Recent Advances of Molecular Pathology on HCC: Review

Molecular Pathogenesis of Hepatocellular Carcinoma

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Abstract

The pathogenesis of hepatocellular carcinoma (HCC) is a multistep process involving the progressive accumulation of molecular alterations pinpointing different molecular and cellular events. The next-generation sequencing technology is facilitating the global and systematic evaluation of molecular landscapes in HCC. There is emerging evidence supporting the importance of cancer metabolism and tumor microenvironment in providing a favorable and supportive niche to expedite HCC development. Moreover, recent studies have identified distinct surface markers of cancer stem cell (CSC) in HCC, and they also put forward the profound involvement of altered signaling pathways and epigenetic modifications in CSCs, in addition to the concomitant drug resistance and metastasis. Taken together, multiple key genetic and non-genetic factors, as well as liver CSCs, result in the development and progression of HCC.

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Recent Applications of Next-Generation Sequencing (NGS) Technology in Capturing Molecular Alterations in Hepatocellular Carcinoma (HCC)

Molecular alterations at both genetic and epigenetic levels have been shown to drive hepatocarcinogenesis. The recent adoption of advanced NGS in analyzing HCC samples from

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Fig. 1. Schematic overview of key events in the molecular pathogenesis of HCC.

patient enables us to revisit and explore the global molecular landscapes of HCC at multiple levels ranging from copy number alterations (CNAs) and somatic mutations at the genome level, to gene expression at transcriptome level, as well as epigenetic changes. Furthermore, the capability of NGS to unbiasedly capture all the viral integration sites provides a whole new way to understand the interplay between hepatitis B virus (HBV) and the host genome (fig. 1).

Genomic Alterations

Large-Scale and Focal CNAs

Genome instability is a key driver in human cancers including HCC, and this may result in CNAs with gain or loss in chromosomes of different extents or somatic mutations in the genomes. In the pre-NGS era, comparative genome hybridization and its array-based derivatives were commonly used to profile and uncover the global picture of chromosomal gains or losses [1, 2]. Recently, three independent studies have evaluated CNAs by comparing the sequencing data generated by whole-genome and whole-exome sequencing in paired HCC samples [3–5]. Their results have allowed the generation of a map with better resolution highlighting the chromosome gains and losses in HCC. Specifically, they collectively show frequent large-scale gains at 1q, 5p, 6p, 8q, 17q, 20q and Xq; and losses at 1p, 4p-q, 6q, 8p, 13p-q, 16p-q, 17p, 21p-q and 22q. Interestingly, focal amplifications at oncogenes including *MET*, *MYC*, *TERT*, *CCND1* and *FGF19*, and focal deletions at tumor suppressors including

PTEN, *TP53*, *CDKN2A* and *CDKN2B* were detected. The overall findings are consistent with the previous reports using different technologies [6].

Common Mutations of Protein Coding Genes

Recently, active NGS-based genome-wide mutational screenings in HCC samples with different etiological backgrounds have been carried out worldwide [3–5, 7–13]. *CTNNB1* and *TP53* are the most commonly mutated genes in HCC. Mutant *CTNNB1* has been shown to be the putative driver for alcoholic HCC, while the mutant *TP53* is likely to be associated with HBV infection [4]. In addition to the previously documented mutant genes, these studies have further identified other putative drivers, e.g. *CDKN2A*, *RB1*, *AXIN1*, *ARID1A*, *ARID1B*, *ARID2*, *NFE2L2*, *KEAP1*, *RPS6KA3*, *MLL*, *MLL3* and *MLL4*, to be recurrently mutated at high frequencies. These novel mutations have been suggested to play deleterious effects on their intrinsic protein function. Pathway analysis has further demonstrated their potential roles in affecting multiple facets of human cellular machinery such as deoxyribonucleic acid (DNA) repair and surveillance (*TP53*, *CDKN2A* and *RB1*), Wnt/ β -catenin signaling (*CTNNB1* and *AXIN1*), chromatin remodeling (*ARID1A*, *ARID1B* and *ARID2*), oncogenic mitogen-activated protein kinase (MAPK) signaling (*RPS6KA3*), oxidative stress (*NFE2L2* and *KEAP1*), and histone modification (*MLL*, *MLL3* and *MLL4*). However, how these mutants functionally cooperate amongst themselves remains to be addressed. Recently, Schulze et al. took an integrative approach to assess the somatic mutations and focal CNAs in human HCCs to look for gene targets which are frequently and significantly altered through different mechanisms [4]. They listed and reported 161 putative HCC driver genes. Further validation of these novel targets, along with the aforementioned mutant genes, on relevant HCC samples and *in vivo* models would significantly help to derive novel molecular insight in the realm of human HCC.

TERT Promoter Mutations

Apart from the aforementioned putative HCC drivers affecting a wide spectrum of cellular processes, emerging evidence has indicated frequent and recurrent somatic mutations in the promoter area of the *TERT* (telomerase reverse transcriptase) in various types of cancer [14, 15]. The hotspot *TERT* promoter mutations (–124G>A and –146G>A; with the numbers that denote the distance from the ATG start site) creates potential binding sites for E-twenty six/ternary complex factor transcription factors, which increase the promoter activity and transcription of *TERT*. As a result, a mutation screening study was undertaken in HCC to investigate the prevalence of *TERT* promoter mutations [16]. It was found that such mutations affected 59% of a large cohort of human HCC samples (n=305). Moreover, *TERT* was found to be the first gene recurrently mutated in preneoplastic lesions in cirrhosis and its promoter mutations was involved in the last step of malignant transformation from hepatocellular adenoma into HCC [16]. Subsequently, another report from the same group [17] revealed that *TERT* promoter mutations were highly related to the step-wise hepatocarcinogenesis, with its frequency increasing progressively from 6% in low-grade dysplastic nodules (n=32), through 19% in high-grade dysplastic nodules (n=16), and which peaked at 61% in early HCCs (n=23) and 42% in small and progressed HCCs (n=17), respectively. It has also been suggested that *TERT* is the earliest somatic alteration, since mutations of 10 other recurrently mutated HCC genes are only identified in 28% of small and progressed HCCs. Somatic *TERT* promoter mutations are believed to be a new predictive marker in highlighting the transformation of a premalignant lesion, on a cirrhotic background, into a HCC.

HBV Integration

Among the identified etiological risk factors for HCC, which include chronic viral infections (HBV and hepatitis C virus), chronic alcohol consumption, and non-alcoholic fatty liver

disease and non-alcoholic steatohepatitis, chronic HBV infection accounts for around 50% of the cases [18]. One of the distinctive features of HBV DNA is that it can integrate into the human genome, which in turn disrupts the endogenous tumor suppressors and other regulatory genes, or enhances the activity of proto-oncogenes. The resultant enhanced survival, proliferation and reduction in apoptosis may lead to an imbalance of the overall oncogenic and tumor suppressive signals, thus supporting HCC development. In the past, biased polymerase chain reaction (PCR)-based methods were employed to detect HBV integration sites [19]. Recent studies have commonly used NGS-based methods such as whole-genome sequencing, whole-transcriptome sequencing or other derivatives [8, 20–25]. *TERT*, *MLL4*, *CCNE1* and *ROCK1* are frequently and recurrently affected by HBV integration. Intriguingly, a recent study has reported the frequent (23.3%) *HBx-LINE1* chimeric transcript resulting from HBV integration in HBV-associated HCC [23]. Such *HBx-LINE1* fusion results in a long non-coding ribonucleic acid (lncRNA) and it is associated with poorer overall patient survival. *HBx-LINE1* expression activates Wnt signaling through β -catenin transactivity. However, such frequent and positionally recurrent *HBx-LINE1* fusion has not been identifiable in a number of genome-wide HBV integration surveys [8, 20–22, 24, 25], suggesting a possible difference in its actual frequency, an uneven ethnic distribution, or possibly technical artifact contamination in its detection. The reason for the discrepancy remains to be elucidated, but further verification is awaited.

Epigenetic Alterations

Epigenetic alterations are reversible modifications at the genome level without involving changes of the DNA sequence. They control the gene expression level through modulations at the gene transcription level (DNA methylation, histone modification, and chromatin remodeling) as well as post-transcriptionally through various non-coding RNAs (ncRNAs) (e.g. microRNAs [miRNAs] and lncRNAs). Existing studies focusing on the epigenetic regulation of individual genes or particular gene sets have been reviewed and summarized elsewhere [26–29]. In a global perspective, systematic array-based and more recently NGS-based studies have revealed that significant numbers of gene targets are affected by aberrant DNA methylation at the gene promoter regions, and this directly increases or decreases the downstream gene transcription activity [6, 30].

Recently, using advanced sequencing technologies, a number of studies have identified and characterized less well-explored ncRNAs in human HCC. In particular, Law et al. identified piR-Hep1, a new class of ncRNAs with between 26–32 nucleotides, being upregulated in nearly half of the HCC tumors that were screened. This upregulation is functionally linked to an increased proliferation rate and tumor invasiveness, likely through maintaining active AKT signaling [26]. Conversely, further analysis of the miRNome (all the miRNAs expressed in a given genome) data has highlighted the significant association of miR-1323 overexpression in cirrhosis. Mizuguchi et al. highlighted the upregulation of miR-21 and miR-34a and downregulation of miR-122 in HCC [27], suggesting the potential use of this molecular signature as a biomarker to detect HCC.

Currently, limited work has been performed to explore the role of lncRNAs in human HCC. Our group utilized a quantitative PCR-based assay to profile a subset of 88 well-annotated lncRNAs and identified a lncRNA, HOTTIP, to be frequently upregulated in HCC. HOTTIP is under the negative regulation of miR-125b, a miRNA with frequent downregulation in HCC. HOTTIP has been demonstrated to play a significant oncogenic role in HCC, possibly through the upregulation of HOXA genes [31]. Given the lack of systematic capture of epi-

genetic regulations at multiple levels (e.g. DNA epigenetic marks, histone marks, expression of epigenetic regulators, and ncRNAs) in the same HCC sample set with defined etiological background, further global studies are awaited to gain a better understanding of how deregulation of the diverse epigenetic events collectively contributes to gene expression changes in HCC development.

Transcriptomic Alterations

Compared with the active global sequencing analysis at the genomic level, only a few NGS-based whole-transcriptomic exploratory analyses have been carried out in human HCC. They revealed the transcriptomic landscape of HCC through comparing tumors against the corresponding non-tumorous liver tissues [32–34]. Many of them had very limited sample sizes in providing a representative overview and identifying critical gene expression changes. Our group reported the use of The Cancer Genome Atlas HCC whole-transcriptome sequencing data, consisting of a relatively large sample cohort, to identify key differential gene expression patterns [32]. Apart from the dysregulations in the expression of *ALG11*, *SERPINA11*, *TMEM82*, *GPC3*, *SPINK1* and *ESM1* which were reported by other studies, other genes such as *CENPF* and *FOXM1* were found to be the top-listing upregulated genes. *CENPF* is an essential protein for kinetochore function and mitotic segregation, while *FOXM1* is a forkhead box protein transcription factor that regulates cell cycle processes and in turn controls cell proliferation and DNA damage repair. Concurrent upregulation of these two proteins in HCC likely suggests their potential functional synergism, as recently suggested in prostate cancer. On the other hand, multiple members of the C-type lectin family (*CLEC4G*, *CLEC1B* and *CLEC4M*) and *CRHBP* (corticotropin-releasing hormone binding protein) were found to be downregulated. C-type lectins are calcium-dependent glycan binding proteins involved in adhesion, and they act as signaling receptors for immune-related processes such as inflammation and immune responses towards tumor and virally infected cells. The downregulation of C-type lectin expression may suggest its involvement in the pathogenesis of HCC. Conversely, corticotropin-releasing factor (CRF) has been shown to modulate tumor progression and angiogenesis in various cancers. Depletion of *CRHBP*, a member of the CRF system, may likewise be involved in hepatocarcinogenesis through similar mechanisms.

Altered Cell Signaling Pathways

The valuable information generated by various genomic, transcriptomic and epigenetic alterations provide us with a comprehensive assessment about how these changes may affect key signaling pathways supporting the development of HCC. Although the current understanding on altered signaling pathways in HCC is far from complete, emerging evidence generated by using different model systems have improved our understanding that a confined subset of cell signaling pathways may be sequentially involved in hepatocarcinogenesis and tumor progression with metastasis [35–37]. In brief, these pathways play critical roles in controlling a wide range of essential biological processes required by the tumors in maintaining survival and adapting to the microenvironmental changes which includes Wnt/ β -catenin, Notch and Hedgehog signaling pathways (differentiation and development), p53/p21 and RB1 signaling pathways (genomic stability and cell cycle regulation), EGF/EGFR, HGF/MET, IGF/IGFR, PI3K/AKT/mTOR and RAS/MAPK growth factor receptor signaling (cell proliferation and survival), VEGF/VEGFR, PDGF/PDGFR and FGF/FGFR signaling pathways (angiogen-

esis), NFE2L2/KEAP1 signaling pathway (oxidative stress), ARID1A/ARID1B/ARID2 and MLL signaling pathways (chromatin regulation), and JAK/STAT signaling pathway (cytokine and growth factor signaling transduction).

To date, the multikinase (VEGFR, PDGFR, B-raf and C-raf) inhibitor sorafenib is the only United States Food and Drug Administration-approved molecular drug for advanced HCC with modest survival benefits due to development of drug resistance. This resistance had been suggested to be contributed to by the upregulation of oncogenic AKT activation [38] and the potential involvement of additional pathways including TGF- β -SMAD [39] and NF- κ B [40] signaling. With the use of a liver specific p19^{Arf} knockout mouse model, Rudalska et al. performed the first unbiased in vivo RNA interference screening for potential targets underlying sorafenib resistance [41]. They performed hydrodynamic injection of a predesigned small hairpin RNA (shRNA) library of 253 shRNAs which specifically targeted genes located in the genome with frequent focal amplification in patients with HCC and cell lines, followed by comparing the individual shRNA enrichment or depletion ratio between the sorafenib and control treated tumors by deep sequencing. *MAPK14* gene, encoding p38 α stress response kinase, was identified to specifically maintain the active Raf-MAPK signaling cascade in phosphorylating the downstream phospho-ATF2 substrate upon sorafenib treatment. Interestingly, p38 α appears to play a highly specific role in conferring sorafenib resistance, as knockdown of p38 α did not show an observable impact on the intrahepatic tumor burden and overall survival in mice. With these data generated by advanced sequencing technologies on patient samples having well-defined clinical backgrounds, it enables the search for clinically relevant gene targets for complementing the use of sorafenib, or it opens up the design of an alternative substitute for a more personalized management for patients with HCC carrying a specific type of molecular signature.

Multi-Step Molecular Pathogenesis Leading to HCC

With efforts from studies pinpointing the multiple aspects of HCC pathogenesis, knowledge has been accumulating on the stochastic molecular alterations (as mentioned in previous sections) targeting different cancer hallmark events [42]. Traditionally, it is believed that HCC emerges from normal hepatocytes, through sequential acquisition of essential molecular alterations that empower them with cancer hallmark capabilities. It is a long and cumulative process under the continued clonal selective pressure that certain subclones of cells possessing growth and survival advantage will dynamically undergo clonal expansion. As a result, the clonal evolution of altered hepatocytes progressively transform into HCC. Recently identified findings also emphasize the importance of cancer metabolism and tumor-promoting microenvironment in augmenting the development of HCC (fig. 1). More importantly, the cancer stem cell (CSC) theory which has emerged in recent decades, together with the traditional clonal evolution model, helps to explain the underlying molecular mechanisms of HCC to a better extent, particularly in the context of tumor relapse, chemoresistance and metastasis (fig. 1).

HCC and Cancer Metabolism

The intimate relationship between tumor microenvironment and cancer metabolism has been explored in a number of studies and new data are rapidly emerging [43]. A spotlight in this area lies in the link between the hypoxic tumor microenvironment and glycolysis [44].

While human cells physiologically generate energy in the form of adenosine triphosphate by oxidative phosphorylation in the presence of oxygen, cancer cells preferentially adopt aerobic glycolysis as a major mechanism for energy production. This is the well-known Warburg effect [45].

In HCC, hypoxia-inducible factor (HIF)-1-induced glycolysis is associated with biological aggressiveness [46]. In 2004, correlation analysis of the expression of hypoxia marker and glycolytic enzyme, hexokinase II, in HCC suggested that HCC cells switched to glycolysis through the mediation of HIF-1 [47]. Apart from hexokinase II, hypoxia also reprograms cancer cells to glycolysis through induction of glucose transporter 1 (GLUT-1). GLUT-1 is a crucial player in the glycolytic pathway and located on the cell surface enhancing glucose uptake into the cell [48, 49]. GLUT-1 protein expression levels have been reported to be associated with higher tumor grades, advanced tumor stages, and higher proliferative index in HCC [50]. It is also a potential therapeutic target for HCC [51]. HIF-1 co-operates with c-myc to facilitate aerobic glycolysis [52]. Other regulators of GLUT-1 include p53 [53] and the PI3K/AKT pathway [54].

Pyruvate kinase isozyme M2 (PKM2) has been an active area for research with respect to HCC glycolysis in recent years. PKM2 expression in HCCs correlates with vascular invasion and advanced tumor stages. PKM2+TRIM35- is an indicator for poorer patient survival [55]. Our group has reported the overexpression of PKM2 in human HCCs, and silencing of PKM2 inhibits HCC aerobic glycolysis, in vitro cell proliferation, as well as in vivo tumor growth and metastasis. Moreover, miR-122 is a negative regulator of PKM2 by directly interacting with the 3'-untranslated region of the latter [56]. Similar findings were reported by Liu et al [57]. As a crucial regulator of HCC glycolysis, several key modulators of PKM2 have been identified in recent years. PARP14 activates PKM2 through inhibition of pro-apoptotic kinase JNK1 [58]. Mineralocorticoid negatively regulates pyruvate kinase by targeting miR-388-3p [59]. Inactivation of Spry2 fosters HCC glycolysis by activating PKM2 in AKT-overexpressing cells [60]. In addition to PKM2, our group reported the role of PIM1 in HCC glycolysis, which is overexpressed in human HCC tissues. Knockdown of PIM1 suppressed cell proliferation and invasion in vitro, as well as tumor growth and metastasis in vivo. Furthermore, PIM1 facilitates glycolysis in HCC cells possibly through AKT [61].

The identification of functional markers for HCC glycolysis would shed light on therapeutic opportunities. Specific inhibitors and monoclonal antibodies might serve as alternatives in some cases. Furthermore, other elements in the tumor microenvironment, such as cancer-associated fibroblasts and immune cells, are potentially involved in the metabolic reprogramming of HCC not limited to glycolysis. This area definitely warrants further investigations.

CSCs of HCC

CSCs or tumor-initiating cells are a subpopulation of tumor cells that possess the ability to self-renew and differentiate [62]. Moreover, CSCs confer chemo- and radio-resistance [63]. As a consequence, CSCs are believed to play a crucial role in tumor initiation and tumor relapse clinically.

In HCC, a recent comprehensive study provided insight on the origin of liver CSCs. Holzbauer et al. elegantly demonstrated that liver CSCs originated from three hepatic lineages – adult differentiated hepatocytes, hepatoblasts and adult hepatic progenitor cells [64]. Among the liver CSCs arising from these hepatic lineages, epithelial-mesenchymal transition and embryonic stem cell (ESC) related genes were found to be significantly upregulated. In particular, tumors that developed from adult hepatocyte-reprogrammed CSCs displayed the greatest number of activated ESC-related genes in which *Myc* ranked first on the list. Although

liver CSCs evolving from different cells of origin can share similar CSC phenotypes, they are genetically different in terms of self-renewal through the specific modulation of signaling pathways. In view of this, there is a need to further subclassify and characterize liver CSCs.

Major and Characterized Liver CSC Surface Markers

Over the past decade, a number of liver CSC surface markers have been identified, as evidenced by the functional CSC properties. Identified liver CSC markers include epithelial cell adhesion molecule (EpCAM), CD133, CD90, CD13, CD44, CD24, CD47, OV6, GEP, and DLK1 [65, 66]. Notably, liver CSCs can be enriched by chemotherapeutic agents; doxorubicin and fluorouracil enriched CD133+ HCC cells [67] and sorafenib enriched EpCAM+ as well as CD47+ HCC cells, to name a few [40, 68].

Signaling Pathways Associated with CSC

In normal hepatic progenitor cells, the Wnt/ β -catenin signaling and STAT3 signaling pathways are responsible for liver specification and liver regeneration in a strictly controlled manner [69–71]. Dysregulation of these two pathways has also been found to be associated with the maintenance of liver CSCs. With regard to the Wnt/ β -catenin signaling pathway, Mokkapatil et al. reported that stabilization of β -catenin in liver stem/progenitor cells using β -catenin overexpressing transgenic mice resulted in the development of HCC with spontaneous lung metastases [72]. Its activation led to the enrichment of EpCAM+ and OV6+ liver CSCs and subsequently to cancer cell growth [73] and chemoresistance [74].

For the STAT3 signaling pathway, two independent studies deciphered the role of CD24+ CSCs in the development of HCC. Lee et al. demonstrated that CD24+ cells endowed CSC properties through the STAT3-mediated NANOG pathway [75], while Liu et al. further reported that Twist2 regulated CD24 expression by direct binding to the E-box region located on the CD24 promoter [76]. The STAT3 signaling pathway is augmented in response to pro-inflammatory cytokines and this may lead to tumor formation. A recent study has shown that the IL6/STAT3 signaling cascade gives rise to the enrichment of CD133+ in HCC [77]. Thus, STAT3 signaling appears to be an important pathway deregulated during early hepatocarcinogenesis. Other signaling pathways implicated in liver CSCs include sonic hedgehog signaling, Ras/Raf/MAPK signaling, the Notch pathway, PI3-K/Akt/mTOR and TGF- β signaling. Deregulation of these cascades has been shown to result in the enrichment of CSCs, typified by various liver CSC surface markers [66].

Epigenetic Regulation of CSC Expression

Apart from the signaling cascades, there is a growing interest in the epigenetic regulation of CSC expression. Epigenetic regulators, histone deacetylase 3 and DNA methyltransferase 1 (DNMT1) have been reported in the maintenance of CSCs [78, 79]. Intriguingly, liver CSCs are generally more hypomethylated than the differentiated cancer cells [80]. It is reported that TGF- β 1 enhanced CD133+ HCC cells by inhibiting DNMT1 and DNMT3 β , which are critical in the maintenance of regional DNA methylation [81]. Moreover, treatment with a DNMT1 inhibitor induced CSC properties in an isolated liver CSC side population [82]. As far as miRNA is concerned, miR-142-3p was reported to inversely correlate with the expression of CD133 in HCC [83], whereas miR-181 expression led to the enrichment of EpCAM+ HCC cells [84].

CSC and Tumor Microenvironment

Since most HCCs develop from a chronic inflammatory background of the liver, it has been reasoned that CSCs can evolve, or their stemness properties are maintained from the interaction with the tumor microenvironment, including stromal cells and extracellular ma-

trix, hypoxia, and immune cells [85]. Increased matrix stiffness caused by chronic inflammation is a common pathological condition occurring in HCC. Schrader et al. demonstrated that an environment with increased stiffness profoundly altered the phenotype and behavior of HCC cells in vitro. Increased matrix stiffness led to enhanced HCC cell proliferation and resistance to cisplatin treatment, whereas a soft physiological environment enriched CSC surface markers and enhance stemness gene expression (CD133, CD44, CXCR4, OCT4 and NANOG) [86]. Furthermore, elevated expression levels of proinflammatory cytokines are detected during inflammation. IL6, a cytokine secreted by tumor-associated macrophages (TAMs), has been detected in HCC samples and recently found to promote the expansion of CD44+ cells in HCCs by co-culturing CD14+ TAMs [87].

NF- κ B signaling pathway is heavily implicated in the inflammatory response in the liver. Literature studies have provided hints for linking NF- κ B from chronic liver injury to HCC upon proinflammatory cytokine stimulation [88]. This pathway has been shown to play a key role in inflammation induced hepatocarcinogenesis [88]. Given that the inflammatory milieu is a potential breeding hub for liver CSCs, there is only scarce information about the role of NF- κ B in liver CSCs. Lo et al. reported that NF- κ B regulated the CD47 overexpression in sorafenib-resistant HCC cells and this endowed the CSC properties [40]. Recently, NF- κ B has been shown to be associated with cancer stemness in HCC [89]. The linkage between NF- κ B and liver CSCs appears to be a promising area of study. Besides, the emergence of three-dimensional tissue organoid system is more structurally relevant and it can recapitulate, to a greater extent, in vivo pathophysiological processes. Hans Clevers' group has successfully cultured injury induced Lgr5+ liver cells in an organoid system and subsequently delineated the Wnt signaling-mediated expansion of Lgr5+ progenitor cells after liver injury [90]. As mentioned previously, since enrichment of liver CSCs partly depends on the interaction with the tumor microenvironment, the organoid system is able to resemble the in vivo conditions and tackle with the drawbacks of the commonly used two-dimensional culture system. By doing so, the picture of the tumorigenesis from the evolvement of CSCs to tumor formation may be unveiled.

Liver CSCs–Therapeutic Opportunities and Applications

Despite the emergence of various therapeutic modalities including chemotherapy, targeted therapy and radiotherapy in recent years, the efficacy in eradicating HCC is still unsatisfactory partially attributed to resistance to treatment conferred by liver CSCs. Residual liver CSCs that survive from therapeutic agents are able to initiate tumor formation and lead to tumor recurrence. Thus, targeting and eliminating liver CSCs is an appealing direction to devise further treatment options. Recent work has illustrated that sorafenib enriches EpCAM+ liver CSCs via the TSC2-AKT pathway [68]. In this context, agents that reduce Akt activity would be a potential therapeutic treatment for the elimination of EpCAM+ liver CSCs induced by sorafenib. Apart from targeting the signaling pathway, the use of monoclonal antibody targeting CSC surface markers is also a potential treatment to eradicate CSCs. As CD47+ HCC cells are enriched in sorafenib-resistant HCC cells, it has been shown that anti-CD47 monoclonal antibody can reduce liver CSCs properties and sensitize HCC cells to both conventional chemotherapeutic drugs and sorafenib treatment [40, 91]. These encouraging findings should prompt further work to characterize CSC genes and associated targeted therapies.

Conclusions

Dissection of hepatocarcinogenesis at multiple molecular levels potentially sheds light on alternative treatment options. Identification of key molecular events and crucial signal-

ing pathways provides insight to devise targeted therapies to treat this cancer. On the other hand, recent studies using NGS technology have facilitated the global and systematic evaluation of molecular landscapes in HCC. Emerging evidence supports the importance of cancer metabolism and tumor microenvironment in providing a favorable and supportive niche to expedite the development of HCC. Moreover, the importance of liver CSCs in HCC and their involvement of altered signaling pathways, epigenetic modifications, concomitant drug resistance and metastasis have highlighted their roles of potential targets of novel treatments. Therefore, future effective novel therapies against specific targets in HCC are anticipated, and we believe there will be better management of patients with HCC from improved treatment efficacy.

Conflict of Interest

The authors declare none.

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