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Original contribution

# Expression of Yes-associated protein modulates Survivin expression in primary liver malignancies☆

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**Summary** Hepatocellular carcinoma and intrahepatic cholangiocarcinoma account for 95% of primary liver cancer. For each of these malignancies, the outcome is dismal; incidence is rapidly increasing, and mechanistic understanding is limited. We observed abnormal proliferation of both biliary epithelium and hepatocytes in mice after genetic manipulation of Yes-associated protein, a transcription coactivator. Here, we comprehensively documented Yes-associated protein expression in the human liver and primary liver cancers. We showed that nuclear Yes-associated protein expression is significantly increased in human intrahepatic cholangiocarcinoma and hepatocellular carcinoma. We found that increased Yes-associated protein levels in hepatocellular carcinoma are due to multiple mechanisms including gene amplification and transcriptional and posttranscriptional regulation. Survivin, a member of the inhibitors-of-apoptosis protein family, has been reported as an independent prognostic factor for poor survival in both hepatocellular carcinoma and intrahepatic cholangiocarcinoma. We found that nuclear Yes-associated protein expression correlates significantly with nuclear Survivin expression for both intrahepatic cholangiocarcinoma and hepatocellular carcinoma. Furthermore, using mice engineered to conditionally overexpress Yes-associated protein in the liver, we found that *Survivin* messenger RNA expression depends upon Yes-associated protein levels. Our findings suggested that

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Yes-associated protein contributes to primary liver tumorigenesis and likely mediates its oncogenic effects through modulating *Survivin* expression.


## 1. Introduction

Hepatocellular carcinoma (HCC) and intrahepatic cholangiocarcinoma (ICC) account for most primary liver malignancies and are among the most lethal and aggressive neoplasms [1,2]. Primary liver cancer is the third most common cause of cancer mortality worldwide [2]. For patients with HCC, although advances have been achieved through development of multiple therapeutic approaches, most patients still lack effective treatment and have a poor prognosis [1]. Curative surgical resection offers the only hope for patients with ICC because it is not responsive to systemic chemotherapy and radiotherapy regimens. However, relatively few patients are suitable candidates for curative surgical resection, and the recurrence rates remain high even after curative surgical resection [2]. Therefore, there is urgent need to advance our understanding of the molecular mechanisms underlying these devastating cancers to devise novel strategies aimed at improving the prognosis.

Yes-associated protein (YAP) is the nuclear effector of the Hippo signaling pathway [3]. As a transcription coactivator, YAP can induce the expression of a class of genes that promote cell proliferation and inhibit cell death [4]. The transcriptional coactivator activity of YAP can be inhibited by the Hippo signaling pathway through phosphorylating the conserved serine 127 residue [4]. This phosphorylation leads to cytoplasmic retention of YAP [4]; therefore, the activity of YAP can be reflected by its subcellular location: active YAP is located in the nucleus, whereas inactive YAP can be found in the cytoplasm. Overexpression of YAP or ablation of upstream tumor suppressors in the Hippo pathway with genetically modified mouse models results in tissue overgrowth, which frequently leads to HCC and ICC [4-9]. *Yap* deficiency in the mouse liver induces defects in bile duct development and proliferation [8], suggesting that YAP plays an important role in biliary tract homeostasis. YAP has been identified as an independent prognostic marker for HCC and lung and ovarian cancer [10-12], and YAP is frequently overexpressed in lung, ovarian, pancreatic, colorectal, and prostate carcinomas and brain malignancies [4,13,14]. However, YAP expression in patients with cholangiocarcinoma has not been well documented. Gene amplification of the *Yap* locus has been reported in a wide spectrum of human and murine malignancies including medulloblastomas; oral squamous cell carcinomas; and carcinomas of the lung, pancreas, esophagus, liver, and mammary gland [15-21]. No other mechanisms have been revealed to contribute to the elevated YAP expression in human tumors. Although a set of genes have been identified as transcriptional targets of YAP through genetically modified mouse models or cell lines, such as the inhibitors-of-apoptosis protein (IAP) family member *BIRC5/Survivin* [4], the secreted Cystein-rich protein *connective tissue growth factor* [22], the epidermal growth factor family member *amphiregulin* [23], and the AXL receptor kinase (*Axl*) [24], none of them has been shown to correlate with YAP expression in human patients with cancer. Thus, the purposes of this study are to investigate the expression of YAP in normal and malignant human liver tissue, to explore the mechanisms that contribute to the elevated YAP expression, and to find YAP-regulated targets in patients with cancer.

## 2. Materials and methods

### 2.1. Human subjects

The use of human tissues in this study was approved by the Johns Hopkins Institution Review Board. All human liver samples are from patients undergoing surgical resection at the Johns Hopkins Hospital, Baltimore, MD. Tumor tissues and adjacent nontumor tissues were collected at the time of surgery and stored at −80°C. Formalin-fixed, paraffin-embedded normal liver sections for interlobular bile duct, hilum, and gallbladder each from 5 patients without liver disease were from pathology archives in the Johns Hopkins University School of Medicine.

### 2.2. Tissue microarray

HCC and biliary cancer tissue microarrays (TMAs) were constructed with the preexisting paraffin-embedded tissues as described [13]. A total of 11 HCC TMAs including 87 HCC patient tumors and adjacent nontumor tissues (each tumor and adjacent nontumor tissue has 4 cores on the same TMA) and 2 biliary cancer TMAs including 10 ICC patient tumors (each tumor has 2 cores on both TMA) were investigated in this study.

### 2.3. Animal procedures

The animal protocols were approved by the Institutional Animal Care and Use Committee of the Johns Hopkins University. The inducible YAP transgenic mice (ApoE/rtTA-YAP) have been generated and described previously [4]. Six-