BIOMEDICAL JOURNAL 44 (2021) S73–S83

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Biomedical Journal

journal homepage: www.elsevier.com/locate/bj

Original Article

Percentage genome change and chromosome 7q amplification predict sorafenib response in advanced hepatocellular carcinoma

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ARTICLE INFO

Article history:
Received 7 September 2019
Accepted 13 July 2020
Available online 15 July 2020

Keywords:
Advanced HCC
Sorafenib
Copy number aberrations
7q amplification
ABCB1

ABSTRACT

Background: Hepatocellular carcinoma (HCC) may arise from genomic instability and has dismal outcome. Sorafenib is the first-line treatment for advanced stage HCC, but its therapeutic efficacy is less than 50%. Biomarkers for predicting the therapeutic efficacy of sorafenib administration to patients with advanced HCC are required. Here, we evaluated the role of chromosomal copy number aberrations (CNAs) in patients with advanced HCC who were treated with sorafenib along with their drug response.

Methods: The response to sorafenib treatment of twenty-three HCC patients who developed advanced recurrence after partial hepatectomy was analyzed using the modified Response Evaluation Criteria in Solid Tumors (mRECIST). Formalin fixed paraffin embedded (FFPE) tissue specimens obtained after tumor resection were analyzed using the Affymetrix OncoScan® FFPE assay.

Results: From the 23 patients analyzed in this study, 7 (30.4%) had complete/partial response to sorafenib (CR/PR), 7 (30.4%) had stable disease (SD), and 9 (39.1%) had progressive disease (PD). The mean genome-wide percentage of genome change acquisition via the OncoScan platform was 19.8% for patients with CR/PR/SD and 50.02% in the PD group ($p = 0.055$). Percentage of genome change above 33% was associated with adverse outcomes for sorafenib treatment in the time-to-progression analysis ($p = 0.007$) and overall survival ($p = 0.096$). Among these CNAs, amplification of chromosome 7q, containing the multidrug resistance gene *ATP Binding Cassette Subfamily B Member 1 (ACBC1)*, significantly associated with poor overall survival ($p = 0.004$) and time-to-progression ($p < 0.001$).

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Peer review under responsibility of Chang Gung University.
https://doi.org/10.1016/j.bj.2020.07.001
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Conclusions: Higher percentage genome change and amplification of chromosome 7q in advanced HCC is associated with sorafenib resistance.

At a glance of commentary

Scientific background

Sorafenib is the first-line treatment for advanced stage HCC, but its therapeutic efficacy is less than 50%. Therefore, biomarkers for predicting the therapeutic efficacy of sorafenib administration to patients with advanced HCC are still required.

What this study adds to the field

In this report, tumor specimen with higher percentage genome change and amplification of chromosome 7q containing ABCB1 drug resistant gene in advanced HCC was associated with sorafenib resistance, which might be applied for copy number analysis before drug administration after partial hepatectomies in these patients.

Hepatocellular carcinoma (HCC) is the fifth most common cancer in the world. The major risk factors for HCC are chronic infection with hepatitis B virus (HBV) and hepatitis C virus (HCV), alcohol intake, smoking, and toxin exposure [1,2]. Curative treatment, including a partial hepatectomy, ablation therapy, and liver transplantation at an early stage, lead to the best outcome, but the risk of recurrence is still more than 50% [3–6]. Most recurrences are intrahepatic; however, some patients develop disseminated spreading or distant metastasis and require systemic treatment [7]. The poor prognosis after HCC resection may arise from increased genomic instability [4,8,9]. Besides, early recurrence has a dismal outcome and, thus, there is an increased need for the development of effective adjuvant therapies [10]. Combined treatment with sorafenib and transcatheter arterial chemoembolization (TACE) was a well-tolerated and efficacious strategy for Barcelona Clinic Liver Cancer (BCLC) stage B in START trials [11]. Although systemic targeted therapy with sorafenib has been assumed as a standard treatment for advanced HCC, a precise and personalized approach is necessary to improve survival [12].

Sorafenib is a multikinase inhibitor that targets the vascular endothelial growth factor receptor (VEGFR), the platelet-derived growth factor receptor (PDGFR), and the downstream RAF/mitogen-activated protein/extracellular signal-regulated kinase (MAPK)/(ERK) cascade [13]. The antiproliferative and antiangiogenic effects of sorafenib reduce tumor growth in several kinds of cancers [13]. The real world treatment showed that half of the patients treated with sorafenib had progressive disease and their quality of life did not benefit because of sorafenib resistance [14]. There are several theories regarding sorafenib resistance: extrahepatic spreading and occurrence of intolerant adverse events [15]; escape of cancer stem cells from treatment-induced apoptosis, leading to tumor repopulation [16]; activation of hypoxia-inducible factor 1 after sorafenib treatment and the subsequent induction of angiogenesis, immune escape, and autophagy in the tumor [17]; activation of the phosphatidylinositol-3-kinase (PI3K)/Akt signaling pathway; and enhancement of epithelial–mesenchymal transition (EMT) [18]. Suppression of the RAF/MEK/ERK signaling pathway and induction of cell cycle arrest in the G2/M phase could help treatment in patients with sorafenib resistance [19]. Changes in the tumor microenvironment has been proven to be correlated with sorafenib resistance [20,21]. Chromosome instability and CNAs in HCC and other solid tumors could lead to the activation of oncogenes and the inactivation of tumor suppressor genes, which induce changes in transcription, translation, and tumor invasiveness [8,9]. A recent study has shown that genome-wide CNAs and VEGFA amplification in circulating cell-free DNA were a biomarker for advanced HCC in patients treated with sorafenib [22]. In addition, Fibroblast Growth Factor 3 and 4 (FGF3/4) amplification detected via fluorescence *in situ* hybridization was associated with lung metastases in sorafenib-treated advanced HCC patients [23]. Amplification of FGF19 has also been reported to be associated with sorafenib response in advanced HCC using next generation sequencing and copy number assay [24]. Therefore, genome instability has been strongly associated with sorafenib treatment response in advanced HCC patients; systematic analysis of CNAs in tumor specimens of patients would improve the survival of patients.

In this study, we assessed the role of chromosomal CNAs in patients with advanced HCC who were treated with sorafenib and also evaluated their drug response. Twenty-three HCC patients had undergone surgical resection and showed advanced recurrence. FFPE tumor specimens of HCC at resection were used for the analysis of chromosomal CNAs using the Affymetrix OncoScan® FFPE assay to decipher the difference between sorafenib sensitive and resistant groups. The long-term outcome of sorafenib treatment in patients with advanced HCC was also examined.

Patients and methods

Patients and samples

This study was approved by the Institute Review Board (IRB) of Chang Gung Memorial Hospital (CGMH), Linkou (IRB 201600707B0C501). Twenty-three HCC patients had curative treatment and developed HCC recurrence. All recurrence was at advanced stage, involved portal vein thrombus (PVT) or extrahepatic spreading (EHS). All patients enrolled was in good performance status with ECOG score between 0 and 1

and Child-Pugh A status, then sorafenib based combination treatment was started. The clinical and pathological variables were collected for analysis and the long-term outcome was compared with log-rank test and Kaplan–Meier survival analysis. The response to sorafenib was measured according to the modified RECIST as best clinical response; complete response (CR), partial response (PR), stable disease (SD), and progressive disease (PD) [25]. The operation FFPE specimens were processed for genomic DNA extraction and OncoScan genechip analysis.

DNA extraction and FFPE sample gene chips analysis

The genomic DNA extraction and data processing after Affymetrix OncoScan genechip hybridization were performed as previously described [9]. The FFPE genomic DNA was extracted using the QIAamp DNA FFPE Tissue Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. DNA samples were further processed at the Genomic Medicine Research Core Laboratory in CGMH for Affymetrix OncoScan® FFPE assay (Santa Clara, CA, United States) to analyze copy number aberrations (CNAs), loss of heterozygosity (LOH), and somatic mutations [26]. The data were analyzed by the Nexus Copy Number software included in the Affymetrix OncoScan FFPE Express Service (Biodiscovery). The percentage genome change showed the number of CNAs of each patient divided by the total of 875 genes designed in OncoScan platform.

Immunohistochemistry

The FFPE specimen were sectioned to 4 μm in thickness and de-paraffinized, rehydrated, and processed for antigen retrieval. The slides were further incubated with appropriate dilutions of the selected antibodies at room temperature for 1–2 h. After incubation, the slides were washed three times in phosphate-buffered saline (PBS), incubated with a horse reddish peroxidase conjugated antibody polymer (Zymed) at room temperature for 10 min, and were then developed by treatment with 3,3′-diaminobenzidine (Roche) at room temperature for 10 min. The titer for ABCB1 (Wuhan Fine Biotech, FNab09805), EGFR (Zeta Corporation, clone EP22) and BRAF (Abcam) was 1:500, 1:500 and 1:100; respectively.

Statistical analysis

Categorical data were analyzed using the chi-square test or Fisher's exact test. Continuous variables were analyzed using with Mann–Whitney U test due to the limited cases without normal distribution in this study. Survival rates in each group were determined by the Kaplan–Meier method and differences between groups were analyzed using log-rank test. The best cut-off value was estimated using the Youden index for maximizing the sum of sensitivity and specificity to discriminate different events. Statistical analyses were performed using SPSS statistical software version 19.0 (SPSS, Inc., Chicago, IL, United States).

Results

The best clinical response was associated with the long term-outcome

Of the 23 patients who were administered sorafenib, 7 (30.4%) showed CR/PR, 7 (30.4%) showed SD, and 9 (39.1%) presented PD in the evaluation of best clinical response [Fig. 1] [Table 1]. The mean age of the patients was 58.7 ± 11.2 years and 22 (95.7%) patients were male. Four patients (17.4%) had stage III HCC and 19 (82.6%) had stage IV HCC. The most common extrahepatic spreading was to bone, lung, and lymph nodes [Table 1]. The mean duration of treatment (DOT) was 12.2, 34.9, 6.8, and 2.73 months in CR, PR, SD, and PD, respectively. The typical sorafenib response in patients with advanced HCC is shown in Fig. 1A–C. The Kaplan–Meier survival curve for patients with advanced HCC treated with sorafenib and the log-rank test indicated a better outcome when the treatment response was CR/PR (tumor shrinkage) as compared with PD and SD ($p = 0.003$) [Fig. 1D]. There is no significant difference by log rank tests for combination treatment, radiotherapy (RT) and transarterial chemoembolization (TACE) in overall survival ($p = 0.777$, 0.119 and 0.120; respectively).

Application of percentage of genome changes for sorafenib response in advanced HCC after resection

All patients with HCC recurrence administered sorafenib were at an advanced HCC stage, with portal vein thrombus (PVT) or extrahepatic spreading (EHS) involvement. Global genomic alterations in recurring HCC were analyzed using the Affymetrix OncoScan® FFPE assay with 875 oncogenes or tumor suppressor genes. CNAs, upon virtual karyotyping of chromosomes showed a remarkable difference in amplification in patients with CR/PR, SD, and PD treatment responses. However, there was a significant increase in amplification in chromosome 7q in HCC patients with PD, and the clinical treatment response of patients was poor when the global percentage genome change was increased in the tumor specimen [Fig. 2]. The comparison between patients with low and high percentage genome change (≤33.0% and >33.0%, respectively) is shown in Table 2. There was no difference in clinical staging and demographic data but the percentage genome change and disease control rate (DCR) were different. The log-rank test indicated there was borderline difference in overall survival between high and low percentage genome change ($p = 0.096$) but the time-to-progression analysis indicated a significantly better outcome in patients with low percentage genome change ($p = 0.004$) [Fig. 3A and B], which indicated higher percentage genome change in tumor

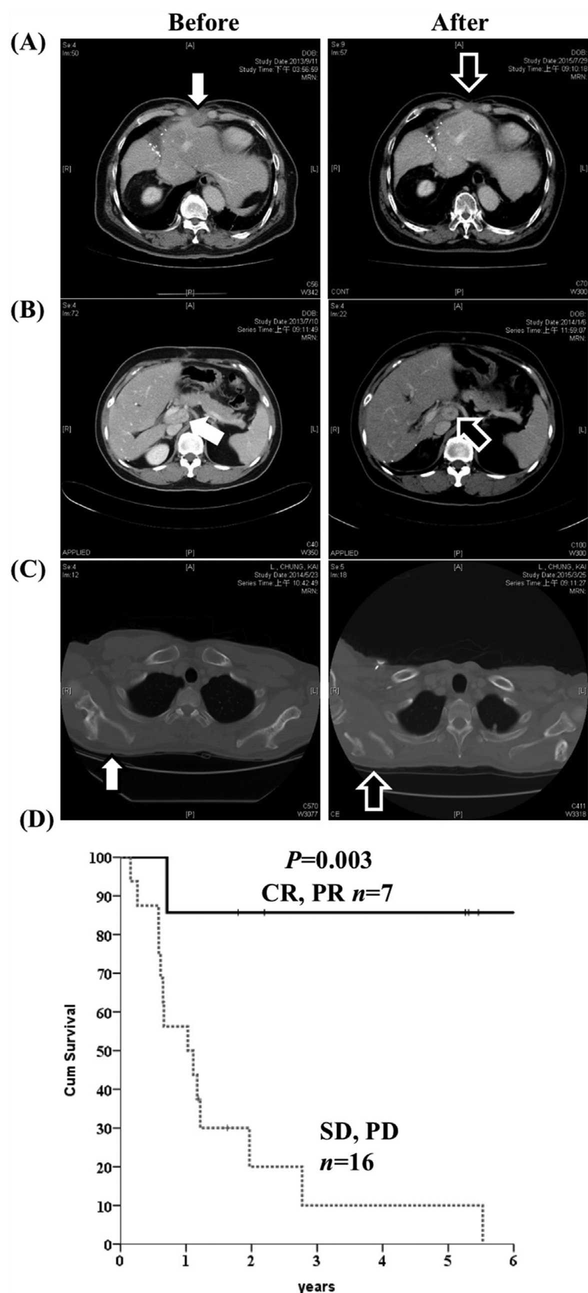

Fig. 1 Clinical treatment of HCC at advanced stage. (A) No. 23, 74 y/o male, developed sternum and liver recurrence. He had complete treatment response to sorafenib, radiotherapy, and TACE. He had been off treatment for 5 years or more. (B) No. 18, 42 y/o female, had regional lymph node metastasis and failed local ablation treatment, but she received a 200–400 mg daily dosage of sorafenib to keep her condition stable. The best response was a partial response. (C) 56 y/o male, had right scapula metastatic destruction and progressive disease. The target lesion is marked with white and black arrows before and 3 months after treatment in the computed tomography scans, respectively. (D) Cumulative survival rates of advanced HCC with sorafenib administration and the log-rank test indicated a better outcome when the treatment was complete/partial response (CR/PR) (tumor shrinkage, black solid line) as compared with stable disease/progressive disease (SD/PD) (gray dotted line) ($p = 0.003$).

specimen of advance HCC indicate adverse prognosis after sorafenib treatment.

Karyotyping analysis of sorafenib response in advanced HCC

The analysis of amplifications and deletions in 875 genes listed in the Affymetrix OncoScan® FFPE assay showed there was a significant increase of amplification at chromosome 7q associated with sorafenib resistance [Table 3]. HCC patients with PD after sorafenib administration had more than 75% amplification at chromosome 7; especially the amplification of 7q21.12-7q31.2 was the most significantly associated with treatment failure [Fig. 2] [Table 3]. Only one patient had chromosome 7q amplification in the CR/PR/SD group. These results imply that the drug response was possibly related to the amplification. Within the amplification region, 7q21.12-7q31.2, was the *ATP Binding Cassette Subfamily B Member 1* (ABCB1) gene, which functions as an ATP-dependent drug efflux pump and has been reported to be associated with multidrug resistance in several kinds of cancers [27,28]. Cyclin Dependent Kinase 6 (CDK6) has been reported to play a role in the proliferation of cancer cells [29]. Besides 7q21.12-7q31.2, other region in chromosome 7 contains epidermal growth factor receptor (*EGFR*) and *INHBA*, which may trigger the MAPK/ERK and PI3K pathways [18,30]. Respectively, and play a role in cell survival, were also amplified in the sorafenib resistant patients. Moreover, the amplification of chromosome 7q was significantly associated with poor outcome in overall survival and time-to-progression analyses [Fig. 3C and D, $p = 0.004$ and $p < 0.001$, respectively]. Therefore, CNA changes in the tumors of patients with advanced HCC may increase the activity of the ATP-dependent drug efflux pump, cell survival, and cell proliferation, establishing resistance to sorafenib treatment.

To correlate whether the amplification of 7q21.12-7q31.2 was associated with elevated its protein level, the expression of ABCB1, EGFR, and BRAF [list on Table 3] were examined via immunohistochemistry in tumor FFPE specimen of seven cases. Even amplification in chromosome 7q21.12-7q31.2 in these cases, the heterogeneity still existed in each specimen; some of specimen containing whole 7q21.12-7q31.2 amplification, some specimen containing only ABCB1, or EGFR amplification [Fig. 4]. Overall speaking, the amplification of ABCB1 region had relatively higher protein expression than those without ABCB1 amplification [Fig. 4], which might be the result for treatment failure.

In summary, the treatment response for sorafenib for recurring HCC could be possibly predicted before drug treatment via acquisition of global CNAs in the tumor tissue using the OncoScan platform. Higher percentage genome change or amplification in chromosome 7q (containing ABCB1 amplification) in tumor specimen of advance HCC indicate resistance to the drug sorafenib.

Discussion

It is still necessary to develop prognostic biomarkers to determine the efficacy and cost-effectiveness of sorafenib

Table 1 Clinical response to sorafenib administration in HCC patients who underwent partial hepatectomy and have recurrent disease.

No	CNA (%)	Age	Sex	stage at diagnosis	Stage at recurrence	Target site	Dosage	DOT	Treatment/combination	Best Clinical Response	Mortality	Survival
1	10.3	53	M	I	IV	Bone	600	2.1	SORA/RT	PD	Yes	7.8
2	14.8	45	M	I	IV	IVC, PV, HV, adrenal	800	1.8	SORA	PD	Yes	1.8
3	29.2	56	M	I	IV	Bone, Liver	800	2.4	SORA/RT	PD	Yes	12.3
4	34.3	39	M	I	IV	Lung, Bone	600	2.8	SORA/RT	PD	Yes	14.6
5	36.9	65	M	I	IV	Duodenum	400	2.3	SORA/RT	PD	Yes	3.1
6	38.4	57	M	I	IV	Bone	600	1.6	SORA	PD	Yes	23.6
7	48.7	66	M	I	IV	IVC, HV, adrenal	400	2.3	SORA	PD	Yes	33.3
8	60.7	49	M	I	IV	Lung, IVC	600	6.4	SORA	PD	Yes	7.0
9	77.2	57	M	I	III	MHV	800	2.7	SORA/RT	PD	Yes	8.0
10	17.8	74	M	I	IV	Bone	400	7.8	SORA/RT	SD	Yes	13.3
11	19.6	43	M	I	IV	LN, PVT	600	4.8	SORA/TACE, RT	SD	Yes	7.0
12	29.2	70	M	II	IV	Pleura	800	1.2	SORA/RT	SD	Yes	66.3[c]
13	30.2	59	M	I	III	Liver	400	12.1	SORA	SD	Yes	14.0
14	33.0	62	M	I	III	Liver	600	14.3	SORA/TACE	SD		14.3
15	36.1	72	M	I	IV	LN	400	5.8	SORA	SD		19.6
16	36.4	48	M	I	IV	Lung	600	1.7	SORA	SD	Yes	7.3
17	0.3	55	M	I	III	Liver	200	33.4	SORA/OP[a]	PR		65.5
18	11.8	42	F	II	IV	LN	400	72.6	SORA	PR		72.6
19	20.8	65	M	I	IV	LN	400	7.3	SORA	PR	Yes	8.5
20	28.0	79	M	I	IV	Lung	600	26.3	SORA	PR		26.3
21	23.5	54	M	I	IV	Lung	600	5.8	SORA/OP[b]	CR		21.5
22	28.5	65	M	II	IV	Bone, Liver	600	11.4	SORA/RT, TACE	CR		63.7
23	31.6	74	M	II	IV	Lung, Sternum	400	19.4	TACE, SORA	CR		63.2

Abbreviations: CNA (%): percentage of genome change in copy number aberration; DOT: duration of treatment; SORA: sorafenib; RT: radiotherapy; CT: chemotherapy; TACE: transarterial chemoembolization.

[a] Liver transplantation.

[b] Lung lobectomy for solitary lesion.

[c] Stable state after radiotherapy.

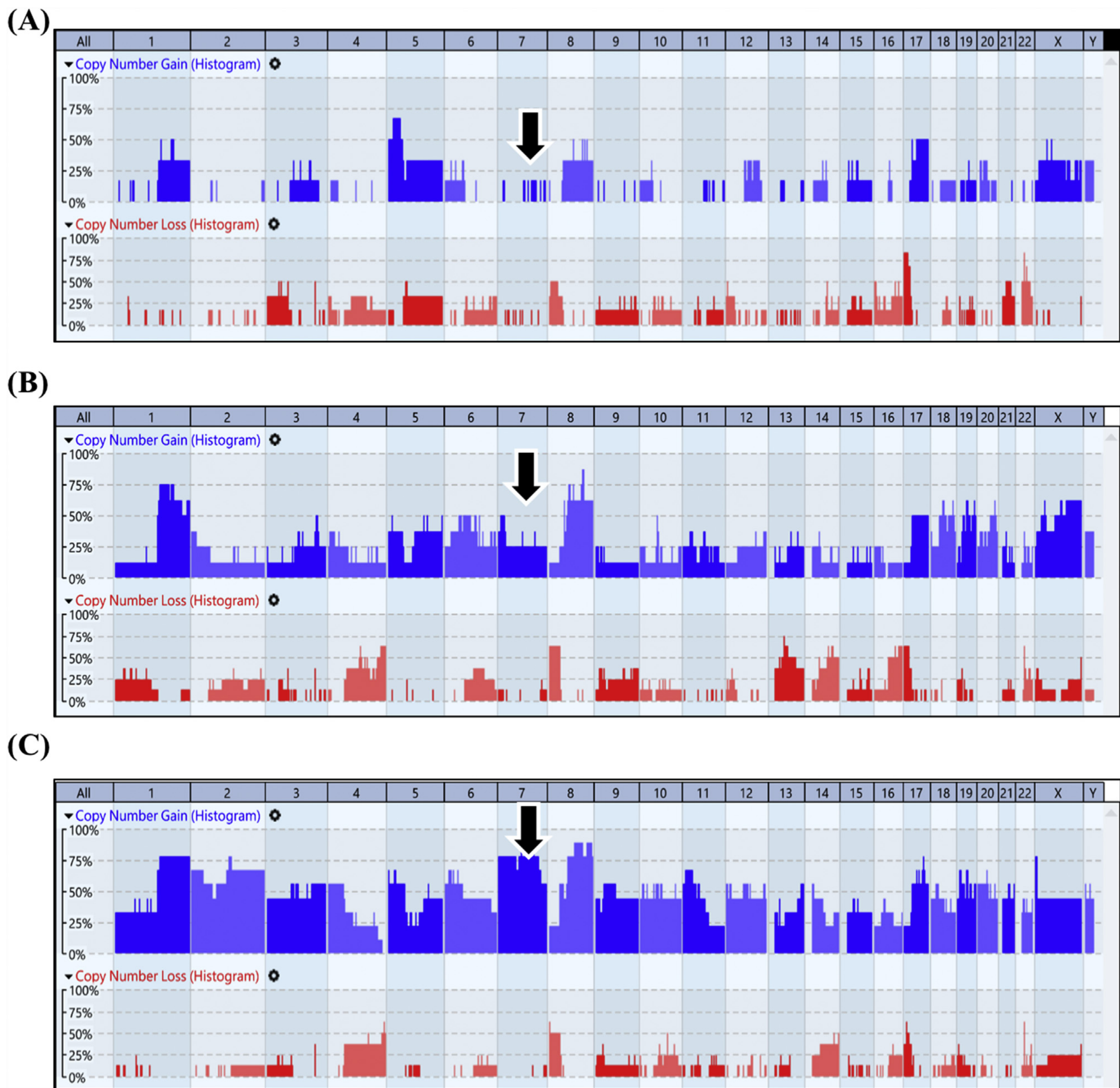

Fig. 2 The global chromosomal change in clinical sorafenib treatment for HCC. There was a remarkable increase in CNAs for the treatment response from CR/PR (A) to SD (B) to PD (C). There was significant increase of amplification at chromosome 7q that was associated with sorafenib resistance (Black arrow).

therapy [31,32]. Elevated levels of the hepatocyte growth factor (HGF), phosphorylated-ERK (p-ERK), and c-Jun have been reported to contribute to the poor response to sorafenib. However, cancer genetic heterogeneity influences the clinical application of these biomarkers as predictors of the response to sorafenib [33–35]. In our study, amplification of chromosome 7, including *ABCB1*, *EGFR*, *BARF*, and *MET*, the upstream receptor kinases for activation of p-ERK and c-Jun, was found in the sorafenib resistant group [Table 3]. The expression of EGFR was not evaluated in these twenty-three patients via immunohistochemistry; therefore, we could not confirm whether EGFR and its downstream RAF/MEK/ERK pathway were activated in sorafenib resistant patients [36]. In a previous study, inhibition of the PI3K/Akt pathway reversed sorafenib resistance in HCC cells, indicating that combinatorial treatment would promote sorafenib treatment efficacy [18,37]. Interestingly, amplification of *INHBA* (encoded activin A) was also found to be associated with sorafenib response in advanced HCC patients [Table 3]. Overexpression of activin A stimulated the non-canonical pathway PI3K/akt signaling to promote cell migration [30]. Therefore, amplification of chromosome 7 contributed to the characteristics of sorafenib resistant HCC cells. Besides, the quantitation of chromosomal alterations as the percentage of genome changed via the

Table 2 Comparison of the clinical characteristics of two groups of the 23 patients with advanced HCC created using a percentage genome change cut off value of 33%.

	Percent genome change ≤33.0% (n = 15)	Percent genome change >33.0% (n = 8)	P
Age (years)	59.7 ± 11.6	56.6 ± 10.9	0.651
Sex (male)	14 (93.3%)	8 (100.0%)	0.652
Stage (Diagnosis)	II 4 (26.7%)	II 0 (0%)	0.154
	I 11 (73.3%)	I 8 (100.0%)	
Stage (Recurrence)	IV 12 (80.0%)	IV 7 (87.5%)	0.565
	III 3 (20.0%)	III 1 (12.5%)	
AFP (ng/ml)	96.0 ± 173.3	8379.9 ± 23287.9	0.071
CNA (%)	21.9 ± 9.5	46.1 ± 15.4	<0.001***
Dosage ≥600 mg	9 (60.0%)	5 (62.5%)	0.633
DCR (%)	7 (46.7%)	0 (0%)	0.026*

Abbreviations: AFP: alpha-fetoprotein; DCR: disease control rate. Age, CNA, and AFP are presented as mean ± S.D. Sex, stage, Dosage≥600 mg and disease control rate (DCR) are presented as number of cases (percentage).*P<0.05, ***P<0.001.

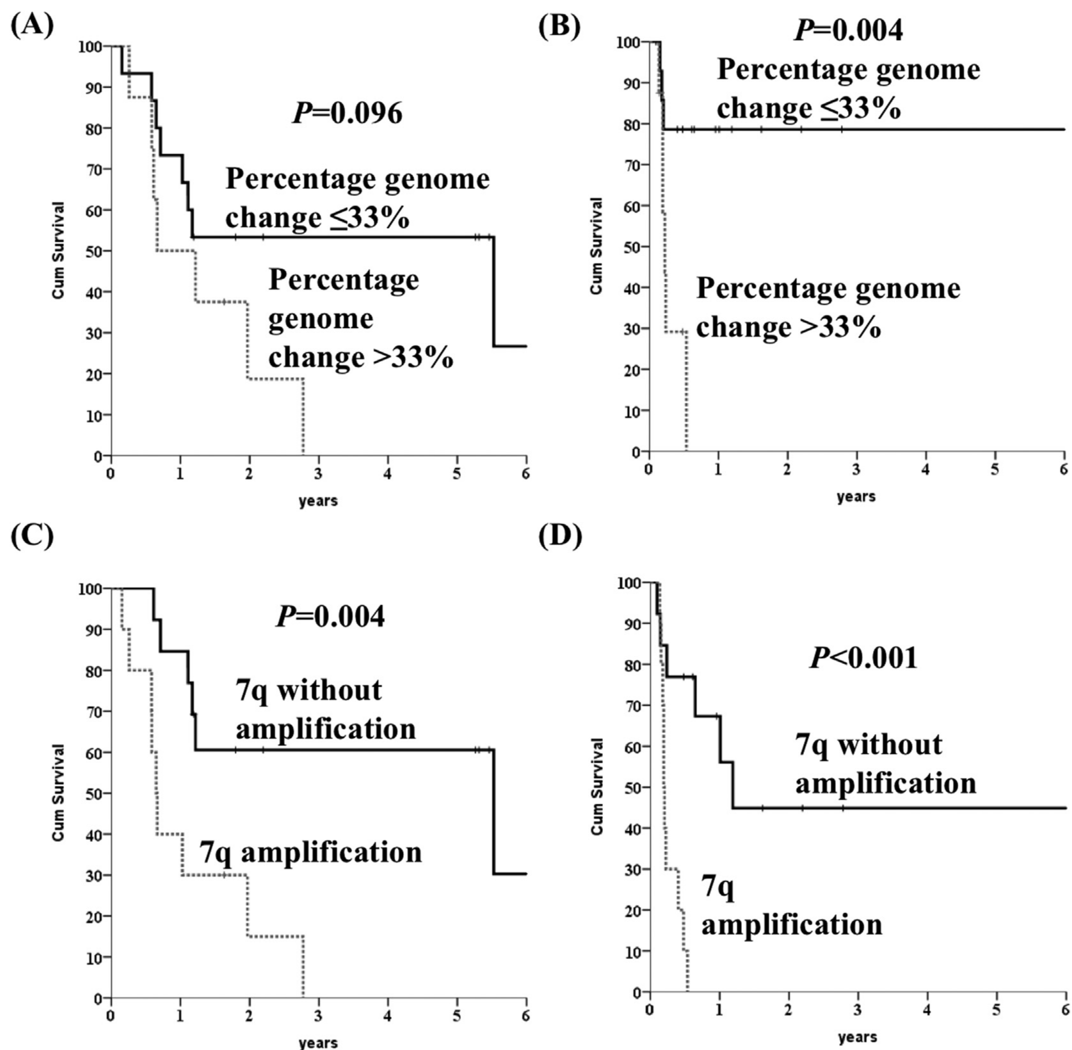

Fig. 3 The percentage genome change and amplification of chromosome 7q in overall survival and time-to progression analysis (A). The cut-off of percentage genome change for outcome prediction was set at ≤33.0% vs. >33.0% and the log-rank test showed no significant difference although it showed a tendency for the same ($p = 0.096$). (B) The time-to-progression analysis indicated a significantly better outcome when percentage genome change was <33% (black solid line) ($p = 0.004$). (C) The amplification of chromosome 7q (dotted line) was significantly associated with poor outcome in overall survival ($p = 0.004$). (D) The amplification of chromosome 7q (dotted line) was significantly associated with poor survival in the time-to progression analysis. ($p < 0.001$).

Table 3 Association of amplification in chromosome 7 with the response to sorafenib.

Gene	Cytogenetic band	Official full name	P
ABCB1	7q21.12	*ATP binding cassette subfamily B member 1*	0.000211
AKAP9	7q21.2	*A-kinase anchor protein 9*	0.000211
CDK6	7q21.2	*Cyclin-dependent kinase 6*	0.000211
LMTK2	7q21.3	*Serine/threonine-protein kinase LMTK2*	0.000211
STAG3	7q22.1	*Stromal antigen 3*	0.000211
PILRB	7q22.1	*Paired immunoglobulin-like type 2 receptor beta*	0.000211
MOSPD3	7q22.1	*Motile sperm domain containing 3*	0.000211
SERPINE1	7q22.1	*serpin E1*	0.000211
PIK3CG	7q22.3	*Phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit gamma isoform*	0.000211
PPP1R3	7q31.1	*protein phosphatase 1 regulatory subunit 3A*	0.000211
MET	7q31.2	*MET proto-oncogene*	0.00031
GRM8	7q31.33	*Glutamate metabotropic receptor 8*	0.001126
FLNC	7q32.1	*Filamin C*	0.000211
SMO	7q32.1	*Smoothened*	0.000211
CREB3L2	7q33	*cAMP responsive element binding protein 3 like 2 Aliases*	0.001126
TRIM24	7q33-q34	*Tripartite motif-containing 24*	0.001126
HNRNPA2B1	7p15.2	*Heterogeneous nuclear ribonucleoproteins A2/B1*	0.004785
JAZF1	7p15.2-p15	*Juxtaposed with another zinc finger protein 1*	0.004785
INHBA	7p14.1	*Inhibin, beta A*	0.004785
GLI3	7p14.1	*GLI family zinc finger 3*	0.004785
HECW1	7p14.1-p13	*HECT, C2 and WW domain containing E3 ubiquitin protein ligase 1*	0.004785
IGFBP1	7p12.3	*Insulin-like growth factor-binding protein 1*	0.004785
IGFBP3	7p12.3	*Insulin-like growth factor-binding protein 3*	0.004785
GRB10	7p12.1	*Growth factor receptor-bound protein 10*	0.006192
EGFR	7p11.2	*Epidermal growth factor receptor*	0.004785
SBDS	7q11.21		0.004785
CLDN3	7q11.23	*Claudin 3*	0.004785
ELN	7q11.23	*Elastin*	0.004785
CLIP2	7q11.23	*CAP-Gly domain-containing linker protein 2*	0.003745
HIP1	7q11.23	*Huntingtin-interacting protein 1*	0.004785
STYXL1	7q11.23	*Serine/threonine/tyrosine-interacting-like protein 1*	0.004785
YWHAG	7q11.23	*14-3-3 protein gamma*	0.004785

Affymetrix OncoScan® FFPE assay for each HCC patient tumor specimen could predict drug responsiveness to sorafenib, which may be applied before drug administration after partial hepatectomies in these patients.

ABCB1 amplification and single nucleotide polymorphisms have been associated with multiple drug resistance in several kinds of cancers [27,28]. Resistance to doxorubicin and 5-fluorouracil have been associated with overexpression of ABCB1 in HCC tumor tissues. Additionally, some reports have suggested that combination with a specific ABCB1 inhibitor would increase sensitivity to drugs [38]. However, the *ABCB1* polymorphism 3435C > T was significantly associated with the lowest sorafenib plasma levels and, thus, with a better therapeutic response [39]. In our study, the amplification of *ABCB1* was significantly associated with resistance to sorafenib. This is the first report showing the correlation between amplification of *ABCB1* and sorafenib resistance in advance HCC patients.

Based on previous reports, amplifications of *VEGFA*, *FGF3/FGF4*, and *FGF19* have been reported to be associated with sorafenib response in advanced HCC using different detection methods [22–24]. As shown in supplementary Table 1, amplification of *VEGFA*, *FGF3*, *FGF4*, and *FGF19* in the SD, PD and PR/CR groups resulted in no statistically significant difference between them upon analysis of OncoScan data; however the probes in *FGF3/4* and *FGF19* were limited in the Affymetrix OncoScan® FFPE assay platform. The amplification of *VEGFA* was also not associated with the sorafenib response; however, the difference of this result with those of other studies might be due to differences in analysis platforms or patient-inclusion criteria. Meanwhile, the amplification or loss of *RPPH1* (RNase P) was also found in our data (supplementary Table 1). The copy number of *RPPH1* is frequently used as an internal control (reference) in commercial copy number assays. Among the 23 cases assessed here, *RPHH1* was amplified in several cases with PD; this could have resulted in a lack of observed *ACBC1* amplification in the analysis of CNAs through copy number PCR assay. Therefore, the internal controls for normalization of copy number PCR results should be carefully chosen.

The limited number of cases analyzed in this study could lead to potential bias and, thus, the results should be carefully interpreted. Further studies using well-designed large cohort could help validate our results.

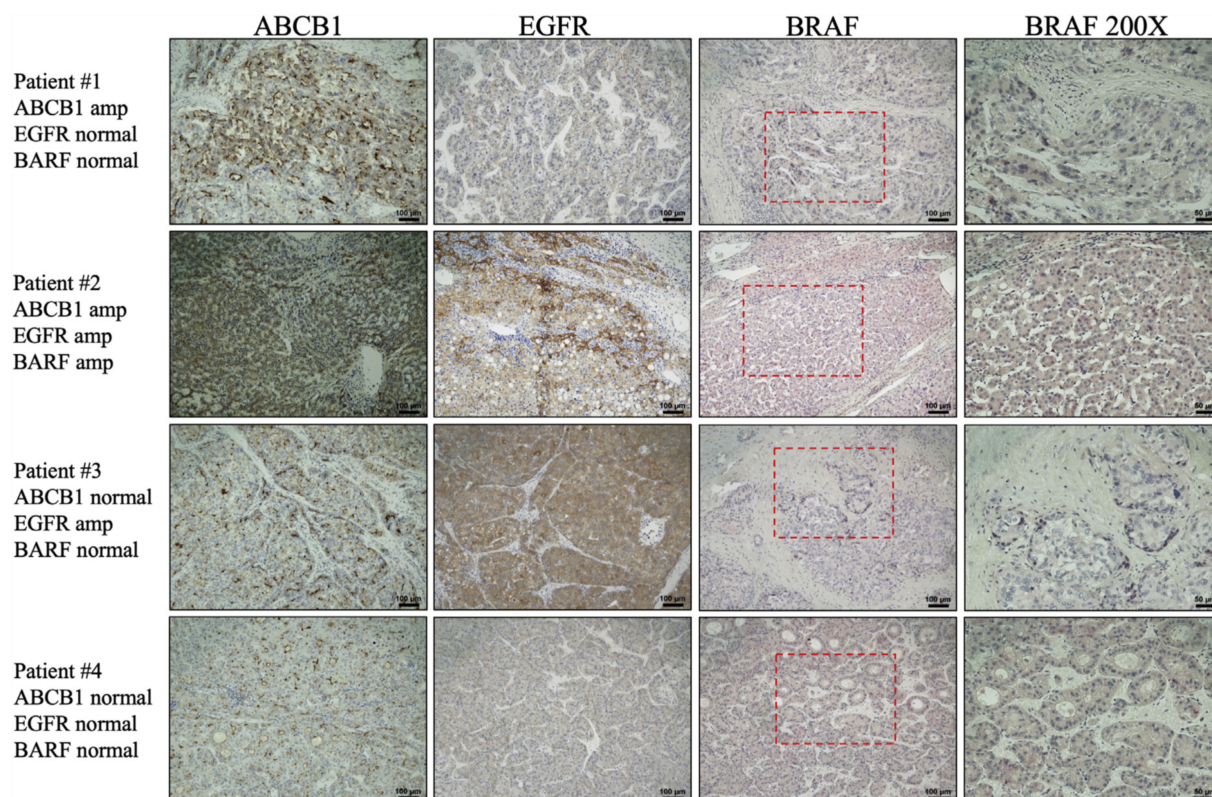

Fig. 4 The expression of ABCB1, EGFR and BRAF in four representative cases were examined via immunohistochemistry in tumor FFPE specimen. The copy number changes in each gene within patient was as described. Amp: amplification (copy number >2), normal (copy number = 2). The staining density of ABCB1, EGFR was higher in specimen with copy number amplification than those are without amplification. The magnification of left three panel was 100X; whereas the magnification of the right panel (BRAF) was 200X.

Conclusion

Although the enrolled case number in this study was limited, the following conclusions could be drawn:

1. The treatment failure for sorafenib could be possibly predicted via higher percentage genome changes in tumor specimen acquisition from Affymetrix OncoScan platform.
2. Among these CNAs, amplification in chromosome 7q that containing drug resistant gene *ACBC1* in tumor specimen tend to resistant to Sorafenib.
3. From tumor specimen of patients with HCC could predict drug responsiveness to sorafenib, which may be applied before drug administration after partial hepatectomies in these patients.

Financial support

The study was supported by Chang Gung Memorial Hospital CMRPG3F1993 and CMRPG1H0131 for Dr. Yu MC; CMRPD1G0611 and CMRPD1H0661 for Dr. Tsai CN.

Conflicts of interest

The authors declare no conflicts of interest.

Acknowledgments

We are grateful for the technological support of the Department of Cancer Center and Genomic Medicine Research Core Laboratory, Chang Gung Memorial Hospital, Linkou branch.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bj.2020.07.001.

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