Hepatocellular carcinoma (HCC) accounts for the majority of primary liver cancer cases, with more than 850,000 new diagnoses per year globally. Recent trends in the United States have shown that liver cancer mortality has continued to increase in both men and women, while 5-year survival remains below 20%. Understanding key mechanisms that drive chronic liver disease progression to HCC can reveal new therapeutic targets and biomarkers for early detection of HCC. In that regard, many studies have underscored the importance of alternative splicing as a source of novel HCC prognostic markers and disease targets. Alternative splicing of pre-mRNA provides functional diversity to the genome, and endows cells with the ability to rapidly remodel the proteome. Genes that control fundamental processes, such as metabolism, cell proliferation, and apoptosis, are altered globally in HCC by alternative splicing. This review highlights the major splicing factors, RNA binding proteins, transcriptional targets, and signaling pathways that are of key relevance to HCC. We highlight primary research from the past 3–5 years involving functional interrogation of alternative splicing in rodent and human liver, using both large-scale transcriptomic and focused mechanistic approaches. Because this is a rapidly advancing field, we anticipate that it will be transformative for the future of basic liver biology, as well as HCC diagnosis and management. (Cell Mol Gastroenterol Hepatol 2020;10:699–712; https://doi.org/10.1016/j.jcmgh.2020.04.018)

Keywords: mRNA; Metabolism; Cancer; Variants.

Liver cancer–associated mortality in the United States has doubled in the past 2 decades, and continues to increase at a faster rate than any other cancer type.1 Hepatocellular carcinoma (HCC) is the most common form of primary liver cancer.2,3 HCC is the end result of chronic hepatocyte injury,4 and generally develops in the context of cirrhosis resulting from viral hepatitis, alcoholic liver disease, or nonalcoholic steatohepatitis (NASH).3 In the United States, chronic hepatitis C (HCV) infection still accounts for the majority of HCC cases, but the burden of HCV has decreased in recent years4 with the approval of all-oral antiviral regimens that have near-complete response rates.6 However, the proportion of NASH-related HCC cases among patients listed on the liver transplant list has increased significantly in the past few years.7 NASH is the most severe form of nonalcoholic fatty liver disease (NAFLD), which affects a quarter of the general population.8 Compared with healthy controls, patients with NAFLD are 7 times more likely to develop HCC, and this risk is higher in those with cirrhosis.9 With an overall 5-year survival rate of 18%, liver cancer represents a significant health burden that will become even greater in the near future because it is projected that, by 2030, it will be the third leading cause of cancer-related deaths.10

Prevention and early detection strategies for HCC are challenging to implement because of the long disease course and the high interindividual variability in tumor growth patterns, as assessed by imaging studies on large cohorts of patients.11 Therefore, targeted interventions are needed urgently to extend the quality of life and increase the survival rates among HCC patients. In that respect, understanding key drivers of chronic liver disease progression to HCC can uncover novel strategies for selective targeting of HCC tumors. There are now numerous examples from the literature that highlight how alternative pre-messenger RNA (mRNA) splicing yields new proteins that have either lost a tumor-suppressor function, or have gained new oncogenic functions in HCC. This review highlights some of the major findings published on this topic within the past 3–5 years.

Abbreviations used in this paper: BIN1, Myc box–dependent–interacting protein; DDC, 3,5-diethoxycarbonyl-1,4-dihydrocollidine; EMT, epithelial–mesenchymal transition; ESRP2, epithelial splicing regulatory protein 2; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; hnRNP, heterogeneous nuclear ribonucleoprotein; KHK, keto–hexokinase; MALAT1, metastasis-associated lung adenocarcinoma transcript 1; MBNL, muscleblind-like; mRNA, messenger RNA; MTR4, Exosome RNA helicase MTR4; Myc, Myc proto-oncogene protein; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; NONO, non-POU domain-containing octamer-binding protein 2; POUSF1, ribose-phosphate pyrophosphokinase 1; RBP, RNA binding protein; SRSF, serine/arginine-rich splicing factor; TAZ, WW domain-containing transcription regulator protein 1; YAP, Transcriptional coactivator YAP1.

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Alternative RNA Splicing Is Dysregulated in Human HCC

Post-transcriptional gene regulation, in particular alternative splicing, is frequently dysregulated in cancer, in part owing to somatic changes in key genes, such as TP53. Alternative splicing is a mechanism for controlled gene expression that involves the production of multiple mRNA transcripts from a single gene. This mechanism is important for the abundance and diversity of protein isoforms, and is particularly critical during development for tissue specification. Alternative splicing and other post-transcriptional control mechanisms, such as mRNA turnover and translation, are integrated with gene transcription to regulate key aspects of cellular metabolism. A prime example of this is the regulation of insulin signaling by a controlled rate of insulin mRNA translation, as glucose-mediated insulin mRNA stabilization, and alternative splicing of the insulin receptor. Insulin, in turn, controls the expression of more than 1000 liver transcripts, including both coding and noncoding RNAs.

Most multi-exon human genes undergo alternative splicing, and different tissues possess unique splicing signatures. Of the more than 13,000 protein-coding genes expressed in the adult human liver, more than >80% were found to undergo alternative splicing to produce 4 or more transcripts. Skipped exon and alternative use of the first exon are the most common splicing events in the liver. Compared with normal liver, there is a high degree of differential splicing in primary HCC tumor tissues and many of these changes correlate with HCC patient survival. A growing number of studies have shown that altered splicing programs in HCC tumor cells give rise to novel protein isoforms that often have distinct, and sometimes opposing, functions from their canonical counterparts. (Table 1).

In a global sense, genes regulating the cell cycle, cell proliferation, DNA repair, metabolism, and the epithelial-mesenchymal transition (EMT) are differentially spliced in HCC tumors compared with nontumor adjacent tissue.

Among these pathways, metabolism-related genes are the most common, particularly those that are associated with carbohydrate processing. Furthermore, some splicing events are linked strongly to the etiology of HCC and can be a source of novel biomarkers of disease activity. For example, a splicing switch in the fibroblast growth factor receptor 2 is associated with the presence of hepatitis B virus or HCV infection, and the tumor-specific fibroblast growth factor receptor 2 splice variant isoform correlates with tumor size.

Changes in RNA-Binding Proteins and Splicing Factors in HCC

One potential mechanism underlying the vast changes in splicing programs between tumor and nontumor tissue in HCC is the differential expression of RNA binding proteins (RBPs) and splicing factors. In HCC tumors, 231 RBP-encoding genes were found to be up-regulated, whereas 55 were downregulated relative to nontumor tissue. Interestingly, a few RBP-encoding genes appear to serve as master regulators of many cellular pathways. For example, SNRPA and RALY each regulate more than 1000 splicing events and are predicted to impact up to 30 different pathways in HCC. This is not surprising because SNRPA encodes a component of the spliceosome, the complex molecular machinery that catalyzes pre-mRNA splicing. The precise function of RALY is not known, but this gene encodes a heterogeneous nuclear ribonucleoprotein (hnRNP) that may be a potential oncogene. Interestingly, there are also a number of RBP genes that were found to regulate fewer splicing events, but those events were linked to a large number of pathways. For example, the gene RBM24 was associated with 200 splicing events, but linked to more than 20 cellular pathways. RBM24 encodes an RNA binding protein that is both a target and regulator of the tumor-suppressor p53, which frequently is mutated in human HCC and known to limit tumor progression in mice. In addition to its function as a tumor suppressor, RBM24 is also a master regulator of alternative splicing.

### Table 1. Examples of Novel Splice Variants Expressed in HCC and Associated Cellular Pathways

| Gene       | Protein                             | Splice variant isoform | Major associated pathway                                      | Literature source                  |
|------------|-------------------------------------|------------------------|----------------------------------------------------------------|-----------------------------------|
| BIN1       | Myc box-dependent-interacting protein-1 | Long variant BIN1L     | Regulation of membrane signaling and Myc                      | Malakar et al, Cancer Res 2017    |
| CCDC50     | Coiled-coil domain-containing protein 50 | Short variant CCDC50S  | Ubiquitin-proteasome, cytoskeleton                            | Wang et al, Hepatology 2019       |
| INSR       | Insulin receptor                     | Short variant IR-A     | Insulin signaling                                             | Chettouh et al, Cancer Res 2013    |
| KHK        | Ketohexokinase                       | Variant KHK-A          | Fructose metabolism                                           | Li et al, Nat Cell Biol 2016       |
| NF2        | Merlin                              | Short variant Δ2-4Merlin | Hippo signaling/cell growth and proliferation                | Luo et al, Nat Commun 2015        |
| NT5E       | Ecto-5’-nucleotidase (CD73)          | Short variant CD73S    | Extracellular adenosine production                            | Snider et al, Mol Biol Cell 2014   |
| NUMB       | Protein numb homolog                 | Long variant PRR (L)   | Tissue morphogenesis                                          | Lu et al, Hepatology 2015         |
| RCAN1      | Calcipressin-1                       | Variant isoform 4      | Regulation of transcription                                   | Jin et al, Gastroenterology 2017   |
| TLL1       | Tolloid-like protein 1               | TLL1 short variant     | Extracellular matrix and cell differentiation                 | Matsuura et al, Gastroenterology 2017 |
suppressors, recent work has shown that wild-type p53 also can act in an oncogenic manner by regulating metabolic reprogramming of HCC cells.\textsuperscript{49} It remains to be determined if RBM24-regulated pathways in HCC can be linked to its effects on p53. Collectively, these transcriptome-wide studies show that alternative splicing is involved in the rewiring of cellular metabolism and other critical pathways in the liver, and that aberrant splicing in HCC tumors can be traced to several master regulators involved in RNA processing.

Alternative Splicing in Liver Development and Maturation

Pathways that normally signal during liver development are reactivated in HCC.\textsuperscript{49–51} Recent studies have shown significant alternative splicing changes during normal fetal-to-adult liver maturation\textsuperscript{52} and during injury-associated adult-to-fetal reversion in hepatocytes.\textsuperscript{53} Analyses of alternative splicing events in mouse liver just before birth, shortly after birth, and in adulthood found that the most dramatic splicing changes (affecting >500 genes) occurred at the switch between the prenatal and postnatal periods, and that many of these genes encoded cytoskeleton and chromatin modification regulators.\textsuperscript{56} Furthermore, comparison of different cell types between P0 and adult mouse showed that more than 50% of postnatal splicing transitions in the liver occurred specifically within hepatocytes.\textsuperscript{52} Many of the genes showing a hepatocyte-specific exon inclusion or exclusion during the transition from fetal to adult liver also are known to be functionally involved in HCC, including \textit{Camkk2},\textsuperscript{54} \textit{Kras},\textsuperscript{55} \textit{Pla2g6},\textsuperscript{56} \textit{Usp4},\textsuperscript{57} \textit{Vps29},\textsuperscript{58} and \textit{Rpa3}.\textsuperscript{59}

Among these genes, developmentally regulated splicing of \textit{Kras} is particularly interesting because of its known involvement in oncogenic hepatocyte signaling.\textsuperscript{60} Although \textit{Kras} mutations in human HCC are not common, the Ras signaling pathway is hyperactivated,\textsuperscript{61} in part via the splicing factor hnRNPA2.\textsuperscript{31} Furthermore, Ras signaling contributes to HCC tumor growth by suppressing the anti-tumorigenic transcription factor \textit{KLF6}.\textsuperscript{62} It is important to note that, at the mRNA abundance level, 3000–5000 mouse liver genes change during the prenatal to postnatal transition periods,\textsuperscript{52,63} which is under the control of the methyltransferases \textit{EZH1} and \textit{EZH2}.\textsuperscript{63} Therefore, alternative splicing could be considered an important fine-tuning mechanism, rather than a major switch during postnatal liver maturation.

A potential caveat is that only approximately 40% of developmentally regulated, alternatively spliced mouse genes were found to be regulated similarly in human liver.\textsuperscript{72} This is an important consideration because there are human-specific splicing events known to be up-regulated in HCC. For example, the production of catalytically impaired splice variants of the nucleotide-regulating enzymes ecto-5’-nucleotidase (\textit{NTSE})\textsuperscript{35} and kynurenine formamidase (\textit{AFMID})\textsuperscript{16} occurs only in humans. In light of that, the most effective strategies for identifying tumor-promoting oncofetal splice variants and mechanisms can come out of integrating data from human HCC cell-based models and tissue specimens with the most appropriate animal models.\textsuperscript{67–69}

Up-regulation of the Oncofetal Splicing Factor Muscleblind-Like 3 in HCC

Muscleblind-like (MBNL) proteins are encoded by 3 genes (\textit{MBNL1–3}) and regulate RNA splicing in a tissue-specific manner.\textsuperscript{70,71} The splicing factor MBNL3 is highly expressed in fetal liver and in HCC, but not in normal adult liver.\textsuperscript{72} Up-regulation of MBNL3 in HCC is considered to be a result of increased activity of several transcription factors, including \textit{NANOG, OCT4}, and \textit{SOX2}.\textsuperscript{72} Increased MBNL3 was associated with the differential splicing of the long non-coding RNA \textit{PXN-AS1} in HCC tumors.\textsuperscript{72} Specifically, MBNL3 promoted the inclusion of exon 4, resulting in the generation of a long isoform (\textit{PXN-AS1-L}). The \textit{PXN-AS1-L} isoform had an opposing function to the short isoform \textit{PXN-AS1-S}, which is expressed in normal liver.\textsuperscript{72} \textit{PXN-AS1-L} bound to the 3’-untranslated region of the paxillin-encoding \textit{PXN} gene, leading to \textit{PXN} mRNA stabilization and increased paxillin expression.\textsuperscript{72} The opposite was true for \textit{PXN-AS1-S}, which inhibited \textit{PXN} mRNA translation and paxillin expression. Paxillin is known to promote HCC cell migration and metastasis, particularly when it is phosphorylated by JNK.\textsuperscript{73} Therefore, the increased paxillin expression in HCC may be part of a splicing program to promote HCC metastasis (Figure 1). In support of that, MBNL3 expression, \textit{PXN} exon 4 retention, and paxillin expression are correlated positively with each other and with poor HCC patient survival.\textsuperscript{72}

Epithelial Splicing Regulatory Protein 2 Splicing Factor Regulates Hippo Signaling in the Liver

Hepatic epithelial injury triggers a strong regenerative response through a variety of mechanisms, including hepatocyte renewal, inflammation, and extracellular matrix remodeling.\textsuperscript{74,75} An essential component of liver regeneration under physiologic and pathologic conditions is the remarkable plasticity of hepatocytes, which are able to dedifferentiate and become progenitor-like.\textsuperscript{76} Furthermore, recent work has shown that the transforming growth factor β signaling pathway is critical for liver regeneration after partial hepatectomy, in part via activating hepatocyte EMT reprogramming in concert with the transcriptional co-activator \textit{YAP}.\textsuperscript{77} However, \textit{YAP}-dependent signaling also can lead to improper regeneration of hepatocytes, marked by overactivation of fetal signaling pathways, thereby contributing to acute liver failure.\textsuperscript{78} \textit{YAP} is a mechanosensitive target of the Hippo signaling pathway, which regulates organ size and tissue regeneration\textsuperscript{79,80} and is dysregulated in HCC.\textsuperscript{81–84} Furthermore, Hippo signaling in the liver is under the control of the master splicing regulator epithelial splicing regulatory protein 2 (\textit{Esrp2}),\textsuperscript{82} suggesting that alternative splicing is an important component of regenerative responses in the liver.
Although Esrp2 regulates the splicing of approximately 20% of mouse liver genes, Esrp2<sup>−/−</sup> mice develop normally, and at 4 months of age do not show any differences in their liver-to-body weight ratios, metabolic homeostasis, or signs of liver injury when compared with wild-type mice. While it appears that Esrp2 is not essential for liver development, it is possible that it could regulate HCC metastasis. This has not been reported to date in vivo, but it would be of interest to examine because ESRP2 is able to support cell-cell adhesion and attenuate the motility of cancer cells in vitro.

Esrp2 is an important stress response factor that changes dynamically during liver injury and recovery, and it is involved in adult-to-fetal reversion in injured hepatocytes (Figure 1). Livers from Esrp2<sup>−/−</sup> mice were marked by the presence of small immature hepatocytes that produced less albumin and showed evidence of hyperproliferation. Furthermore, Esrp2 was down-regulated significantly in mice challenged with the hepatotoxicant 3,5-diethoxycarbonyl-1,4-dihydrocollidine (DDC), and restored during the recovery period after DDC challenge. This is likely an adaptive response to injury because DDC-treated Esrp2<sup>−/−</sup> mice presented with significantly more hepatocyte proliferation and increased hepatomegaly. In the absence of Esrp2 (triggered by DDC feeding or genetic deletion), there was an adult-to-neonatal isoform switching of several Hippo pathway genes, including Yap1, Nf2, Csnk1d, and Tead1. The resulting protein variants downregulated Hippo signaling, allowing mature hepatocytes to exit their quiescent state and become proliferative (Figure 1).

The mouse model of DDC liver injury reflects some aspects of chronic hepatocyte stress associated with alcoholic hepatitis, such as hepatomegaly and the presence of Mallory–Denk bodies. Recent work has extended on the findings in Esrp2<sup>−/−</sup> mice to provide evidence for ESRP2 function in human alcoholic liver injury. Hyun et al. showed that ESRP2 is down-regulated significantly in severe human liver injury associated with alcoholic hepatitis, potentially via transcriptional down-regulation by the inflammatory cytokines tumor necrosis factor α and interleukin 1β (Figure 1). In addition to reduced overall

Figure 1. Alternative splicing rewires Hippo signaling during chronic liver injury and HCC. (A) The splicing regulator ESRP2 is important for the maintenance of a differentiated adult hepatocyte population via activation of the Hippo pathway. When Hippo signaling is active (On), the transcriptional co-regulators YAP and TAZ, which promote cell proliferation, are degraded (Off). This pathway is modulated during liver injury and recovery via the dynamic regulation of ESRP2 expression. Proinflammatory cytokines, such as tumor necrosis factor α and interleukin 1α, promote down-regulation of Esrp2 in human hepatocytes. The absence or down-regulation of Esrp2 leads to altered splicing and hypoactivation of Hippo kinases. This promotes the expression of YAP/TAZ target genes, resulting in hepatocyte hyperproliferation and hepatomegaly. (B) The gene NF2, which encodes the protein Merlin, is a direct target of ESRP2, which is implicated in HCC via a mechanism involving the production of a tumor-promoting protein variant (Δ<sup>2-4</sup>Merlin). The Δ<sup>2-4</sup>Merlin variant up-regulates the expression of stem cell transcription factors (stemness genes) such as SOX2. Expression of SOX2 and other stemness genes induces MBNL3, a splicing factor that is expressed in fetal liver and HCC. A major target of MBNL3 is PXN-AS1, a long noncoding RNA that regulates expression of the protein paxillin. Alternative splicing of PXN-AS1 promotes the production of the long variant PXN-AS1-L, which stabilizes PXN mRNA, leading to increased paxillin expression. Paxillin regulates cell adhesion and migration and promotes HCC metastasis.
expression, ESRP2 protein was mislocalized to the cytoplasm in liver tissue from alcoholic hepatitis patients. This resulted in hypoactivation of Hippo kinases and derepression of the downstream transcriptional co-activators YAP and TAZ, which are known to promote cell proliferation. Ultimately, in the absence of ESRP2, there was reversion of splicing to a fetal-like program that produced functionally compromised, proliferative immature hepatocytes that could lead to hepatic insufficiency associated with alcoholic hepatitis. It would be interesting to determine how this mechanism applies to the spectrum of alcoholic liver disease. Furthermore, as downstream effectors of Hippo signaling, YAP/TAZ have been linked to HCC development via several key pathways, including inflammation, metabolic reprogramming, and chromosomal instability. Therefore, this splicing switch potentially could be harnessed to mitigate the risk of HCC development because patients with alcohol-related HCC in general have a worse prognosis compared with other etiologies.

**Aberrant Splicing of a Core Hippo Pathway Component in HCC**

The tumor-suppressor gene NF2 encodes the moesin-ezrin-radixin-like protein merlin, an upstream regulator of Hippo signaling that is mutated in patients with neurofibromatosis type 2, a rare disease involving benign tumors of the nervous tissue. Inactivating mutations in NF2 also occur in HCC. Recently, it was shown that merlin directly contributes to HCC metastasis via a dominant-negative splice variant isoform (Figure 1). Canonical merlin and the closely related ezrin, radixin, and moesin proteins function primarily at the plasma membrane to assemble multiprotein complexes of receptors, adapter proteins, and Rho guanosine triphosphatase modulators, which associate with the cortical cytoskeleton. Liver-specific deletion of NF2 in mice leads to a hyperproliferative response in the progenitor cell population and development of both cholangiocarcinoma and metastatic HCC via overactivation of the epidermal growth factor receptor. Although it was recognized more than 2 decades ago that aberrant splicing of NF2 via exon skipping promoted merlin inactivation, only recently was a specific merlin splice variant (of >9 different isoforms) implicated in HCC. Exclusion of exons 2, 3, and 4 led to the production of the Δ2-4merlin variant, which acted in a dominant-negative fashion to canonical merlin to promote tumor metastasis in HCC. Expression of the Δ2-4merlin variant, shown by the use of an isoform-specific antibody, was increased significantly in human HCC tumors and portal vein tumor thrombi relative to nontumor tissue. This was in stark contrast to canonical merlin, which was found to be expressed most highly in nontumor liver tissue. Canonical merlin (but not Δ2-4merlin) inhibited HCC cell migration and expression of EMT markers (such as TWIST and SNAIL), while Δ2-4merlin promoted the expression of stemness genes, such as EpCAM, SOX2, and KLF4 (Figure 1) and supported the formation of HCC cell spheroids in culture. The latter function was attributed to the inability of Δ2-4merlin to support plasma membrane anchoring of β-catenin and ezrin, radixin, and moesin proteins, resulting in decreased expression of β-catenin at the plasma membrane.

**The RNA Binding Protein SLU7 Controls Hepatic Metabolism**

The RNA binding protein SLU7 acts as a stabilizing component of the spliceosome to ensure fidelity in splice-site recognition. SLU7 is normally expressed in the nuclei of mature hepatocytes, but is down-regulated in HCC tumors. Knock-down of SLU7 perturbed nearly 600 splicing events and also led to major gene expression changes in the human PLC/PRF/5 hepatoma cell line. Among the genes that were affected, both at the level of splicing and expression, were many lipid and carbohydrate metabolism regulators, implicating SLU7 in metabolic homeostasis in hepatocytes. In support of that, diminished hepatic expression of Slu7 in mice (via adenovirus-mediated knockdown) was correlated strongly with down-regulation of the rate-limiting gluconeogenic genes Pepeck and G6pc. This resulted in decreased hepatic glucose production after pyruvate or glucagon injection, and blunted hepatic insulin responses during fasting/refeeding. Slu7 depletion also up-regulated the expression of Hk2 and Pkm2, which are linked to aerobic glycolysis and a tumor-like metabolic state. Interestingly, SLU7 regulates the splicing of Sirt1, which encodes the HCC-promoting NAD+-dependent sirtuin-1 deacetylase enzyme. Therefore, the metabolic reprogramming that accompanies HCC development may be linked, at least in part, to the loss of the RNA binding protein SLU7.

Precisely how the loss of Slu7 leads to altered metabolism is still an open question. However, one potential mechanism is via its ability to regulate the alternative splicing of a key splicing factor: SRSF3 (Figure 2). The serine/arginine-rich splicing factors (SRSFs) are a conserved group of nuclear RNA binding proteins that regulate multiple aspects of pre-mRNA splicing and are crucial for mammalian development. In humans, there are 12 nonredundant SRSF proteins (SRSF 1–12), and several have been implicated in HCC, as discussed in the next section.

**SRSF RNA Binding Proteins Differentially Regulate Hepatic Genomic and Metabolic Stability**

The importance of SRSF proteins in liver biology is reflected in findings that hepatocyte differentiation during the early postnatal period and lipid homeostasis are under the control of Srsf3. Liver-specific deletion of Srsf3 in mice led to significant perinatal death, and the mice that survived had significantly lower body weight, liver weight, and liver/body weight ratios. Highly abnormal, enlarged hepatocytes with irregular nuclei were present in 1-month-old mice lacking hepatic Srsf3, along with a high rate of cell proliferation and apoptosis. Not surprisingly, compromised hepatocyte maturation ultimately
led to hepatic insufficiency with impaired glucose production. At the molecular level, gene expression changes and missplicing were observed in the Srsf3-null livers when compared with wild-type livers. The most notable effects were observed for Hnf1a, where aberrant splicing led to the exclusion of exon 2, predicted to cause non-sense-mediated decay of the transcript. Consistent with that, a number of Hnf1a target genes were down-regulated, including Ghr, leading to growth hormone insensitivity. Interestingly, 100% of the Srsf3-null mice that survived to 24 months developed spontaneous HCC, with lung metastasis noted in approximately a quarter of the HCC tumor-bearing mice. Furthermore, development of a fibrotic phenotype was noted as early as 1 month of age, and attributable in part to aberrant splicing of the Fn1 gene. When the younger mice were challenged further with the profibrotic agent carbon tetrachloride, they developed precancerous lesions. The primed tumor phenotype in these mice was attributed to aberrant splicing of EMT genes and abnormal activity of the Wnt/β-catenin pathway.

These results have translational importance to human HCC because SRSF3 is absent or down-regulated significantly in more than half of HCC cases. In cases in which SRSF3 was present in HCC, it was mislocalized to the cytoplasm, suggesting diminished activity as a splicing regulator. Interestingly, truncated variants of SRSF3, produced by aberrant splicing in the absence of SLU7, were found to act in a dominant-negative fashion and to interfere with proper cell division. SRSF3 degradation was independent of the ubiquitin-proteasome pathway, but controlled by another ubiquitin-like modification: neddylation. These results have translational importance to human HCC because SRSF3 is absent or down-regulated significantly in more than half of HCC cases. Consistent with that, a number of Hnf1a target genes were down-regulated, including Ghr, leading to growth hormone insensitivity. Interestingly, 100% of the Srsf3-null mice that survived to 24 months developed spontaneous HCC, with lung metastasis noted in approximately a quarter of the HCC tumor-bearing mice. Furthermore, development of a fibrotic phenotype was noted as early as 1 month of age, and attributable in part to aberrant splicing of the Fn1 gene. When the younger mice were challenged further with the profibrotic agent carbon tetrachloride, they developed precancerous lesions. The primed tumor phenotype in these mice was attributed to aberrant splicing of EMT genes and abnormal activity of the Wnt/β-catenin pathway.

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although there is some cross-over between the neddylation and ubiquitination pathways.\textsuperscript{116} The NEDD8-mediated down-regulation of SRSF3 bears relevance to HCC because multiple components of the neddylation machinery are up-regulated and correlate with shorter survival in HCC patients.\textsuperscript{117}

SRSF2, unlike SRSF3, is up-regulated in HCC and correlates with poor prognosis in patients.\textsuperscript{111} Previous work has shown that Srsf2 is essential for liver homeostasis and survival because liver-specific deletion of Srsf2 in mice caused liver failure and death within the first 2–4 weeks.\textsuperscript{118} Srsf2-null hepatocytes showed markers of severe ER and oxidative stress, together with aberrant splicing of multiple autophagy and stress response genes.\textsuperscript{118} The splicing program regulated by SRSF2 in HCC tumors is not well described, although in Huh7 cells it was linked to the splicing of genes regulating the cell cycle, DNA repair, and chromatin modifications.\textsuperscript{117} Therefore, the evidence to date suggests that SRSF2 serves both a protective homeostatic function as well as a tumor-promoting function in the mammalian liver. This dual function may be attributed, in part, to the ability of Srsf2 to serve as a transcription factor for cholesterol and bile acid metabolism genes.\textsuperscript{110}

Mice with a liver-specific deletion of Srsf1 have a normal liver function and life span, suggesting that SRSF1 is dispensable for liver homeostasis.\textsuperscript{118} However, SRSF1 is a known transcriptional target of Myc\textsuperscript{119} and functions as a proto-oncogene.\textsuperscript{120} It was shown several years ago that SRSF1 promotes HCC cell growth via the splicing of KLF6, acting upstream of the cell-cycle regulator p21.\textsuperscript{110} More recently, it was shown that expression of SRSF1 is also under the control of the long noncoding RNA metastasis-associated lung adenocarcinoma transcript 1 (MALAT1), which is overexpressed in HCC\textsuperscript{121} (Figure 2). When MALAT1 levels were high, the expression and nuclear activity of SRSF1 were enhanced, and there was increased production of several splice variant isoforms of SRSF1 target genes that control apoptosis, cell proliferation, and protein synthesis.\textsuperscript{121} Specifically, MALAT1 overexpression was associated with the inclusion of exon 12A in the gene encoding the Myc suppressor box-dependent-interacting protein-1 (BIN1).\textsuperscript{121} The HCC tumor-associated BIN1 variant isoform containing exon 12A encodes a longer protein that lacks this tumor-suppressive activity against Myc.\textsuperscript{122} SRSF1 also regulates the Hippo signaling pathway via the inclusion of exon 5 in the gene encoding the transcription factor TEAD1, the primary target of YAP/TAZ transcriptional co-activators.\textsuperscript{123} This also was under the control of MALAT1 and led to enhanced TEAD1-mediated cell proliferation.\textsuperscript{121} Another consequence of MALAT1 overexpression in HCC is the increased production of the isoform 2 of ribosomal protein S6 kinase \(\beta1\) (S6K1), leading to mammalian target of rapamycin complex 1 activation. The latter is of particular relevance to HCC because mammalian target of rapamycin is overactivated and represents a central target for limiting tumor growth and recurrence.\textsuperscript{124} Collectively, these studies highlight the importance of SRSF proteins in balancing hepatic genomic and metabolic stability, and open several potential avenues for selective targeting of tumor cells (Figure 2).

**The RNA Binding Protein NONO Controls Glucose Metabolism Genes in HCC**

Interestingly, there appears to be some functional redundancy between the MALAT1-SRSF1 axis and another RNA binding protein, the non-POU domain-containing octamer-binding protein (NONO). NONO is an RNA binding protein that regulates the splicing of hepatic metabolic genes, including the glucose transporter Glut2 and Gck.\textsuperscript{125} NONO function is important for hepatic nutrient metabolism in coordination with the circadian clock.\textsuperscript{125} In the context of HCC, NONO interacts with an adenosine triphosphate-dependent RNA helicase (DHX9) and a splicing factor (SFPQ) to promote exon 12A retention in BIN1\textsuperscript{126} (Figure 2). This, again, leads to the synthesis of the BIN1 long protein product that lacks the ability to suppress Myc.\textsuperscript{126} In this case, the long BIN1 isoform also stabilized the serine/threonine kinase PLK1 by preventing its degradation by the ubiquitin/proteasome system.\textsuperscript{126} PLK1 is known to be tumor-promoting in HCC.\textsuperscript{127} High expression of NONO was associated with poor survival and increased recurrence of HCC after surgery, while deletion of NONO reduced HCC cell growth in vitro and in vivo.\textsuperscript{126}

Another manner in which NONO could affect HCC progression is through interacting with another RNA helicase, MTR4.\textsuperscript{126} MTR4 is a component of the nuclear exosome targeting complex, which regulates RNA turnover.\textsuperscript{129} MTR4 is a direct target of Myc that promotes HCC cell proliferation in vitro and tumor growth in mice. Analysis of splicing in MTR4-deficient cells has shown a number of differentially spliced genes, including the metabolic regulators GLUT1 and PKM2. Similar to NONO, MTR4 is up-regulated in HCC tissues and is associated with poor survival in patients.\textsuperscript{130} Although they were shown to interact,\textsuperscript{126} it remains to be tested whether NONO and MTR4 work in tandem to reprogram glucose metabolism in HCC.

**Altered Fructose Metabolism via hnRNP H1 and H2 Proteins**

Recent work has shown a novel isoform switching mechanism favoring de novo nucleotide biosynthesis at the expense of fructose metabolism in HCC.\textsuperscript{131} Ketohexokinase (KHK) phosphorylates fructose to form fructose-1-phosphate, which undergoes further metabolism to generate substrates for glycolysis.\textsuperscript{132} The KHK gene contains the mutually exclusive exons 3A and 3C.\textsuperscript{133} Retention of exon 3A results in the expression of KHK-A, which is associated primarily with fetal development\textsuperscript{133} and has a low affinity for fructose.\textsuperscript{134} In contrast, retention of exon 3C yields the high-affinity KHK-C that is expressed primarily in the liver and is the main isoform involved in normal hepatic fructose metabolism.\textsuperscript{134} Splicing of KHK pre-mRNA is under the control of the RNA binding protein A1CF, which generates the KHK-C isoform and promotes metabolic homeostasis in the normal
liver. 135 Liver-specific deletion of A1cf results in complete loss of KHK-C protein, while re-expression of A1cf leads to KHK-C protein restoration 135 in mice. Furthermore, A1CF activity is antagonized by the RNA binding proteins hnRNPH1 and hnRNPH2. 136 These proteins belong to a large family of RNA regulators that control alternative splicing, transcription, translation, and mRNA stability, 136 and are transcriptionally up-regulated by Myc. 137 Increased expression of hnRNPH1 and H2 promotes KHK-A over KHK-C production in HCC tumor cells, leading to a reduction in fructose metabolism. 131 KHK-A leads to the phosphorylation and activation of phosphoribosyl pyrophosphate synthetase 1 (PRPS1) and enhanced de novo synthesis of nucleic acids to fuel tumor cell growth and proliferation. 131 PRPS1 is essential for nucleotide biosynthesis and sensitive to feedback inhibition by phosphate and nucleotide concentrations in normal hepatocytes. This feedback inhibition was lost in HCC cells and PRPS1 remained in a constant “on” state. 131 High expression of Myc, hnRNPH1/2, KHK-A, and phospho-PRPS1 all were correlated with poor HCC patient survival, 131 suggesting that this pathway could be targeted to restore metabolic balance.

Conclusions and Future Directions

Understanding of the cellular and molecular mechanisms involved in HCC and other forms of primary liver cancer has expanded rapidly in the past several years. 13 These advancements have been fueled by large-scale genomic, transcriptomic, proteomic, and metabolomics studies, which have identified new players and regulatory networks. 138 Transcriptomic profiling of primary human HCC tumors coupled with mechanistic studies in cells and animal models have unveiled novel disease targets that arise in response to dysregulated processing of RNA via alternative splicing. Alternative splicing is recognized as a critical mechanism involved in the tumorigenesis process across cancer types. 139 Detailed insight into the regulation and function of novel transcript and protein variants in HCC is useful on many fronts, such as discovery of novel biomarkers for early detection and molecular targets for intervention. However, timely and effective translation of these novel molecular findings to the clinic hinges on the ability to classify patients based on the molecular features of their tumors and tailor their therapy accordingly. Although some alternative splicing pathways may be linked strongly to the etiology of HCC, 140 others may be related to the presence of cirrhosis and otherwise independent of the underlying major risk factor. 141 As such, detecting and modulating alternative splicing events at the premalignant stage also could be used as an approach for HCC prevention. The latter would be an ideal scenario to lessen the global burden of this highly common and deadly cancer type. 142

References

1. Cronin KA, Lake AJ, Scott S, Sherman RL, Noone AM, Howlader N, Henley SJ, Anderson RN, Firth AU, Ma J, Kohler BA, Jemal A. Annual report to the nation on the status of cancer, part I: national cancer statistics. Cancer 2018;124:2785–2800.

2. Llovet JM, Zucman-Rossi J, Pikarsky E, Sangro B, Schwartz M, Sherman M, Gores G. Hepatocellular carcinoma. Nat Rev Dis Primers 2016;2:16018.

3. El-Serag HB. Hepatocellular carcinoma. N Engl J Med 2011;365:1118–1127.

4. Sia D, Villanueva A, Friedman SL, Llovet JM. Liver cancer cell of origin, molecular class, and effects on patient prognosis. Gastroenterology 2017;152:745–761.

5. Goldberg D, Ditah IC, Saeian K, Lalehzari M, Aronsohn A, Gorospe EC, Charlton M. Changes in the prevalence of hepatitis C virus infection, nonalcoholic steatohepatitis, and alcoholic liver disease among patients with cirrhosis or liver failure on the waitlist for liver transplantation. Gastroenterology 2017;152:1090–1099 e1.

6. Hepaticit C (HCV) agents. In: LiveTox: clinical and research information on drug-induced liver injury. Bethesda, MD: 2012.

7. Younossi Z, Younossi Z, Stepanova M, Ong JP, Jacobson IM, Bugianesi E, Duseja A, Eguchi Y, Wong VW, Negro F, Yilmaz Y, Romero-Gomez M, George J, Ahmed A, Wong R, Younossi Z, Ziyae M, Afendy A. Global Nonalcoholic Steatohepatitis Council Nonalcoholic steatohepatitis is the fastest growing cause of hepatocellular carcinoma in liver transplant candidates. Clin Gastroenterol Hepatol 2019; 17:748–755 e3.

8. Younossi Z, Anstee QM, Marietti M, Hardy T, Henry L, Eslam M, George J, Bugianesi E. Global burden of NAFLD and NASH: trends, predictions, risk factors and prevention. Nat Rev Gastroenterol Hepatol 2018; 15:11–20.

9. Kanwai F, Kramer JR, Mapakshi S, Natarajan Y, Chayanupatkul M, Richardson PA, Li L, Desiderio R, Thrift AP, Asch SM, Chu J, El-Serag HB. Risk of hepatocellular cancer in patients with non-alcoholic fatty liver disease. Gastroenterology 2018;155:1828–1837 e2.

10. Rahib L, Smith BD, Aizenberg R, Rosenzweig AB, Flesham JM, Matrisian LM. Projecting cancer incidence and deaths to 2030: the unexpected burden of thyroid, liver, and pancreas cancers in the United States. Cancer Res 2014;74:2913–2921.

11. Rich NE, John BV, Parikh ND, Rowe I, Mehta N, Khatri G, Thomas SM, Anis M, Mendiattlala M, Hernandez C, Odewole M, Sundaram LT, Konjeti VR, Shetty S, Shah T, Zhu H, Yopp AC, Hoshida Y, Yao FY, Marrero JA, Singal AG. Hepatocellular carcinoma demonstrates heterogeneous growth patterns in a multi-center cohort of patients with cirrhosis. Hepatology 2020.

12. Kahles A, Lehmann KV, Toussaint NC, Huser M, Stark SG, Sachsenberg T, Stegle O, Kohlbacher O, Sander C, Cancer Genome Atlas Research N, Ratsch G. Comprehensive analysis of alternative splicing across tumors from 8,705 patients. Cancer Cell 2018; 34:211–224 e6.

13. Climente-Gonzalez H, Faro-Pardo E, Godzik A, Eyras E. The functional impact of alternative splicing in cancer. Cell Rep 2017;20:2215–2226.

14. Danan-Gotthold M, Golan-Gerstl R, Eisenberg E, Meir K, Karri R, Levanon EY. Identification of recurrent regulated
alternative splicing events across human solid tumors. Nucleic Acids Res 2015;43:5130–5144.

15. Shiraishi Y, Fujimoto A, Furuta M, Tanaka H, Chiba K, Boroveich KA, Abe T, Kawakami Y, Ueno M, Gotoh K, Arizumi S, Shibuya T, Nakano K, Sasaki A, Maejima K, Kitada R, Hayami S, Shige kawa Y, Marubashi S, Yamada T, Kubo M, Ishikawa O, Aikata H, Arihiko K, Ohdan H, Yamamoto M, Yamaue H, Chayama K, Tsunoda T, Miyano S, Nakagawa H. Integrated analysis of whole genome and transcriptome sequencing reveals diverse transcriptomic aberrations driven by somatic genomic changes in liver cancers. PLoS One 2014; 9:e114263.

16. Lee Y, Rio DC. Mechanisms and regulation of alternative pre-mRNA splicing. Annu Rev Biochem 2015;84:291–323.

17. Floor SN, Doudna JA. Tunable protein synthesis by transcript isoforms in human cells. Elife 2016;5.

18. Liu Y, Gonzalez-Porta M, Santos S, Brazma A, Marioni JC, Aebersold R, Venkitaraman AR, Wickramasinghe VO. Impact of alternative splicing on the human proteome. Cell Rep 2017;20:1229–1241.

19. Baralle FE, Giudice J. Alternative splicing as a regulator of development and tissue identity. Nat Rev Mol Cell Biol 2017;18:437–451.

20. Kalsotra A, Cooper TA. Functional consequences of developmentally regulated alternative splicing. Nat Rev Genet 2011;12:715–729.

21. Arif W, Datar G, Kalsotra A. Intersections of post-transcriptional gene regulatory mechanisms with intermediary metabolism. Biochim Biophys Acta Gene Regul Mech 2017;1860:349–362.

22. Itoh N, Okamoto H. Translational control of proinsulin synthesis by glucose. Nature 1980;283:100–102.

23. Tillmar L, Carlsson C, Welsh N. Control of insulin mRNA stability in rat pancreatic islets. Regulatory role of a 3'-untranslated region pyrimidine-rich sequence. J Biol Chem 2002;277:1099–1106.

24. Seino S, Bell GI. Alternative splicing of human insulin receptor messenger RNA. Biochem Biophys Res Comm 1989;159:312–316.

25. Batista TM, Garcia-Martin R, Cai W, Konishi M, O'Neill BT, Sakaguchi M, Kim JH, Jung DY, Kim JK, Kahn CR. Multi-dimensional transcriptional remodeling by physiological insulin in vivo. Cell Rep 2019; 26:3429–3443 e3.

26. Merkin J, Russell C, Chen P, Burge CB. Evolutionary dynamics of gene and isoform regulation in mammalian tissues. Science 2012;338:1593–1599.

27. Li S, Hu Z, Zhao Y, Huang S, He X. Transcriptome-wide analysis reveals the landscape of aberrant alternative splicing events in liver cancer. Hepatology 2019; 69:359–375.

28. Chen H, Gao F, He M, Ding XF, Wong AM, Sze SC, Yu AC, Sun T, Chan AW, Wang X, Wong N. Long-read RNA sequencing identifies novel splice variants in hepatocellular carcinoma and tumor-specific isoforms. Hepatology 2019, Epub ahead of print.

29. Zhu GQ, Zhou YJ, Qiu LX, Wang B, Yang Y, Liao WT, Luo YH, Shi YH, Zhou J, Fan J, Dai Z. Prognostic alternative mRNA splicing signature in hepatocellular carcinoma: a study based on large-scale sequencing data. Carcinogenesis 2019, Epub ahead of print.

30. Jin H, Wang C, Jin G, Ruan H, Gu D, Wei L, Wang H, Wang N, Arunachalam E, Zhang Y, Deng X, Yang C, Xiong Y, Feng H, Yao M, Fang J, Gu J, Cong W, Qin W. Regulator of calcineurin 1 gene isoform 4, down-regulated in hepatocellular carcinoma, prevents proliferation, migration, and invasive activity of cancer cells and metastasis of orthotopic tumors by inhibiting nuclear translocation of NFAT1. Gastroenterology 2017; 153:799–811 e33.

31. Shiloh A, Ben Hur V, Denichenko P, Stein I, Pikarsky E, Rauch J, Kolch W, Zender L, Kamri R. Splicing factor hnRNP A2 activates the Ras-MAPK-ERK pathway by controlling A-Raf splicing in hepatocellular carcinoma development. RNA 2014;20:505–515.

32. Wang H, Zhang CZ, Lu SX, Zhang MF, Liu LL, Luo RZ, Yang X, Wang CH, Chen SL, He YF, Xie D, Xu RH, Yun JP. A coiled-coil domain containing 50 splice variant is modulated by serine/arginine-rich splicing factor 3 and promotes hepatocellular carcinoma in mice by the Ras signaling pathway. Hepatology 2019;69:179–195.

33. Lu Y, Xu W, Ji J, Feng D, Sourbier C, Yang Y, Qu J, Zeng Z, Wang C, Chang X, Chen Y, Mishra A, Xu M, Lee MJ, Lee S, Trepel J, Linehan WM, Wang X, Yang Y, Neckers L. Alternative splicing of the cell fate determinant Numb in hepatocellular carcinoma. Hepatology 2015;62:1122–1131.

34. Matsuura K, Sawai H, Ikeo K, Ogawa S, Iio E, Isogawa M, Shimada N, Komori A, Toyoda H, Kumada T, Namisaki T, Yoshiji H, Sakamoto N, Nakagawa M, Asahina Y, Kurosaki M, Izumi N, Enomoto N, Kusakabe A, Kajiwara E, Itoh Y, Ide T, Tamori A, Matsubara M, Kawada N, Shirabe K, Tomita E, Honda M, Kaneko S, Nishina S, Suetsugu A, Hiasa Y, Watanabe H, Genda T, Sakaida I, Nishiguchi S, Takaguchi K, Tanaka E, Sugihara J, Shimada M, Kondo Y, Kawai Y, Kojima K, Nagasaki M, Tokunaga K, Tanaka Y. Genome-wide association study identifies TLL1 variant associated with development of hepatocellular carcinoma after eradication of hepatitis C virus infection. Gastroenterology 2017;152:1383–1394.

35. Snider NT, Altshuler PJ, Wan S, Welling TH, Cavalcioni J, Omary MB. Alternative splicing of human NT5E in cirrhosis and hepatocellular carcinoma produces a negative regulator of ecto-5'-nucleotidase (CD73). Mol Biol Cell 2014;25:4024–4033.

36. Lin KT, Ma WK, Scharner J, Liu YR, Krainer AR. A human-specific switch of alternatively spliced AFMID isoforms contributes to TP53 mutations and tumor recurrence in hepatocellular carcinoma. Genome Res 2018, Epub ahead of print.

37. Zhang L, Liu X, Zhang X, Chen R. Identification of important long non-coding RNAs and highly recurrent aberrant alternative splicing events in hepatocellular carcinoma through integrative analysis of multiple RNA-Seq datasets. Mol Genet Genomics 2016; 291:1035–1051.
Conti F, Praz F, Housset C, Rosmorduc O, Desbois-Mouthon C. Mitogenic insulin receptor-A is overexpressed in human hepatocellular carcinoma due to EGFR-mediated dysregulation of RNA splicing factors. Cancer Res 2013;73:3974–3986.

39. Tremblay MP, Armero VE, Allaire A, Boudreault S, Martenon-Brodeur C, Durand M, Lapointe E, Thibault P, Tremblay-Letourneau M, Perreault JP, Scott MS, Bisaillon M. Global profiling of alternative RNA splicing events provides insights into molecular differences between various types of hepatocellular carcinoma. BMC Genomics 2016;17:683.

40. Lin KT, Shann YJ, Chau GY, Hsu CN, Huang CY. Identification of latent biomarkers in hepatocellular carcinoma by ultra-deep whole-transcriptome sequencing. Oncogene 2014;33:4786–4794.

41. Wang H, Lekbaby B, Fares N, Augustin J, Attout T, Schnuriger A, Cassard AM, Panasyuk G, Perlemuter G, Bieche I, Vacher S, Selves J, Peron JM, Bancel B, Merle P, Kremsdorf D, Hall J, Chemin I, Soussan P. Alteration of splicing factors’ expression during liver disease progression: impact on hepatocellular carcinoma outcome. Hepatol Int 2019;13:454–467.

42. Will CL, Luhmann R. Spliceosome structure and function. Cold Spring Harb Perspect Biol 2011;3.

43. Rossi A, Moro A, Tebaldi T, Cornella N, Gasperini L, Lunelli L, Quattrone A, Viero G, Macchi P. Identification and dynamic changes of RNAs isolated from RALY-containing ribonucleoprotein complexes. Nucleic Acids Res 2017;45:6775–6792.

44. Zhang M, Zhang Y, Xu E, Mohibi S, de Anda DM, Jiang Y, Zhang J, Chen X. Rbm24, a target of p53, is necessary for proper expression of p53 and heart development. Cell Death Differ 2018;25:1118–1130.

45. Hussain SP, Rossi A, Tebaldi T, Cornella N, Merle P, Kremsdorf D, Hall J, Soussan P, Conti F, Praz F, Housset C, Rosmorduc O, Desbois-Mouthon C. Indian Hedgehog links obesity to development of hepatocellular carcinoma. Oncogene 2014;33:4786–4794.

46. Schulze K, Imbeaud S, Letouze E, Alexandrov LB, Hussain SP, Schwank J, Staib F, Wang XW, Harris CC. p53 tumor suppressor activity in murine liver carcinomas. Nature 2007;445:656–660.

47. Schulz C, Zhen X, Zeng T, Zhao M, Chen L, Wu J, Zeng R, Chen L. Dysfunction of PLA2G6 and CYP2C44-associated network signals imminent carcinogenesis from chronic inflammation to hepatocellular carcinoma. J Mol Cell Biol 2017;9:489–503.

48. Ye H, Zhang C, Wang BJ, Pan L. Replication protein A 3 is associated with hepatocellular carcinoma tumorigenesis and poor patient survival. Dig Dis 2018;36:26–32.

49. Li T, Yan B, Ma Y, Weng J, Yang S, Zhao N, Wang X, Sun X. Ubiquitin-specific protease 4 promotes hepatocellular carcinoma progression via cyclophilin A stabilization and deubiquitination. Cell Death Dis 2018;9:148.

50. Wang H, Lekbaby B, Fares N, Augustin J, Attout T, Schnuriger A, Cassard AM, Panasyuk G, Perlemuter G, Bieche I, Vacher S, Selves J, Peron JM, Bancel B, Merle P, Kremsdorf D, Hall J, Chemin I, Soussan P. Alteration of splicing factors’ expression during liver disease progression: impact on hepatocellular carcinoma outcome. Hepatol Int 2019;13:454–467.

51. Steinway SN, Zanudo JG, Ding W, Rountree CB, Feith DJ, Loughran TP, Jr, Albert R. Network modeling of TGFbeta signaling in hepatocellular carcinoma epithelial-to-mesenchymal transition reveals joint sonic hedgehog and Wnt pathway activation. Cancer Res 2014;74:5963–5977.

52. Bhate A, Parker DJ, Bebee TW, Ahn J, Arif W, Rashan EH, Chorghade S, Chau A, Lee JH, Anakk S, Carstens RP, Xiao X, Kalsotra A. ESPR2P controls an adult splicing programme in hepatocytes to support postnatal liver maturation. Nat Commun 2015;6:8768.

53. Huo X, Li H, Li Z, Yan C, Mathavan S, Liu J, Gong Z. p53-dependent suppression of oxidative phosphorylation in hepatocellular carcinoma. Gastroenterology 2006;130:1117–1128.

54. Chong YC, Lim TE, Fu Y, Shin EM, Tergaonkar V, Han W. Indian Hedgehog links obesity to development of hepatocellular carcinoma. Oncogene 2019;38:2206–2222.

55. Zhang M, Zhang Y, Xu E, Mohibi S, de Anda DM, Jiang Y, Zhang J, Chen X. Rbm24, a target of p53, is necessary for proper expression of p53 and heart development. Cell Death Differ 2018;25:1118–1130.

56. Li T, Yan B, Ma Y, Weng J, Yang S, Zhao N, Wang X, Sun X. Ubiquitin-specific protease 4 promotes hepatocellular carcinoma progression via cyclophilin A stabilization and deubiquitination. Cell Death Dis 2018;9:148.

57. Huo X, Li H, Li Z, Yan C, Mathavan S, Liu J, Gong Z. p53-dependent suppression of oxidative phosphorylation in hepatocellular carcinoma. Gastroenterology 2006;130:1117–1128.
Sasazuki T, Martignetti JA, Llovet JM, Friedman SL. Ras promotes growth by alternative splicing-mediated inactivation of the KLF6 tumor suppressor in hepatocellular carcinoma. Gastroenterology 2008;134:1521–1531.

63. Grindheim JM, Nicetto D, Donahue G, Zaret KS. Polycomb repressive complex 2 proteins EZH1 and EZH2 regulate timing of postnatal hepatocyte maturation and fibrosis by repressing genes with euchromatic promoters in mice. Gastroenterology 2019;156:1834–1848.

64. Hirschfield H, Bian CB, Higashi T, Nakagawa S, Zeleke TZ, Nair VD, Fuchs BC, Hoshida Y. In vitro modeling of hepatocellular carcinoma molecular subtypes for anti-cancer drug assessment. Exp Mol Med 2018;50:e419.

65. Liu M, Yan Q, Sun Y, Nam Y, Hu L, Loong JH, Ouyang Q, Zhang Y, Li HL, Kong FE, Li L, Li Y, MI, Cheng W, Jiang LX, Fang S, Yang XD, Mo JQ, Gong YF, Tang YQ, Li Y, Yuan YF, Ma NF, Lin G, Ma S, Wang JG, Guan YX. A hepatocyte differentiation model reveals two subtypes of liver cancer with different oncogenic properties and therapeutic targets. Proc Natl Acad Sci U S A 2020, Epub ahead of print.

66. Caruso S, Calatayud AL, Pilet J, La Bella T, Rekik S, Imbeaud S, Letouze E, Meunier L, Bayard Q, Calderaro J, Lin G, Yuan YF, Ma NF, Lin G, Ma S, Wang JG, Guan YX. A hepatocyte differentiation model reveals two subtypes of liver cancer with different oncogenic properties and therapeutic targets. Proc Natl Acad Sci U S A 2020, Epub ahead of print.

67. Brown ZJ, Heinrich B, Greten TF. Mouse models of hepatocellular carcinoma: an overview and highlights for immunotherapy research. Nat Rev Gastroenterol Hepatol 2016;15:536–548.

68. Caviglia JM, Schwabe RF. Mouse models of liver cancer. Methods Mol Biol 2015;1267:165–183.

69. Febbraio MA, Reibe S, Shalapour S, Ooi GJ, Watt MJ, Carin M. Preclinical models for studying NASH-driven HCC: how useful are they? Cell Metab 2019;29:18–26.

70. Ho TH, Charlet BN, Poulos MG, Singh G, Swanson MS, Cooper TA. Muscleblind proteins regulate alternative splicing. EMBO J 2004;23:3103–3112.

71. Konieczny P, Stepniak-Konieczna E, Sobczak K. MBNL proteins and their target RNAs, interaction and splicing regulation. Nucleic Acids Res 2014;42:10873–10887.

72. Yuan JH, Liu XN, Wang TT, Pan W, Tao QF, Zhou WP, Wang F, Sun SH. The MBNL3 splicing factor promotes hepatocellular carcinoma by increasing PXN expression through the alternative splicing of IncRNA-PXN-AS1. Nat Cell Biol 2017;19:820–832.

73. Ching YP, Leong VY, Lee MF, Xu HT, Jin DY, Ng IO. P21-activated protein kinase is overexpressed in hepatocellular carcinoma and enhances cancer metastasis involving c-Jun NH2-terminal kinase activation and paxillin phosphorylation. Cancer Res 2007;67:3601–3608.

74. Cordero-Espinoza L, Huch M. The balancing act of the liver: tissue regeneration versus fibrosis. J Clin Invest 2018;128:85–96.

75. Bangru S, Kalsotra A. Cellular and molecular basis of liver regeneration. Semin Cell Dev Biol 2020;100:74–87.

76. Chen Y, Wong PP, Sjaklocha L, Steer CJ, Sahin MB. Mature hepatocytes exhibit unexpected plasticity by direct dedifferentiation into liver progenitor cells in culture. Hepatology 2012;55:563–574.

77. Oh SH, Swiderska-Syn M, Jewell ML, Premont RT, Diehl AM. Liver regeneration requires Yap1-TGFbeta-dependent epithelial-mesenchymal transition in hepatocytes. J Hepatol 2018;69:359–367.

78. Hyun J, Oh SH, Premont RT, Guy CD, Berg CL, Diehl AM. Disregulated activation of fetal liver programme in acute liver failure. Gut 2019;68:1076–1087.

79. Ma S, Meng Z, Chen R, Guan KL. The Hippo pathway: biology and pathophysiology. Annu Rev Biochem 2019;88:577–604.

80. Dong J, Feldmann G, Huang J, Wu S, Zhang N, Comerford SA, Gayyed MF, Anders RA, Maitra A, Pan D. Elucidation of a universal size-control mechanism in Drosophila and mammals. Cell 2007;130:1120–1133.

81. Lee KP, Lee JH, Kim TS, Kim TH, Park HD, Byun JS, Kim MC, Jeong WI, Calvisi DF, Kim JM, Lim DS. The Hippo-Salvador pathway restrains hepatic oval cell proliferation, liver size, and liver tumorigenesis. Proc Natl Acad Sci U S A 2010;107:8248–8253.

82. Patel SH, Camargo FD, Yimlamai D. Hippo signaling in the liver regulates organ size, cell fate, and carcinogenesis. Gastroenterology 2017;152:533–545.

83. Kim W, Khan SK, Gvozdenovic-Jeremic J, Kim Y, Dahlman J, Kim H, Park O, Ishitani T, Jho EH, Gao B, Yang Y. Hippo signaling interactions with Wnt/beta-catenin and Notch signaling repress liver tumorigenesis. J Clin Invest 2017;127:137–137.

84. Zhang S, Zhou D. Role of the transcriptional coactivators YAP/TAZ in liver cancer. Curr Opin Cell Biol 2019;61:64–71.

85. Ishii H, et al. Epithelial splicing regulatory proteins 1 (ESRP1) and 2 (ESRP2) suppress cancer cell motility via different mechanisms. J Biol Chem 2014;289:27386–27399.

86. Bangru S, Saioth M, Sakamoto K, Kondo T, Kato R, Tanaka S, Motizuki M, Masuyama K, Miyazawa K. Alternative splicing rewrites Hippo signaling pathway in hepatocytes to promote liver regeneration. Nat Struct Mol Biol 2018;25:928–939.

87. Snider NT, Griggs NW, Singla A, Moons DS, Weerasinghe SV, Lok AS, Ruan C, Burant CF, Conjeevaram HS, Omary MB. CD73 (ecto-5’-nucleotidase) hepatocyte levels differ across mouse strains and contribute to Mallory-Denk body formation. Hepatology 2013;58:1790–1800.

88. Yuan WC, Pepe-Mooney B, Galli GG, Dill MT, Huang HT, Hao M, Wang Y, Li HL, Kong FE, Li L, Li Y, Mei W. NUAK2 is a critical YAP target in liver cancer. Nat Commun 2018;9:4835.
cell proliferation and chemoresistance in hepatocellular carcinoma. Oncogene 2014;33:1468–1474.

90. Kim W, Khan SK, Liu Y, Xu R, Park O, He Y, Cha B, Gao B, Yang Y. Hapotic Hippo signaling inhibits protumoral microenvironment to suppress hepatocellular carcinoma. Gut 2018;67:1692–1703.

91. Ji S, Liu Q, Zhang S, Chen Q, Wang C, Zhang W, Xiao C, Li Y, Nian C, Li J, Li J, Geng J, Hong L, Xie C, He Y, Chen X, Li X, Yin ZY, You H, Lin KH, Wu Q, Yu C, Johnson RL, Wang L, Chen L, Wang F, Zhou D. FGF15 activates Hippo signaling to suppress bile acid metabolism and liver tumorigenesis. Dev Cell 2019; 48:460–474 e9.

92. Jeong SH, Kim HB, Kim MC, Lee JM, Lee JH, Kim JH, Kim JW, Park WY, Kim SY, Kim JB, Kim H, Kim JM, Choi HS, Lim DS. Hippo-mediated suppression of IRS2/ AKT signaling prevents hepatic steatosis and liver cancer. J Clin Invest 2018;128:1010–1025.

93. Weiler SME, Pinna F, Wolf T, Lutz T, Geng J, Zhang W, Lu Z, Yin ZY, Lin KH, Wu Q, Li Q, Nakayama K, Nakayama KI, Deng X, Johnson RL, Zhu L, Gao D, Chen L, Zhou D. Hippo signaling suppresses cell ploidy and tumorigenesis through Skp2. Cancer Cell 2017;31:669–684 e7.

94. Zhang S, Chen Q, Liu Q, Li Y, Sun X, Hong L, Ji S, Liu C, Geng J, Zhang W, Lu Z, Yin ZY, Zeng Y, Lin KH, Wu Q, Li Q, Nakayama K, Nakayama KI, Deng X, Johnson RL, Zhu L, Gao D, Chen L, Zhou D. Hippo signaling suppresses cell ploidy and tumorigenesis through Skp2. Cancer Cell 2017;31:669–684 e7.

95. Ganne-Carrie N, Nahon P. Hepatocellular carcinoma in the setting of alcohol-related liver disease. J Hepatol 2019;70:284–293.

96. Petrilli AM, Fernandez-Valle C. Role of Merlin/NF2 inactivation in tumor biology. Oncogene 2016;35:537–548.

97. Pineau P, Marchio A, Nagamori S, Seki S, Tiollais P, Ganne-Carrie N, Nahon P. Hepatocellular carcinoma in the setting of alcohol-related liver disease. J Hepatol 2003;37:852–861.

98. Luo ZL, Cheng SQ, Shi J, Zhang HL, Zhang CZ, Chen HY, Qiu BJ, Tang L, Hu CL, Wang HY, Li Z. A splicing variant of Merlin promotes metastasis in hepatocellular carcinoma. Nat Commun 2015;6:8457.

99. McClatchey AI, Fehon RG. Merlin and the ERM proteins—regulators of receptor distribution and signaling at the cell cortex. Trends Cell Biol 2009; 19:198–206.

100. Benhamouche S, Curto M, Saotome I, Gladden AB, Liu CH, Giovannini M, McClatchey AI. Nf2/Merlin controls progenitor homeostasis and tumorigenesis in the liver. Genes Dev 2010;24:1718–1730.

101. Bianchi AB, Mitsunaga SI, Cheng JQ, Klein WM, Jhanwar SC, Seizinger B, Kley N, Klein-Szanto AJ, Testa JR. High frequency of inactivating mutations in the neurofibromatosis type 2 gene (NF2) in primary malignant mesotheliomas. Proc Natl Acad Sci U S A 1995; 92:10854–10858.

102. Fica SM, Oubridge C, Wilkinson ME, Newman AJ, Nagai K. A human postcatalytic spliceosome structure reveals essential roles of metazoan factors for exon ligation. Science 2019;363:710–714.

103. Elizalde M, Urtasun R, Azkona M, Latasa MU, Goni S, Garcia-Irigoyen O, Uriarte I, Segura V, Collantes M, Di Scala M, Lujambio A, Prieto J, Avila MA, Berasain C. Splicing regulator Slu7 is essential for maintaining liver homeostasis. J Clin Invest 2014;124:2909–2920.

104. Wang J, Kainrad N, Shen H, Zhou Z, Rote P, Zhang Y, Nagy LE, Wu J, You M. Hepatic knockdown of splicing regulator Slu7 ameliorates inflammation and attenuates liver injury in ethanol-fed mice. Am J Pathol 2018; 188:1807–1819.

105. Portmann S, Fahrner R, Lechleiter A, Keogh A, Overney S, Laemmle A, Mikami K, Montani M, Tschan MP, Candinas D, Stroka D. Antitumor effect of SIRT1 inhibition in human HCC tumor models in vitro and in vivo. Mol Cancer Ther 2013;12:499–508.

106. Cancer Genome Atlas Research Network. Comprehensive and integrative genomic characterization of hepatocellular carcinoma. Cell 2017;169:1327–1341 e23.

107. Jimenez M, Urtasun R, Elizalde M, Azkona M, Latasa MU, Uriarte I, Arechederra M, Alignani D, Barcena-Varela M, Alvarez-Sola G, Colyn L, Santamaria E, Sangro B, Rodriguez-Ortigosa C, Fernandez-Barrena MG, Avila MA, Berasain C. Splicing events in the control of genome integrity: role of SLU7 and truncated SRSF3 proteins. Nucleic Acids Res 2019;47:3450–3466.

108. Manley JL, Krainer AR. A rational nomenclature for serine/arginine-rich protein splicing factors (SR proteins). Genes Dev 2010;24:1073–1074.

109. Jumaa H, Wei G, Nielsen PJ. Blastocyst formation is blocked in mouse embryos lacking the splicing factor Sprrp20. Curr Biol 1999;9:899–902.

110. Munoz U, Puche JE, Hannivoort R, Lang UE, Cohen- Naftaly M, Friedman SL. Hepatocyte growth factor enhances alternative splicing of the Kruppel-like factor 6 (KLF6) tumor suppressor to promote growth through SRSF1. Mol Cancer Res 2012; 10:1216–1227.

111. Luo C, Cheng Y, Liu Y, Chen L, Liu L, Wei N, Xie Z, Wu W, Feng Y. SRSF2 regulates alternative splicing to drive hepatocellular carcinoma development. Cancer Res 2017;77:1168–1178.

112. Ma K, He Y, Zhang H, Fei Q, Niu D, Wang D, Ding X, Xu H, Chen X, Zhu J. DNA methylation-regulated miR-193a-3p dictates resistance of hepatocellular carcinoma to 5-fluorouracil via repression of SRSF2 expression. J Biol Chem 2012;287:5639–5649.

113. Sen S, Langewicz M, Jumaa H, Webster NJ. Deletion of serine/arginine-rich splicing factor 3 in hepatocytes predisposes to hepatocellular carcinoma in mice. Hepatology 2015;61:171–183.

114. Sen S, Jumaa H, Webster NJ. Splicing factor SRSF3 is crucial for hepatocyte differentiation and metabolic function. Nat Commun 2013;4:1336.
Osborn O, Webster NJ. Degradation of splicing factor SRSF3 contributes to progressive liver disease. J Clin Invest 2019;130:4477–4491.

116. Enchev RI, Schulman BA, Peter M. Protein neddylation: beyond cullin-RING ligases. Nat Rev Mol Cell Biol 2015;16:30–44.

117. Yu J, Huang WL, Xu QG, Zhang L, Sun SH, Zhou WP, Yang F. Overactivated neddylation pathway in human hepatocellular carcinoma. Cancer Med 2018, Epub ahead of print.

118. Cheng Y, Luo C, Wu W, Xie Z, Fu X, Feng Y. Liver-specific deletion of SRSF2 caused acute liver failure and early death in mice. Mol Cell 2016;36:1628–1638.

119. Das S, Anczukow O, Akerman M, Krainer AR. Oncogenic splicing factor SRSF1 is a critical transcriptional target of MYC. Cell Rep 2012;1:110–117.

120. Karmi R, de Stanchina E, Lowe SW, Sinha R, Mu D, Krainer AR. The gene encoding the splicing factor SF2/ASF is a proto-oncogene. Nat Struct Mol Biol 2007;14:185–193.

121. Malakar P, Shilo A, Mogilevsky A, Stein I, Pikarsky E, Nevo Y, Benyami H, Elgavish S, Zong X, Prasanth KV, Karmi R. Long noncoding RNA MALAT1 promotes hepatocellular carcinoma development by SRSF1 upregulation and mTOR activation. Cancer Res 2017;77:1155–1167.

122. Ge K, DuHadaway J, Du W, Herlyn M, Rodeck U, Prendergast GC. Mechanism for elimination of a tumor suppressor: aberrant splicing of a brain-specific exon causes loss of function of Bin1 in melanoma. Proc Natl Acad Sci U S A 1999;96:9689–9694.

123. Mesrouze Y, Bokhovchuk F, Meyerhofer M, Fontana P, Zimmermann C, Martin T, Delaunay C, Erdmann D, Schmelzle T, Chene P. Dissection of the interaction between the intrinsically disordered YAP protein and the transcription factor TEAD. Elife 2017;6.

124. Matter MS, Decaens T, Andersen JB, Thorgeirsson SS. Targeting the mTOR pathway in hepatocellular carcinoma: current state and future trends. J Hepatol 2014;60:855–865.

125. Benegiamo G, Mure LS, Erikson G, Le HD, Moriggi E, Brown SA, Panda S. The RNA-binding protein NONO coordinates hepatic adaptation to feeding. Cell Metab 2018;27:404–418 e7.

126. Hu Z, Li S, Li Z, Yejun Q, Li Y, Ding J, Chen Z, Yangjun W, Wang Z, Huang S, Gao Q, Zhao Y, He X. Splicing regulator p54(nrb) /NONO enhances carcinogenesis through oncogenic isoform switch of BIN1 in hepatocellular carcinoma. Hepatology 2019, Epub ahead of print.

127. Pellegrino R, Calvisi DF, Ladu S, Ehemann V, Staniscia T, Evert M, Dombrowski F, Schirmacher P, Longerich T. Oncogenic and tumor suppressive roles of polo-like kinases in human hepatocellular carcinoma. Hepatology 2010;51:857–868.

128. Ogami K, Richard P, Chen Y, Hoque M, Li W, Moresco JJ, Yates JR, 3rd, Tian B, Manley JL. An Mtr4/ZFC3H1 complex facilitates turnover of unstable nuclear RNAs to prevent their cytoplasmic transport and global translational repression. Genes Dev 2017;31:1257–1271.

129. Lubas M, Christensen MS, Kristiansen MS, Domanski M, Falkenby LG, Lykke-Andersen S, Andersen JS, Dziembowski A, Jensen TH. Interaction profiling identifies the human nuclear exosome targeting complex. Mol Cell 2011;43:624–637.

130. Yu L, Kim J, Jiang L, Feng B, Ying Y, Ji KY, Tang Q, Chen W, Mai T, Dou W, Zhou J, Xiang LY, He YF, Yang D, Li Q, Fu X, Xu Y. MTR4 drives liver tumorigenesis by promoting cancer metabolic switch through alternative splicing. Nat Commun 2020;11:708.

131. Li X, Qian X, Peng LX, Jiang Y, Hawke DH, Zheng Y, Xia Y, Lee JH, Cote G, Wang H, Wang L, Qian CN, Lu Z. A splicing switch from ketohexokinase-C to ketohexokinase-A drives hepatocellular carcinoma formation. Nat Cell Biol 2016;18:561–571.

132. Heinz F, Lamprecht W, Kirsch J. Enzymes of fructose metabolism in human liver. J Clin Invest 1968;47:1826–1832.

133. Hayward BE, Bonthron DT. Structure and alternative splicing of the ketohexokinase gene. Eur J Biochem 1998;257:85–91.

134. Ishimoto T, Lanaspa MA, Le MT, Garcia GE, Diggle CP, Maclean PS, Jackman MR, Asipu A, Roncal-Jimenez CA, Kosugi T, Rivard CJ, Maruyama S, Rodriguez-Irurbe B, Sanchez Lozada LG, Bonthron DT, Sautin YY, Johnson RJ. Opposing effects of fructokinase C and A isoforms on fructose-induced metabolic syndrome in mice. Proc Natl Acad Sci U S A 2012;109:4320–4325.

135. Nikolaou KC, Vatandaslar H, Meyer C, Schmid MW, Tuschi T, Stoffel M. The RNA-binding protein A1CF regulates hepatic fructose and glycerol metabolism via alternative RNA splicing. Cell Rep 2019;29:83–90.

136. Geuens T, Bouhy D, Timmerman V. The hnRNP family: insights into their role in health and disease. Hum Genet 2016;135:851–867.

137. Rauch J, Moran-Jones K, Albrecht V, Schwarzl T, Hunter K, Gires O, Kolch W. c-Myc regulates RNA splicing of the A-Raf kinase and its activation of the ERK pathway. Cancer Res 2011;71:4664–4674.

138. Chen B, Garmire L, Calvisi DF, Chua MS, Kelley RK, Chen X. Harnessing big ’omics’ data and AI for drug discovery in hepatocellular carcinoma. Nat Rev Gastroenterol Hepatol 2020;17:238–251.

139. Obeng EA, Stewart C, Abdel-Wahab O. Altered RNA processing in cancer pathogenesis and therapy. Cancer Discov 2019;9:1493–1510.

140. Jin Y, Byun S, Han S, Chamberlin J, Kim D, Kim MJ, Lee Y. Differential alternative splicing regulation among hepatocellular carcinoma with different risk factors. BMC Med Genomics 2019;12:175.

141. Nakagawa S, Wei L, Song WM, Higashi T, Ghoshal S, Kim RS, Bian CB, Yamada S, Sun X, Venkatesh A,
Goossens N, Bain G, Lauwers GY, Koh AP, El-Abtah M, Ahmad NB, Hoshida H, Erstad DJ, Gunasekaran G, Lee Y, Yu ML, Chuang WL, Dai CY, Kobayashi M, Kumada H, Beppu T, Baba H, Mahajan M, Nair VD, Lanuti M, Villanueva A, Sangiovanni A, Iavarone M, Colombo M, Llovet JM, Subramanian A, Tager AM, Friedman SL, Baumert TF, Schwarz ME, Chung RT, Tanabe KK, Zhang B, Fuchs BC, Hoshida Y. Molecular liver cancer prevention in cirrhosis by organ transcriptome analysis and lysophosphatidic acid pathway inhibition. Cancer Cell 2016;30:879–890.

Yang JD, Hainaut P, Gores GJ, Amadou A, Plymoth A, Roberts LR. A global view of hepatocellular carcinoma: trends, risk, prevention and management. Nat Rev Gastroenterol Hepatol 2019; 16:589–604.

Received March 11, 2020. Accepted April 27, 2020.

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Seung Eun Lee and Natasha T. Snider performed a literature review and wrote the first draft of the manuscript and generated the figures; Karel P. Alcedo and Hong Jin Kim provided comments and edits on the first draft; and Natasha T. Snider revised and finalized the manuscript and figures.

Conflicts of interest
The authors disclose no conflicts.

Funding
This work was supported by National Institutes of Health grant DK110355 (N.T.S.) and by an institutional training grant from the University of North Carolina Cancer Cell Biology Training Program (K.P.A.).