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Tumorigenic role of YAP in hepatocellular carcinogenesis is involved in SHP2 whose function is different *in vitro* and *in vivo*

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ABSTRACT

Yes-associated protein (YAP) is a nuclear effector of the cell-density sensing Hippo pathway and interacts with Src homology phosphotyrosine phosphatase 2 (SHP2), which controls cell proliferation and survival. The tumor promoting/suppressing activities of YAP and SHP2 during liver tumorigenesis remain controversial. This study aimed to investigate the tumorigenic roles of YAP and SHP2 in hepatocellular carcinogenesis. Cell density associated subcellular distributions of YAP and SHP2 in normal human hepatocytes (THLE-2) and hepatocellular carcinoma (HCC) cells (SK-Hep1, SNU-182) were investigated by Western blotting and cell block immunohistochemistry. The effects of YAP knockdown on proliferation, migration and invasion were studied using YAP-specific siRNAs. The prognostic significance of YAP and SHP2 expressions was investigated immunohistochemically using a tissue microarray (TMA) from 50 HCC cases. High-cell density decreased the nuclear expression of YAP and SHP2 in normal hepatocytes as compared with low-cell density. However, in HCC cells, nuclear YAP and SHP2 were observed regardless of cell density. Nuclear YAP influenced SHP2 expression and cell proliferation. In particular, YAP knockdown impacted nuclear levels of SHP2 protein in SK-Hep1 cells. In HCC tissues, nuclear YAP expression was elevated and cytoplasmic SHP2 expression was diminished as compared with adjacent non-tumor tissues. Notably, these expressions were found to be significantly associated with poor recurrence-free and overall survival rate in patients with HCC. Consequently, the tumor promoting role of YAP is involved in SHP2 which functions as a tumor promoter *in vitro* but as a tumor suppressor *in vivo*. YAP and SHP2 can be unfavorable prognostic markers in HCC.

1. Introduction

Hepatocellular carcinoma (HCC) is the sixth most common malignancy and the third leading cause of cancer-related mortality worldwide [1]. HCC is characterized by multicentric development, recurrence, and high metastatic potential, which contributes to its dismal prognosis [2]. To aid the development of therapeutic strategies for HCC, the molecular mechanisms responsible for the liver tumorigenesis have been intensively studied, and the Hippo pathway has recently been implicated in the pathogenesis of HCC [3].

The Hippo pathway senses cell density and by regulating cell proliferation and apoptosis is involved in the control of organ size [4]. Yes-associated protein (YAP) is a nuclear effector in the Hippo pathway, and functions as a key regulator of cell growth, cell proliferation and survival [5,6]. Recently, YAP was suggested to be a candidate oncoprotein, and that its expression in cancer was linked with poor prognosis [7–9]. Elevated YAP protein levels have been reported in various types of cancers including hepatic, colorectal, ovarian, and breast cancers [10–14]. On the other hand, some researchers regard YAP a tumor suppressor in some cancers including hepatocellular carcinoma, and to be associated with better prognosis, and complete loss of YAP has been reported to predict poorer survival in cancer [15–20].

Src homology phosphotyrosine phosphatase 2 (SHP2) is encoded by *PTPN11* and a ubiquitously expressed intracellular protein that promotes cell proliferation and motility [21,22]. Interestingly, it was recently reported SHP2 interacts with YAP/TAZ complex, a major target of the Hippo signaling pathway, and that this interaction is essential for the cell density associated nuclear function of SHP2 [23]. Furthermore, SHP2 has been suggested to act as an oncoprotein in a variety of malignancies [22,24–26], although recent studies suggest it has a tumor

Abbreviations: YAP, Yes, associated protein; SHP2, Src homology phosphotyrosine phosphatase 2; HCC, hepatocellular carcinoma; TMA, tissue microarray; RFS, recurrence-free survival; OS, overall survival; SD, standard deviation; W, whole cell lysates; N, nuclear lysates; C, cytosolic lysates; siRNA, small interfering RNAs

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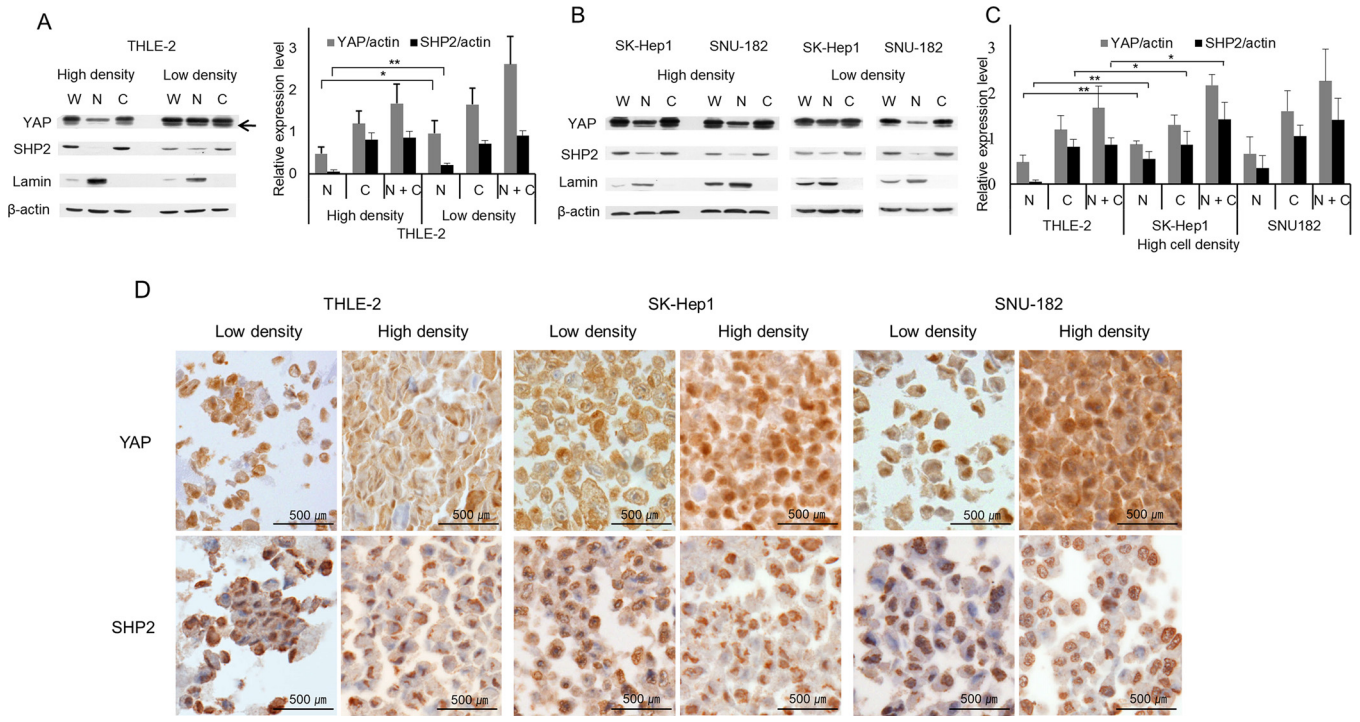

Fig. 1. Subcellular distributions of YAP and SHP2 proteins and their dependence on cell density in human liver cells. (A) In human normal hepatocytes (THLE-2 cells) YAP and SHP2 were present in nuclei and cytoplasm under the low-density (10×10^4 cells/well in 6-well plates) condition. However, under the high-density (90×10^4 cells/well in 6-well plates) condition the nuclear expression levels of YAP and SHP2 were significantly lowered (*, $P < 0.05$; **, $P < 0.01$). (B) In human HCC cells (SK-Hep1 and SNU-182), YAP and SHP2 were observed in nuclear as well as cytoplasmic compartments regardless of cell density. (C) The nuclear expressions of YAP and SHP2 were increased more in HCC cells than in normal hepatocytes (*, $P < 0.05$; **, $P < 0.01$). (D) Cell block immunohistochemical staining showed lower nuclear expressions of YAP and SHP2 in normal hepatocytes at high-density than those at low-density, and increased nuclear expressions of YAP and SHP2 more in HCC cells than in normal hepatocytes. The arrow indicates YAP isoforms.

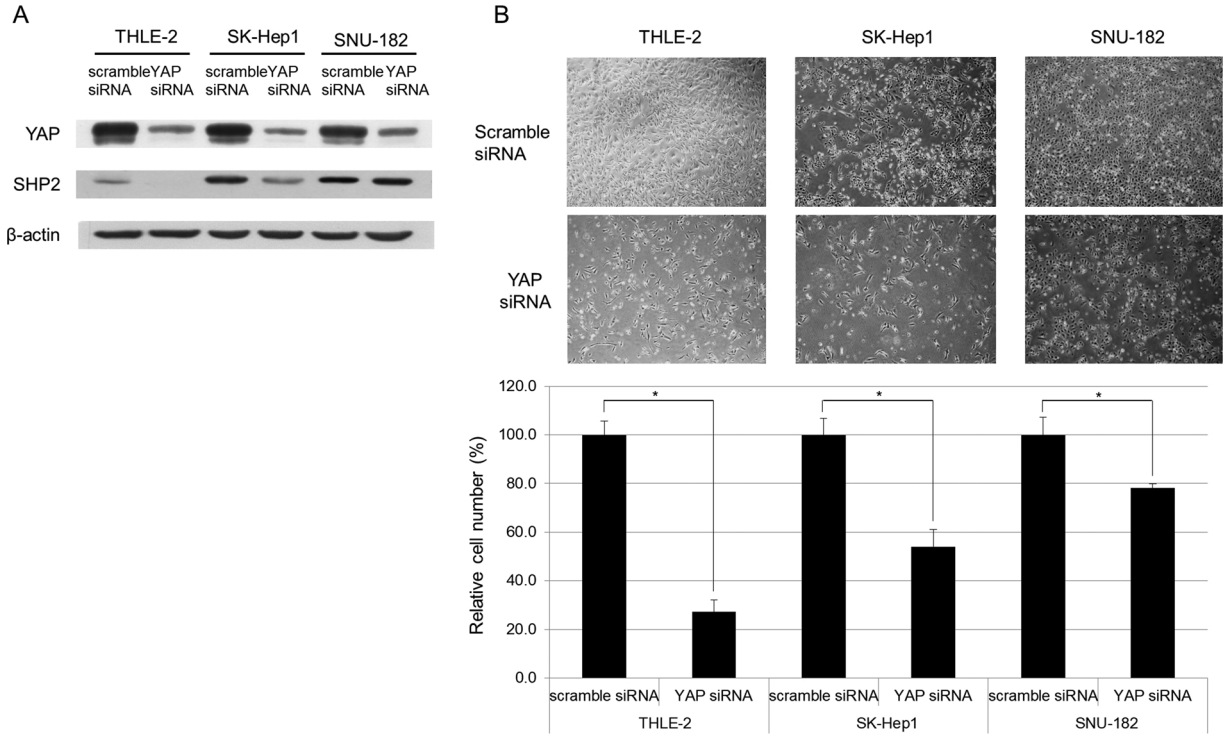

Fig. 2. Impact of YAP inhibition on SHP2 expression and cell proliferation. (A) YAP knockdown reduced SHP2 expression in THLE-2 and SK-Hep1 cells, whereas it had little effect on SHP2 levels in SNU-182 cells. (B) YAP knockdown significantly suppressed the proliferation of THLE-2, SK-Hep1, and SNU-182 cells, although it failed to down-regulate SHP2 protein levels in SNU-182 cells. The results shown are representative of three separate experiments. *, $P < 0.05$.