Copy Number Aberrations of Multiple Genes Identified as Prognostic Markers for Extrahepatic Metastasis-free Survival of Patients with Hepatocellular Carcinoma*

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Summary: Extrahepatic metastasis confers unfavorable patient prognosis in patients with hepatocellular carcinoma (HCC), however, reliable markers allowing prediction of extrahepatic metastasis at the time of initial diagnosis are still lacking. This study was to identify gene-level copy number aberrations (CNAs) related to extrahepatic metastasis-free survival of HCC patients, and further examine the associations between CNAs and gene expression. Array comparative genomic hybridization (aCGH) and expression array were used to analyze gene CNAs and expression levels, respectively. The associations between CNAs of a panel of 20 genes and extrahepatic metastasis-free survival were analyzed in 66 patients with follow-up period of 1.6–90.5 months. The gene expression levels between HCCs with and without gene CNA were compared in 109 patients with HCC. We observed that gains at MDM4 and BCL2L1, and losses at APC and FBXW7 were independent prognostic markers for extrahepatic metastasis-free survival of HCC patients. Integration analysis of aCGH and expression data showed that MDM4 and BCL2L1 were significantly upregulated in HCCs with gene gain, while APC and FBXW7 were significantly downregulated in HCCs with gene loss. We concluded that gene gains at MDM4 and BCL2L1, and losses at APC and FBXW7, with concordant expression changes, were associated with extrahepatic metastasis-free survival of HCC patients and have potential to act as novel prognostic markers.
Key words: hepatocellular carcinoma; extrahepatic metastasis-free survival; gene; copy number aberration; expression

Hepatocellular carcinoma (HCC) is one of the most lethal malignancies worldwide and in China 422 100 people died from this disease in 2015[1, 2]. Surgical resection remains the first-line treatment for early-stage HCC nowadays, whereas postoperative extrahepatic metastasis occurs frequently, which confers unfavorable patient prognosis. However, predictive factors for the development of extrahepatic metastasis continue to be incompletely defined. Therefore, discovery of metastasis biomarkers, especially those useful in guiding clinicians to elaborate treatment strategies at the time of initial diagnosis, is warranted.

DNA copy number aberration (CNA) is an important subclass of somatic mutations, with aberrant chromosomal regions of gain or loss commonly associated with overexpressed oncogenes or loss of tumor suppressor genes, respectively[3]. As one of the major features of solid tumors including HCC[4], CNA has been proved to play an important role in tumor development, progression and patient outcomes[5–7]. Previous studies of HCC have identified a number of recurrent chromosomal CNAs and some of them have

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been associated with advanced tumor stage, presence of venous infiltration, low differentiation, higher AFP level, intrahepatic recurrence, and poor overall and disease-free survivals of HCC patients[8–12]. However, whether these CNAs are associated with extrahepatic metastasis of HCC has not been thus far elucidated. In addition, the correlation between CNAs and extrahepatic metastasis has not been determined on gene level.

In the present study, the CNA status of a panel of 20 genes was detected in 66 HCC samples to identify extrahepatic metastasis-related gene CNAs. In addition, we also examined the gene expression levels related to the gene CNAs potential correlated with extrahepatic metastasis.

1 MATERIALS AND METHODS

1.1 Patients

A total of 179 patients with HCC were enrolled from the Eastern Hepatobiliary Surgery Hospital, Second Military Medical University, Shanghai, China. Final diagnosis of HCC was pathologically confirmed and only patients with samples containing ≥80% tumor cells were enrolled. None of enrolled patients received radiotherapy or chemotherapy before surgery. Tumors were immediately cut from livers, frozen in liquid nitrogen, and stored at −80°C until DNA/RNA extraction. Written informed consent was obtained from all patients, and the study protocol was approved by the Institutional Review Board of the Eastern Hepatobiliary Surgery Hospital.

Of the 179 patients enrolled, 66 were enrolled between December 2007 and January 2008 (group Ⅰ) to identify gene CNAs correlated with extrahepatic metastasis-free survival. The remaining 113 patients (group Ⅱ) were enrolled between January and March 2007 to identify differentially expressed genes in the same direction as CNAs (i.e. upregulation and gain, or downregulation and loss). For group Ⅱ, experimental analyses were unsuccessful for 8 and 5 cases for array comparative genomic hybridization (aCGH) and expression array, respectively, leaving 100 patients with matched aCGH and expression data. Nine patients in group Ⅰ also had expression data, and thus a total of 109 patients with matched aCGH and expression data were included in the final analyses of the associations between gene CNAs and expression levels.

1.2 Follow-up

All 66 patients in group Ⅰ were followed up for the development of extrahepatic metastasis by clinical examination, serum alpha fetoprotein (AFP), imaging modalities (CT, MRI, bone ECT, angiography, PET/CT, etc.), and/or pathological examination. Serum AFP was measured once a month, and imaging modalities were performed once every 3–6 months, or when necessary. The median follow-up time was 37.2 months (range, 1.6–90.5 months) with a total analysis time of 2633.4 months, and during the follow-up extrahepatic metastasis occurred in 25 patients (37.9%). Extrahepatic metastasis-free survival was expressed as the number of the months from the date of surgery to the date of diagnosis of extrahepatic metastasis or the last follow-up.

1.3 Gene Selection

A panel of 20 genes, including oncogenes, tumor suppressor genes and some other genes potentially associated with tumor metastasis, were included in the present study. These genes were selected based on: i) previously published association of the gene CNA with metastasis in various tumors, thus increasing the chance of screening the genes with extrahepatic metastasis of HCC; ii) gene CNA (gain or loss) frequency ≥10% in HCC, according to the published data; and iii) successful probe coverage of the gene in the Agilent 244K aCGH platform we used in this study.

1.4 Array CGH Analysis for Gene CNAs

Tumor DNA was extracted using the Genomic DNA Purification Kit (Qiagen, Valencia, USA). Array CGH analysis for gene CNA was performed using the Agilent Human Genome Microarray Kit 244K (Hu-244A; Agilent Technologies, Santa Clara, USA), as detailed previously[9–11]. An absolute $\log_2$ ratio >0.5 was used as the threshold for the gain or loss in gene copy number.

1.5 Gene Expression Analysis

Total RNA in tumor was extracted and purified using the RNeasy Mini Kit (Qiagen, China). Gene expression levels in tumors were measured using Affymetrix GeneChip Human Genome U133 Plus 2.0 array (Affymetrix, Santa Clara, USA), which has been detailed elsewhere[9–11]. All data were $\log_2$ transformed for display and further analysis.

1.6 Statistical Analysis

A univariate Cox regression model was applied to explore the associations of clinicopathological features and gene CNAs with extrahepatic metastasis-free survival. To identify independent prognostic gene CNAs, a multivariate Cox regression model was performed with adjustment for variables that have been associated with metastasis-free survival in univariate analysis, including tumor encapsulation (present, absent), hypertension history (no, yes), platelet count (≥100×10^9/L, <100×10^9/L), prothrombin time (≤12, >12 s), and TNM stage (Ⅰ/Ⅱ, Ⅲ). As a sensitivity analysis, we further adjusted for age (≤50, >50 years), sex, serum AFP (≤20, >20 μg/L), Child-Pugh classification (A, B), liver cirrhosis (no, yes), Edmondson-Steiner grade (II, III), and vascular invasion (no, yes), but yielded no meaningful differences (data not shown). Extrahepatic metastasis-free survival curves were estimated using the Kaplan-Meier method and compared with the

log-rank test. The Mann-Whitney *U* test was used to compare the gene expression levels between HCCs with and without gene CNA. A *P* value <0.05 was considered statistically significant, and all of the tests were two-tailed. Statistical analyses were performed with Stata 13.0 (Stata, College Station, USA).

2 RESULTS

2.1 Associations between Clinicopathological Features and Extrahepatic Metastasis-free Survival

The associations between clinicopathological features and extrahepatic metastasis-free survival in 66 HCC patients in group Ⅰ are summarized in table 1. Hypertension history (*P*=0.007), the absence of tumor encapsulation (*P*=0.019), and advanced TNM stage (*P*=0.026) were associated with poor extrahepatic metastasis-free survival, while lowered platelet count (*P*=0.018) and prolonged prothrombin time (*P*=0.024) were associated with favorable metastasis-free survival. No significant association was observed between age, sex, serum AFP, Child-Pugh classification, liver cirrhosis, Edmondson-Steiner grade, or vascular invasion and metastasis-free survival.

Table 1 Association of clinicopathological features with extrahepatic metastasis of hepatocellular carcinoma

	n	Person-months	Metastasis (*n*)	*HR* (95% *CI*)*	*P* value*
Age (years)					
≤50	32	1245.9	11	1.0 (Reference)	
>50	34	1387.5	14	1.10 (0.50–2.45)	0.808
Sex					
Female	18	669.9	10	1.0 (Reference)	
Male	48	1963.5	15	0.52 (0.23–1.15)	0.107
HBsAg					
Negative	3	124.6	2	1.0 (Reference)	
Positive	63	2508.8	23	0.60 (0.14–2.57)	0.494
Anti-HCV					
Negative	64	2462.0	25	1.0 (Reference)	
Positive	1	90.1	0	–	–
AFP (μg/L)					
≤20	20	759.9	8	1.0 (Reference)	
>20	46	1873.5	17	0.88 (0.38–2.04)	0.765
Child-Pugh classification					
A	55	2299.3	23	1.0 (Reference)	
B	11	334.1	2	0.58 (0.14–2.47)	0.462
Liver cirrhosis					
No	27	1188.6	13	1.0 (Reference)	
Yes	39	1444.8	12	0.75 (0.34–1.64)	0.467
Tumor encapsulation					
Present	23	1301.3	6	1.0 (Reference)	
Absent	43	1332.1	19	3.08 (1.21–7.87)	0.019
Edmondson-Steiner grade					
Ⅱ	14	708.0	6	1.0 (Reference)	
Ⅲ	52	1925.4	19	1.16 (0.46–2.92)	0.755
Hypertension history					
No	59	2477.1	20	1.0 (Reference)	
Yes	7	156.3	5	3.94 (1.47–10.58)	0.007
Platelet count (10^9/L)					
≥100	44	1650.0	22	1.0 (Reference)	
<100	22	983.5	3	0.23 (0.07–0.78)	0.018
Prothrombin time (s)					
≤12	44	1575.2	21	1.0 (Reference)	
>12	22	1058.2	4	0.29 (0.10–0.85)	0.024
Vascular invasion					
No	32	1580.5	12	1.0 (Reference)	
Yes	34	1052.9	13	1.58 (0.72–3.49)	0.258
TNM stage					
Ⅰ / Ⅱ	41	2011.3	14	1.0 (Reference)	
Ⅲ	25	622.1	11	2.49 (1.12–5.55)	0.026

HR, hazard ratio; CI, confidence interval; AFP, alpha fetoprotein; TNM, tumor-node-metastasis
*Univariate Cox regression model

2.2 Gene CNAs Correlated with Extrahepatic Metastasis-free Survival

Univariate Cox analyses of the associations between gene CNAs in HCC and extrahepatic metastasis-free survival showed that gains at MDM4 [hazard ratio (*HR*)=3.28, 95% confidence interval (*CI*)=1.45–7.44] and BCL2L1 (*HR*=3.94, 95% *CI*=1.46–10.63), and losses at APC (*HR*=4.29, 95% *CI*=1.51–12.17), FBXW7 (*HR*=0.29, 95% *CI*=0.12–0.73), and ANXA10 (*HR*=0.34, 95% *CI*=0.14–0.86) were significantly associated extrahepatic metastasis-free survival (table 2). In the multivariate Cox model, with adjustment for tumor encapsulation, hypertension history, platelet count, prothrombin time, and TNM stage, MDM4 gain (*HR*=2.74, 95% *CI*=1.18–6.37), BCL2L1 gain (*HR*=3.45, 95% *CI*=1.13–10.52), APC loss (*HR*=4.92, 95% *CI*=1.69–14.31), and FBXW7 loss (*HR*=0.32, 95% *CI*=0.12–0.89) were independent prognostic factors for extrahepatic metastasis-free survival, where ANXA10 loss lost statistical significance (table 2). The patient extrahepatic metastasis-free survival curves were significantly different according to the status of MDM4 gain, APC loss, BCL2L1 gain, and FBXW7 loss (fig. 1).

Of the 66 samples, 47 had intrahepatic metastases, including 20 cases of intrahepatic and extrahepatic metastases, and 27 cases of intrahepatic metastasis only. Statistical analysis showed no significant correlation between intrahepatic metastasis and extrahepatic metastasis (Chi-square test, *P*=0.270). Inclusion of the intrahepatic metastasis (no/yes) as a potential confounding factor in the multivariate Cox model did not significantly affect the associations between gene CNA and extrahepatic metastasis-free survival (data not shown).

2.3 Associations between Expression Levels and Gene CNAs Correlated with Extrahepatic Metastasis-free Survival

The expression levels of genes MDM4, APC, BCL2L1 and FBXW7 were further compared between HCCs with and without CNA, based on the 109 HCCs with matched aCGH and expression data. As shown in fig. 2, MDM4 (*P*=0.0034) and BCL2L1 (*P*=0.0109) were significantly upregulated in HCCs with CNA gain, while APC (*P*=0.0032) and FBXW7 (*P*<0.0001) were significantly downregulated in HCCs with CNA loss, compared to those without CNA.

3 DISCUSSION

In the present study, we screened out 4 gene CNAs correlated with extrahepatic metastasis-free survival of postoperative HCC, including poor prognostic factors of MDM4 gain, APC loss, and BCL2L1 gain, and favorable prognostic factor of FBXW7 loss. Our findings suggest that determination of the status of these gene CNAs at the time of diagnosis could be useful for identification of the patients at higher risk of developing extrahepatic metastasis, which may aid oncologists in selecting treatment and follow-up

Table 2 Associations of gene copy number aberrations (CNAs) with extrahepatic metastasis-free survival of patients with hepatocellular carcinoma

Gene	CNA		Univariate Cox model		Multivariate Cox model*	
	Type	*n* (%)	*HR* (95% *CI*)	*P* value	*HR* (95% *CI*)	*P* value
MDM4	Gain	24 (36.4)	3.28 (1.45–7.44)	0.005	2.74 (1.18–6.37)	0.019
APC	Loss	7 (10.6)	4.29 (1.51–12.17)	0.006	4.92 (1.69–14.31)	0.003
BCL2L1	Gain	7 (10.6)	3.94 (1.46–10.63)	0.007	3.45 (1.13–10.52)	0.030
FBXW7	Loss	29 (43.9)	0.29 (0.12–0.73)	0.009	0.32 (0.12–0.89)	0.029
ANXA10	Loss	27 (40.9)	0.34 (0.14–0.86)	0.022	0.55 (0.20–1.50)	0.245
SRC	Gain	11 (16.7)	2.24 (0.89–5.64)	0.086	1.39 (0.50–3.86)	0.527
PTEN	Loss	8 (12.1)	1.95 (0.73–5.24)	0.186	2.33 (0.84–6.50)	0.104
MYCN	Gain	9 (13.6)	1.65 (0.65–4.19)	0.295	1.82 (0.58–5.66)	0.304
PARK2	Loss	11 (16.7)	1.63 (0.65–4.10)	0.299	1.07 (0.37–3.04)	0.906
HDGF	Gain	37 (56.1)	1.46 (0.64–3.33)	0.363	1.42 (0.60–3.36)	0.430
PTPRD	Loss	20 (30.3)	0.68 (0.27–1.72)	0.419	0.91 (0.35–2.35)	0.843
MCL1	Gain	36 (54.6)	1.28 (0.56–2.90)	0.561	0.69 (0.29–1.63)	0.397
CDH13	Loss	30 (45.5)	1.24 (0.57–2.72)	0.591	1.19 (0.50–2.81)	0.697
CDH1	Loss	22 (33.3)	0.80 (0.35–1.86)	0.609	0.99 (0.41–2.40)	0.982
MAP2K4	Loss	26 (39.4)	0.84 (0.37–1.91)	0.678	1.14 (0.49–2.67)	0.757
MYC	Gain	20 (30.3)	1.11 (0.44–2.81)	0.826	0.74 (0.27–2.03)	0.554
CCND1	Gain	14 (21.2)	1.10 (0.41–2.93)	0.853	1.71 (0.58–5.07)	0.334
MTAP	Loss	20 (30.3)	0.92 (0.38–2.22)	0.861	1.94 (0.71–5.33)	0.199
CDKN2B	Loss	12 (18.2)	1.06 (0.40–2.83)	0.907	1.94 (0.64–5.90)	0.243
WWOX	Loss	28 (42.4)	0.96 (0.44–2.13)	0.928	1.33 (0.57–3.11)	0.506

HR, hazard ratio; CI, confidence interval.

*Adjusted by tumor encapsulation, hypertension history, platelet count, prothrombin time, and TNM stage

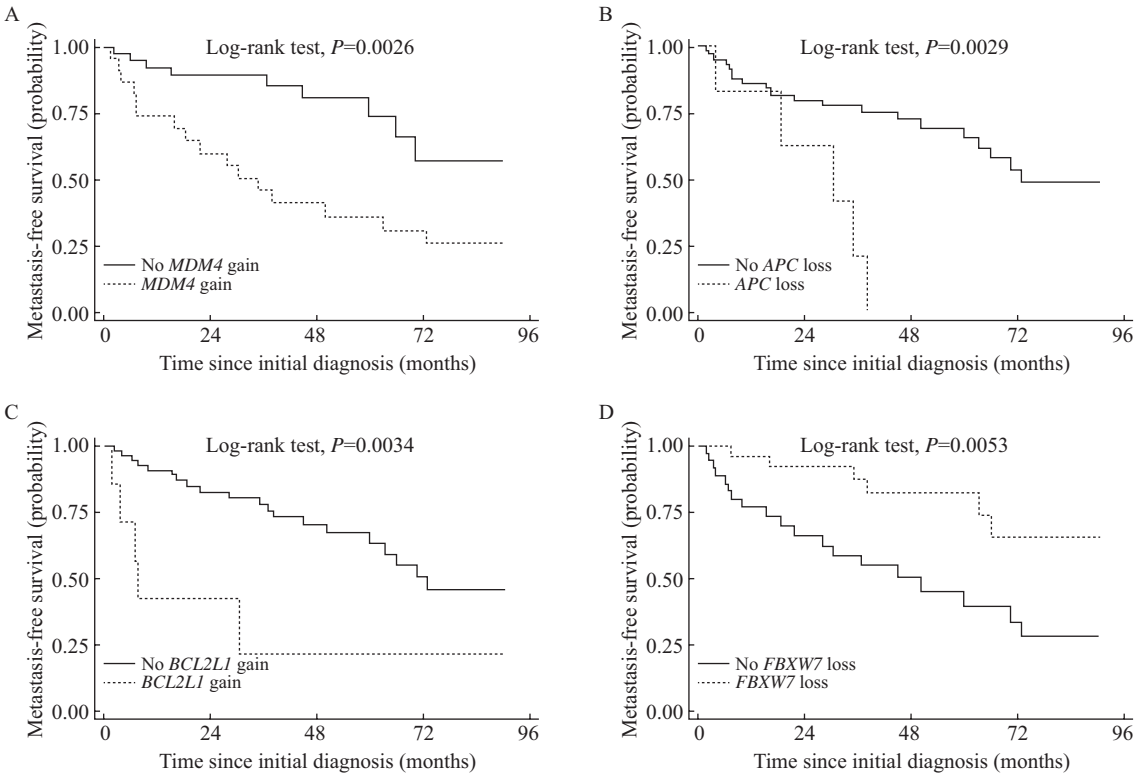

Fig. 1 Extrahepatic metastasis-free survival of patients with hepatocellular carcinoma according to the status of MDM4 gain (A), APC loss (B), BCL2L1 gain (C), and FBXW7 loss (D)

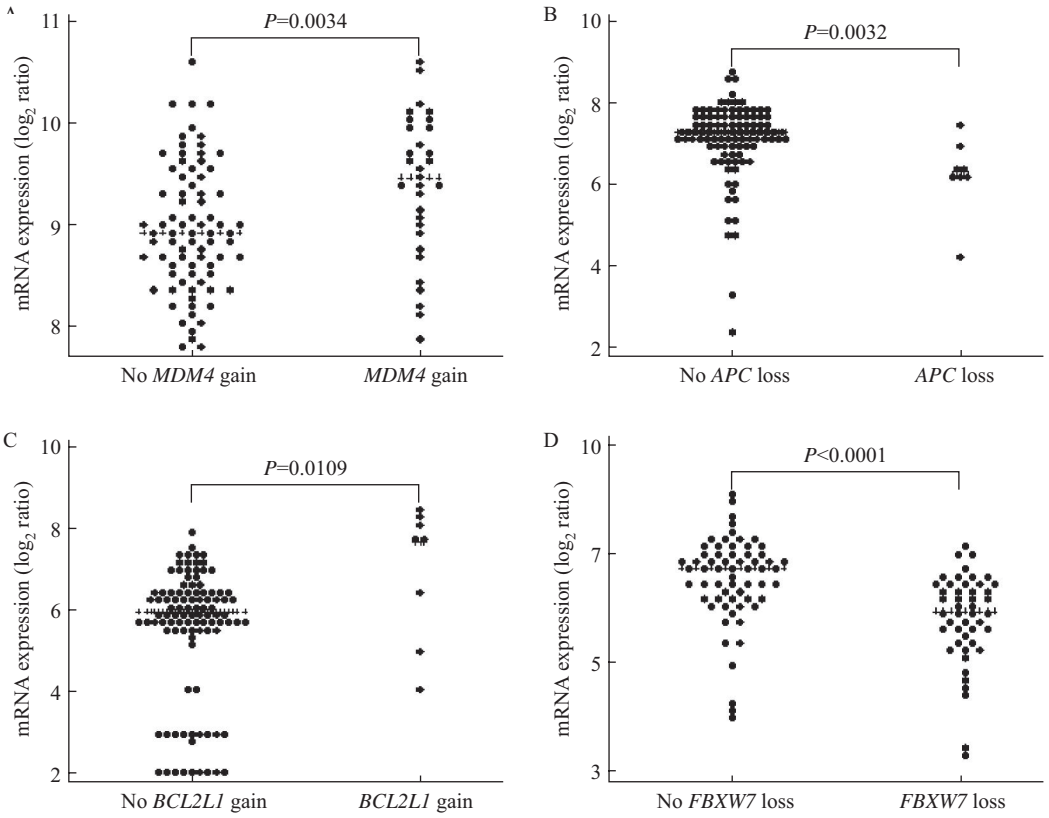

Fig. 2 Associations between gene copy number aberrations and expression levels of MDM4 (A), APC (B), BCL2L1 (C), and FBXW7 (D)

strategies for postoperative HCC patients. Further, we showed that MDM4 and BCL2L1 were significantly upregulated in HCCs with CNA gain, while APC and FBXW7 were significantly downregulated in HCCs with CNA loss.

MDM4, a critical negative regulator of the tumor suppressor p53, has been identified as an oncogene in cancers including HCC, and its overexpression seems to drive tumor progression and prognosticates poor outcome[13]. MDM4 gain, as a frequent molecular event in a variety of cancers, has been proposed as the main target for 1q32 amplification in malignant gliomas[14]. It has been reported that MDM4 gain is correlated with aggressive tumor growth, lymph node metastasis, advanced tumor stage and worse overall survival in salivary gland cancer[15]. Defect in programmed cell death, or apoptosis, is a hallmark of cancer, and the anti-apoptotic BCL2L1 overexpression has been characterized as a key survival factor in multiple cancers[16–18]. It has been reported that BCL2L1 gain could upregulate its expression in colorectal cancer[19], and chromosome fragment gain of 20q11.1 where BCL2L1 located, has been associated with progression-free survival in giant-cell tumor of bone[20]. APC is a tumor suppressor, and loss of functional APC protein may result in activation of canonical Wnt/β-catanin signaling and thus contribute to tumor progression[21]. It has been reported that APC promoter hypermethylation was associated with distant metastasis and decreased 3-year survival in familial breast cancer [22]. Moreover, APC loss has been associated with lymph node or distant metastasis in a study of colorectal cancer[23]. Our data revealed significant associations of MDM4 gain, BCL2L1 gain, and APC loss with extrahepatic metastasis-free survival in HCC, and thus corroborate previous findings in other cancer.

FBXW7 is a substrate recognition subunit of the SCF ubiquitin ligase, which has been reported to be responsible for targeted ubiquitylation and subsequent proteasomal degradation of an array of oncoproteins, and thus functions as a tumor suppressor[24]. It has been reported that FBXW7 was downregulated in HCC, and decreased FBXW7 expression correlated significantly with metastasis and worse 5-year survival of HCC patients[25]. In a study of esophageal squamous cell carcinoma, copy number loss of FBXW7 has been associated with poor prognosis[26]. However, we observed in the present study that FBXW7 loss was a favorable prognostic factor for extrahepatic metastasis-free survival in HCC. The reason for the discrepancy between our findings and previous findings is not clear. Three different FBXW7 isoforms (α, β and γ) have been identified in mammals and isoform specific interactions with several accessory proteins have been reported[27]. To date, defined FBXW7 substrates of oncoproteins are reported to be degraded by the FBXW7-α/γ isoforms[28], whereas FBXW7-β specific substrates still await discovery. Thus the possibility that FBXW7-β exerts oncogene function cannot be excluded. In supporting of this hypothesis, Akhoondi *et al* reported that inactivation of FBXW7-β expression by promoter hypermethylation is associated with favorable prognosis in breast cancer[29].

Gene CNA in cancer cells is one of the underlying genetic mechanisms leading to gene deregulation by altering DNA dosage. By integration of aCGH and expression data, we observed that MDM4 and BCL2L1 were upregulated in HCCs with CNA gain, and APC and FBXW7 were downregulated in HCCs with gene loss. These data suggested that these gene CNAs may contribute to extrahepatic metastasis by altering gene expression levels. On the other hand, gene CNAs at MDM4, BCL2L1, APC, and FBXW7 were each associated with expression levels in the same direction (i.e. upregulation and gain, or downregulation and loss), suggesting that the transcriptional deregulation of the genes could be ascribed partially to their CNAs, at least in HCC.

Our data also showed that hypertension history was associated with poor extrahepatic metastasis-free survival of HCC patients. Similarly, Gao *et al* reported that colorectal cancer patients with hypertension history were more likely to develop lymph node or distant metastasis[30]. The biological mechanisms for these findings are unknown. Calcium-channel blocker has been associated with increased cancer recurrence in head and neck squamous cell carcinoma[31], indicating that antihypertensive drug use might in part explain the results. It should be noted that, given the relatively small sample size of our study, significant findings by chance cannot be excluded. Larger studies are needed to validate our findings.

Our study has several limitations, including the retrospective design and the relatively small sample size for analyzing the associations of gene CNAs with extrahepatic metastasis-free survival. In addition, 91.7% (100/109) samples for expression array lacked follow-up data, which hindered our ability to examine the associations of the gene expression levels with extrahepatic metastasis-free survival. Finally, most of the HCC samples in our study were HBV positive (63/66, 95.5%), which might make our results less applicable to HCCs with other etiological background.

In conclusion, we showed that gains at MDM4 and BCL2L1, and losses at APC and FBXW7, with concordant expression changes, were associated with extrahepatic metastasis-free survival of HCC patients and may be potentially used as novel prognostic markers. Due to limited number of the samples analyzed and lack of the validation in an independent sample set, further studies are needed to verify our findings.

Conflict of Interest Statement

The authors declare that there are no competing interests associated with the manuscript.

REFERENCES

1 Siegel RL, Miller KD, Jemal A. Cancer Statistics, 2017. CA Cancer J Clin, 2017,67(1):7-30

2 Chen W, Zheng R, Baade PD, *et al*. Cancer statistics in China, 2015. CA Cancer J Clin, 2016,66(2):115-132

3 Pinkel D, Albertson DG. Array comparative genomic hybridization and its applications in cancer. Nat Genet, 2005,37 Suppl:S11-17

4 Beroukhim R, Mermel CH, Porter D, *et al*. The landscape of somatic copy-number alteration across human cancers. Nature, 2010,463(7283):899-905

5 Ali H, Bitar MS, Al Madhoun A, *et al*. Functionally-focused algorithmic analysis of high resolution microarray-CGH genomic landscapes demonstrates comparable genomic copy number aberrations in MSI and MSS sporadic colorectal cancer. PLoS One, 2017,12(2):e0171690

6 Wang P, Shan L, Xue L, *et al*. Genome wide copy number analyses of superficial esophageal squamous cell carcinoma with and without metastasis. Oncotarget, 2017,8(3):5069-5080

7 Castagnola P, Zoppoli G, Gandolfo S, *et al*. Genomic DNA Copy Number Aberrations, Histological Diagnosis, Oral Subsite and Aneuploidy in OPMDs/OSCCs. PLoS One, 2015,10(11):e0142294

8 Qi LN, Li LQ, Chen YY, *et al*. Genome-wide and differential proteomic analysis of hepatitis B virus and aflatoxin B1 related hepatocellular carcinoma in Guangxi, China. PLoS One, 2013,8(12):e83465

9 Wang D, Zhu ZZ, Jiang H, *et al*. Multiple genes identified as targets for 20q13.12-13.33 gain contributing to unfavorable clinical outcomes in patients with hepatocellular carcinoma. Hepatol Int, 2015,9(3):438-446

10 Chen L, Zhu ZZ, Liu SF, *et al*. Loss at 16q22.1 identified as a risk factor for intrahepatic recurrence in hepatocellular carcinoma and screening of differentially expressed genes. Neoplasma, 2016,63(1):114-120

11 Zhao K, Zhao Y, Zhu JY, *et al*. A Panel of Genes Identified as Targets for 8q24.13-24.3 Gain Contributing to Unfavorable Overall Survival in Patients with Hepatocellular Carcinoma. Curr Med Sci, 2018,38(4):590-596

12 Furuta M, Ueno M, Fujimoto A, *et al*. Whole genome sequencing discriminates hepatocellular carcinoma with intrahepatic metastasis from multi-centric tumors. J Hepatol, 2017,66(2):363-373

13 Markey MP. Regulation of MDM4. Front Biosci (Landmark Ed), 2011,16:1144-1156

14 Riemenschneider MJ, Knobbe CB, Reifenberger G. Refined mapping of 1q32 amplicons in malignant gliomas confirms MDM4 as the main amplification target. Int J Cancer, 2003,104(6):752-757

15 Ach T, Schwarz-Furlan S, Ach S, *et al*. Genomic aberrations of MDM2, MDM4, FGFR1 and FGFR3 are associated with poor outcome in patients with salivary gland cancer. J Oral Pathol Med, 2016,45(7):500-509

16 Watanabe J, Kushihata F, Honda K, *et al*. Prognostic significance of Bcl-xL in human hepatocellular carcinoma. Surgery, 2004,135(6):604-612

17 Karczmarek-Borowska B, Filip A, Wojcierowski J, *et al*. Estimation of prognostic value of Bcl-xL gene expression in non-small cell lung cancer. Lung Cancer, 2006,51(1):61-69

18 Espana L, Fernandez Y, Rubio N, *et al*. Overexpression of Bcl-xL in human breast cancer cells enhances organ-selective lymph node metastasis. Breast Cancer Res Treat, 2004,87(1):33-44

19 Zhang H, Xue J, Hessler P, *et al*. Genomic analysis and selective small molecule inhibition identifies BCL-X(L) as a critical survival factor in a subset of colorectal cancer. Mol Cancer, 2015,14:126

20 Smith LT, Mayerson J, Nowak NJ, *et al*. 20q11.1 amplification in giant-cell tumor of bone: Array CGH, FISH, and association with outcome. Genes Chromosomes Cancer, 2006,45(10):957-966

21 Masuda M, Sawa M, Yamada T. Therapeutic targets in the Wnt signaling pathway: Feasibility of targeting TNIK in colorectal cancer. Pharmacol Ther, 2015,156:1-9

22 Debouki-Joudi S, Trifa F, Khabir A, *et al*. CpG methylation of APC promoter 1A in sporadic and familial breast cancer patients. Cancer Biomark, 2017,18(2):133-141

23 Fang Z, Xiong Y, Li J, *et al*. Detection of APC gene deletions in colorectal malignancies using quantitative PCR in a Chinese population. Pathol Oncol Res, 2011,17(3): 657-661

24 Cao J, Ge MH, Ling ZQ. Fbxw7 Tumor Suppressor: A Vital Regulator Contributes to Human Tumorigenesis. Medicine (Baltimore), 2016,95(7):e2496

25 Wang X, Zhang J, Zhou L, *et al*. Fbxw7 regulates hepatocellular carcinoma migration and invasion via Notch1 signaling pathway. Int J Oncol, 2015,47(1):231-243

26 Yokobori T, Mimori K, Iwatsuki M, *et al*. Copy number loss of FBXW7 is related to gene expression and poor prognosis in esophageal squamous cell carcinoma. Int J Oncol, 2012,41(1):253-259

27 Sionov RV, Netzer E, Shaulian E. Differential regulation of FBXW7 isoforms by various stress stimuli. Cell Cycle, 2013,12(22):3547-3554

28 Davis RJ, Welcker M, Clurman BE. Tumor suppression by the Fbw7 ubiquitin ligase: mechanisms and opportunities. Cancer Cell, 2014,26(4):455-464

29 Akhoondi S, Lindstrom L, Widschwendter M, *et al*. Inactivation of FBXW7/hCDC4-beta expression by promoter hypermethylation is associated with favorable prognosis in primary breast cancer. Breast Cancer Res, 2010,12(6):R105

30 Gao Q, Zhang J, Liu J. Pathological analysis of colorectal cancer complicated with primary hypertension. Mod Pract Med (Chinese), 2013,29(8):1268-1270

31 Kim SA, Moon H, Roh JL, *et al*. Postdiagnostic use of beta-blockers and other antihypertensive drugs and the risk of recurrence and mortality in head and neck cancer patients: an observational study of 10,414 person-years of follow-up. Clin Transl Oncol, 2017,19(7):826-833

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