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**Role of chromosome 1q copy number variation in hepatocellular carcinoma**

Jacobs NR *et al*. Chromosome 1q and HCC

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**Abstract**

Chromosome 1q often has been observed to be amplified in hepatocellular carcinoma. This review summarizes literature reports of multiple genes that have been proposed as possible 1q amplification drivers. These largely fall within 1q21-1q23. In addition, publicly available copy number alteration data from The Cancer Genome Atlas project were used to identify additional candidate genes involved in carcinogenesis. The most frequent location for gene amplification was 1q22, consistent with the results of the literature search. The genes *TPM3* and *NUF2* were found to be candidates whose amplification and/or mRNA up-regulation was most highly associated with poorer hepatocellular carcinoma outcomes.

**Key Words:** Liver cancer; Chromosomal amplification; Hepatocellular carcinoma; The Cancer Genome Atlas

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**Core Tip:** A list of candidate chromosome 1q amplification driver genes was compiled from the existing literature by PubMed search. Bioinformatics tools were used to identify additional candidates using publicly available genomics and transcriptomics data. Genes identified this way were largely distinct from those identified from the literature. Thus, these two strategies can be used in a complementary manner.

**INTRODUCTION**

Hepatocellular carcinoma (HCC) is one of the leading causes of cancer related deaths worldwide. Liver cancers are the fourth most common cause of cancer related deaths (the sixth most commonly diagnosed type of cancer), and HCC accounts for between 75% and 85% of primary liver cancer cases[1]. About 54% of HCC cases worldwide are attributed to the hepatitis B virus (HBV) while 31% of cases are attributed to hepatitis C virus (HCV) infections[2]. Given the fact that chronic HBV infection presents as a significant risk factor for HCC, vaccination against HBV is recommended as a way to prevent HCC[3].

**COMMON GENOMIC ALTERATIONS IN HCC**

More recently, technological advances have permitted the sequencing of the genomes and transcriptomes of numerous cancers. Mutations in several genes have been detected repeatedly in HCC[4]. Common somatic changes include mutations to beta-catenin and p53, resulting in activation of the Wnt signaling pathway and dysregulation of the cell cycle, respectively. Mutations activating *TERT* gene expression are also common. Patterns of genetic alterations in individual tumors have been examined with the goal of classifying them, to predict outcome and potentially guide therapeutic decisions[5].

Over the past few decades, a significant amount of research has shown an association between HCC and specific chromosomal abnormalities. In particular, chromosomal gains have been noted on 1q, 6p, 8q, 17q, and 20q. Similarly, chromosomal losses have been detected on 1p, 4q, 6q, 8p, 13q 16p, and 17q[6-8]. Amplification of chromosome 1q21-23 has been identified as the most frequent chromosomal alteration associated with HCC[9]. Thus, we were interested in considering the evidence for which gene or genes is critical for driving this chromosomal abnormality.

**AMPLIFICATION OF CHROMOSOME 1q GENES**

During the past two decades, several genes within or near the 1q21-23 range have been highlighted as potentially significant to HCC[10]. Many of these are highlighted in Table 1. In 2003, Wong *et al*[11]studied the 1q21-1q22 region using positional mapping by interphase cytogenetics. They identified significantly increased levels of gene expression of the *JTB*, *SHC1*, *CCT3*, and *COPA* genes in five cases of HCC compared to paired adjacent non-malignant liver tissues, and they concluded that these genes may represent targets in HCC progression[11]. More recently, *JTB* (Jumping Translocation Breakpoint) has been identified as a protein that negatively regulates the apoptotic process by affecting the activation of caspase 9[12]. *SHC1* is involved in signal transduction from receptor tyrosine kinases to various downstream proteins and has been identified in mitogenic signaling[13-15]. *CCT3* is involved in cell cycle regulation[16]. *COPA* is the α-subunit of the coatomer protein complex I which plays a role in retrograde protein trafficking from the Golgi to the endoplasmic reticulum[17].

In 2004, Midorikawa *et al*[8] used an expression imbalance map analysis [which they confirmed using genomic quantitative real-time polymerase chain reaction (qPCR)] to demonstrate amplification of the 1q21-12 region in HCC tumor samples. Moreover, they identified two new genes (*HAX-1* and *CKS1B*) as being as being highly expressed in HCC tissue compared with noncancerous tissues. They also described the amplification of *SHC1* and *CCT3* (previously identified by Wong *et al*[11]). *HAX-1* (*HCLS1* associated protein X-1, gene name *HAX1*) has been associated with activation of tyrosine kinases[18]. Like *CCT3*, *CKS1B* (CDC28 protein kinase regulatory subunit) plays an essential role in mediating a cell’s progression through the cell cycle[19]. To further support the conclusions of Midorikawa *et al*[8], Shen *et al*[20] demonstrated that HCC cells had increased levels of *CKS1B* mRNA and protein compared to adjacent non-tumor liver tissue. Elevated *CKS1B* expression was also positively associated with poor differentiation features[20].

In 2008, Inagaki *et al*[21]analyzed a 700-kb DNA region located at 1q21 in 19 HCC-derived cell lines. Using high-density SNP microarray analysis, fluorescence in situ hybridization (FISH), and real-time quantitative PCR, they identified a significant increase in copy number at the 1q21 region. Using reverse transcriptase PCR, they identified three genes (*CREB3L4*, *INTS3*, and *SNAPAP*) that were significantly overexpressed in samples taken from HCC tumors[21]. Based on these findings, they concluded that these three genes are likely targets for the amplification mechanism, and they may be involved in HCC progression. *CREB3L4* (cyclic amplification responsive element binding protein 3-like 4) is part of the CREB/ATF family of transcriptional factors, and it is primarily expressed in the prostate gland in humans as well as prostate and breast cancer cell lines[22]. *CREB3L4* has been shown (by immunostaining) to have a higher expression level in cancerous prostate cells than in adjacent noncancerous cells[22] and it has also been shown to contribute to the progression of breast cancer[23]. INTS3 (integrator complex subunit 3) is part of the Integrator complex which is associated with the C-terminal domain of RNA polymerase II[24]. SNAPAP (snare-associated protein, gene name *SNAPIN*) is part of the SNARE complex of proteins that is involved in the docking and fusion of synaptic vessel[25]. At this point, little is known about the relationship of either INTS3 or SNAPAP with tumorigenesis.

Later in 2008 Ma *et al*[26] used microdissected DNA from 1q21 and hybrid selection to isolate ALC1 (also known as CHD1L) as a candidate oncogene. After confirming the amplification of *ALC1* using FISH, they transfected it into human liver cell lines resulting in the cells being able to form more colonies than blank vector-transfected cells when grown in soft agar[26]. They also demonstrated that *ALC1* overexpression plays a role in facilitating DNA synthesis, down-regulating p53 expression, promoting G1/S phase transition, and inhibiting apoptosis.

More recently, in 2016 Elsemman *et al*[27] were interested in S-adenosylmethionine (SAMe) which has been described by Lu *et al*[28] as playing a significant role in hepatic diseases including HCC. SAMe is synthesized from ATP and methionine by methionine adenosyl transferase genes including MAT1A which is significantly downregulated in HCC. Elsemman *et al*[27] analyzed reactions containing SAMe, and using copy number variation analysis they identified five methyltransferase genes (*ASH1L*, *METTL13*, *TARBP1*, *SMYD2*, and *SMYD3*) located on chromosome 1q, all of which were amplified in samples of HCC relative the healthy tissue samples. ASH1L is a histone methyltransferase protein which is involved in the regulation of gene expression[29]. *METTL13* (gene name EEF1AKNMT) has been shown repeatedly to promote tumor growth and metastasis and is negatively associated with survival among lung and pancreatic cancer patients[30,31]. *TARBP1* is a double-stranded RNA binding protein that promotes the replication of human immunodeficiency virus-1 and -2 as well as HCV[32]. It has also been directly correlated with decreased survival rates in patients with HCC[33]. SMYD2 and SMYD3 are both members of the protein lysine methyltransferase family of proteins[34], and each has been associated with a variety of cancer types. SMYD2 has been shown to be overexpressed in esophageal squamous carcinoma, gastric cancer, and pediatric acute lymphoblastic leukemia[35-37]. SMYD3 is overexpressed in cancers including breast, liver, and colorectal cancer[38,39].

**ANALYSIS OF GENOMIC AND TRANSCRIPTOMIC DATA**

We were interested in what more recent genomic and transcriptomic studies have revealed about chromosome 1q amplification and HCC. The Cancer Genome Atlas (TCGA) Project has accumulated an important, publicly available genomic and mRNA expression data set which includes multiple cancers types including HCC (data set Liver Hepatocellular Carcinoma, LIHC)[40]. There is also a more recent version of this data, which is part of TCGA Pan-Cancer Clinical Data Resource[41], a subset of the LIHC data set that has been curated to include four major clinical outcome endpoints. We chose to use this data set to try to identify additional candidate amplification driver genes. This version of the LIHC patient cohort (PanCan-LIHC) has the following patient characteristics: 251 males/121 female with 241 living, and 131 deceased. Most individuals had a total of 10-140 mutations genome wide; 23 had 140-190, 18 had greater than 190, and 2 had fewer than 10 (14 did not have data available). Most PanCan-LIHC individuals exhibited genome alterations, with gains in 1q being the most common alteration: 225 individuals (60.5%) exhibited 1q gains, with 23.7% called as diploid and 15.9% with data not available).

The original publication reporting the LIHC cohort analyses identified copy number alterations (CNAs) in several likely driver genes spread across several chromosomes[40]. However, the only driver gene listed for 1q is MCL1 at 1q21.3. They also reported a short stretch of four genes that were significantly amplified at 1q22, but no candidate genes were indicated. In a report on the analysis of aneuploidy across TCGA cancer types, strong 1q amplification was noted in the PanCan-LIHC cohort (as well as in other epithelial breast and lung tumors)[42]. Using the Oncoprint tool at the cBioPortal for Cancer Genomics (https://www.cbioportal.org/), we could see that all of the genes listed in Table 1 were amplified in 7%-13% of tumors, with mRNAs overexpressed in 9%-41% of tumors (data not shown), consistent with the earlier reports described above.

**STRATEGY TO IDENTIFY ADDITIONAL CANDIDATE DRIVER GENES**

To further explore possible 1q amplification driver candidates, the frequency of CNAs in the Pan-Cancer version of LIHC sample set was explored using the cBioPortal suite of tools[43,44]. First, the CNA data set for all genes in the PanCan-LIHC was downloaded and imported into in an Excel spreadsheet. Second, all genes that had been scored as having an amplification or homozygous deletion with a frequency of at least 5% of tumor samples were sorted from those with lower frequency. This resulted in a list of 1871 genes meeting these criteria. Finally, this set of 1871 altered genes was sorted by chromosome and further restricted to those that were annotated as Cancer genes according OncoKB[45].

These steps produced a list of 49 candidate genes localized to chromosome 1q (not shown). These fell into two groups, a centromere proximal group spanning intervals 1q21.2-1q25.2 (28 genes), and a second group covering the distal interval of 1q31.1-1q44 (21 genes). Across the 1q region, the gene amplified in the highest percentage of tumors was *MUC1* located at 1q22 (11.7% amplification). This might correspond to the short stretch identified at 1q22 by the TCGA-LIHC paper referred to above. The overall frequency of amplification was greater in the proximal group of genes (mean of 10.29%, range of 8.2%-11.7%) *vs* the distal set (mean of 6.41%, range of 5.4%-7.4%). Of the 15 genes listed in Table 1, only two were present in the list of 49, *CKS1B* and *SMYD3*.

**ANALYSIS OF NEW CANDIDATES**

Using the Oncoprint visualization tool at cBioPortal, all 49 genes were examined to determine the putative CNAs from GISTIC2.0 calls[46], as well as the presence of non-synonymous mutations and altered mRNA expression (*z*-score threshold of +/- 2.0 relative to diploid samples). The total alteration percentages ranged from < 10% to 50% for the individual genes, with few non-synonymous mutations (not shown). The total number of genes under consideration was narrowed down to 12 by focusing on those with at least 25% of samples with one or more of the various alterations (Figure 1). All but one of these genes was derived from the centromere proximal half of the 1q arm (the exception was PARP1 at 1q42.12). All 12 genes exhibited numerous instances of mRNA upregulation, both with and without DNA amplification. Note that COP1 in Figure 1 at 1q25.1 is not the same as COPA at 1q23.3 (Table 1).

Each of the 12 genes was examined individually using the cBioPortal Comparison and Survival tools to determine whether the presence of alterations was associated with survival outcomes. There were only two genes where amplification, or mRNA increase, or both were associated with reduced survival compared with the samples without either type of alteration. These two were *TPM3* at 1q21.3 and NUF2 at 1q23.3 (Table 2, scores designated “all”). However, when the CNAs were examined separately from increased mRNA levels, amplification alone was not associated with any survival or outcome measure (not shown). Instead, the mRNA elevations clearly had a more significant correlation with patient outcome, as can be seen from the Logrank test q-values (Table 2, “mRNA”). Patients with *TPM3* mRNA elevation had an overall median survival of 25.15 mo *vs* 80.74 mo for those without the elevation. Patients with *NUF2* mRNA elevation had an overall median survival of 23.38 mo *vs* 70.06 mo for the unaltered group. Thus, altered expression of these two genes may contribute to clinical outcome.

**COMPARING THE FREQUENCY OF TPM3 AND NUF2 ALTERATIONS IN HCC WITH OTHER CANCERS**

We were interested whether *TPM3* and *NUF2* alterations were common in other types of cancer besides HCC. To explore the alteration frequencies in other cancer types, the entire Pan-Cancer patient cohort was analyzed using the cBioPortal suite of tools[41]. All 32 cancer types included in the Pan-Cancer sample set were selected, and the *TPM3* and *NUF2* genes were searched individually. The Cancer Types Summary produced a display showing the frequency of gene alterations (amplifications, deep deletions, non-synonymous mutations, structural variants) in all 32 types of cancer as well as the types of alterations identified (Figure 2). The PanCan-LIHC HCC dataset had the second highest percentage of TPM3 alterations and the third highest percentage of *NUF2* alterations. In the case of both genes, amplification of *TPM3* and *NUF2* was the most common type of alteration seen in the HCC patient sample. Interestingly, *NUF2* had a relatively higher frequency of mutations than amplifications in some cancer types.

**PREVIOUSLY REPORTED ASSOCIATION BETWEEN *TPM2* OR *NUF2* AND HCC**

Despite the low q-values, it remains possible that associate between *TPM3* and *NUF2* gene expression and patient survival is random. Therefore, we searched the literature to find whether either *TPM3* or *NUF2* genes had been associated previously with HCC. Kim *et al* examined chromosomal alterations in 76 HCC, finding frequent gain of 1q[47]. They found *TPM3* mRNA was elevated in tumors compared to normal tissue, and proposed that it might represent an oncogene in HCC, consistent with our analysis. A follow up study found that knock down of *TPM3* in HCC cells reduced migration and invasion capabilities[48].

*NUF2* elevation was reported in micro-dissected malignant hepatocytes derived from HBV-associated tumors[49]. Analysis of the Gene Expression Omnibus HCC data also revealed upregulation of NUF2 in HCC compared with healthy colon epithelial cells[50]. An analysis of the original TCGA-LIHC data set, which has subtantial overlap with the PanCan-LIHC samples that we explored, also found that *NUF2* was overexpressed compared with normal liver samples[51], and that overexpression was significantly associated with overall median survival. Other independent analyses of the same data set also reported *NUF2* upregulation and association with poorer prognosis[52-54]. It has been suggested that *NUF2* may represent a biomarker for early recurrence after HCC resection[55], and that it might represent a potential therapeutic target[56].

**IMPLICATIONS OF *TPM3* AND/OR *NUF2* OVEREXPRESSION**

The product of the TPM3 gene is tropomyosin3, an actin binding protein. The four TPM genes TPM genes produce 40 distinct protein isoforms by use of alternative promoters and extensive alternative mRNA splicing[57]. Changes in isoform production have been associated with cellular transformation[48,58]. The specific role of increased *TPM3* in cancer cells is unclear, as the protein is involved in numerous activities related to the actin cytoskeleton. Despite this, it is worth noting that small molecules that block the binding of isoform TPM3.1 to actin showed promise in perturbing the growth of cancer cells[59,60].

The protein encoded by the *NUF2* gene, along with those encoded NDC80, SPC24 and SPC25 form the Nuclear Division Cycle 80 complex. This complex plays an important role in mitotic spindle formation and chromosome segregation[61]. Over expression of other complex members, especially NDC80, has also been observed frequently in multiple cancers, and it has been proposed that overexpression of NDC80 complex proteins leads to defective mitosis and may promote aneuploidy[62]. Screening in epithelial ovarian carcinoma cells of an siRNA library has identified *NUF2* as one of four genes that reduced cell viability and increased apoptosis when knocked down[63]. This study also found a correlation between *NUF2* mRNA elevation and poorer prognosis in ovarian carcinoma patients. *NDC80* (also known as Hec1) interacts directly with *NUF2* and may represent a therapeutic target. A screen of a small molecule library for inhibitors of the interaction between *NDC80* and mitotic kinase Nek2 identified a compound named INH1 as being able to disrupt the protein-protein interaction[64]. This study also showed that INH1 decreased proliferation of breast cancer cells in culture and in a mouse xenograft assay.

**CONCLUSION**

In conclusion, our review of the literature and independent analysis of the TCGA-LIHC PanCancer data set identified two non-overlapping sets of genes that reside on chromosome 1q and frequently undergo amplification in HCC (compare Figure 1 and Table 1). We found what appears to be a significant correlation between amplification and/or increased expression of *TPM3* and *NUF2* and poorer prognosis, which is consistent with previous reports in the literature. Amplification of 1q also is observed frequently in other cancers. One limitation to our strategy to identify additional driver genes is that only genes previously identified as involved in cancer by OncoKB were considered. The absence of many genes in Table 1 suggests more candidate genes may still be identified. In the case of large chromosomal CNAs such as seen with 1q, it is truly challenging to identify the critical driver mutations involved. Further studies will be needed to understand the contributions of numerous genes amplified on chromosome 1q so as to effectively target therapeutics.

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**Figure Legends**



**Figure 1 Oncoprint of genetic alterations and mRNA elevations.** The alterations to the 12 genes identified by the amplification driver gene identification strategy were visualized using the cBioPortal Oncoprint tool. The nature of the alterations is indicated by the key below the Oncoprint. Note that some individuals display both amplification (solid red) plus elevated mRNA (red outline). Each vertical set of lines reflects the alterations occurring in a single hepatocellular carcinoma patient. Individuals with no alterations detected in any of the 12 genes are not shown.



**Figure 2 Frequency of *TPM3* or *NUF2* alterations in other cancers.** Analyses from cBioPortal show the percent of cancer cases that include a *TPM3* gene alteration (upper plot) or a *NUF2* gene alteration. The results were generated by first selecting all 32 of The Cancer Genome Atlas PanCancer Atlas studies[41] from the cBioPortal Query page, and then searching for DNA alterations in the *TPM3* gene and *NUF2* gene individually. Graphs shown in this figure were taken from the Cancer Types Summary results page that is produced by cBioPortal following this search. The colors above represent the following: green, mutation; purple, fusion; red, amplification; blue, deep deletion; grey, multiple alterations.

**Table 1 Amplified genes within/near 1q21-23 that have been associated with hepatocellular carcinoma**

|  |  |  |  |
| --- | --- | --- | --- |
| **Gene1** | **Location2** | **Description of protein product** | **Ref.** |
| *JTB* | 1q21.3 | Promotes cell resistance to apoptosis | Wong *et al*[11] and Kanome *et al*[12] |
| *SHC1* | 1q21.3 | Downstream signaling from receptor tyrosine kinases | Wong *et al*[11], Midorikawa *et al*[8]*,* Pelicci *et al*[13], Kavanaugh and Williams[14], and van der Geer *et al*[15] |
| *CCT3* | 1q22 | Associated with cell cycle regulation | Wong *et al*[11], Midorikawa *et al*[8]*,* Won *et al*[16] |
| *COPA* | 1q23.2 | Assists in retrograde vesicular transport from Golgi to endoplasmic reticulum | Wong *et al*[11] and Vece *et al*[17] |
| *CKS1B* | 1q21.2 | Associated with cell cycle regulation | Midorikawa *et al*[8] and Ganoth *et al*[19] |
| *HAX-1* (*HAX1*) | 1q21.3 | Plays a role in the activation of receptor tyrosine kinases | Midorikawa *et al*[8] and Suzuki *et al*[18] |
| *CREB3L4* | 1q21.3 | Associated with androgen receptor signaling | Inagaki *et al*[21] and Qi *et al*[22] |
| *INTS3* | 1q21.3 | Associated with RNA polymerase II | Inagaki *et al*[21] and Baillat *et al*[24] |
| *SNAPAP* (*SNAPIN*) | 1q21.3 | Part of SNARE complex (docking and fusion of synaptic vesicles) | Inagaki *et al*[21] and Ilardi *et al*[25] |
| *ALC1* (*CHD1L*) | 1q21.1 | Facilitates DNA synthesis and cell cycle when over expressed | Ma *et al*[26] |
| *ASH1L* | 1q22 | Histone methyltransferase involved in gene expression | Elsemman *et al*[27] and An *et al*[29] |
| *METTL13* (*EEF1AKNMT*) | 1q24.3 | Regulates protein synthesis in cancer cells; promotes tumor growth and metastasis | Elsemman *et al*[27]*;* Liu *et al*[30], and Li *et al*[31] |
| *TARBP1* | 1q42.2 | Double-stranded RNA binding protein; Promotes HIV-1 and -2 and HCV replication | Elsemman *et al*[27], Zhang *et al*[50], and Christensen *et al*[32] |
| *SMYD2* | 1q32.2 | Part of the protein lysine methyltransferase family of enzymes | Elsemman *et al*[27] and Leinhart and Brown[34] |
| *SMYD3* | 1q44 | Part of the protein lysine methyltransferase family of enzymes | Elsemman *et al*[27] andLeinhart and Brown[34] |

1Alternative gene designation provided in parentheses (see text). 2Chromosome locations are as found on the Genome Browser at http://genome.ucsc.edu[65]. HIV: Human immunodeficiency virus; HCV: Hepatitis C virus.

**Table 2 Correlation between *TPM3* and *NUF2* alterations and prognosis**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Gene** | ***n* (affected)** | **Overall1** | **Disease-specific1** | **Progression free1** | **Disease free1** |
| *TPM3* (all) | 92 | 1.283e-3 | 1.263e-3 | 0.108 | 0.0242 |
| *NUF2* (all) | 87 | 4.931e-3 | 2.357e-4 | 6.231e-4 | 4.931e-3 |
| *TPM3* (mRNA) | 78 | 4.046e-5 | 4.046e-5 | 1.231e-3 | 2.238e-3 |
| *NUF2* (mRNA) | 65 | 3.898e-4 | 1.441e-5 | 3.374e-4 | 1.520e-3 |

1All Logrank test survival *P* and *q* values were generated using the Comparison/Survival tool at cBioPortal to compare survival rates between groups of patients. Various types of survival values were calculated for individuals with any type of alteration (all) or only those with altered mRNA levels (mRNA). The q-values shown were derived from the initially *P* values using the Benjamini-Hochberg False Discovery Rate correction procedure.