The pattern of gene copy number variations (CNVs) in hepatocellular carcinoma; in silico analysis

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Research

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Abstract

Recent studies showed that genetic lost or gain in the genome can predispose cells toward malignancy. Hepatocellular carcinoma (HCC) is the most common type of liver cancer which occurs predominantly in patients with underlying chronic liver disease and cirrhosis. Prognosis of HCC is strongly connected with diagnostic delay. To date, no ideal screening modality has been developed for HCC. Recent findings demonstrated that Copy number variation (CNVs) can lead to activation of oncogenes and inactivation of tumor suppressor genes in cancers. In this study, CNV profile of 361 HCC samples was evaluated to reveal the potent - chromosomal regions involved in the disease. The obtained data showed that the chr1q and chr8p were two hotspot regions for gene amplifications and deletions in studied samples respectively. In this research, *YY1AP1 (Yin Yang-1 Associated Protein 1*) on chr1q22 was the most amplified gene in HCC samples and showed the positive correlation with tumor grade. Deletion of *CHMP7 (Charged Multivesicular Body Protein 7)* on chr8p21.3 was another frequently observed CNV among HCC patients. Both genes were interacted with variety of well-known oncogenes and tumor suppressor genes including YY1 (Yin Yang 1), CCND1 (Cyclin D1), HDAC1 (Histone deacetylase 1), VHL (von Hippel-Lindau tumor suppressor), MAD2L2 (Mitotic Arrest Deficient 2 Like 2), CEBPA (CCAAT/enhancer-binding protein alpha), CHMP4A, CHMP5, CHMP2A, CHMP3 and ENSG00000249884 (RNF103-CHMP3 gene), all of them are well-known in carcinogenesis. Although this study was based on *in silico* evaluations, our findings can open a new window for researchers of HCC to focus on such candidate genes during experimental assays.

Background

Hepatocellular carcinoma (HCC) is the most common type of liver cancer that occurs predominantly in patients with underlying chronic liver disease and cirrhosis. The well-established risk factors of HCC are hepatitis B virus infection, alcoholism and metabolic disorders [1, 2]. Prognosis of HCC is strongly connected with diagnostic delay and HCC is usually diagnosed after development of end-stage clinical symptoms [3]. To date, no ideal screening modality has been developed for HCC. The Liver lesions are usually seen on *computed tomography (CT)*, many HCC tumors are asymptomatic and will not be diagnosed in time. Recent findings demonstrated that Copy number variation (CNV) can lead to activation of oncogenes and inactivation of tumor suppressor genes in cancers and consequently, can predispose the cells toward malignancy. These genetic variations are included with variety of genetic gains and losses which can deviate the genome of the cells from normal diploid state [4, 5]. Such cytogenetic changes influence the stability of genome and push the cells toward tumorigenesis [6]. Chromosomal aberrations are contributed to tumor age of onset, tumor metastatic state, drug resistant and tumor failure phenotypes [7]. Recent advances in functional genomics techniques like comparative genomic hybridization (CGH) or microarray open a new window to characterize the cytogenetic signatures of the malignant cells. This breakthrough seems to be promising in diagnostic and therapeutic area, these days [8]. Here, by means of an *in silico* analysis we have evaluated the pattern of CNVs in HCC samples which could be an interesting analysis to define the experimental researches on HCC

Results

As illustrated in Fig. 1, chr1q and chr8p were detected as the hotspot loci of copy number variation in the studied HCC samples. The obtained data will be discussed below.

Cytobands chr1q and chr8q were strongly associated with gene amplification in HCC samples

Of 24776 examined genes, 1024 (4.1%) were amplified in studied samples (linear copy number values cut-off ≥0.5). These genes were mapped on chr1p (1.3%; n=13), chr1q (86%; n=880) and chr8q (13%; n=131) (Fig. 2A). The highest scores were belonged to 34 genes including *ADAM15, FLAD1, ZBTB7B, PYGO2, DCST2, LENEP, SHC1, PBXIP1, PMVK, DCST1, KCNN3, SLC50A1, DPM3, SCAMP3, FAM189B, MTX1, KRTCAP2, GBA, GBAP1, MUC1, THBS3, TRIM46, ASH1L, RUSC1, CLK2, HCN3, FDPS, PKLR, YY1AP1, MSTO1, GON4L, RIT1, SYT11* and *KIAA0907.* Most of these amplified genes were located on chr1q22 (67.6%; n=23), and the remaining genes were belonged to chr1q21.3 (32.4%; n=11) (Fig. 2A). These proteins were belonged to 15 signaling pathways which have been illustrated in Fig. 2B. Around 12.5 % of proteins were associated with Cholesterol biosynthesis. The amplification of 13 out of these genes were associated with tumor grade (Table 1). No association was found between gene amplification and other clinicopathologic parameters of HCC including tumor size, stage and metastasis. Although, all the 34 selected genes were amplified in HCC samples, only the expression of oncogene *YY1AP1 (Yin Yang-1 AssociatedProtein 1*) showed a strong correlation with their corresponding CNVs in 72% of studied samples (r>0.6). The obtained data from STRING Interaction Network showed that 25 proteins directly interacted with *YY1AP1* gene among which NeuroG3 (transcription factor), SS18L2 (transcription coactivator), ZMYM4 (cell morphology and cytoskeletal organization), ZNF496 (transcription factor) and ZNF576 (presumably transcriptional regulation) are the top five proteins interacted with *YY1AP1* gene (Fig. 3A). As illustrated in this figure, among the remaining 20 proteins, well-established oncogenes like, YY1 (Yin Yang 1), CCND1 (Cyclin D1), HDAC1 (Histone deacetylase 1) and tumor suppressors like VHL (Von Hippel-Lindau), MAD2L2 (Mitotic Arrest Deficient 2 Like 2) and CEBPA (CCAAT/enhancer-binding protein alpha) are observable.

Enrichment analysis through pathway common webserver showed that some of these 25 genes were belonged to the critical cellular pathway including Nucleolar Remodeling Complex (NoRC) which negatively regulates rRNA expression and regulation of cell cycle and DNA damage repair system like NER (Table2).

We also found that *hsa-miR-375*, *hsa-miR-222-3p*, are two miRNAs that target YY1AP1, therefore, are able to regulate the concentration of YY1AP1 protein in the cell (Table3).

Cytoband chr8p was strongly associated with gene deletion in HCC samples

Of 24776 examined genes, 172 (0.69%) were lost in studied samples (n=361) (with linear copy number values cut-off ≥0.5) which were mostly located on chr8p21-23. The most deletion scores were belonged to 17 genes including *TNFRSF10B, RHOBTB2, PEBP4, TNFRSF10C, CHMP7, TNFRSF10A, ENTPD4, EGR3,*

BIN3, PDLIM2, R3HCC1, LOXL2, STC1, PIWIL2, SLC25A37, TNFRSF10D and *CSMD1* that were considered for further analysis-. Among these, 16 genes were mapped on chr8p21.3 (94%) and - one gene chr8p23.2 (6%) (Fig. 4A). These genes are transcription factors and cytoskeletal proteins that act in apoptosis, EGF, FGF and P53 signaling pathways (Fig. 4B). Deletions of none of the mentioned genes were associated with tumor metastasis or grade. Although all of the mentioned genes were downregulated in studied cancerous tissues, the correlation with CNV was not significant expect for *CHMP7* gene whose expression showed the moderate correlation (r=0.5) with corresponding CNV in 70% of studied samples. The obtained data from STRING Interaction Network showed that 10 proteins directly interacted with *CHMP7 (Charged Multivesicular Body Protein 7)* gene among which CHMP4A, CHMP5, CHMP2A, CHMP3 and ENSG00000249884 (*RNF103-CHMP3* gene) are the top five proteins interacted with *CHMP7* gene (Fig. 3B). Enrichment analysis through pathway common webserver showed that some of these genes were belonged to the vital cellular pathway including spindle organization, sister chromatid segregation, centrosome duplication, cytokinesis, Nucleus organization, Nuclear envelope reassembly and Vacuolar transport. (Table2).

We also found that *hsa-miR-375*, *hsa-miR-222-3p*, are two miRNAs that target CHMP7, therefore they are capable of regulating the concentration of CHMP7 protein in the cell (Table3).

Discussion

Liver cancer is one of the human cancers with poor prognosis which largely originates from its genetic nature. Therefore, identifying the key genetic components and new therapeutic targets is a major step, especially in case of hepatocellular carcinoma (HCC) affected patients who are ineligible for surgical resection or liver transplant. However, the heterogenous nature of HCC has complicated this approach [9]. It is truly amazing that exponential advances in genomic sequencing during the past 10 years, along with the emergence of bioinformatic tools, enables the scientists to translate such high-throughput data, speeds up the discovery of hundreds of new potential targets for human diseases especially in cancer, and finally, offers a perspective to propose new prognostic, diagnostic and therapies approaches [10]. However, there is an urgent need to work on such putative targets experimentally to reveal the druggable candidates [9]. Copy number variations are one kind of DNA mutations that seem to have high impact in cancer pathogenesis. These genetic variations are also valuable tools for finding the hotspot regions in cancers [11].

In this study, the authors carried out genome-wide chromosomal CNV analysis in 361 HCC patients whose both CNV and RNA-seq data were available on highly cited bioportals. Around 24776 genes were screened in this study. As chromosomal CNVs may affect the level of gene expression in HCC, the RNA sequencing profile of target genes were also considered in parallel. The observed data on 361 HCC samples showed that the chr1q and chr8p were the most important regions for gene amplification and deletion respectively. In study by Takafumi Nishimura*et al*, they showed that chromosomal gain at chr1q is one of the most common features of HCC [12, 13]. Chen and coworker introduced chr1q as host spot regions of gene amplification in HCC. In this study, the regions of chr1q12–q22, chr1q23.3–q25.3 and

chr1q23.1–q43 were reported as minimal amplified region on chr1q [14]. The chr8p region has also been highlighted for HCC in two independent studies by Roesseler [15] and Qin [16]. Another research demonstrates that HCC may develop from cirrhotic cells carrying chr8p loss [17]. In case of 34 found amplified genes, 13 genes were associated with tumor grade. Growing body of evidences showed that amplifications of chr1q and chr8q have been strongly connected with tumor grade and size [4]. We also found that all of selected genes were involved in pathways which repeatedly reported as critical routs in HCC cells [18]. These include Wnt signaling pathway, angiogenesis signaling pathway, CCKR signaling map pathway, Ras signaling, cholesterol biosynthesis pathway, EGF receptor signaling pathway, FGF signaling pathway, flavin signaling pathway, glycolysis pathway, inflammation mediated by chemokine and cytokine signaling pathway, integrin signaling pathway, interleukin signaling pathway, PDGF signaling pathway, pyruvate metabolism pathway, RAS signaling pathway, and synaptic vesicle trafficking. We also found that the gain of *YY1AP1* and lose of the *CHMP7* were observed in most patients (around 70%) and resulted in upregulation and downregulation of corresponding transcripts respectively. It has also been found that YY1AP1 interact with NeuroG3, SS18L2, ZMYM4, ZNF496 and ZNF576 - the proteins that apart from the first two, altered expressions in HCC is reported in literatures [19-21]. Regarding the two microRNAs that target YY1AP1, hsa-miR-375 is a tumor suppressor [22] while hsa-miR-222-3p shows oncogenic properties [23]. The YY1AP1 is a component of the INO80 chromatin remodeling complex, which is responsible for transcriptional regulation, DNA repair, and replication [24]. In a recent study by Zhao X *et al*, they found that YY1AP1 may serve as a key molecular target for EpCAM(+) AFP(+) HCC subtype which was attributed with poor prognosis and stem cell-like phenotype [9]. They also showed that YY1AP1 is connected with stem cell features of this subtype and silencing of YY1AP1 eliminates the oncogenic feature of the cells through altering the chromatin landscape and triggering massive apoptosis *in vitro* and *in vivo* [9].

In case of CHMP7, the five top interacting proteins are CHMP4A, CHMP5, CHMP2A, CHMP3 and ENSG00000249884, all of which are involved in endosomal transport. Apart from CHMP4A, involvement of the rest was previously shown in HCC [19, 25]. We also found that four microRNAs target CHMP7: hsa-miR-26b-5p, hsa-miR-505-5p, hsa-miR-484 and hsa-miR-15b-5p. hsa-miR-26b-5p is involved in hepatitis B virus mediated HCC [26]; hsa-miR-484 shows both oncogenic and tumor-suppressor properties depending on the interacting partners [27, 28] and miR-15b-5p is a potential tumor suppressor [29, 30]. This suggests that CHMP7 could play important role in mediating HCC. no publication was found for *CHMP7* gene in HCC that shows its potency for further analysis in HCC samples, our enrichment analysis showed that CHMP7 promotes nuclear envelope sealing and mitotic spindle disassembly during late anaphase. It plays a role in the endosomal sorting pathway too. Altogether, considering the observed data, we suggest that candidate regions chr1q and chr8p in HCC could be subjects of further researches, with a major emphasis on the role of two genes *YY1AP1* and *CHMP7*.

Methods

The TCGA CNA raw data possessing 24777 genes in 361 samples of Liver Hepatocellular Carcinoma (TCGA, Provisional) was extracted from cBioPortal (http://www.cbioportal.org) and analyzed in R v3.5

using the cgdsr extension package (cran.rproject.org/web/packagescgdsr/). The linear copy number values cut-off ≥ 0.5 and cut-off ≤ -0.5 were considered as thresholds for gene amplification and deletion respectively. Filtered genes were selected as target/candidate genes for more analysis. The frequency of CNV for target genes was also calculated in HCC samples. Interaction of target proteins with other proteins and their involvement in various pathways was obtained from STRINGDB (https://string-db.org/). Using the PNTHER classification system (http://www.pantherdb.org), the protein classes and the pathway involvements for target proteins were estimated. The association of CNV variants with pathological tumor grade and stage was also examined in R.

Consequently, we obtained the raw data of RNA-seq for Liver Hepatocellular Carcinoma (TCGA, Provisional) and extracted the relevant information for these candidate genes in order to examine if any correlation exists between CNV of target genes and their corresponding expression values. As in strong positive correlation, the linear correlation coefficient (r) is close to +1, the results were filtered based on the r > 0.7. The interaction of target protein was traced using the UCSC genome browser (https://genome.ucsc.edu). Two databases, one for oncogenes (http://ongene.bioinfo-minzhao.org/) and one for tumor suppressor genes (https://bioinfo.uth.edu/TSGene/), were used to identify if any of the selected genes are listed oncogenes/tumor suppressor genes. Enrichment analysis was performed for selected amplified/deleted genes using Pathway Commons webserver (https://apps.pathwaycommons.org/). MiRWalk analysis helped to predict the possible presence of seed sequence in the selected gene.

Declarations

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Authors' contributions

Study conception and design: Maryam Tahmasebi-Birgani; Acquisition of data: Arman Shahrisa; Analysis and interpretation of data: Arman Shahrisa, Maryam Tahmasebi-Birgani; Drafting of manuscript: Hossein Ansari, Zahra Mohammadi and Javad Mohammadi-Asl; Critical revision: Vinicio Carloni;

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Availability of data

The datasets used and/or analyzed during the current study are available on cbioportal.org and are also accessible through cgdsr R package.

Ethics approval and consent to participate

The present study was approved by Ethical Committee of Ahvaz Jundishapur University of Medical Sciences research affair, Ahvaz, Iran (Grant number: CMRC-9613 and code of ethics: IR.AJUMS.REC.1396.367) and experimentally performed in Department of Medical Genetics.

Consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interest.

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Tables

Table 1 The association of copy number variations with clinicopathologic parameters of 361 HCC samples. Only candidate genes with significant association have been presented in the table.

Gene symbols (n=14)	CNV type	clinical parameters
YY1AP1, FLAD1, FAM189B, DCST2, ZBTB7B, TRIM46, SYT11, SHC1, SCAMP3, RIT1, LENEP, MSTO1, KIAA0907	AMP	Tumor grade

Tag	Genes Shared with Entered List
YY1AP1- interacted proteins	
FOXO-mediated transcription of oxidative	*HDAC1, HDAC2, SIN3A*
Transcriptional Regulation by MECP2	*HDAC1, HDAC2, SIN3A*
Ectodermal placode formation	*HDAC1, HDAC2*
Protein deacetylation	*HDAC1, HDAC2, SIN3A, SIN3B*
Regulation of cyclin-dependent protein serine/threonine kinase activity	*CCND1, CCND2, CCND3, CEBPA*
SUMOylation	*HDAC1, HDAC2, SIN3A, VHL*
Mitotic G1 phase and G1/S transition	*CCND1, CCND2, CCND3, HDAC1*
Positive regulation of cell cycle G1/S phase transition	*CCND1, CCND2, CCND3*
NoRC negatively regulates rRNA expression	*HDAC1, HDAC2, SIN3A, SIN3B*
DNA Damage Recognition in GG-NER	*INO80C, INO80E, YY1*
P75NTR negatively regulates cell cycle via SC1	*HDAC1, HDAC2*
CHMP7- interacted proteins	
Endosome transport via multivesicular body sorting pathway	*CHMP2A, CHMP3*
Positive regulation of viral release from host cell	*CHMP2A, CHMP3*
Macro autophagy	*CHMP2A, CHMP3, CHMP4A, CHMP7*
Sister chromatid segregation	*CHMP2A, CHMP4A, CHMP5, CHMP7*
Mitotic cytokinesis	*CHMP2A, CHMP3, CHMP4A, CHMP5, CHMP7*
Regulation of microtubule cytoskeleton organization	*CHMP2A, CHMP3, CHMP5*
Regulation of spindle organization	*CHMP2A, CHMP5*
Regulation of centrosome cycle	*CHMP2A, CHMP3, CHMP5*
Vacuolar transport	*CHMP2A, CHMP3, CHMP4A, CHMP5, CHMP7*
Nucleus organization	*CHMP2A, CHMP4A, CHMP5, CHMP7*
Nuclear envelope reassembly	*CHMP2A, CHMP7*
Exit from mitosis	*CHMP2A, CHMP7*

Table 2. Enrichment analysis for *YY1AP1* and *CHMP7* interacted proteins using Pathway common webserver (https://apps.pathwaycommons.org/)

Table 3. Human miRNAs target human YY1AP1 and CHMP7. Data were extracted from mirWalk dataset (http://mirtarbase.cuhk.edu.cn/php/search.php).

ID	miRNA	Target
MIRT004478	hsa-miR-375	YY1AP1
MIRT046762	hsa-miR-222-3p	YY1AP1
MIRT030249	hsa-miR-26b-5p	CHMP7
MIRT037936	hsa-miR-505-5p	CHMP7
MIRT042391	hsa-miR-484	CHMP7
MIRT046411	hsa-miR-15b-5p	CHMP

Figures

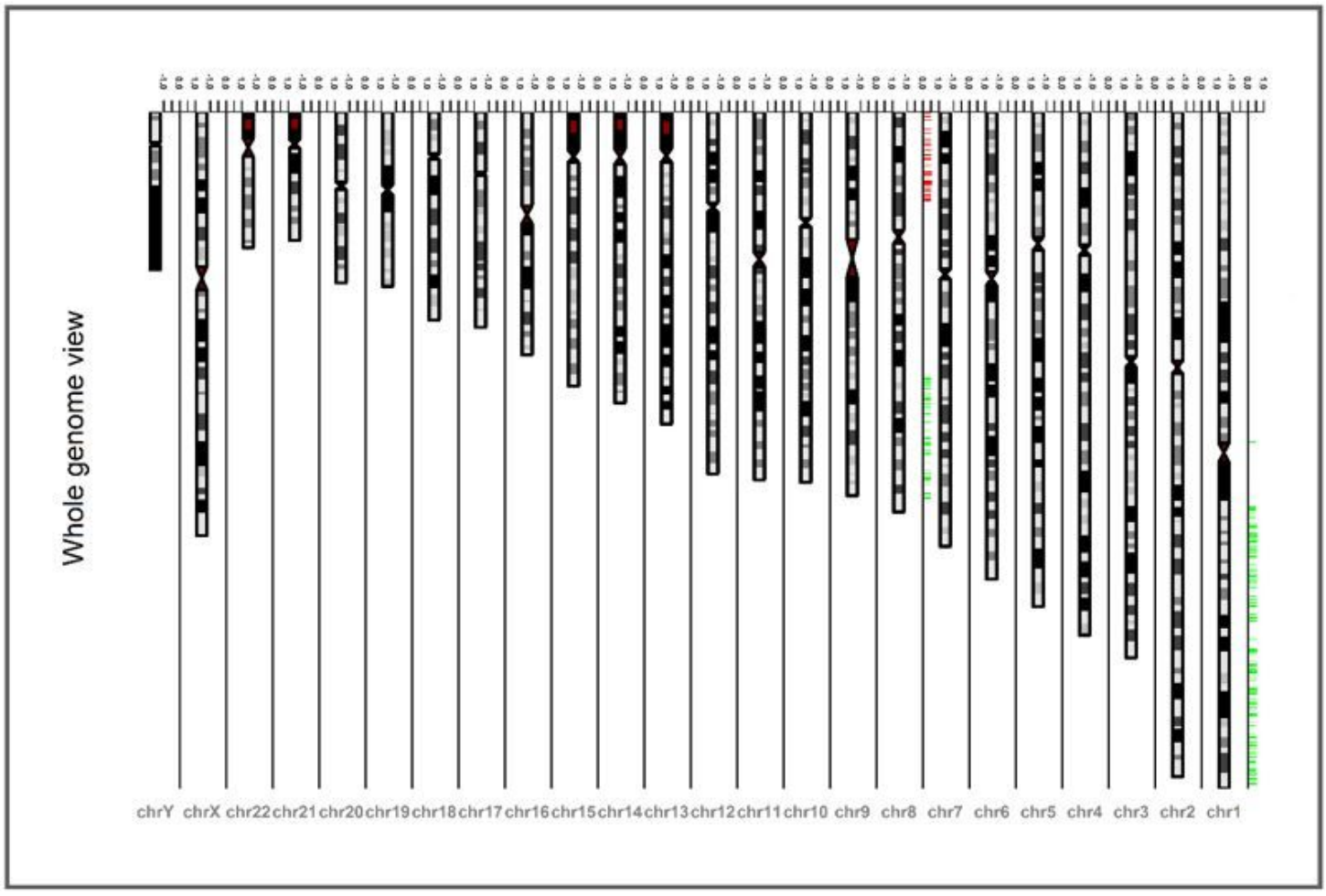


Figure 1

Human chromosome ideograms showing regions of copy number variation (deletion and/or amplification) in a high resolution (244K) whole genome hybridization (aCGH) analysis of 361 subjects

with HCC. Deletions are shown in red and amplification in green. Data was extracted from cBioPortal dataset (https://www.cbioportal.org) through Bioconductor package cgdsr and the ideogram was generated by Bioconductor package IdeoViz.

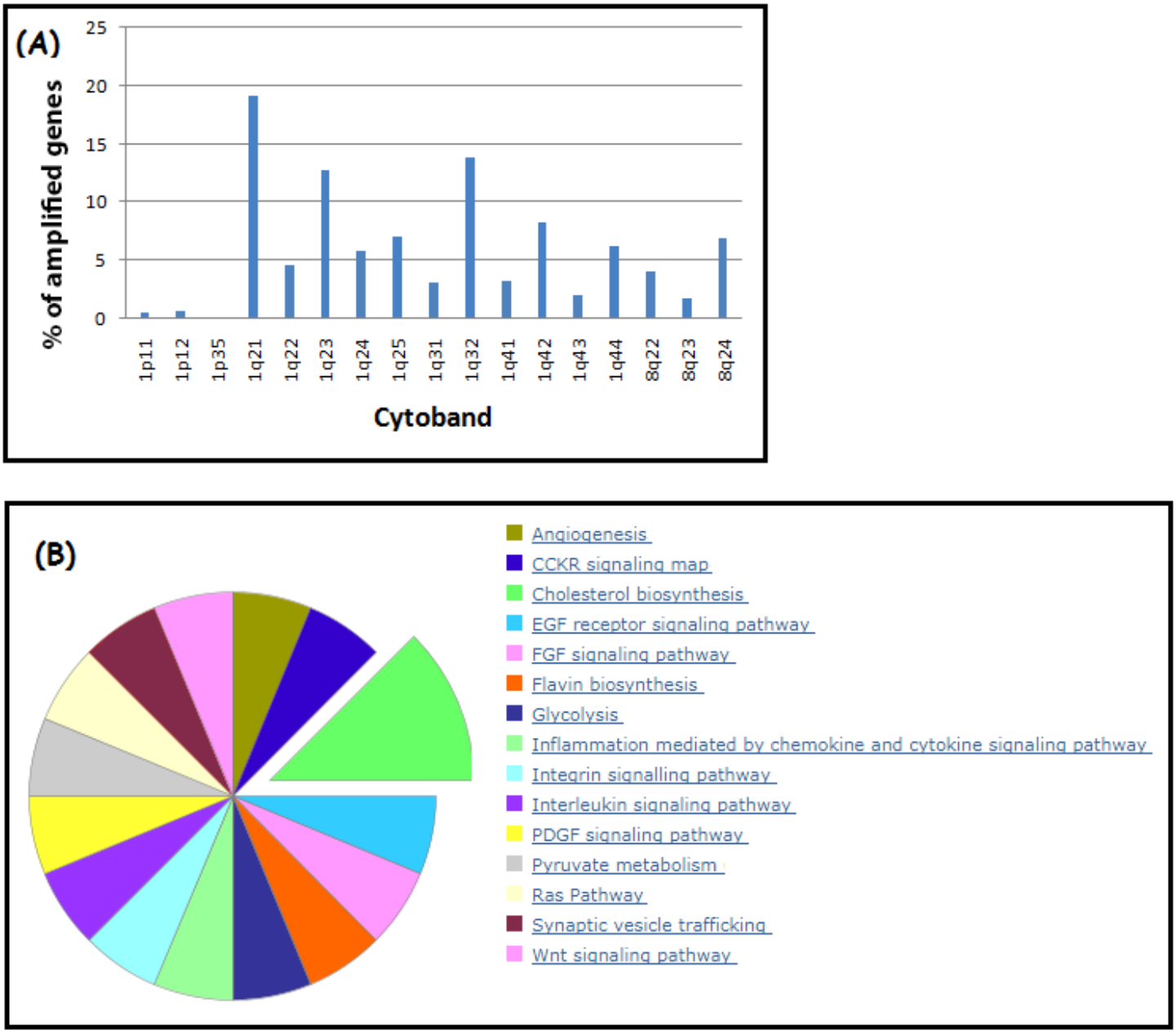


Figure 2

(A) Presentation of hotspot regions for all amplified genes on chr 1 and chr 8. 361 HCC samples posessing both expression data as well as CNV data were used to draw this plot. Data was extracted from cBioPortal dataset (https://www.cbioportal.org). (B) Signaling pathways associated with the selected 34 amplified genes among HCC samples. Data was extracted from PANTER dataset (http://www.pantherdb.org).

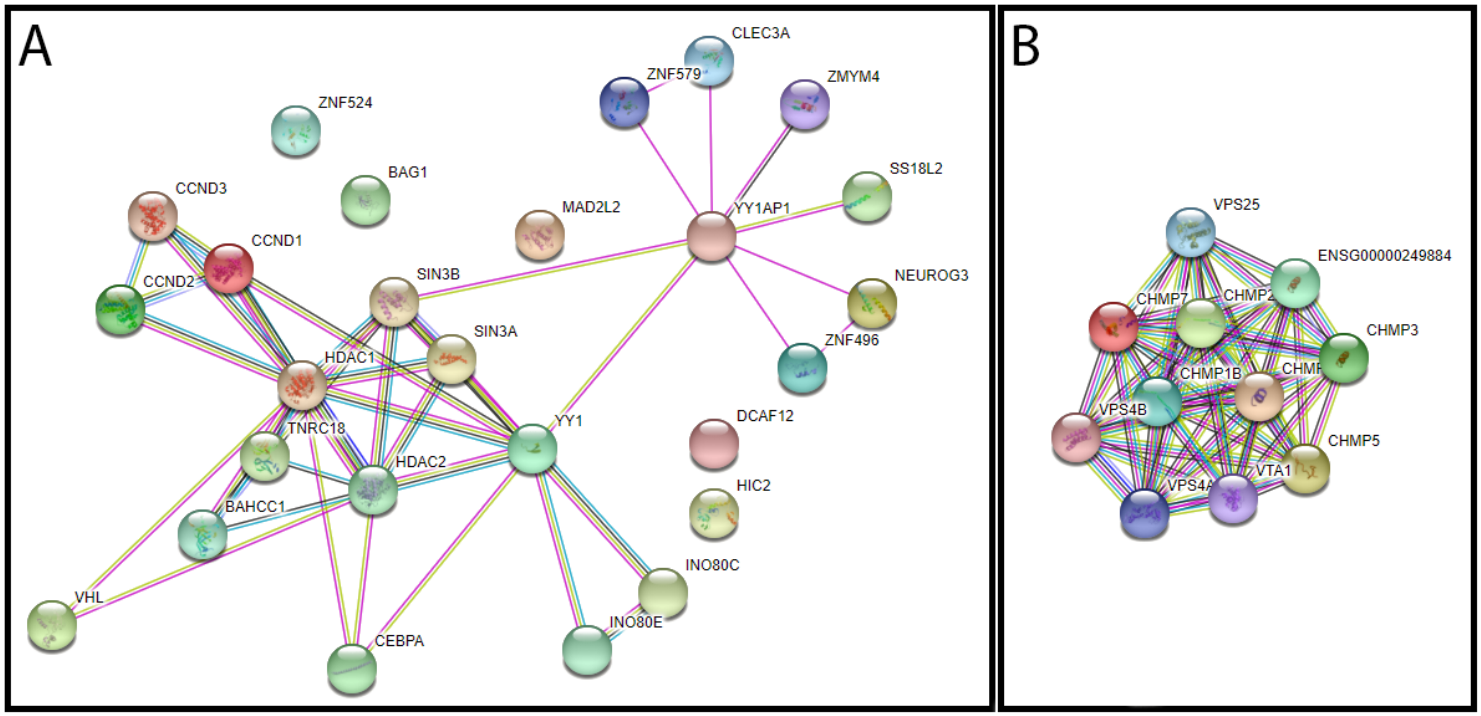
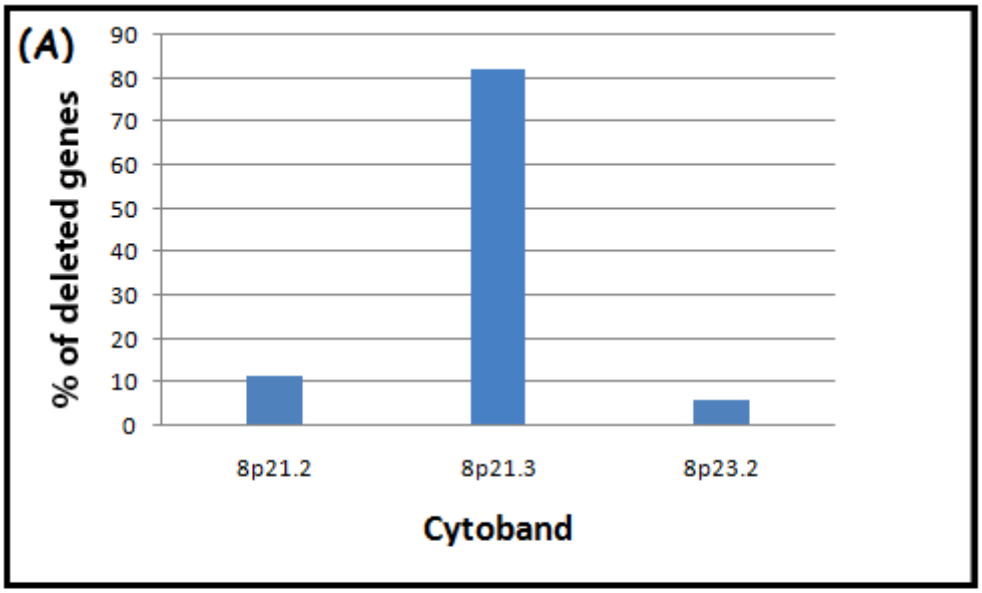


Figure 3

(A) The top protein intractions with YY1AP1 protein, obtained from STRING version 11 (https://string-db.org/cgi/network?taskId=bIxVofnnvI2q&sessionId=bIJOb9Bp4XNj) (B) The top protein interactions with CHMP7 (https://string-db.org/cgi/network?taskId=b7ZM8I4EGZ21&sessionId=bwHanJLjODoR)



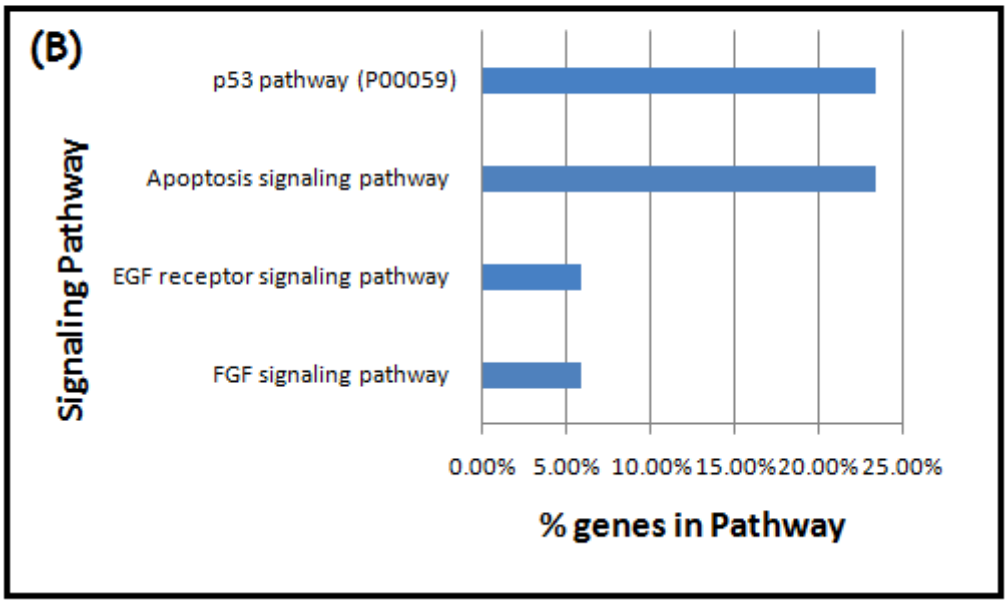


Figure 4

(A) The frequency of cytogenetic bands involved in all deleted genes on chromosme 8. 361 HCC samples posessing both expression data as well as CNV data were used to draw this plot. Data was extracted from cBioPortal dataset (https://www.cbioportal.org). (B) The signaling pathways associated with deleted genes. Data was extracted from PANTER dataset (http://www.pantherdb.org).