doi:10.1093/jmcb/mjy086
Published online January 4, 2019

Review

It takes a team: a gain-of-function story of p53-R249S

Huai Wang[1,2,3], Peng Liao[1], Shelya X. Zeng[1], and Hua Lu[1,*]

[1] Department of Biochemistry and Molecular Biology, Tulane Cancer Center, Tulane University School of Medicine, New Orleans, LA 70112, USA
[2] School of Public Health, Nanchang University, Nanchang 330006, China
[3] Jiangxi Provincial Key Laboratory of Preventive Medicine, Nanchang University, Nanchang 330006, China
* Correspondence to: Hua Lu, E-mail: hlu2@tulane.edu

Edited by Chandra S. Verma

Gain-of-function (GOF), the most malicious oncogenic activity of a cancer-promoting protein, is well illustrated to three hotspot p53 mutations at R248, R175, and R273 with distinct molecular mechanisms. Yet, less is known about another hotspot p53 mutant, R249S (p53-R249S). p53-R249S is the sole hotspot mutation in hepatocellular carcinoma (HCC) that is highly associated with chronic hepatitis B virus (HBV) infection and dietary exposure to aflatoxin B1 (AFB1). Its GOF is suggested by the facts that this mutant is associated with earlier onset of HCC and poorer prognosis of cancer patients and that its overexpression drives HCC proliferation and tumorigenesis. By contrast, simply knocking in this mutant in normal mice did not show apparent GOF activity. Hence, the GOF activity for p53-R249S and its underlying mechanisms have been elusive until recent findings offered some new insights. This review will discuss these findings as well as their clinical significance and implications for the development of a strategy to target multiple molecules as a therapy for p53-R249S-harboring HCC.

Keywords: p53-R249S, Liver cancer, HCC, CDK4/cyclin D1, PIN1, c-Myc, FBW7a

Introduction of hepatocellular carcinoma

Liver cancer is the second most lethal cancer worldwide with ~700000 annual deaths globally in recent years (The Cancer Genome Atlas Research Network, 2017). This accounts for nearly 70% of cancer deaths in developing countries: western and central Africa, east and south-east Asia (Ferlay et al., 2015). As the most common primary malignancy of the liver, hepatocellular carcinoma (HCC) is the fifth most common cancer worldwide in men, and seventh among women (Mittal and El-Serag, 2013). Because of the lack of symptoms in the early stages and the rapid growth rate of tumors, most cases of HCC are diagnosed at an advanced stage. The 5-year survival rate for HCC individuals is <5% (Mikhail et al., 2014).

There are a number of etiological risk factors for liver cancer, including Hepatitis B virus (HBV), hepatitis C virus (HCV), and hepatitis D virus (HDV) (Ghouri et al., 2017). Also, AFB1 exposure has been shown to be highly related to the increasing incidences of liver cancer (Kew, 2013). Chronic HBV infection combined with dietary exposure toxin in the high-risk regions, such as central Africa and southeast Asia, is the main reason for the geographic distribution of much higher incidences of HCC. Interestingly, these two risk factors (high AFB1 exposure and/or HBV infection) are also highly associated with the high incidence of a missense mutation of TP53 in HCC patients (Gouas et al., 2012; Weng et al., 2017). This mutation is a single base substitution at the third base of codon 249 (AGG to AGT), resulting in the amino acid substitution of serine for arginine (p53-R249S). More interestingly, p53-R249S is the main hotspot mutant identified in overall 30% of HCCs that contain p53 mutations, and in >96% of HCCs in the high-risk regions (Hsu et al., 1991; Staib et al., 2003; Hussain et al., 2007; Qi et al., 2015). These epidemiological results suggest that p53-R249S must play an important role in the development, progression and metastasis of HCCs with positive HBV infection and AFB1 exposure, which will be further described below.

Can p53-R249S be a biomarker for HCC?

Although HCC surveillance programs have been implemented in specific high-risk populations, early diagnosis of HCC is still a

Received July 8, 2018. Revised November 3, 2018. Accepted January 3, 2019.

challenging task due to the low sensitivity of current screening methods (Bruix and Sherman, 2011). For instance, serum α-fetoprotein (AFP) is the most widely used biomarker in the screening of liver cancer, but its sensitivity is 40%–60% for all stages with the sensitivity for early liver cancer being 40%–50% (Trevisani et al., 2001; Moriya et al., 2013). Therefore, there is an urgent need for more sensitive biomarkers specific for the early diagnosis of HCC.

Like other cancers, HCC also harbors some drastic alterations in the human genome (Jones and Baylin, 2002; Vogelstein and Kinzler, 2004; Ozen et al., 2013). The identification of DNA modifications in HCC, including mutation and methylation, could be utilized for diagnosis of liver cancers. As mentioned above, one of the highly representative alterations in HCC is the mutation of the tumor suppressor gene TP53 at codon 249 (Gouas et al., 2009). In the high incidence areas of HBV infection and AFB1 exposure, the TP53 mutation occurred in >50% of the cases, among whom there is a high proportion of HCC patients with p53-R249S (Szymańska and Hainaut, 2003). This mutation accounts for 26% of the TP53 mutations described in liver cancer, but is surprisingly rare (<2%) in other types of human cancers (Szymańska et al., 2004).

Epidemiological studies have shown that p53-R249S is by far the predominant mutation in the areas of high HCC incidence, such as Egypt, Brazilian, Gambia, Mexico, Nigeria, Senegal, Turkey, Thailand, Southern India, as well as Guangxi and Qidong from China (Coursaget et al., 1993; Soini et al., 1996; Kirk et al., 2000; Stern et al., 2001; Huang et al., 2003; Kuang et al., 2005; Hosny et al., 2008; Igetei et al., 2008; Nogueira et al., 2009; Ozdemir et al., 2010; Pandima Devi et al., 2010). It has been shown that aflatoxin metabolites can induce this mutation *in vitro* and *in vivo* (Hainaut and Vahakangas, 1997; Mace et al., 1997). It has also been suggested that p53-R249S might promote carcinogenesis in liver (Szymańska and Hainaut, 2003), but not in other tissues. This raises the possibility of using p53-R249S as a biomarker for diagnosis of HCC in the above high-risk areas. Earlier studies using patients' cancer tissues and blood samples showed that free plasma DNA often contains mutated DNA originated from the tumor lesions (Anker et al., 1999). Thus, it has been attempted to examine plasma DNA for p53-R249S mutation from HCC patients. In the Gambian study, the incidence of p53-R249S mutation in plasma DNA increased with the severity of liver diseases, and this supports the idea that p53-R249S is selected during hepatocarcinogenesis associated with AFB1 exposure (Szymańska et al., 2004). This study also showed that the p53-R249S mutation could be detected in the plasma DNA of 38% of the patients with liver cancer (Szymańska et al., 2004). Another study detected plasma R249S-mutated DNA prior to HCC diagnosis in a small number of HCC patients from Qidong, a high-incidence area in China (Jackson et al., 2003). In addition, quantitative studies demonstrated a strong correlation between the high copy number of R249S mutation in plasma and HCC in Gambian patients, as there were >2500 copies of R249S/ml in plasma for ~50% of these HCC patients (Lleonart et al., 2005). It was also found that HCC is closely correlated with the R249S-containing plasma DNA level with >10000 copies/ml (Lleonart et al., 2005). Taken together, these studies suggest that p53-R249S mutation detected in plasma DNA could serve as one biomarker for HBV- and AFB1-associated HCC that harbor this p53 mutant.

Why is p53-R249S solely selected in HCC?

It has been puzzling why HBV infection and AFB1 exposure specially select the p53-R249S mutation. One possibility would be that AFB1 might selectively target the codon 249 of TP53. AFB1 is a toxin produced by fungi called Aspergillus flavus that grows on improperly stored grain, and metabolized by cytochrome P450 in the liver. It generally causes DNA damage by forming covalent and promutagenic DNA adducts. Specifically, AFB1 metabolites could cause the G→T transversion at the codon 249 of TP53, leading to transfer from AGG to AGT in 50% (Africa) to 90% (Qidong of China) of HCC patients in highly exposed AFB1 region (Bressac et al., 1991; Hsu et al., 1991; Aguilar et al., 1993; Shen and Ong, 1996), which was not detected in other hotspot mutation regions of TP53. Supporting this is an *in vitro* study showing that the context of AGG sequence is a favorable adductive site for AFB1 metabolites (Besaratinia et al., 2009). Hence, this specificity suggests that p53-R249S might have a selective advantage in the development of liver cancer. Then, how is this mutation highly associated with HBV infection in HCC?

It is possible that p53-R249S might also be picked up via a biological selection in HBV-associated HCC. Previous studies suggested that HBV-encoded x protein (HBx) might promote HCC proliferation and growth by interplaying with p53-R249S (Gouas et al., 2010; Kew, 2011), as silencing either p53-R249S or HBx in HCC cells that harbor both of the proteins retarded cell proliferation and growth. Also, HBx could form a complex with p53-R249S. These results suggest that HBx might execute its oncogenic function by specifically binding to this p53 mutant. However, HBx can also promote cell cycle progression and HCC growth by suppressing and interacting with other negative growth regulators, such as wild-type p53. Thus, the specificity for selecting p53-R249S mutation in HBV-infected HCC could not be interpreted by the interaction of HBx with this p53 mutant.

Another explanation is that HBV infection might endorse the formation of AFB1-induced 249 codon mutation. Chronic inflammation caused by viral replication and infection, such as HBV infection, can lead to oxidative stress in hepatocytes. This might be related to the mutation load of TP53's codon 249 in hepatocytes (Hussain et al., 1994). Also, since the expression of CYP 450 enzyme that metabolizes and activates AFB1 is elevated in HBV surface antigen (HBsAg) transgenic mice (Kirby et al., 1994), the mutagenic ability of this chemical carcinogen would be more drastically potentiated by HBV infection. Consistent with this conjecture is that the concentration of AFB1 adducts in children and adolescents infected with chronic HBV in Gambia is greater than that of those without this infection (Kew, 2003). Moreover, HBV may boost the mutation rate through indirect mechanisms, such as by inducing chronic inflammation, thereby

increasing the transformation rate of hepatocytes and the risk of obtaining mutations, such as TP53-R249S (Gouas et al., 2009). Although all of these possibilities discussed above seem reasonable, the evidence supporting these speculations is indirect, descriptive, and less convincing. Thus, it still remains mysterious about why HBV infection and AFB1 exposure solely select p53-R249S, rather than other hotspot mutations of p53, in HCC.

Does p53-R249S possess gain-of-function activity in HCC?

Similar to other hotspot p53 mutants, p53-R249S displays both loss of function (LOF) and dominant negative (DN) effects crucial for HCC cell proliferation (Goh et al., 2011; Lee et al., 2012). However, unlike R175, R273 and R248 that clearly display their gain-of-function (GOF) in each of their knockin mouse models (Sigal and Rotter, 2000; Hanel et al., 2013; Muller and Vousden, 2013), genetic knockin of mouse R246S (equivalent to human R249S) only showed LOF and DN effects on tumor development in mice (Lee and Sabapathy, 2008; Lee et al., 2012). In the R246S knockin study, $TP53^{R246S/wt}$, $TP53^{-/-}$, and $TP53^{R249S/R249S}$ mice showed similar survival rates, and homozygous mutations of p53 at this amino acid, unlike R172H, did not promote further tumorigenesis (Lee et al., 2012). Also, expressing different levels of R246S in the presence of wild-type p53 in mice displayed marked DN effect on acute p53 activation and radiosensitivity in cell-specific and dose-dependent manners (Lee et al., 2012). Clearly, p53-R249S did not show GOF in its knockin mice, although previous studies suggested that p53-R249S might possess a GOF activity in human HCC cells (Lee et al., 2000; Gouas et al., 2010). Perhaps, p53-R249S might exert its GOF activity in a context dependent manner (Lee et al., 2012), because the increase of mutant p53 level simply due to Mdm2's absence or DNA damage in tumors is insufficient to convey GOF activity to this mutant p53. Also, the failure of the R249S mutant to inhibit p73 seems to be one of the reasons for its lack of GOF. Finally, p53-R249S does not exhibit GOF properties in other cell types, both in the primary and transformed tissues, and even in the absence of Mdm2. Hence, it remains questionable if p53-R249S possesses a GOF activity key for proliferation, growth, and tumorigenesis of HCC cells. If so, what would be the underlying molecular mechanism, and why its GOF could not be found in p53-R249S knockin mice (Lee and Sabapathy, 2008; Lee et al., 2012)? To address these questions, recent studies offered some new insights as further described below.

SETDB1 methylates p53-R249S and enhances its GOF activity

Earlier on, p53-R249S was shown to be regulated by a methyltransferase called SETDB1 (SET domain bifurcated 1, SET domain in the C-terminal region) (Fei et al., 2015). SETDB1 was originally thought to be a protein containing a SET domain (Harte et al., 1999), and its gene is located on human chromosome 1q21.3 with 38.6Kb. It belongs to the family of SET-domain (Su(var)3–9, E(z), Trithorax) protein methyltransferase (Schultz et al., 2002). There are two main functional domains: The N-terminal Tudor domain involved in the interaction of proteins and the central MBD domain that mediates the binding of methyl with CpG. It has been shown that SETDB1 can mediate trimethylation of H3K9, leading to gene silencing or transcriptional suppression of some tumor suppressors, such as RASSF1A and p53-binding protein 2 (P53BP2), and thus to play an important role in promoting tumor initiation and progression (Wang et al., 2003; Lu et al., 2016). Although it has been shown that SETDB1 is a possible target for early treatment of Huntington's disease and associated with embryonic development (Matsui et al., 2010), it is also often up-regulated in human HCCs through multiple mechanisms, such as at chromosomal (amplification), transcriptional and post-transcriptional levels (Wong et al., 2016). Through a set of bioinformatics, cellular, molecular, and biochemical studies, it has been shown that SETDB1 can regulate p53-R249S stability as well (Fei et al., 2015).

Interestingly, the high copy number and RNA level of the SETDB1 gene were well correlated with the p53-R249S status in HCC tissues. The proportion of p53-R249S mutation increased significantly in HCC specimens that carried more SETDB1 copies or higher RNA levels as examined by exon sequencing, human SNP array 6.0, and RNA expression microarrays. Also, SETDB1 was required for the growth of HCC cells that harbor p53-R249S. R249S, but not wild-type, p53, determined the cell growth sensitivity to SETDB1 knockout, and also rendered HCC cells more dependent on SETDB1. Additionally, SETDB1 tended to form more complexes with p53-R249S than with wild-type p53 as determined by immunoprecipitation. Remarkably, SETDB1 could di-methylate p53-R249S at K370 and stabilize the mutant p53 perhaps by preventing its MDM2-mediated ubiquitination and degradation, whereas the attenuation of SETDB1 activity reduced the level of p53-R249S K370me2 and enhanced MDM2-facilitated proteasomal turnover of this mutant p53 (Fei et al., 2015). This study revealed SETDB1 as an upstream regulator of p53-R249S, whose activity for p53 methylation is likely required for the GOF activity of the mutant p53 in HCC (Figure 1). However, this study does not address how this mutant p53 executes its GOF activity in HCC.

p53-R249S acquires its GOF activity through a wide array of molecules

That issue has remained unaddressed until a more recent study (Liao et al., 2017) unveiled a unique signaling pathway involving multiple proteins for p53-R249S to execute its GOF activity in HCC cells. Remarkably, all of these proteins have been shown to be involved in tumorigenesis of various cancers either as an oncoprotein, such as CDK4/cyclin D1, Pin1 or c-Myc, or as a tumor suppressor, such as FBW7a (Wang et al., 2012).

CDK4 plays a key role in the G1/S phase of the cell cycle by forming a complex with cyclin D or A family (Johnson and Walker, 1999). The CDK4/cyclin D1 complex is often utilized by tumor cells for their survival and growth advantages, as it is highly expressed in various human cancers (Sherr, 1996; Malumbres and Barbacid, 2009). One of the important target substrates for this cell cycle-regulated kinase is Rb that is a

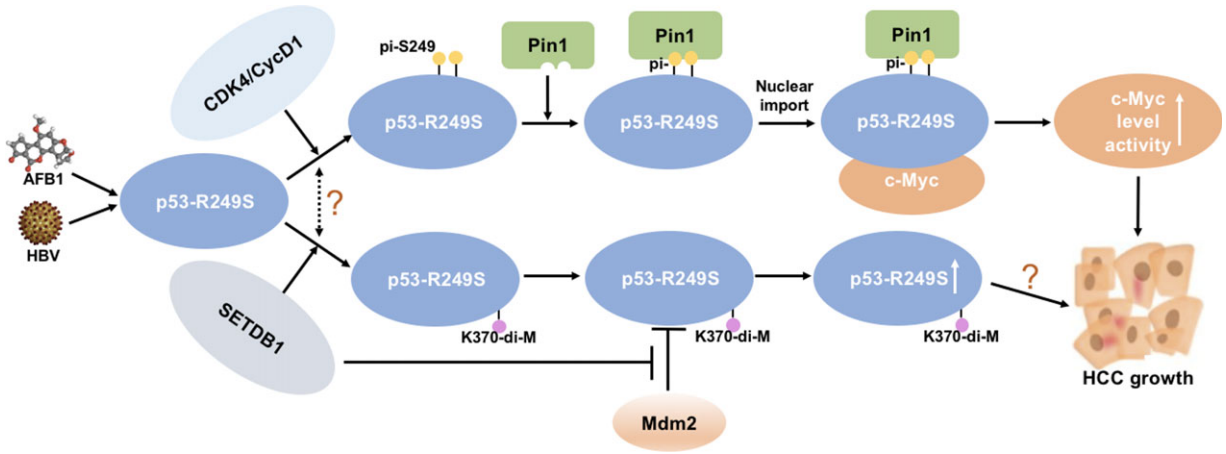

Figure 1. A schematic showing how p53-R249S may acquire its GOF activity in HCC. Arrows indicate activation, while bars indicate suppression.

tumor suppressor and can suppress the progression of the cell cycle by inhibiting the transcriptional activity of E2F or its analogs (Giacinti and Giordano, 2006). By phosphorylating Rb, CDK4/cyclin D1 inhibits its activity toward E2F, thus boosting the cell cycle progression and proliferation (Kitagawa et al., 1996). In addition, CDK4/cyclin D1 promotes the cell cycle progression and inhibits cell senescence and apoptosis by phosphorylating other proteins as well (Sheppard and McArthur, 2013). It is through the influence on the cell cycle progression CDK4/cyclin D1 can drive tumorigenic events, including hepatocellular carcinogenesis (Asghar et al., 2015). Interestingly, HBx has also been shown to increase CDK4 activity in the development of HBV-related liver cancer (Kremsdorf et al., 2006; Gearhart and Bouchard, 2010).

c-Myc is a nuclear transcription factor that is very important for the proliferation and renewal of stem cells as well as the survival of cancer (stem) cells (Bouchard et al., 1998; Takahashi and Yamanaka, 2006; Gordan et al., 2007; Kim et al., 2010). One key function of c-Myc is to activate the expression of genes involved in ribosomal biogenesis and protein synthesis (Grandori et al., 2005). This oncoprotein is highly expressed in nearly 80% of human cancers (Dang, 2012) via multiple mechanisms, including chromosomal translocation, rearrangement, gene amplification, and so on (Chen et al., 2014). It also plays a more global role in regulation of gene transcription crucial for cell proliferation and survival as well as tumorigenesis (Fernandez et al., 2003; Li et al., 2003; Lin et al., 2012). Hence, c-Myc is an important oncoprotein essential for the growth and progression of various types of cancer cells including HCC. This oncogenic activity of c-Myc is prevented by FBW7a, a tumor suppressor protein with an intrinsic E3 ubiquitin ligase activity, which can ubiquitinate the former and lead to its degradation (Yada et al., 2004).

Pin1, which is also highly expressed in human cancer, plays a carcinogenic role by converting inactive proteins into active oncoproteins, such as p53 mutants or c-Myc (Yeh and Means, 2007; Girardini et al., 2011; Farrell et al., 2013). Pin1 possesses a peptidyl-prolyl cis-trans isomerase activity (Lu et al., 1996), through which it can convert a target protein from its cis conformation to its trans conformation by binding to a phosphorylated Ser-Pro motif (Albert et al., 1999; Arevalo-Rodriguez et al., 2000; Hsu et al., 2001). It is through this activity Pin1 could modulate multiple cellular events, including nuclear import, gene regulation, RNA processing, cell proliferation, and differentiation, thus promoting cell proliferation, survival and tumorigenesis. Intriguingly, it has been shown that Pin1 also interplays with HBx and promotes the development of HBV-associated HCC (Datta et al., 2007).

Amazingly, the study by Liao et al. (2017) connected all of the aforementioned cancer-related proteins in one single pathway that leads to the 'materialization' of p53-R249S's GOF activity in HCC. Interestingly, mutation of Arg249 to Ser249 in p53 converted this cancer-derived serine residue to a substrate specific for CDK4/cyclin D1, but not for other cell cycle-regulated kinase complexes, during the G1 phase of the cell cycle of HCC cells. Once phosphorylated, p53-R249S became a preferred target with a phosphor-Ser249-Pro250 motif for Pin1, and the latter then bound to p53-R249S at this motif and facilitated its nuclear import. More interestingly, the nuclear-phosphorylated p53-R249S bound to FBW7a and c-Myc and prevented the interaction between the latter two. As a result, p53-R249S inhibited FBW7a ubiquitination of c-Myc, stabilized the latter, and activated its activity, consequently augmenting c-Myc-dependent cell proliferation and survival. Hence, these cellular and biochemical studies unravel that these proteins work in concert to form a unique signaling pathway that equips p53-R249S with a new GOF activity critical for HCC proliferation and growth (Figure 1).

Indeed, this signaling pathway has been confirmed in primary human HCC tissues (Liao et al., 2017). First, higher protein levels of p53-R249S were detected in all of the eight HCC tissues that were HBV positive. Correspondingly, c-Myc, CDK4, and Pin1 levels, as well as p53-R249S phosphorylation, were also relatively higher than that in HCC tissues without the p53-R249S mutation. Notably, the p53-R249S–CDK4–c-Myc complex was also detected in these p53-R249S-harboring HCC tissues. These results demonstrate a

novel and unique CDK4/cyclin D1–Pin1–p53-R249S–c-Myc pathway that is crucial for HCC proliferation, survival and carcinogenesis in the HBV-infected environment.

Ending remarks

Since the identification of p53-R249S as the sole hotspot mutation of TP53 in human HCCs that are highly associated with HBV infection and AFB1 exposure in 1991 (Bressac et al., 1991; Hsu et al., 1991), a great progress has been made to better understand why this mutant is specifically selected in this specific type of HCCs and how it executes its GOF activity in the HCC (Hussain et al., 1994; Kirby et al., 1994; Besaratinia et al., 2009; Gouas et al., 2009, 2010; Kew, 2011, 2013; Fei et al., 2015; Liao et al., 2017). Despite the fact that p53-R249S exhibits its GOF characteristics in a context dependent manner (Lee et al., 2012), from a series of epidemiological, genetic, biochemical, cellular and bioinformatics studies (Bressac et al., 1991; Hsu et al., 1991; Aguilar et al., 1993; Coursaget et al., 1993; Shen and Ong, 1996; Soini et al., 1996; Anker et al., 1999; Kirk et al., 2000; Stern et al., 2001; Huang et al., 2003; Jackson et al., 2003; Szymańska et al., 2004; Kuang et al., 2005; Lleonart et al., 2005; Hosny et al., 2008; Igetei et al., 2008; Nogueira et al., 2009; Ozdemir et al., 2010; Pandima Devi et al., 2010; Fei et al., 2015; Liao et al., 2017), we have now learned that p53-R249S indeed acquires its GOF activity via specific chemical targeting of R249S by AFB1, biological selection by HBV that creates a microenvironment or molecular environment and reprograms oncogenic signaling pathways leading to unusual posttranslational modifications, such as methylation at K370 by SETDB1 (Fei et al., 2015) and phosphorylation at S249 by CDK4/cyclin D1 (Liao et al., 2017) (Figure 1). However, there are still several outstanding issues that remain to be addressed. First, would K370 methylation and S249 phosphorylation cross talk with each other in implementing the GOF activity of p53-R249S in HCC (Figure 1)? Also, would p53-R249S indeed be able to drive HCC development and progression in a more biological setting, such as in an animal model system? Would the CDK4/cyclin D1–Pin1–p53-R249S–c-Myc signaling pathway be more prevalent in a much larger HCC population in China or Africa? Would the GOF activity of p53-R249S play a role in metastasis and drug resistance of late-stage HCCs harboring this mutant p53? If so, this would lead to the last question, i.e. is it possible to co-target them as a combined strategy to develop more effective therapies for HCCs that harbor this p53 mutant, such as co-targeting mutant p53 and CDK4, or even triple-targeting c-Myc, CDK4, and Pin1, and so on? Addressing these issues will certainly further advance our knowledge about the role of p53-R249S in HCC development and provide us with more useful information for developing therapies for this type of cancer, beneficial to human health.

Acknowledgements

We thank Caiyue Li for proofreading.

Funding

H.W. was supported in part by China Scholarship Council (CSC No. 201706825042). H.L. and S.X.Z. are supported in part by NIH/NCIR01CA095441, R01CA172468, R01CA127724, and R21CA190775 grants.

Conflict of interest: none declared.

References

Aguilar, F., Hussain, S.P., and Cerutti, P. (1993). Aflatoxin B1 induces the transversion of G→T in codon 249 of the p53 tumor suppressor gene in human hepatocytes. Proc. Natl Acad. Sci. USA *90*, 8586–8590.

Albert, A., Lavoie, S., and Vincent, M. (1999). A hyperphosphorylated form of RNA polymerase II is the major interphase antigen of the phosphoprotein antibody MPM-2 and interacts with the peptidyl-prolyl isomerase Pin1. J. Cell Sci. *112*, 2493–2500.

Anker, P., Mulcahy, H., Chen, X.Q., et al. (1999). Detection of circulating tumour DNA in the blood (plasma/serum) of cancer patients. Cancer Metastasis Rev. *18*, 65–73.

Arevalo-Rodriguez, M., Cardenas, M.E., Wu, X., et al. (2000). Cyclophilin A and Ess1 interact with and regulate silencing by the Sin3-Rpd3 histone deacetylase. EMBO J. *19*, 3739–3749.

Asghar, U., Witkiewicz, A.K., Turner, N.C., et al. (2015). The history and future of targeting cyclin-dependent kinases in cancer therapy. Nat. Rev. Drug Discov. *14*, 130–146.

Besaratinia, A., Kim, S.I., Hainaut, P., et al. (2009). In vitro recapitulating of TP53 mutagenesis in hepatocellular carcinoma associated with dietary aflatoxin B1 exposure. Gastroenterology *137*, 1127–1137.

Bouchard, C., Staller, P., and Eilers, M. (1998). Control of cell proliferation by Myc. Trends Cell Biol. *8*, 202–206.

Bressac, B., Kew, M., Wands, J., et al. (1991). Selective G to T mutations of p53 gene in hepatocellular carcinoma from southern Africa. Nature *350*, 429–431.

Bruix, J., and Sherman, M. (2011). Management of hepatocellular carcinoma: an update. Hepatology *53*, 1020–1022.

Chen, B.J., Wu, Y.L., Tanaka, Y., et al. (2014). Small molecules targeting c-Myc oncogene: promising anti-cancer therapeutics. Int. J. Biol. Sci. *10*, 1084–1096.

Coursaget, P., Depril, N., Chabaud, M., et al. (1993). Highprevalence of mutations at codon 249 of the p53 gene in hepatocellular carcinomas from Senegal. Br. J. Cancer *67*, 1395–1397.

Dang, C.V. (2012). MYC on the path to cancer. Cell *149*, 22–35.

Datta, S., Banerjee, A., Chandra, P.K., et al. (2007). Pin1–HBx interaction: a step toward understanding the significance of hepatitis B virus genotypes in hepatocarcinogenesis. Gastroenterology *133*, 727–728.

Farrell, A.S., Pelz, C., Wang, X., et al. (2013). Pin1 regulates the dynamics of c-Myc DNA binding to facilitate target gene regulation and oncogenesis. Mol. Cell. Biol. *33*, 2930–2949.

Fei, Q., Shang, K., Zhang, J., et al. (2015). Histone methyltransferase SETDB1 regulates liver cancer cell growth through methylation of p53. Nat. Commun. *6*, 8651.

Ferlay, J., Soerjomataram, I., Dikshit, R., et al. (2015). Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. Int. J. Cancer *136*, E359–E386.

Fernandez, P.C., Frank, S.R., Wang, L., et al. (2003). Genomic targets of the human c-Myc protein. Genes Dev. *17*, 1115–1129.

Gearhart, T.L., and Bouchard, M.J. (2010). The hepatitis B virus X protein modulates hepatocyte proliferation pathways to stimulate viral replication. J. Virol. *84*, 2675–2686.

Ghouri, Y.A., Mian, I., and Rowe, J.H. (2017). Review of hepatocellular carcinoma: epidemiology, etiology, and carcinogenesis. J. Carcinog. *16*, 1.

Giacinti, C., and Giordano, A. (2006). RB and cell cycle progression. Oncogene *25*, 5220–5227.

Girardini, J.E., Napoli, M., Piazza, S., et al. (2011). A Pin1/mutant p53 axis promotes aggressiveness in breast cancer. Cancer Cell *20*, 79–91.

Goh, A.M., Coffill, C.R., and Lane, D.P. (2011). The role of mutant p53 in human cancer. J. Pathol. *223*, 116–126.

Gordan, J.D., Thompson, C.B., and Simon, M.C. (2007). HIF and c-Myc: sibling rivals for control of cancer cell metabolism and proliferation. Cancer Cell *12*, 108–113.

Gouas, D., Shi, H., and Hainaut, P. (2009). The aflatoxin-induced TP53 mutation at codon 249 (R249S): biomarker of exposure, early detection and target for therapy. Cancer Lett. *286*, 29–37.

Gouas, D.A., Shi, H., Hautefeuille, A.H., et al. (2010). Effects of the TP53p. R249S mutant on proliferation and clonogenic properties in human hepatocellular carcinoma cell lines: interaction with hepatitis B virus X protein. Carcinogenesis *31*, 1475–1482.

Gouas, D.A., Villar, S., Ortiz-Cuaran, S., et al. (2012). TP53 R249S mutation, genetic variations in HBX and risk of hepatocellular carcinoma in The Gambia. Carcinogenesis *33*, 1219–1224.

Grandori, C., Gomez-Roman, N., Felton-Edkins, Z.A., et al. (2005). c-Myc binds to human ribosomal DNA and stimulates transcription of rRNA genes by RNA polymerase I. Nat. Cell Biol. *7*, 311–318.

Hainaut, P., and Vahakangas, K. (1997). p53 as a sensor of carcinogenic exposures: mechanisms of p53 protein induction and lessons from p53 gene mutations. Pathol. Biol. *45*, 833–844.

Hanel, W., Marchenko, N., Xu, S., et al. (2013). Two hotspot mutant p53 mouse models display differential gain of function in tumorigenesis. Cell Death Differ. *20*, 898–909.

Harte, P.J., Wu, W., Carrasquillo, M.M., et al. (1999). Assignment of a novel bifurcated SET domain gene, SETDB1, to human chromosome band 1q21 by in situ hybridization and radiation hybrids. Cytogenet. Cell Genet. *84*, 83–86.

Hosny, G., Farahat, N., Tayel, H., et al. (2008). Ser-249 TP53 and CTNNB1 mutations in circulating free DNA of Egyptian patients with hepatocellular carcinoma versus chronic liver diseases. Cancer Lett. *264*, 201–208.

Hsu, T., McRackan, D., Vincent, T.S., et al. (2001). Drosophila Pin1 prolyl isomerase Dodo is a MAP kinase signal responder during oogenesis. Nat. Cell Biol. *3*, 538–543.

Hsu, I.C., Metcalf, R.A., Sun, T., et al. (1991). Mutational hotspot in the p53 gene in human hepatocellular carcinomas. Nature *350*, 427–428.

Huang, X.H., Sun, L.H., Lu, D.D., et al. (2003). Codon 249 mutation in exon 7 of p53 gene in plasma DNA: maybe a new early diagnostic marker of hepatocellular carcinoma in Qidong risk area, China. World J. Gastroenterol. *9*, 692–695.

Hussain, S.P., Aguilar, F., Amstad, P., et al. (1994). Oxy-radical induced mutagenesis of hotspot codons 248 and 249 of the human p53 gene. Oncogene *9*, 2277–2281.

Hussain, S.P., Schwank, J., Staib, F., et al. (2007). TP53 mutations and hepatocellular carcinoma: insights into the etiology and pathogenesis of liver cancer. Oncogene *26*, 2166–2176.

Igetei, R., Otegbayo, J.A., Ndububa, D.A., et al. (2008). Detection of p53 codon 249 mutation in Nigerian patients with hepatocellular carcinoma using a novel evaluation of cell-free DNA. Ann. Hepatol. *7*, 339–344.

Jackson, P.E., Kuang, S.Y., Wang, J.B., et al. (2003). Prospective detection of codon 249 mutations in plasma of hepatocellular carcinoma patients. Carcinogenesis *24*, 1657–1663.

Johnson, D.G., and Walker, C.L. (1999). Cyclins and cell cycle checkpoints. Annu. Rev. Pharmacol. Toxicol. *39*, 295–312.

Jones, P.A., and Baylin, S.B. (2002). The fundamental role of epigenetic events in cancer. Nat. Rev. Genet. *3*, 415–428.

Kew, M.C. (2003). Synergistic interaction between aflatoxin B1 and hepatitis B virus in hepatocarcinogenesis. Liver Int. *23*, 405–409.

Kew, M.C. (2011). Hepatitis B virus x protein in the pathogenesis of hepatitis B virus-induced hepatocellular carcinoma. J. Gastroenterol. Hepatol. *26*, 144–152.

Kew, M.C. (2013). Aflatoxins as a cause of hepatocellular carcinoma. J. Gastrointestin. Liver Dis. *22*, 305–310.

Kim, J., Woo, A.J., Chu, J., et al. (2010). A Myc network accounts for similarities between embryonic stem and cancer cell transcription programs. Cell *143*, 313–324.

Kirby, G.M., Chemin, I., Montesano, R., et al. (1994). Induction of specific cytochrome P450s involved in aflatoxinB1 metabolism in hepatitis B virus transgenic mice. Mol. Carcinog. *11*, 74–80.

Kirk, G.D., Camus-Randon, A.M., Mendy, M., et al. (2000). Ser-249 p53 mutations in plasma DNA of patients with hepatocellular carcinoma from the Gambia. J. Natl Cancer Inst. *92*, 148–153.

Kitagawa, M., Higashi, H., Jung, H.K., et al. (1996). The consensus motif for phosphorylation by cyclin D1-Cdk4 is different from that for phosphorylation by cyclin A/E-Cdk2. EMBO J. *15*, 7060–7069.

Kremsdorf, D., Soussan, P., Paterlini-Brechot, P., et al. (2006). Hepatitis B virus-related hepatocellular carcinoma: paradigms for viral-related human carcinogenesis. Oncogene *25*, 3823–3833.

Kuang, S.Y., Lekawanvijit, S., Maneekarn, N., et al. (2005). Hepatitis B 1762T/1764A mutations, hepatitis C infection, and codon 249 p53 mutations in hepatocellular carcinomas from Thailand. Cancer Epidemiol. Biomarkers Prev. *14*, 380–384.

Lee, Y.I., Lee, S., Das, G.C., et al. (2000). Activation of the insulin-like growth factor II transcription by aflatoxin B1 induced p53 mutant 249 is caused by activation of transcription complexes; implications for a gain-of-function during the formation of hepatocellular carcinoma. Oncogene *19*, 3717–3726.

Lee, M.K., and Sabapathy, K. (2008). The R246S hot-spot p53 mutant exerts dominant-negative effects in embryonic stem cells in vitro and in vivo. J. Cell Sci. *121*, 1899–1906.

Lee, M.K., Teoh, W.W., Phang, B.H., et al. (2012). Cell-type, dose, and mutation-type specificity dictate mutant p53 functions in vivo. Cancer Cell *22*, 751–764.

Li, Z., Van Calcar, S., Qu, C., et al. (2003). A global transcriptional regulatory role for c-Myc in Burkitt's lymphoma cell. Proc. Natl Acad. Sci. USA *100*, 8164–8169.

Liao, P., Zeng, S.X., Zhou, X., et al. (2017). Mutant p53 gains its function via c-Myc activation upon CDK4 phosphorylation at serine 249 and consequent PIN1 binding. Mol. Cell *68*, 1134–1146.

Lin, C.Y., Lovén, J., Rahl, P.B., et al. (2012). Transcriptional amplification in tumor cells with elevated c-Myc. Cell *151*, 56–67.

Lleonart, M.E., Kirk, G.D., Villar, S., et al. (2005). Quantitative analysis of plasma TP53 249Ser-mutated DNA by electrospray ionization mass spectrometry. Cancer Epidemiol. Biomarkers Prev. *14*, 2956–2962.

Lu, K.P., Hanes, S.D., and Hunter, T. (1996). A human peptidyl-prolyl isomerase essential for regulation of mitosis. Nature *380*, 544–547.

Lu, J.W., Shen, C.K., and Tzeng, T.Y. (2016). Epigenetics of cancer: the role of histone methyltransferase, SETDB1, in cancer metastasis. Transl. Cancer Res. *5*, S139–S141.

Mace, K., Aguilar, F., Wang, J.S., et al. (1997). Aflatoxin B1-induced DNA adduct formation and p53 mutations in CYP450-expressing human liver cell lines. Carcinogenesis *18*, 1291–1297.

Malumbres, M., and Barbacid, M. (2009). Cell cycle, CDKs and cancer: a changing paradigm. Nat. Rev. Cancer *9*, 153–166.

Matsui, T., Leung, D., Miyashita, H., et al. (2010). Proviral silencing in embryonic stem cells requires the histone methyltransferase ESET. Nature *464*, 927–931.

Mikhail, S., Cosgrove, D., and Zeidan, A. (2014). Hepatocellular carcinoma: systemic therapies and future perspectives. Expert Rev. Anticancer Ther. *14*, 1205–1218.

Mittal, S., and El-Serag, H.B. (2013). Epidemiology of hepatocellular carcinoma: consider the population. J. Clin. Gastroenterol. *47*, S2–S6.

Moriya, S., Morimoto, M., Numata, K., et al. (2013). Fucosylated fraction of α-fetoprotein as a serological marker of early hepatocellular carcinoma. Anticancer Res. *33*, 997–1001.

Muller, P.A., and Vousden, K.H. (2013). p53 mutations in cancer. Nat. Cell Biol. *15*, 2–8.

Nogueira, J.A., Ono-Nita, S.K., Nita, M.E., et al. (2009). 249 TP53 mutation has high prevalence and is correlated with larger and poorly differentiated HCC in Brazilian patients. BMC Cancer *9*, 204–211.

Ozdemir, F.T., Tiftikci, A., Sancak, S., et al. (2010). The prevalence of the mutation in codon 249 of the P53 gene in patients with hepatocellular carcinoma (HCC) in Turkey. J. Gastrointest. Cancer *41*, 185–189.

Ozen, C., Yildiz, G., Dagcan, A.T., et al. (2013). Genetics and epigenetics of liver cancer. New Biotechnol. *30*, 381–384.

Pandima Devi, K., Sivamaruthi, B., Kiruthiga, P.V., et al. (2010). Study of p53 codon 72 polymorphism and codon 249 mutations in Southern India in relation to age, alcohol drinking and smoking habits. Hum. Exp. Toxicol. *29*, 451–458.

Qi, L.N., Bai, T., Chen, Z.S., et al. (2015). The p53 mutation spectrum in hepatocellular carcinoma from Guangxi, China: role of chronic hepatitis B virus infection and aflatoxin B1 exposure. Liver Int. *35*, 999–1009.

Schultz, D.C., Ayyanathan, K., Negorev, D., et al. (2002). SETDB1: a novel KAP-1-associated histone H3, lysine 9-specific methyltransferase that contributes to HP1-mediated silencing of euchromatic genes by KRAB zinc-finger proteins. Genes Dev. *16*, 919–932.

Shen, H.M., and Ong, C.N. (1996). Mutations of the p53 tumor suppressor gene and ras oncogenes in aflatoxin hepatocarcinogenesis. Mutat. Res. *366*, 23–44.

Sheppard, K.E., and McArthur, G.A. (2013). The cell-cycle regulator CDK4: an emerging therapeutic target in melanoma. Clin. Cancer Res. *19*, 5320–5328.

Sherr, C.J. (1996). Cancer cell cycles. Science *274*, 1672–1677.

Sigal, A., and Rotter, V. (2000). Oncogenic mutations of the p53 tumor suppressor: the demons of the guardian of the genome. Cancer Res. *60*, 6788–6793.

Soini, Y., Chia, S.C., Bennett, W.P., et al. (1996). An aflatoxin-associated mutational hotspot at codon 249 in the p53 tumor suppressor gene occurs in hepatocellular carcinomas from Mexico. Carcinogenesis *17*, 1007–1012.

Staib, F., Hussain, S.P., Hofseth, L.J., et al. (2003). TP53 and liver carcinogenesis. Hum. Mutat. *21*, 201–216.

Stern, M.C., Umbach, D.M., Yu, M.C., et al. (2001). Hepatitis B, aflatoxin B(1), and p53 codon 249 mutation in hepatocellular carcinomas from Guangxi, People's Republic of China, and a meta-analysis of existing studies. Cancer Epidemiol. Biomarkers Prev. *10*, 617–625.

Szymańska, K., and Hainaut, P. (2003). TP53 and mutations in human cancer. Acta Biochim. Pol. *50*, 231–238.

Szymańska, K., Lesi, O.A., Kirk, G.D., et al. (2004). Ser-249TP53 mutation in tumour and plasma DNA of hepatocellular carcinoma patients from a high incidence area in the Gambia, West Africa. Int. J. Cancer *110*, 374–379.

Takahashi, K., and Yamanaka, S. (2006). Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell *126*, 663–676.

The Cancer Genome Atlas Research Network. (2017). Comprehensive and integrative genomic characterization of hepatocellular carcinoma. Cell *169*, 1327–1341.

Trevisani, F., D'Intino, P.E., Morselli-Labate, A.M., et al. (2001). Serum α-fetoprotein for diagnosis of hepatocellular carcinoma in patients with chronic liver disease: influence of HBsAg and anti-HCV status. J. Hepatol. *34*, 570–575.

Vogelstein, B., and Kinzler, K.W. (2004). Cancer genes and the pathways they control. Nat. Med. *10*, 789–799.

Wang, H., An, W., Cao, R., et al. (2003). mAM facilitates conversion by ESET of dimethyl to trimethyl lysine 9 of histone H3 to cause transcriptional repression. Mol. Cell *12*, 475–487.

Wang, Z., Inuzuka, H., Zhong, J., et al. (2012). Tumor suppressor functions of FBW7 in cancer development and progression. FEBS Lett. *586*, 1409–1418.

Weng, M.W., Lee, H.W., Choi, B., et al. (2017). AFB1 hepatocarcinogenesis is via lipid peroxidation that inhibits DNA repair, sensitizes mutation susceptibility and induces aldehyde-DNA adducts at p53 mutational hotspot codon 249. Oncotarget *8*, 18213–18226.

Wong, C.M., Wei, L., Law, C.T., et al. (2016). Up-regulation of histone methyltransferase SETDB1 by multiple mechanisms in hepatocellular carcinoma promotes cancer metastasis. Hepatology *63*, 474–487.

Yada, M., Hatakeyama, S., Kamura, T., et al. (2004). Phosphorylation-dependent degradation of c-Myc is mediated by the F-box protein Fbw7. EMBO J. *23*, 2116–2125.

Yeh, E.S., and Means, A.R. (2007). PIN1, the cell cycle and cancer. Nat. Rev. Cancer *7*, 381–388.