Hepatocellular Carcinoma Column: Editorial

Amplification of *MPZL1/PZR* gene in hepatocellular carcinoma

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Abstract: Hepatocellular carcinoma (HCC) is the fifth leading cause of cancer mortality worldwide. It is noted that metastasis is a fundamental biological behavior of HCC and the main cause of treatment failure. The identification of somatic alterations and their specific inhibitors may contribute to reduce side effects and prolong patient survival in HCC. Chromosomal copy number alterations (CNAs) are important subclasses of somatic mutations and can be used as an effective method of identifying driver genes with causal roles in carcinogenesis. Jia *et al*. identified a novel recurrent focal amplicon, 1q24.1-24.2, targets the *MPZL1* gene in HCC. They also found that MPZL1 may recruit the SHP-2 and subsequently activate/phosphorylate Src kinase at Tyr426, promoting phosphorylation of cortactin and migration of HCC cells. It is noted that phosphorylation of Tyr416 in the activation loop of the kinase domain up-regulates enzyme activity of Src. In addition, the active state of c-Src, p-Tyr416-c-Src, is an independent prognostic marker of poor patient survival in HCC. Therefore, c-Src signaling may be a druggable target and c-Src targeted therapy may improve patient outcome in this specific subtype of HCC patient with a gain of the recurrent focal amplicon, 1q24.1-24.2.

Keywords: Hepatocellular carcinoma (HCC); chromosomal copy number alterations (chromosomal CNAs) ; c-Src

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Hepatocellular carcinoma (HCC) is the fifth leading cause of cancer mortality, causing an estimated half a million deaths annually. Surgical resection is the most successful treatment for early stage HCC. However, fewer than 30% of HCC patients are qualified for curative resection. Metastasis is a fundamental biological behavior of HCC and the main cause of treatment failure. In a clinical setting, the most common site of distant spread is the lung, owing to dissemination of tumor cells via the bloodstream, hemodynamic features of the liver and the intrinsic biological characteristics of the tumor, such as increased proliferation, invasion and motility (1). Recent successes in cancer targeted therapy arising from the identification of somatic alterations and their specific inhibitors are associated with reduced side effects and prolonged patient survival. Although no specific drug target has been identified for HCC, FDA approved the multi-kinase inhibitor sorafenib for treatment of advanced HCC, due to a favorable overall patient survival. Nevertheless, HCC patients receiving sorafenib showed marginal benefits, with a prolonged survival of 3-4 months on average (2). With limited improvement of HCC patient survival, identification of recurrent and altered somatic genes through integrated genomic approaches is vital to better understand HCC molecular tumorigenesis, to develop early diagnostic markers and methods, and to find additional druggable targets for the improvement of HCC management.

Chromosomal copy number alterations (CNAs) are important subclasses of somatic mutations, with aberrant chromosomal regions of amplification or deletion commonly associated with overexpressed oncogenes or loss of tumor suppressor genes (3). Although certain regions of recurrent CNAs harbor a single gene, most of these regions include several genes. It is generally thought that increased CNAs are closely correlated with expression levels of target genes in both transcripts and proteins. Nevertheless, researchers usually cannot find direct or significant correlation of CANs with the expression levels of target genes, as shown

in this study (4). There are many other uncertain situations involved. Epigenetic events such as methylation of promoter illustrated by them (4) or micro-RNAs (miRNAs) may be involved. Copy number gain at 1q is one of the most frequently detected alterations in HCC (58-78%) and has been suggested to be an early genomic event in the process of HCC development (5). Jia *et al*. further identified a novel recurrent focal amplicon, 1q24.1-24.2, targets the *MPZL1* gene in HCC (4). They also found a positive correlation between the expression levels of *MPZL1* transcripts and intrahepatic metastasis of the HCC specimens, critically suggesting MPZL1 might serve as pro-metastatic gene in HCC. Rather, it is difficult to see if the direct correlation of CNAs of this gene with proteins, transcripts, and most importantly, with intrahepatic metastasis of the matched HCC specimens. Although they provided *in vitro* evidence that MPZL1 could significantly enhance the migratory and metastatic potential of the HCC cells, they did not present the correlation of amplicon, 1q24.1-24.2, with the protein and mRNA level of *MPZL1* gene in six HCC cell lines. Notably, the protein level of *MPZL1* was relatively low in HepG2, Hep3B, HUH-7 and SMMC-7721 cells, which have no or low metastatic potential, whereas the protein level of the *MPZL1* gene was relatively high in SK-HEP-1 and Li-7 cells, which have high metastatic potential.

C-Src, a cellular homologue of the Rous sarcoma virus-transforming gene (*v-Src*), has been clearly demonstrated in contributing to malignant transformation. This non-receptor type of tyrosine kinase is expressed ubiquitously in a variety of tissues and, when activated, plays roles in a variety of signaling networks that regulate angiogenesis, proliferation and invasion. C-Src activity is regulated by tyrosine phosphorylation at two distinct sites, but their phosphorylation has opposite effects. Phosphorylation of Tyr416 in the activation loop of the kinase domain up-regulates enzyme activity, whereas phosphorylation of Tyr527 in the carboxyl terminal tail renders the enzyme less active (6). In general, an intermolecular auto-phosphorylation mediated by another Src kinase molecule at Tyr416 promotes kinase activity. The catalytic activity of c-Src is suppressed by phosphorylation on the tyrosine residue Tyr527, which is catalyzed by c-terminal Src Kinase (Csk). Down-regulation of Src kinase activity by adenovirus-mediated *Csk* gene transfer abrogates the highly metastatic phenotype of colon cancer cells (7), implicating an important role of c-Src in metastatic potential of human cancers. Notably, under basal conditions *in vivo*, 90-95% of Src is phosphorylated at Tyr527 (8). In addition, the Tyr527Phe Src mutant is more active than the wildtype and can induce anchorage-independent growth *in vitro* and *in vivo* (9,10). Of note, although higher c-Src kinase activity was detected by an *in vitro* kinase assay in HCC (11) and c-Src activation has been shown to be involved in the cancerous behaviors of HCC cells (12,13), positive staining for the activated c-Src is more frequently observed in well or moderately differentiated carcinoma (14). These studies implicated a more complicated nature or network of c-Src signaling in clinic. Our previous study (15) showed that the active state of c-Src, p-Tyr416-c-Src, was not significantly correlated with any characteristic in HCC. The expression of p-Tyr416-c-Src was tended to positively correlate with tumor grade (P=0.062) but inversely correlate with vascular invasion (P=0.071). The inactive state of c-Src, p-Tyr527-c-Src, was significantly decreased in male patients but increased HCV-infected patients. The Kaplan-Meier survival curve further showed that increased p-Tyr416-c-Src was significantly associated with poor patient survival. A multivariable COX regression model showed that p-Tyr416-c-Src expression was an effective predictor for patient survival in HCC. Our previous results suggest that the active state of c-Src, p-Tyr416-c-Src, may serve as an independent prognostic marker of patient survival in HCC. Relative levels of other phosphorylated or non-phosphorylated c-Src kinases may also present different statuses during HCC development. In addition, the impacts of c-Src on HCC may be a late event in contributing to advanced development or attenuating therapeutic effectiveness.

The authors found that Src kinase mediates the phosphorylation and activation of cortactin induced by *MPZL1* overexpression. Notably, MPZL1 was previously identified as a SHP-2-binding partner in epithelial cells, and the intracellular portion of MPZL1 has two immunoreceptor tyrosine-based inhibition motifs that specifically interact with SHP-2, an SH2 domain-containing tyrosine phosphatase with a crucial role in cell signaling (16,17). MPZL1 serves not only as a specific anchor protein of SHP-2 on the plasma membrane but also as a physiological substrate of the enzyme. It has been also reported that the SHP-2 tyrosine phosphatase activates the Src tyrosine kinase via a non-enzymatic mechanism (18). This result suggested that the activation of Src kinase by overexpression of *MPZL1* is probably associated with the recruitment and activation of SHP-2. Furthermore, the expression of p-Tyr416-c-Src was significantly increased upon *MPZL1* overexpression, while the expression of

p-Tyr527-c-Src was significantly decreased in *MPZL1*-overexpressing cells. They also found that siRNA-mediated knockdown of the *SHP-2* gene significantly attenuated the increased phosphorylation of the active form of p-Tyr416-c-Src and p-Tyr421-cortactin caused by overexpression of *MPZL1* in HUH-7 cells.

Cortactin was originally identified as a substrate for the Src family kinases, which are major kinases in the tyrosine phosphorylation of cortactin. It has been demonstrated that MPZL1 is able to activate Src kinase upon stimulation of cells with extracellular stimuli (19). Therefore, the authors propose that a novel MPZL1/Src/cortactin signaling cascade may exist and functions in the process of HCC cell migration. Rather, with clinical specimens, they seemed not to successfully find the direct correlation among the protein levels of *MPZL1* and the phosphorylation levels of cortactin and Src.

Taken together, the study (4) suggests that *MPZL1* is a novel pro-metastatic gene targeted by a recurrent region of copy number amplification at 1q24.1-24.2 in HCC. Activation of c-Src related signaling such as phosphorylation of cortactin may imply the pro-metastatic role of MPZL1 in HCC. HCC remains a highly lethal cancer due to the lack of biomarkers for early diagnosis, molecular classification and efficient therapeutic interventions. Efforts to develop specific inhibitors for these aberrant pathways and reveal better therapeutic targets in HCC are urgently needed. CNAs can be one of effective methods of identifying driver genes with causal roles in carcinogenesis is the detection of genomic regions that undergo frequent alterations in cancers. This approach may provide information allowing discovery of druggable targets and further development of corresponsive therapies in HCC. In this study, c-Src is appeared to be a druggable target in this subtype of HCC with a recurrent region of copy number amplification at 1q24.1-24.2. A recent study suggest that cell line models maintain the molecular background of HCC and that subtype may be important for selecting patients for response to novel therapies (20). In addition, it highlights a potential role for Src family signaling in this progenitor subtype of HCC. Sensitivity to dasatinib, a Src/Abl inhibitor, was associated with a progenitor subtype. Dasatinib was effective at inducing cell cycle arrest and apoptosis in "progenitor-like" cell lines but not in resistant lines. Although the study provides only *in vitro* data, it emphasizes that c-Src signaling can be a druggable target and c-Src targeted therapy may improve patient outcome in a specific subtype of HCC.

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