LETTER TO THE EDITOR

A CRISPR screen identifies CDK7 as a therapeutic target in hepatocellular carcinoma

Cell Research (2018) 28:690–692; <https://doi.org/10.1038/s41422-018-0020-z>

Dear Editor,

Liver cancer is one of the most frequent malignancies and the second leading cause of cancer-related mortality worldwide.^{1,2} In the last decade, our understanding of the genetic landscape of hepatocellular carcinoma (HCC) has improved significantly through whole-exome sequencing studies. However, unlike *EGFR* mutation in lung cancer and *BRAF* mutation in melanoma, the most frequent mutations in HCC are currently undruggable. Sorafenib, a small-molecule RAF kinase and VEGF receptor kinase inhibitor, was approved for the treatment of advanced HCC patients. However, it only provides limited survival benefit for HCC patients: 2.8 months overall survival benefit in the SHARP trial and 2.3 months overall survival benefit for Asia-Pacific patients.^{3,4} It is therefore urgent to identify new therapeutic approaches for HCC.

CRISPR/Cas9-based functional genetic screens represent a powerful tool to identify cancer-relevant genes.^{5,6} To systematically probe kinase-driven pathways required for cell viability in HCC, we screened a gRNA library representing the full complement of human kinases in two HCC cell lines (Supplementary information, Table S1). Hep3B and Huh7 cells were infected with the lentiviral kinome gRNA collection and cultured for 14 days. Changes in library representation were determined after 14 days of culture by next-generation sequencing of the gRNA inserts in three biological replicates (Fig. 1a and Supplementary information, Figure S1a). To identify essential genes in HCC, we focused follow-up experiments on the significantly depleted gRNAs identified in both cell lines. We identified a set of cyclin-dependent kinases (CDKs), including CDK7, CDK9, CDK12 and CDK13 as hits in our screen (Fig. 1b, Supplementary information, Figure S1b and Table S2). These CDKs play important roles in transcription initiation and elongation.⁷ We evaluated the expression levels of these CDKs in the GSE14520 cohort ($n = 213$) and the TCGA database ($n = 50$), which provide data on gene expression for both paired non-tumor and HCC tissues. Of the identified hits, only CDK7 expression was selectively upregulated in tumor tissue in both cohorts (paired t -test, $P < 0.001$, Fig. 1c and Supplementary information, Figure S1c). Next, we analyzed CDK7 expression using a tissue microarray (TMA) containing 80 HCC specimens by immunohistochemistry analysis. Kaplan–Meier analysis indicated that HCC patients with high expression of CDK7 exhibited worse overall survival as compared to patients with low expression of CDK7 ($n = 80$, $P = 0.048$, log-rank test, Fig. 1d). We therefore selected CDK7 for more detailed analysis. We infected Hep3B and Huh7 cells with two CDK7 shRNAs, both of which reduced CDK7 levels (Fig. 1e). As expected, inhibition of CDK7 impaired proliferation of Hep3B and Huh7 cells in both short-term IncuCyte® cell proliferation assays and long-term colony formation assays (Fig. 1f, g). We also analyzed the growth inhibitory effects of shCDK7 in five additional cell lines (SNU398, JHH1, SNU449, PLC/PRF/5 and MHCC97H). We found that SNU398 and JHH1 are also

very sensitive to CDK7 knockdown, whereas SNU449, PLC/PRF/5 and MHCC97H cells are resistant to CDK7 knockdown (Supplementary information, Figure S2a, b).

Next, we focused our attention on THZ1, a covalent CDK7 inhibitor.⁸ Recent data indicate that THZ1 has a therapeutic effect in several types of cancer, including T cell acute lymphoblastic leukemia, *MYCN*-amplified neuroblastoma, small cell lung cancer and triple-negative breast cancer.^{8–11} To test the sensitivity of HCC cells to THZ1, we treated the panel of 10 HCC cell lines with increasing concentrations of THZ1 for about 2 weeks in colony formation assays. As shown in Fig. 1h, the panel of HCC cell lines exhibited differential responses to CDK7 inhibition. Comparable results were observed in IncuCyte® short-term cell proliferation assays (Supplementary information, Figure S3a, b). CDK7 inhibition induced apoptosis in the sensitive cells (Hep3B, Huh7, HepG2, JHH1 and SNU398), as indicated by the IncuCyte® caspase-3/7 apoptosis assay, but not in the insensitive cell lines (SNU387, SNU449, SNU475, PLC/PRF/5 and MHCC97H) (Fig. 1i). THZ1 treatment also failed to induce apoptosis in non-transformed human BJ fibroblasts and RPE-1 cell lines (Supplementary information, Figure S3c). Together, our data indicate that there may be a subgroup of liver cancer cells with a profound dependence on CDK7 for survival and CDK7 may represent a novel therapeutic target in this subgroup.

CDK7 regulates transcriptional processing through phosphorylating RNAPII C-terminal domain (RNAPII CTD).¹² We observed that RNAPII CTD phosphorylation was equally suppressed by THZ1 in both sensitive and insensitive cell lines (Supplementary information, Figure S4a, b). These results indicate that there is no correlation between cell fate induced by CDK7 inhibitor and the activity of CDK7 on direct downstream targets. Recent evidence indicates that the MYC transcriptional network has an important role in the response to CDK7 inhibition in *MYCN*-amplified neuroblastoma and small cell lung cancer.^{8,9} In our HCC cell line panel, we also observed that the presence of high level of MYC protein was associated with high sensitivity to CDK7 inhibition (Fig. 1j, k). Nuclear MYC protein levels showed a similar correlation in the panel of liver cancer cell lines, with the exception of HepG2 cells (Supplementary information, Figure S4c). THZ1 efficiently suppressed the high expression of MYC in the sensitive cells (Hep3B and Huh7), but at best modest changes in MYC expression were observed in the insensitive cell lines having lower intrinsic MYC levels (PLC/PRF/5 and MHCC97H) (Supplementary information, Figure S4d). Interestingly, HCC cell lines also showed a similar response to MYC knockdown as was seen with CDK7 inhibition (Fig. 1l). Ectopic expression of MYC in PLC/PRF/5 cells led to a greater sensitivity to THZ1 as compared to vector control-transfected cells (Supplementary information, Figure S4e). These data suggest that MYC level may serve as a useful biomarker to predict the response to CDK7 inhibition.

Received: 9 October 2017 Revised: 17 January 2018 Accepted: 6 February 2018
Published online: 5 March 2018

Fig. 1 CDK7 is a potential therapeutic target for liver cancer. **a** Schematic outline of the stress lethality screen. Pooled kinome CRISPR screens were performed in Hep3B and Huh7 cells. **b** A set of CDKs was identified as stress lethal hits in both Hep3B and Huh7 cells. **c** Gene expression levels of *CDK7* in the cohort of GSE14520 ($n = 213$) and TCGA database ($n = 50$). **d** Association of *CDK7* expression with prognosis in HCC patients ($n = 80$). **e** The level of *CDK7* knockdown in HCC cell lines was measured by western blot. **f, g** Viability was assessed by IncuCyte assay and colony formation assay. **h** Liver cancer cell lines were treated with increasing concentrations of THZ1 for about 2 weeks. Viability was assessed by colony formation assay. **i** Representative images of control and THZ1-treated cells in the presence of a caspase-3/7 activatable dye. **j** Immunoblotting analysis of MYC level in a panel of HCC cell lines. **k** Short-term growth inhibition assay of THZ1 in the panel of HCC cell lines. **l** Knockdown of MYC impairs cell proliferation in MYC high-expressed cells. **m** THZ1 can selectively suppress tumor growth in MYC high-expressed xenograft models (10 mg/kg intraperitoneal, twice daily). Mean tumor volumes $\pm$ SEM are shown ($n = 5-7$ mice per group). * $P < .05$, *** $P < .001$.

To assess whether the in vitro findings can be recapitulated in vivo, Huh7 and SNU398 (high MYC expression), and MHCC97H (low MYC expression) cells were injected into nude mice. Upon tumor establishment, xenografts were treated with either vehicle or THZ1 for 14–21 days. THZ1 treatment significantly impaired tumor growth of MYC high HCC xenografts (Huh7 and SNU398), whereas

no anti-tumor effect was observed in MYC low (MHCC97H) xenografts (Fig. 1m). Moreover, biochemical analysis indicated that THZ1 suppressed the expression of MYC in the tumors from Huh7 and SNU398 xenografts. However, the MYC expression is very low in tumors from MHCC97H xenografts and no change was observed upon THZ1 treatment (Supplementary information, Figure S4f).

MYC deregulation is a common event in liver cancer. We analyzed the correlation between CDK7 and MYC mRNA levels in two clinical HCC cohorts (GSE14520 $n = 225$ and TCGA database $n = 371$). As shown in Supplementary information, Figure S5, we observed a significant correlation between CDK7 and MYC mRNA expression in both GSE14520 ($R = 0.274$, $P < 0.001$) and TCGA database ($R = 0.348$, $P < 0.001$). Two previous studies have shown that MYC proteins are potential biomarkers of response to CDK7 inhibitors in small cell lung cancer and neuroblastoma.^{8,9} Our work now identifies CDK7 inhibition as vulnerability of HCC tumors having high MYC expression. The recent start of a clinical study with a CDK7 inhibitor (NCT03134638) may enable clinical validation of this notion in the near future.

ACKNOWLEDGEMENTS

This work was supported by grants from the Cancer Genomic Center Netherlands, the Dutch Cancer Society (KWF), the National Key Basic Research Program of China (973 Program: 2015CB553905), the National Natural Science Foundation of China (81672933, 81421001), Shanghai Jiao Tong University School of Medicine (YG2014MS44 and PYXJS16-004), and Shanghai Municipal Commission of Health and Family Planning (2017YQ064 and 201640007).

ADDITIONAL INFORMATION

Supplementary information accompanies this paper at <https://doi.org/10.1038/s41422-018-0020-z>.

Competing interests: The authors declare no competing interests.

Cun Wang^{1,2}, Haojie Jin², Dongmei Gao³, Liqin Wang¹, Bastiaan Evers¹, Zheng Xue¹, Guangzhi Jin⁴, Cor Liefink¹, Roderick L. Beijersbergen¹, Wenxin Qin² and René Bernards¹
¹Division of Molecular Carcinogenesis, Oncode Institute, Cancer Genomics Centre Netherlands, The Netherlands Cancer Institute, Plesmanlaan 121, 1066 CX Amsterdam, The Netherlands; ²State Key Laboratory of Oncogenes and Related Genes, Shanghai Cancer Institute, Renji Hospital, Shanghai Jiao Tong University School of

Medicine, Shanghai 200032, China; ³Liver Cancer Institute, Zhongshan Hospital, Fudan University, Key Laboratory of Carcinogenesis and Cancer Invasion, Ministry of Education, Shanghai 200032, China and ⁴Department of Pathology, Eastern Hepatobiliary Surgery Hospital, Second Military Medical University, Shanghai 200438, China

These authors contributed equally: Cun Wang, Haojie Jin, and Dongmei Gao.

Correspondence: Wenxin Qin (wxqin@sjtu.edu.cn) or René Bernards (r.bernards@nki.nl)

REFERENCES

- Llovet, J. M. et al. Hepatocellular carcinoma. *Nat. Rev. Dis. Primers* **2**, 16018, (2016).
- Zucman-Rossi, J., Villanueva, A., Nault, J. C. & Llovet, J. M. Genetic landscape and biomarkers of hepatocellular carcinoma. *Gastroenterology* **149**, 1226–1239, (2015).
- Llovet, J. M. et al. Sorafenib in advanced hepatocellular carcinoma. *N. Engl. J. Med.* **359**, 378–390, (2008).
- Cheng, A. L. et al. Efficacy and safety of sorafenib in patients in the Asia-Pacific region with advanced hepatocellular carcinoma: a phase III randomised, double-blind, placebo-controlled trial. *Lancet Oncol.* **10**, 25–34, (2009).
- Shalem, O. et al. Genome-scale CRISPR-Cas9 knockout screening in human cells. *Science* **343**, 84–87, (2014).
- Evers, B. et al. CRISPR knockout screening outperforms shRNA and CRISPRi in identifying essential genes. *Nat. Biotechnol.* **34**, 631–633, (2016).
- Zhou, Q., Li, T. & Price, D. H. RNA polymerase II elongation control. *Annu. Rev. Biochem.* **81**, 119–143, (2012).
- Chipumuro, E. et al. CDK7 inhibition suppresses super-enhancer-linked oncogenic transcription in MYCN-driven cancer. *Cell* **159**, 1126–1139, (2014).
- Christensen, C. L. et al. Targeting transcriptional additions in small cell lung cancer with a covalent CDK7 inhibitor. *Cancer Cell* **26**, 909–922, (2014).
- Kwiatkowski, N. et al. Targeting transcription regulation in cancer with a covalent CDK7 inhibitor. *Nature* **511**, 616–620, (2014).
- Wang, Y. et al. CDK7-dependent transcriptional addiction in triple-negative breast cancer. *Cell* **163**, 174–186, (2015).
- Glover-Cutter, K. et al. TFIIH-associated Cdk7 kinase functions in phosphorylation of C-terminal domain Ser7 residues, promoter-proximal pausing, and termination by RNA polymerase II. *Mol. Cell Biol.* **29**, 5455–5464, (2009).