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Invited review

Cytogenetic and molecular genetic alterations in hepatocellular carcinoma

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Key words

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Abstract

Specific chromosome aberrations are frequently detected during the development of hepatocellular carcinoma. Molecular cytogenetic approaches such as comparative genomic hybridization and loss of heterozygosity analyses have provided fruitful information on changes in HCC cases at the genomic level. Mapping of chromosome gains and losses have frequently resulted in the identification of oncogenes and tumor suppressors, respectively. In this review, we summarize some frequently detected chromosomal aberrations reported for hepatocellular carcinoma cases using comparative genomic hybridization and loss of heterozygosity studies. Focus will be on gains of 1q, 8q, and 20q, and losses of 4q, 8p, 13q, 16q, and 17p. We then examine the candidate oncogenes and tumor suppressors located within these regions, and explore their possible functions in hepatocarcinogenesis. Finally, the impact of microarray-based screening platforms will be discussed.

Introduction

Hepatocellular carcinoma (HCC) is one of the most common human malignant neoplasms, with a particularly high incidence in Chinese and African populations[1]. In 2000, HCC ranked as the fifth most frequent cancer, but the third leading cause of cancer death worldwide[2]. Unlike other cancers, the main causative agents for HCC, hepatitis B virus (HBV), hepatitis C virus (HCV) and aflatoxin (AFB), are well studied. Yet little is known about the molecular pathogenesis of HCC[3]. In fact, the majority of HCC are associated with a background of chronic liver disease. Therefore, hepatocarcinogenesis is believed to be a long-term process that involves multiple genetic alterations.

Recurrent chromosome alterations

Chromosome aberrations are a hallmark of solid tumors and it has been known for decades that chromosome rearrangements exist in most if not all human tumors[4]. Additionally, cytogenetic study followed by molecular analysis of recurring chromosome changes has greatly facilitated the identification of crucial oncogenes and tumor suppressors[5]. For example, the tumor suppresor gene Rb was identified based on the observation of chromosome deletion del(13)(q14) in retinoblastoma[6] and the proto-oncogene myc was shown to be involved in the chromosome translocation t(8;14) in human Burkett's lymphoma[7]. The conventional comparative genomic hybridization (CGH) method provides a powerful means of global analysis of segmental chromosome gains or losses[8]. Since it was developed, CGH has been widely applied to the detection of recurrent chromosomal alterations in various cancers, including HCC[9–13]. Frequent non-random chromosomal gains and losses detected by CGH are summarized in Table 1, including gains of 1q, 6p, 8q, 17q, and 20q, and losses of 1p, 4q, 5q, 6q, 8p, 9p, 10q, 13q, 16q, 17p, 19p, and 22q[9–13]. In addition, the loss of heterozygosity (LOH) assay is used to define chromosomal regions with allelic deletions, and results revealed that LOH was frequently detected in 1p, 4q, 6q, 8p, 13q, 16q, and 17p[14–16]. These studies suggest the presence of multiple oncogenes or tumor suppressor genes in regions of recurrent gain or loss, respectively.

To avoid overlap with comprehensive reviews that have been published recently[3,17], this review will mainly focus on chromosomal gains on 1q, 8q, and 20q, and losses on 4q, 8p, 13q, 16q, and 17p in HCC. For simplicity, gene symbols will

Table 1. Summary of chromosomal gains and losses revealed by comparative genomic hybridization analyses

	Marchio *et al*[9]	Wong *et al*[10]	Kusano *et al*[11]	Guan *et al*[12]	Chang *et al*[13]
Gains					
1q	25/43 (58%)	48/67 (72%)	32/41 (78%)	33/50 (66%)	19/22 (86%)
8q	26/43 (60%)	32/67 (48%)	27/41 (66%)	24/50 (48%)	17/22 (77%)
20q	10/43 (23%)	25/67 (37%)	6/41 (14%)	10/50 (20%)	1/22 (5%)
Losses					
4q	30/43 (70%)	29/67 (43%)	13/41 (32%)	20/50 (40%)	13/22 (59%)
8p	28/43 (65%)	25/67 (37%)	12/41 (29%)	16/50 (32%)	17/22 (77%)
13q	16/43 (37%)	25/67 (37%)	15/41 (37%)	8/50 (16%)	6/22 (27%)
16q	23/43 (54%)	20/67 (30%)	19/41 (46%)	35/50 (70%)	11/22 (50%)
17p	22/43 (51%)	7/67 (10%)	21/41 (51%)	26/50 (52%)	10/22 (45%)

be used for all genes described throughout the review (details for all genes are summarized in Table 2).

Chromosome gains

Gain of 1q Gain of 1q is one of the most frequently detected alterations in HCC and has been suggested as an early genomic lesion in the process of HCC development[18], although the target oncogene within the amplified region has not been identified. CGH studies showed that the gain of 1q was detected in 58%–86% of HCC cases[9–13], with about 10% high-copy number amplification[12]. Several minimal amplifying regions (MAR) were mapped including 1q12-q22[12,19], 1q23.3-q25.3[20] and 1q24.2-1q43[21]. In an attempt

Table 2. Summary of candidate cancer-related genes within abnormal chromosomal regions

Gene name	Symbol	Locus	Reference
Oncogenes			
Jumping translocation breakpoint	JTB	1q21-q22	22
SHC (Src homology 2 domain containing) transforming protein 1	SHC1	1q21-q22	22
Chaperonin containing TCP1, subunit 3 (gamma)	CCT3	1q21-q22	22
Coatomer protein complex, subunit alpha	COPA	1q21-q22	22
V-myc myelocytomatosis viral oncogene homolog (avian)	c-Myc	8q24.12-q24.13	18
Protein tyrosine kinase 2	PTK2	8q23-q24	29
Eukaryotic translation initiation factor 3, subunit 3 gamma	EIF3S3	8q23-q24	29
Nuclear receptor coactivator 3	AIB1	20q12	18, 31
Tumor suppressors			
PR domain containing 5	PRDM5	4q25-q26	37
PIN2-interacting protein 1	PINX1	8p23	38
Fibrinogen-like 1	FGL1	8p22-p21.3	39
Deleted in liver cancer 1	DLC1	8p22	40–42
Platelet-derived growth factor receptor-like	PDGFRL	8p22-p21.3	43
Tumor suppressor candidate 3	TUSC3	8p22	44
Retinoblastoma 1 (including osteosarcoma)	RB	13q14.2	6
Inhibitor of growth family, member 1	P33ING1	13q34	45, 46
START domain containing 13 (Deleted in liver cancer 2)	DLC2	13q12-q13	47
Cadherin 1, type 1, E-cadherin (epithelial)	CDH1	16q22.1	49, 50
HSV-1 stimulation-related gene 1	HSRG1	16q23.1	51
WW domain containing oxidoreductase	WWOX	16q23.3-q24.1	52
Tumor protein p53 (Li-Fraumeni syndrome)	TP53	17p13.1	53
Chromosome 17 open reading frame 25	C17orf25	17p13.3	58

to identify candidate oncogene(s) at 1q12-q22, Wong *et al* examined the expression level of several candidate oncogenes in this region using reverse transcription-polymerase chain reaction (RT-PCR). Significant overexpression of 4 genes known as *JTB*, *SHC1*, *CCT3*, and *COPA* were observed in tumors as compared with paired adjacent liver tissues[22].

Gain of 8q Gain of 8q is frequently observed in HCC (48%–77%)[9–13]. The gain of 8q is also frequently detected in many other solid tumors including those of the prostate[23], lung[24], esophagus[25], nasopharynx[26], ovary[27] and breast[28]. Amplification and overexpression of the c-myc oncogene has been reported in various solid tumors, including HCC. In our recent study, amplification of *c-myc* was correlated with HCC tumor size, tumor metastasis and recurrence. Amplification of *c-myc* was found in 11/13 (85%), 9/25 (36%) and 4/12 (33%) cases of large HCC (>9 cm), medium HCC (4–9 cm) and small HCC (<4 cm), respectively. In addition, a higher frequency of c-myc amplification was detected in metastatic tumors (45%) than in their primary HCC (29%), and in recurrent tumors (60%) than in their primary HCC (38%)[18]. Because the gain of 8q involved the entire long arm in this study, it is highly likely that one or more novel oncogenes are involved in HCC development apart from *c-myc*. In a recent report, Okamoto *et al* suggested 2 candidate oncogenes as amplification targets at 8q23-q24 known as *PTK2* and *EIF3S3*. *PTK2* encodes focal adhesion kinase, while *EIF3S3* encodes the p40 subunit of eukaryotic translation initiation factor 3. Overexpression of these genes may be involved in HCC progression[29].

Gain of 20q Gain of 20q was detected in 3/5 CGH studies with a frequency of 20%–37% [9,10,12]. Gain of 20q was also frequently detected in breast cancer, and a steroid receptor coactivator, *AIB1*, has been identified within the commonly amplified region at 20q12[30,31]. Interestingly, gain of 20q is significantly associated with clinical stages and tumor size of HCC. Guan *et al* reported that gain of 20q occurred in 10/40 (25%) of stage II and III HCC but in 0/10 stage I HCC[12]. In addition, Wong *et al* reported that significantly more 20q gain was found in non-cirrhotic HCC. Strikingly, gain of 20q was observed in 9/12 (75%) non-cirrhotic, while only in 16/55 (29%) cirrhotic HCC (P=0.003)[10]. In our recent study, amplification of the *AIB1* gene was correlated with HCC metastasis and recurrence. The amplification frequency of AIB1 was significantly higher in metastatic HCC (41%) than in matched primary HCC (23%), as well as being higher in recurrent HCC (60%) than in matched primary HCC (29%) ($P < 0.05$)[18].

Chromosome losses

Loss of 4q Loss of chromosome 4q was detected by CGH in 32%–70% of HCC cases[9–13]. Deletion of 4q has been well described because of its specificity for HCC. Several minimal deletion regions (MDR) on 4q have been detected by LOH including 4q12-q23, 4q13.1-q21.23, 4q13-q34, 4q21-q25, 4q24-q28, 4q28.2-q34.3, and 4q35[32–34]. In addition, Yeh *et al* reported recently the mapping of a 17.5 cM MDR between D4S1534 and D5S1572 by screening 149 HCC: a few candidate genes were found to be downregulated[35]. Biological evidence for tumor suppressor genes on 4q comes from a study that reversed the tumorigenic phenotype of a teratoma cell line by introducing a normal chromosome 4 into malignant teratoma cells using microcell-mediated chromosome transfer[36]. To date, candidate tumor suppressor genes correlated with 4q loss have remained unidentified. A recent report by Deng *et al* described the role of *PRDM5* at 4q26 as a suppressor in various tumors including HCC. *PRDM5* encodes a protein belonging to the PR-domain protein family. Epigenetic silencing was evident for this gene and its overexpression resulted in proapoptotic and growth suppressive functions[37].

Loss of 8p Loss of 8p is well documented in HCC with a frequency of 29%–77%[9–13]. By analyzing 50 HCC cases, we observed a correlation of 8p loss with advanced stage of disease and tumor size; most strikingly, 8p loss was present in 8/13 (62%) of large, 7/25 (28%) of medium, but only 1/12 (8%) of small HCC cases (P=0.014)[12]. Furthermore, 8p deletion was associated with HCC metastasis, suggesting the presence of one or more candidate gene(s) are involved in HCC progression[19]. Using the high-resolution LOH strategy, Jou *et al* reported that 50% of HCC showed LOH in chromosome 8p and the percentages of 3 MDR at 8p23.3-p23.1 (D8S504–D8S277), 8p22-p21.3 (D8S1106–D8S258) and 8p21.3-p12 (D8S258–D8S283) were 71%, 69%, and 63%, respectively[34]. Several candidate tumor suppressors have been isolated within these regions, including *PINX1* at 8p23[38], *FGL1* at 8p22[39] and *DLC1*[40] at 8p22-p21.3 regions. In the 8p23 region, Liao *et al* observed the under-expression of *PINX1* (*LPTS*) in HCC cells and tissues, while ectopic expression of this gene in liver cancer cells SMMC-7721 resulted in growth suppression[38]. At 8p22, Yan *et al* reported reduced or undetectable expression of *FGL1* in HCC specimens, and found a significant correlation with tumor differentiation[39]. Interestingly, Wong *et al* reported the significant under-expression of DLC1 mRNA and promoter hypermethylation was found in 24% of primary HCC cases, suggesting a role of epigenetic silencing in *DLC1* downregulation[41]. *DLC-1* belongs to the rho family GTPase-activating proteins (RhoGAP) specific to RhoA and cdc42, which are implicated in cell migration control. Re-expression of *DLC1*

in HCC cells resulted in caspase-3-dependent apoptosis, inhibition of cell growth and invasiveness *in vitro*, and reduction of tumor formation in nude mice[42]. Furthermore, candidate tumor suppressors known as *PDGFRL*[43] and *TUSC3*[44] were identified in the 8p22-p21.3 region.

Loss of 13q Loss of 13q was detected in 16%–37% of HCC cases[9–13]. LOH analyses also showed a high percentage of allelic deletion on 13q (40%–55%)[14–16]. Jou *et al* identified 5 MDR with high frequencies of LOH including 13q12.11-q12.3 (71%), 13q13.1-q22.1 (79%), 13q31.3-q32.2 (67%), 13q32.2-q33.3 (73%) and 13q33.3-q34 (75%)[34]. MDR in 13q13.1-q22.1 (D13S171–D13S156) showed the highest frequency of LOH, and well-documented tumor suppressors including *BRCA2* and *RB* are located in this region. In 13q33.3-q34 MDR, one candidate tumor suppressor known as *P33ING1* has been isolated[45]. Overexpression of *p33ING1* inhibited cell cycle progression and the repression function of *p33ING1* was enhanced by the Ras/Raf pathway[46]. Recently, another candidate tumor suppressor known as deleted in liver cancer 2 (*DLC2*) was identified on 13q12.3. It shows homology to tumor suppressor gene *DLC1* located at 8p22-p21.3. *DLC2* encodes a protein with RhoGAP, SAM and START domains and is significantly underexpressed in only 8 of 45 (18%) HCC, as compared with its ubiquitous expression in normal tissues[47].

Loss of 16q One of the most frequent losses in HCC cases is located in the long arm of chromosome 16. Loss of 16q was detected in 30%–70% of HCC cases by CGH studies[9–13]. LOH also revealed allelic deletions in 55%–59 % of HCC[14–16]. Two MDR at 16q in HCC have been reported to include 16q12.1-q23.1 (71%) and 16q23.1-q24.1 (71%)[33]. Several candidate tumor suppressors have been reported in recent publications. The loss of 16q has also been reported as a frequent genomic alteration in ovarian[27] and prostate cancer[48]. The loss of 16q24.1-q24.2 in prostate cancer has been associated with aggressive behavior of the disease, recurrent growth, poor differentiation of the tumor, and a poor prognosis for the patient[48]. The best-studied tumor suppressor gene is E-cadherin (*CDH1*) at 16q22.1. Interestingly, expression of the E-cadherin gene is reduced by a hypermethylation mechanism although no somatic mutations of the E-cadherin gene in HCC have yet been described[49,50]. Bando *et al* identified a distinct commonly deleted region at 16q24.1-24.2 (D16S534 and D16S3091) near novel sequence *HSRG1*[51]. Another candidate tumor suppressor known as WWOX was identified at 16q23.3-q24.1: absence or reduced expression of WWOX was observed in 60% of HCC cell lines compared with normal liver[52].

Loss of 17p Loss of 17p is one of the most frequent chromosomal alterations in HCC as determined by both CGH[2,11–13] and LOH[14–16]. Deletion of 17p is common in various cancers including breast[53] and colon cancer[54]. The frequent deletion of 17p may affect the tumor suppressor gene *TP53* on 17p13.1. *TP53* is frequently inactivated in various types of malignant tumors, including HCC[55]. However, Yumoto *et al* showed that loss of 17p occurred in 18/31 HCC cases (58%) by LOH, whereas *TP53* mutation was only observed in 8/31 HCC cases (26%)[56]. Our recent study revealed that loss of one allele at 17p13.3 distal to the *TP53* gene was observed in 48/94 HCC (51%), whereas LOH at 17p13.1, near the *TP53* gene, was detected in 30/94 HCC (32%) and p53 mutation was only detected in 22/94 HCC (23%)[57]. These results suggest that another tumor suppressor gene at 17p13.3 may be involved in the pathogenesis of HCC. One candidate suppressor, *C17orf25*, has been isolated from this region[58]. Downregulated expression of *C17orf25* was found in HCC samples matched to adjacent non-tumor liver tissues. Furthermore, transfection of *C17orf25* into the hepatocellular carcinoma cell SMMC-7721 inhibited cell growth[58].

Conclusion

Molecular cytogenetic approaches such as CGH and LOH provide important clues to the identification of novel genes involved in the multistage development of HCC. In this review, we discussed some of the most common chromosome aberrations, including gains of 1q, 8q, and 20q, and loss of 4q, 8p, 13q, 16q, and 17p, followed by candidate oncogenes and tumor suppressors that may be involved within these regions. Due to the heterogeneity of HCC, it has been suggested that gains or losses of specific chromosomal loci rarely affect more than half of them, and specific HCC-related genes have not been identified in most of the above-mentioned chromosomal regions[3]. Results obtained with traditional approaches such as cytogenetic CGH and LOH studies are relatively low in resolution and are labor intensive. Advances in microarray-based approaches are beginning to reverse this situation by providing an integrated view of genetic alterations during tumorigenesis and progression[59].

Different microarray-based CGH (aCGH) platforms are emerging that allow high-resolution genomic profiling[60–62]. For instance, Hashimoto *et al* reported the use of cytogenetic CGH and aCGH in the identification of genes involved in HCV-related HCC[63]. In a recent article, Ishkanian *et al* reported the construction of submegabase resolution tiling genomic arrays (SMRT arrays) containing 32 433 overlapping BAC clones. This allows the detection of cryptic chro-

mosomal changes down to microgain and microloss resolution[64]. In addition, some groups have used oligonucleotide arrays for high-resolution CGH and LOH studies. Single nucleotide polymorphism (SNP) arrays have been employed for high-resolution LOH studies in small cell lung cancer samples[65] and have permitted the simultaneous detection of copy number changes and LOH in a single experiment[66].

In another approach, chromosomal bias of gene expression signature was studied in HCC cases[67,68]. Using a recently developed technique known as expression balance map analysis, Midorikawa *et al* identified regions with frequent chromosomal aberrations in HCC based solely on expression data. Common alterations, including gain of 1q21-q23 and loss of 4q13, were found in 74% and 48% of HCC cases, respectively[67]. These matched well with previous CGH analyses. In conclusion, a combination of these powerful technologies provides a more comprehensive view of the development of HCC, and identification of novel gene candidates may provide better diagnosis and prognostication of HCC.

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