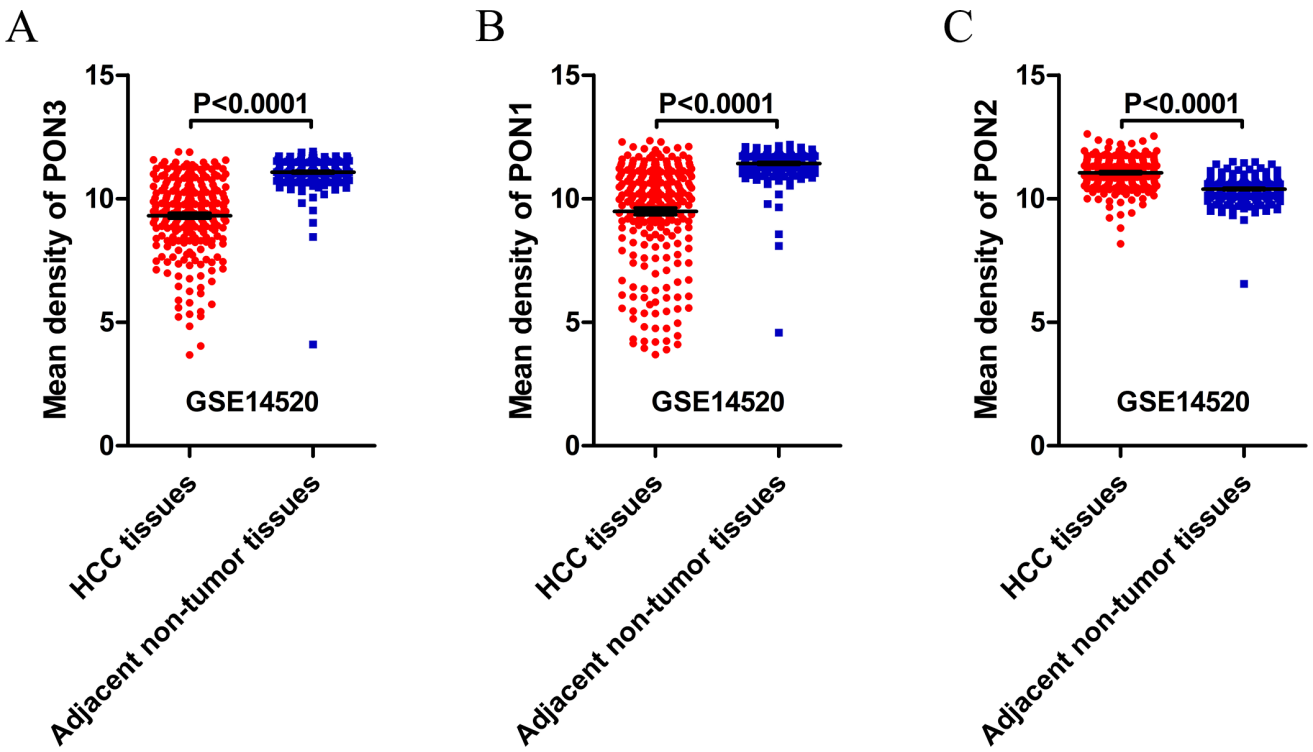
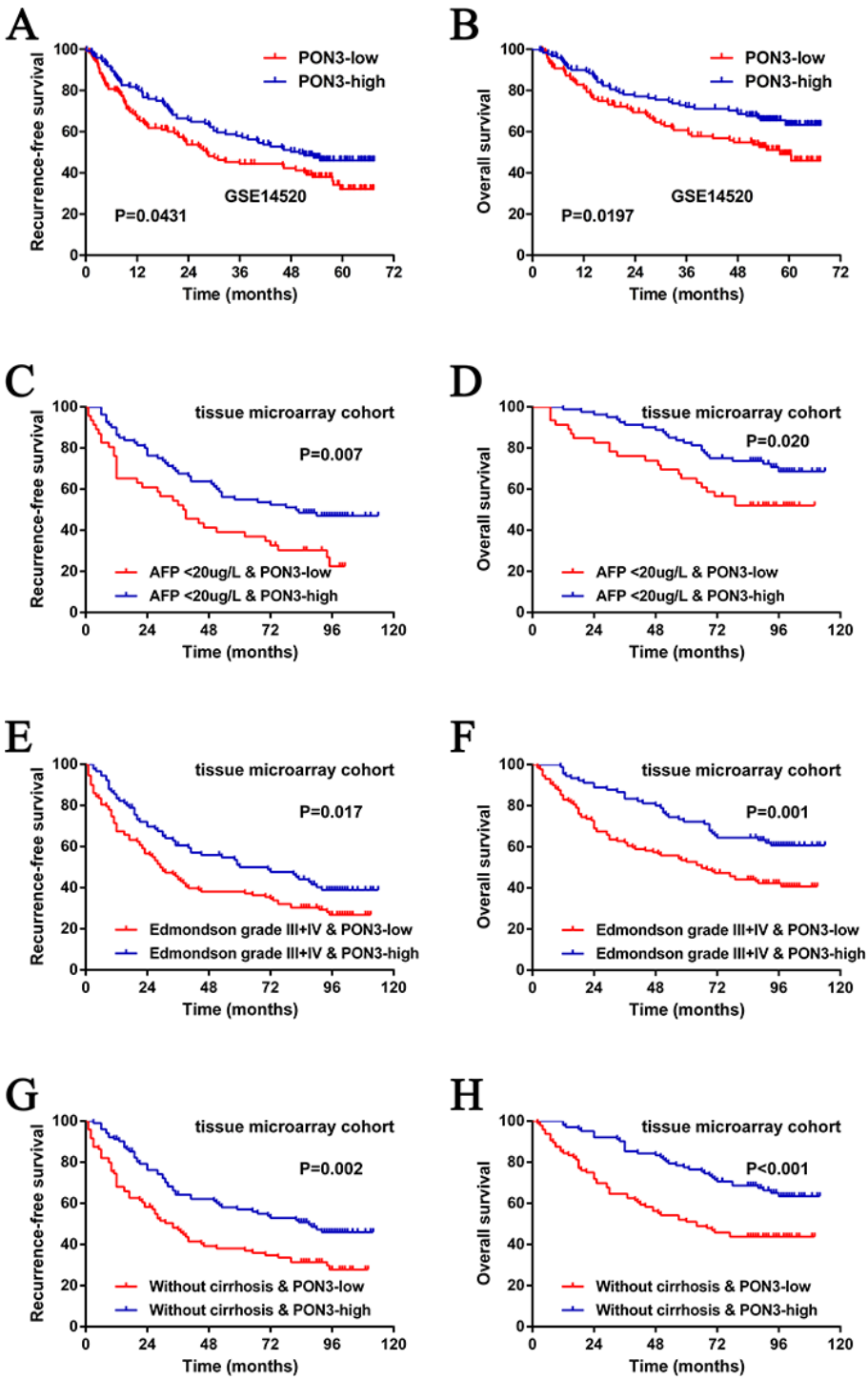
# Paraoxonase 3 inhibits cell proliferation and serves as a prognostic predictor in hepatocellular carcinoma

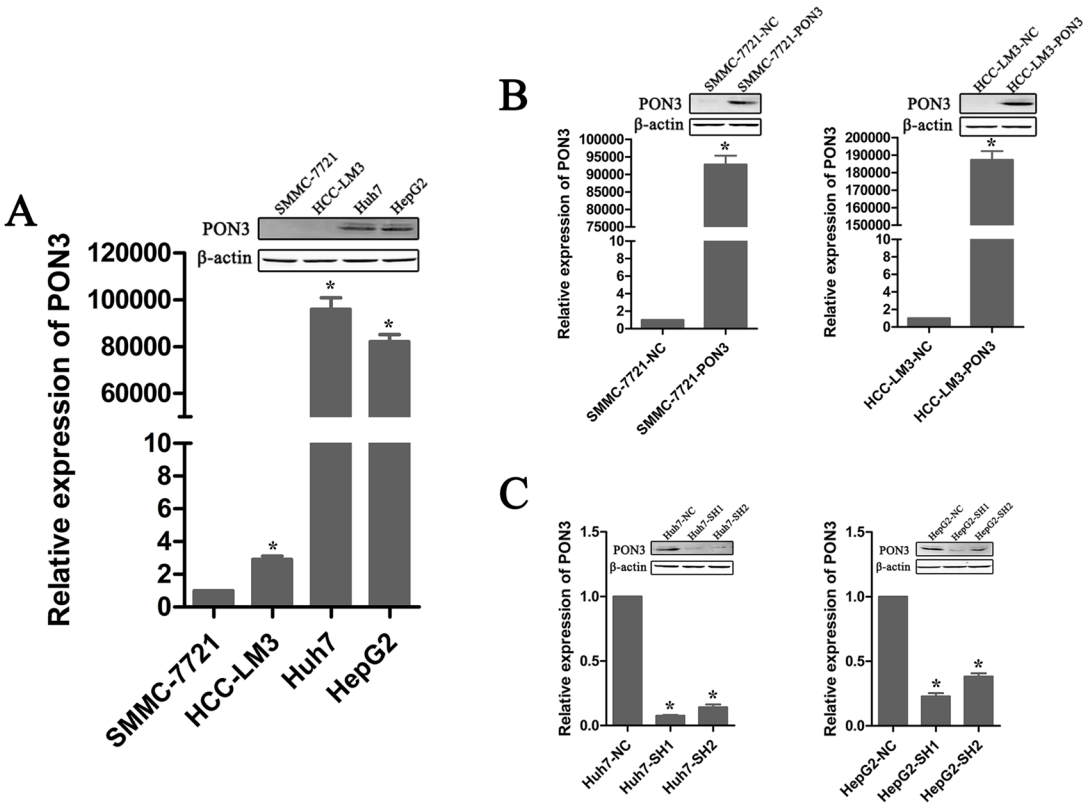
## Supplementary Materials



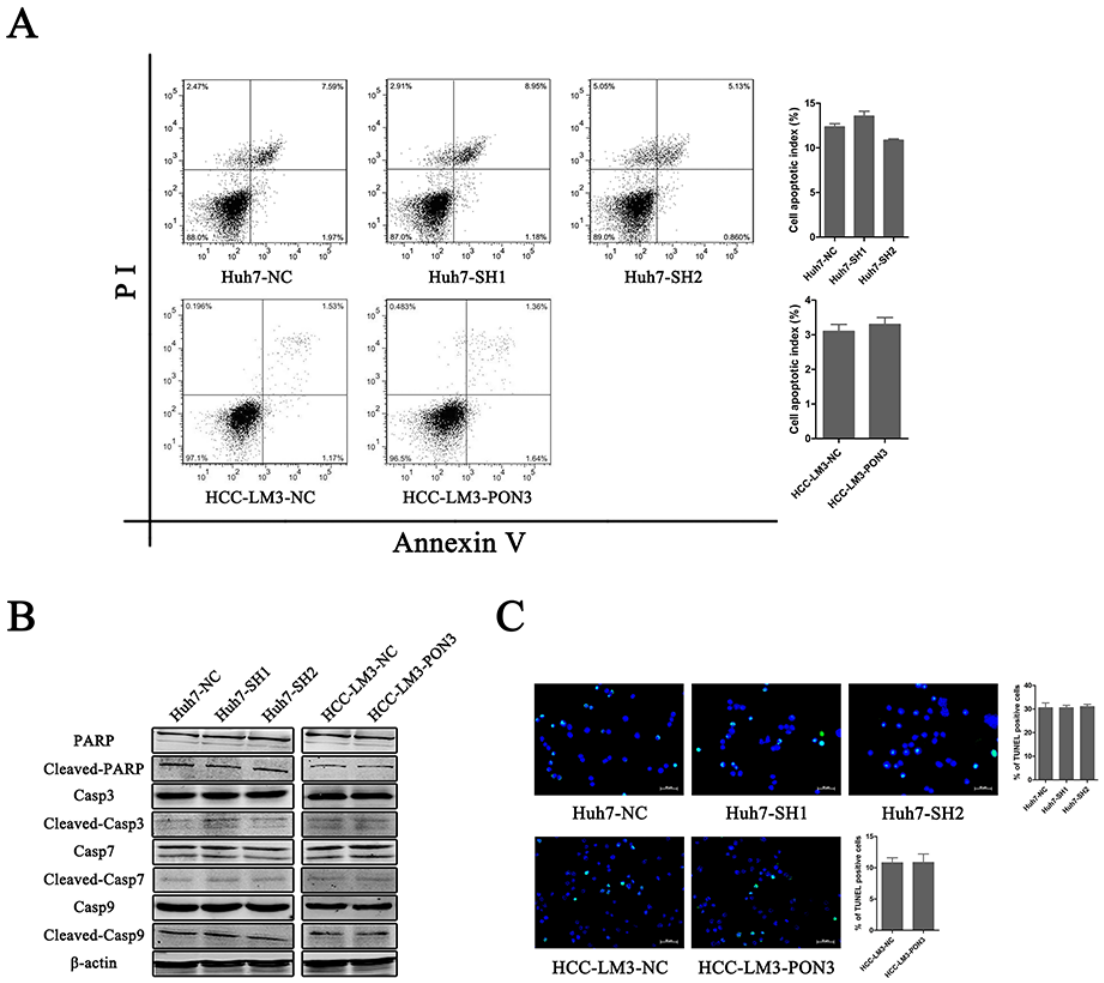

**Supplementary Figure S1: GEO DataSets (GSE14520) analyses of PON genes expression in HCC.** Statistical significance was determined by student's *t*-tests. Data are shown as mean ± SD.




**Supplementary Figure S2:** (**A** and **B**) The subgroup with low PON3 mRNA level had significantly shorter RFS and OS than the subgroup with high PON3 mRNA level in an online microarray data (GSE14520). The prognostic value was also observed in patients with serum AFP < 20 μg/L (**C** and **D**), poor differentiation (**E** and **F**), and cirrhosis absence (**G** and **H**). Statistical significance was assessed by two-sided log-rank tests.




**Supplementary Figure S3:** (**A**) PON3 expression level in HCC cell lines. (**B** and **C**) The efficiency of PON3 overexpression or knockdown in stable transfected cells. Data are shown as mean ± SD. Significance ($p < 0.05$) was determined from three independent experiments and assessed by student's *t*-test.




**Supplementary Figure S4: Anti-apoptotic function of PON3 was not observed in HCC cells.** (**A**) A flow cytometry analysis of apoptosis using annexin V-FITC and PI staining in PON3 overexpression and knockdown cells. (**B**) Western-blot analysis of apoptosis-related proteins PARP, caspase-3, -7, -9 and their respective cleavage fragments in PON3 overexpression and knockdown cells. β-actin was used as an internal control. (**C**) Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) staining of PON3 overexpression and knockdown cells. Nuclei were detected with Hoechst 33342 in blue and TUNEL-positive cells were in green. Magnification, 200×; scale bars = 50 μm. Cells were pretreated with apoptosis-inducers A (Apopida) and B (Apobid) (1:1,000) for 6 h for apoptosis analyses. The experiments were performed in triplicate and data are expressed as the mean ± SD. Significance was assessed by student's *t*-test.

**Supplementary Table S1: Univariate analysis of the post-hepatectomy outcomes of HCC patients in tissue microarray cohort**

| Variable | Recurrence-Free Survival (%)▲ | *P*-value | Overall Survival (%)▲ | *P*-value |
|---|---|---|---|---|
| Age (year), ≥ 50: < 50 | 36.232:36.486 | 0.589 | 53.623:52.027 | 0.764 |
| Gender, Male: Female | 35.827:40.625 | 0.533 | 50.394:71.875 | 0.045★ |
| Hepatocirrhosis, Present: Absent | 27.273:40.404 | 0.018★ | 48.863:54.545 | 0.437 |
| Edmondson grade, III + IV: I + II | 35.616:38.806 | 0.234 | 49.772:62.687 | 0.036★ |
| Tumor size (cm), ≥ 5: < 5 | 36.842:36.126 | 0.154 | 42.105:58.115 | 0.001★ |
| Capsule, Absent: Present | 41.975:34.146 | 0.476 | 49.383:54.146 | 0.216 |
| Microvascular invasion, Present: Absent | 35.000:36.466 | 0.443 | 40.000:53.759 | 0.051 |
| Satellite lesions, Present: Absent | 22.727:37.500 | 0.020★ | 50.000:53.030 | 0.598 |
| TNM stage, II-IV: I | 26.230:39.111 | 0.001★ | 36.066:57.333 | 0.001★ |
| HBeAg, Positive: Negative | 35.922:36.612 | 0.708 | 49.515:54.645 | 0.298 |
| Serum AFP (ug/L), ≥ 20: < 20 | 33.750:39.683 | 0.031★ | 44.375:63.492 | 0.000★ |
| PON3 expression, Low: High | 32.168:40.559 | 0.007★ | 44.056:61.538 | 0.000★ |

▲The time follow-up ended is used to calculate the Recurrence-Free Survival and Overall Survival
★$p < 0.05$ by Long-rank test.

**Supplementary Table S2: The list of primers sequences used in study**

| Gene symbol | Sequence |
|---|---|
| **PON3** | Forward: GAGAAGTGGAGCCAGTAGA |
| | Reverse: CAGGAGGGAGTTGGTAAA |
| **MKI67** | Forward: AATTGAACCTGCGGAAGA |
| | Reverse: CTCTCCTCTGCCACCTT |
| **PAX6** | Forward: GAAGCAAGAATACAGGTATGG |
| | Reverse: TTGTGGAATTGGTTGGTAGA |
| **IL18** | Forward: TGAATCCTCCTGATAACATC |
| | Reverse: TAGTCTTCGTTTTGAACAGT |
| **IGFBP2** | Forward: CCAAGAAGCTGCGACCAC |
| | Reverse: GGGATGTGCAGGGAGTAGAG |
| **MMP7** | Forward: ACGGATGGTAGCAGTCTA |
| | Reverse: ATGGAGTGGAGGAACAGT |
| **NUPR1** | Forward: ACAGAGCTGGAGATGAGG |
| | Reverse: TGCCGTGCGTGTCTATT |
| **CENPF** | Forward: GCAGAAGACACGGAAGG |
| | Reverse: GGTTCTCGGAGGATGGT |
| **β-actin** | Forward: GGGAAATCGTGCGTGACATTAAG |
| | Reverse: TGTGTTGGCGTACAGGTCTTTG |