



Review Article

Unraveling Key Signaling Pathways Altered in Hepatocellular Carcinoma



Varsha D. Shiragannavar, Shreyas H. Karunakara, Lakshana D. Puttananumantharayappa, Nirmala G. Sannappa Gowda and Prasanna K. Santhekadur*

Department of Biochemistry, Center of Excellence in Molecular Biology & Regenerative Medicine, JSS Medical College, JSS Academy of Higher Education and Research, Mysore, Karnataka, India

Received: October 31, 2022 | Revised: January 17, 2023 | Accepted: February 22, 2023 | Published online: March 22, 2023

Abstract

Hepatocellular carcinoma (HCC) is becoming one of the major health problems, and the leading cause of cancer-related mortality globally. Its multifactorial risk factors remain as paramount challenges to the treatment of this deadly disease. The conventionally known risk factors that trigger HCC include hepatitis C virus (HCV), hepatitis B virus (HBV), excess alcohol consumption, and environmental toxins, such as aflatoxin, aristolochic acid, *etc.* All these risk factors activate the oncogenic signaling in the liver, and transform the normal liver into an HCC liver. Recently, globalization and the Western sedentary lifestyle have newly emerged as risk factors for HCC, which include obesity, metabolic syndrome, and associated clinical and pathological modalities. In addition, a number of cellular signaling pathways are derailed in HCC, and these pathways, which are altered in HCC, are known to be directly controlled by oncogenes, such as KRAS, BRAF, c-MYC, astrocyte elevated gene-1 (AEG-1), staphylococcal nuclease domain containing 1 (SND1), late SV40 factor (LSF), apoptosis-antagonizing transcription factor (AATF), WNT/ β -catenin, TGF- β , *etc.* All these oncogenes activate the oncogenic signaling in HCC, and suppress the important cellular tumor suppressor protein activity, playing a prominent role in hepatocarcinogenesis, and its development and progression. The present review established a novel interconnected network between all these oncogene-associated proteins, in order to determine its role in deregulating normal cellular signaling pathways, and transforming these into oncogenic signaling. A number of these oncogenes regulate the miRNA-RISC-associated oncogenic signaling, and trigger oncogenic miRNA singling by downregulating various tumor suppressor genes.

Therefore, therapeutically targeting these oncogenes and associated proteins would aid in the development of new drugs to treat HCC.

Keywords: Hepatocellular carcinoma; AEG-1; SND1; Inflammation; Proliferation; Metastasis.

Abbreviations: AATF, apoptosis-antagonizing transcription factor; AEG-1, astrocyte elevated gene 1; AFB1, Aflatoxin B1; Ago2, argonaute 2; EMT, epithelial-to-mesenchymal transition; ERK, extracellular signal-regulated kinase cascade; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; Hh, Hedgehog; HIF, hypoxia-inducible factors; IGF, Insulin-like growth factor; LSF, late SV40 factor; MAPK, mitogen-activated protein kinase; MCP-1, monocyte chemoattractant protein-1; MEK, mitogen-activated protein kinase cascade; MMP-7, matrix metalloproteinase 7; mTOR, mammalian target of rapamycin; Mst1/2, mammalian sterile 20-like 1/2; NAFLD, non-alcoholic fatty liver disease; PCBs, polychlorinated biphenyls; PI3K, phosphatidylinositol 3-kinase; PTCH, patched; RISC, RNA-induced silencing complex; PTEN, phosphatase and tensin homolog; PLZF, promyelocytic zinc finger protein; TGF- β , transforming growth factor-beta; TNF- α , tumour necrosis factor α ; RAS, rat sarcoma; RAF, rapidly accelerated fibrosarcoma; RTKs, receptor tyrosine kinase; Smo, smoothened; SMAD, suppressor of mothers against decapentaplegic; SND1, staphylococcal nuclease domain containing 1; SREBP1-c, Sterol regulatory element-binding transcription factor 1; VEGF, vascular endothelial growth factor; VEGFR, vascular endothelial growth factor receptor; YAP, yes associated protein.

*Correspondence to: Prasanna K. Santhekadur, Department of Biochemistry, Center of Excellence in Molecular Biology and Regenerative Medicine, JSS Medical College, JSS Academy of Higher Education and Research, Sri Shivarathreshwara Nagar, Mysore-570015, Karnataka, India. ORCID: <https://orcid.org/0000-0003-3338-375X>. Tel: +91 9108655013, E-mail: prasannakumars@jssuni.edu.in

How to cite this article: Shiragannavar VD, Karunakara SH, Puttananumantharayappa LD, Sannappa Gowda NG, Santhekadur PK. Unraveling Key Signaling Pathways Altered in Hepatocellular Carcinoma. *Gene Expr* 2023;22(1):28–40. doi: 10.14218/GE.2022.00009S.

Introduction

Hepatocellular carcinoma (HCC) refers to a form of primary liver carcinoma, which has recently emerged as the third most paramount cause of cancer-associated mortality worldwide.¹ Various risk factors, such as the occurrence of oncogenic viruses, including hepatitis C virus (HCV) and hepatitis B virus (HBV), excess consumption of alcohol, environmental toxins, autoimmune hepatitis and hemochromatosis, are associated with this multifactorial complex disease.² The process of HCC initiation, development and progression involves various complex cellular signaling pathways and networks. These pathways and networks are controlled by a plethora of oncogenes and tumor suppressor genes, and its protein products.³ The most common oncogenes that are involved in HCC, and control conventional oncogenic signaling pathways have been well-studied and established, such as KRAS, BRAF, c-MYC, *etc.* However, these conventionally labeled oncogenes are controlled by newly emerged oncogenes, such as astrocyte elevated gene 1 (AEG-1), staphylococ-

cal nuclease domain containing 1 (SND1), late SV40 factor (LSF), apoptosis-antagonizing transcription factor (AATF), WNT/ β -catenin and transforming growth factor-beta (TGF- β), in various derailed signaling pathways in HCC.⁴⁻⁷ All these newly emerged oncogenes have the potential ability to transform normal cells into cancer cells, without the aid of conventionally established oncogenes or any known carcinogens. Furthermore, these control almost all the hallmarks of cancer, either by activating oncogenic signaling, or by suppressing the tumor suppressor ability of tumor suppressor genes.⁸ These oncogenes activate various conventionally known cellular signaling pathways in cancer cells, including the PI3K-AKT/mTOR, ERK, AP1, NF- κ B and c-MYC pathways, thereby inducing various inflammatory cytokines and growth factors. These molecules would aid in its proliferation, migration, invasion, angiogenesis and multi-drug resistance, and induce metastasis.⁹⁻¹¹ There are various other complex networks of signaling pathways involved in HCC, and these interconnected pathways are activated by a number of newly established oncogenes. One of the major leading pathways associated with this oncogenic signaling is the RNA-induced silencing complex (RISC). As a highly established multi-protein complex, RISC plays a major role in gene regulation and expression, including oncogene regulation and expression in cancer cells. RISC-associated component proteins, such as SND1 and AEG-1, have highly expressed oncogenes in almost all types of cancers. These regulate the expression of various miRNA target genes by targeting or downregulating tumor suppressor genes.¹² Almost all genes in the human genome are regulated or controlled by thousands of miRNAs through RISC. Therefore, RISC has a major role in controlling the initiation, development and progression of hepatocarcinogenesis by controlling signaling pathways that are altered in the transformed liver.

Major signaling pathways altered in hepatocellular carcinoma

There are various oncogenic and tumor suppressor genes, and the signaling pathways are altered in HCC. The number of abnormal and deregulated cellular signaling pathways contribute to the uncontrolled rapid cell division and proliferation, failed cellular differentiation, and long survival, which may lead to the immortality of these cells.¹³ In addition, this abnormal cellular signaling may sometimes trigger programmed cell death or apoptosis. All these deregulated cellular processes may induce various biomarkers in the oncogenic process. The proper and experimental understanding of these altered pathways would aid in the targeting of important master regulators, preventing HCC initiation, development and progression.¹⁴ The present study discusses some of the very important pathways that are altered, and play a prominent role in HCC initiation, development and metastasis. The detailed understanding and interpretation of all these cellular signaling pathways may help to better understand the disease features, and identify suitable and specific targets to treat HCC.

PI3K-AKT/mTOR

One of the highly active, well-established, and more studied intracellular signal transduction pathways in HCC is the phosphatidylinositol 3-kinase (PI3K)-AKT (also known as protein kinase B)/mTOR (the mammalian target of rapamycin) pathway. This is activated by various extracellular cues and growth factors, such as the epidermal growth factor receptor (also known as ERBB1, HER1 and EGFR), and hormones, including insulin and insulin-like growth factor (IGF).^{15,16} This promotes cellular metabolism, proliferation, cell survival, growth, and tumor angiogenesis.¹⁷ This pathway is actively involved in HCC initiation, development, tu-

mor progression, and metastasis.¹⁸ Furthermore, this overactive PI3K-AKT/mTOR pathway is targeted and negatively regulated by a tumor suppressor protein, which is known as phosphatase and tensin homolog (PTEN), in normal cells. The PTEN expression is downregulated in HCC cells.¹² The PTEN tumor suppressor gene is a target gene of miR-221, which is an oncogenic miRNA, and is highly overexpressed in HCC.¹⁹ Therefore, miR-221 aids in the degradation of the PTEN protein, and indirectly activates the PI3K-AKT/mTOR cellular signaling pathway in this cancer. The rapid division of cancer cells require huge amounts of energy and structural components, such as lipids and proteins, for cell and nuclear membrane synthesis. All these essential requirements are positively supported and provided by the activation of PI3K-AKT/mTOR in these cancer cells.¹⁸ Therefore, highly proliferative cancer cells take the advantage of this pathway during vigorous cell division. Recently, a number of reports have established the role of the mTOR pathway in nutrient sensing and cancer cellular metabolism.¹⁷ Cancer cells have a higher rate of metabolism, and these require a huge amount of nutrition. Therefore, the PI3K-AKT/mTOR pathway aids in providing the required amount of nutrients and other essential cellular building blocks. All these studies reveal the importance and major role of the PI3K-activated AKT/mTOR pathway in HCC initiation, development and metastasis.

The PI3K-AKT/mTOR pathway mainly functions through some protein networks, which include various serine or threonine kinases, and a range of downstream protein substrates. The major players of these essential and vital cellular pathways are receptor tyrosine kinase (RTKs). RTKs act as high-affinity cell surface receptors. Together with these receptors, a number of growth factors, cytokines and hormones (insulin) are also involved in this pathway.²⁰ Phosphatidylinositol 3-kinase (PI3K) is also an important mediator of this pathway. This belongs to the kinase family of proteins, and phosphorylates phosphatidylinositol. Furthermore, PI3K can sometimes be activated by another major signaling protein in cancer cells, called RAS (an oncogene). Lipids, such as phosphatidylinositol-4,5-bisphosphate and associated phosphatidylinositol-3,4,5-bisphosphate, are the minor phospholipid components of cell membranes. These play a prominent role, have been involved in this pathway, and aid in the recruitment of Akt/protein kinase B in the plasma membrane during the signaling process.²¹

This pathway has various prominent and essential downstream effects for the proliferation of HCC cells, and this is regulated by various factors in a balanced and sophisticated manner. A number of proteins negatively regulate this pathway. Very importantly, PTEN and protein phosphatase 2A have a negative role, together with feedback inhibition or auto-regulation.²² By suppressing the PTEN expression, the transcription factor (NF- κ B) will activate this pathway during inflammation and cancer, along with tumour necrosis factor α (TNF- α).²³ It is very rare for this activated NF- κ B to positively regulate the peroxisome proliferator-activated receptor delta, along with the known agonists.²⁴ The mTOR and S6K kinases are involved in cellular protein synthesis. During insulin signaling, the mTORC1 complex (Raptor, mTOR, Depton and G β L) negatively regulates the PI3K-AKT/mTOR pathway via S6K1 activation. S6K1 phosphorylates insulin receptor 1 in various serine residues, which in turn, leads to the prevention of insulin receptor 1 binding to RTKs, and the inhibition of PI3K activation.²⁵

The Ras/Raf/Mitogen-activated protein kinase cascade (MEK) and associated extracellular signal-regulated kinase cascade (ERK) pathway

The Ras/Raf/MEK/ERK cellular signaling pathway is one of the

most common pathways activated in the pathophysiology of HCC initiation, development and metastasis. Furthermore, this is an important regulator and mediator of various types of tumor cell proliferation, migration, invasion and apoptosis. Ras (rat sarcoma virus protein) is an oncogene, which undergoes various types of mutation during the initiation of hepatocarcinogenesis.²⁶ This is mainly expressed as a cytosolic protein, and undergoes posttranslational modification which results in the prenylation of the protein. This post translational modification results in the translocation of the Ras protein to the inner surface of the cellular membrane, which leads to the anchoring of the oncoprotein to the cytoplasmic membrane. The prenylation of the Ras protein may add additional stability to the protein in cancer cells. However, this modification (prenylation) is not necessary for Ras activity in normal cells.²⁷

In a number of human HCC cases, the Ras gene is mutated, and this mutated gene acts as a primary hit during HCC initiation. The process of activation of the normal cellular Ras/Raf/MEK/ERK pathway is initiated by several physiological cytokines, inflammatory factors, hormones, growth factors, and associated specific tyrosine-kinases receptors. This triggers and leads to ligand-specific receptor dimerization and autophosphorylation, which further transduces signaling by activating the downstream intracellular signal cascade via the Ras protein. Therefore, the Ras protein acts as a master mediator of cellular signaling.²⁸ The RTK activation by vascular endothelial growth factor (VEGF) and platelet-derived growth factor results to the activation of the RAS pathway in HCC, which is important for cell proliferation, survival, migration and progression.²⁹ Ras isoforms, such as H-Ras, K-Ras and N-Ras, are members of the Ras family of small GTPases, which play a crucial role in the development and progression of HCC.³⁰ These proteins act as molecular switches, alternating between an inactive GDP-bound state, and an active GTP-bound state. Furthermore, these regulate various cellular processes, including cell proliferation, differentiation and survival.³¹ In HCC, the activation of H-Ras, K-Ras and N-Ras can lead to abnormal cellular growth and survival. This activation can occur through various mechanisms, including genetic mutations in the Ras genes, post-translational modifications of the Ras protein, and the activation of upstream signaling pathways that regulate Ras activity. Once activated, the Ras protein binds to and activates the downstream signaling molecules, such as Raf kinases, MEK kinases, and ERK kinases. This leads to the activation of the mitogen-activated protein kinase (MAPK) pathway, which is involved in regulating cellular proliferation, differentiation and survival. Overactive MAPK signaling has been implicated in the development and progression of HCC, because this can lead to the induction of cellular proliferation, and the suppression of apoptosis (programmed cell death).^{32,33} According to the latest research, microRNAs (miRNAs) play an important role in the progression of cancer. The miR-30a is a microRNA that has been implicated in the regulation of the rat sarcoma (RAS) pathway in HCC. The molecular mechanism by which miR-30a regulates the RAS pathway in HCC involves the post-transcriptional regulation of target genes through binding to the 3' untranslated regions (3' UTRs). Furthermore, miR-30a functions as a negative regulator of target gene expression by binding to the 3' UTR of target mRNAs, and inhibiting the translation into proteins. In HCC, miR-30a has been shown to target genes involved in the RAS pathway, including KRAS, HRAS and NRAS, among others. By binding to the 3' UTRs of these genes, and suppressing the expression, miR-30a effectively downregulates the RAS pathway, and reduces the oncogenic activity. This results in the inhibition of HCC cell proliferation, and promotion of apoptosis, which ulti-

mately leads to the reduction of tumor growth and progression.³⁴ Another miRNA that acts as a tumor suppressor miRNA is Let-7. The Let-7 miRNA is a small non-coding RNA molecule that functions as a post-transcriptional regulator of gene expression. This is involved in the regulation of multiple cellular processes, including cellular differentiation, apoptosis and tumorigenesis. The molecular mechanism by which the Let-7 miRNA regulates the RAS pathway in HCC involves the direct targeting of RAS effector proteins, such as RASGAP and RAF1. In HCC, a loss of Let-7 expression is often observed, which contributes to the activation of the RAS pathway and tumorigenesis. The restoration of Let-7 expression has been shown to reduce the expression of RAS effector proteins, and suppress the proliferation of HCC cells. By targeting the RAS pathway, the Let-7 miRNA acts as a tumor suppressor in HCC.³⁵

Another very important protein in this signaling pathway is Raf. Raf stands for rapidly accelerated fibrosarcoma, and the name itself shows that it is an oncogene, and a downstream effector of the Ras oncoprotein. Furthermore, this is a phosphorylating enzyme (a serine/threonine protein kinase) positioned as the starting and first signaling protein of the MAPK pathway. The Raf protein is encoded by the rapidly accelerated fibrosarcoma (RAF) gene, and has three closely correlated similar proteins in its kinase family: serine/threonine-protein kinase A-Raf, serine/threonine-protein kinase B-Raf (B-Raf), and serine/threonine-protein kinase C-Raf.³⁶ All these isoforms have a distinct tissue-specific expression profile, and all three isoforms can be found in normal human liver cells. Furthermore, significant evidence has shown that these are overexpressed, or highly induced or mutated in HCC tissues.³⁷ The very well-known and only available FDA-approved commercial drug, sorafenib, is a potent inhibitor of these RAF proteins.³⁸ To date, this is the only known drug for HCC therapy. Furthermore, this is a potent multi-kinase inhibitor, which inhibits HCC-associated tumor angiogenesis by targeting the activity of the RAF/MEK/ERK signaling cascade. In this pathway, Ras activates Raf, which further phosphorylates and activates other downstream kinases, such as MEK1 and MEK2, and in turn, phosphorylates and activates ERK1/2. ERK1/2 regulates several transcription factors, including Sp1, NF- κ B, STAT3, c-Myc, Ap1 (c-Jun, c-Fos), ETS and ELK-1, directly or indirectly through the 90-kDa ribosomal s6 kinases protein.^{39–41} Finally, all these kinases would aid in the expression of various growth factors, inflammatory cytokines, and oncogenes in HCC. This would lead to rapid cell division, uncontrolled proliferation, and epithelial-to-mesenchymal transition (EMT), which in turn, helps in the invasion, migration and metastasis of HCC cells (Fig. 1).⁴²

AEG-1, SND1, Ago2 and RISC in HCC

AEG-1 is a bonafide oncogene, and a scaffold protein. This is overexpressed in almost all types of cancers, and is upregulated in HCC. In HCC, this controls virtually all the hallmarks of cancers, and plays a major role in HCC carcinogenesis and progression.⁴³ Recently, it was found that this is a major component of RISC, which interacts with very important ribonucleases of RISC, such as SND1 and Ago2.¹² Indeed, it is well-known that RISC acts as a master regulator of post-transcriptional gene regulation. There are thousands of miRNAs involved in this master gene regulation machinery. AEG-1 was first isolated and cloned by Kang and Su *et al*. It was initially considered that this is a PHFA-associated gene. Subsequently, data-driven studies revealed that AEG-1 is a major oncogene in every type of cancer.^{44,45} Furthermore, AEG-1 is known by other names, such as metadherin and LYRIC.^{46,47} The AEG-1 gene is positioned on human chromosome number 8q22.1,

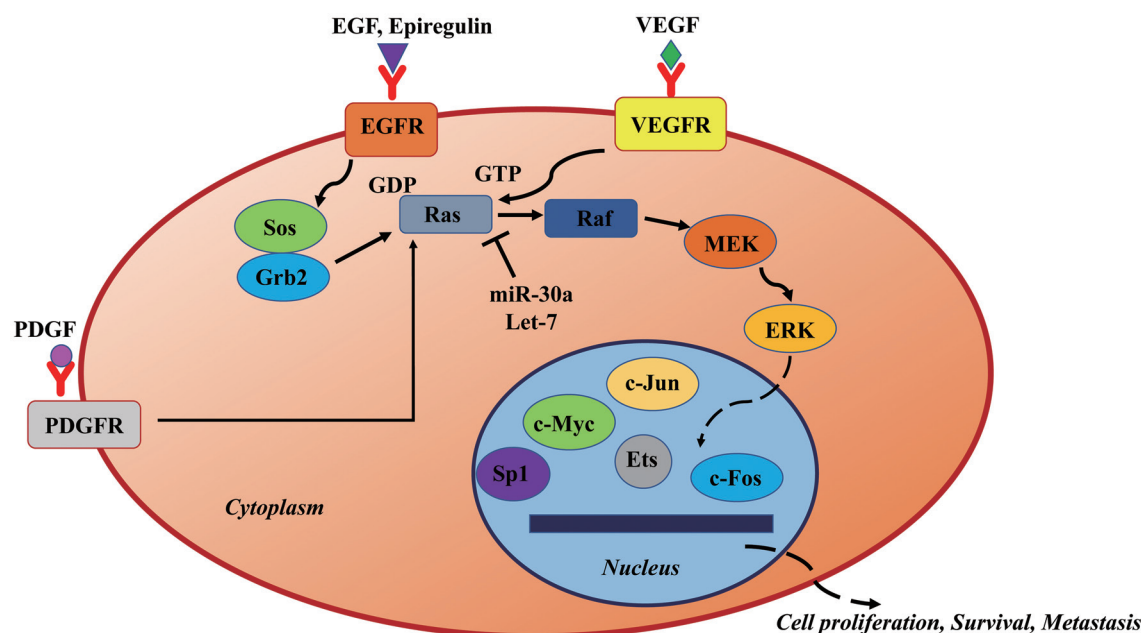


Fig. 1. Activation of the most important Ras/Raf/MEK/ERK signaling cascade in HCC through growth factors and hormones. These are the key proteins involved in the EGFR, VEGFR and PDGFR pathways. EGFR, epidermal growth factor receptor; ERK, extracellular signal-regulated kinase; Grb2, growth factor receptor-bound protein 2; MEK, mitogen-activated protein kinase; PDGFR, platelet-derived growth factor receptor; SOS, mammalian son-of-sevenless; VEGFR, vascular endothelial growth factor receptor.

and chromosome number 15, 13.98 in mice. Structurally, AEG-1 has various domains, including the nuclear localization signal domain and transmembrane domain. Therefore, AEG-1 has been reported and known to localize in the nucleus, cytoplasm, and cell membrane. The molecular function and mechanism of action of AEG-1 depends mainly on its localization. Its spatial location inside the cell determines its interacting partners. Based on the interacting partners, the function of AEG-1 specifically varies in cells and tissues.⁴⁸

In the nucleus, AEG-1 interacts with the p65 subunit of NF- κ B, and induces the expression of various cancer-associated genes, inflammatory cytokines, and growth factors. Furthermore, this regulates the c-Myc expression by preventing the recruitment of tumor suppressor promyelocytic zinc finger protein (PLZF) to the promoter of the c-Myc gene, thereby increasing its expression. Moreover, AEG-1 is involved in BRCA2 and CDKN1A-interacting protein tumor suppressor protein degradation via ubiquitin-associated proteasomal degradation.^{49,50}

Due to its scaffold protein nature, AEG-1 interacts with a number of proteins, and two of the most important interacting partners include SND1 and Ago2. SND1 and Ago2 constitutes the very important enzymatic parts of the RISC machinery, which regulate thousands of gene expression, along with thousands of miRNAs. These two ribonuclease enzymes play a prominent role in the degradation of miRNA-bound target mRNAs, allowing these to undergo downregulation, and preventing the translation of these mRNA messages into a functional protein. These RISC components are highly overexpressed in HCC, and the overall RISC activity itself is highly elevated in HCC.¹²

SND1 is a multifaceted and highly conserved protein involved in RISC activity and function, RNA splicing, and transcription.^{12,19} This is known by other names, such as Tudor SN, p100 and TSN1. The SND1 gene is located on chromosome number 7q32.1 in

humans, and in chromosome number 6.28.48 in mice.⁵¹ SND1, together with AEG-1 and Ago2, constitute the very important components of RISC members, which degrade a number of tumor suppressor genes in HCC.¹² Furthermore, SND1 is involved in steatosis, inflammation, fibrosis and angiogenesis in HCC. Moreover, it is known that this activates the NF- κ B and TGF- β signaling pathways.^{52,53} Both SND1 and AEG-1 are involved in nonalcoholic fatty liver development and HCC progression.^{54,55} In addition, the SND1 protein has different domains. The staphylococcal nuclease domain plays an important role in ribonuclease activity. The Tudor domain is involved in methyl-lysine and methyl-arginine binding, and helps in diverse cellular functions, such as DNA transcription, RNA degradation and metabolism, epigenetic regulation, and the maintenance of genomic and protein stability.⁵⁶

Another very important component of the RISC complex is Ago2 (argonaute RISC catalytic component), which is encoded by a gene called, EIF2C2, and is located on chromosome number 8q24.3 in humans, and chromosome 15:73-731 in mice. Furthermore, Ago2 is overexpressed in HCC, and non-alcoholic fatty liver disease (NAFLD).⁵⁷ It is known that Ago2, SND1 and AEG-1 together form a complex, and plays a major role in RNA interference and RISC activity in HCC.⁵⁸ The knockdown of any of the components of the RISC will result in the suppression of HCC growth and development.¹²

Transcription factor late SV40 factor (LSF) is another very important mediator of HCC development and progression.⁵⁹ Initially, in HCC cells, LSF was identified as one of the very important downstream genes of the AEG-1 oncogene. Subsequently, it was revealed that LSF is involved in drug resistance, and plays a major role in AEG-1-associated resistance to nucleotide analog 5-fluorouracil in HCC cells.⁶⁰ LSF is a transcription factor, and it got its name from the SV40 virus. Since this LSF is involved in virus replication, it is commonly known as TFPC2.⁶¹ Structurally,

this belongs to the CP2 family related to the Grainy head family of proteins. Furthermore, this is functionally involved in various complex biological events, including the regulation of various cellular and viral gene promoters, cell division, proliferation, cell cycle, DNA synthesis, cancer initiation, development and metastasis, and has a role in Alzheimer's disease.^{61,62} Various elegant and important studies have strongly established the oncogenic role of this protein in HCC. Furthermore, this is highly overexpressed in human HCC, when compared to its normal counterpart.

In eukaryotes, the LSF transcription factor induces the expression of thymidylate synthase (TS), which is an enzyme involved in thymidine synthesis, and catalyzes the transformation of deoxyuridine monophosphate to another form (deoxythymidine monophosphate).⁶⁰ Therefore, the role of LSF is very crucial, and its major role in DNA synthesis in HCC cells is a druggable one.⁶² Furthermore, LSF induces the expression of osteopontin, which in turn, activates the hepatocyte growth factor/c-met pathway in HCC cells.⁶³ Therefore, the AEG-1, SND1, Ago2 and RISC signaling pathways control various secondary signaling, and control the initiation, development and progression of HCC via secondary oncogenes.⁶⁴

Apoptosis-antagonizing transcription factor (AATF)

The recent collaborative study conducted by the investigators elucidated the oncogenic nature of AATF in obesity-induced NAFLD-mediated HCC.⁶⁵ In general and in clinic, it was proven that obesity plays a major role in the development of NAFLD, and the long-term progression of NAFLD would further lead to the initiation of hepatocarcinogenesis. The novel study conducted by the investigators established the role of AATF in obesity-mediated NAFLD-associated HCC development. Initially, AATF was considered to be the regulator of cell cycle checkpoints.⁶⁶ However, a number of elegant studies have deduced the various functions of AATF in regulating the hallmarks of cancer.⁶⁷ AATF is a highly conserved protein in eukaryotes. This has various names, and is encoded by the Che-1 gene, which is located on chromosome 17q12 in humans and chromosome number 11:84.42 in mice. Furthermore, this has various protein domains. These domains help in the interaction with various other proteins, including transcription factors, cell cycle-associated proteins, adaptor proteins, nuclear receptors, leucine zippers, *etc.* All these domains help in the multifaceted role of AATF, including the role of a transcriptional cofactor and anti-apoptotic factor, proliferation, migration, invasion, metastasis, the response to a different kind of stress, cellular stress, *etc.*⁶

AATF was initially reported and cloned by two different groups simultaneously in 2000.^{68,69} Even after almost two decades after its discovery, AATF remains to be significant in cancer research, particularly in HCC, where the molecular aspects of AATF needs to be elucidated in detail. One of the master inflammatory cytokines, TNF- α , is commonly known to be elevated in obesity and NAFLD. In the study conducted by the investigators, the overexpression of TNF- α positively induced the AATF expression in obesity-associated NAFLD-mediated HCC. The bioinformatic analysis of the AATF gene promoter conducted by the investigators revealed the binding sites for sterol regulatory element binding transcription factor 1-c, which is generally denoted as the SREBP1-c, Sterol regulatory element-binding transcription factor 1 (SREBP-1c) protein. SREBP-1c is a very commonly known transcription factor involved in fatty acid synthesis and lipogenesis. Furthermore, SREBP-1c induces the AATF expression in NAFLD-associated HCC, and the AATF transcription factor interacts with another transcription factor called, STAT3. Together, these two transcrip-

tion factors induces the expression of monocyte chemoattractant protein-1 (MCP-1). MCP-1 is known for macrophage recruitment, and plays a prominent role in the AATF-induced development and progression of hepatocarcinogenesis.^{65,66}

Wnt/ β -catenin

The Wnt/ β -catenin signaling cascade and pathway is one of the highly conserved cell signaling pathways, which regulates stem cell properties, including pluripotency and cell fate decisions, during the normal developmental process.⁷⁰ This Wnt ligand is usually secreted as a glycoprotein, and binds to its specific receptors called, Frizzled receptors. This ligand-receptor interaction leads to the formation of a protein-protein complex with other proteins, such as LRP5/6.⁷¹ This induces the Wnt receptor to be activated, and trigger the displacement of a multifunctional and multifaceted kinase known as, GSK-3 β , from a different regulatory multi-protein complex (APC/axin/GSK-3 β -complex). The Wnt-mediated activation of β -catenin would result in its nuclear translocation from the cytoplasm, form a complex with various transcription factors, including LEF1/TCF1 and FoxO, and induce various Wnt cascade target genes (mainly c-Myc, CD44, Cyclin D1 and matrix metalloproteinase 7 (MMP-7)).⁷² These genes are known to play a major role in hepatocarcinogenesis. Various emerging shreds of evidence have demonstrated that obesity-associated metabolic signaling pathways can deregulate local and global epigenetic changes in NAFLD. This would result in the modification and activation of a number of gene expression programs, which in turn, would contribute to the hepatocarcinogenesis. Therefore, the obesity-induced aberrant epigenetic changes and activation of the Wnt/ β -catenin signaling pathway play a critical and paramount role in NAFLD development, and this is associated with hepatocarcinogenesis.⁷⁰

TGF- β

Obesity and NAFLD-mediated HCC development mainly depend on inflammation and associated fibrosis. Simple fatty liver or steatosis (non-alcoholic fatty liver) due to the accumulation of extra fat and activation of various inflammatory signals would lead to non-alcoholic steatohepatitis. At the late stage of non-alcoholic steatohepatitis, a hyperactivated fibrotic signal may be present in the liver, which would further lead to cirrhosis of the liver, finally resulting in the development of HCC. TGF- β is the master regulator of liver fibrosis, and plays a major role in EMT in several cancers.⁷⁰ Although this plays a major role in liver fibrosis and EMT, the TGF- β pathway is very complex to understand, and difficult to target for therapeutic purposes. There are various proteins present in the TGF- β superfamily, which include TGF- β proteins, activins, bone morphogenetic proteins, and a number of growth and differentiation factors, transcription factors, coactivators, and adaptor proteins.^{53,73,74}

The TGF- β family of proteins bind to the specific cell surface type 1 and type 2 receptors, which in turn, leads to the activation of suppressor of mothers against decapentaplegic (SMAD)-dependent and SMAD-independent signaling cascades, and the induction of TGF- β target gene expression. These target genes include collagen 1, collagen 2, smooth muscle alpha-actin, and EMT-associated genes. Therefore, the activation of TGF- β signaling not only helps in liver fibrosis and HCC development, but also aids in HCC tumor metastasis.⁵³

Hippo signaling pathway

The Hippo/yes associated protein (YAP) signaling pathway plays a significant role in organogenesis and organ size development by

controlling the cell proliferation and differentiation.^{75,76} In mammalian cells, mammalian Ste20-like kinases 1/2 and the associated adaptor protein, which is known as, Sav family WW domain-containing protein 1, are essential components of this pathway. This ligand-binding kinase domain is under the regulation of tumor suppressor genes, such as mammalian sterile 20-like 1/2 (Mst1/2). Mst1/2 initiates the downstream kinase domain, thereby initiating the oncoprotein YAP activity, and this enables its physical interaction with target genes in tumorigenesis.⁷⁷ In animal models, slight changes in YAP expression and activity, and the impaired modulation of several other primary Hippo pathway-associated components, including Mst1/2, large tumor suppressor homolog 1/2, Mob1, Salvador and Mer, would result in carcinogenesis.⁷⁸ Therefore, the slight dysregulation of the Hippo pathway would result in the unregulated initiation of YAP, which further leads to the growth of a wide variety of human tumor forms, even HCC. The revocation of Mst1/2-mediated YAP phosphorylation allows the YAP protein to enter the nucleus in animal models, and be reinstated in human cancers, mainly in HCC. A number of chemoresistance pathways in HCC tumors are YAP-dependent. Therefore, the increase in expression of YAP was observed in various HCC studies, and YAP appears to be a significant predictor for illness in HCC patients.⁷⁹

Hedgehog signaling pathway

Hedgehog proteins are segment polarity proteins. In mammals, these have three homologs: Sonic Hedgehog (Shh), Indian Hedgehog (Ihh), and Desert Hedgehog (Dhh). All these Hedgehog proteins are involved in the Hedgehog signaling pathway, and the Hedgehog pathway itself is involved in embryological development, tissue patterning, and cell differentiation and proliferation.^{80–82} From flies to vertebrates, this signaling system comprises of seven key components: Hedgehog (Hh) protein, GLI proteins, Patched (PTCH), kinesin-like protein costal 2, fused (Fu), smoothened (Smo), and suppressor of fused.⁸¹ The Hh signaling pathway is abnormally activated in various cancers, and this is particularly compelled in HCC through the mutation of Hh pathway components.⁸³ Elegant studies have revealed that the overexpression of Hh components, such as PTCH1, Gli and SMO, is responsible for the onset of tumorigenesis.^{84,85} Shh stimulation in the liver has been shown to promote liver fibrosis, and cause HCC.⁸⁶ Stimulated Shh signaling enhances the cyclin B1 and cyclin-dependent kinase 1 protein activity and expression, which in turn, stimulates tumor growth, and promotes hepatocarcinogenesis by promoting the G2/M transition.⁸⁷ Furthermore, in human HCC, Smo increases the expression and abnormal ratio of Smo-to-PTCH mRNA activity, which are linked to tumor size. Moreover, the increase in SMO-mediated c-Myc expression contributes to HCC growth.⁸⁴ In the context of HCC, the Gli1 overexpression has been shown to promote proliferation, invasion and metastasis via the induction of epithelial-mesenchymal transition.⁸⁸

Notch signaling pathway

This Notch signaling cascade-associated pathway is involved in cellular homeostasis, and is a strongly conserved signaling pathway that plays a predominant role in embryonic development and morphogenesis. Furthermore, the signaling pathway has been relevant, in terms of liver regeneration and carcinogenesis. The Notch pathway in mammals comprises of four Notch-associated receptors (Notch 1-4), and five canonical Notch ligands, such as Jagged 1-2 and Delta-like (Dll proteins 1, 3 and 4).⁸⁹ The abnormal Notch signaling cascade plays a key role in the onco-transformation of hepatocytes and proliferation of HCC tumors. In contrast to nor-

mal liver tissues, HCC tissues often contain mutated Notch genes. In liver cancer cells, the four Notch protein receptors are highly expressed, with different distributions of subcellular components. HCC tissues have been reported to have higher levels of Notch1 protein in the cytoplasm and Notch4 protein in the nucleus, respectively, but these were reported to have lower levels of Notch2 in the cytoplasm, when compared to non-tumor adjacent tissues.⁹⁰ Likewise, a similar Jagged1 expression in HCC implied that the downregulation of the Notch1/Jagged1 signaling may maintain the HCC progression.⁹¹ Hepatitis B virus X protein antigen and an oncoprotein (HBx) may induce the malignant transformation of human hepatic L02 cells, and activate Notch1. Consequently, the upregulation of Notch target genes, mainly HES1 and JAGGED1 through virus antigen HBx, has been known to be a major contributor to hepatocarcinogenesis.^{92–94} The analysis of clinically relevant tumor samples revealed the abnormally elevated abundance in Notch3 protein and Hes, suggesting that higher Notch3 expression levels in these tumors can lead to the induction of HES1 gene expression.⁹⁵ In multipotent cells in the liver, the Notch target gene, mainly Sox9, is highly expressed. During hepatocarcinogenesis, the Notch signaling pathway can be activated by expanding the population of pre-existing progenitor-like cells, or providing progenitor-like capabilities to these differentiated cells. In HCC, Notch1 and Jagged1 expression levels were found to be elevated, and the rate of HCC metastasis was frequently linked to higher Notch1 mRNA levels.^{96,97} Advanced TMN (tumor node metastasis) classification and positive angiogenesis-associated vascular invasion in the liver were linked to high variations in Jagged1 gene copy number.⁹⁸ Furthermore, Notch2 has been shown to be involved in HCC development and metastasis. Moreover, the aggressiveness and metastasis of HCC have been linked to Notch4.⁹⁹

Vascular endothelial growth factor signaling pathway

Tumorigenesis is supported by hypervascularization. In HCC, several pro-angiogenic factors are overexpressed in both HCC cells and the tumor microenvironment. The most influential and well-studied regulators of angiogenesis (VEGF and vascular endothelial growth factor receptors [VEGFRs]) are essential for HCC growth and development. The VEGF family consists of important members, such as VEGF-A, VEGF-B, VEGF-C, VEGF-D and VEGF-E, and the placental growth factor. The VEGF receptors family includes membrane receptors, such as VEGFR-1 (flt1), very common VEGFR-2 (flk-1/KDR), and VEGFR-3 (flt-4). The process for the angiogenesis and maintenance of mature blood vessels were aided by the VEGF-A signaling.¹⁰⁰ Immature liver tumor vessels, which are highly leaky, also induce irregular blood flow. In HCC, this causes extreme hypoxia, which leads to the secretion of growth factors, such as hypoxia-inducible factors 1 (HIF-1) and hypoxia-inducible factors 2 (HIF-2), as well as insulin-like growth factor 2 (IGF2). HIF-1 and IGF2 promotes tumor angiogenesis, which lead to tumor proliferation and metastases.¹⁰¹ Hypoxia and acidosis separately control the VEGF expression in tissues. Oncogenic gene defects, hormones, cytokines, and various signaling molecules all influence the VEGF expression.¹⁰² The activation of Rho GTPase and VEGFR-2 is essential for angiogenesis processes, which include vascular permeability, extracellular matrix breakdown, cellular migration, and invasion.¹⁰³

Tumor suppressor genes in HCC

Tumor suppressor genes are genes that help regulate cell growth and division, and prevent the development of cancer. In HCC, several

tumor suppressor genes have been identified to play a role in the development of the disease. Some examples are presented below.

The p53 tumor suppressor gene

The p53 tumor suppressor gene plays a crucial role in the development of HCC. This is a well-known “guardian of the genome”, which helps regulate cell division, and prevent the formation of cancer by recognizing DNA damage, and triggering the appropriate response to repair or eliminate a damaged cell.¹⁰⁴ In HCC, mutations in the p53 gene are common, occurring in approximately 50–60% of cases.¹⁰⁵ These mutations can lead to loss of function of the p53 protein, which can no longer act as a tumor suppressor. The loss of function of p53 can lead to the accumulation of mutations in other genes, eventually leading to the development of cancer.^{104,106} Furthermore, the p53 pathway can also be targeted by other genetic changes, such as the mutations in genes involved in the regulation of the p53 pathway (such as MDM2 and MDM4), or by the epigenetic silencing of the p53 gene.¹⁰⁷ The loss of function of p53 can also be caused by viral infections, such as hepatitis B and C, which can lead to the development of HCC. Mutations in p53 are common in HCC, and can lead to loss of function of the p53 protein. This would promote cell survival and proliferation, and lead to the development of cancer.¹⁰⁸

The retinoblastoma (RB1) gene

The retinoblastoma (RB1) gene is a tumor suppressor gene that plays a crucial role in the regulation of cell division, and the maintenance of genomic stability. The protein encoded by this gene, pRb, binds to the E2F family of transcription factors, and are responsible for the regulation of genes involved in cell growth and DNA replication.¹⁰⁹ When pRb is phosphorylated by cyclin-dependent kinases, this releases E2F, allowing for cell cycle progression.¹¹⁰ In HCC, mutations in the RB1 gene are associated with poor prognosis. The loss of function of the RB1 gene leads to the increase in cell proliferation, and promotes angiogenesis, which in turn, leads to the development of HCC.^{111,112} This loss of function occurs due to genetic changes, such as mutations or deletions in the RB1 gene, or due to epigenetic silencing. Furthermore, pRb has a role in DNA damage response by recruiting repair proteins to the damaged site, and inhibiting the activity of the pro-apoptotic protein p53.^{111,113} Therefore, when RB1 is lost, cells become more prone to DNA damage, and less likely to undergo apoptosis.

Epigenetic alterations

It has been reported that HCC is one of the most lethal cancers in the world, and has long been recognized as a genomic disorder supplemented by a wide range of epigenetic aberrations. Epigenetic changes typically result in the activation and overexpression of oncogenes, or the inactivation or decrease in expression of tumor suppressor genes. All of these correlates to cancerous tumor characteristics. Epigenetic modifications are emerging as a significant mechanism in cancer development and growth, according to mounting evidence.¹¹⁴ As a result, metabolic risk factors, such as morbid obesity, heavy alcohol intake, and viral hepatitis insults, induce hepatic epigenome disruption.¹¹⁵ The changes in the epigenome, which include changes in genomic alterations induced by DNA methylation, chromatin remodeling, miRNAs and lncRNAs, might cause uncontrolled cell division and invasion capabilities, and perhaps even the development of liver cancer, through systemic inflammation, fibrosis and mutation.¹¹⁶

Environmental exposure

Hepatitis B and C virus

Chronic virus infections, mainly HBV or HCV, results in liver cirrhosis, and finally leads to the development of HCC. HBV and HCV have been found to be oncogenic viruses. A viral genome can integrate its DNA into the host genome, and directly cause HCC mutations.¹¹⁷ The integration of HBV and HCV may trigger chromosomal instability by inducing massive inverted duplications, deletions, amplifications and translocations, among the other mutations.¹¹⁸ The viral DNA gets incorporated into target genes, such as human telomerase gene *hTERT*, *MAPK1* and cyclin A, which regulate cell proliferation and differentiation.¹¹⁹ In chronic hepatitis B infection, a different HBV protein called, the HBV spliced protein, is produced. This variant has been shown to trigger apoptosis, and modulate through the TGF β signaling, suggesting a novel pathway in stimulating liver fibrosis and hepatocarcinogenesis.¹²⁰ The HCV core protein is involved in the formation of full virions, and the aggregation of viral particles. In general, major metabolic, molecular and cellular processes, such as transcription initiation, lipid metabolism and apoptosis, are all influenced by the core protein.¹¹⁹ One of the major mechanisms of HCV is the induction of oxidative stress through the production of more reactive oxygen species, further inducing steatosis.¹²¹

Aflatoxin

Mycotoxins predominantly formed by *Aspergillus flavus* are known as aflatoxins. Aflatoxin B1 (AFB1), a major strain of aflatoxin, is a powerful carcinogen found in a variety of foods, including peanuts, rice and grains. The liver is the primary site of aflatoxin metabolism, and the main human cytochrome p450 enzyme is involved in its metabolism. Evident studies have revealed that AFB1 metabolites bind the DNA through the alkylation of bases, inducing cell cycle disruption, and the mutation of tumor suppressor gene p53.¹²² Aflatoxin is converted to aflatoxin-8,9-epoxide through p450 enzymes in the liver, and promutagenic AFB1 (AFB1-N7-Gua) is formed by binding to the DNA. The apurinic site and AFB1-formamidopyrimidine (AFB1-FABY) are the two forms derived by AFB1-N7-Gua.¹²³ DNA mutation occurs when AFB1-FABY is incorporated into the DNA, and blocks replication. These mutations are extremely reactive with DNA, resulting in modifications that can lead to malignant transformation over time.¹²⁴

Alcohol consumption

Multiple variables are involved in molecular pathways, through which alcohol consumption increases the risk of HCC. The breakdown of alcohol produces reactive oxygen species, which causes oxidative stress and damage to liver cells, leading to DNA damage and genetic abnormalities.¹²⁵ Furthermore, chronic alcohol use can activate immune cells in the liver, resulting in chronic inflammation and further liver cell damage.¹²⁶ Moreover, alcohol metabolism produces acetaldehyde, which is toxic to liver cells, and can cause DNA, protein and lipid damage, resulting in oxidative stress and inflammation.¹²⁷ In addition, alcohol intake might change hormone levels, such as insulin, which can raise the risk of HCC.¹²⁸ The regulation of several genes plays a role in the development of HCC in individuals who consume alcohol heavily, and for long periods of time. Alcohol consumption can suppress the expression of tumor suppressor genes, such as TP53, and activate oncogenes, such as MYC and HIF1A, promoting the growth and survival of cancer cells.^{129,130} Furthermore, this can alter the expression of inflammatory cytokines, such as TNF- α and IL-6, which promote

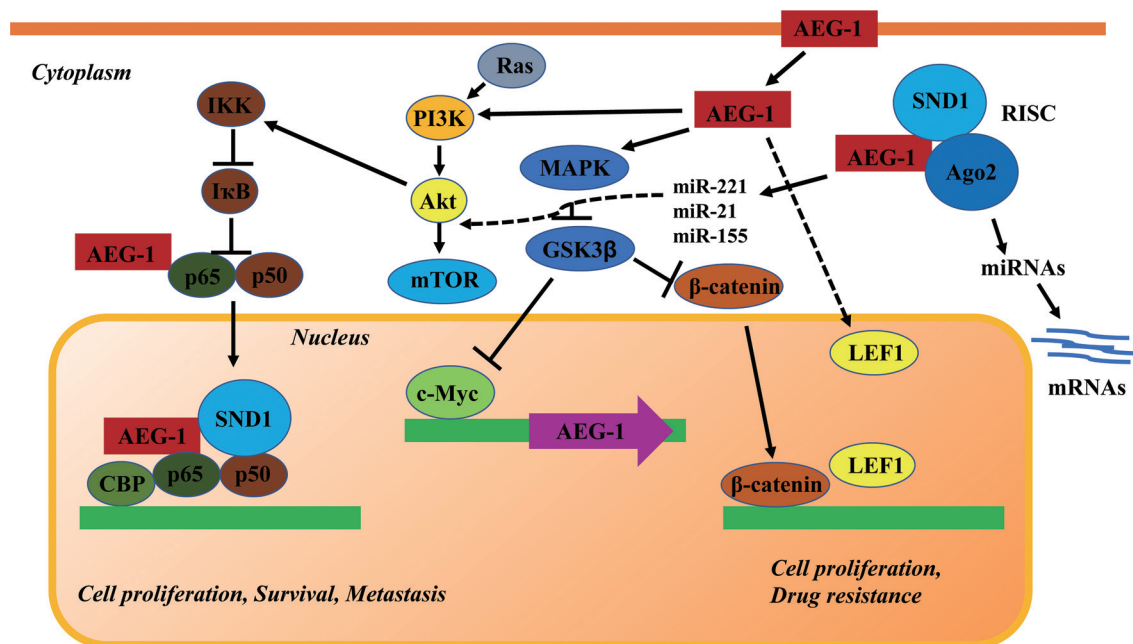


Fig. 2. The role of AEG-1, SND1, Ago2 and RISC in HCC development and progression via the crosstalk between multiple signaling pathways. All these signaling pathways play a major role in cell proliferation survival and metastasis. AEG-1, astrocyte elevated gene 1; Ago2, argonaute 2; HCC, hepatocellular carcinoma; RISC, RNA-induced silencing complex; SND1, staphylococcal nuclease domain containing 1.

chronic inflammation, and increase the risk of HCC.¹³¹ Alcohol consumption can affect the expression of metabolic genes, such as CYP2E1, and play a role in alcohol metabolism, and the production of toxic by-products.¹³² In addition, this can alter the expression of cell cycle regulators, such as cyclin D1 and CDK4, which control the progression of cells through the cell cycle, and promote the development of cancer.^{133,134} These changes in gene expression contribute to the development of liver damage and HCC, making it important to limit or avoid alcohol consumption, in order to reduce the risk of this disease.

Cigarette smoking

The molecular mechanisms involved in HCC induced by smoking can lead to changes in gene expression that contribute to the development of the disease. The oxidative stress caused by smoking can activate signaling pathways involved in oxidative stress response, such as Nrf2 and p53, leading to the alteration of genes involved in antioxidant defense, DNA repair, and cell death.^{135,136} Chronic inflammation triggered by smoking can activate pathways involved in inflammation regulation, such as the NF- κ B pathway, altering the expression of genes involved in inflammation and oxidative stress.^{137,138} The accumulation of DNA mutations caused by cigarette smoking can alter the expression of genes involved in DNA repair and cell death. Epigenetic changes, such as changes in DNA methylation and histone modification patterns, can alter the expression of genes involved in cell proliferation, differentiation, and cell death.¹³⁹ These gene expression alterations can contribute to the development of HCC by disrupting normal cellular processes, leading to the accumulation of genetic changes that drive the development of the disease.

Chemical exposure

Some industrial chemicals have been found to cause HCC. One example of the industrial chemical linked to HCC, vinyl chloride,

which is used in the manufacturing of certain plastics.¹⁴⁰ Vinyl chloride exposure can cause oxidative stress and DNA damage in the liver, which leads to mutations and altered gene regulation, and may contribute to the development of HCC.¹⁴¹ Another example is polychlorinated biphenyls (PCBs), which are mixtures of chemicals used in a variety of industrial and commercial applications. The exposure to PCBs has been associated with an increased risk of HCC, which is likely due to the ability of these chemicals to cause oxidative stress, inflammation, and DNA damage to the liver. The specific genes altered by chemical exposure that may contribute to the development of HCC can have significant effects on the regulation of cell growth, differentiation, and death.^{142,143} The altered expression of these genes has been observed in HCC, suggesting that exposure to certain chemicals may contribute to the development and progression of the disease by altering the metabolism of these chemicals in the liver. The specific genes altered by chemical exposure that may contribute to the development of HCC play critical roles in regulating cellular processes, such as cell growth, differentiation, and death. Further research is needed to fully understand the molecular mechanisms involved in the development and progression of HCC.

The crosstalk between various signaling pathways

Cellular signaling is a complex network of various proteins, which include several kinases and phosphatases, membrane receptors, nuclear receptors, secondary messengers, adaptor proteins, transcription factors, and epigenetic modifiers. All these major signaling pathways, at one or other points in time, are interconnected, and there will be the existence of crosstalk between these proteins (Fig. 2). A crosstalk exists between PI3K-AKT/mTOR and AEG-1, SND1, Ago2 and RISC via miR-221, miR-21 and miR-155 in HCC.¹² Furthermore, there is an interaction between the Ras/Raf/MEK/ERK pathway and SND1, AT1R, ERK and TGF- β signal-

Table 1. Altered signaling pathways and its role in hepatocellular carcinoma

Related Pathways	Genes/Proteins	Role	References
PI3K-AKT/mTOR signaling pathway	PI3K; mTOR; EGFR; IGF; PIP2; PIP3	Promotes cellular metabolism, proliferation, cell survival, growth, tumor angiogenesis and metastasis	15–17,21
Ras/Raf/MEK/ERK pathway	Ras (H-, K-, N-); Raf (B-, A-, C-); MEK; ERK; MAPK	Activation associated with HCC development and progression	29,31–33,36–38
RNA-induced silencing complex	SND1; Ago2; MTDH	Involved in steatosis, inflammation, fibrosis, migration, invasion and angiogenesis in HCC	49,50,55
AATF	TNF- α ; SREBP-1c; STAT3; MCP-1	Regulator of cell cycle checkpoints. Transcriptional cofactor, anti-apoptotic factor, proliferation, migration, invasion, metastasis, response to a different kind of stress, cellular stress	6,7,63
Wnt/ β -catenin pathway	β -catenin; c-Myc; CD44; Cyclin D1; MMP-7	Involved in the initiation, growth, survival, migration, differentiation, and apoptosis of HCC	69
TGF- β signaling pathway	SMAD; TGFB R1/R2; Col1A1; Col2A1; Col3A1; α -SMA	Promotes fibrosis, tumor growth, invasion, progression and metastasis, and regulates immune response	50,70,71
Hippo signaling pathway	SAV1; Mst1/2; YAP/TAZ; Lats1/2	Promotes growth and progression.	74–76
Hedgehog signaling pathway	Cos2; Smo; SuFu; Cyclin B1; CDK1; c-Myc; Gli1	Promotes liver fibrosis. Responsible for the onset of tumorigenesis and hepatocarcinogenesis.	78,81,82,85
Notch signaling pathway	Notch receptors (Notch1,2,3,4); Jagged1,2; Notch3; Hes-1; Sox9; Notch4	Regulates cell proliferation, differentiation and apoptosis. Promotes tumorigenesis and progression.	93,94,96
VEGF signaling pathway	VEGF; VEGFR; Flt1; HIF-1/2	Promotes and regulates tumor angiogenesis, growth and progression	97–99

AATF, apoptosis-antagonizing transcription factor; Ago2, argonaut 2; CDK1, cyclin-dependent kinase 1; EGFR, Epidermal growth factor receptor; ERK, extracellular signal-regulated kinase cascade; HIF-1/2, hypoxia-inducible factors 1/2; IGF, Insulin-like growth factor; Lats1/2, large tumor suppressor homolog 1/2; MAPK, mitogen-activated protein kinase; MCP-1, monocyte chemoattractant protein-1; MEK, mitogen-activated protein kinase cascade; Mst1/2, mammalian sterile 20-like 1/2; MMP-7, matrix metalloproteinase 7; MTDH, metadherin; mTOR, mammalian target of rapamycin; PI3K, phosphatidylinositol 3-kinase; PIP2, phosphatidylinositol-4,5-bisphosphate; PIP3, phosphatidylinositol-3,4,5-bisphosphate; RAF, rapidly accelerated fibrosarcoma; RAS, rat sarcoma; SAV1, Sav family WW domain-containing protein 1; α -SMA, smooth muscle alpha-actin; SMAD, suppressor of mothers against decapentaplegic; SND1, staphylococcal nuclease domain containing 1; Smo, Smoothened; SuFu, suppressor of fused; SREBP-1c, Sterol regulatory element-binding transcription factor 1; STAT 3, signal transducer and activator of transcription 3; TAZ, transcriptional co-activator with PDZ-binding motif; TNF- α , tumour necrosis factor α ; VEGF, vascular endothelial growth factor; VEGFR, vascular endothelial growth factor receptor.

ing.⁵³ SND1 has been very well-studied for its trans-activating role on SREBP transcription factors (including SREBP-1c), which in turn, results in AATF induction and expression.^{65,144,145} Both AEG-1 and SND1 activate NF- κ B, and are involved in the c-Myc (oncogene), cyclin D1 (cell cycle-associated protein) and MMP-7 (matrix degrading enzyme) expression, which are the known target genes of the Wnt/ β -catenin pathway.¹⁹ Furthermore, SND1 regulates the expression of mTOR, SREBP-1c and cyclin D1 in bovine mammary epithelial cells.¹⁴⁶ Moreover, both AEG-1 and SND1 activate the Wnt/ β -catenin pathway in different types of cancer cells. Therefore, all these very important and prominent cellular signaling pathways may interact in one way or the other, and may play a major role in HCC carcinogenesis (Table 1).^{6,7,15–17,21,29,31–33,36–38,43,49,50,55,63,69–71,74–76,78,81,82,85,93,94,96–99,147}

Conclusions

HCC is a multifactorial disease. This has a complex network of cellular signaling pathways, and all these pathways together aid in the various pathophysiology of the disease. Although there are various independent signaling conduits involved in disease development and progression, there are multistep networks and crosstalk that regulate every protein function during this signaling. The proper understanding of all crosstalk and signaling is needed through elegant experimental studies. With the increasing discoveries and the

proper understanding of all these signals, and the knowledge of potential target proteins, more effective and highly potential treatment strategies can be generated using biological, immunological, chemical, and molecular approaches to combat the deadly HCC. Overall, these are the most common signaling pathways that are altered in HCC. Targeting these through specific and lifesaving drugs would become the ray of hope for HCC therapy.

Acknowledgments

This work was supported by the Ramalingaswami Re-entry fellowship, Department of Biotechnology (DBT), Govt. of India to PKS, and salary support to VDS.

Funding

This review article did not receive any specific grant from funding agencies in the public, commercial, or nonprofit sectors.

Conflict of interest

PKS has been an editorial board member of *Gene Expression* since August 2022. The authors have no other conflict of interests related to this publication.

Author contributions

PKS: conceptualization of the review; VDS, SHK, LDP and NGS: collection of the review information; VDS and PKS: initial drafting of the manuscript; SHK, NGS, LDP and PKS: critical assessment of the manuscript, and provision of intellectual input. All authors approved the final draft.

References

- [1] Villanueva A. Hepatocellular Carcinoma. *N Engl J Med* 2019;380(15):1450–1462. doi:10.1056/NEJMra1713263, PMID:30970190.
- [2] Sanyal AJ, Yoon SK, Lencioni R. The etiology of hepatocellular carcinoma and consequences for treatment. *Oncologist* 2010;15(Suppl 4):14–22. doi:10.1634/theoncologist.2010-S4-14, PMID:21115577.
- [3] Whittaker S, Marais R, Zhu AX. The role of signaling pathways in the development and treatment of hepatocellular carcinoma. *Oncogene* 2010;29(36):4989–5005. doi:10.1038/ncr.2010.236, PMID:20639898.
- [4] Li L, Zhao GD, Shi Z, Qi LL, Zhou LY, Fu ZX. The Ras/Raf/MEK/ERK signaling pathway and its role in the occurrence and development of HCC. *Oncol Lett* 2016;12(5):3045–3050. doi:10.3892/ol.2016.5110, PMID:27899961.
- [5] Robertson CL, Srivastava J, Rajasekaran D, Gredler R, Akiel MA, Jariwala N, *et al*. The role of AEG-1 in the development of liver cancer. *Hepat Oncol* 2015;2(3):303–312. doi:10.2217/hep.15.10, PMID:26798451.
- [6] Iezzi S, Fanciulli M. Discovering Che-1/AATF: a new attractive target for cancer therapy. *Front Genet* 2015;6:141. doi:10.3389/fgene.2015.00141, PMID:25914721.
- [7] Kumar DP, Santhekadur PK, Seneshaw M, Mirshahi F, Uram-Tuculescu C, Sanyal AJ. A Regulatory Role of Apoptosis Antagonizing Transcription Factor in the Pathogenesis of Nonalcoholic Fatty Liver Disease and Hepatocellular Carcinoma. *Hepatology* 2019;69(4):1520–1534. doi:10.1002/hep.30346, PMID:30394550.
- [8] Pyrzynska B, Pilecka I, Miaczynska M. Endocytic proteins in the regulation of nuclear signaling, transcription and tumorigenesis. *Mol Oncol* 2009;3(4):321–338. doi:10.1016/j.molonc.2009.06.001, PMID:19577966.
- [9] Kawamura N, Nagai H, Bando K, Koyama M, Matsumoto S, Tajiri T, *et al*. PTEN/MMAC1 mutations in hepatocellular carcinomas: somatic inactivation of both alleles in tumors. *Jpn J Cancer Res* 1999;90(4):413–418. doi:10.1111/j.1349-7006.1999.tb00763.x, PMID:10363579.
- [10] Chen HJ, Hu MH, Xu FG, Xu HJ, She JJ, Xia HP. Understanding the inflammation-cancer transformation in the development of primary liver cancer. *Hepatology* 2018;4:29. doi:10.20517/2394-5079.2018.18.
- [11] Cervello M, McCubrey JA, Cusimano A, Lampiasi N, Azzolina A, Montalto G. Targeted therapy for hepatocellular carcinoma: novel agents on the horizon. *Oncotarget* 2012;3(3):236–260. doi:10.18632/oncotarget.466, PMID:22470194.
- [12] Yoo BK, Santhekadur PK, Gredler R, Chen D, Emdad L, Bhutia S, *et al*. Increased RNA-induced silencing complex (RISC) activity contributes to hepatocellular carcinoma. *Hepatology* 2011;53(5):1538–1548. doi:10.1002/hep.24216, PMID:21520169.
- [13] Nwosu ZC, Megger DA, Hammad S, Sitek B, Roessler S, Ebert MP, *et al*. Identification of the consistently altered metabolic targets in human hepatocellular carcinoma. *Cell Mol Gastroenterol Hepatol* 2017;4(2):303–323.e1. doi:10.1016/j.jcmgh.2017.05.004, PMID:28840186.
- [14] Wu M, Liu Z, Zhang A, Li N. Identification of key genes and pathways in hepatocellular carcinoma: A preliminary bioinformatics analysis. *Medicine (Baltimore)* 2019;98(5):e14287. doi:10.1097/MD.00000000000014287, PMID:30702595.
- [15] Matter MS, Decaens T, Andersen JB, Thorgeirsson SS. Targeting the mTOR pathway in hepatocellular carcinoma: current state and future trends. *J Hepatol* 2014;60(4):855–865. doi:10.1016/j.jhep.2013.11.031, PMID:24308993.
- [16] Zhou Q, Lui VW, Yeo W. Targeting the PI3K/Akt/mTOR pathway in hepatocellular carcinoma. *Future Oncol* 2011;7(10):1149–1167. doi:10.2217/fon.11.95, PMID:21992728.
- [17] Advani SH. Targeting mTOR pathway: A new concept in cancer therapy. *Indian J Med Paediatr Oncol* 2010;31(4):132–136. doi:10.4103/0971-5851.76197, PMID:21584218.
- [18] Grabinski N, Ewald F, Hofmann BT, Staufer K, Schumacher U, Nashan B, *et al*. Combined targeting of AKT and mTOR synergistically inhibits proliferation of hepatocellular carcinoma cells. *Mol Cancer* 2012;11:85. doi:10.1186/1476-4598-11-85, PMID:23167739.
- [19] Santhekadur PK, Das SK, Gredler R, Chen D, Srivastava J, Robertson C, *et al*. Multifunction protein staphylococcal nuclease domain containing 1 (SND1) promotes tumor angiogenesis in human hepatocellular carcinoma through novel pathway that involves nuclear factor κB and miR-221. *J Biol Chem* 2012;287(17):13952–13958. doi:10.1074/jbc.M111.321646, PMID:22396537.
- [20] Lemmon MA, Schlessinger J. Cell signaling by receptor tyrosine kinases. *Cell* 2010;141(7):1117–1134. doi:10.1016/j.cell.2010.06.011, PMID:20602996.
- [21] Liu P, Cheng H, Roberts TM, Zhao JJ. Targeting the phosphoinositide 3-kinase pathway in cancer. *Nat Rev Drug Discov* 2009;8(8):627–644. doi:10.1038/nrd2926, PMID:19644473.
- [22] Yu HC, Hou DR, Liu CY, Lin CS, Shiau CW, Cheng AL, *et al*. Cancerous inhibitor of protein phosphatase 2A mediates bortezomib-induced autophagy in hepatocellular carcinoma independent of proteasome. *PLoS One* 2013;8(2):e55705. doi:10.1371/journal.pone.0055705, PMID:23383345.
- [23] Vasudevan KM, Gurumurthy S, Rangnekar VM. Suppression of PTEN expression by NF-κB prevents apoptosis. *Mol Cell Biol* 2004;24(3):1007–1021. doi:10.1128/MCB.24.3.1007-1021.2004, PMID:14729949.
- [24] Wagner N, Wagner KD. PPAR beta/delta and the hallmarks of cancer. *Cells* 2020;9(5):1133. doi:10.3390/cells9051133, PMID:32375405.
- [25] Draznin B. Molecular mechanisms of insulin resistance: serine phosphorylation of insulin receptor substrate-1 and increased expression of p85α: the two sides of a coin. *Diabetes* 2006;55(8):2392–2397. doi:10.2337/db06-0391, PMID:16873706.
- [26] Yang S, Liu G. Targeting the Ras/Raf/MEK/ERK pathway in hepatocellular carcinoma. *Oncol Lett* 2017;13(3):1041–1047. doi:10.3892/ol.2017.5557, PMID:28454211.
- [27] Baranyi M, Buday L, Hegedűs B. K-Ras prenylation as a potential anticancer target. *Cancer Metastasis Rev* 2020;39(4):1127–1141. doi:10.1007/s10555-020-09902-w, PMID:32524209.
- [28] Donninger H, Schmidt ML, Mezzanotte J, Barnoud T, Clark GJ. Ras signaling through RASSF proteins. *Semin Cell Dev Biol* 2016;58:86–95. doi:10.1016/j.semcdb.2016.06.007, PMID:27288568.
- [29] Moawad AW, Szklaruk J, Lall C, Blair KJ, Kaseb AO, Kamath A, *et al*. Angiogenesis in Hepatocellular Carcinoma; Pathophysiology, Targeted Therapy, and Role of Imaging. *J Hepatocell Carcinoma* 2020;7:77–89. doi:10.2147/JHC.S224471, PMID:32426302.
- [30] Dietrich P, Giza A, Wormser L, Fritz V, Hellerbrand C, Bosserhoff AK. Neuroblastoma RAS viral oncogene homolog (NRAS) is a novel prognostic marker and contributes to sorafenib resistance in hepatocellular carcinoma. *Neoplasia* 2019;21(3):257–268. doi:10.1016/j.neo.2018.11.011, PMID:30685691.
- [31] Simanshu DK, Nissley DV, McCormick F. RAS Proteins and Their Regulators in Human Disease. *Cell* 2017;170(1):17–33. doi:10.1016/j.cell.2017.06.009, PMID:28666118.
- [32] Fernández-Medarde A, Santos E, Ras in cancer and developmental diseases. *Genes Cancer* 2011;2(3):344–358. doi:10.1177/1947601911411084, PMID:21779504.
- [33] Santarpia L, Lippman SM, El-Naggar AK. Targeting the MAPK-RAS-RAF signaling pathway in cancer therapy. *Expert Opin Ther Targets* 2012;16(1):103–119. doi:10.1517/14728222.2011.645805, PMID:22239440.
- [34] Zhou K, Luo X, Wang Y, Cao D, Sun G. MicroRNA-30a suppresses tumor progression by blocking Ras/Raf/MEK/ERK signaling pathway in hepatocellular carcinoma. *Biomed Pharmacother* 2017;93:1025–1032. doi:10.1016/j.biopha.2017.07.029, PMID:28732393.
- [35] Ma Y, Shen N, Wicha MS, Luo M. The Roles of the Let-7 Family of MicroRNAs in the Regulation of Cancer Stemness. *Cells* 2021;10(9):2415. doi:10.3390/cells10092415, PMID:34572067.
- [36] Leicht DT, Balan V, Kaplun A, Singh-Gupta V, Kaplun L, Dobson M, *et al*. Raf kinases: function, regulation and role in human cancer. *Bio-*

- chim Biophys Acta 2007;1773(8):1196–1212. doi:10.1016/j.bbamcr.2007.05.001, PMID:17555829.
- [37] Gnani A, Licchetta A, Memeo R, Argentiero A, Solimando AG, Longo V, *et al.* Role of BRAF in hepatocellular carcinoma: A rationale for future targeted cancer therapies. *Medicina (Kaunas)* 2019;55(12):754. doi:10.3390/medicina55120754, PMID:31766556.
 - [38] Méndez-Sánchez N, Vázquez-Fernández F, Zamora-Valdés D, Uribe M. Sorafenib, a systemic therapy for hepatocellular carcinoma. *Ann Hepatol* 2008;7(1):46–51. PMID:18376365.
 - [39] Weng CJ, Chau CF, Hsieh YS, Yang SF, Yen GC. Lucidenic acid inhibits PMA-induced invasion of human hepatoma cells through inactivating MAPK/ERK signal transduction pathway and reducing binding activities of NF-kappaB and AP-1. *Carcinogenesis* 2008;29(1):147–156. doi:10.1093/carcin/bgm261, PMID:18024477.
 - [40] Chen Y, Lin C, Liu Y, Jiang Y. HMGB1 promotes HCC progression partly by downregulating p21 via ERK/c-Myc pathway and upregulating MMP-2. *Tumour Biol* 2016;37(4):4399–408. doi:10.1007/s13277-015-4049-z, PMID:26499944.
 - [41] Pascale RM, Feo CF, Calvisi DF, Feo F. Deregulation of methionine metabolism as determinant of progression and prognosis of hepatocellular carcinoma. *Transl Gastroenterol Hepatol* 2018;3:36. doi:10.21037/tgh.2018.06.04, PMID:30050996.
 - [42] Chen L, Guo P, He Y, Chen Z, Chen L, Luo Y, *et al.* HCC-derived exosomes elicit HCC progression and recurrence by epithelial-mesenchymal transition through MAPK/ERK signalling pathway. *Cell Death Dis* 2018;9(5):513. doi:10.1038/s41419-018-0534-9, PMID:29725020.
 - [43] Yoo BK, Emdad L, Su ZZ, Villanueva A, Chiang DY, Mukhopadhyay ND, *et al.* Astrocyte elevated gene-1 regulates hepatocellular carcinoma development and progression. *J Clin Invest* 2009;119(3):465–477. doi:10.1172/JCI36460, PMID:19221438.
 - [44] Su ZZ, Kang DC, Chen Y, Pekarskaya O, Chao W, Volsky DJ, *et al.* Identification and cloning of human astrocyte genes displaying elevated expression after infection with HIV-1 or exposure to HIV-1 envelope glycoprotein by rapid subtraction hybridization, RaSH. *Oncogene* 2002;21(22):3592–3602. doi:10.1038/sj.onc.1205445, PMID:12032861.
 - [45] Kang DC, Su ZZ, Sarkar D, Emdad L, Volsky DJ, Fisher PB. Cloning and characterization of HIV-1-inducible astrocyte elevated gene-1, AEG-1. *Gene* 2005;353(1):8–15. doi:10.1016/j.gene.2005.04.006, PMID:15927426.
 - [46] Brown DM, Ruoslahti E. Metadherin, a cell surface protein in breast tumors that mediates lung metastasis. *Cancer Cell* 2004;5(4):365–374. doi:10.1016/s1535-6108(04)00079-0, PMID:15093543.
 - [47] Britt DE, Yang DF, Yang DQ, Flanagan D, Callanan H, Lim YP, *et al.* Identification of a novel protein, LYRIC, localized to tight junctions of polarized epithelial cells. *Exp Cell Res* 2004;300(1):134–148. doi:10.1016/j.yexcr.2004.06.026, PMID:15383321.
 - [48] Yoo BK, Emdad L, Lee SG, Su ZZ, Santhekadur P, Chen D, *et al.* Astrocyte elevated gene-1 (AEG-1): A multifunctional regulator of normal and abnormal physiology. *Pharmacol Ther* 2011;130(1):1–8. doi:10.1016/j.pharmthera.2011.01.008, PMID:21256156.
 - [49] Thirkettle HJ, Mills IG, Whitaker HC, Neal DE. Nuclear LYRIC/AEG-1 interacts with PLZF and relieves PLZF-mediated repression. *Oncogene* 2009;28(41):3663–3670. doi:10.1038/onc.2009.223, PMID:19648967.
 - [50] Ash SC, Yang DQ, Britt DE. LYRIC/AEG-1 overexpression modulates BCCIPalpha protein levels in prostate tumor cells. *Biochem Biophys Res Commun* 2008;371(2):333–338. doi:10.1016/j.bbrc.2008.04.084, PMID:18440304.
 - [51] Ochoa B, Chico Y, Martínez MJ. Insights Into SND1 Oncogene Promoter Regulation. *Front Oncol* 2018;8:606. doi:10.3389/fonc.2018.00606, PMID:30619748.
 - [52] Arretxe E, Armengol S, Mula S, Chico Y, Ochoa B, Martínez MJ. Profiling of promoter occupancy by the SND1 transcriptional coactivator identifies downstream glycerolipid metabolic genes involved in TNFα response in human hepatoma cells. *Nucleic Acids Res* 2015;43(22):10673–10688. doi:10.1093/nar/gkv858, PMID:26323317.
 - [53] Santhekadur PK, Akiel M, Emdad L, Gredler R, Srivastava J, Rajasekaran D, *et al.* Staphylococcal nuclease domain containing-1 (SND1) promotes migration and invasion via angiotensin II type 1 receptor (AT1R) and TGFβ signaling. *FEBS Open Bio* 2014;4:353–361. doi:10.1016/j.fob.2014.03.012, PMID:24918049.
 - [54] Garcia-Arcos I, Rueda Y, González-Kotter P, Palacios L, Ochoa B, Fresno do O. Association of SND1 protein to low density lipid droplets in liver steatosis. *J Physiol Biochem* 2010;66(1):73–83. doi:10.1007/s13105-010-0011-0, PMID:20414760.
 - [55] Srivastava J, Siddiq A, Emdad L, Santhekadur PK, Chen D, Gredler R, *et al.* Astrocyte elevated gene-1 promotes hepatocarcinogenesis: novel insights from a mouse model. *Hepatology* 2012;56(5):1782–1791. doi:10.1002/hep.25868, PMID:22689379.
 - [56] Gutierrez-Beltran E, Denisenko TV, Zhivotovsky B, Bozhkov PV. Tudor staphylococcal nuclease: biochemistry and functions. *Cell Death Differ* 2016;23(11):1739–1748. doi:10.1038/cdd.2016.93, PMID:27612014.
 - [57] Höck J, Meister G. The Argonaute protein family. *Genome Biol* 2008;9(2):210. doi:10.1186/gb-2008-9-2-210, PMID:18304383.
 - [58] Zhang C, Seo J, Murakami K, Salem ESB, Bernhard E, Borra VJ, *et al.* Hepatic Ago2-mediated RNA silencing controls energy metabolism linked to AMPK activation and obesity-associated pathophysiology. *Nat Commun* 2018;9(1):3658. doi:10.1038/s41467-018-05870-6, PMID:30201950.
 - [59] Yoo BK, Emdad L, Gredler R, Fuller C, Dumur CI, Jones KH, *et al.* Transcription factor Late SV40 Factor (LSF) functions as an oncogene in hepatocellular carcinoma. *Proc Natl Acad Sci USA* 2010;107(18):8357–8362. doi:10.1073/pnas.1000374107, PMID:20404171.
 - [60] Yoo BK, Gredler R, Vozhilla N, Su ZZ, Chen D, Forcier T, *et al.* Identification of genes conferring resistance to 5-fluorouracil. *Proc Natl Acad Sci USA* 2009;106(31):12938–12943. doi:10.1073/pnas.0901451106, PMID:19622726.
 - [61] Santhekadur PK, Rajasekaran D, Siddiq A, Gredler R, Chen D, Schaus SE, *et al.* The transcription factor LSF: a novel oncogene for hepatocellular carcinoma. *Am J Cancer Res* 2012;2(3):269–85. PMID:22679558.
 - [62] Santhekadur PK, Gredler R, Chen D, Siddiq A, Shen XN, Das SK, *et al.* Late SV40 factor (LSF) enhances angiogenesis by transcriptionally up-regulating matrix metalloproteinase-9 (MMP-9). *J Biol Chem* 2012;287(5):3425–3432. doi:10.1074/jbc.M111.298976, PMID:22167195.
 - [63] Yoo BK, Gredler R, Chen D, Santhekadur PK, Fisher PB, Sarkar D. c-Met activation through a novel pathway involving osteopontin mediates oncogenesis by the transcription factor LSF. *J Hepatol* 2011;55(6):1317–1324. doi:10.1016/j.jhep.2011.02.036, PMID:21703197.
 - [64] Santhekadur PK, Kumar DP. RISC assembly and post-transcriptional gene regulation in Hepatocellular Carcinoma. *Genes Dis* 2020;7(2):199–204. doi:10.1016/j.gendis.2019.09.009, PMID:32215289.
 - [65] Asgharpour A, Cazanave SC, Pacana T, Seneshaw M, Vincent R, Bani BA, *et al.* A diet-induced animal model of non-alcoholic fatty liver disease and hepatocellular cancer. *J Hepatol* 2016;65(3):579–588. doi:10.1016/j.jhep.2016.05.005, PMID:27261415.
 - [66] Srinivas AN, Suresh D, Mirshahi F, Santhekadur PK, Sanyal AJ, Kumar DP. Emerging roles of AATF: Checkpoint signaling and beyond. *J Cell Physiol* 2021;236(5):3383–3395. doi:10.1002/jcp.30141, PMID:33145763.
 - [67] Kaiser RWJ, Erber J, Höpker K, Fabretti F, Müller RU. AATF/Che-1-An RNA Binding Protein at the Nexus of DNA Damage Response and Ribosome Biogenesis. *Front Oncol* 2020;10:919. doi:10.3389/fonc.2020.00919, PMID:32587828.
 - [68] Fanciulli M, Bruno T, Di Padova M, De Angelis R, Iezzi S, Iacobini C, *et al.* Identification of a novel partner of RNA polymerase II subunit 11, Che-1, which interacts with and affects the growth suppression function of Rb. *FASEB J* 2000;14(7):904–912. doi:10.1096/fasebj.14.7.904, PMID:10783144.
 - [69] Lindfors K, Halttunen T, Huotari P, Nupponen N, Vihinen M, Visakorpi T, *et al.* Identification of novel transcription factor-like gene from human intestinal cells. *Biochem Biophys Res Commun* 2000;276(2):660–666. doi:10.1006/bbrc.2000.3480, PMID:11027528.
 - [70] Tian Y, Mok MT, Yang P, Cheng AS. Epigenetic Activation of Wnt/β-Catenin Signaling in NAFLD-Associated Hepatocarcinogenesis. *Cancers (Basel)* 2016;8(8):76. doi:10.3390/cancers8080076, PMID:27556491.
 - [71] Liu LJ, Xie SX, Chen YT, Xue JL, Zhang CJ, Zhu F. Aberrant regulation of Wnt signaling in hepatocellular carcinoma. *World J Gastroenterol* 2016;22(33):7486–7499. doi:10.3748/wjg.v22.i33.7486, PMID:2767271.
 - [72] Liu YC, Yeh CT, Lin KH. Cancer Stem Cell Functions in Hepatocellular Carcinoma and Comprehensive Therapeutic Strategies. *Cells* 2020;9(6):1331. doi:10.3390/cells9061331, PMID:32466488.
 - [73] Chen J, Gingold JA, Su X. Immunomodulatory TGF-β Signaling in

- Hepatocellular Carcinoma. *Trends Mol Med* 2019;25(11):1010–1023. doi:10.1016/j.molmed.2019.06.007, PMID:31353124.
- [74] van Zijl F, Zulehner G, Petz M, Schneller D, Kornauth C, Hau M, *et al.* Epithelial-mesenchymal transition in hepatocellular carcinoma. *Future Oncol* 2009;5(8):1169–1179. doi:10.2217/fon.09.91, PMID:19852728.
- [75] Zhao B, Wei X, Li W, Udan RS, Yang Q, Kim J, *et al.* Inactivation of YAP oncoprotein by the Hippo pathway is involved in cell contact inhibition and tissue growth control. *Genes Dev* 2007;21(21):2747–2761. doi:10.1101/gad.1602907, PMID:17974916.
- [76] Boopathy GTK, Hong W. Role of Hippo Pathway-YAP/TAZ Signaling in Angiogenesis. *Front Cell Dev Biol* 2019;7:49. doi:10.3389/fcell.2019.00049, PMID:31024911.
- [77] Meng Z, Moroishi T, Guan KL. Mechanisms of Hippo pathway regulation. *Genes Dev* 2016;30(1):1–17. doi:10.1101/gad.274027.115, PMID:26728553.
- [78] Bae SJ, Luo X. Activation mechanisms of the Hippo kinase signaling cascade. *Biosci Rep* 2018;38(4):BSR20171469. doi:10.1042/BSR20171469, PMID:30038061.
- [79] Valero V 3rd, Pawlik TM, Anders RA. Emerging role of Hpo signaling and YAP in hepatocellular carcinoma. *J Hepatocell Carcinoma* 2015;2:69–78. doi:10.2147/JHC.S48505, PMID:27508196.
- [80] Nybakken K, Perrimon N. Hedgehog signal transduction: recent findings. *Curr Opin Genet Dev* 2002;12(5):503–511. doi:10.1016/s0959-437x(02)00333-7, PMID:12200154.
- [81] Della Corte CM, Viscardi G, Papaccio F, Esposito G, Martini G, Ciardiello D, *et al.* Implication of the Hedgehog pathway in hepatocellular carcinoma. *World J Gastroenterol* 2017;23(24):4330–4340. doi:10.3748/wjg.v23.i24.4330, PMID:28706416.
- [82] Zheng X, Zeng W, Gai X, Xu Q, Li C, Liang Z, *et al.* Role of the Hedgehog pathway in hepatocellular carcinoma (review). *Oncol Rep* 2013;30(5):2020–2026. doi:10.3892/or.2013.2690, PMID:23970376.
- [83] Lu JT, Zhao WD, He W, Wei W. Hedgehog signaling pathway mediates invasion and metastasis of hepatocellular carcinoma via ERK pathway. *Acta Pharmacol Sin* 2012;33(5):691–700. doi:10.1038/aps.2012.24, PMID:22543708.
- [84] Sicklick JK, Li YX, Jayaraman A, Kannangai R, Qi Y, Vivekanandan P, *et al.* Dysregulation of the Hedgehog pathway in human hepatocarcinogenesis. *Carcinogenesis* 2006;27(4):748–757. doi:10.1093/carcin/bgi292, PMID:16339184.
- [85] Cheng WT, Xu K, Tian DY, Zhang ZG, Liu LJ, Chen Y. Role of Hedgehog signaling pathway in proliferation and invasiveness of hepatocellular carcinoma cells. *Int J Oncol* 2009;34(3):829–836. doi:10.3892/ijo.00000209, PMID:19212688.
- [86] Chung SI, Moon H, Ju HL, Cho KJ, Kim DY, Han KH, *et al.* Hepatic expression of Sonic Hedgehog induces liver fibrosis and promotes hepatocarcinogenesis in a transgenic mouse model. *J Hepatol* 2016;64(3):618–627. doi:10.1016/j.jhep.2015.10.007, PMID:26471504.
- [87] Cai H, Li H, Li J, Li X, Li Y, Shi Y, *et al.* Sonic hedgehog signaling pathway mediates development of hepatocellular carcinoma. *Tumour Biol* 2016;37(12):16199–16205. doi:10.1007/s13277-016-5463-6, PMID:27744627.
- [88] Matsuo N, Shiraha H, Fujikawa T, Takaoka N, Ueda N, Tanaka S, *et al.* Twist expression promotes migration and invasion in hepatocellular carcinoma. *BMC Cancer* 2009;9:240. doi:10.1186/1471-2407-9-240, PMID:19615090.
- [89] Yamamoto S, Schulze KL, Bellen HJ. Introduction to Notch signaling. *Methods Mol Biol* 2014;1187:1–14. doi:10.1007/978-1-4939-1139-4_1, PMID:25053477.
- [90] Gao J, Song Z, Chen Y, Xia L, Wang J, Fan R, *et al.* Deregulated expression of Notch receptors in human hepatocellular carcinoma. *Dig Liver Dis* 2008;40(2):114–121. doi:10.1016/j.dld.2007.08.001, PMID:17920003.
- [91] Wang M, Xue L, Cao Q, Lin Y, Ding Y, Yang P, *et al.* Expression of Notch1, Jagged1 and beta-catenin and their clinicopathological significance in hepatocellular carcinoma. *Neoplasma* 2009;56(6):533–41. doi:10.4149/neo_2009_06_533, PMID:19728763.
- [92] Sun Q, Wang R, Luo J, Wang P, Xiong S, Liu M, *et al.* Notch1 promotes hepatitis B virus X protein-induced hepatocarcinogenesis via Wnt/ β -catenin pathway. *Int J Oncol* 2014;45(4):1638–1648. doi:10.3892/ijo.2014.2537, PMID:25017705.
- [93] Giovannini C, Gramantieri L, Chicco P, Minguzzi M, Lago F, Pianetti S, *et al.* Selective ablation of Notch3 in HCC enhances doxorubicin's death promoting effect by a p53 dependent mechanism. *J Hepatol* 2009;50(5):969–979. doi:10.1016/j.jhep.2008.12.032, PMID:19304334.
- [94] Wang F, Zhou H, Yang Y, Xia X, Sun Q, Luo J, *et al.* Hepatitis B virus X protein promotes the growth of hepatocellular carcinoma by modulation of the Notch signaling pathway. *Oncol Rep* 2012;27(4):1170–1176. doi:10.3892/or.2012.1620, PMID:22218807.
- [95] Zhang Q, Lu C, Fang T, Wang Y, Hu W, Qiao J, *et al.* Notch3 functions as a regulator of cell self-renewal by interacting with the β -catenin pathway in hepatocellular carcinoma. *Oncotarget* 2015;6(6):3669–3679. doi:10.18632/oncotarget.2898, PMID:25668819.
- [96] Furuyama K, Kawaguchi Y, Akiyama H, Horiguchi M, Kodama S, Kuhara T, *et al.* Continuous cell supply from a Sox9-expressing progenitor zone in adult liver, exocrine pancreas and intestine. *Nat Genet* 2011;43(1):34–41. doi:10.1038/ng.722, PMID:21113154.
- [97] Villanueva A, Alsinet C, Yanger K, Hoshida Y, Zong Y, Toffanin S, *et al.* Notch signaling is activated in human hepatocellular carcinoma and induces tumor formation in mice. *Gastroenterology* 2012;143(6):1660–1669.e7. doi:10.1053/j.gastro.2012.09.002, PMID:22974708.
- [98] Kawaguchi K, Honda M, Yamashita T, Okada H, Shirasaki T, Nishikawa M, *et al.* Jagged1 DNA Copy Number Variation Is Associated with Poor Outcome in Liver Cancer. *Am J Pathol* 2016;186(8):2055–2067. doi:10.1016/j.ajpath.2016.04.011, PMID:27315779.
- [99] Ahn S, Hyeon J, Park CK. Notch1 and Notch4 are markers for poor prognosis of hepatocellular carcinoma. *Hepatobiliary Pancreat Dis Int* 2013;12(3):286–294. doi:10.1016/s1499-3872(13)60046-6, PMID:23742774.
- [100] Amini A, Masoumi Moghaddam S, Morris DL, Pourgholami MH. The critical role of vascular endothelial growth factor in tumor angiogenesis. *Curr Cancer Drug Targets* 2012;12(1):23–43. doi:10.2174/156800912798888956, PMID:22111836.
- [101] Muto J, Shirabe K, Sugimachi K, Maehara Y. Review of angiogenesis in hepatocellular carcinoma. *Hepatol Res* 2015;45(1):1–9. doi:10.1111/hepr.12310, PMID:24533487.
- [102] Amarapurkar AD, Amarapurkar DN, Vibhav S, Patel ND. Angiogenesis in chronic liver disease. *Ann Hepatol* 2007;6(3):170–173. PMID:17786144.
- [103] van der Meel R, Symons MH, Kudernatsch R, Kok RJ, Schiffrers RM, Storm G, *et al.* The VEGF/Rho GTPase signalling pathway: a promising target for anti-angiogenic/anti-invasion therapy. *Drug Discov Today* 2011;16(5-6):219–228. doi:10.1016/j.drudis.2011.01.005, PMID:21262381.
- [104] Rivlin N, Brosh R, Oren M, Rotter V. Mutations in the p53 Tumor Suppressor Gene: Important Milestones at the Various Steps of Tumorigenesis. *Genes Cancer* 2011;2(4):466–474. doi:10.1177/1947601911408889, PMID:21779514.
- [105] Link T, Iwakuma T. Roles of p53 in extrinsic factor-induced liver carcinogenesis. *Hepatoma Res* 2017;3:95–104. doi:10.20517/2394-5079.2017.07, PMID:30123836.
- [106] Claudia K, Marika H, Sebastian H, Sophie S, Martina M. The p53 family in hepatocellular carcinoma. *Transl Cancer Res* 2016;5(6):632–636. doi:10.21037/11030.
- [107] Nag S, Qin J, Srivenugopal KS, Wang M, Zhang R. The MDM2-p53 pathway revisited. *J Biomed Res* 2013;27(4):254–271. doi:10.7555/JBR.27.20130030, PMID:23885265.
- [108] de Fromental CC, Levrero M. p53 functional loss, stemness and hepatocellular carcinoma. *Hepatoma Res* 2020;6:80. doi:10.20517/2394-5079.2020.77.
- [109] Knudsen ES, Wang JY. Targeting the RB-pathway in cancer therapy. *Clin Cancer Res* 2010;16(4):1094–1099. doi:10.1158/1078-0432.CCR-09-0787, PMID:20145169.
- [110] Heilmann AM, Dyson NJ. Phosphorylation puts the pRb tumor suppressor into shape. *Genes Dev* 2012;26(11):1128–1130. doi:10.1101/gad.195552.112, PMID:22661226.
- [111] Linn P, Kohno S, Sheng J, Kulathunga N, Yu H, Zhang Z, *et al.* Targeting RB1 Loss in Cancers. *Cancers (Basel)* 2021;13(15):3737. doi:10.3390/cancers13153737, PMID:34359636.
- [112] Indovina P, Pentimalli F, Casini N, Vocca I, Giordano A. RB1 dual role in proliferation and apoptosis: cell fate control and implications for cancer therapy. *Oncotarget* 2015;6(20):17873–17890. doi:10.18632/

- oncotarget.4286, PMID:26160835.
- [113] Anwar SL, Krech T, Hasemeier B, Schipper E, Schweitzer N, Vogel A, *et al.* Deregulation of RB1 expression by loss of imprinting in human hepatocellular carcinoma. *J Pathol* 2014;233(4):392–401. doi:10.1002/path.4376, PMID:24838394.
 - [114] Momparler RL. Cancer epigenetics. *Oncogene* 2003;22(42):6479–6483. doi:10.1038/sj.onc.1206774, PMID:14528271.
 - [115] Wilson CL, Mann DA, Borthwick LA. Epigenetic reprogramming in liver fibrosis and cancer. *Adv Drug Deliv Rev* 2017;121:124–132. doi:10.1016/j.addr.2017.10.011, PMID:29079534.
 - [116] Toh TB, Lim JJ, Chow EK. Epigenetics of hepatocellular carcinoma. *Clin Transl Med* 2019;8(1):13. doi:10.1186/s40169-019-0230-0, PMID:31056726.
 - [117] Bonilla Guerrero R, Roberts LR. The role of hepatitis B virus integrations in the pathogenesis of human hepatocellular carcinoma. *J Hepatol* 2005;42(5):760–777. doi:10.1016/j.jhep.2005.02.005, PMID:15826727.
 - [118] Kremsdorf D, Soussan P, Paterlini-Brechot P, Brechot C. Hepatitis B virus-related hepatocellular carcinoma: paradigms for viral-related human carcinogenesis. *Oncogene* 2006;25(27):3823–3833. doi:10.1038/sj.onc.1209559, PMID:16799624.
 - [119] Fung J, Lai CL, Yuen MF. Hepatitis B and C virus-related carcinogenesis. *Clin Microbiol Infect* 2009;15(11):964–970. doi:10.1111/j.1469-0691.2009.03035.x, PMID:19874379.
 - [120] Bréchet C. Pathogenesis of hepatitis B virus-related hepatocellular carcinoma: old and new paradigms. *Gastroenterology* 2004;127(5 Suppl 1):S56–S61. doi:10.1053/j.gastro.2004.09.016, PMID:15508104.
 - [121] Okuda M, Li K, Beard MR, Showalter LA, Scholle F, Lemon SM, *et al.* Mitochondrial injury, oxidative stress, and antioxidant gene expression are induced by hepatitis C virus core protein. *Gastroenterology* 2002;122(2):366–375. doi:10.1053/gast.2002.30983, PMID:11832451.
 - [122] Szymański P, Olszewska P, Mikiciuk-Olasik E, Różalski A, Maszewska A, Markiewicz Ł, *et al.* Novel tetrahydroacridine and cyclopentaquinoline derivatives with fluorobenzoic acid moiety induce cell cycle arrest and apoptosis in lung cancer cells by activation of DNA damage signaling. *Tumour Biol* 2017;39(3):1010428317695011. doi:10.1177/1010428317695011, PMID:28351316.
 - [123] Hussain SP, Schwank J, Staib F, Wang XW, Harris CC. TP53 mutations and hepatocellular carcinoma: insights into the etiology and pathogenesis of liver cancer. *Oncogene* 2007;26(15):2166–2176. doi:10.1038/sj.onc.1210279, PMID:17401425.
 - [124] Brown RL, Chen ZY, Cleveland TE, Russin JS. Advances in the development of host resistance in corn to aflatoxin contamination by *aspergillus flavus*. *Phytopathology* 1999;89(2):113–7. doi:10.1094/PHYTO.1999.89.2.113, PMID:18944783.
 - [125] Osna NA, Donohue TM Jr, Kharbanda KK. Alcoholic Liver Disease: Pathogenesis and Current Management. *Alcohol Res* 2017;38(2):147–161. PMID:28988570.
 - [126] Szabo G, Saha B. Alcohol's effect on host defense. *Alcohol Res* 2015;37(2):159–170. PMID:26695755.
 - [127] Setshedi M, Wands JR, Monte SM. Acetaldehyde adducts in alcoholic liver disease. *Oxid Med Cell Longev* 2010;3(3):178–185. doi:10.4161/oxim.3.3.12288, PMID:20716942.
 - [128] Matsushita H, Takaki A. Alcohol and hepatocellular carcinoma. *BMJ Open Gastroenterol* 2019;6(1):e000260. doi:10.1136/bmj-gast-2018-000260, PMID:31139422.
 - [129] Ramadori P, Cubero FJ, Liedtke C, Trautwein C, Nevzorova YA. Alcohol and Hepatocellular Carcinoma: Adding Fuel to the Flame. *Cancers (Basel)* 2017;9(10):130. doi:10.3390/cancers9100130, PMID:28946672.
 - [130] Erstad DJ, Fuchs BC, Tanabe KK. Molecular signatures in hepatocellular carcinoma: A step toward rationally designed cancer therapy. *Cancer* 2018;124(15):3084–3104. doi:10.1002/cncr.31257, PMID:29663340.
 - [131] Neuman MG, Maor Y, Nanau RM, Melzer E, Mell H, Opris M, *et al.* Alcoholic Liver Disease: Role of Cytokines. *Biomolecules* 2015;5(3):2023–2034. doi:10.3390/biom5032023, PMID:26343741.
 - [132] Zakhari S. Overview: how is alcohol metabolized by the body? *Alcohol Res Health* 2006;29(4):245–254. PMID:17718403.
 - [133] Bisteau X, Caldez MJ, Kaldis P. The Complex Relationship between Liver Cancer and the Cell Cycle: A Story of Multiple Regulations. *Cancers (Basel)* 2014;6(1):79–111. doi:10.3390/cancers6010079, PMID:24419005.
 - [134] Javed A, Malagrabá G, Yarmohammadi M, Perelló-Reus CM, Barceló C, Rubio-Tomás T. Therapeutic potential of mitotic kinases' inhibitors in cancers of the gastrointestinal system. *Future Pharmacology* 2022;2(3):214–237. doi:10.3390/futurepharmacol2030015.
 - [135] Marra M, Sordelli IM, Lombardi A, Lamberti M, Tarantino L, Giudice A, *et al.* Molecular targets and oxidative stress biomarkers in hepatocellular carcinoma: an overview. *J Transl Med* 2011;9:171. doi:10.1186/1479-5876-9-171, PMID:21985599.
 - [136] Raghunath A, Sundarraj K, Arfuso F, Sethi G, Perumal E. Dysregulation of Nrf2 in Hepatocellular Carcinoma: Role in Cancer Progression and Chemoresistance. *Cancers (Basel)* 2018;10(12):481. doi:10.3390/cancers10120481, PMID:30513925.
 - [137] Chen L, Deng H, Cui H, Fang J, Zuo Z, Deng J, *et al.* Inflammatory responses and inflammation-associated diseases in organs. *Oncotarget* 2017;9(6):7204–7218. doi:10.18632/oncotarget.23208, PMID:29467962.
 - [138] Lee J, Taneja V, Vassallo R. Cigarette smoking and inflammation: cellular and molecular mechanisms. *J Dent Res* 2012;91(2):142–149. doi:10.1177/0022034511421200, PMID:21876032.
 - [139] Zong D, Liu X, Li J, Ouyang R, Chen P. The role of cigarette smoke-induced epigenetic alterations in inflammation. *Epigenetics Chromatin* 2019;12(1):65. doi:10.1186/s13072-019-0311-8, PMID:31711545.
 - [140] Brandt-Rauf PW, Li Y, Long C, Monaco R, Kovvali G, Marion MJ. Plastics and carcinogenesis: The example of vinyl chloride. *J Carcinog* 2012;11:5. doi:10.4103/1477-3163.93700, PMID:22529741.
 - [141] Mastrangelo G, Fedeli U, Fadda E, Valentini F, Agnesi R, Magarotto G, *et al.* Increased risk of hepatocellular carcinoma and liver cirrhosis in vinyl chloride workers: synergistic effect of occupational exposure with alcohol intake. *Environ Health Perspect* 2004;112(11):1188–1192. doi:10.1289/ehp.6972, PMID:15289165.
 - [142] Robertson LW, Ludewig G. Polychlorinated Biphenyl (PCB) carcinogenicity with special emphasis on airborne PCBs. *Gefährst Reinhalt Luft* 2011;71(1-2):25–32. PMID:21686028.
 - [143] Ludewig G, Robertson LW. Polychlorinated biphenyls (PCBs) as initiating agents in hepatocellular carcinoma. *Cancer Lett* 2013;334(1):46–55. doi:10.1016/j.canlet.2012.11.041, PMID:23211541.
 - [144] Armengol S, Arretxe E, Enzunza L, Llorente I, Mendibil U, Navarro-Imaz H, *et al.* SREBP-2-driven transcriptional activation of human SND1 oncogene. *Oncotarget* 2017;8(64):108181–108194. doi:10.18632/oncotarget.22569, PMID:29296233.
 - [145] Wang X, Xin L, Duan Z, Zuo Z, Wang Y, Ren Y, *et al.* Global Tudor-SN transgenic mice are protected from obesity-induced hepatic steatosis and insulin resistance. *FASEB J* 2019;33(3):3731–3745. doi:10.1096/fj.201801253RR, PMID:30521378.
 - [146] Ao J, Wei C, Si Y, Luo C, Lv W, Lin Y, *et al.* Tudor-SN regulates milk synthesis and proliferation of bovine mammary epithelial Cells. *Int J Mol Sci* 2015;16(12):29936–29947. doi:10.3390/ijms161226212, PMID:26694361.
 - [147] Tsuchiya N, Ochiai M, Nakashima K, Ubagai T, Sugimura T, Nakagama H. SND1, a component of RNA-induced silencing complex, is up-regulated in human colon cancers and implicated in early stage colon carcinogenesis. *Cancer Res* 2007;67(19):9568–76. doi:10.1158/0008-5472.CAN-06-2707, PMID:17909068.