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Review

Chromosome 1q21 amplification and oncogenes in hepatocellular carcinoma

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Hepatocellular carcinoma (HCC) is among the most lethal of human malignancies. During human multistep hepatocarcinogenesis, genomic gain represents an important mechanism in the activation of proto-oncogenes. In many circumstances, activated oncogenes hold clinical implications both as prognostic markers and targets for cancer therapeutics. Gain of chromosome 1q copy is one of the most frequently detected alterations in HCC and 1q21 is the most frequent minimal amplifying region (MAR). A better understanding of the physiological and pathophysiological roles of target genes within 1q21 amplicon will significantly improve our knowledge in HCC pathogenesis, and may lead to a much more effective management of HCC bearing amplification of 1q21. Such knowledge has long term implications for the development of new therapeutic strategies for HCC treatment. Our research group and others, focused on the identification and characterization of 1q21 target genes such as *JTB*, *CKS1B*, and *CHD1L* in HCC progression. In this review, we will summarize the current scientific knowledge of known target genes within 1q21 amplicon and the precise oncogenic mechanisms of *CHD1L* will be discussed in detail.

Keywords: amplification; 1q21; oncogene; CHD1L; hepatocellular carcinoma

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Introduction

Primary liver cancer mainly refers to hepatocellular carcinoma (HCC), cholangiocarcinoma, and angiosarcoma. As the fifth most common neoplasm, HCC accounts for 85% to 95% of all primary liver cancers[1] and ranks as the third-leading cause of cancer-related deaths worldwide[2–4]. The causes of HCC are perhaps better understood than those of any other major human cancer. The main risk factors include infections of hepatitis B virus (HBV) and hepatitis C virus (HCV), and intake of aflatoxin B1 (AFB1), which are responsible for about 80% HCC cases[5]. Other etiological factors, such as microcystin-contaminated drinking water, tobacco smoking, non-alcoholic steatohepatitis resulting from obesity and/or diabetes, and inherited metabolic diseases such as hereditary hemochromatosis[6–8], have also been proposed to lead to HCC, although at a lower frequency. The incidence of HCC also increases progressively with age, suggesting that the accumulation of genetic alterations over time contributes to HCC development. HCC has a very poor prognosis, with a life expectancy of about 6 months from time of diagnosis, and a <3% 5-year survival rate for untreated cancer. Death often occurs due to liver failure associated with cirrhosis and/or rapid outgrowth of multilobular HCC. Although early HCC could be cured by surgical resection, the fact that many tumors are asymptomatic means that most patients at risk for HCC will not be diagnosed in time. These patients often present with inoperable HCC, usually with the large and/or multiple HCC nodules.

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Detection of recurrent alterations in HCC

Genomic abnormalities play important roles in carcinogenesis. Over last several decades, the importance of chromosome aberrations in pathogenesis of cancer has been widely elucidated[9]. Like most cancers, HCC is a heterogeneous tumor with a complex variety of genetic changes[10]. Progressive genetic alteration causes a spectrum of events initiating with cell proliferation and progression from hyperplasia to dysplasia and ultimately to cancer[11, 12]. This model has become widely accepted and is applicable to many types of cancers, including HCC. The molecular analysis of HCC clarifies that the multistage process of hepatocarcinogenesis is complex and depends on a combination of genetic, viral, and environmental factors. In cancer cells, chromosomes are broken, truncated, deleted, amplified or translocated to other chromosomes. Some of aberrations are specific for tumorigenesis, whereas

most of them happen randomly. Chromosomal abnormalities may lead to the inactivation of tumor suppressor genes (TSGs) or activation of oncogenes via amplification. As listed in Table 1, chromosomal losses are frequently detected in HCC patients at 1p (36%–37%), 4q (32%–70%), 6q (19%–37%), 8p (26%–77%), 13q (16%–55%), 16p (14%–70%), and 17p (10%–60%) while gains are often detected at 1q (46%–86%), 6p (20%–33%), 8q (31%–83%), 17q (29%–48%), and 20q (5%–37%)[13-22]. In particular, gain of chromosome 1q21–23 and 8q22–24 has been associated with the early development of HCC[16, 23], whereas gain of 3q has been linked to tumor recurrence and poor overall patient survival[24]. In addition, deletion of 8p has been correlated with HCC metastasis[25].

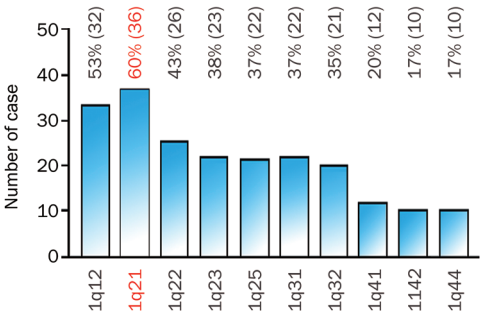

Figure 1. Amplification of different regions along chromosome 1q in 60 clinical HCC specimens. Amplification of 1q21 is most frequent chromosomal alteration in chromosome 1q in HCC.

Identification of minimal amplified region on chromosome 1q

Using comparative genomic hybridization (CGH), we and other groups, have demonstrated that copy number gain of chromosome 1q is one of the most frequently detected alterations in HCC[13–22] and has been suggested as an early genomic alteration during HCC progression[26]. Several minimal amplified regions (MARs) on 1q were mapped including 1q12–q22[17, 25], 1q23.3–q25.3[27] and 1q23.1–1q43[28]. Gains of 1q21–q25 have been linked to a more aggressive phenotype with metastatic potential in renal clear cell carcinoma and squamous cell carcinomas of lung[29, 30]. In ovarian cancer and neuroblastoma, 1q21–q22 amplification has been suggested to be correlated with a drug-resistant phenotype[31, 32]. In HCC, regional 1q21–q22 gains were found in 40% of advanced metastatic HCC cases[26]. In the past three years, our research group showed that 1q21 was the most frequently amplified region in chromosome 1q and its amplification has been detected in 36/60 (60%) of HCC specimens[33] (Figure 1). This implies that 1q21 may harbor one or more oncogenes whose overexpression caused by amplification plays an important role in HCC pathogenesis.

Identification of cancer-related genes within 1q21 amplicon

In human solid tumors, the copy number alterations are believed to contribute to the tumorigenesis by affecting the functions of cancer-related genes in the altered chromosomal regions[34]. In HCC, isolation and identification of putative cancer-related oncogenes can improve our understanding of hepatocarcinogenesis. Inagaki *et al* reported a novel 1q21 amplicon in HCC-derived cell lines using high-density single nucleotide polymorphism (SNP) array[35]. Using fluorescence *in situ* hybridization (FISH) and real-time quantitative PCR, they identified three candidate targets *CREB3L4*, *INTS3*, and *SNAPAP* within 700-kb amplified 1q21 region and found these

Table 1. Summary of chromosomal gains and losses in hepatocellular carcinoma.

	Detected By Comparative Genomic Hybridization (CGH)									By EIM
Chr	Marchio *et al*[13]	Kusano *et al*[14]	Sakakura *et al*[15]	Wong *et al*[16]	Guan *et al*[17]	Chang *et al*[18]	Niketeghad *et al*[19]	Shiraishi *et al*[20]	Balsara *et al*[21]	Midorikara *et al*[22]
Gains										
1q	58	78	46	72	66	86	57	77	73	74
6p	33		20							
8q	60	66	31	48	48	77	52	52	83	48
17q	33		43	30			29			48
20q	23	14		37	20	5	29			22
Losses										
1p			37		36					
4q	70	32	48	43	40	59	33	35	40	48
6q	37		23					19		
8p	65	29	28	37	32	77		26	44	35
13q	37	37	20	37	16	27	19	55		32
16p	54	46	33	30	70	50	14	52	63	
17p	51	51	37	10	52	45		52	60	29

Value means percentage of positive region. The number in square bracket corresponds with reference number.
Abbreviation: EIM, expression imbalance map.

targets were all significantly overexpressed in tumors from HCC patients[35] (Table 2). Wong *et al* found that the expression levels of *JTB* and *SHC1* in 1q21 amplicon were significantly higher than the paired adjacent non-malignant liver tissues[36] (Table 2). In addition, Midorikawa et al. demonstrated that a regional 1q21-23 gain (74%) was significantly associated with HCC by expression imbalance map (EIM) analysis which can detect mRNA expression imbalance linked to chromosomal regions, and they also identified two candidate genes *SHC1* and *CKS1B* within 1q21 region[22]. Recently, our research group isolated a novel candidate oncogene *CHD1L* by using microdissected DNA from 1q21 and hybrid selection[33]. We found that amplification of *CHD1L* at genomic level and overexpression of CHD1L at protein level were detected in 50.6% (86/170) and 52.4% (163/311) of informative HCC tissues, respectively[33] (Table 2).

Multistep progression of hepatocarcinogenesis

The development and progression of HCC is typical of a multistage process. Malignant hepatocytes result from step-by-step changes that accumulate in mature hepatocytes or can be derived from stem cells[37]. The most widely accepted hypothesis describes a sequential process in which this stepwise transformation begins in the liver tissue that undergoing chronic hepatitis or cirrhosis caused by external stimuli (HBV or HCV infection, alcohol or metabolic diseases), progresses through a series of hyperplastic and dysplastic stages (low- and high-grade dysplastic) stages, and progresses further to become more malignant, making metastases possible[38, 39]. This malignant transformation of a normal cell into a cancer cell is thought to be caused by aberrant gene expression critical to cellular processes such as cell cycle control, cell growth, differentiation, apoptosis, cell adhesion and other functions at the cellular, molecular, and genetic levels. The tumor cells of early HCC are well differentiated and highly proliferative, and they go on to form less differentiated HCCs that frequently show a nodule-in-nodule appearance, a signature of advanced HCC. At this advanced stage, angiogenesis, tissue invasion, and metastases occur in up to 25% of cases[37]. Later on, the neoplastic cells become undifferentiated and are able to invade vessels and spread outside the liver, characteristics that define end-stage disease.

Roles of oncogenes in 1q21 during hepatocarcinogenesis

During human multistep hepatocarcinogenesis, DNA amplification represents an important mechanism in the activation of proto-oncogenes. In many circumstances, activated oncogenes hold clinical implications both as prognostic markers and targets for cancer therapeutics. Next, we will summarize the current findings of known target genes within 1q21 amplicon in HCC, and the role of a novel oncogene *CHD1L* identified by our laboratory during hepatocarcinogenesis will be described in detail.

CREB3L4, INT3, SNAPAP, and JTB

CREB3L4 (cyclic AMP responsive element binding protein3-like 4), belongs to the CREB/ATF family of transcriptional factor[40]. Immunostaining analysis showed that the expression of CREB3L4 at protein level were higher in prostate cancer than the adjacent noncancerous tissues[40], whereas the functional role of CREB3L4 in HCC remains to be revealed. JTB (Jumping Translocation Breakpoint) is a transmembrane protein which suffers an unbalanced translocation in various types of cancers[41]. Wild-type JTB confers resistance to apoptosis induced by TGF-β1[42]. The average level of JTB expression in HCC tumor cells was 1.9-fold higher than paired nontumor liver tissues[36]. To elucidate the role of JTB during hepatocarcinogenesis, Pan *et al* found that JTB could interact with HBs (Hepatitis B surface) and overexpression of JTB inhibited ultraviolet radiation-induced apoptosis which was compromised by co-overexpression of HBs[41]. However, little is known about the possible functional association of *INTS3* and *SNAPAP* with HCC tumorigenesis.

SHC1

Huebner *et al* assigned the *SHC1* gene to 1q21[43]. The SHC gene encodes 2 widely expressed overlapping proteins of 46 and 52 kilodalton (kDa) as well as a 66-kDa isoform, $p66^{Shc}$.

Table 2. Candidate target genes in 1q21 amplicon in HCC.

Gene	Location	Function	Overexpression in tumor[a]	Reference
CREB3L4	1q21.3	Androgen receptor signaling	78% (14/18)	Inagaki *et al*[35]; Qi *et al*[40]
INTS3	1q21.3	Genome instability; DNA damage response	78% (14/18)	Inagaki *et al*[35]; Zhang *et al*[59]; Huang *et al*[60]
SNAPAP	1q21.3	Control exocytosis processes	72% (13/18)	Inagaki *et al*[35]; Ruder *et al*[58]
JTB	1q21.3	Apoptosis resistance		Pan *et al*[41]; Wong *et al*[36]; Kanome *et al*[42]
SHC1	1q21.3	Cell proliferation; apoptosis; tyrosine phosphorylation; signaling		Wong *et al*[36]; Huebner *et al*[43]; Kraut-Cohen *et al*[44]; Pinton *et al*[45]
CKS1B	1q21.2	Cell cycle regulation	63% (30/48)	Midorikawa *et al*[22]; Wang *et al*[47]; Ganoth *et al*[48]; Shen *et al*[50]; Calvisi *et al*[51]
CHD1L	1q21.1	Cell proliferation; apoptosis; transcription regulation; invasion and metastasis; DNA repair	52% (163/311)	Ma *et al*[33]; Ahel *et al*[54]; Chen *et al*[56]; Chen *et al*[57]

[a] The percentage of HCC cases with higher expression of target gene than their adjacent nontumor tissues.

Shc is involved in signal transduction from receptor tyrosine kinases to downstream signal recipients such as Ras[43, 44]. The mitochondrial pro-apoptotic activity of $p66^{Shc}$ has been reported to be associated with its phosphorylation caused by protein kinase C β under oxidative conditions[45]. The expression level of $p66^{Shc}$ in tumor tissue has been reported to inversely correlate with an increased risk of disease recurrence in early-stage breast cancer[46]. Wong *et al* reported the expression of *SHC1* gene at mRNA level was significantly higher than the adjacent nontumor liver tissues[36]. However, the oncogenic mechanism of *SHC1* during HCC initiation and progression remains unclear.

CKS1B in 1q21.2

CKS1B (CDC28 protein kinase regulatory subunit 1B), also named Cks1 (cyclin kinase subunit 1), is a member of the highly conserved family of Cks/Suc 1 proteins[47]. Human Cks1 is required for SCF^{Skp2}-mediated ubiquitination and degradation of $p27^{kip1}$ that is indispensable for G_1/S transition during cell cycle progression[48]. In this process, Cks1 interacts with Skp2, the substrate recognition component of SCF, thereby increasing its association with phosphorylated $p27^{kip1}$, Skp2-bound $p27^{kip1}$ is then degraded via ubiquitin-proteasome pathway[49]. So far, several groups have identified the potential role of Cks1 in HCC[50, 51]. Shen *et al* demonstrated that expression of Cks1 at mRNA and protein levels was significantly higher in HCC than those in the adjacent non-tumor liver tissue, and Cks1 expression was closely associated with poor differentiation and also negatively associated with $p27^{kip1}$ in HCC[50].

CHD1L in 1q21.1

CHD1L (Chromodomain Helicase/ATPase DNA Binding Protein 1-Like, also known as Amplified in Liver Cancer 1, *ALC1*) belongs to the SNF2-like subfamily of the Snf2 (sucrose non-fermenting 2) family. The full-length mRNA (2.98 kb) of *CHD1L* encodes an 897aa protein. The CHD1L protein contains highly conserved helicase motifs, which are also found in other family members such as Snf2, ISWI, and CHD1[52]. The sequence homology of the helicase domain (107 aa) between CHD1L and CHD1 is 59%[33]. Unlike CHD1, however, CHD1L does not contain a chromo domain, which can recognize methylated histone tails. Instead, CHD1L contains a Macro domain, which is an ADP-ribose/PAR-binding element[53]. Ahel *et al* demonstrated that CHD1L, a chromatin remodeling enzyme, functions as a DNA damage-response protein via its association with known DNA factors and its rapid poly(ADP-ribose)-dependent recruitment to DNA damage sites[54].

In 2008, the functional role of *CHD1L* in human tumorigenesis was first reported by our laboratory. We have demonstrated that 1) *CHD1L*-transfected cells are able to form more colonies in soft agar and caused tumor formation in nude mice; 2) the transformation ability of *CHD1L* is effectively inhibited by small interference RNA (siRNA) against *CHD1L*; 3) CHD1L can promote the G_1/S transition and inhibit apoptosis in response to cellular stress[33]. Importantly, there is now a transgenic mouse model with ubiquitous CHD1L expression[55]. Spontaneous tumor formation was found in 10/41 (24.4%) of *CHD1L*-transgenic mice including 4 mice with HCCs, and no cases were seen in their 39 wild-type littermates[55]. Further, our mechanistic studies of *CHD1L* in the past two years have delineated CHD1L-involved hepatocarcinogenesis during HCC progression and metastasis. As a member of SNF2-like subfamily, *CHD1L* is a unique gene because most of its family members are likely to act as tumor suppressor genes (TSGs). Based on the identification of CHD1L target genes by means of yeast-two hybrid and chromatin immunoprecipitation (ChIP)-based cloning approaches, the underlying anti-apoptotic and metastatic mechanisms of *CHD1L* were well elucidated[56, 57]. Chen *et al* revealed that the anti-apoptotic ability of CHD1L was found to be linked to its interaction with Nur77, a critical member of a p53-independent apoptotic pathway[56]. As the first cellular protein identified to bind with Nur77, CHD1L was able to inhibit the nucleus-to-mitochondria translocation of Nur77, thereby causing the hindrance of cytochrome *c* (Cyt *c*) release into the cytoplasm, activation of caspase 9 and 3, as well as the initiation of apoptosis (Figure 2). Further, the C-terminal domain of CHD1L is proved to be responsible for the interaction with Nur77 and the inhibition of Nur77-mediated apoptosis. CHD1L was also found to be endowed with DNA binding activity[57]. A ChIP-based cloning strategy was conducted to isolate 35 CHD1L regulated genes, one of which was *ARHGEF9*, a guanine nucleotide exchange factor (GEF) for the Rho small GTPase Cdc42. *ARHGEF9* was transcriptionally activated by CHD1L. Both in vitro and in vivo functional studies found that CHD1L could increase cell motility, induce filopodia formation and the epithelial-mesenchymal transition (EMT) via ARHGEF9-mediated Cdc42 activation, all contributing to tumor cell invasion and metastasis[57] (Figure 3). Our further investigation of clinical HCC specimens exhibited significant association of CHD1L expression with not only clinicopathological features such as microsatellite tumor formation, venous infiltration and advanced tumor stage, but also the overall survival time and the disease-free survival rate (unpublished data). Furthermore, CHD1L was found to be significantly correlated with chemotherapy responsiveness and survival of recurrent HCC patients (unpublished data). Interestingly, the involvement of Nur77-mediated pathway in chemotherapeutic agent-induced apoptosis can dictate if CHD1L could confer resistance to chemotherapy (unpublished data). Taken together, we delineated two novel molecular mechanisms, CHD1L/Nur77 and CHD1L/ARHGEF9/Cdc42/EMT, during HCC progression and metastasis (Figure 4).

Conclusions and future directions

During HCC progression, regional chromosomal gain represents a major mechanism in the activation of proto-oncogenes. Gain of 1q is one of the most frequently detected alterations in HCC and has been identified as an genomic event associated with early development of HCC[25]. Due to the highest frequency of copy number gain at 1q21, much effort has been devoted to identifying target genes which are responsible

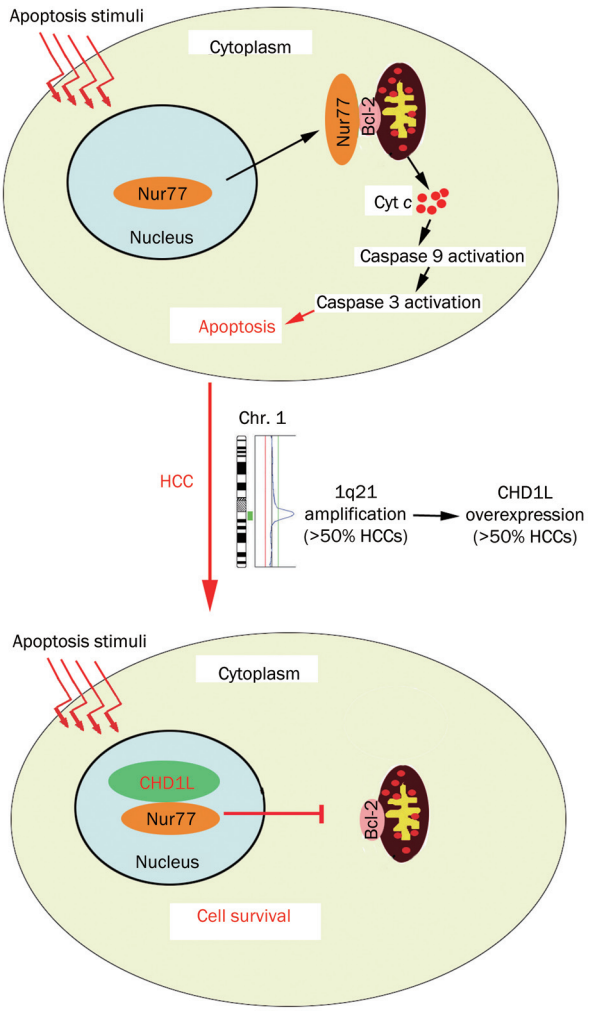

Figure 2. The role of CHD1L in Nur77-mediated pathway during hepatocarcinogenesis. In normal condition, Nur77 localizes in nucleus. In response to apoptosis stimuli, Nur77 translocates from nucleus to mitochondria where it binds to Bcl-2 and induces its conformational change, thereby leading to cytochrome *c* (Cyt *c*) release into cytoplasm and the initiation of caspase activation and apoptosis. In HCC, CHD1L, which is overexpressed caused by 1q21 amplification, are able to inhibit the nucleus-to-mitochondria translocation of Nur77, resulting in the hindrance of cytochrome *c* (Cyt *c*) release, caspase activation and apoptosis.

for 1q21 amplicon. However, the extensive and complicated nature of chromosome aberrations makes difficult for identifying the biologically relevant alterations and their functional significance. In this decade, some research groups focused on the identification and characterization of 1q21 target genes such as *CHD1L*, *CKS1B*, and *SHC1* in HCC progression. Based on our recent work, CHD1L is proved to be amplified and overexpression in HCC cases. By using molecular biological techniques, we demonstrated two molecular pathways (CHD1L)-(Nur77)-(Cyt *c*) and (CHD1L)-(ARHGEF9)-(Cdc42)-(EMT), which led to evasion of apoptosis, the decreased sensitivity to chemotherapy and the promotion of tissue invasion and metastasis. Although more and more candidate target genes within 1q21 amplicon have been identified, for majority of target genes, the precise mechanisms underlying HCC initiation and progression however, remain unclear. It is also possible that coactivation of these target genes leads to HCC development and progression. To better elucidate the relationship between genomic aberrations and hepatocarcinogenesis, functional and mechanistic studies are needed to clarify the roles of these target genes.

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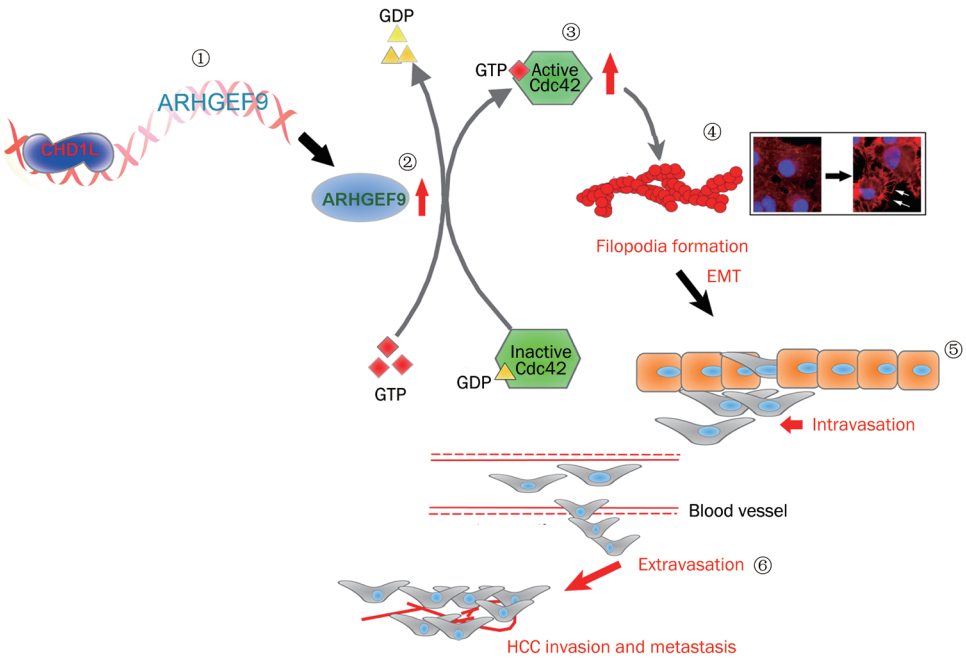

Figure 3. During HCC progression, CHD1L is overexpressed and activates ARHGEF9 transcription via a protein-DNA interaction (①). CHD1L enhances the GTP loading onto Rho GTPase protein Cdc42 (the increased level of Cdc42-GTP) (②) through the up-regulation of ARHGEF9 (③). CHD1L/ARHGEF9-induced Cdc42 activation leads to more filopodia formation as a characteristic of actin cytoskeletal rearrangement and the concomitant EMT phenotype (④). CHD1L-dependent EMT may result in a sequential process including the loss of cell-cell contact, the penetration of basement membrane (BM) and the entry of tumor cells into blood circulation (intravasation) (⑤), finally the growth of tumor cells in the secondary sites in liver (intrahepatic metastases or microsatellite formation) or new organs (distant metastases) (⑥).

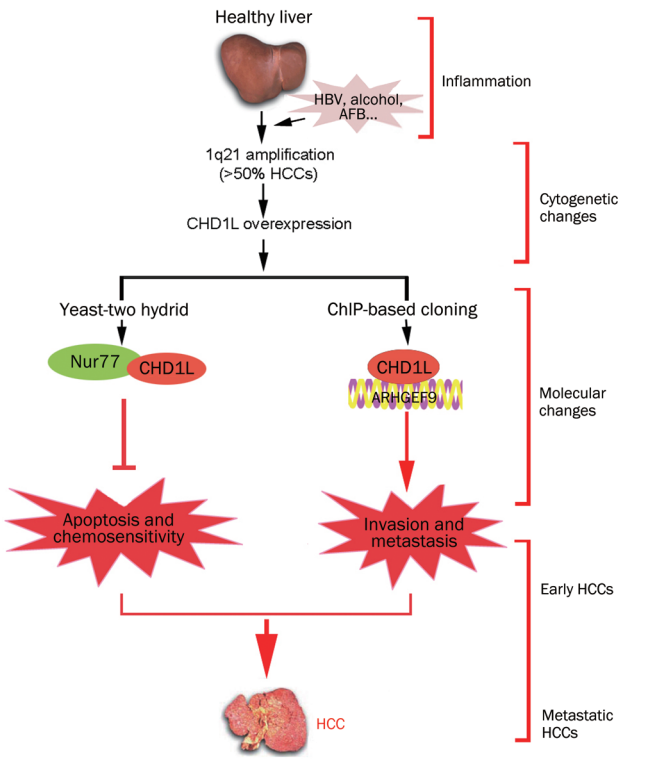

Figure 4. Healthy liver that undergoes chronic hepatitis or cirrhosis, progresses through hyperplastic and dysplastic stages. During these stages, cytogenetic alterations often occur including amplication of 1q21 which has been detected in over 50% of HCC specimens. Therefore, CHD1L is amplified and overexpression in HCC cases. By using molecular biological techniques, we delineated two molecular pathways (CHD1L)-(Nur77)-(Cyt *c*) and (CHD1L)-(ARHGEF9)-(Cdc42)-(EMT), which led to evasion of apoptosis, the decreased sensitivity to chemotherapy and the promotion of tissue invasion and metastasis.

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