[Frontiers in Bioscience, Landmark, 20, 946-963, June 1, 2015]

Epigenetic regulation of hepatic tumor-initiating cells

Jia Ding[1,2], Jian Wu[1,3,4]

[1]Key Laboratory of Medical Molecular Virology, Ministries of Education and Public Health, Fudan University Shanghai Medical College, Shanghai 200032, China; [2]Department of Gastroenterology, Shanghai Jing'an District Central Hospital, Shanghai 200040, China; [3]University of California, Davis Medical Center, Dept. of Internal Medicine, Division of Gastroenterology & Hepatology, Sacramento, CA 95817, USA; [4]Shanghai Institute of Liver Diseases, Fudan University Affiliated Zhongshan Hospital, Shanghai 200032, China

TABLE OF CONTENTS

1. ABSTRACT

Hepatocellular carcinoma (HCC) is the third most lethal cancer and resistant to common chemotherapy. Tumor-initiating cells (T-ICs) that are thought to be responsible for tumorigenesis share surface markers and signaling pathways similar to normal tissue stem cells. Identification of T-ICs and elucidation of aberrant epigenetic modulation and self-renewal pathways may provide new insights into hepatic carcinogenesis, metastasis and chemotherapeutic resistance. Histone modification, DNA methylation and microenvironmental changes are considered as key elements to promote the derivation and function of T-ICs. In this review, we intend to compare the similarity and difference between normal liver stem cells and T-ICs, and to define the intrinsic and micro-environmental factors that lead to the transformation from normal liver stem cells to hepatic T-ICs. We believe that etiology, microenvironmental alteration, epigenetic modification and epithelial-mesenchymal transition play a fundamental role in initiating the transformation. Strategies targeting signaling molecules critical in modulating these processes may offer a personalized therapy for HCC in the future.

2. INTRODUCTION

The stochastic and the hierarchic models are two hypotheses of carcinogenesis. Different from the traditional stochastic model, the hierarchic model

Table 1. Epigenetic modification of histones and DNA

Kinds of modification	Catalytic enzymes	Biologic effects
Histone acetylation	Histone acetyltransferase (HAT)	Reducing nucleosome stability and DNA accessibility (9)
Histone deacetylation	Histone deacetylase (HDAC)	Overexpression of HDAC was found in HCC tissue, and HDAC inhibitors suppress oncogenic potential (12)
Histone methylation	Histone methyltransferase (HMT)	Net consequence of methylation depends on the specific lysine residues to be methylated
Histone demethylation	Histone demethylase (HDM), such as JmjC family, which removes methyl group in lysine residues	Demethylase LSD1 in HCC tissue is negative (120)
Histone phosphorylation	Protein kinase	Phosphorylation of histone 3 leads to chromosome condensation & segregation (16)
Histone dephosphorylation	Phosphatase	For the balance of histone phosphorylation status
DNA methylation	DNA methyltransferase (DMNTs)	Hypermethylation leads to inactivation of TSGs
Methylation of CpG islands	DMNTs	In general, methylation of CpG islands results in the silence of specific gene involved.
Demethylation	Demethylase	Hypomethylation leads to genomic instability (25)

postulates that a subpopulation of tumor-initiating cells (T-ICs), also called cancer stem cells (CSCs), gives rise to heterogeneous populations in a tumor mass. Discovery of stem cells within primary intestinal adenomas, an intestinal precancerous lesion by lineage tracing, provides direct evidence for the presence of T-ICs (1). T-ICs possess many fundamental similarities to tissue stem cells, including self-renewal, differentiation and proliferation capacity. In addition to these properties, T-ICs may evolve to subpopulations which are capable of metastasis and resistance to conventional chemotherapy. Over the past decade, T-ICs have been identified in the hematopoietic malignancies, neural, breast and other solid tumors (2). Therapeutic strategies targeting T-ICs not only promise to eliminate cancers more efficiently, but also reduce the risk of tumor relapse and metastasis.

Hepatocellular carcinoma (HCC) is the third most lethal cancer in the world (3), while most of patients are inoperable as detected at an advanced stage. It is conceivable that approaches that could eradicate hepatic T-ICs in combination with conventional therapies will effectively improve the outcomes of HCC treatment. Several cell types in the liver have a long life-span, including liver stem/progenitor cells, hepatocytes and cholangiocytes. Evidence from human mammary epithelial cells demonstrated that differentiated normal and neoplastic non-stem cells could spontaneously convert to a stem-like state under circumstances such as inflammation, chemotherapy or irradiation, and that the conversion without genetic manipulation may involve epigenetic modifications (4). Epigenetic modulations include histone modification, DNA methylation and chromatin remodeling, and may alter promoter activity and transcription status of individual genes; and even result in reprogramming with activation of specific transcription factors, such as pluripotent genes without any changes in the DNA sequence. It is hypothesized that once liver cells overcome specific epigenetic barriers, they may gain “stemness” or a pluripotent trait. Thus, hepatic T-ICs may originate from liver stem/progenitor cells or dedifferentiation of mature hepatocytes (5, 6). However, how epigenetic modulations manipulate the fate of liver stem cells towards malignancy remains unknown. In-depth elucidation of epigenetic regulation in hepatic T-ICs will aid in understanding the pathogenesis of HCC and formulating potential remedies. This review focuses on the role of epigenetic regulation in the transformation from normal stem cells to T-ICs.

3. COMMON EPIGENETIC REGULATIONS OF HCC

Key epigenetic modifications include histone modifications, DNA methylation and chromatin remodeling, which could result in activation or repression of specific genes, reprogramming at a transcriptional level or modifications at a post-translational stage (Table 1). Genetic and epigenetic elements interact in multiple steps during a progressive process in liver carcinogenesis. Half of primary HCC is estimated to undergo chromatin remodeling and epigenetic modifications (7).

3.1. Histone modifications

As long linear DNA forms chromosomes by wrapping histone and other proteins, histone modifications directly affect chromatin structure and function. Eight kinds of post-translational modifications including acetylation, methylation, phosphorylation, sumoylation, ubiquitination, biotinylation and ADP-ribosylation may occur in histones (8). Histone acetylation or de-acetylation is the modification of multiple lysine

residues catalyzed by histone acetyltransferase (HAT) and histone deacetylase (HDAC), respectively. As acetylation removes the positive charge on the histones; hence, the affinity between histone and negatively charged DNA is weakened. Therefore, the degree of histone acetylation may affect nucleosome stability and DNA accessibility (9). HDAC-4 expression was often up-regulated in HCC specimens compared with adjacent noncancerous liver tissues (10). Overexpression of HDAC-1 in HCC is associated with a high incidence of invasion, chemo-resistance and poor prognosis (11); whereas, inhibition of HDAC-1 suppressed cell growth, induced autophagic cell death, and reduced tumorigenic potential of hepatoma Hep3B cells (12). Histone methylation is the modification of arginine or lysine residues in histone by the addition of methyl groups. Histone methylation status is determined by the balanced action of histone methyltransferase (HMT) and histone demethylase (HDM). The net consequence of histone methylation depends on the modification at specific lysine residues. For instance, histone H3 lysine 27 (H3K27) tri-methylation and histone H3 lysine 9 (H3K9) methylation result in transcriptional repression; whereas methylation at histone H3 lysine 4 (H3K4) causes transcriptional activation (13). High expression of H3K27me3 was observed in 63.2 percent of HCC tissues by immunohistochemistry, and the expression levels positively or negatively correlated with poor differentiation, advanced clinical stage, vascular invasion and shortened survival duration (14). In a different study tissue microarrays exhibited that high expression of H3K4me3 was observed in 45~50 percent of HCC specimens and was a valid predictor of reduced overall survival (15). Histone phosphorylation occurs at serine, threonine or tyrosine residues catalyzed by a number of protein kinase, and dephosphorylated by phosphatases. Phosphorylation of histone 3 is associated with chromosome condensation and segregation (16). In a diethylnitrosamine (DEN)-induced rodent HCC model, DEN increased phosphorylation of histone H3 at serine 10 residue within the promoter region of RNA polymerase III-dependent genes to modulate their transcription, which led to hepatocellular proliferation and transformation (17).

3.2. DNA methylation

DNA methylation occurs at the 5'-promoter region containing cytosine-guanine dinucleotide (CpG) islands in a specific gene. The demethylated status of these genes switches them into a transcriptional activation state; whereas the methylated status in general refers to transcriptional silence (18). The major DNA methyltransferases (DNMTs) consist of DNMT-1, DNMT-3a and DNMT-3b. DNMT-1 maintains the DNA methylation status during DNA replication; whereas DNMT-3a and DNMT-3b are involved in establishing *de novo* DNA methylation (19). Hypermethylation in CpG islands at the promoter region of tumor suppressor genes (TSG) may lead to their inactivation. In order to identify specifically altered DNA methylation, quantitative methylation analyses at the promoter regions and in a genome-wide fashion have been employed in HCC, cirrhotic and normal livers. A number of hypermethylated TSGs were selected to predict the emergence of HCC, including *GSTP1*, *p16INK4-alpha* (20), *SHP* (21), *HIC1*, *SOCS1*, *RASSF1*, *CDKN2A*, *APC*, *RUNX3*, *PRDM2* (22), *Lefty* (23), *SMPD3* and *NEFH* (24). On the contrary, DNA hypomethylation increases genomic instability and occurs in the regions where it is normally hypermethylated. For instance, the human telomerase reverse transcriptase (hTERT) promoter was heavily methylated in normal liver tissue; whereas it was hypomethylated in most HCC specimens. The status of DNA methylation and histone modifications affected the ability of c-myc binding to the hTERT promoter, and regulated hTERT expression levels. Epigenetic reactivation of hTERT, which was seen in nearly 70 percent of HCC tissues, promoted the occurrence and progression of HCC (25). Therefore, it appears that both global DNA hypomethylation and TSG hypermethylation play crucial roles in hepatic carcinogenesis by governing specific gene expression levels, such as activation of hTERT or inactivation of tumor suppressive genes.

Polycomb group protein (PGP) forms polycomb repressive complexes (PRCs) to silence differentiation-triggering genes, and maintains pluripotency of embryonic stem cells (ESCs) through H3K27me3. These developmental genes are repressed in ESCs and activated during differentiation (26). On the other hand, PRC target genes (such as pluripotency factors) were hypermethylated in HCC as compared to non-cancerous tissues, which implied that aberrant methylation is a mechanism for hepatic T-ICs to maintain the "stemness" (27, 28). It is known that hypermethylation may occur in an aging liver; whereas methylation in HCC exhibits in a gene-specific and disease-specific manner (29).

4. LIVER STEM CELLS AND PUTATIVE HEPATIC TUMOR-INITIATING CELLS

4.1. Normal liver progenitor cells and surface markers

During an embryonic development stage, the liver bud from the definite endoderm develops to bipotential liver progenitor cells (LPCc). LPCs and rodent oval cells express stem cell markers, such as epithelial cell adhesion molecule (EpCAM), oval cell marker 6 (OV-6) and neural cell adhesion molecule (NCAM), as well as hepatocyte-specific (albumin) and cholangiocyte-specific markers, such as cytokeratin-19 (CK-19) (30). As a continuation of LPCs, hepatoblasts further mature to hepatocyte and cholangiocyte lineages. Hepatoblasts uniquely express the combination of EpCAM, intercellular cell adhesion molecule (ICAM-1), CK-19, albumin and alpha-fetoprotein (AFP). Mature hepatocytes and

Table 2. Origins of hepatic T-ICs

• Reprogramming of normal liver progenitor cells
• Dedifferentiation of matured hepatocyte or cholangiocytes
• Transformation from hepatoblasts, hepatocytes or cholangiocytes
• Deviation from bulk tumor cells into T-ICs or CTCs for tumor relapse or metastases
• Originated from stromal or mesenchymal cells by reprogramming or transformation
• Side population with a unclear origin of cell types

cholangiocytes no longer express stem cell markers, but only their lineage-specific markers (31). Four main (Wnt, Notch, Hedgehog and transforming growth factor-beta (TGF-beta)) signaling pathways are involved during the liver developmental stage, and they are responsible for hepatic lineage specification and morphogenesis (32). In adult human, multipotent stem/progenitor cells exist in the periductular space within the biliary tress. They express classic endodermal transcription factors (SOX-17, SOX-9, HNF-6 and SALL-4) and stem/progenitor surface markers (EpCAM, NCAM, CD133 and CXCR4) (33). These intermediate hepatobiliary cells proliferate primarily in regenerative responses when acute or chronic liver damage occurs or there is a loss of liver mass (34).

4.2. Definition of tumor-initiating cells (T-ICs) or cancer stem cells (CSCs)

To define a T-IC subpopulation from HCC tissue or a hepatoma cell line, it is crucial to have reliable cell surface markers which can be used for separating them from bulk of cancer cells. Throughout the recent studies, a group of surface markers have been selected based on following criteria: First, the expression of selected surface markers is observed in human HCC specimens or circulating tumor cells (CTCs)(35), and the selected surface markers overlap with those for putative LPSc (36). Second, cells from hepatoma with positive markers exhibit higher tumorigenicity ability *in vitro* and *in vivo* than those with negative markers. Moreover, further serial transplantation of hepatoma cells with the positive markers from tumor xenografts could continuously generate tumors in the second and third batch of immunodeficient mice. Third, hepatoma cells with the positive markers not only possess self-renewal capacity, but also are capable of differentiating into both positive and negative marked cells. Fourth, hepatoma cells with the positive markers express genes important for the proliferation, self-renewal and differentiation of stem cells (37). Fifth, blockage of these markers could significantly inhibit cellular invasion, spheroid formation and tumorigenicity. According to these standards, CD133 (38), CD90 (39, 40) and EpCAM (41, 42) have been chosen as useful markers of liver T-ICs. However, the origins and definition of T-ICs are not well specified, and studies with different patient specimens, hepatoma cell lines or primary tumors from animal models may have a variety of cell sources which could potentially become the origins for T-ICs under particular circumstances as listed in Table 2.

4.3. Side subpopulation

Another subpopulation not in full accordance with above criteria is side population (SP) cells. These cells are defined by their ability to efflux the DNA-binding dye Hoechst 33342 through an adenosine triphosphate (ATP)-binding cassette (ABC) membrane transporter associated with multidrug resistance (43). SP cells were found to display higher proliferation activity, apoptotic tolerance and oncogenic capacity in serial NOD-SCID xenograft transplantation than non-SP cells (44). Hepatic progenitor markers (CD44, EpCAM and Bmi) were upregulated in these SP cells. SP cells isolated from a myc-driven mouse HCC model were resistant to chemotherapy (doxorubicin and paclitaxel) through multidrug resistance gene 1 (MDR1) transporter (45). It is claimed that T-ICs are a subset of the SP cells and contribute to resistance and relapse after chemotherapy or irradiation (45). However, SP cells that have stem cell potential and generate all cell types in a tumor usually do not have the same surface marker profile as T-ICs. Whether they are the same subpopulation as those selected by T-ICs markers need to be verified.

5. ROLE OF ETIOLOGY AND MICROENVIRONMENTAL CHANGES IN EPIGENETIC MODIFICATIONS

HCC is heterogeneous in terms of oncogenic pathways, molecular regulation, progressive and metastatic capability due to the fact that it derives from various base diseases. Therefore, tumors from various individuals may arise from a different cell of origin. Undoubtedly, etiology plays an important role in epigenetic modulation of hepatocytes from which HCC may be derived. Viral infection, inflammation and oxidative stress could change the microenvironment niche and induce epigenetic instability, and the latter in turn fosters the appearance of tumor-initiating cells.

5.1. Hepatitis B viral infection

Hepatitis B viral infection is the leading cause of HCC in the Asian and African regions and the mechanisms of HBV-associated HCC have been studies for decades, however they largely remain to be poorly understood. Recent studies revealed that the HBV X protein (HBx) is responsible for the aberrant epigenetic regulation, and resulted in a malignant transformation. HBx not only functioned as a transcription factor to enhance the expression of DNMT-1, DNMT-3A1 and DNMT-3A2, which in turn repress host tumor suppressor genes by hypermethylation of their promoters (46), but also upregulated tumor-initiating genes through hypomethylation, such as aldehyde dehydrogenase 1 (ALDH-1), plasma retinol-binding protein precursor

(RBP), and cellular retinol-binding protein 1 (CRBP1) (47). Moreover, HBx downregulated the chromatin remodeling protein suppressor of zeste 12 homolog (Suz12) and zinc finger protein, MYM-type 2 (Znf198) (48). Suz12 is a component of the polycomb repressive complexes PRC2 which mediates H3K27 trimethylation (49); whereas Znf198 stabilizes the lysine-specific demethylase 1-corepressor of RE1-silencing transcription factor histone deacetylase-1 (LSD1-CoREST-HDAC-1) complex, and the latter removes histone modifications affecting transcriptional activation (50). Repression of Suz12 enhanced the EpCAM expression and promoted cell proliferation (51). Immunohistochemical analysis of HBV-infected HCC tissue showed that HBx was associated with upregulation of "stemness" transcription factors: Oct-4, Nanog and KLF-4, as well as "stemness" marker: EpCAM. Consistent with stem cell behavior, HBx-transfected HepG2 cells displayed pronounced tumorigenic ability *in vitro* and *in vivo*. Thus, HBx-induced epigenetic alterations, including aberrant histone modifications, DNA methylation and critical transcription factors, such as Suz12 and Znf98 or a repressor, facilitate the differentiation of HBV-infected cells towards hepatic T-ICs.

5.2. Hepatitis C viral infection

Hepatitis C virus is the second to HBV that causes chronic hepatitis and an increased risk for HCC. In a chimeric mouse model, a long period of HCV infection elicited methylation of 237±110 genes, some of which were increased in HCC specimens compared to noncancerous tissues (52). Wnt/beta-catenin signaling plays an important role in the development, cell differentiation or proliferation and metabolism of the liver. Epigenetic modification of a Wnt antagonist, secreted frizzled related proteins (SFRP), may result in abnormal activation of Wnt signaling. The HCV core protein directly increased the expression and binding of DNMT-1 and HDAC-1 to the transcriptional start site of the SFRP-1 promoter, which led to hypermethylation of SFRP-1 and silence of its expression, and eventually the accelerated growth and aggressiveness of HCC (53). In HCV core gene transgenic mice fed alcohol model, enhanced DNA hypomethylation, histone acetylation and demethylation elicited the generation of TLR4-Nanog dependent T-ICs (54). Thus, the HCV core protein directly or indirectly affects epigenetic alterations that may expedite the oncogenic transformation in the host cells.

5.3. Chronic inflammation

Chronic inflammation enables hepatic carcinogenesis by induction of mutations and chromosomal instability, and by the release of a wide range of cytokines, such as TNF-alpha, interleukins and TGF-beta, that mediate interactions between inflammatory pathways and epigenetic modifications (55). Among these cytokines, TGF-beta is responsible for suppressing the proliferation of LPCs to acquire characteristics of T-ICs (56). In human cirrhotic liver, increased TGF-beta levels were correlated with OV-6-positive LPCs that co-express CD133, CD90 and EpCAM. After treatment with TGF-beta, rat pluripotent LPC-like WB-F344 cells constantly exhibited mesenchymal characteristics and expressed more CD90 and CD133 than control cells. Moreover, TGF-beta-1-treated cells generated xenograft tumors in NOD-SCID mice. TGF-beta-1 increased CD133 expression by inducing demethylation of the CD133 promoter-1. After TGF-beta-1 bound to the TGF-beta receptor, both Smad2 and Smad3 were phosphorylated, and they formed a hetero-complex. The latter was translocated into the nucleus and suppressed DNMT1 and DNMT-3-beta expression. Decreased DNMT-1 and DNMT-3-beta finally led to demethylation of the CD133 promoter-1(57). In albumin-cre transgenic mice lacking p53 ($Trp53^{KO}$) model, inactivation of p53 and TGF-beta receptor II reduced the phosphorylation of downstream Smad3 and extracellular signal-regulated kinase (ERK)1/2, which in consequence down-regulated TGF-beta target genes. Thus, it is not difficult to understand that the disruption of the TGF-beta pathway triggered the emergence of T-IC, and accelerated HCC progression through p53 inactivation (58).

5.4. Oxidative stress

Sustained HBV or HCV infection, alcohol liver toxicity or NAFLD causes chronic liver damage and oxidative stress, and eventually HCC develops in these base diseases. Excess of endogenous and exogenous reactive oxygen species (ROS) results in DNA damage and alteration of the methylation status. H_2O_2 induced hypermethylation of the E-cadherin promoter through upregulation of snail expression. Snail elicited E-cadherin hypermethylation through recruiting DNMT-1 and HDAC-1 (59). Besides inactivation of TSGs through DNA hypermethylation, ROS activates a positive feedback loop that exacerbates a chronic state of inflammation. As mentioned above, chronic liver inflammation initiates epigenetic instability and in turn results in abnormal hepatocellular proliferation and oncogenic transformation in an inflammatory microenvironment where oxidant stress is constantly overwhelmed, such as NASH, alcoholic liver injury, ion-over load and drug toxicity, even without cirrhosis (60).

6. EPIGENETIC MODIFICATION FACILITATES THE TRANSFORMATION TOWARDS T-ICS

Overexpression of four transcription factors (Oct-4, Sox-2, KLF-4 and C-myc) could reprogram fibroblasts into induced pluripotent stem cells (iPSC) (61). Similarly, introduction of these four factors into gastrointestinal cells may reprogram them into a pluripotent state and sensitize them to a malignant transformation (62). In a carcinogen-induced AH130 hepatoma model, o-aminoazotoluene exposure accomplished a series of reprogramming and

tumorigenesis step-wisely. Eventually 100 percent of AH130 cells expressed immature marker CD133, and three transcription factors: Nanog, KLF-4 and c-myc (63). Expression of these stemness and oncogenic genes was probably the primary intrinsic force driving the oncogenic transformation. Thus, an initiating event such as viral infection, inflammation or drug toxicity, may activate the "epigenetic switch" that resets the long-term memory of hepatocytes or liver stem cells, and provoke their transformation to hepatic T-ICs. The following are examples of how epigenetic modifications lead to oncogenic reprogramming towards T-ICs.

6.1. Histone ubiquitination and acetylation

As a core element of PRC1, B lymphoma moloney murine leukemia virus insertion region 1 homolog (Bmi-1) plays an important role in the self-renewal of LPCs. Forced over-expression of Bmi-1 by lentiviral vectors enhanced self-renewal capacity of LPCs. Transplantation of Bmi-1-transduced single cells into NOD-SCID mice resulted in hepatic carcinogenesis (64). Chromatin immunoprecipitation (ChIP) assays and microarray analyses demonstrated that LPCs achieve their tumorigenic potential through reducing the specific promoter activity, by suppressing *Ink4a/Arf* tumor suppressor gene and Sox-17 gene expression, and increasing levels of monoubiquitinylated histone H2A (65).

Levels of histone 3/4 acetylation were higher in Nanog-negative Huh7 and patient-derived primary HCC T115 cells than Nanog-positive CSC cells. High expression of HDAC-3 significantly correlated with poor prognosis of HCC patients and expression of Nanog and CD133 in primary human HCC tissues. Either HDAC inhibitors (TSA) or knock-down of HDAC-3 suppressed cell growth and self-renewal of $CD133^{+}$ and $Nanog^{+}$ T-ICs cells. Meanwhile, the same treatment induced differentiation of $Nanog^{+}$ T-ICs cells through increasing levels of histone-3/4 acetylation, and decreased expression of pluripotent transcription factors, such as Nanog, Sox-2 and Oct-4 (66). Therefore, modification of HDAC-3 activity may enhance pluripotency of $Nanog^{+}$ T-ICs.

SALL-4 is one of classic endodermal transcription factors, and causes upregulated expression of LPC markers (EpCAM, CK19 and CD44), and enhanced spheroid formation capacity of $EpCAM^{+}$ cells. It was found that its activity was mediated through modification of HDAC activity, because HDAC inhibitors suppressed the proliferation of $SALL\text{-}4^{+}$ cells (67). In addition to interaction with HDAC-1, SALL-4 could suppress target gene expression through direct interaction with DNMTs and mediate activity of the gene product, HDAC-1 (68).

In summary, it appears that pluripotent transcription factors, such as Nanog, Sox-2, Oct-4 or SALL-4, affect the fate of genetic reprogramming by regulating activity of specific target genes via the activity change of HDAC-1 or DNMTs at epigenetic levels.

6.2. DNA methylation and demethylation

Global genomic DNA hypomethylation has been observed as a frequent event in various cancers. Methylation of long interspersed nucleotide element-1 (LINE-1) was reported to represent as a surrogate marker for the global DNA methylation and a molecular marker of prognosis for many solid tumors (69, 70). It is demonstrated in the tamoxifen-induced rat hepatic carcinogenic model that exposure to tamoxifen resulted in LINE-1 hypomethylation and increased expression of LINE-1 and c-myc. Tamoxifen-containing diet up-regulated regenerative cell proliferation of rat hepatocytes (71). In human HCC specimens, LINE-1 demethylation correlated to elevated CD133 expression and shorter cumulative survival (72). Therefore, it is speculated that LINE-1 demthylation could be a trigger for the transformation of mature hepatocytes to oncogenic T-ICs in this model.

6.3. Epigenetic modification of transcription factors affecting pluripotency

Epigenetic regulation of transcription factors, such as Nanog, Oct-4, Sox-2, c-Myc and KLF-4 provokes pluripotency of cancer cells. Hypomethylation of the Nanog promoter was observed in primary HCC specimens and $CD133^{+high}$ colorectal carcinoma cells. In accordance with this, the upregulation of Nanog was associated with demethylation of the Oct-4 promoter and enhanced Oct-4 mRNA expression (73). Oct-4 overexpression in turn activated the Oct-4-AKT-ABCG2 pathway, and the latter eventually led to chemo-resistance. Furthermore, these chemo-resistant HCC cells displayed stem cell features (74). Thus, demethylation of pluripotent genes contributed to epigenetic reprogramming and initiation of T-ICs.

6.4. Aberrant hedgehog signaling in hepatic T-ICs

Hedgehog (Hh) signaling governs the fate of progenitor cells during embryogenesis, and helps orchestrate liver development and repair. Reactivation of Hh in adulthood occurs during liver regeneration, inflammation, fibrogenesis and vascular remodeling (75-77). In NAFLD, the level of Hh signaling activation in the liver tissue paralleled with the extent of steatohepatitis and fibrosis. The level of Hh signaling activity was also strongly correlated with clinical parameters of metabolic syndrome (age, BMI, waist circumference, log HOMA-insulin resistance and hypertension) (78). A retrospective analysis demonstrated that 65 percent (20/31) of individuals with metabolic syndrome developed HCC without significant fibrosis (79), which implied that the Hh signaling may be of paramount importance in carcinogenesis in these patients. The Hh signaling had been identified to be

crucial for the maintenance of T-ICs in multiple myeloma and myeloid leukemia (80,81). In HBV/HCV-associated HCC, Hh-responsive cells, including T-ICs, expanded during cirrhosis and HCC (82). Hypermethylation of the Hh-interacting protein (HHIP) gene, the negative regulator of Hh signaling, led to down-regulation of the HHIP at a transcriptional level in hepatoma cells and HCC tissues. Based on growing evidence, it appears to be most likely that hypermethylation elicited activation of the Hh pathway, which in turn stimulated liver transformation and HCC development (83).

6.5. Abnormal activation of Wnt-beta-catenin signaling activity

In active necrotic inflammation, a strong activation of the Wnt signaling stimulated the proliferation of LPCs (84). In Huh7 cells and primary HCC tissues, OV6-positive progenitor cells expressed high levels of β-catenin and displayed increased proliferation capacity. General activation of the Wnt/beta-catenin pathway has been observed in one third of HCCs, and activation of the Wnt/beta-catenin pathway provoked expression of EpCAM, enhanced self-renewal potential and chemotherapy resistance (85, 86). The increased Wnt/beta-catenin signaling in HCC samples and hepatoma cell lines was caused by hypermethylation and histone H3 lysine 27 trimethylation of Wnt antagonists, which lead to reduced expression of Wnt antagonists (87, 88). Epigenetic down-regulation of the Wnt signaling antagonists would in turn lead to hyperactivation of the Wnt/beta-catenin pathway into a positive feedback loop, and consequently enhanced proliferation of HCC.

7. ROLE OF MICRORNAS IN MODULATING EPIGENETIC STATUS

miRNAs are a class of non-coding RNAs that regulate gene expression by binding to 3'-untranslated regions (UTR) of target mRNAs. Tremendous efforts have been dedicated to identify specific miRNAs that play oncogenic or suppressive roles in HCC (89, 90). Up to date, miRNAs that have been referred as tumor-suppressors of HCC included miRNA-101 (91), miRNA-152 (92), miRNA-125b (93, 94), miRNA-503 (95), miRNA-200b (96) and let-7c (97). In contrast, miRNA-519d (98), miRNA-224 (99), miRNA-21 and miRNA-17-92 are considered as oncogenic for HCC (100, 101).

In addition to functioning as translational repressors, miRNAs may regulate the expression of target genes through interaction with epigenetic components. Enhancer of zeste homolog 2 (EZH2), the H3K27 tri-methylating enzyme, was upregulated in 69.5 percent (41/59) of primary HCCs, and multiple tumor-suppressor miRNAs were epigenetically silenced by EZH2. miRNAs that were silenced in this fashion included miR-139-5p, miR-125b, miR-101, let-7c and miR-200b (102). In addition to interaction with the H3K27 tri-methylating enzyme, miR-152 targeted 3'UTR of DNMT-1 and caused increased hypermethylation of two tumor suppressor genes (*GSTP1* and *CDH1*) in HBV-related HCC (92). Thus, miRNAs not only modulate target gene expression at translational levels, but also affect oncogenes or tumor suppressive genes at transcriptional levels via epigenetic modification. The following is a list of miRNAs which alter expression of target oncogenic or pluripotent genes through epigenetic modulation.

7.1. miR-148a

MiR-148a is identified as a liver-specific miRNA highly expressed in mature hepatocytes, and frequently down-regulated in human HCC lines and tissues. In mouse fetal hepatoblasts, miR-148a facilitated hepatic differentiation through DNMT-1 inhibition. MiR-148a directly regulated DNMT-1 expression by complementary binding to its 3'-UTR element. In Hepa 1-6 hepatoma cells, miR-148a suppressed invasive properties of HCC cells by indirectly inhibiting hepatocyte growth factor receptor (c-Met) (103). As IL-6 increased the expression of DNMT-1 through miR-148a in human cholangiocarcinoma cell lines, miR-148a provides a link between inflammation-associated cytokine and oncogenesis in cholangiocarcinoma (104).

7.2. miR-122

MiR-122 is a critical miRNA abundant in the liver, and is involved in hepatic carcinogenesis, lipid metabolism and HCV replication. MiR-122 was highly enriched in differentiated human hepatocytes, but its expression decreased in hESCs and HCC. miR-122 functions as a modulator to suppress self-renewal and proliferation of hESCs and HCC through inhibiting the expression of *Pkm2*. In human primary hepatocytes, the promoter of miR-122 was hypo-methylated, which facilitated RNA polymerase II to bind and initiate transcription. Whereas, hypermethylation of the same region in hESCs and HCC made it impossible for RNA polymerase II to bind and in turn inhibited miR-122 transcription (105). Treating the HepG2 and Hep3B cells with a DNA methylation inhibitor (5'aza-2'deoxycytidine) restored the expression of miR-122. In addition, peroxisome proliferator activated receptor-gamma (PPAR-gamma) and retinoid X receptor alpha (RXR-alpha) complex could bind to the miR-122 promoter and enhance its transcription (106).

7.3. miR-214

The attenuated expression of miR-214 was often observed in HCC tissue and highly associated with tumor early recurrence. miR-214 directly targeted 3'-UTR sequence of EZH2 and β-catenin mRNA, and inhibited their protein expression. Knockdown miR-214 expression increased $EpCAM^+$ stem-like HLE cells through activating β-catenin signaling. Thus, miR-214 induced stem-like features of HCC, and may be closely involved in early tumor recurrence (107).

In summary, a group of miRNAs are oncogenic and their expression is often suppressed in hepatic malignancy, whereas other miRNAs act as tumor-suppressors by inhibiting expression of genes involved in oncogenesis. These tumor-suppressive miRNAs are often down-regulated in HCC. The influence of miRNAs on epigenetic changes depends on the target gene specificity of individual miRNAs, and their effects range from differentiation, aggressive behavior, drug resistance to metastasis. The dissection and verification of each miRNA function in these processes will enhance our understanding of critical but complicated interplay of multiple miRNAs in oncogenesis, metastasis or relapse.

8. EPITHELIAL MESENCHYMAL TRANSITION AND EPIGENETIC MODIFICATIONS

Epithelial-mesenchymal transition (EMT) is defined as a process by which epithelial cells lose cell adhesion and baso-apical polarity, meanwhile acquire mesenchymal features. The molecular characteristic of EMT is the down-regulation of E-cadherin and upregulation of mesenchymal markers, including N-cadherin, vimentin and fibronectin. EMT occurs in such cases as normal organ development and embryogenesis, tissue damage, inflammation, fibrosis and tumor metastasis (108-110). Expression of EMT-related transcription factors, such as snail and twist, was upregulated in scirrhous HCC, a rare variant of HCC which is characterized by abundant fibrous stroma and expression of several LPC markers (such as EpCAM and CK19) (111). In a Pten loxp/loxp/Alb-Cre^{+} mouse model, a second round of xenograft transplantation expanded from CD133^{+} cells isolated from Pten$^{-/-}$ mice demonstrated epithelial and mesenchymal phenotypes. Within fibroblastoid-like cells, E-cadherin and keratins were decreased, while mesenchymal markers (MMP2/3, snail1 and zeb1/2) were significantly increased. Mesenchymal phenotypic cells secreted a high level of HGF to stimulate proliferation of epithelial cells. In turn, stimulated epithelial cells changed to fibroblastoid-like cells and became more aggressive and invasive (112). CTCs isolated from a mouse HCC model exhibited an EMT status, and increased expression of HGF and its receptor (c-Met) was demonstrated in these cells. Demethylation of HGF and c-Met increased expression of HGF, which in turn switched CTC to display EMT characters, increased tumorigenicity and metastatic potential (113). Increasing matrix stiffness drove HCC cells to acquire the mesenchymal properties and to be resistant to chemotherapy *in vitro*. On contrary, a soft environment enhanced the colony initiating capacity of chemotherapy-treated cells, which was associated with increased “stemness” gene expression, including CD133, CD44, OCT-4 and NANOG (114). In summary, EMT facilitates disseminated tumor cells to emigrate from the primary tumor microenvironment and to establish micrometastases at distal sites.

9. CONCLUSIONS AND PERSPECTIVES

Pathogenic processes, such as viral infection, inflammation, oxidative stress and drug toxicity alter the micro-environment in the liver and elicit abnormal epigenetic modifications, including histone acetylation or deacetalytion, and DNA hyper- or hypomethylation. In these processes, miRNAs participate in the regulation of their target gene expression in addition to their translational repression. The aberrant epigenetic modifications facilitate the transformation of normal LPCs to hepatic T-ICs in the response to changes of the microenvironmental niche; whereas the activation of “stemness” transcription factors, such as KLF-4, Nanog, Oct-4, Bmi-1 or Sox-2 or 17, as well as oncogenic signaling molecules, such as TGF-beta-1, hedgehog, beta-catinen, met or c-myc results in a genetic/epigenetic reprogramming. These signaling molecules may be considered as intrinsic forces in driving the malignant transformation and the appearance of T-ICs. Although the effects and regulation underlying the complex interplays of each epigenetic modification on histone and other nuclear proteins, signaling molecules remain largely unexplored, the net consequence is the occurrence and expansion of T-ICs in various disease bases. These T-ICs probably are the origins of many cell types in a tumor mass. It is also conceivable that T-ICs may be induced during various stages of cancer development and treatment, and these T-ICs are mainly responsible for chemo- or radiation-resistance, and are believed to be the cell origins for relapse (34). EMT has been suggested as a critical phenotypic change for cancer cells to be chemo-resistant and metastatic. Given the fact that the markers used for T-IC isolation and characterization are different from various studies, it is understandable that T-ICs are not the same from different disease bases or at various stages of HCC development and progression. Whether a unique T-IC population will be identified for HCC depends on the success of a large cohort of clinical trials by multi-center collaboration with a defined combination of surface markers, such as CD133, CD90 or EpCAM, etc. (115, 116). It is foreseeable that such a trial aimed at defining the most representative “hepatic T-ICs” by multi-disciplinary investigators is warranted in the near future. For a better understanding of this complex interplay, a schematic illustration is shown as Figure 1.

On the other hand, the identification of critically abnormal epigenetic modificatio`ns in HCC development and progression offers molecular targets for fine-tuning therapeutics and personalized therapy strategies. Increasing evidence demonstrates that a therapeutic strategy targeting a single pathway is insufficient to eliminate HCC. Due to critical roles of epigenetic modulations on hepatic T-IC function and differentiation, the epigenetic therapy may point to a novel direction in the development of next generation of therapeutics. For instance, as a self-renewal regulator, EZH2, modulates the self-renewal and

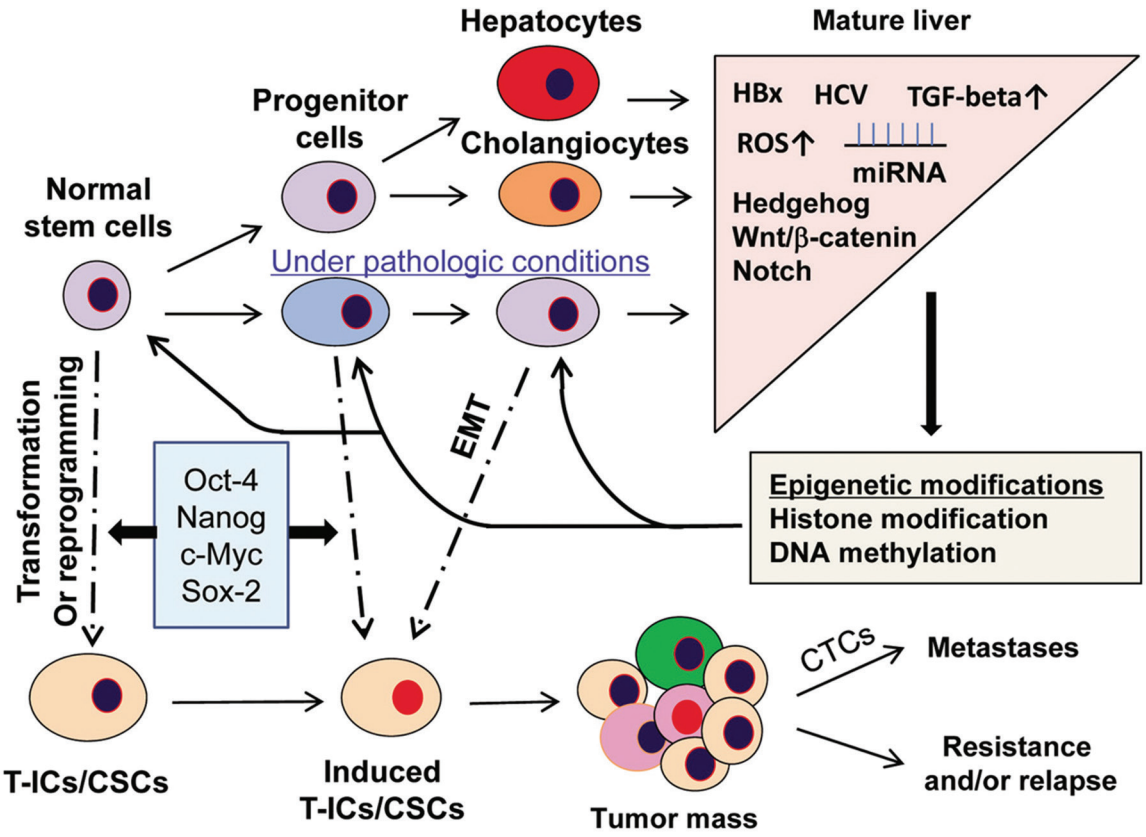

Figure 1. Schematic illustration of how epigenetic modifications affect the transformation of normal liver stem cells, progenitor cells or matured hepatocytes or cholangiocytes into malignant T-ICs. As shown in Table 1, various cell types in the liver under a variety of pathologic conditions could be the sources for transformation caused by reprogramming, genetic or epigenetic modifications. The interplays of how specific epigenetic changes affect critical transcription factors controlling reprogramming remain largely unexplored. However, the pathologic alternations leading to changes of the microenvironmental niche in the liver provoke the epigenetic modifications of critical signaling molecules or transcription factors controlling reprogramming or EMT, miRNA expression. New T-ICs may be induced during chemotherapy or irradiation for drug-resistance, and may be responsible for relapse. Circulating tumor cells (CTCs) are the cells of disseminated in the bloodstream, and may form new metastatic lesions in distal sites or return to the original tumor sites after resection or implants after transplantation (reseeding). The abbreviations used in the illustration are the same as in the text.

differentiation of hepatic stem cells. 3-Deazaneplanocin A (DZNep), an EZH2 pharmacological inhibitor, decreased $EpCAM^+$ T-IC subpopulation and suppressed tumorigenesis in NOD-SCID mice (117). In this context, the use of EZH2 inhibitors, such as an S-adenosylhomocysteine hydrolase inhibitor, DZNep, might be a promising strategy through targeting hepatic T-ICs (117). A demethylating agent, zebularine, increased self-renewal and tumorigenicity of low density HCC cells. But treated HCC cells at high density with zebularine decreased self-renewal and tumorigenicity. Thus, the dual effect of this DNMT1 inhibitor on T-ICs characters depended on HCC cellular density (118). A multicenter phase I/II trial assessed the efficacy of a histone deacetylase inhibitor, belinostat, on unresectable HCC. The rate of partial response and stable disease of 42 patients was 2.4 and 45.2 percent, respectively. The progression-free and overall survival was 2.6 and 6.6 months, respectively. Epigenetic therapy with belinostat displayed well-tolerated and disease stabilization, and more studies are needed to further determine the efficacy and benefits with belinostat treatment in HCC patients (119). Obviously, questions remain regarding whether epigenetic therapy alone or in combination with conventional chemotherapy will be more effective in eradicating T-IC than currently available adjuvant therapeutics for the improvement of therapeutic outcome.

10. ACKNOWLEDGEMENTS

The present work was partially supported by the Natural Science Foundation of China (NSFC #81272436) and the Fudan University Starting Fund to Dr. Jian Wu.

11. REFERENCES

1. A. G. Schepers, H. J. Snippert, D. E. Stange, M. van den Born, J. H. van Es, M. van de Wetering and H. Clevers: Lineage tracing reveals Lgr5+ stem cell activity in mouse intestinal adenomas. *Science*, 337(6095), 730-5 (2012)
DOI: 10.1126/science.1224676

2. C. T. Jordan, M. L. Guzman and M. Noble: Cancer stem cells. *N Engl J Med*, 355(12), 1253-61 (2006)
DOI: 10.1056/NEJMra061808

3. A. Jemal, R. Siegel, E. Ward, Y. Hao, J. Xu, T. Murray and M. J. Thun: Cancer statistics, 2008. *CA Cancer J Clin*, 58(2), 71-96 (2008)
DOI: 10.3322/CA.2007.0010

4. C. L. Chaffer, I. Brueckmann, C. Scheel, A. J. Kaestli, P. A. Wiggins, L. O. Rodrigues, M. Brooks, F. Reinhardt, Y. Su, K. Polyak, L. M. Arendt, C. Kuperwasser, B. Bierie and R. A. Weinberg: Normal and neoplastic nonstem cells can spontaneously convert to a stem-like state. *Proc Natl Acad Sci U S A*, 108(19), 7950-5 (2011)
DOI: 10.1073/pnas.1102454108

5. T. Roskams: Liver stem cells and their implication in hepatocellular and cholangiocarcinoma. *Oncogene*, 25(27), 3818-22 (2006)
DOI: 10.1038/sj.onc.1209558

6. R. Bjerkvig, B. B. Tysnes, K. S. Aboody, J. Najbauer and A. J. Terzis: Opinion: the origin of the cancer stem cell: current controversies and new insights. *Nat Rev Cancer*, 5(11), 899-904 (2005)
DOI: 10.1038/nrc1740

7. Z. Zhang: Genomic landscape of liver cancer. *Nat Genet*, 44(10), 1075-7 (2012)
DOI: 10.1038/ng.2412

8. T. Kouzarides: Chromatin modifications and their function. *Cell*, 128(4), 693-705 (2007)
DOI: 10.1016/j.cell.2007.02.005

9. C. D. Allis, S. L. Berger, J. Cote, S. Dent, T. Jenuwien, T. Kouzarides, L. Pillus, D. Reinberg, Y. Shi, R. Shiekhattar, A. Shilatifard, J. Workman and Y. Zhang: New nomenclature for chromatin-modifying enzymes. *Cell*, 131(4), 633-6 (2007)
DOI: 10.1016/j.cell.2007.10.039

10. J. H. Yuan, F. Yang, B. F. Chen, Z. Lu, X. S. Huo, W. P. Zhou, F. Wang and S. H. Sun: The histone deacetylase 4/SP1/microrna-200a regulatory network contributes to aberrant histone acetylation in hepatocellular carcinoma. *Hepatology*, 54(6), 2025-35 (2011)
DOI: 10.1002/hep.24606

11. T. Rikimaru, A. Taketomi, Y. Yamashita, K. Shirabe, T. Hamatsu, M. Shimada and Y. Maehara: Clinical significance of histone deacetylase 1 expression in patients with hepatocellular carcinoma. *Oncology*, 72(1-2), 69-74 (2007)
DOI: 10.1159/000111106

12. H. J. Xie, J. H. Noh, J. K. Kim, K. H. Jung, J. W. Eun, H. J. Bae, M. G. Kim, Y. G. Chang, J. Y. Lee, H. Park and S. W. Nam: HDAC1 inactivation induces mitotic defect and caspase-independent autophagic cell death in liver cancer. *PLoS One*, 7(4), e34265 (2012)
DOI: 10.1371/journal.pone.0034265

13. J. W. Han and Y. S. Yoon: Epigenetic landscape of pluripotent stem cells. *Antioxid Redox Signal*, 17(2), 205-23 (2012)
DOI: 10.1089/ars.2011.4375

14. M. Y. Cai, J. H. Hou, H. L. Rao, R. Z. Luo, M. Li, X. Q. Pei, M. C. Lin, X. Y. Guan, H. F. Kung, Y. X. Zeng and D. Xie: High expression of H3K27me3 in human hepatocellular carcinomas correlates closely with vascular invasion and predicts worse prognosis in patients. *Mol Med*, 17(1-2), 12-20 (2011)
DOI: 10.2119/molmed.2010.00103

15. C. He, J. Xu, J. Zhang, D. Xie, H. Ye, Z. Xiao, M. Cai, K. Xu, Y. Zeng, H. Li and J. Wang: High expression of trimethylated histone H3 lysine 4 is associated with poor prognosis in hepatocellular carcinoma. *Hum Pathol*, 43(9), 1425-35 (2012)
DOI: 10.1016/j.humpath.2011.11.003

16. D. Rossetto, N. Avvakumov and J. Cote: Histone phosphorylation: a chromatin modification involved in diverse nuclear events. *Epigenetics*, 7(10), 1098-108 (2012)
DOI: 10.4161/epi.21975

17. Q. Zhong, G. Shi, Q. Zhang, Y. Zhang, D. Levy and S. Zhong: Role of phosphorylated histone H3 serine 10 in DEN-induced deregulation of Pol III genes and cell proliferation and transformation. *Carcinogenesis*, 34(11), 2460-9 (2013)
DOI: 10.1093/carcin/bgt219

18. S. B. Baylin: DNA methylation and gene silencing in cancer. *Nat Clin Pract Oncol*, 2 Suppl 1, S4-11 (2005)
DOI: 10.1038/ncponc0354

19. H. J. Park, E. Yu and Y. H. Shim: DNA methyltransferase expression and DNA hypermethylation in human hepatocellular

carcinoma. *Cancer Lett*, 233(2), 271-8 (2006) DOI: 10.1016/j.canlet.2005.03.017

20. J. Harder, O. G. Opitz, J. Brabender, M. Olschewski, H. E. Blum, S. Nomoto and H. Usadel: Quantitative promoter methylation analysis of hepatocellular carcinoma, cirrhotic and normal liver. *Int J Cancer*, 122(12), 2800-4 (2008) DOI: 10.1002/ijc.23433
21. N. He, K. Park, Y. Zhang, J. Huang, S. Lu and L. Wang: Epigenetic inhibition of nuclear receptor small heterodimer partner is associated with and regulates hepatocellular carcinoma growth. *Gastroenterology*, 134(3), 793-802 (2008) DOI: 10.1053/j.gastro.2008.01.006
22. N. Nishida, M. Kudo, T. Nagasaka, I. Ikai and A. Goel: Characteristic patterns of altered DNA methylation predict emergence of human hepatocellular carcinoma. *Hepatology*, 56(3), 994-1003 (2012) DOI: 10.1002/hep.25706
23. A. Saito, H. Ochiai, S. Okada, N. Miyata and T. Azuma: Suppression of Lefty expression in induced pluripotent cancer cells. *FASEB J*, 27(6), 2165-74 (2013) DOI: 10.1096/fj.12-221432
24. K. Revill, T. Wang, A. Lachenmayer, K. Kojima, A. Harrington, J. Li, Y. Hoshida, J. M. Llovet and S. Powers: Genome-wide methylation analysis and epigenetic unmasking identify tumor suppressor genes in hepatocellular carcinoma. *Gastroenterology*, 145(6), 1424-35 e1-25 (2013) DOI: 10.1053/j.gastro.2013.08.055
25. D. Iliopoulos, M. Satra, A. Drakaki, G. A. Poultsides and A. Tsezou: Epigenetic regulation of hTERT promoter in hepatocellular carcinomas. *Int J Oncol*, 34(2), 391-9 (2009)
26. T. I. Lee, R. G. Jenner, L. A. Boyer, M. G. Guenther, S. S. Levine, R. M. Kumar, B. Chevalier, S. E. Johnstone, M. F. Cole, K. Isono, H. Koseki, T. Fuchikami, K. Abe, H. L. Murray, J. P. Zucker, B. Yuan, G. W. Bell, E. Herbolsheimer, N. M. Hannett, K. Sun, D. T. Odom, A. P. Otte, T. L. Volkert, D. P. Bartel, D. A. Melton, D. K. Gifford, R. Jaenisch and R. A. Young: Control of developmental regulators by Polycomb in human embryonic stem cells. *Cell*, 125(2), 301-13 (2006) DOI: 10.1016/j.cell.2006.02.043
27. O. Ammerpohl, J. Pratschke, C. Schafmayer, A. Haake, W. Faber, O. von Kampen, M. Brosch, B. Sipos, W. von Schonfels, K. Balschun, C. Rocken, A. Arlt, B. Schniewind, J. Grauholm, H. Kalthoff, P. Neuhaus, F. Stickel, S. Schreiber, T. Becker, R. Siebert and J. Hampe: Distinct DNA methylation patterns in cirrhotic liver and hepatocellular carcinoma. *Int J Cancer*, 130(6), 1319-28 (2012) DOI: 10.1002/ijc.26136
28. Y. L. Chen, C. J. Ko, P. Y. Lin, W. L. Chuang, C. C. Hsu, P. Y. Chu, M. Y. Pai, C. C. Chang, M. H. Kuo, Y. R. Chu, C. H. Tung, T. H. Huang, Y. W. Leu and S. H. Hsiao: Clustered DNA methylation changes in polycomb target genes in early-stage liver cancer. *Biochem Biophys Res Commun*, 425(2), 290-6 (2012) DOI: 10.1016/j.bbrc.2012.07.084
29. N. Nishida, T. Nagasaka, T. Nishimura, I. Ikai, C. R. Boland and A. Goel: Aberrant methylation of multiple tumor suppressor genes in aging liver, chronic hepatitis, and hepatocellular carcinoma. *Hepatology*, 47(3), 908-18 (2008) DOI: 10.1002/hep.22110
30. E. Schmelzer, E. Wauthier and L. M. Reid: The phenotypes of pluripotent human hepatic progenitors. *Stem Cells*, 24(8), 1852-8 (2006) DOI: 10.1634/stemcells.2006-0036
31. L. Zhang, N. Theise, M. Chua and L. M. Reid: The stem cell niche of human livers: symmetry between development and regeneration. *Hepatology*, 48(5), 1598-607 (2008) DOI: 10.1002/hep.22516
32. Y. Wang, Y. Hl, C. Barbier, E. Wauthier, C. Cb and N. Moss: Lineage-dependent epithelial–mesenchymal paracrine signals dictate growth versus differentiation of human hepatic stem cells to adult fates. *Hepatology*, 52, 1443-54 (2010)
33. V. Cardinale, Y. Wang, G. Carpino, C. B. Cui, M. Gatto, M. Rossi, P. B. Berloco, A. Cantafora, E. Wauthier, M. E. Furth, L. Inverardi, J. Dominguez-Bendala, C. Ricordi, D. Gerber, E. Gaudio, D. Alvaro and L. Reid: Multipotent stem/progenitor cells in human biliary tree give rise to hepatocytes, cholangiocytes, and pancreatic islets. *Hepatology*, 54(6), 2159-72 (2011) DOI: 10.1002/hep.24590
34. J. M. Rosen and C. T. Jordan: The increasing complexity of the cancer stem cell paradigm.

Science, 324(5935), 1670-3 (2009)
DOI: 10.1126/science.1171837

35. L. J. Wu, Y. D. Pan, X. Y. Pei, H. Chen, S. Nguyen, A. Kashyap, J. Liu and J. Wu: Capturing circulating tumor cells of hepatocellular carcinoma. *Cancer Lett*, 326(1), 17-22 (2012)
DOI: 10.1016/j.canlet.2012.07.024

36. X. R. Yang, Y. Xu, B. Yu, J. Zhou, S. J. Qiu, G. M. Shi, B. H. Zhang, W. Z. Wu, Y. H. Shi, B. Wu, G. H. Yang, Y. Ji and J. Fan: High expression levels of putative hepatic stem/progenitor cell biomarkers related to tumour angiogenesis and poor prognosis of hepatocellular carcinoma. *Gut*, 59(7), 953-62 (2010)
DOI: 10.1136/gut.2008.176271

37. J. S. Lee, J. Heo, L. Libbrecht, I. S. Chu, P. Kaposi-Novak, D. F. Calvisi, A. Mikaelyan, L. R. Roberts, A. J. Demetris, Z. Sun, F. Nevens, T. Roskams and S. S. Thorgeirsson: A novel prognostic subtype of human hepatocellular carcinoma derived from hepatic progenitor cells. *Nat Med*, 12(4), 410-6 (2006)
DOI: 10.1038/nm1377

38. S. Ma, K. W. Chan, L. Hu, T. K. Lee, J. Y. Wo, I. O. Ng, B. J. Zheng and X. Y. Guan: Identification and characterization of tumorigenic liver cancer stem/progenitor cells. *Gastroenterology*, 132(7), 2542-56 (2007)
DOI: 10.1053/j.gastro.2007.04.025

39. Z. F. Yang, D. W. Ho, M. N. Ng, C. K. Lau, W. C. Yu, P. Ngai, P. W. Chu, C. T. Lam, R. T. Poon and S. T. Fan: Significance of CD90+ cancer stem cells in human liver cancer. *Cancer Cell*, 13(2), 153-66 (2008)
DOI: 10.1016/j.ccr.2008.01.013

40. Z. F. Yang, P. Ngai, D. W. Ho, W. C. Yu, M. N. Ng, C. K. Lau, M. L. Li, K. H. Tam, C. T. Lam, R. T. Poon and S. T. Fan: Identification of local and circulating cancer stem cells in human liver cancer. *Hepatology*, 47(3), 919-28 (2008)
DOI: 10.1002/hep.22082

41. T. Yamashita, M. Forgues, W. Wang, J. W. Kim, Q. Ye, H. Jia, A. Budhu, K. A. Zanetti, Y. Chen, L. X. Qin, Z. Y. Tang and X. W. Wang: EpCAM and alpha-fetoprotein expression defines novel prognostic subtypes of hepatocellular carcinoma. *Cancer Res*, 68(5), 1451-61 (2008)
DOI: 10.1158/0008-5472.CAN-07-6013

42. T. Yamashita, J. Ji, A. Budhu, M. Forgues, W. Yang, H. Y. Wang, H. Jia, Q. Ye, L. X. Qin, E. Wauthier, L. M. Reid, H. Minato, M. Honda, S. Kaneko, Z. Y. Tang and X. W. Wang: EpCAM-positive hepatocellular carcinoma cells are tumor-initiating cells with stem/progenitor cell features. *Gastroenterology*, 136(3), 1012-24 (2009)
DOI: 10.1053/j.gastro.2008.12.004

43. M. A. Goodell, K. Brose, G. Paradis, A. S. Conner and R. C. Mulligan: Isolation and functional properties of murine hematopoietic stem cells that are replicating in vivo. *J Exp Med*, 183(4), 1797-806 (1996)

44. T. Chiba, K. Kita, Y. W. Zheng, O. Yokosuka, H. Saisho, A. Iwama, H. Nakauchi and H. Taniguchi: Side population purified from hepatocellular carcinoma cells harbors cancer stem cell-like properties. *Hepatology*, 44(1), 240-51 (2006)
DOI: 10.1002/hep.21227

45. E. K. Chow, L. L. Fan, X. Chen and J. M. Bishop: Oncogene-specific formation of chemoresistant murine hepatic cancer stem cells. *Hepatology*, 56(4), 1331-41 (2012)
DOI: 10.1002/hep.25776

46. I. Y. Park, B. H. Sohn, E. Yu, D. J. Suh, Y. H. Chung, J. H. Lee, S. J. Surzycki and Y. I. Lee: Aberrant epigenetic modifications in hepatocarcinogenesis induced by hepatitis B virus X protein. *Gastroenterology*, 132(4), 1476-94 (2007)
DOI: 10.1053/j.gastro.2007.01.034

47. A. Tong, L. Gou, Q. C. Lau, B. Chen, X. Zhao, J. Li, H. Tang, L. Chen, M. Tang, C. Huang and Y. Q. Wei: Proteomic profiling identifies aberrant epigenetic modifications induced by hepatitis B virus X protein. *J Proteome Res*, 8(2), 1037-46 (2009)
DOI: 10.1021/pr8008622

48. O. M. Andrisani: Deregulation of epigenetic mechanisms by the hepatitis B virus X protein in hepatocarcinogenesis. *Viruses*, 5(3), 858-72 (2013)
DOI: 10.3390/v5030858

49. S. L. Squazzo, H. O'Geen, V. M. Komashko, S. R. Krig, V. X. Jin, S. W. Jang, R. Margueron, D. Reinberg, R. Green and P. J. Farnham: Suz12 binds to silenced regions of the genome in

a cell-type-specific manner. *Genome Res*, 16(7), 890-900 (2006)
DOI: 10.1101/gr.5306606

50. C. B. Gocke and H. Yu: ZNF198 stabilizes the LSD1-CoREST-HDAC1 complex on chromatin through its MYM-type zinc fingers. *PLoS One*, 3(9), e3255 (2008)
DOI: 10.1371/journal.pone.0003255

51. L. L. Studach, S. Menne, S. Cairo, M. A. Buendia, R. L. Hullinger, L. Lefrancois, P. Merle and O. M. Andrisani: Subset of Suz12/PRC2 target genes is activated during hepatitis B virus replication and liver carcinogenesis associated with HBV X protein. *Hepatology*, 56(4), 1240-51 (2012)
DOI: 10.1002/hep.25781

52. Y. Okamoto, K. Shinjo, Y. Shimizu, T. Sano, K. Yamao, W. Gao, M. Fujii, H. Osada, Y. Sekido, S. Murakami, Y. Tanaka, T. Joh, S. Sato, S. Takahashi, T. Wakita, J. Zhu, J. P. Issa and Y. Kondo: Hepatitis virus infection affects DNA methylation in mice with humanized livers. *Gastroenterology*, 146(2), 562-72 (2014)
DOI: 10.1053/j.gastro.2013.10.056

53. H. Quan, F. Zhou, D. Nie, Q. Chen, X. Cai, X. Shan, Z. Zhou, K. Chen, A. Huang, S. Li and N. Tang: Hepatitis C virus core protein epigenetically silences SFRP1 and enhances HCC aggressiveness by inducing epithelial-mesenchymal transition. *Oncogene*, 33(22), 2826-35 (2014)
DOI: 10.1038/onc.2013.225

54. K. Machida, C. L. Chen, J. C. Liu, C. Kashiwabara, D. Feldman, S. W. French, L. Sher, J. J. Hyeongnam and H. Tsukamoto: Cancer stem cells generated by alcohol, diabetes, and hepatitis C virus. *J Gastroenterol Hepatol*, 27 Suppl 2, 19-22 (2012)
DOI: 10.1111/j.1440-1746.2011.07010.x

55. M. Martin and Z. Herceg: From hepatitis to hepatocellular carcinoma: a proposed model for cross-talk between inflammation and epigenetic mechanisms. *Genome Med*, 4(1), 8 (2012)
DOI: 10.1186/gm307

56. L. N. Nguyen, M. H. Furuya, L. A. Wolfraim, A. P. Nguyen, M. S. Holdren, J. S. Campbell, B. Knight, G. C. Yeoh, N. Fausto and W. T. Parks: Transforming growth factor-beta differentially regulates oval cell and hepatocyte proliferation. *Hepatology*, 45(1), 31-41 (2007)
DOI: 10.1002/hep.21466

57. H. You, W. Ding and C. B. Rountree: Epigenetic regulation of cancer stem cell marker CD133 by transforming growth factor-beta. *Hepatology*, 51(5), 1635-44 (2010)
DOI: 10.1002/hep.23544

58. S. M. Morris, J. Y. Baek, A. Koszarek, S. Kanngurn, S. E. Knoblaugh and W. M. Grady: Transforming growth factor-beta signaling promotes hepatocarcinogenesis induced by p53 loss. *Hepatology*, 55(1), 121-31 (2012)
DOI: 10.1002/hep.24653

59. S. O. Lim, J. M. Gu, M. S. Kim, H. S. Kim, Y. N. Park, C. K. Park, J. W. Cho, Y. M. Park and G. Jung: Epigenetic changes induced by reactive oxygen species in hepatocellular carcinoma: methylation of the E-cadherin promoter. *Gastroenterology*, 135(6), 2128-40, 2140 e1-8 (2008)
DOI: 10.1053/j.gastro.2008.07.027

60. N. Nishida and M. Kudo: Oxidative stress and epigenetic instability in human hepatocarcinogenesis. *Dig Dis*, 31(5-6), 447-53 (2013)
DOI: 10.1159/000355243

61. K. Takahashi, K. Tanabe, M. Ohnuki, M. Narita, T. Ichisaka, K. Tomoda and S. Yamanaka: Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell*, 131(5), 861-72 (2007)
DOI: 10.1016/j.cell.2007.11.019

62. N. Miyoshi, H. Ishii, K. Nagai, H. Hoshino, K. Mimori, F. Tanaka, H. Nagano, M. Sekimoto, Y. Doki and M. Mori: Defined factors induce reprogramming of gastrointestinal cancer cells. *Proc Natl Acad Sci U S A*, 107(1), 40-5 (2010)
DOI: 10.1073/pnas.0912407107

63. I. Marzi, M. G. Cipolleschi, M. D'Amico, T. Stivarou, E. Rovida, M. C. Vinci, S. Pandolfi, P. Dello Sbarba, B. Stecca and M. Olivotto: The involvement of a Nanog, Klf4 and c-Myc transcriptional circuitry in the intertwining between neoplastic progression and reprogramming. *Cell Cycle*, 12(2), 353-64 (2013)
DOI: 10.4161/cc.23200

64. T. Chiba, Y. W. Zheng, K. Kita, O. Yokosuka, H. Saisho, M. Onodera, H. Miyoshi, M. Nakano, Y. Zen, Y. Nakanuma, H. Nakauchi, A. Iwama

and H. Taniguchi: Enhanced self-renewal capability in hepatic stem/progenitor cells drives cancer initiation. *Gastroenterology*, 133(3), 937-50 (2007)
DOI: 10.1053/j.gastro.2007.06.016

65. T. Chiba, A. Seki, R. Aoki, H. Ichikawa, M. Negishi, S. Miyagi, H. Oguro, A. Saraya, A. Kamiya, H. Nakauchi, O. Yokosuka and A. Iwama: Bmi1 promotes hepatic stem cell expansion and tumorigenicity in both Ink4a/Arf-dependent and -independent manners in mice. *Hepatology*, 52(3), 1111-23 (2010)
DOI: 10.1002/hep.23793

66. C. Liu, L. Liu, J. Shan, J. Shen, Y. Xu, Q. Zhang, Z. Yang, L. Wu, F. Xia, P. Bie, Y. Cui, X. Zhang, X. Bian and C. Qian: Histone deacetylase 3 participates in self-renewal of liver cancer stem cells through histone modification. *Cancer Lett*, 339(1), 60-9 (2013)
DOI: 10.1016/j.canlet.2013.07.022

67. S. S. Zeng, T. Yamashita, M. Kondo, K. Nio, T. Hayashi, Y. Hara, Y. Nomura, M. Yoshida, T. Hayashi, N. Oishi, H. Ikeda, M. Honda and S. Kaneko: The transcription factor SALL4 regulates stemness of EpCAM-positive hepatocellular carcinoma. *J Hepatol*, 60(1), 127-34 (2014)
DOI: 10.1016/j.jhep.2013.08.024

68. J. Yang, T. R. Corsello and Y. Ma: Stem cell gene SALL4 suppresses transcription through recruitment of DNA methyltransferases. *J Biol Chem*, 287(3), 1996-2005 (2012)
DOI: 10.1074/jbc.M111.308734

69. A. S. Yang, M. R. Estecio, K. Doshi, Y. Kondo, E. H. Tajara and J. P. Issa: A simple method for estimating global DNA methylation using bisulfite PCR of repetitive DNA elements. *Nucleic Acids Res*, 32(3), e38 (2004)
DOI: 10.1093/nar/gnh032

70. L. Sigalotti, E. Fratta, E. Bidoli, A. Covre, G. Parisi, F. Colizzi, S. Coral, S. Massarut, J. M. Kirkwood and M. Maio: Methylation levels of the "long interspersed nucleotide element-1" repetitive sequences predict survival of melanoma patients. *J Transl Med*, 9, 78 (2011)
DOI: 10.1186/1479-5876-9-78

71. V. P. Tryndyak, O. Kovalchuk, L. Muskhelishvili, B. Montgomery, R. Rodriguez-Juarez, S. Melnyk, S. A. Ross, F. A. Beland and I. P. Pogribny: Epigenetic reprogramming of liver cells in tamoxifen-induced rat hepatocarcinogenesis. *Mol Carcinog*, 46(3), 187-97 (2007)
DOI: 10.1002/mc.20263

72. C. Zhang, Y. Xu, J. Zhao, L. Fan, G. Jiang, R. Li, Y. Ling, M. Wu and L. Wei: Elevated expression of the stem cell marker CD133 associated with Line-1 demethylation in hepatocellular carcinoma. *Ann Surg Oncol*, 18(8), 2373-80 (2011)
DOI: 10.1245/s10434-011-1599-1

73. X. Q. Wang, R. K. Ng, X. Ming, W. Zhang, L. Chen, A. C. Chu, R. Pang, C. M. Lo, S. W. Tsao, X. Liu, R. T. Poon and S. T. Fan: Epigenetic regulation of pluripotent genes mediates stem cell features in human hepatocellular carcinoma and cancer cell lines. *PLoS One*, 8(9), e72435 (2013)
DOI: 10.1371/journal.pone.0072435

74. X. Q. Wang, W. M. Ongkeko, L. Chen, Z. F. Yang, P. Lu, K. K. Chen, J. P. Lopez, R. T. Poon and S. T. Fan: Octamer 4 (Oct4) mediates chemotherapeutic drug resistance in liver cancer cells through a potential Oct4-AKT-ATP-binding cassette G2 pathway. *Hepatology*, 52(2), 528-39 (2010)
DOI: 10.1002/hep.23692

75. A. Omenetti, S. Choi, G. Michelotti and A. M. Diehl: Hedgehog signaling in the liver. *J Hepatol*, 54(2), 366-73 (2011)
DOI: 10.1016/j.jhep.2010.10.003

76. G. Xie, S. S. Choi, W. K. Syn, G. A. Michelotti, M. Swiderska, G. Karaca, I. S. Chan, Y. Chen and A. M. Diehl: Hedgehog signalling regulates liver sinusoidal endothelial cell capillarisation. *Gut*, 62(2), 299-309 (2013)
DOI: 10.1136/gutjnl-2011-301494

77. B. Ochoa, W. K. Syn, I. Delgado, G. F. Karaca, Y. Jung, J. Wang, A. M. Zubiaga, O. Fresnedo, A. Omenetti, M. Zdanowicz, S. S. Choi and A. M. Diehl: Hedgehog signaling is critical for normal liver regeneration after partial hepatectomy in mice. *Hepatology*, 51(5), 1712-23 (2010)
DOI: 10.1002/hep.23525

78. C. D. Guy, A. Suzuki, M. Zdanowicz, M. F. Abdelmalek, J. Burchette, A. Unalp, A. M. Diehl and C. R. N. Nash: Hedgehog pathway activation parallels histologic severity of injury and fibrosis in human nonalcoholic fatty liver disease. *Hepatology*, 55(6), 1711-21 (2012)
DOI: 10.1002/hep.25559

79. V. Paradis, S. Zalinski, E. Chelbi, N. Guedj, F. Degos, V. Vilgrain, P. Bedossa and J. Belghiti: Hepatocellular carcinomas in patients with metabolic syndrome often develop without significant liver fibrosis: a pathological analysis. *Hepatology*, 49(3), 851-9 (2009) DOI: 10.1002/hep.22734

80. C. D. Peacock, Q. Wang, G. S. Gesell, I. M. Corcoran-Schwartz, E. Jones, J. Kim, W. L. Devereux, J. T. Rhodes, C. A. Huff, P. A. Beachy, D. N. Watkins and W. Matsui: Hedgehog signaling maintains a tumor stem cell compartment in multiple myeloma. *Proc Natl Acad Sci U S A*, 104(10), 4048-53 (2007) DOI: 10.1073/pnas.0611682104

81. C. Zhao, A. Chen, C. H. Jamieson, M. Fereshteh, A. Abrahamsson, J. Blum, H. Y. Kwon, J. Kim, J. P. Chute and D. Rizzieri: Hedgehog signalling is essential for maintenance of cancer stem cells in myeloid leukaemia. *Nature*, 458(7239), 776-779 (2009) DOI: 10.1038/nature07737

82. A. Pereira Tde, R. P. Witek, W. K. Syn, S. S. Choi, S. Bradrick, G. F. Karaca, K. M. Agboola, Y. Jung, A. Omenetti, C. A. Moylan, L. Yang, M. E. Fernandez-Zapico, R. Jhaveri, V. H. Shah, F. E. Pereira and A. M. Diehl: Viral factors induce Hedgehog pathway activation in humans with viral hepatitis, cirrhosis, and hepatocellular carcinoma. *Lab Invest*, 90(12), 1690-703 (2010) DOI: 10.1038/labinvest.2010.147

83. M. Tada, F. Kanai, Y. Tanaka, K. Tateishi, M. Ohta, Y. Asaoka, M. Seto, R. Muroyama, K. Fukai, F. Imazeki, T. Kawabe, O. Yokosuka and M. Omata: Down-regulation of hedgehog-interacting protein through genetic and epigenetic alterations in human hepatocellular carcinoma. *Clin Cancer Res*, 14(12), 3768-76 (2008) DOI: 10.1158/1078-0432.CCR-07-1181

84. B. Spee, G. Carpino, B. A. Schotanus, A. Katoonizadeh, S. Vander Borght, E. Gaudio and T. Roskams: Characterisation of the liver progenitor cell niche in liver diseases: potential involvement of Wnt and Notch signalling. *Gut*, 59(2), 247-57 (2010) DOI: 10.1136/gut.2009.188367

85. W. Yang, H. X. Yan, L. Chen, Q. Liu, Y. Q. He, L. X. Yu, S. H. Zhang, D. D. Huang, L. Tang, X. N. Kong, C. Chen, S. Q. Liu, M. C. Wu and H. Y. Wang: Wnt/beta-catenin signaling contributes to activation of normal and tumorigenic liver progenitor cells. *Cancer Res*, 68(11), 4287-95 (2008) DOI: 10.1158/0008-5472.CAN-07-6691

86. T. Yamashita, A. Budhu, M. Forgues and X. W. Wang: Activation of hepatic stem cell marker EpCAM by Wnt-beta-catenin signaling in hepatocellular carcinoma. *Cancer Res*, 67(22), 10831-9 (2007) DOI: 10.1158/0008-5472.CAN-07-0908

87. P. Kaur, S. Mani, M. P. Cros, J. Y. Scoazec, I. Chemin, P. Hainaut and Z. Herceg: Epigenetic silencing of sFRP1 activates the canonical Wnt pathway and contributes to increased cell growth and proliferation in hepatocellular carcinoma. *Tumour Biol*, 33(2), 325-36 (2012) DOI: 10.1007/s13277-012-0331-5

88. A. S. Cheng, S. S. Lau, Y. Chen, Y. Kondo, M. S. Li, H. Feng, A. K. Ching, K. F. Cheung, H. K. Wong, J. H. Tong, H. Jin, K. W. Choy, J. Yu, K. F. To, N. Wong, T. H. Huang and J. J. Sung: EZH2-mediated concordant repression of Wnt antagonists promotes beta-catenin-dependent hepatocarcinogenesis. *Cancer Res*, 71(11), 4028-39 (2011) DOI: 10.1158/0008-5472.CAN-10-3342

89. R. Li, N. Qian, K. Tao, N. You, X. Wang and K. Dou: MicroRNAs involved in neoplastic transformation of liver cancer stem cells. *J Exp Clin Cancer Res*, 29, 169 (2010) DOI: 10.1186/1756-9966-29-169

90. C. M. Wong, C. C. Wong, J. M. Lee, D. N. Fan, S. L. Au and I. O. Ng: Sequential alterations of microRNA expression in hepatocellular carcinoma development and venous metastasis. *Hepatology*, 55(5), 1453-61 (2012) DOI: 10.1002/hep.25512

91. L. Wang, X. Zhang, L. T. Jia, S. J. Hu, J. Zhao, J. D. Yang, W. H. Wen, Z. Wang, T. Wang, J. Zhao, R. A. Wang, Y. L. Meng, Y. Z. Nie, K. F. Dou, S. Y. Chen, L. B. Yao, D. M. Fan, R. Zhang and A. G. Yang: c-Myc-mediated epigenetic silencing of microRNA-101 contributes to dysregulation of multiple pathways in hepatocellular carcinoma. *Hepatology*, 59(5), 1850-63 (2014) DOI: 10.1002/hep.26720

92. J. Huang, Y. Wang, Y. Guo and S. Sun:

Down-regulated microRNA-152 induces aberrant DNA methylation in hepatitis B virus-related hepatocellular carcinoma by targeting DNA methyltransferase 1. *Hepatology*, 52(1), 60-70 (2010)
DOI: 10.1002/hep.23660

93. L. Liang, C. M. Wong, Q. Ying, D. N. Fan, S. Huang, J. Ding, J. Yao, M. Yan, J. Li, M. Yao, I. O. Ng and X. He: MicroRNA-125b suppressesed human liver cancer cell proliferation and metastasis by directly targeting oncogene LIN28B2. *Hepatology*, 52(5), 1731-40 (2010)
DOI: 10.1002/hep.23904

94. D. N. Fan, F. H. Tsang, A. H. Tam, S. L. Au, C. C. Wong, L. Wei, J. M. Lee, X. He, I. O. Ng and C. M. Wong: Histone lysine methyltransferase, suppressor of variegation 3-9 homolog 1, promotes hepatocellular carcinoma progression and is negatively regulated by microRNA-125b. *Hepatology*, 57(2), 637-47 (2013)
DOI: 10.1002/hep.26083

95. B. Zhou, R. Ma, W. Si, S. Li, Y. Xu, X. Tu and Q. Wang: MicroRNA-503 targets FGF2 and VEGFA and inhibits tumor angiogenesis and growth. *Cancer Lett*, 333(2), 159-69 (2013)
DOI: 10.1016/j.canlet.2013.01.028

96. P. A. Gregory, A. G. Bert, E. L. Paterson, S. C. Barry, A. Tsykin, G. Farshid, M. A. Vadas, Y. Khew-Goodall and G. J. Goodall: The miR-200 family and miR-205 regulate epithelial to mesenchymal transition by targeting ZEB1 and SIP1. *Nat Cell Biol*, 10(5), 593-601 (2008)
DOI: 10.1038/ncb1722

97. S. M. Johnson, H. Grosshans, J. Shingara, M. Byrom, R. Jarvis, A. Cheng, E. Labourier, K. L. Reinert, D. Brown and F. J. Slack: RAS is regulated by the let-7 microRNA family. *Cell*, 120(5), 635-47 (2005)
DOI: 10.1016/j.cell.2005.01.014

98. F. Fornari, M. Milazzo, P. Chieco, M. Negrini, E. Marasco, G. Capranico, V. Mantovani, J. Marinello, S. Sabbioni, E. Callegari, M. Cescon, M. Ravaioli, C. M. Croce, L. Bolondi and L. Gramantieri: In hepatocellular carcinoma miR-519d is up-regulated by p53 and DNA hypomethylation and targets CDKN1A/p21, PTEN, AKT3 and TIMP2. *J Pathol*, 227(3), 275-85 (2012)
DOI: 10.1002/path.3995

99. Y. Wang, H. C. Toh, P. Chow, A. Y. Chung, D. J. Meyers, P. A. Cole, L. L. Ooi and C. G. Lee: MicroRNA-224 is up-regulated in hepatocellular carcinoma through epigenetic mechanisms. *FASEB J*, 26(7), 3032-41 (2012)
DOI: 10.1096/fj.11-201855

100. E. Connolly, M. Melegari, P. Landgraf, T. Tchaikovskaya, B. C. Tennant, B. L. Slagle, L. E. Rogler, M. Zavolan, T. Tuschl and C. E. Rogler: Elevated expression of the miR-17-92 polycistron and miR-21 in hepadnavirus-associated hepatocellular carcinoma contributes to the malignant phenotype. *Am J Pathol*, 173(3), 856-64 (2008)
DOI: 10.2353/ajpath.2008.080096

101. J. L. Mott: MicroRNAs involved in tumor suppressor and oncogene pathways: implications for hepatobiliary neoplasia. *Hepatology*, 50(2), 630-7 (2009)
DOI: 10.1002/hep.23010

102. S. L. Au, C. C. Wong, J. M. Lee, D. N. Fan, F. H. Tsang, I. O. Ng and C. M. Wong: Enhancer of zeste homolog 2 epigenetically silences multiple tumor suppressor microRNAs to promote liver cancer metastasis. *Hepatology*, 56(2), 622-31 (2012)
DOI: 10.1002/hep.25679

103. L. Gailhouste, L. Gomez-Santos, K. Hagiwara, I. Hatada, N. Kitagawa, K. Kawaharada, M. Thirion, N. Kosaka, R. U. Takahashi, T. Shibata, A. Miyajima and T. Ochiya: miR-148a plays a pivotal role in the liver by promoting the hepatospecific phenotype and suppressing the invasiveness of transformed cells. *Hepatology*, 58(3), 1153-65 (2013)
DOI: 10.1002/hep.26422

104. C. Braconi, N. Huang and T. Patel: MicroRNA-dependent regulation of DNA methyltransferase-1 and tumor suppressor gene expression by interleukin-6 in human malignant cholangiocytes. *Hepatology*, 51(3), 881-90 (2010)
Http://dx.doi.org/ 10.1002/hep.23381

105. C. J. Jung, S. Iyengar, K. R. Blahnik, T. P. Ajuha, J. X. Jiang, P. J. Farnham and M. Zern: Epigenetic modulation of miR-122 facilitates human embryonic stem cell self-renewal and hepatocellular carcinoma proliferation. *PLoS One*, 6(11), e27740 (2011)
DOI: 10.1371/journal.pone.0027740

106. K. Song, C. Han, J. Zhang, D. Lu, S. Dash, M. Feitelson, K. Lim and T. Wu: Epigenetic regulation of MicroRNA-122 by peroxisome proliferator activated receptor-gamma and hepatitis b virus X protein in hepatocellular carcinoma cells. *Hepatology*, 58(5), 1681-92 (2013)
DOI: 10.1002/hep.26514

107. H. Xia, L. L. Ooi and K. M. Hui: MiR-214 targets beta-catenin pathway to suppress invasion, stem-like traits and recurrence of human hepatocellular carcinoma. *PLoS One*, 7(9), e44206 (2012)
DOI: 10.1371/journal.pone.0044206

108. S. S. Choi and A. M. Diehl: Epithelial-to-mesenchymal transitions in the liver. *Hepatology*, 50(6), 2007-13 (2009)
DOI: 10.1002/hep.23196

109. M. Pinzani: Epithelial-mesenchymal transition in chronic liver disease: fibrogenesis or escape from death? *J Hepatol*, 55(2), 459-65 (2011)
DOI: 10.1016/j.jhep.2011.02.001

110. L. J. Talbot, S. D. Bhattacharya and P. C. Kuo: Epithelial-mesenchymal transition, the tumor microenvironment, and metastatic behavior of epithelial malignancies. *Int J Biochem Mol Biol*, 3(2), 117-36 (2012)

111. J. Y. Seok, D. C. Na, H. G. Woo, M. Roncalli, S. M. Kwon, J. E. Yoo, E. Y. Ahn, G. I. Kim, J. S. Choi, Y. B. Kim and Y. N. Park: A fibrous stromal component in hepatocellular carcinoma reveals a cholangiocarcinoma-like gene expression trait and epithelial-mesenchymal transition. *Hepatology*, 55(6), 1776-86 (2012)
DOI: 10.1002/hep.25570

112. W. Ding, H. You, H. Dang, F. LeBlanc, V. Galicia, S. C. Lu, B. Stiles and C. B. Rountree: Epithelial-to-mesenchymal transition of murine liver tumor cells promotes invasion. *Hepatology*, 52(3), 945-53 (2010)
DOI: 10.1002/hep.23748

113. O. O. Ogunwobi, W. Puszyk, H. J. Dong and C. Liu: Epigenetic upregulation of HGF and c-Met drives metastasis in hepatocellular carcinoma. *PLoS One*, 8(5), e63765 (2013)
DOI: 10.1371/journal.pone.0063765

114. J. Schrader, T. T. Gordon-Walker, R. L. Aucott, M. van Deemter, A. Quaas, S. Walsh, D. Benten, S. J. Forbes, R. G. Wells and J. P. Iredale: Matrix stiffness modulates proliferation, chemotherapeutic response, and dormancy in hepatocellular carcinoma cells. *Hepatology*, 53(4), 1192-205 (2011)
DOI: 10.1002/hep.24108

115. S. Lingala, Y. Y. Cui, X. Chen, B. H. Ruebner, X. F. Qian, M. A. Zern and J. Wu: Immunohistochemical staining of cancer stem cell markers in hepatocellular carcinoma. *Exp Mol Pathol*, 89(1), 27-35 (2010)
DOI: 10.1016/j.yexmp.2010.05.005

116. X. Chen, S. Lingala, S. Khoobyari, J. Nolta, M. A. Zern and J. Wu: Epithelial mesenchymal transition and hedgehog signaling activation are associated with chemoresistance and invasion of hepatoma subpopulations. *J Hepatol*, 55(4), 838-45 (2011)
DOI: 10.1016/j.jhep.2010.12.043

117. T. Chiba, E. Suzuki, M. Negishi, A. Saraya, S. Miyagi, T. Konuma, S. Tanaka, M. Tada, F. Kanai, F. Imazeki, A. Iwama and O. Yokosuka: 3-Deazaneplanocin A is a promising therapeutic agent for the eradication of tumor-initiating hepatocellular carcinoma cells. *Int J Cancer*, 130(11), 2557-67 (2012)
DOI: 10.1002/ijc.26264

118. C. Raggi, V. M. Factor, D. Seo, A. Holczbauer, M. C. Gillen, J. U. Marquardt, J. B. Andersen, M. Durkin and S. S. Thorgeirsson: Epigenetic reprogramming modulates malignant properties of human liver cancer. *Hepatology*, 59(6), 2251-62 (2014)
DOI: 10.1002/hep.27026

119. W. Yeo, H. C. Chung, S. L. Chan, L. Z. Wang, R. Lim, J. Picus, M. Boyer, F. K. Mo, J. Koh, S. Y. Rha, E. P. Hui, H. C. Jeung, J. K. Roh, S. C. Yu, K. F. To, Q. Tao, B. B. Ma, A. W. Chan, J. H. Tong, C. Erlichman, A. T. Chan and B. C. Goh: Epigenetic therapy using belinostat for patients with unresectable hepatocellular carcinoma: a multicenter phase I/II study with biomarker and pharmacokinetic analysis of tumors from patients in the Mayo Phase II Consortium and the Cancer Therapeutics Research Group. *J Clin Oncol*, 30(27), 3361-7 (2012)
DOI: 10.1200/JCO.2011.41.2395

120. C. Magerl, J. Ellinger, T. Braunschweig, E. Kremmer, L. K. Koch, T. Holler, R. Buttner, B. Luscher and I. Gutgemann: H3K4

dimethylation in hepatocellular carcinoma is rare compared with other hepatobiliary and gastrointestinal carcinomas and correlates with expression of the methylase Ash2 and the demethylase LSD1. *Hum Pathol*, 41(2), 181-9 (2010)
DOI: 10.1016/j.humpath.2009.08.007

Abbreviations: ABC, adenosine triphosphate (ATP)-binding cassette, ALDH-1, aldehyde dehydrogenase-1, Bmi-1, B lymphoma moloney murine leukemia virus insertion region 1 homolog, ChIP, chromatin immunoprecipitation, CpG, cytosine-guanine dinucleotide, CRBP-1, cellular retinol-binding protein-1, CSCs, cancer stem cells, DNMT, DNA methyltransferase, EMT, epithelial-mesenchymal transition, EpCAM, epithelial cell adhesion molecule, ERK, extracellular signal-regulated kinase, EZH2, enhancer of zeste homolog-2, HAT, histone acetyltransferase, HBx, hepatitis B viral X protein, HCC, hepatocellular carcinoma, HDAC, histone deacetylase, HDM, histone demethylase, HGF, hepatocyte growth factor, HMT, histone methyltransferase, hTERT, human telomerase reverse transcriptase, ICAM-1, intercellular cell adhesion molecule-1, iPSC, induced pluripotent stem cells, LINE-1, long interspersed nucleotide element-1, LPC, liver progenitor cells, MDR-1, multidrug resistance gene-1, NAFLD, non-alcoholic fatty liver disease, NCAM, neural cell adhesion molecule, OV-6, oval cell marker-6, PGP, polycomb group protein, PPAR-gamma, peroxisome proliferator activated receptor-gamma, PRC, polycomb repressive complex, PTEN, phosphatase and tensin homolog deleted on chromosome-10, RBP, retinol-binding protein precursor, ROS, reactive oxygen species, RXR-alpha, retinoid X receptor-alpha, SFRP, secreted frizzled related protein, SP, side population, TGF-beta, transforming growth factor-beta, T-ICs, tumor-initiating cells, TSG, tumor suppressor gene, UTR, untranslated region

Key Words: Tumor-initiating cells; Cancer stem cells; Epigenetic regulation; Hepatocellular carcinoma, Review

Send correspondence to: Jian Wu, Key Laboratory of Medical Molecular Virology, Fudan University Shanghai Medical College, 138 Yi Xue Yuan Road, Shanghai 200032, China, Tel: 86-21-5423-7705, Fax: 86-21-6422-7201, E-mail: jdwu@ucdavis.edu