Materials and methods

Human tissue samples. HCC tissues and paired adjacent tissues were obtained from 18 patients (all males, 51-67 years of age) undergoing surgical procedures at Henan Provincial People's Hospital, between March 2019 and September 2019. All samples were stored at -80°C in order to avoid degradation of RNA. Before the use of samples for research, written informed consent was obtained from all patients. The use of tissue samples was approved by the Human Ethics Committee of Henan Provincial People's Hospital.

Wound healing assay. Wound healing assay was performed for analysis of cell migration in vitro. Briefly, the HCC cell lines (Huh7, Hep3B, SNU-449 and SNU-387) were cultured in 6-well plates (5x105/well) and incubated overnight. After transfection with H19 siRNA and the NC siRNA, a sterile pipette tip was used to scrape uniform wounds. At 0 and 24 h after scratch formation, images were obtained using an inverted microscope at a magnification of x40 and then the results were photographed.

Invasion assay. For the cell invasion assay, the upper culture chamber was covered with BD MatrigelTM Matrix (BD Biosciences). At 24 h after transfection with H19 siRNA and NC siRNA, the HCC cell lines (Huh7, Hep3B, SNU-449, SNU-387) in serum-free medium (100 μ l) were plated into the upper chamber at 37°C. Meanwhile, 600 μ l of complete medium was added to the lower chamber. At 24 h after incubation at 37°C, the uninvaded cells in the upper chamber were removed by a cotton tip. The invading cells were fixed in 4% polyoxymethylene and then stained with 1% crystal violet solution for 30 min at 25°C. Images of the invasive cells per well were captured and the cells were counted in random fields using a light microscope at x40 magnification.

Figure S1. Upregulation of long non-coding RNA (lncRNA) H19 and miR-675 in HCC tissue samples. Statistical analysis of the relative expression level of (A) H19 and (B) miR-675 in HCC tissues and adjacent tissues as determined by the RT-qPCR assay. $^*P<0.05$ and $^{***}P<0.001$. HCC, hepatocellular carcinoma.

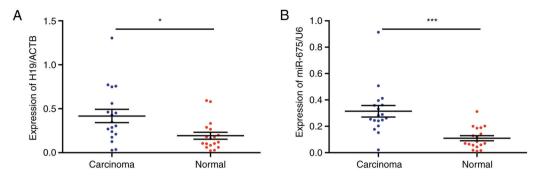


Figure S2. Downregulation of long non-coding RNA (lncRNA) H19 suppresses the migration and invasive abilities of the HCC (Huh7, Hep3B, SNU-449, SNU-387) cell lines. (A) Scratch wound healing and (B) Matrigel invasion assays revealed that the migration and invasive abilities of HCC cells transfected with H19 siRNA were evidently suppressed compared with the NC siRNA group. ***P<0.001 vs. control. HCC, hepatocellular carcinoma; NC, negative control.

