Yes-Associated Protein Is Crucial for Constitutive Androstane Receptor-Driven Hepatocyte Proliferation But Not for Induction of Drug Metabolism Genes in Mice

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BACKGROUND AND AIMS: Constitutive androstane receptor (CAR) agonists, such as 1,4-bis [2-(3,5-dichloropyridyloxy)]benzene (TCPOBOP), are known to cause robust hepatocyte proliferation and hepatomegaly in mice along with induction of drug metabolism genes without any associated liver injury. Yes-associated protein (Yap) is a key transcription regulator that tightly controls organ size, including that of liver. Our and other previous studies suggested increased nuclear localization and activation of Yap after TCPOBOP treatment in mice and the potential role of Yap in CAR-driven proliferative response. Here, we investigated a direct role of Yap in CAR-driven hepatomegaly and hepatocyte proliferation using hepatocyte-specific Yap-knockout (KO) mice.

APPROACH AND RESULTS: Adeno-associated virus 8-thyroxine binding globulin promoter-Cre recombinase vector was injected to Yap-floxed mice for achieving hepatocyte-specific Yap deletion followed by TCPOBOP treatment. Yap deletion did not decrease protein expression of CAR or CAR-driven induction of drug metabolism genes (including cytochrome P450 [Cyp] 2b10, Cyp2c55, and UDP-glucuronosyltransferase 1a1 [Ugt1a1]). However, Yap deletion substantially reduced TCPOBOP-induced hepatocyte proliferation. TCPOBOP-driven cell cycle activation was disrupted in Yap-KO mice because of delayed (and decreased) induction of cyclin D1 and higher expression of p21, resulting in decreased phosphorylation of retinoblastoma protein. Furthermore, the induction of other cyclins, which are sequentially involved in progression through cell cycle (including cyclin E1, A2, and B1), and important mitotic regulators (such as Aurora B kinase and polo-like kinase 1) was remarkably reduced in Yap-KO mice. Microarray analysis revealed that 26% of TCPOBOP-responsive genes that were mainly related to proliferation, but not to drug metabolism, were altered by Yap deletion. Yap regulated these proliferation genes through alerting expression of Myc and forhead box protein M1, two critical transcriptional regulators of CAR-mediated hepatocyte proliferation.

CONCLUSIONS: Our study revealed an important role of Yap signaling in CAR-driven hepatocyte proliferation; however, CAR-driven induction of drug metabolism genes was independent of Yap. (Hepatology 2021;73:2005-2022).

Constitutive androstane receptor (CAR) is conventionally considered to be a xenobiotic-sensing nuclear receptor that is activated by several drugs (e.g., phenobarbital) and environmental chemicals. Activation of CAR and its nuclear
translocation induces drug-disposition enzymes, including phase I conjugation enzymes (e.g., cytochrome P450s), phase II conjugation enzymes (e.g., sulfotransferases and UDP-glucuronosyltransferases), and drug transporters, as an adaptive mechanism to eliminate foreign substances.\(^{(1)}\) In nucleus, CAR binds with RXR to directly induce transcription of genes that encode these enzymes. More recently, CAR has also emerged as an important regulator of energy metabolism in the liver.\(^{(1)}\) Apart from this, CAR activation also induces robust hepatocyte proliferation and hepatomegaly.\(^{(2)}\) This phenomenon is shared by some other nuclear receptors (including thyroid hormone receptor, retinoic acid receptor, and peroxisome proliferator-activated receptor \(\alpha\)) and differs from regenerative response after surgical or chemical hepatectomy, as it does not involve any underlying tissue loss or liver injury. This CAR-driven proliferative response is linked with promotion of hepatocellular carcinogenesis.\(^{(3)}\) However, in the context of regenerative therapy, it has been exploited to stimulate liver regeneration and protect liver from failure following extensive tissue loss (>90% hepatectomy).\(^{(4)}\) 1,4-Bis[2-(3,5-dichloropyridyloxy)] benzene (TCPOBOP) is a prototypical CAR ligand that induces robust hepatocyte proliferation comparable with partial hepatectomy (PHx) in mouse liver and is the strongest known chemical mitogen for the liver.\(^{(5)}\) Liver size doubles within a week after single treatment with TCPOBOP in mice andplateaus thereafter up to 4 weeks.\(^{(3)}\) This indicates the unique ability of CAR to reset hepatostat in the liver, but underlying mechanisms are not extensively explored.

Yes-associated protein (Yap) is a major transcription factor that strictly controls organ size, including that of the liver, and thus is considered an important regulator of hepatostat.\(^{(6-8)}\) Yap activity is negatively regulated by the Hippo signaling pathway. The core of the Hippo pathway is comprised of mammalian sterile-20-like kinase (Mst) 1/2 and large tumor suppressor kinase (Lats) 1/2 (serine/threonine kinases) proteins, upstream of Yap. When Hippo pathway is active, phosphorylated Mst (active form) phosphorylates and activates Lats, which in turn phosphorylates Yap and targets it for degradation. However, when Hippo pathway is inactive, Yap enters the nucleus and causes transcription of its target genes.\(^{(7,8)}\) Overexpression of Yap in normal mouse liver or inactivation of its upstream regulators (Mst, Lats, and neurofibromin 2) results in hepatocyte proliferation and hepatomegaly, a phenotype similar to CAR activation.\(^{(6,9-12)}\) However, liver-specific deletion of endogenous Yap (and its analogue transcriptional coactivator with PDZ-binding motif [Taz]) in mice from birth does not show the reverse phenotype; surprisingly, these mice also display hepatomegaly secondary to some liver injury at basal level.\(^{(13)}\)

Published evidence points toward the potential role of Yap in CAR-driven hepatocyte proliferation and hepatomegaly.\(^{(2,14,15)}\) Overexpression of mutated Yap (active form) increased hepatocyte proliferation following a repeat dose of TCPOBOP (a week after first
dose) in mice, which normally does not cause proliferation because of plateauing of CAR-driven proliferative response after the first dose. However, a direct role of endogenous Yap in acute TCPOBOP-driven hepatocyte proliferation was not investigated in this study. In a recent in vitro study using alpha mouse liver 12 (AML12) cells, down-regulation of Yap using small interfering RNA significantly decreased TCPOBOP-driven proliferation. However, in the same study, treatment with verteporfin in mice (to inhibit Yap) slightly but not significantly altered induction of proliferation genes or Yap target genes after TCPOBOP treatment, whereas the effect on proliferation per se was not investigated. Studies have shown that verteporfin does not specifically inhibit Yap and has several nonspecific effects, which can confound interpretation of these findings. Our previous study revealed transient increase in nuclear accumulation of Yap in mice liver, specifically during proliferative phase after TCPOBOP treatment. Concomitant decrease in phospho (p-)Yap (inactive form) levels was also observed in this study. Similar increased nuclear accumulation of Yap after TCPOBOP treatment in mice has also reported by other group. However, direct causal relationship of endogenous Yap with CAR-driven proliferation in vivo is not yet demonstrated.

Based on this background information, here, we investigated the direct role of hepatocyte-specific Yap in TCPOBOP-induced proliferative response by acute deletion of Yap in adult mice using adeno-associated virus 8 (AAV8)-thyroxine binding globulin (TBG) promoter-Cre recombinase vector (AAV8-TBG-CRE vector) administration to Yap-floxed mice. Here, we provide direct and specific evidence that hepatocyte-specific Yap is crucial for CAR-driven proliferative response in mice but not for CAR-driven induction of drug-disposition enzymes.

Materials and Methods

ANIMALS, TREATMENTS, STUDY DESIGN, AND TISSUE HARVESTING

Female YAPfl/fl mice who were 8-12 weeks old (n = 3-6) were administered AAV8.TBG.PI.eGFP.WPRE.bGH8 (2.5 x 10^{11} viral particles per mouse, IP) to obtain hepatocyte-specific Yap-knockout (KO) mice. AAV8.TBG.PI.cGFP.WPRE.bGH8 (2.5 x 10^{11} viral particles per mouse, IP) administered to YAPfl/fl mice served as wild-type (WT) control. Female mice were used because TCPOBOP-induced proliferative response is known to be stronger in female mice. Single dose of TCPOBOP (3 mg/kg; prepared in corn oil) was administered by oral gavage 10 days after AAV8 injection. Mice were sacrificed at various time points (0, 1, 2, 5, and 7 days) after TCPOBOP administration. Day 0 animals did not receive any treatment except AAV8 administration. Livers were obtained and processed further for immunohistochemical, western blot, real-time quantitative PCR, and microarray analysis. For PHx study, Yap-KO or WT mice were sacrificed at 0 hours, 40 hours, 72 hours, 7 days, and 14 days after 2/3 surgical liver resection, and liver tissues were harvested for immunohistochemical analysis. All studies were performed according to the “Guide for the Care and Use of Laboratory Animals” published by the National Institutes of Health and were approved by The Institutional Animal Care and Use Committee at the University of Pittsburgh.

STATISTICAL ANALYSIS

Data presented in the form of bar graphs show mean ± SEM. Significant difference between WT and Yap-KO groups was determined using Student t test. Difference between groups was considered statistically significant at P < 0.05.

Other methodological details are provided in the Supporting Information.

Results

EFFECT OF HEPATOCYTE-SPECIFIC YAP DELETION ON TCPOBOP-INDUCED HEPATOMEGALY AND HEPATOCYTE PROLIFERATION

Deletion of Yap in Yap-floxed mice by AAV8-TBG-CRE treatment was confirmed using western blot analysis for Yap and phospho-Yap (Fig. 1A). As expected, Yap and phospho-Yap expression was massively decreased in Yap-KO mice at basal levels and
FIG. 1. Effect of hepatocyte-specific Yap deletion on TCPOBOP-induced hepatomegaly and hepatocyte proliferation. (A) Western blot analysis of Yap and p-Yap. (B) Representative photomicrographs of Ki67-stained (brown nuclei) liver sections (magnification: ×400). (C) Percentage of hepatocytes in DNA synthesis (Ki67-positive nuclei). Bar graphs showing (D) LW/BW and (E) percent increase in LW/BW compared with basal values (i.e., Day 0). (F) Hepatocyte volume at Day 5 versus Day 0 in WT and Yap-KO mice calculated based on number of hepatocytes per unit area. All liver samples (n = 3–6) were collected from WT and Yap-KO mice at specified time points after TCPOBOP treatment. *Significant difference with regard to (w.r.t.) WT mice at particular timepoint; P < 0.05.
after TCPOBOP treatment (Fig. 1A). p-Yap (inactive form) levels were decreased after TCPOBOP treatment compared with its basal expression in WT mice, consistent with our previous report (Fig. 1A and Supporting Fig. S2B). Furthermore, ingenuity pathway analysis (IPA) of transcriptome data indicated significant activation of Yap transcriptional signature (based on expression profile of downstream genes) in WT mice at Day 2 versus Day 0 after TCPOBOP treatment (activation z-score: 2.42, P value: 2.01 × 10^{-24}) (Supporting Figs. S1A and S2A). In fact, Yap was among the top three transcription factors altered by TCPOBOP treatment in WT mice based on P value (Supporting Fig. S1A). These data are consistent with reports (by our group and others) showing increased nuclear accumulation of Yap after TCPOBOP treatment.(2,15) Ki67 (a marker for S-phase) immunohistochemical staining was performed to study hepatocyte proliferation. Robust hepatocyte proliferation was observed in WT mice at Days 1 and 2, which peaked at Day 2 (~55% Ki67-positive hepatocytes) and receded by Day 5 (only 5% Ki67-positive hepatocytes) after TCPOBOP treatment (Fig. 1B,C). There was a striking decrease in hepatocyte proliferation (~80% reduction) in Yap-KO mice consistently at both Days 1 and 2 after TCPOBOP treatment (Fig. 1B,C). The effect of Yap deletion on CAR-driven proliferative response was further corroborated by remarkably lower induction of proliferating cell nuclear antigen protein (another proliferative marker) in Yap-KO mice after TCPOBOP treatment (Fig. 3A). Liver to body weight ratio (LW/BW) increased consistently with time from Day 0 to Day 5 in WT mice, attaining a maximum of ~2-fold elevation, but plateaued after Day 5 (Fig. 1D,E). LW/BW was also increased in Yap-KO mice with time compared with basal levels, but the percent increase in LW/BW with regard to basal levels remained significantly lower in Yap-KO mice compared with WT mice at all the time points after TCPOBOP treatment (Fig. 1D,E). However, the effect of Yap deletion on liver size appears to be lower in comparison with the effect on proliferative response. Increase in liver size after TCPOBOP treatment depends on both hepatocyte proliferation and increase in hepatocyte size (hypertrophy).(18) Average hepatocyte volume (calculated based on number of hepatocytes per unit area) increased to around 200% of basal levels at Day 5 after TCPOBOP treatment in both WT and Yap-KO with no significant difference between two groups (Fig. 1F). This suggests that Yap deletion does not alter TCPOBOP-induced hypertrophy but alters only hepatocyte proliferation, resulting in an overall lower impact on increase in liver size. Lastly, Yap deletion did not alter proliferation or LW/BW ratio at basal level (Fig. 1B-D). Furthermore, Yap-KO mice appear to be normal with no signs of toxicity and displayed normal liver histology at the basal level.

**CAR expression and its activity associated with induction of drug metabolism in response to TCPOBOP treatment remained intact in Yap-KO mice**

Because TCPOBOP-induced hepatocyte proliferation is a CAR-dependent process, the effect of Yap deletion on CAR expression and its activity was investigated. CAR protein expression was comparable in WT and Yap-KO at both basal level and various time points after TCPOBOP treatment (Fig. 2A). To assess CAR activity, we investigated expression of classical CAR target genes using real-time PCR. As expected, cytochrome P450 (Cyp) 2b10, which is one of the best-characterized targets of CAR involved in drug metabolism, was strongly induced (up to 90-fold) after TCPOBOP treatment in WT mice (Fig. 2B). Interestingly, similar induction was also observed in Yap-KO mice at all the time points (1, 2, and 5 days after TCPOBOP treatment) without any significant difference compared with WT mice (Fig. 2B). We also investigated expression of other classical CAR target genes involved in drug metabolism, which represent both strong (Cyp2c55) and weak (UDP-glucuronosyltransferase 1A1 [Ugt1a1]) CAR-driven transcriptional induction (Fig. 2C,D). Induction of both these genes was not significantly altered in Yap-KO mice, similar to Cyp2b10 (Fig. 2C,D). Furthermore, microarray analysis revealed that several other CAR-inducible drug-disposition genes remain highly induced even in Yap-KO mice after TCPOBOP treatment, and overall induction of these genes was comparable with WT mice (represented by heatmaps in Supporting Fig. S3A,B). Furthermore, IPA of microarray data to investigate the altered
canonical signaling pathway showed significant activation of “xenobiotic metabolism CAR signaling pathway” in both WT (z-score: 2.343) and Yap-KO (z-score: 3.402) mice at Day 2 compared with basal levels (Supporting Fig. S4). IPA also revealed that overall induction of CAR target genes was similar...
in both groups at Day 2 compared with basal levels (z-score for CAR activation: WT: 4.216; Yap-KO: 4.435) (Fig. 2F). The high order of P value (10^-19 to 10^-24) for overlap between CAR target genes induced in both experimental groups versus CAR target genes in the reference genome demonstrate robust activation of CAR in both groups (Fig. 2F). In fact, CAR was among the top 10 transcription factors altered by TCPOBOP treatment in both WT and Yap-KO mice at Day 2 versus Day 0 (Supporting Fig. S1A,B).

Lastly, we also investigated expression of another known CAR target gene, growth arrest and DNA damage inducible beta (Gadd45b), which is reported to be important for CAR-driven hepatocyte hypertrophy and liver growth. (18) Similar to other CAR target genes (described previously), Gadd45b was strongly induced (up to 40-fold) after TCPOBOP treatment in WT mice, but its induction was comparable in Yap-KO mice (Fig. 2E). It is interesting to note that Gadd45b has also recently been identified as a Yap target gene in biliary cells. (19) However, it was not altered by hepatocyte-specific Yap deletion at basal levels or after TCPOBOP treatment in our study (Fig. 2E). This suggests differential transcriptional regulation of Gadd45b in hepatocytes and biliary cells. In hepatocytes, Gadd45b might be strongly regulated by other transcription factors, including CAR.

**IMPAIRMENT OF CELL CYCLE ACTIVATION IN YAP-KO MICE**

Next, activation of core cell cycle machinery was assessed to further explore the mechanisms for impaired proliferative response in Yap-KO mice. Cyclin (Ccn) d1 is a key protein that governs entry into the cell cycle. As expected, Ccn1 expression increased remarkably after TCPOBOP treatment compared with basal levels in WT mice (Fig. 3A). Ccn1 expression peaked at Day 1 and returned to basal levels at Day 5 in WT mice. Ccn1 expression was notably lower in Yap-KO mice compared with WT mice at Day 1. However, Ccn1 expression appeared higher in Yap-KO compared with WT mice at Days 2 and 5 (Fig. 3A). Ccn1 binds and activates cyclin-dependent kinase (Cdk) 4, which phosphorylates retinoblastoma (Rb) protein. Inhibition of Rb protein through its phosphorylation frees transcription factors of the E2F family, ultimately resulting in induction of several cell cycle genes, including other cyclins (E1, A2, and B1), which sequentially control progression through the cell cycle. (20) Similar to Ccn1 expression, phosphorylation of Rb peaked at Day 1, remained greatly elevated at Day 2, and declined to basal levels by Day 5 in WT mice (Fig. 3A). Despite delayed induction of Ccn1 in Yap-KO mice, phosphorylation of Rb remained persistently lower in Yap-KO at all the time points after TCPOBOP treatment (Fig. 3A). Similar significantly lower induction was also observed for other later-stage cyclins (E1, A2, and B1) and Cdk1 consistently during the entire proliferative phase in Yap-KO mice (Fig. 3B-E). Furthermore, microarray-based IPA of TCPOBOP-dependent genes that were altered by Yap deletion (as described in the Materials and Methods section) revealed E2F proteins (E2F1, E2F2, and E2F3) among the top inhibited upstream regulators in Yap-KO mice at Day 2 (Fig. 5D). In fact, E2F3 was the second-most altered (inhibited) upstream regulator in Yap-KO mice (activation z-score: -3.841 and P value of overlap: 2.23 × 10^-17) (Fig. 5D). Most of its target genes were down-regulated in Yap-KO mice at Day 2, as displayed by the network in Fig. 3F.

p21 is one of the major cell cycle inhibitory proteins that inhibits activity of Ccn1-Cdk4 complex, causing decreased Rb phosphorylation. (20) p21 protein expression was greatly elevated in Yap-KO mice compared with WT mice at Day 2, which is consistent with decreased Rb phosphorylation, despite higher Ccn1 expression at Day 2 in Yap-KO mice (Fig. 3A). IPA of microarray data further substantiated activation of p21 signaling in Yap-KO mice; several genes that are known to be negatively regulated by p21 were indeed down-regulated in Yap-KO mice compared with WT mice at Day 2 (Fig. 3F). In fact, p21 (or Cdkn1a) was the topmost altered upstream regulator (predicted to be activated; activation z-score: 3.354 and P value of overlap: 2.66 × 10^-18) in Yap-KO mice compared with WT mice based on the upstream regulator analysis using IPA (Fig. 5D).

**DOWN-REGULATION OF MYC-FORKHEAD BOX PROTEIN M1 SIGNALING AXIS IN YAP-KO MICE**

Forkhead box protein M1 (Foxm1) controls transcription of crucial proliferative genes involved in cell cycle regulation and is considered critical for driving TCPOBOP-mediated hepatocyte proliferation. (4,20-22)
**FIG. 3.** Impairment of cell cycle activation in Yap-KO mice. (A) Western blot analysis of proliferating cell nuclear antigen (PCNA), phospho-retinoblastoma protein (p-Rb), Ccnd1, p21, and p27. Messenger RNA expression of (B) Ccne1, (C) Ccna2, (D) Ccnb1, and (E) Cdk1 in liver. All samples \( n = 3-4 \) were collected at 0, 1, 2, and 5 days after TCPOBOP treatment in WT and Yap-KO mice. (F) IPA of microarray data showing alteration of E2F3 and p21 downstream gene network in Yap-KO vs WT mice at Day 2 after TCPOBOP treatment. Negative z-score represents predicted inhibition and positive z-score represents predicted activation of upstream regulator activity (absolute z-score > 2 considered as significant) based on expression profile of downstream genes. *Significant difference with regard to WT group at particular time point; \( P < 0.05 \).
Boxm1 was notably induced at all the time points after TCPOBOP treatment in WT mice following a temporal expression pattern similar to TCPOBOP-driven proliferative response (Fig. 4A). Boxm1 induction was significantly attenuated in Yap-KO mice throughout the entire proliferative phase (Fig. 4A). IPA of microarray data further substantiated down-regulation of Boxm1 activity in Yap-KO mice compared with control mice; several Boxm1-inducible genes were greatly down-regulated in Yap-KO mice at Day 2 (Boxm1 downstream gene network is shown in Fig. 4B, and individual genes in this network with expression values are listed in Supporting Table 1). In fact, Boxm1 was among the top 10 upstream regulators altered (predicted to be inhibited; activation z-score: $\text{−}4.587$, $P$ value: $1.79 \times 10^{-11}$) in Yap-KO mice compared with WT mice based on the upstream regulator analysis using IPA (Fig. 5D). Furthermore, down-regulation of Boxm1 was confirmed by investigating expression of several Boxm1 target genes (Aurora B kinase [AurkB], polo-like kinase 1 [Plk1], Survivin [Birc5], Cdc20, centromere protein A [Cenpa], kinesin family member 20a [Kif20a], and Ccnb2) as analyzed using real-time PCR. (D) Western blot analysis of Myc. All samples ($n = 3–4$) were collected at 0, 1, 2, and 5 days after TCPOBOP treatment in WT and Yap-KO mice. IPA of microarray data showing inhibition of (B) Boxm1 and (E) Myc downstream gene network in Yap-KO vs WT mice at Day 2 after TCPOBOP treatment. Negative z-score in (B) and (E) represents predicted inhibition of transcription factor activity (absolute z-score $> 2$ considered as significant) based on expression profile of downstream genes. $P$ value signifies extent of overlap between set of downstream target genes of a given transcription factor in data set compared with all known downstream target genes of that transcription factor in the reference genome (red shapes: up-regulated genes; green shapes: down-regulated genes, with intensity of color reflecting extent of up-regulation or down-regulation) *Significant difference with regard to WT mice at particular time point; $P < 0.05$.

**EFFECT ON HEPATOCYTE NUCLEAR FACTOR 4 ALPHA EXPRESSION AND ITS TRANSCRIPTIONAL SIGNATURE IN YAP-KO MICE**

Hepatocyte nuclear factor 4 alpha (HNF4α) is one of the most strongly activated transcription factors in quiescent liver, which is not only important for maintaining differentiated state in hepatocytes but also for inhibiting proliferative genes.\(^{(23)}\) Furthermore, deletion of HNF4α in the liver results in robust hepatocyte proliferation similar to CAR activation.\(^{(24,25)}\) Several studies have demonstrated functional inhibitory cross talk between CAR and HNF4α by direct competition for the same DNA binding sites and cotranscription factors.\(^{(22,26,27)}\) CAR-driven inhibition of HNF4α transactivation has also been reported to be one of the factors important for TCPOBOP-induced proliferative response in the liver.\(^{(22)}\) Our recent study also indicated down-regulation of HNF4α during TCPOBOP-driven proliferative response.\(^{(2)}\) HNF4α expression was normalized by combined disruption of MET and epidermal growth factor receptor (EGFR) signaling, which also resulted in complete inhibition of TCPOBOP-driven proliferative response.\(^{(2)}\) Consistent with our previous findings, HNF4α gene expression was repressed after TCPOBOP treatment in WT mice at all the time points compared with basal levels (Supporting Fig. S5). However, HNF4α...
was similarly down-regulated in Yap-KO mice after TCPOBOP treatment with no significant difference compared with WT mice at any of the investigated time points (Supporting Fig. S5). Furthermore, IPA of microarray data showed significant inhibition of HNF4α transcriptional activity (based on expression profile of downstream genes) in WT mice at Day 2 versus Day 0 (activation z-score: -2.53, P value: 1.13 \times 10^{-20}) (Supporting Figs. S6 and S1A). HNF4α transcriptional activity remained significantly inhibited (activation z-score: -2.35, P value: 1.21 \times 10^{-19}), even in Yap-KO mice (Supporting Figs. S7 and S1B). In fact, HNF4α was among the top 10 transcription factors altered by TCPOBOP treatment in both WT and Yap-KO mice at Day 2 versus Day 0 (Supporting Fig. S1A,B). This indicates that Yap does not regulate CAR-driven alteration of HNF4α activity, which is one of the transcription factors that govern TCPOBOP-induced proliferative response. This might be one of the reasons why TCPOBOP-induced proliferative response was not completely blocked (but only dampened) in Yap-KO mice.

GLOBAL CHANGES IN GENE EXPRESSION PROFILE AFTER HEPATOCYTE-SPECIFIC YAP DELETION

We further analyzed Yap-driven genome-wide changes in gene expression profile (obtained through microarray) in our study. To filter out Yap-regulated genes relevant to TCPOBOP treatment, we specifically looked at genes that were significantly up-regulated or down-regulated and at least 2-fold altered at 2 days after TCPOBOP treatment compared with basal levels in WT mice and were, at the same time, also significantly altered (at least 2-fold) in Yap-KO mice compared with WT mice at Day 2. At 2 days after TCPOBOP treatment, 2,497 genes were significantly altered compared with basal levels in WT mice. Interestingly, out of these 2,497 genes, around 27% (665 genes) were significantly altered in Yap-KO mice compared with WT mice at Day 2 (Fig. 5A). This demonstrates significant genome-wide impact of Yap deletion on TCPOBOP response. These mostly included genes that were induced by TCPOBOP and down-regulated in Yap-KO mice (350 genes; top 50 listed in Supporting Table 4), along with genes that were repressed by TCPOBOP but up-regulated in Yap-KO mice compared with WT mice (311 genes; top 50 listed in Supporting Table 5). Analysis of these TCPOBOP-dependent genes, which were altered by Yap deletion in the opposite direction using DAVID database, revealed that these genes were significantly enriched for biological processes mainly related to cell proliferation (such as cell cycle [specific genes pertaining to this Gene Ontology term, along with their expression values, are listed in Supporting Table 3], mitotic nuclear division, cell division, DNA replication, cytokinesis, mitotic sister chromatid segregation, and DNA replication) but not drug metabolism (Fig. 5B). Analysis of all these genes using IPA, taking directionality of change into account, predicted several canonical pathways to be significantly altered in Yap-KO mice versus WT mice, including activation of G2/M DNA damage checkpoint regulation and ataxia telangiectasia mutated kinase signaling, and inhibition of signaling related to pyrimidine deoxyribonucleotide de novo biosynthesis, mitotic role of polo-like kinase, cyclins and cell cycle regulation, and nucleotide excision repair pathway (Fig. 5C). Furthermore, based on the downstream gene signatures, IPA predicted alteration of several upstream regulators (Top 20 listed in Fig. 5D) relevant to cell proliferation, including significant activation of CDKN1A (i.e., p21) and p53 (affected downstream gene network shown in Fig. 5E), and inhibition of Myc, Foxm1, and E2F proteins in Yap-KO mice.

ASSOCIATION OF CAR, YAP, AND MYC GENOMIC BINDING

Next, the association of CAR, Yap, and Myc genomic binding sites was explored by analyzing published chromatin immunoprecipitation sequencing (ChIPseq) data sets. The data consisted of compiled peak sets of CAR binding, histone H4 acetylated at lysine 5 (H4K5Ac), deoxyribonuclease I hypersensitivity (DHS), and promoter-binding histone H3 trimethylated at lysine 4 from control and TCPOBOP-treated liver(28), newly compiled peak sets from ChIPseq libraries of TEA domain transcription factor (Tead) from untreated liver; and doxycycline-induced Yap and Myc from liver with tetracycline-regulated transgenes.(29) From these data, enhancers were characterized as small regions of transcription-factor binding and DHS, surrounded by H4K5Ac. It is important to note, however, that the data were
A

WT (2 day vs 0 day) vs YAP KO (2 day)

B

| GO term (biological processes) | No. of genes | p value  |
|-------------------------------|--------------|---------|
| mitotic nuclear division       | 28           | 9.40E-10|
| cell division                 | 32           | 2.90E-09|
| cell cycle                    | 41           | 1.50E-08|
| DNA replication               | 13           | 4.60E-05|
| mitotic sister chromatid segmenation | 6   | 1.90E-04|
| cytokinesis                   | 7            | 2.40E-04|
| chromosome condensation       | 5            | 4.90E-04|
| DNA metabolic process         | 5            | 1.20E-03|
| DNA unwinding involved in DNA replication | 4  | 1.50E-03|
| phosphatidylcholine metabolic process | 4 | 3.40E-03|

D

| Upstream Regulator | Predicted Activation State | Activation z-score | p-value of overlap |
|--------------------|----------------------------|--------------------|--------------------|
| CDKN1A             | Activated                  | 3.354              | 2.66E-18           |
| E2F3               | Inhibited                  | -3.841             | 2.23E-17           |
| E2F4               | Inhibited                  | -2.8E-17           |                    |
| AREG               | Inhibited                  | -3.771             | 5.32E-12           |
| MYC                | Inhibited                  | -3.727             | 7.43E-12           |
| TP53               | Activated                  | 3.516              | 1E-11              |
| Irgm1              | Activated                  | 3.208              | 1.35E-11           |
| ZBTB17             | Activated                  | 3.947              | 1.37E-11           |
| FOXM1              | Inhibited                  | -4.587             | 1.79E-11           |
| RABL6              | Inhibited                  | -4                 | 4.18E-11           |
| E2F1               | Inhibited                  | -4.135             | 5.96E-11           |
| ERBB2              | Inhibited                  | -5.027             | 1.88E-10           |
| RRP1B              | Inhibited                  | -4                 | 4.36E-10           |
| TBX2               | Inhibited                  | -4                 | 6.66E-10           |
| CDKN2A             | Activated                  | 3.173              | 1.27E-09           |
| YY1                | Activated                  | 1.73E-09           |                    |
| RB1                | Activated                  | 2.551              | 3.36E-09           |
| let7               | Activated                  | 3.969              | 1.41E-08           |
| E2F2               | Inhibited                  | -2.646             | 1.51E-08           |
| LIN9               | Inhibited                  | -2.791             | 2.03E-08           |

E

Activation z-score: 3.516
p-value of overlap: 1.0E-11
obtained from different experimental conditions (Yap and Myc data sets used an overexpression system), so the analysis shows binding positions but not whether Yap, Tead, CAR, and Myc are bound at the same time. The analysis began with visualization of candidate genes, starting with Myc, a critical target of both Yap and CAR and an important focus of this paper (Fig. 6). Myc has an extended regulatory region that includes plasmacytoma variant translocation 1 (Pvt1), a noncoding RNA gene with minimal liver expression. TCPOBOP induces CAR-binding, H4K5Ac and DHS at +343 kb, the most active enhancer region near Myc. The distance is surprising, but Myc and Pvt1 are in a region that is devoid of other genes, and even more distant Myc-regulating enhancers are known. Interestingly, Yap and Tead both bind the same enhancer region (+343 kb) as CAR, which also weakly binds Myc (Fig. 6). However, the main Myc binding site is at the Pvt1 promoter (+73 kb). The other candidate gene, Ccnd1, also bound CAR, Yap/Tead, and Myc but at separate enhancers (Supporting Fig. S8).

To characterize full transcriptional effects, peak libraries were compiled for each transcription factor and then restricted to peaks within regions of acetylated histone. MEME-ChIP analysis confirmed that each set had the expected binding specificity because Yap binds as a coactivator at Tead sites (Fig. 7A). Positional analysis indicated that CAR and Yap usually bound to separate enhancers. Their binding to the same Myc +343 kb site was therefore exceptional. In contrast, CAR and Myc frequently bound to the same enhancers, suggesting a direct positive interaction (Fig. 7A).

Binding sites were linked to ~2,700 of 4,300 genes that showed altered expression in response to either TCPOBOP treatment or Yap ablation (Fig. 7B). The Yap set was smallest but still associated with 300 regulated genes, whereas Myc binding was associated with almost 2,500 genes. The numbers of gene linkages were proportional to the size of each set. Overall, gene regulation was associated with combinations of separate enhancers that bind CAR, Yap, Myc, or CAR and Myc (Fig. 7B).

**EFFECT OF HEPATOCYTE-SPECIFIC YAP DELETION ON LIVER REGENERATION AFTER PHx**

Considering the significant role of Yap in CAR-driven proliferative response, we investigated whether hepatocyte-specific Yap deletion affects hepatocyte proliferation in classical liver regeneration model of PHx as well. However, no difference was observed in LW/BW ratio in Yap-KO and WT mice at various time points (40 hours, 72 hours, 7 days, and 14 days) up to 14 days after PHx (Fig. 8A). No obvious difference in hepatocyte proliferation was observed on analysis of Ki67-stained liver section between Yap-KO and WT mice during the entire proliferative phase after PHx (Fig. 8B). Furthermore, quantification of Ki67-stained liver sections at peak proliferative time points (40 and 72 hours) revealed that hepatocyte proliferation was comparable between Yap-KO and WT mice (Fig. 8C). This suggests a differential role of Yap in regulating hepatocyte proliferation during CAR-driven augmentative hepatomegaly versus PHx.
Discussion

In a recent study, we demonstrated that CAR-driven adaptive response of induction of drug-metabolism genes can be dissociated from proliferative response by the combined disruption of MET and EGFR signaling in the liver. Here, we report very similar findings after acute hepatocyte-specific deletion of Yap in adult liver.
FIG. 7. Characterization of Yap, CAR, and Myc genomic binding and its correlation to gene expression. (A) Characterization of Yap, CAR, and Myc peak sets. The overlaps represent binding within the same enhancer. Overlap set P values were calculated with Fisher’s exact test. The strongest binding motif calculated by MEME-ChIP from each set is also displayed along with corresponding e value. (B) Association of binding sites with Yap-regulated and TCPOBOP-regulated local genes. The diagram shows the number of sites within 100 kb of regulated genes, and the overlaps represent separate enhancers linked to the same gene. Compilation of ChIPseq libraries, limitation of peak sets, and composition of the set of regulated genes are described in the Supporting Methods.

FIG. 8. Effect of hepatocyte-specific Yap deletion on liver regeneration after partial hepatectomy. (A) Bar graphs showing LW/BW. (B) Representative photomicrographs of Ki67-stained (brown nuclei) liver sections (magnification: ×400). (C) Percentage of hepatocytes in DNA synthesis (Ki67-positive nuclei). All liver samples (n = 3) were collected from WT and Yap-KO mice at specified time points after partial hepatectomy.
mice. Although combined disruption of MET and EGFR signaling almost completely eliminated CAR-driven proliferative response, Yap-KO mice displayed dampened proliferative response (hepatectic proliferation was decreased by 80%). Similar to MET and EGFR signaling inhibition, Yap-KO did not impact CAR-induced drug-metabolism response. The finding that Yap-KO cannot completely abolish hepatocyte proliferation indicates that other Yap-independent pathways can also contribute to CAR-driven proliferation. For instance, CAR-driven inhibition of HNF4α expression and its downstream transcriptional signature remain unaffected by Yap deletion, which is known to regulate proliferative actions of CAR.\(^{22}\) Nevertheless, our study revealed that endogenous hepatocyte-specific Yap is one the crucial factors that selectively regulates CAR-driven hepatocyte proliferation but not drug metabolism. Interestingly, a recent report showed that Yap knockdown in mice can specifically inhibit proliferative response driven by pregnane X receptor (PXR), a xenobiotic-sensing receptor closely related to CAR, without impacting PXR-driven induction of drug-disposition genes.\(^{32}\) This indicates that PXR and CAR might share common mechanisms involving Yap that selectively drive their proliferative response. This study also showed that PXR can directly bind to Yap. Furthermore, it showed that activation of PXR can regulate Yap activity by controlling its nuclear accumulation and reduce cytoplasmic phosphorylated Yap without altering classical upstream regulators (Hippo pathway kinases) of Yap.\(^{13}\) Thus, Yap-PXR interaction may alter availability of Yap for phosphorylation by upstream kinases. Studies have shown increased nuclear accumulation of Yap after CAR activation in mice, consistent with alteration of Yap downstream gene network and decreased phosphorylation of Yap after TCPOBOP treatment in this study.\(^{2,15}\) However, no consistent pattern of alterations was observed in activity (i.e., phosphorylation) of upstream Hippo-pathway kinases (Lats and Mst) in this study (data not shown). Similar to PXR, whether CAR can directly bind Yap and alter its activation is an issue that needs further investigation. In vitro gene reporter assay in AML12 cells showed that CAR can enhance Yap-driven transcriptional activity and vice versa, suggesting potential interaction of CAR and Yap.\(^{15}\) Strikingly, our analysis of published ChIPseq data revealed strong binding of Yap, TEAD, and CAR to the same enhancer of Myc, an important driver of TCPOBOP-induced proliferation. Moreover, genome-wide analysis of CAR and Yap binding suggested that they can coregulate many genes through separate enhancers, including Ccnd1, an important regulator in TCPOBOP-driven proliferative response. One of the limitations of this in silico analysis was that it was conducted on data sets that used a Yap overexpression system. In the future, further ChIPseq studies of Yap and CAR specifically in a TCPOBOP model will likely provide more insight into Yap and CAR interactions.

Our study also suggested that Yap only regulates CAR-driven proliferative response but not hypertrophy and thus has less of an impact on overall liver size than one would have expected from the Ki67 results alone (Fig. 1). The overall effect on liver size is weaker because it is dependent on a combination of both hepatocyte hypertrophy and proliferation. Increase in hepatocyte size starts even before hepatocyte proliferation after TCPOBOP treatment and is governed by highly elevated synthesis of drug-metabolism enzymes (and associated organelle),\(^{18}\) which was not altered by Yap deletion in our study. Disconnect between TCPOBOP-induced liver growth and hepatocyte proliferation has been demonstrated in Gadd45b-KO mice, which displayed increased propensity of hepatocyte proliferation but lower liver growth compared with WT mice after TCPOBOP treatment.\(^{18}\)

In our study, one of the mechanisms by which Yap-regulated TCPOBOP-driven proliferative response was by altering Myc-Foxm1 signaling axis. Yap-KO displayed impaired induction of Myc protein expression, and activation of its downstream gene network, which is known to be crucial for driving CAR-induced hepatocyte proliferation. Myc-KO mice have been reported not only to display attenuated hepatocyte proliferation and induction of proliferative genes after TCPOBOP treatment but, more importantly, induction of drug disposition and hypertrophy were not affected in Myc-KO mice.\(^{21}\) Furthermore, some degree of proliferation was still present in Myc-KO mice following TCPOBOP treatment, similar to Yap-KO mice in our study.\(^{21}\) This similarity in phenotype between Myc-KO mice and our Yap-KO mice further substantiates that Yap acts through Myc to regulate CAR-driven proliferative genes but is not required for induction of drug-metabolism genes and hypertrophy. Moreover, analysis of the published ChIPseq data indicated that Myc binds to ~35% of
the same enhancers that CAR binds, suggesting a direct positive interaction. There was also a striking overlap of genes regulated by CAR and Myc through separate enhancers (Fig. 7). Apart from direct activation of the proliferative gene network, Yap might also be involved in negatively regulating antiproliferative signaling pathways, including p21 and p53 after TCPOBOP treatment, as predicted by our microarray analysis of Yap-KO mice and elevated p21 expression in Yap-KO mice during the peak of proliferation. This antiproliferative effect in Yap-KO mice might also be mediated through Myc, as increased p21 expression in response to TCPOBOP treatment has been also reported in Myc-KO mice. 

In response to TCPOBOP treatment, Myc has been reported to directly bind to Foxm1 promoter and induce its transcription. Consistent with this study, in addition to impaired Myc induction, Yap-KO mice also displayed dampened induction of Foxm1 in response to TCPOBOP treatment. Foxm1 is a master transcriptional regulator of cell cycle progression that regulates proliferation by regulating transcription of important cell cycle genes, including Cdc25 phosphatases (which activate cyclin-dependent kinases), cyclins (such as Ccnb1), and several mitotic regulators (such as Aurora B kinase, survivin, and polo-like kinase-1). Consistent with lower induction of Foxm1, induction of all these proliferation genes and overall Foxm1 downstream gene network was decreased in Yap-KO mice in response to TCPOBOP treatment. Foxm1 also negatively regulates expression of cell cycle inhibitors, such as p21, which was increased in Yap-KO mice in response to TCPOBOP treatment. The critical role of Foxm1 in CAR proliferative actions can be highlighted by the fact that TCPOBOP-driven hepatocyte proliferation that causes regenerative response and survival after extensive liver resection (86%) model was lost after Foxm1 knockdown in mice. Overall, our study demonstrated that Yap-KO mice have impaired activation of Myc-Foxm1 signaling, which is required for CAR-driven proliferation.

Although Yap remains inactivated in hepatocytes in quiescent liver, inactivation of Mst/Lats kinases, nuclear localization of Yap, and activation of Yap target genes were also observed during proliferation after PHx in mice. Furthermore, Yap signaling was normalized following restoration of liver mass after PHx. However, in our study, hepatocyte-specific deletion of Yap in adult mice only altered CAR-driven proliferative response but did not alter hepatocyte proliferation and restoration of liver mass after PHx at any time during the entire time course of regeneration. Hepatocyte-specific Yap might still have some contribution to timely regenerative response after PHx, but its elimination is dispensable for regeneration and might be compensated by some other proregenerative pathways. Taz might compensate for Yap functions during regeneration after PHx, as a recent study has demonstrated that combined deletion of Yap and Taz in liver significantly decreases hepatocyte proliferation and delays regeneration after PHx in mice. This difference from our findings might also arise because of utilization of different experimental approaches: Yap/Taz was deleted in both hepatocytes and cholangiocytes, prenatally, in this study using Alb-CRE mice, whereas Yap was acutely deleted specifically in hepatocytes in adult mice using AAV8-TBG-CRE in our study. Furthermore, Yap/Taz-deleted mice displayed liver injury and consequent hepatomegaly at basal level, which might affect regenerative response. On the other hand, acute Yap-KO mice did not show any apparent injury or overt histology in our study.

In conclusion, we provide direct and specific evidence that hepatocyte-specific Yap is crucial for CAR-driven proliferative response in mice liver but not for CAR-driven induction of drug-disposition enzymes. Furthermore, Yap-KO mice displayed impaired activation of Foxm1/Myc signaling pathway in response to CAR activation, which is essential for CAR-driven proliferative response. Lastly, hepatocyte-specific Yap was dispensable for liver regeneration after partial hepatectomy.

Author Contributions: Study concept and design: B.B.; Acquisition of data: B.B., J.L., L.M., K.K., J.W.S., W.M.M., S.B., A.O., S.P.; Analysis and interpretation of data: B.B. and J.L.; Writing – original draft: B.B; Writing – review & editing: B.B., G.K.M., J.L., S.P.M. All authors approved the final submission.

REFERENCES

1) Kazantseva YA, Pustylnyak YA, Pustylnyak VO. Role of nuclear constitutive androstane receptor in regulation of hepatocyte proliferation and hepatocarcinogenesis. Biochemistry (Mosc) 2016;81:338-347.
2) Bhushan B, Stoops JW, Mars WM, Orr A, Bowen WC, Paranjpe S, et al. TCPOBOP-induced hepatomegaly and hepatocyte

2021
proliferation are attenuated by combined disruption of MET and EGFR signaling. Hepatology 2019;69:1702-1718.

3) Dong B, Lee JS, Park YY, Yang F, Xu G, Huang W, et al. Activating CAR and beta-catenin induces uncontrolled liver growth and tumorigenesis. Nat Commun 2015;6:5944.

4) Tschuor C, Kachaylo E, Limani P, Raptis DA, Liececker M, Tian Y, et al. Constitutive androstan receptor (CAR)-driven regeneration protects liver from failure following tissue loss. J Hepatol 2016;65:66-74.

5) Ledda-Columbano GM, Pibiri M, Loi R, Perra A, Shinozuka H, Columbano A, et al. Early increase in cyclin-D1 expression and accelerated entry of mouse hepatocytes into S phase after administration of the mitogen 1, 4-Bis[2-(3,5-Dichloropyridyloxy)] benzene. Am J Pathol 2000;156:91-97.

6) Dong J, Feldmann G, Huang J, Wu S, Zhang N, Comerford SA, et al. Elucidation of a universal size-control mechanism in Drosophila and mammals. Cell 2007;130:1120-1133.

7) Yu FX, Zhao B, Guan KL. Hippo pathway in organ size control, tissue homeostasis, and cancer. Cell 2015;163:811-828.

8) Zheng Y, Pan D. The Hippo signaling pathway in development and disease. Dev Cell 2019;50:264-282.

9) Camargo FD, Gokhale S, Johnnidiis JB, Fu D, Bell GW, Jaenisch R, et al. YAP1 increases organ size and expands undifferentiated progenitor cells. Curr Biol 2007;17:2054-2060.

10) Zhou D, Conrad C, Xia F, Park JS, Payer B, Yin Y, et al. Mst1 and Mst2 maintain hepatocyte quiescence and suppress hepatocellular carcinoma development through inactivation of the Yap1 oncogene. Cancer Cell 2009;16:425-438.

11) Zhang N, Bai H, David KK, Dong J, Zheng Y, Cai J, et al. The Merlin/NF2 tumor suppressor functions through the Yap oncoprotein to regulate tissue homeostasis in mammals. Dev Cell 2010;19:27-38.

12) Benhamouche S, Curto M, Saotome I, Gladden AB, Liu CH, Giovannini M, et al. N2F/Merlin controls progenitor cell homeostasis and tumorigenesis in the liver. Genes Dev 2010;24:1718-1730.

13) Lu L, Finegold MJ, Johnson RL. Hippo pathway coactivators Yap and Taz are required to coordinate mammalian liver regeneration. Exp Mol Med 2018;50:e423.

14) Kowalik MA, Saliba C, Pibiri M, Perra A, Ledda-Columbano GM, Sarotto I, et al. Yes-associated protein regulation of adaptive liver enlargement and hepatocellular carcinoma development in mice. Hepatology 2011;53:2086-2096.

15) Abe T, Amaike Y, Shizu R, Takahashi M, Kano M, Hosaka T, et al. Role of YAP activation in nuclear receptor CAR-mediated proliferation of mouse hepatocytes. Toxicol Sci 2018;165:408-419.

16) Gibault F, Corvaisier M, Bailly F, Huet G, Ménynk P, Cotelle P. Non-photoinduced biological properties of verteporfin. Curr Med Chem 2016;23:1171-1184.

17) Ledda-Columbano GM, Pibiri M, Concadas D, Molotu F, Simbula G, Cosso C, et al. Sex difference in the proliferative response of mouse hepatocytes to treatment with the CAR ligand. TCPOBOP. Carcinogenesis 2003;24:1059-1065.

18) Tian J, Huang H, Hoffman B, Liebermann DA, Ledda-Columbano GM, Columbano A, et al. Gadd45β is an inducible coactivator of transcription that facilitates rapid liver growth in mice. JClin Invest 2011;121:4491-502.

19) Pepe-Mooney BJ, Dill MT, Alemany A, Ordovas-Montanes J, Matsushita Y, Rao A, et al. Single-cell analysis of the liver epithelium reveals dynamic heterogeneity and an essential role for Yap in homeostasis and regeneration. Cell Stem Cell 2019;25:23-38.e28.

20) Costa RH, Kalinichenko VV, Tan Y, Wang IC. The CAR nuclear receptor and hepatocyte proliferation. Hepatology 2005;42:1004-1008.

21) Blanco-Bose WE, Murphy MJ, Ehninger A, Öffner S, Dubey C, Huang W, et al. C-Myc and its target FoxM1 are critical downstream effectors of constitutive androstan receptor (CAR) mediated direct liver hyperplasia. Hepatology 2008;48:1302-1311.

22) Kazantseva YA, Yarushkin AA, Mostovich LA, Pustylnyak VA, Pustylnyak VO. Xenosensor CAR mediates down-regulation of miR-122 and up-regulation of miR-122 targets in the liver. Toxicol Appl Pharmacol 2015;288:26-32.

23) Waley C, Apte U. Role of hepatocyte nuclear factor 4α (HNF4α) in cell proliferation and cancer. Gene Expr 2015;16:101-108.

24) Waley C, Edwards G, Borude P, Gunawerdana S, O’Neil M, Yoo B, et al. Hepatocyte nuclear factor 4α deletion promotes diethyltinolamine-induced hepatocellular carcinoma in rodents. Hepatology 2013;57:2480-2490.

25) Waley C, Gunawerdana S, Terverieler EF, Edwards G, Borude P, Apte U. Hepatocyte-specific deletion of hepatocyte nuclear factor-4α in adult mice results in increased hepatocyte proliferation. Am J Physiol Gastrointest Liver Physiol 2013;304:G26-G37.

26) Miao J, Fang S, Bae Y, Kemper JK. Functional inhibitory cross-talk between constitutive androstan receptor and hepatic nuclear factor-4 in hepatic lipid/glucose metabolism is mediated by competition for binding to the DR1 motif and to the common coactivators, GRIP-1 and PGC-1α. J Biol Chem 2006;281:14537-14546.

27) Yarushkin AA, Kachaylo EM, Pustylnyak VO. The constitutive androstan receptor activator 4-[4(R,6R)-4,6-dioxan-2-yl]-N,N-dimethylaniline inhibits the gluconeogenic genes PEPCk and G6Pase through the suppression of HNF4α and FOXO1 transcriptional activity. Br J Pharmacol 2013;168:1923-1932.

28) Tian J, Marino R, Johnson C, Locker J. Binding of drug-activated CAR/Nrho1 alters metabolic regulation in the liver. iScience 2018;9:209-228.

29) Croci O, De Fazio S, Biagioni F, Donato E, Caganovala M, Curti L, et al. Transcriptional integration of mitogenic and mechanical signals by Myc and YAP. Genes Dev 2017;31:2017-2022.

30) Cho SW, Xu J, Sun R, Mumbach MR, Carter AC, Chen YG, et al. Promoter of lncRNA gene PVT1 is a tumor-suppressor DNA boundary element. Cell 2018;173:1398-1412.e1322.

31) Uslu VV, Petretich M, Ruf S, Langenfeld K, Fonesca NA, Marioni JC et al. Long-range enhancers regulating Myc expression are required for normal facial morphogenesis. Nat Genet 2014;46:753-758.

32) Jiang Y, Feng D, Ma X, Fan S, Gao Y, Fu K, et al. Pregnan X receptor regulates liver size and liver cell fate by yes-associated protein activation in mice. Hepatology 2019;69:343-358.

33) Grijalva JL, Huizenga M, Mueller K, Rodriguez S, Brazzo J, et al. Additional Supporting Information may be found at onlinelibrary.wiley.com/doi/10.1002/hep.31521/suppinfo.