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## The expression of YAP1 is increased in high-grade prostatic adenocarcinoma but is reduced in neuroendocrine prostate cancer

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### Abstract

**BACKGROUND**—After long-term androgen deprivation therapy, 25–30% prostate cancer (PCa) acquires an aggressive neuroendocrine (NE) phenotype. Dysregulation of YAP1, a key transcription coactivator of the Hippo pathway, has been related to cancer progression. However, its role in neuroendocrine prostate cancer (NEPC) has not been assessed.

**METHODS**—Immunohistochemistry and bioinformatics analysis were conducted to evaluate YAP1 expression levels during PCa initiation and progression.

**RESULTS**—YAP1 expression was present in the basal epithelial cells in benign prostatic tissues, lost in low grade PCa, but elevated in high grade prostate adenocarcinomas. Interestingly, the expression of YAP1 was reduced/lost in both human and mouse NEPC.

**CONCLUSIONS**—The expression of YAP1 is elevated in high grade prostate adenocarcinomas but lost in NEPC.

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CONFLICT OF INTEREST

We declare no conflicts of interest.

## INTRODUCTION

Prostate cancer (PCa) is the most commonly diagnosed cancer among American men.<sup>1</sup> Although androgen deprivation is an effective therapy for advanced and recurrent PCa, most of the prostate tumors eventually become resistant to treatment and progress to castrate-resistant prostate cancer (CRPC).<sup>2</sup> In the disease progression, PCa cells may lose the prostatic adenomatous features and trans-differentiate into neuroendocrine prostate cancer (NEPC).<sup>3</sup> NEPC is an aggressive pathological type that lacks the responsiveness to androgen deprivation therapy. The survival of the patients with NEPC has been markedly reduced in comparison to adenocarcinomas. Therefore, there is a pressing need for studying the molecular mechanisms that drive the NE differentiation of PCa cells and developing novel agents that could effectively suppress the emergence of this lethal phenotype.

YAP1 and its homologous protein TAZ are key transcriptional coactivators involved in the modulation of several processes in mammalian cells, such as glucose uptake, proliferation, spreading, apoptosis and differentiation.<sup>4</sup> The activity of these transcriptional coactivators is mainly modulated by the Hippo pathway, which when activated impairs the translocation of YAP1 and TAZ to the nucleus and promotes their proteasome-mediated degradation.<sup>4</sup> Alternatively, when the Hippo pathway is inactivated, YAP1/TAZ can enter the nucleus and activate the transcription of target genes.<sup>4</sup> Additionally, their translocation to the nucleus can be modulated by Wnt/ $\beta$ -Catenin signaling in a Hippo-independent manner,<sup>5</sup> and the loss of YAP1/TAZ can induce  $\beta$ -Catenin-dependent gene expression,<sup>5</sup> suggesting that the Hippo and the Wnt/ $\beta$ -Catenin pathways are interconnected to develop an integrated response.<sup>6</sup>

Dysregulation of YAP1 has been related to cancer initiation and progression.<sup>7,8</sup> In CRPC, YAP1 is associated with cancer proliferation and invasiveness.<sup>9,10</sup> However, the significance of YAP1 in NEPC has not been evaluated. In the present study, we showed that the expression of YAP1 was increased in high-grade prostate adenocarcinoma (AdPCa) but was lost in NEPC, suggesting a role of loss of YAP1 in NEPC development.

## MATERIAL AND METHODS

### Sample collection

De-identified human prostate tissue specimens were obtained from LSU Health-Shreveport Biorepository Core, Overton Brooks VA Medical Center, Ochsner Health System Biorepository and Tissue for Research. These specimens consisted of 22 benign prostate hyperplasia, 4 Gleason score (GS) 6, 24 GS 7, 5 GS 8, 14 GS 9, 7 GS 10 prostate adenocarcinoma, and 12 NEPC primary tissues. Most tissues were from prostatectomies except for five NEPC cases that were from biopsies. All the tissues were used in accordance with LSU Health-Shreveport IRB protocols. TRAMP, 12T-10 LADY, and NE10 archived tumor sections were used for this study.

### Immunohistochemistry and Immunofluorescence staining

Immunostaining was performed using Vectastain elite ABC peroxidase kit (Vector Laboratories, Burlingame, CA) as described previously.<sup>11</sup> Primary antibodies of YAP1 and Chromogranin A (CHGA) were purchased from Santa Cruz Biotechnology (sc-101199 and

sc-1488 respectively, Dallas, TX), Synaptophysin (SYP) (611880, BD biosciences, San Jose, CA), p63 and FOXA2 (ab735 and ab108422, respectively, Abcam, Cambridge, MA). The tissue sections were counterstained, mounted, and imaged with a Zeiss microscope (White Plains, NY). The percentage of cells stained was evaluated on a scale of 1+ (1–25%), 2+ (25–50%), 3+ (50–75%), and 4+ (75–100%) and the intensity of expression, 0 (negative), 1+ (weak), 2+ (moderate), and 3+ (strong). To assess the correlation between the expression of YAP1 and the Gleason score of tumor samples, immunohistochemistry results were further evaluated by a semiquantitative H-score in a blinded manner. For immunofluorescence staining, YAP1 was co-stained with cytokeratin 5 (CK5) or 14 (CK14) (904801 and 905501 respectively, BioLegend, San Diego, CA) and imaged with a Nikon fluorescence microscope (Melville, NY).

### **Bioinformatics analyses**

The mRNA levels of differentially expressed genes were extracted from RNA-seq datasets deposited in GEO and cBioportal, including GSE32967, GSE41192, GSE3325, SU2C, Cancer Cell Line Encyclopedia, and Beltran's Neuroendocrine Prostate Cancer datasets. The gene expression was analyzed by using RStudio software.<sup>12</sup>

### **Gene silencing**

PCa cells were transfected with siRNA to knockdown the expression of YAP1. Western blot was conducted to assess the siRNA knockdown efficiency. RNA-seq was conducted using RNA extracted from PC3/Scramble siRNA and PC3/siYAP1 cells (each containing three biological replicates) at the bioinformatic core of Indiana University School of Medicine. Ingenuity Pathway Analysis was conducted to identify the top pathways that are altered using the list of genes that are up- or down-regulated in the YAP1 knockdown cells. ShinyGO was used for Gene Ontology enrichment analysis.<sup>13</sup>

### **Luciferase assay**

The human PCa cell lines PC3 and 22Rv1 were purchased/authenticated from American type culture collection. TOPFlash is a Wnt/ $\beta$ -Catenin-responsive promoter driven luciferase construct.<sup>14</sup> It is used to monitor Wnt/ $\beta$ -Catenin signaling in transfected cells. PCa cells were co-transfected with TOPflash plasmid and scramble siRNA or siRNAs against YAP1 (Santa Cruz Biotechnology) by using X-tremeGENE Transfection Reagent (Sigma Aldrich, St. Louis, MO). Luciferase activity was measured by using Promega Luciferase Assay kit (Promega Biotechnology, Madison, WI).

### **Western blotting**

Equal amounts of protein were subjected to SDS-PAGE and then transferred to a PDVF membrane (Bio-Rad, Hercules, CA). Subsequently, membranes were incubated with primary antibodies and HRP-conjugated secondary antibody (Cell Signaling, Beverly, MA). Proteins were revealed by using ProSignal® Dura ECL Reagent (Genesee Scientific, San Diego, CA) and visualized in a Chemidoc™ Touch Imaging System (Bio-Rad).

## Statistical analyses

Differential gene expression was evaluated using Chi-Square and two-sided Student's *t*-test. The correlation between the expression of YAP1 (H-score) and the Gleason score was assessed using the Spearman correlation test. A p-value of 0.05 was considered statistically significant.

## RESULTS

### YAP1 expression in normal prostate

We used immunohistochemistry to assess the expression of YAP1 in benign prostatic tissues. YAP1 expression was detected in the nuclei of the prostatic p63-positive basal epithelial cells, as well as stromal cells, but it was absent in the p63-negative luminal glandular epithelial cells (Figs. 1A & 1B). Additionally, dual immunofluorescence staining of YAP1 and CK5 or CK14 that highlights prostatic basal cells was performed to corroborate the YAP1 co-localization pattern in benign basal prostatic epithelium. In concordance with previous data, YAP1 immunostaining was mainly present in the nuclei of the cells that were positive for CK5 and CK14 (Figs. 1C to 1H). These results indicate that the expression of YAP1 is localized in basal epithelial cells.

### Expression of YAP1 increased in PIN but lost in mouse prostate NEPC

We used TRAMP and LADY, the two widely used PCa mouse models,<sup>15,16</sup> to evaluate YAP1 expression during PCa progression. TRAMP mice develop PIN and a subset of tumors progress into NEPC.<sup>17</sup> We found that YAP1 expression was absent in the luminal epithelial cells in wild-type prostates (Fig. 2A, n=3) but present in PIN of TRAMP mice (Fig. 2B, n = 3). Rare clusters of PIN cells stained positive for FOXA2, a marker of NEPC (Fig. 2C). In the TRAMP tumors that developed NEPC (n=5), YAP1 was expressed in PIN lesions within the NEPC tumors (Fig. 2D). However, YAP1 was undetectable in the NEPC cells (Fig. 2D) which were highlighted by NEPC marker chromogranin A (CHGA) (Fig. 2E). The NEPC marker FOXA2 showed diffuse immunoreactivity in NEPC cells in contrast to its rare-to-negative stain in PIN tissues (Figs. 2C & 2F).

We also examined the expression of YAP1 in LADY mice including 12T-10, a NEPC model,<sup>18</sup> and NE10, a xenograft line derived from 12T-10 NEPC.<sup>19</sup> In 12T-10 LADY tumors, YAP1 expression was present in focal PIN lesions but was absent in the FOXA2-positive NEPC cells (Figs. 2G & 2H). YAP1 expression was lost in NE10 tumors (Fig. 2I). Additionally, YAP1 immunostaining was absent in NEPC metastases to the liver and lung, which expressed NEPC markers FOXA2 and CHGA (Figs. 2J to 2O). These results indicate that mouse-derived NEPC tumors exhibit a marked reduction in YAP1 expression compared to the non-NE tumors.

### The expression of YAP1 increased in human high-grade prostate adenocarcinomas but decreased in NEPC

YAP1 expression levels were also evaluated in human prostatic tissues including benign prostatic hyperplasia, low grade adenocarcinomas (AdPCa), high grade AdPCa, and NEPC. The results were summarized in Fig. 3 and suppl. Table 1. YAP1 expression was detected in

fibromuscular stromal cells in most cases. However, YAP1 expression in PCa was altered during progression of the disease (Figs. 3A to 3J). YAP1 was expressed in basal epithelial cells as well as fibromuscular stromal cells in benign prostatic tissues but absent from the luminal epithelial cells except of one case of benign prostatic glandular tissues (Fig. 3A, n=22). YAP1 expression was also absent in human high-grade PIN (2 of 2 cases), which was in contrast to the positive expression in mouse prostatic PIN. Of the 4 low-grade AdPCas with GS 6, 3 cases demonstrated absence of YAP1 expression in the acini of AdPCa (Fig. 3B) and only one tumor showed weak (1+) positive nuclear staining of YAP1. In AdPCa with GS 7, a subset of tumors (10 of 24 cases, 42%) exhibited positive YAP1 nuclear localization. Approximately 1–25% of cancer cells demonstrated various degrees of intensity (from 1+ weak to 3+ strong) of YAP1 staining. Of the 5 AdPCa with GS 8, 4 cases (80%) were positive, with one tumor showing strong YAP1 immunostain of 3+ in 50–75% of cancer cells and 2+ in the remaining cells. In AdPCa with GS 9, 10 of 14 (71%) tumors showed ~ 50–75% of cells with moderate (2+) to strong (3+) YAP1 immunostaining while 4 of 7 (57%) AdPCas with GS 10 exhibited as many as 3+ (50–75%) of cancer cells with 2+ (moderate) to 3+ (strong) YAP1 expression. Overall, the expression of YAP1 (H-score) was associated with the Gleason score of AdPCa (Spearman correlation test,  $r=0.4962$ ,  $p<0.0001$ ).

However, YAP1 expression was absent in 6/12 NEPC (Figs. 3D & 3G, suppl. Table 1, n=12), including one AdPCa with Paneth-like NE differentiation, one large cell NEPC, one unspecified NEPC and three small cell NEPC. In the NEPC with focal adenocarcinoma (Fig. 3D), YAP1 expression was present in the adenocarcinoma cells but absent in NEPC components. Chromogranin A (CHGA) (Fig. 3E), FOXA2 (Fig. 3F), and Synaptophysin (SYP) (Fig. 3H) were used as markers for NEPC. These results demonstrate that YAP expression is lost in NEPC.

To further validate the reduction of YAP1 expression in NEPC, we analyzed mRNA levels of YAP1 in an RNA-seq dataset of 46 LuCaP, PCa patient-derived xenograft models (PDXs). The expression levels of YAP1, TAZ, AR, and NEPC markers including FOXA2, SOX2, SYP and CHGA were displayed as a heatmap. As shown in Fig. 4A, YAP1 expression was lost in all 8 NEPC LuCaP PDXs as well as the ampicrine LuCaP 77CR line that is AR<sup>positive</sup> NE<sup>positive</sup>. However, loss of YAP1 expression only occurred in 1 of 35 AR<sup>positive</sup> AdPCa. YAP1 expression was detectable in the double negative LuCaP 173.2 as well as the AR<sup>low</sup>/NE<sup>negative</sup> LuCaP 176 PDX. In contrast to the reduced YAP1 expression in NEPC, TAZ expression in these LuCaP PDXs did not show any consistent patterns (Fig. 4A). Statistical analysis revealed that the loss of YAP1 expression correlated with NE phenotype (Chi-Square test,  $p<0.001$ ). Taken together, these data further support the lack of expression of YAP1 in NEPC.

Results of analysis of the RNA-seq of human tumor dataset published by Beltran *et al*<sup>20</sup> corroborated that NEPC tumors exhibited reduced YAP1 expression (Fig. 4B, *t*-test,  $p<0.01$ ). However, this trend was not followed by TAZ (data not shown). Similar results were also observed in additional datasets including GSE32967, GSE41192, GSE3325, and the SU2C datasets (Figs. 4C–Figs. 4F).<sup>21</sup>

In order to provide additional supportive data, we analyzed the mRNA transcript levels of YAP1 and TAZ in PCa cell lines by using publicly available RNA-seq data.<sup>22</sup> The expression level of YAP1 was the lowest in H660 cells, the only NEPC cell line, among all the PCa cell lines tested (Fig. 4G). The reduced expression of YAP1 in H660 was confirmed by Western blot (Fig. 4H). However, TAZ displayed a different expression pattern and its mRNA level did not show consistent decrease in H660 cells (Fig. 4G).

### YAP1 loss in AdPCa cells induces Wnt/ $\beta$ -Catenin responsive promoter

Hippo/YAP1 pathway has a close crosstalk with Wnt/ $\beta$ -Catenin signaling,<sup>5,6</sup> and previous studies have linked the activation of Wnt/ $\beta$ -Catenin signaling with NE differentiation of PCa.<sup>23,24</sup> Therefore, we assessed the effects of YAP1 knockdown on the Wnt/ $\beta$ -Catenin activity. The activity of Wnt/ $\beta$ -Catenin increased in PCa PC3 cells as well as in 22Rv1 cells after YAP1 knockdown (sFig. 1). To further explore the downstream events mediated by the loss of YAP1 expression, we conducted RNA-Seq experiments followed by IPA analysis of the genes that are up- or down- regulated in PC3/siYAP1 cells. The results indicated that the top pathways changed following YAP1 knockdown include axonal guidance, hepatic fibrosis, protein kinase A, RhoA, and PI3K/AKT signaling. However, Wnt/ $\beta$ -Catenin signaling was not enriched in YAP1 knockdown cells. YAP1 knockdown did not cause significant changes in the expression of NEPC markers either (sFig. 2 and sTable2).

## DISCUSSION

The development of NEPC is a clinically important issue. We need to elucidate the pathogenesis of NEPC and develop an effective therapy for this aggressive disease.<sup>3</sup> In this study, we reported that the Hippo pathway coactivator YAP1 was reduced/lost in NEPC, and a reduction of YAP1 provided a mechanism to activate Wnt/ $\beta$ -Catenin signaling in PCa cells. These results suggest a role of loss of YAP1 in the pathogenesis of NEPC.

In cancer, YAP1 usually functions as an oncogene, promoting cell proliferation and invasion.<sup>4</sup> However, YAP1 also exerts tumor suppressive role in certain cellular contexts. For example, YAP1 overexpression reduces small cell lung carcinoma (SCLC) growth.<sup>25</sup> Inversely, its loss stimulates breast cancer cell proliferation and mobility, protecting them from anoikis.<sup>26</sup> These data suggest that YAP1 exerts a dual-role during tumorigenesis. Therefore, it would be interesting to monitor the changes in its expression during PCa progression. In this study, we demonstrated that the expression of YAP1 was limited to the basal cells in benign prostatic glandular epithelia and was absent in the luminal glandular cells, supporting the previous proposal that YAP1 may serve as a biomarker for prostatic basal epithelial cells.<sup>27</sup> YAP1 expression was absent in the cancer cells of early stage AdPCa where there were no basal cells in the glandular epithelium. Its expression was increased; however, in advanced stages of AdPCa. The increased levels of YAP1 expression was associated with Gleason score of AdPCa (Fig. 3J) and advanced tumor-node-metastasis (TNM) stages.<sup>28,29</sup> Additionally, the inhibition of this protein in high-grade PCa cells not only impaired cellular proliferation, but also enhanced cellular sensitivity to ADT,<sup>9,30</sup> supporting that inhibition of YAP1 could control AdPCa progression.



However, the expression of YAP1 was decreased/lost in cell line, human, and mouse NEPC tumor samples (Figs. 2 to 4). These findings were consistent with that demonstrated in SCLC cells, where the YAP1 expression is reduced in SCLC and its downregulation is essential to NE differentiation.<sup>8,31</sup> However, the molecular mechanism that relates the loss of YAP1 with the acquisition of NE phenotype has not been elucidated. Prior studies in lung cancer have demonstrated that YAP1 knockdown induces the expression of several neuroendocrine markers such as Rab3A to achieve NE differentiation.<sup>8</sup> In this study, we showed that downregulation of YAP1 activated Wnt/ $\beta$ -Catenin responsive promoter. The activation of Wnt/ $\beta$ -Catenin signaling has been previously related to the induction of NE differentiation of PCa.<sup>23,24</sup> Additionally, previous research has shown that YAP1/TAZ inhibit the activation of Wnt/ $\beta$ -Catenin signaling by recruiting  $\beta$ -TrCP to the  $\beta$ -Catenin destruction complex, an essential step for inducing  $\beta$ -Catenin's degradation by the ubiquitin-proteasome system.<sup>5</sup> Consequently, the downregulation of YAP1 could induce the nuclear translocation of  $\beta$ -Catenin resulting in the activation of Wnt/ $\beta$ -Catenin signaling.<sup>6</sup> In consistent with the published findings,<sup>32,33</sup> our studies demonstrated that Wnt/ $\beta$ -Catenin activity increased after YAP1 knockdown, supporting the idea that loss of YAP1 provides a mechanism to activate Wnt/ $\beta$ -Catenin signaling in AdPCa cells. However, RNAseq analysis indicated that transient YAP1 knockdown was not sufficient to activate endogenous Wnt/ $\beta$ -Catenin signaling or induce the expression of NEPC markers. More studies would follow to determine whether long-term loss of YAP1 could promote Wnt/ $\beta$ -Catenin signaling or induce NE differentiation of AdPCa cells.

Although the levels of YAP1 decreased/lost in NEPC, the closely-related TAZ did not display consistent changes in prostatic tissues (Figs. 4A & 4G). Despite the high homology of YAP1 and TAZ proteins, they have different structures, are involved in differential protein-protein interactions, and have different regulation and downstream functions.<sup>34,35</sup> Although YAP1 and TAZ were thought to be functionally redundant, inactivation of YAP1 or TAZ alone can result in significant changes in multiple cellular processes and it has been shown that YAP1 knockout has a greater functional impact.<sup>34,35</sup> We show here that YAP1 and TAZ display different expression patterns in PCa and RNAseq results indicate that the relative mRNA abundance for YAP1 is higher than TAZ in non-NE PCa cells (Fig. 4G). These results indicate that they may have distinct roles in NEPC and that YAP1 is more important than TAZ. This concept is also supported by the observation that YAP1 knockdown alone is sufficient to activate the Wnt/ $\beta$ -Catenin responsive promoter in PCa cells.

Taken together, our results highlighted the alteration of YAP1 expression during PCa progression. Although previous studies have linked YAP1's overexpression with increased AdPCa malignancy, we showed here YAP1 is down regulated in NEPC. We also have evidence supporting that YAP1 downregulation activates the Wnt/ $\beta$ -Catenin signaling, which could lead to NE differentiation. However, more studies would be necessary to determine how the loss of YAP1 affects NE differentiation, as well as if YAP1 inhibitors could be safely used as therapeutic adjuvants for treating AdPCa.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

## ACKNOWLEDGMENT

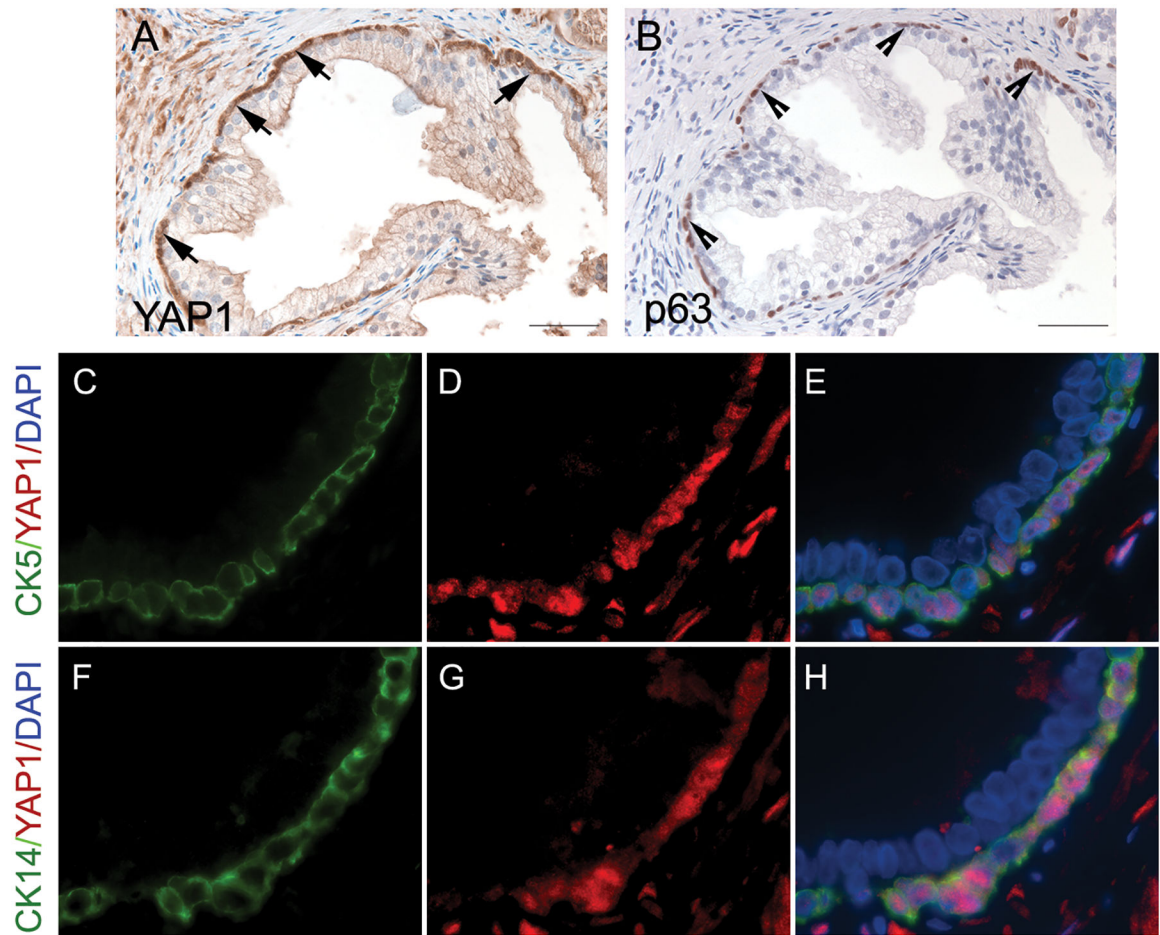
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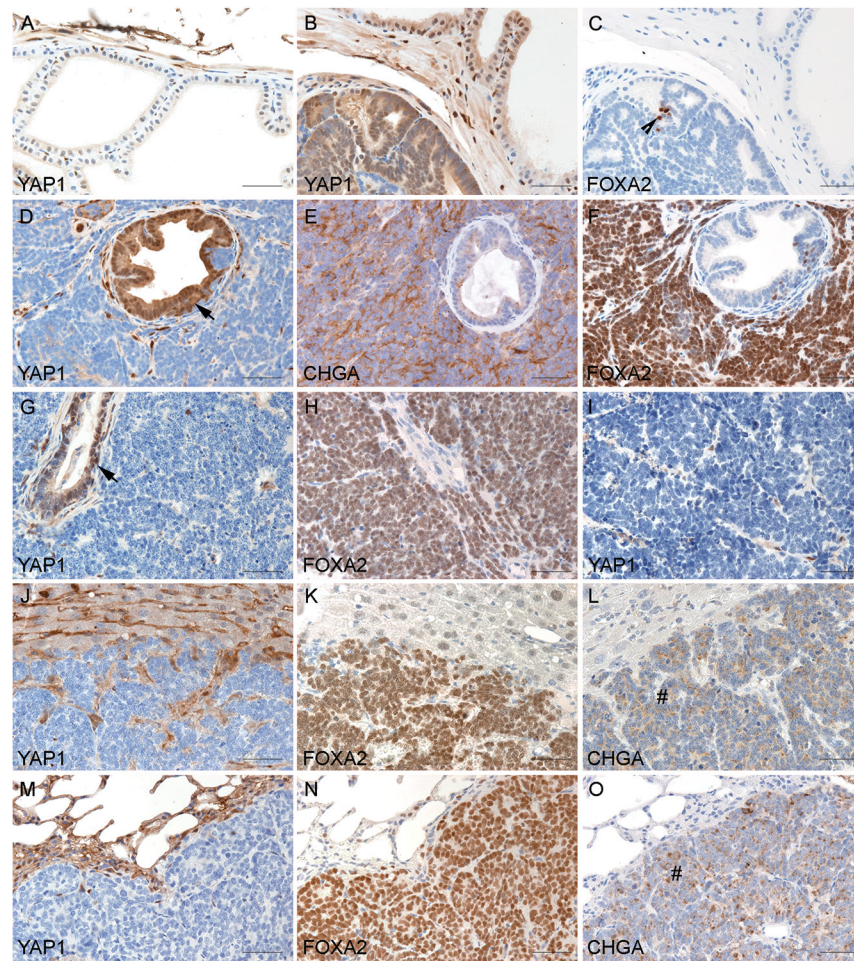


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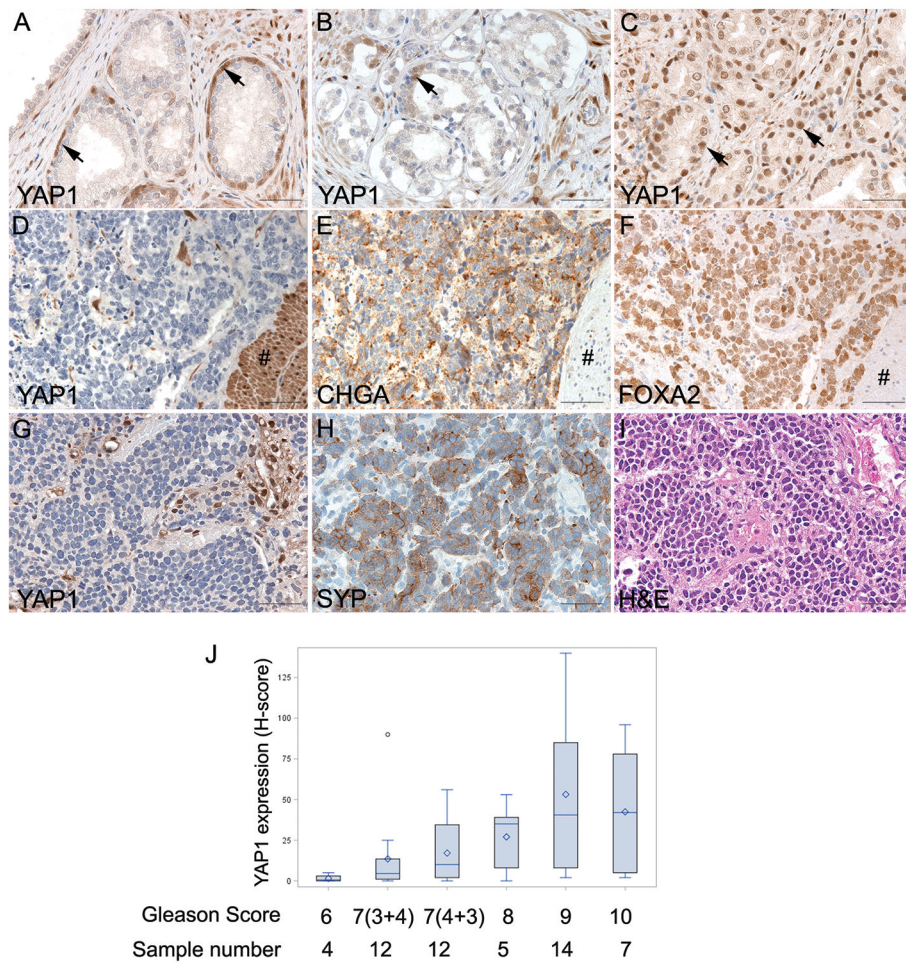
**Fig. 1.**

The expression of YAP1 in benign prostatic hyperplasia. (A) YAP1 displayed positive staining in basal cells (positive nuclear staining, indicated by the arrows) and negative staining in the prostatic luminal epithelial cells. (B) Immunohistochemical staining of p63 highlighted basal cells (indicated by the arrowheads) in the periphery of the prostatic gland. (C to H) Dual immunofluorescence staining of YAP1 and cytokeratin 5 (CK5) or cytokeratin 14 (CK14) confirmed the presence of YAP1 expression in prostatic basal epithelial cells.

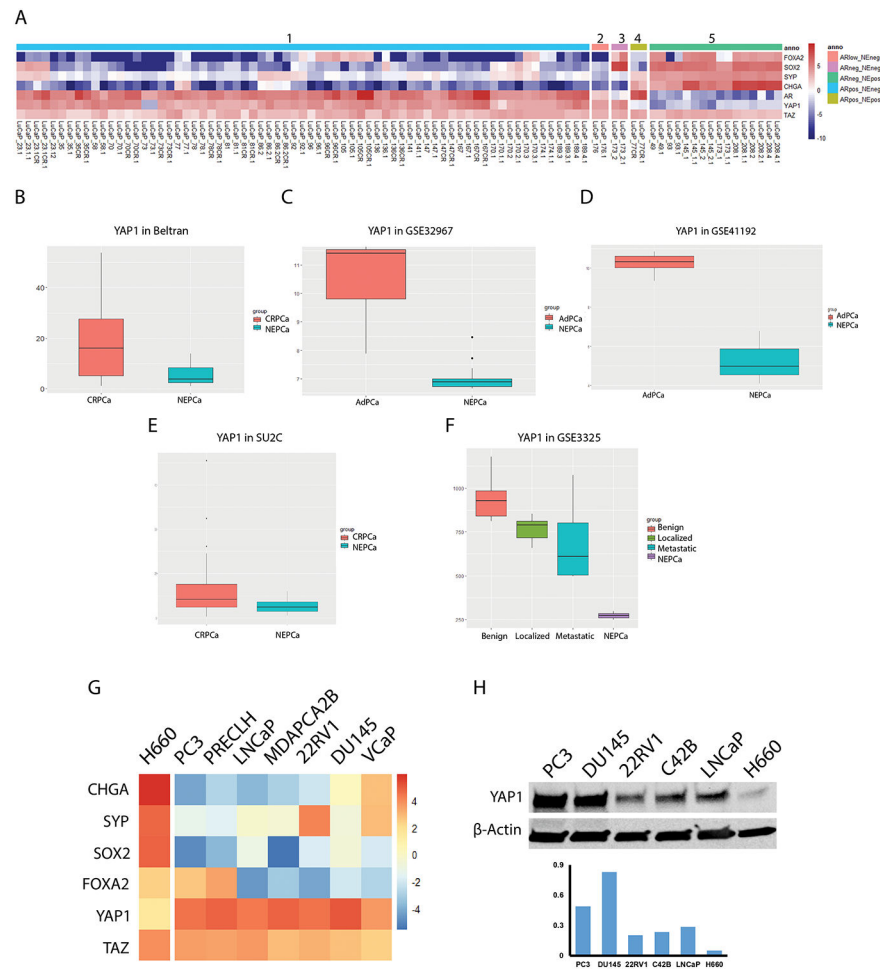


**Fig. 2.** Microscopic examination of the prostate of TRAMP and LADY mice revealed that the expression of YAP1 increased in PIN but decreased in NEPC. (A) Wild-type prostate demonstrating positive staining of YAP1 in the occasional basal epithelia but negative staining in luminal epithelia. (B&C) Serial sections of a PIN tumor of TRAMP demonstrating diffuse positive staining of YAP1 and the rare FOXA2-positive cells in PIN (indicated by the arrowhead). (D to F) Serial sections of a NEPC tumor of TRAMP demonstrating diffuse positive staining of YAP1 in PIN components (indicated by the arrow) but negative in NEPC cells. NEPC markers chromogranin A (CHGA) and FOXA2 were stained positive in NEPC cells but negative in PIN. (G & H) Serial sections of a 12T-10 NEPC tumor demonstrating negativity of YAP1 in NEPC cells in contrast to the positive staining in focal PIN (indicated by the arrow). Positive staining of FOXA2 was seen in the NEPC cells. (I) NE10 tumor showed negative YAP1 staining. (J to O) The expression of YAP1 in NEPC metastases. Sections of the liver (J to L) and lung (M to O) with metastasis of NEPC demonstrated negative YAP1 staining in metastatic NEPC. In contrast, FOXA2 and CHGA were positive in NEPC metastases (indicated by the # signs).



**Fig. 3.**

The expression of YAP1 in human prostatic tissues. **(A)** Benign prostatic tissue demonstrated positive YAP1 staining in the stroma cells and basal cells (indicated by the arrows). **(B)** Low-grade prostate adenocarcinoma. YAP1 expression was present in stromal cells and occasional basal cells (indicated by the arrow) but was absent in adenocarcinoma cells. **(C)** High-grade prostate adenocarcinoma. YAP1 was expressed in prostate adenocarcinomas (indicated by the arrows) with a Gleason score of 8 or higher. **(D to F)** Serial sections of a NEPC with focal adenocarcinoma. YAP1 protein was expressed in high-grade adenocarcinoma components (indicated by the # sign), but the expression was lost in NEPC cells. CHGA and FOXA2 were diffusely expressed in NEPC but not in adenocarcinoma cells. **(G to I)** Serial sections of a small cell carcinoma demonstrating negative YAP1 and positive synaptophysin (SYP) staining in NEPC cells. **(J)** Distribution of YAP1 expression (H-score) among PCa with various Gleason grade. The expression of YAP1 was associated with Gleason score (Spearman correlation test,  $r=0.4962$ ,  $p<0.0001$ ).



**Fig. 4.** The differential expression of YAP1 in PCa. **(A)** The expression of YAP1 in LuCaP PDXs. The expression levels of YAP1, TAZ, AR, and NEPC markers (FOXA2, SOX2, CHGA, and SYP) were extracted from RNAseq data derived from 46 LuCaP PDXs including (from left to right) AR<sup>+</sup>/NE<sup>-</sup> (group 1, n=35), AR<sup>low</sup>/NE<sup>-</sup> (group 2, n=1), AR<sup>-</sup>/NE<sup>-</sup> (group 3, n=1), AR<sup>+</sup>/NE<sup>+</sup> (group 4, n=1), and AR<sup>-</sup>/NE<sup>+</sup> tumors (group 5, n=8). YAP1 expression was lost in all the NE positive LuCaP PDXs. Whereas, YAP1 was expressed in all but one prostate adenocarcinoma cases. **(B - F)** Expression of YAP1 in CRPC vs NEPC. The mRNA levels of YAP1 were extracted from RNA-seq/RNA microarray data of Neuroendocrine Prostate Cancer. The levels of YAP1 was decreased in NEPC. **(G)** Heatmap of differentially expressed genes in PCa cell lines. The mRNA levels of YAP1, TAZ, and NEPC markers (CHGA, SYP, SOX2, and FOXA2) were extracted from RNA-seq data of Cancer Cell Line Encyclopedia. The mRNA level of YAP1 was the lowest in NEPC H660 cells. But TAZ displayed no consistent patterns among PCa cell lines. **(H)** Western blot in assessing the protein levels of YAP1 in PCa cell lines. Lower panel is the quantification of Western blot result.