer clinical data resource. The stronger the correlation, the darker is the color, and the larger is the dot.

Figure S2

Figure S2. Correlation analysis of all helicases coding genes and drug sensitivity (IC50) analysis in HCC, where red represents positive correlations and blue represents negative correlations. We used the drug susceptibility data from Genomics of Drug Sensitivity in Cancer (GDSC) database (A) and Cancer Therapeutics Response Portal (CTRP) database (B).

Figure S3

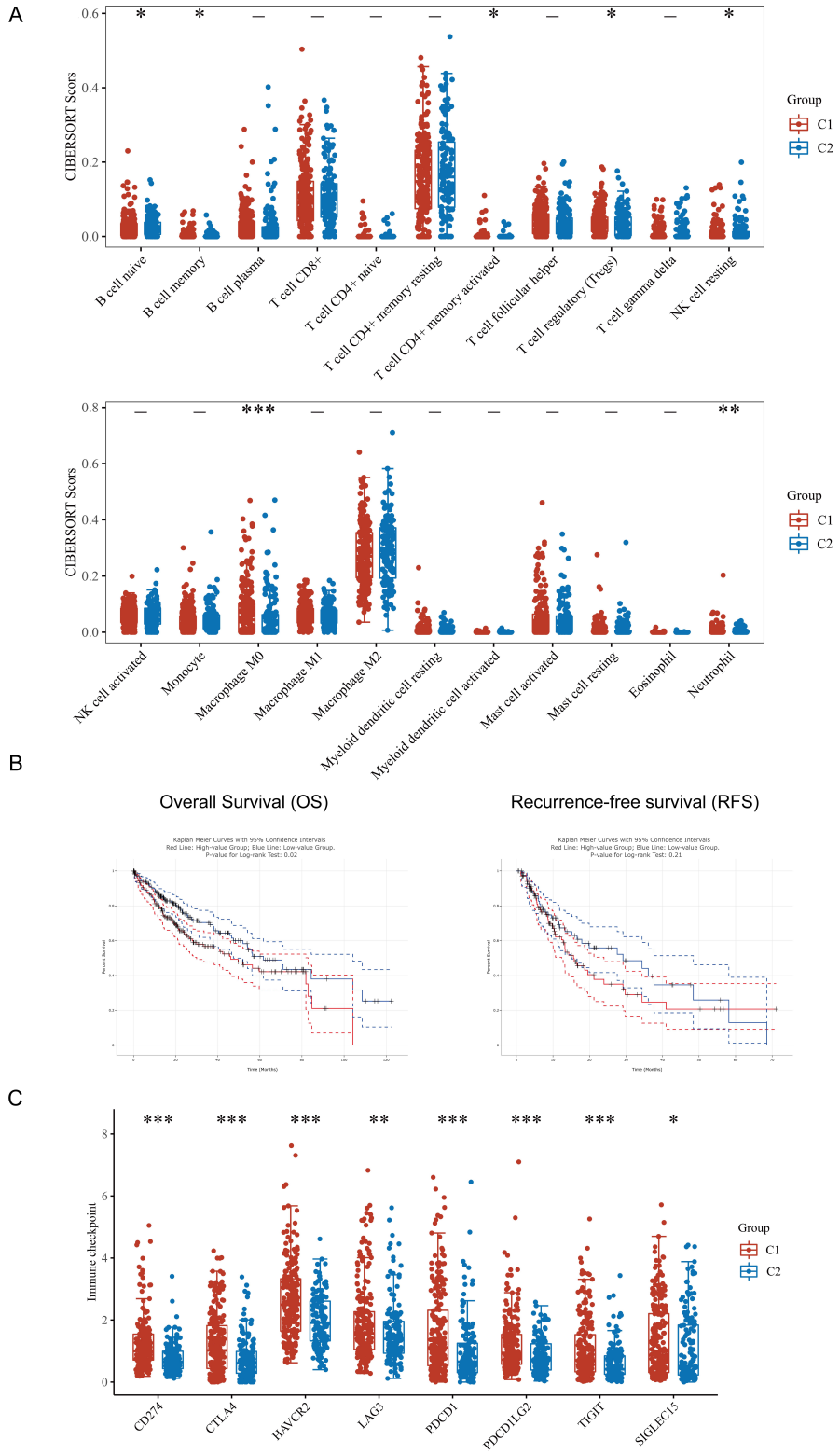

Figure S3. Relationship between the abundance of immune cell infiltration and two different Helicases Level Patterns. (A) Score distribution of immune cells in 2 unique modification patterns of LIHC associated with helicase, where the horizontal axis represents different groups of samples, the vertical axis represents the gene expression distribution, C1 showed helicases low expression levels, and C2 showed high helicases

expression levels. P-values are indicated in the upper left corner and asterisks represent the level of significance. (B) overall survival (OS) and Recurrence-free survival (RFS) analysis by Kaplan Meier method for the two clusters. (C) Common immune checkpoints expression between the two clusters (Kruskal–Walli's test). (*P < 0.05, **P < 0.01, ***P < 0.001).

Figure S4

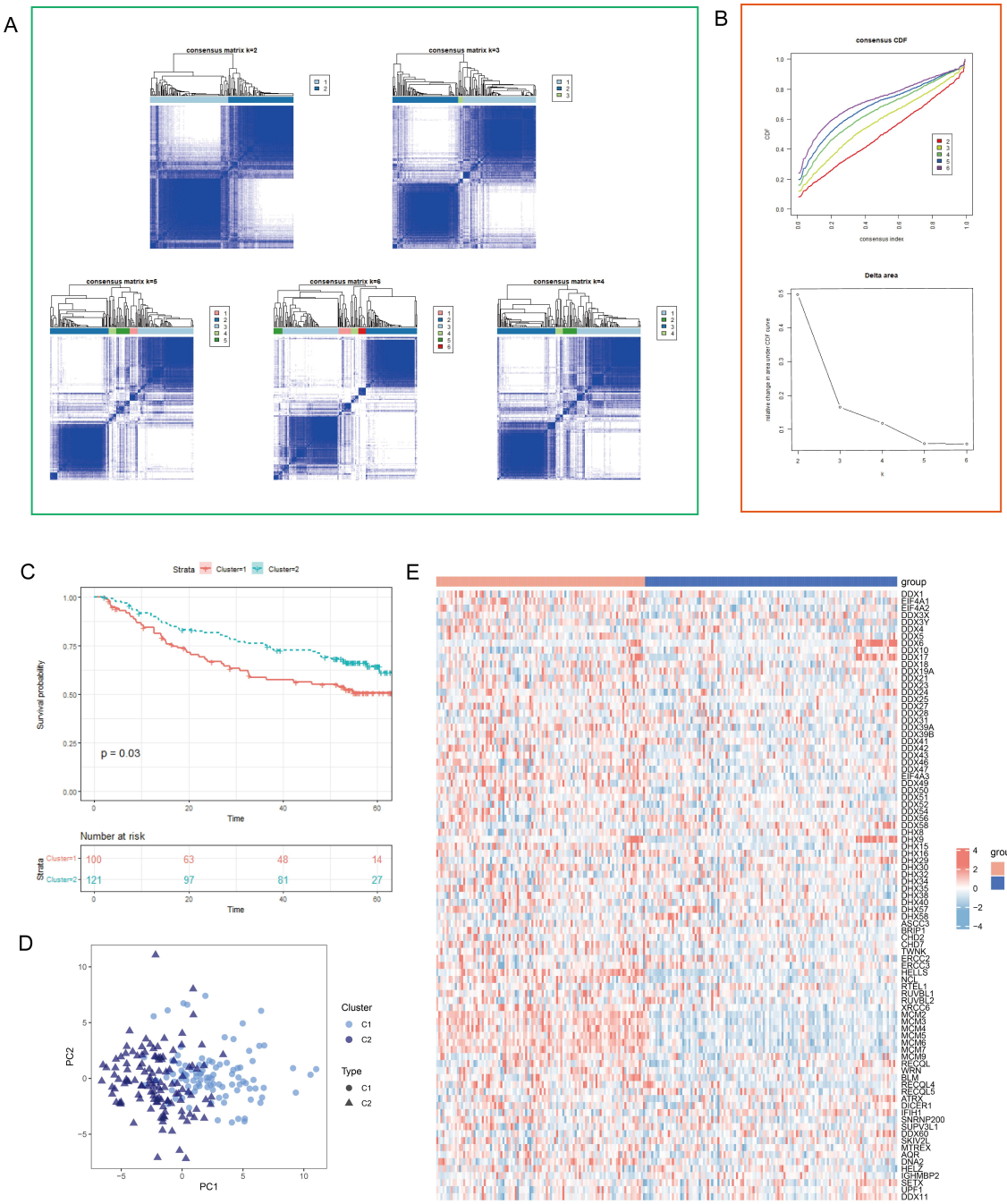

Figure S4 (A)The consensus score matrix of all samples when k = 2-6. (B)The cumulative distribution function (CDF) curves in consensus cluster analysis. CDF curves of consensus scores by different subtype numbers (k = 2, 3, 4, 5, and 6) were displayed. Relative change in area under the CDF curve for k = 2–6. (C) Survival

analysis (OS) based on two subtypes. (D) The PCA distribution of GSE14520 by expression profile of Helicases. Each point represents a single sample; different colors represent the C1 and C2 subtypes respectively. (E) Expression distribution of 112 helicases coding-genes between two subtypes.

Figure S5

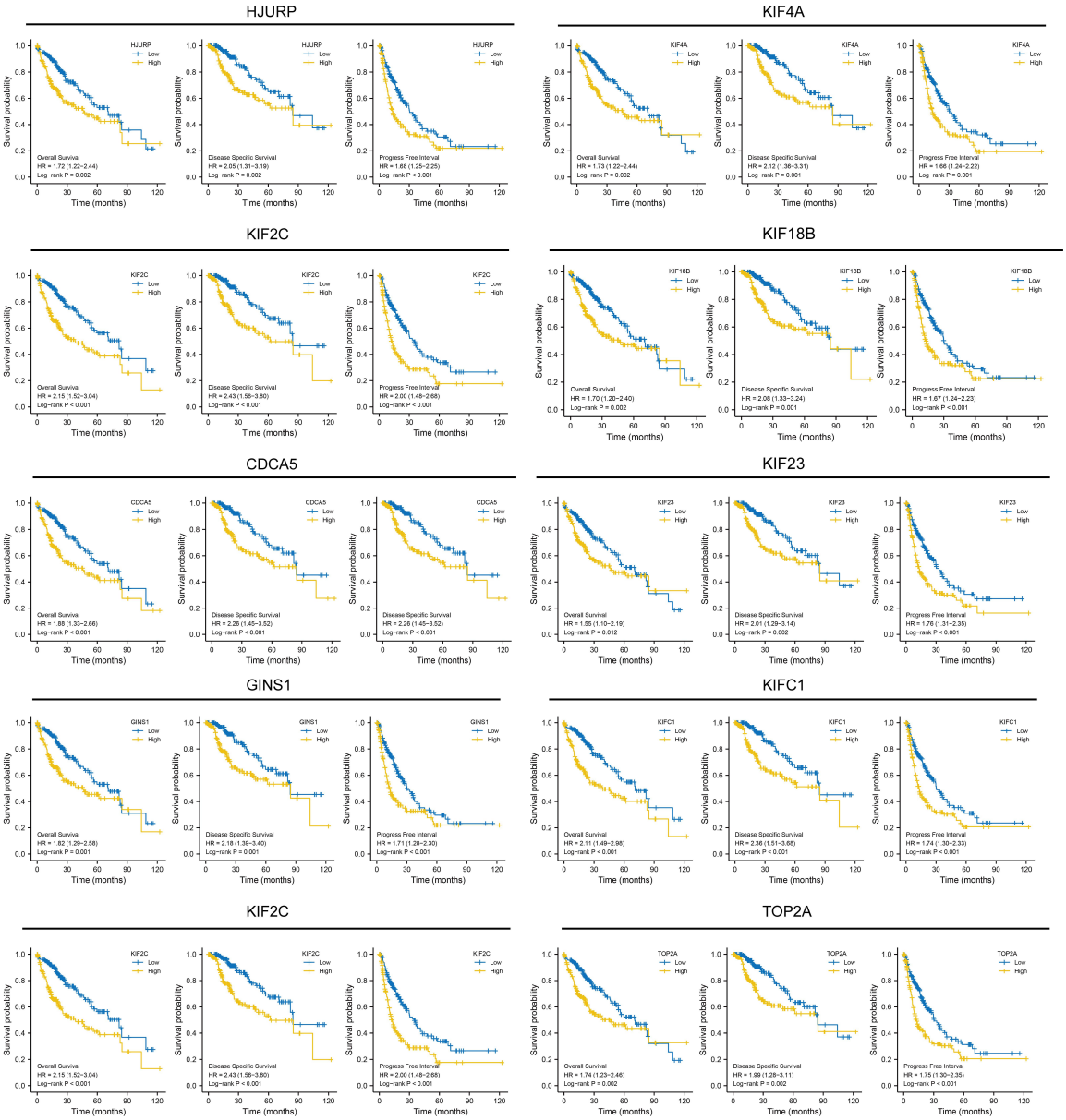

Figure S5. Prognosis value of 10 hub genes. The Kaplan-Meier survival analysis revealed that 10 hub genes were significantly associated with survival outcomes by using external TCGA-LIHC database (n =374).

Supplementary Table S1

Antibodies and Reagents	Manufacturer, Country, Cat number	Concentration
Anti-DDX56/DDX21	Abcam, Cambridge, UK, #ab97648	IHC 1:500

Antibody		WB 1:1000
Anti-Ki67 Antibody	Abcam, Cambridge, UK, #ab156956	IHC 1:1000
β-actin Antibody	Thermo Fisher Scientific, US, #PA5-85490	WB 1:5000
Goat Anti-Rabbit IgG H&L (HRP)	Abcam, Cambridge, UK, #ab205718	IHC 1:2000 WB 1:5000
Bicinchoninic acid (BCA) Assay Kit	Thermo Fisher Scientific, US, #23227	-

Sample Collection and Immunohistochemical Staining (IHC)

The use of specimens was carried out with the informed consent of the patients in accordance with the principles of the Declaration of Helsinki. Our research was approved by the ethics committee of the Zhangjiagang TCM Hospital Affiliated to Nanjing University of Chinese Medicine (Ethics Approval No.2022-03-14). All patient records/information was anonymized and de-identified prior to analysis. Each sample was collected frozen and immediately stored in liquid nitrogen. Inclusion criteria: (1) All patients were diagnosed as HCC for the first time by pathology. (2) Patients with HCC who were hospitalized in the Zhangjiagang TCM Hospital Affiliated to Nanjing University of Chinese Medicine from 2020-01-01 to 2022-05-01. Also, pathological specimens from each patient were kept for independent re-diagnostic confirmation by two doctors. (3) All patients had complete clinical information. Exclusion criteria: (1) Patients with a clinical diagnosis of a tumor that has metastasized to the Liver from another site. (2) Exclusion of any other serious chronic disease or other visceral tumors. (3) Incomplete clinical data. Finally, a total of 40 patients with HCC were analyzed. IHC was performed on the paraffin-embedded tissue sections. According to the standard protocol, after incubation at 60°C for 10 minutes, the tissue sections were dewaxed twice in xylene for 10 minutes each, hydrated in decreasing grades of ethanol (100%, 90%, 75% and 50% ethanol for 5 minutes each), immersed in distilled water for 5 minutes and blocked for 1 h using 4.0% rabbit and 2.0% goat serum. Subsequently, the slides were incubated with primary antibodies overnight at 4°C. Three 10-min TBS washes were carried out, and the samples were incubated with 1:2000 of secondary

biotin-conjugated antibody for 2 hours. All slides contained duplicate sections from which one served as a control for secondary antibody-binding specificity. Micrographs were acquired by a NIKON Eclipse Ni-E microscope (original magnification, ×400) (NIKON, Japan). The extent and intensity of staining were assessed by two independent investigators with the H-SCORE (higher scores indicating stronger positive staining, range 0–300). Staining intensities were graded as 0 (none), 1 (weak), 2 (moderate), and 3 (strong). Staining extent was graded as 0 (no positively stained cells), 1 (less than 10%), 2 (10–50%), and 3 (over 50%). The H-SCORE, representing both the proportion of stained cells and the degree of staining, was determined as described in routine clinical trials[1].

1. Yuan, Y., et al., *miR-410 induces both epithelial-mesenchymal transition and radioresistance through activation of the PI3K/mTOR pathway in non-small cell lung cancer.* Signal Transduct Target Ther, 2020. **5**(1): p. 85.

Cell Culture and Western Blotting

Five human HCC cell lines were used in this study. Those human hepatocarcinoma cell lines (SNU-182/SNU-387/HepG2/Huh7/SK-Hep-1) were purchased from Wuhan Pu-nuo-sai Life Technology Co. Ltd. (Wuhan, China). They were cultivated in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% Fetal bovine serum (FBS). All the cells were maintained in a humidified 5% CO_2 atmosphere at 37°C. Cells were lysed in RIPA buffer, and protein concentration was measured using a BCA Assay Kit. 20 μg proteins were electrophoresed by SDS-polyacrylamide gels and transferred onto anitrocellulose membrane. The membrane was then blocked in non-fat milk for 1 hour at room temperature and subsequently incubated with the primary antibodies overnight at 4°C. After washing with PBST (phosphate-buffered saline containing 0.05% Tween 20) for three or four times, the membrane was incubated with the secondary antibody for 1 hour at room temperature. Protein bands were visualized using the Odyssey Infrared Imaging System (Li-COR Biosciences). The expression levels of each protein were normalized relative to β-actin protein expression and are shown as relative protein

levels. Relative protein expression = gray value of target protein band/gray value of β-actin band.