Impact of *TP53* Mutations in Hepatocellular Carcinoma on Carcinogenesis, Diagnosis, Prognosis and Treatment

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Abstract: With almost 800.000 annual deaths, hepatocellular carcinoma (HCC) has the fourth highest cancer mortality rate, owed mainly to late stage diagnosis and poor response to treatment. In average, approx. 30% of cases exhibit mutations within the *TP53* gene, with much higher frequencies in Africa and China, where a specific hepatitis B virus (HBV) and aflatoxin B1 (AFB1) related mutation (R249S) is present in 50% and 90% of cases, respectively, making *TP53* the most frequently mutated tumor suppressor gene in HCC.

Mutant p53 has a direct impact on tumorigenesis via distortion of numerous regulatory pathways, including activation of c-myc, and inactivation of TAp63, with the latter being related to initiation of a pro-invasive transcription program. Abrogation of p53-induced cell cycle arrest and apoptosis is esp. pronounced in HBV positive cases through interference of the HBx protein. Generally, *TP53* mutations are related to a poor prognosis, making them an important prognostic factor. In a pilot study, an immunoprosthetic model based on differential expression of immune-related genes in HCC cases with WT vs. mutant p53 was superior to clinicopathologic risk factors alone in predicting the risk of poor clinical outcome.

In high-risk areas with high levels of exposure to HBV and AFB1 like Sub-Saharan Africa and China, the predominant R249S mutation can be used as biomarker for early cancer detection, assessed as circulating free DNA from peripheral blood.

A new concept of a two-step therapeutic scheme specifically targets cells with mutant p53 for induction of senescence in a first step, and initiation of apoptosis in these cells in a second step. Another new approach inhibits the E3 ubiquitin ligase ARF-BP1 resulting in growth suppression and G2/M phase cell cycle arrest regardless of the *TP53* status. Restoring of WT p53 activity through gene therapy is a valuable and promising approach esp. in patients without p53 expression.

This review summarizes recent studies and presents their findings in one common context, interconnecting their ideas and approaches in order to markup new possible ways to tackle HCC on all levels, from surveillance, diagnosis and risk stratification to therapy.

Keywords: p53, mutant, Hepatocellular carcinoma, Cancer, Tumorigenesis, Diagnosis, Treatment.

1. INCIDENCES AND ETIOLOGY OF HEPATOCELLULAR CARCINOMA

With an incidence of 841,080 new cases in 2018, liver cancer is the seventh most common cancer worldwide, with the fourth highest cancer mortality rate (781.631 deaths; 8.2%), after lung cancer (1.761.007 deaths; 18.4%), colorectal cancer (861.663 deaths; 9%), and stomach cancer (782.685 deaths; 8.2%) [1]. The incidence of liver cancer has a wide geographical variation due to the large heterogeneity of the penetration of risk factors within the population [2].

Hepatocellular carcinoma (HCC), the predominant form of liver cancer, has several known risk factors; in fact, any substance that induces chronic liver damage,

and eventually cirrhosis, has an oncogenic potential. The most relevant risk factors include chronic hepatitis B virus (HBV) and hepatitis C virus (HCV) infections, cirrhosis, fibrosis, exposure to dietary aflatoxin B1 (AFB1) as co-factor with HBV, vinyl chloride, alcohol abuse, autoimmune hepatitis, diabetes mellitus, obesity (nonalcoholic steatohepatitis), iron overload (whether caused by hemochromatosis or by dietary intake is still under debate) and several other metabolic diseases [3-6].

These risk factors create an environment that triggers progressive inflammation, ultimately leading to cycles of necrosis and regeneration that may end up with the induction of preneoplastic lesions such as dysplastic nodules. Subsequently, genetic and epigenetic alterations in key genes controlling apoptosis or survival may accumulate and provide an advantage to premalignant cells with respect to proliferation, survival or invasion. This eventually favors

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the transformation of dysplastic cells into fully-manifested HCC [7-10]. It's tale telling that the geographic hotspots for HCC are Southern China and sub-Saharan Africa, where also the worldwide highest level of dietary AFB1 intake and the highest HBV incidences are being observed [3-5].

In Western countries and Japan in contrast, HCV infection is the main cause of HCC. While HBV infection has a direct oncogenic effect, regardless of the degree of underlying liver fibrosis, HCC rarely occurs in HCV-infected patients without advanced fibrosis. The etiology of HCC is nicely reviewed in detail by Refolo *et al.* [11] and Rosetto *et al.* [12].

2. PROMINENT MUTATIONS IN HCC

HCC is a very heterogeneous tumor and great effort had been undertaken to identify genetic alterations at all levels, including base substitution mutations, small insertions and deletions (indels), copy number variants (CNV's), and changes in methylation patterns.

With respect to chromosome stability status, hepatic tumors can be assigned to either of two groups: Tumors with a tendency to chromosome instability are typically HBV positive and poorly differentiated and frequently associated with mutations of the tumor suppressor gene *TP53* (MIM# 191170) [13-16], while tumors that exhibit stable chromosomes are usually non-HBV and well-differentiated and are frequently found to be related to activated β -catenin [17].

Also the genetic alterations found in HCC can be assigned to two groups: the first group encompasses genetic alterations that are specific of hepatocellular tumor risk factors such as integration of HBV DNA or the G to T transversion at the third position of codon 249 of the *TP53* gene in AFB1-exposed patients, resulting in a substitution of a serine for an arginine (R249S). *KRAS* mutations are believed to be related to increased reactive oxygen species and fibrosis associated to hepatocellular adenomas and adenomatous polyposis coli (APC) germline mutations predisposing to hepatoblastomas.

The second group of genetic alterations is etiological nonspecific and includes recurrent gains and losses of chromosomes, alterations of *TP53* other than R249S, activation of the WNT/ β -catenin pathway through activating mutations of *CTNNB1* (β -catenin) and *AXIN* (axis inhibition protein) mutations, inactivation of retinoblastoma and IGF2R (insulin-like growth factor 2 receptor) pathways through inactivation of the retinoblastoma gene (*RB1*), P16 and IGF2R [17].

However, *TERT* promoter mutations activating telomerase expression found in 44 - 60% of cases are the most common somatic mutation in HCC according to recent whole genome and whole exome sequencing studies [18, 19]. They seem to be related to early stages as they are already present in dysplastic nodules; the *TERT* promoter is also a recurrent insertion site for the HBV genome.

The most frequently mutated tumor suppressor gene is *TP53* (25% - 31% of cases), followed by *AXIN1* (8%) and *RB1*, the retinoblastoma tumor suppressor gene [14, 15, 20-22]. *RB1* mutations are found in 20 to 25% as a single gene mutation, but together with *TP53* mutations they have been identified in 80 to 86% of HCC [23].

Mutations within the p53 pathway were identified even in a total of 49% of cases [24]. Like for *TP53*, also abnormalities in *RB1* are frequently observed in advanced HCC [25].

CTNNB1 is the most frequently mutated oncogene found in 15.9 – 27% of cases [14, 19-21]. Yet, activation of the WNT/ β -catenin pathway has been found in 54% of tumors [24] and nuclear overexpression occurs in 10–90% of HCC [3]. A deregulated β -catenin pathway may transcriptionally activate the target genes of the Wnt-pathway, such as c-myc, cyclinD1, and PPAR δ . Of course, transactivation of these target genes has the potential to promote tumor progression by stimulation of cell proliferation [26, 27].

A number of oncogenes such as N-ras, c-myc, or c-fos are frequently found to be overexpressed, yet they are rarely mutated. The overexpression however can be explained by the aforementioned activation of the Wnt/ β -catenin-pathway [23, 25].

3. PHYSIOLOGICAL FUNCTIONS OF P53

The p53 protein is composed of 393 amino acids which make up a number of distinct domains such as two N-terminal transactivation domains (TAD1 and TAD2), a proline-rich domain, a core DNA-binding domain (DBD), an oligomerization domain (OD), and an unstructured C-terminal domain (CTD) with a non-sequence specific DNA-binding ability. The two TADs fulfill different functions and interact with a number of transcriptional cofactors in order to determine the function of p53 in a specific context [28].

Initially, p53 had been identified in a complex with the SV40 tumor-virus oncoprotein [29-32] and was described as an oncogene [32, 33]. When it was realized that this work had been done on a mutant protein, efforts were shifted towards uncovering the physiologic function of WT p53, which ultimately was reclassified as a major tumor suppressor and even was referred to as 'Guardian of the Genome' [34].

Its prominent role in human cancer prevention is underlined by the finding that the *TP53* gene is mutated in approx. 50% of human cancers [35, 36] and that the inheritance of a mutant *TP53* allele is causative of the Li-Fraumeni syndrome of familial cancer predisposition [37, 38]. More than 80% are missense mutations [39] and more than 90% of them occur in the central DBD [40] with six mutation hotspots at R175, G245, R248, R249, R273 and R282 [41].

The fact that *TP53* mainly undergoes missense mutations that do not entirely abolish all p53 biochemical activities, unlike conventional tumor suppressors, which are not expressed in human cancers due to nonsense mutation or gene deletion, may explain why there is no strict correlation at the individual level between type of mutation and clinical outcome [42-44].

As a tumor suppressor, p53 acts as a transcription factor that binds target genes through p53 response elements (REs) as a tetramer. Various cellular stresses, such as DNA damage, activation of oncogenes, and metabolic stress, activate p53 which in turn launches a very complex anti-proliferative response program including cell cycle arrest, DNA repair, senescence, and apoptosis in order to maintain the integrity of the genome [45-48].

The complex activation, regulation, and activity of the p53 network have nicely been reviewed by Edward R. Kasthuber & Scott W. Lowe [49], and M. Fischer [50]. Here, only the 'classic' pathways are discussed in brief.

DNA Damage and Metabolic Stress Response

DNA damage responses (DDR) activate the stress sensor kinases ataxia telangiectasia mutated (ATM) or ataxia telangiectasia and Rad3-related (ATR), which activate p53/p21^{cip1} by stabilization of p53 levels through phosphorylation of p53 and its ubiquitin ligase MDM2 (mouse double minute protein 2) [51, 52].

In its maybe most prominent role p53 maintains the G1 cell cycle checkpoint and initiates a G1 cell cycle arrest through p21/WAF, while transactivating genes involved in DNA repair like GADD45 (Growth Arrest and DNA Damage), as well as pro-apoptotic proteins like Puma and Noxa [39].

There is solid evidence that p53 possesses alternative biological functions beyond its transcription and transactivation abilities, which play a significant role in the tumor-suppressive activity of p53. They seem to be mainly mediated by the TAD and CTD which represent multi-functional binding sites for an ever-growing interactome. Also these domains are subject to extensive post-translational modifications, which can regulate protein stability, turnover, and cellular localization and subsequently, protein-protein interactions [53, 54].

As a component of the replisome machinery at active replication forks, p53 is an important player in replication stress response. The DBD and CTD of p53 can recognize various DNA damage-associated structures. The CTD for instance has a strong affinity for stretches of DNA with single-stranded gaps, as for triple-stranded DNA, and DNA duplexes with either free ends or mismatches caused by insertion-deletion lesions [28].

P53 also possesses 3' to 5' exonuclease activity [55-57] and proofreading ability [58], and regulates initiation of nucleotide excision repair (NER), base excision repair (BER), and mismatch repair (MMR) events. Through direct interaction with RAD51 it also regulates RAD51 oligomerization at sites of double-strand breaks (DSBs) and blocks continued strand exchange, suppressing both spontaneous and damage-induced homologous recombination (HR) [59]. P53 can directly rejoin or ligate DNA with DSBs [60, 61] and it was demonstrated that it has an inhibitory effect on error-prone or microhomology-mediated non-homologous end-joining (alt-NHEJ) [62].

Besides DNA damage, p53 is also activated in response to metabolic stress, a diverse group of stress factors that include oxidative stress, starvation, hypoxia and deprivation of growth factors [40] and that seems to play a key role in responding to starvation by mobilizing alternative nutrient sources and catabolic pathways. In this context transient activation of p21 is important. In case of serine starvation for instance, mounting a cell cycle arrest through p21 will determine

serine from de novo synthesis preferably to the glutathione production pathway, rather than using it for nucleotide synthesis. Also activation of autophagy and fatty acid oxidation by p53 as alternative catabolic pathways may allow cells to temporarily survive in the absence of exogenous nutrients, while at any time p53 has the potential to doom cells that cannot recover [63, 64].

Senescence

Senescence can be viewed as adaptation of individual cells and whole organisms to inferior environmental conditions and is defined as a cell state characterized by prolonged and generally irreversible cell-cycle arrest. Triggers can be intrinsic factors, such as oxidative stress, telomere shortening, hyperproliferation, or activation of oncogenes, as well as environmental factors, including exposure to UV-light, gamma or X-ray, as well as chemotherapeutic drugs; any of these factors triggers a DNA damage response, with the most extensively studied pathways being p53/p21^{cip1} and/or p16^{INK4A}/Rb [51, 65].

Mild DNA damage and transient stimuli normally induce a transient cell cycle arrest, allowing the cell to attempt repairing the damage, while initiation of the senescence program seems to require a stable and more severe stimulus. Severe injury can activate the death programs instead [66].

p53 is Mainly Regulated by MDM2 and ARF (p14^{ARF})

Regulation of p53 is partly achieved by controlling its protein levels through continuous ubiquitination and degradation, and partly by complex translational and post-translational modifications, such as phosphorylation, acetylation and methylation which contribute to the regulation of its functions and may also modulate the ultimate outcome of the response [67].

One of the main negative regulators of p53 is the E3 ubiquitin ligase MDM2, which blocks the recruitment of factors necessary for induction of gene expression by binding the transcriptional activation domain of p53 [68]. Moreover MDM2 targets p53 for proteasomal degradation by acting as an E3 ubiquitin ligase [69]. MDM2 is constitutively expressed but further transactivated by p53. Thus, p53 and MDM2 form an auto-regulatory loop in which p53 positively regulates MDM2 expression and MDM2 negatively regulates p53 [70, 71].

The p53 level increases in response to stresses because of extended half-life and possibly due to an enhanced rate of translational initiation of p53 mRNA [40]. Stress induced kinases such as ATM and ATR phosphorylate p53 in order to modulate its binding to MDM2; small proteins like ARF (p14^{ARF}) or ribosomal protein L11 (RPL11) bind and inactivate MDM2 [72], resulting in p53 stabilization and cell cycle arrest or apoptosis [73, 74]. Phosphorylation of MDM2 by AKT in contrast, enhances p53 degradation in response to growth stimulatory signals [75].

Regulation on the mRNA Level by Initiation of Alternative Translation Start Codons

Depending on the cellular stressor, two p53 isoforms are synthesized by alternative translation initiation of the p53 mRNA. Full length p53 (p53FL) is synthesized following activation of the ATM kinase by translation initiation at +1 AUG. The full-length p53 includes TAD1 which is required for induction of p53 target genes that ultimately lead to G1 cell cycle arrest or apoptosis.

Activation of the Unfolded Protein Response (UPR) pathway following stress to the endoplasmic reticulum activates the PERK kinase and results in initiation of the p53/47 isoform at the second in frame AUG at position +120. P53/47 lacks TAD1 but retains TAD2 and causes G2/M arrest [76].

P53 can be Co-Expressed in Different Isoforms

The human *TP53* gene on chromosome 17p13.1 comprises 13 exons; 2 of which are subject to alternative splicing. In humans, alternative splicing can occur to produce variants retaining intron 2 and intron 9. Moreover, p53 isoforms may be generated by a combination of the use of alternative promoters (P1 and P2), alternative splicing and alternative initiation of translation.

Canonical *TP53* transcription initiates at promoter P1 which is located upstream of exon1, while the alternative promoter P2 is situated within intron4. Alternative splicing and the use of alternative translation initiation sites, as afore mentioned, may lead to the co-expression of p53 protein isoforms with different protein-interacting domains and activities [77].

4. TP53 MUTATIONS IN HCC

Recent studies have provided evidence that *TP53* plays a major role in hepatocarcinogenesis [3, 5, 78,

79]. HBV-associated HCC cases exhibit the highest *TP53* mutation rates and show nearly exclusively a specific *TP53* mutation: the afore mentioned G to T transversion at the third position of codon 249, leading to transfer from AGG to AGT which ultimately results in a substitution of serine for arginine (R249S). This specific mutation is present in 50% of HCC patients from Africa and in 90% of cases from Qidong (China), both areas with a high exposure to AFB1 [80, 81]. In western countries, where there is no exposure to AFB1, *TP53* mutations are found in approximately 20% of HCC cases, without specific hotspot mutations [78].

These results contrast with the pattern of *TP53* mutations found in other human cancers, where the mutations are primarily scattered over four of the five evolutionarily conserved domains [81]. Hence the presence of such a specific mutational hotspot implicates environmental carcinogens and endogenous processes in the etiology of the respective cancer entity. Strikingly, geographic areas with high prevalence of HBV infections also experience a high exposure to AFB1 [3, 4].

Staib *et al.* [3] could show that the dose of dietary AFB1 exposure is directly correlated to the frequency of *TP53* R249S mutations in three different ethnic populations on three continents. Furthermore, the R249S mutation is also detectable in nontumorous liver cells in high risk areas where it also demonstrates a dose dependent relationship with the intake of AFB1 [82].

The mycotoxin AFB1 is produced by the fungus *Aspergillus flavus* on improperly stored grain and peanuts. It is metabolized via cytochrome P450 in the liver [80]. Several studies demonstrated that the secondary AFB1-formamidopyrimidine (AFB1-FAPY) binds very efficiently to the sequence context of *TP53* codon 249 (AGGCC) and induces a G to T transversion at a high rate, making the FAPY adduct the prime candidate for the mutagenicity of aflatoxin that ultimately may lead to liver cancer [83-85].

The Histone Methyltransferase SETDB1 Stabilizes p53-R249S

Mutant p53-R249S protein is stabilized by SETDB1 (SET domain bifurcated 1), a histone H3K9 methyltransferase that has a critical role in early development. In HCC SETDB1 is frequently over-expressed with moderate copy number gain, and the high copy number and RNA level of the SETDB1 gene

were well correlated with the p53-R249S status in HCC tissues, suggesting a cooperative mode of action. This is also indicated by the observation that inactivation of SETDB1 in HCC cell lines bearing the R249S mutation suppresses cell growth.

It could be shown that SETDB1 forms a complex with p53-R249S and catalyses p53K370di-methylation, which stabilizes the mutant p53 mostly by preventing its MDM2-mediated ubiquitination and degradation [86].

HBx Forms a Complex with p53-R249S Enhancing Proliferation

The X gene of HBV (HBx) is the most common open reading frame integrated into the host genome in HCC [3].

Experiments using HCC cell lines showed that p53-R249S mutants predictably lost their capacity to bind to p53 response elements and to transactivate p53 target genes, and that in p53-null Hep3B cells, stable transfection of p53-R249S did not induce significant changes in cell proliferation and survival after cytotoxic stress. But in a cell line that constitutively expresses both p53-R249S and the hepatitis B virus antigen HBx, silencing of either gene by RNA interference slowed down proliferation, with no additive effects when both factors were silenced. This finding suggested that p53-R249S is selected for in HBV-associated HCC since HBV-encoded x protein likely forms a complex with p53-R249S conferring a subtle growth advantage at early steps of the transformation process [87].

Hepatocytes transfected with *TP53* R249S exhibited impaired induction of apoptosis by the HBx protein and TNF- α , indicating that blocking of apoptosis through enhanced production of IGF-II possibly provides a survival advantage which helps selecting transformed hepatocytes [88].

5. IMPAIRMENT OF WT P53 FUNCTION IN HCC

HBx Inhibits WT p53-Induced Apoptosis

The HBx protein can also form a complex with WT p53 and thereby inhibiting its DBD and transcriptional transactivator activity. Moreover, HBx suppresses p53-induced apoptosis by preventing p53 to bind to protein factors that are involved in initiation of apoptosis. Since p53-mediated apoptosis is important for tissue homeostasis and prevention of neoplastic transformation, inactivation of p53-mediated apoptosis by HBx could impede normal cellular surveillance

mechanisms for removing damaged cells, which likely results in the accumulation of genetically abnormal cells, from which clones with a survival advantage would be selected. Thus, reduction of p53-mediated apoptosis by HBx may contribute to the development of human hepatocellular carcinoma [89].

The Product of a Nonsense Mutation in the HBV Large S Gene Downregulates WT p53

Nonsense mutations in the HBV large S (LHBs) gene frequently found in the liver from HBV core antigen-positive HCC patients [90] showed to be oncogenic in mouse xenograft models. In an in-vitro study, Chiu *et al.* [91] could demonstrate that SW182*, a carboxy terminally truncated protein from a nonsense mutant (stop codon at tryptophane-182) of the LHBs gene, downregulated tumor suppressors p53 and Smad4 when expressed in a liver cell line (Huh-7).

The authors also showed that c-jun activation domain-binding protein 1 (Jab1) physically interacts with SW182*, but not WT LHBs. RNA interference (RNAi) of Jab1 was able to restore the levels of the downregulated p53 and Smad4, pointing to the direct involvement of Jab1 in the impairment of p53 and Smad4. Consistently, RNAi of Jab1 restored the levels of the downregulated p53 and Smad4. The downregulation was also reversed by the proteasome inhibitor MG132, implying the involvement of proteasome-based protein degradation in the observed regulation of the tumor suppressors [91].

These results indicate that the LHBs nonsense mutant counteracts the tumor suppressor pathways through Jab1 in the liver contributing to HCC development and suggest an alternative way of impairing p53 in HCC cases with WT *TP53*.

6. DOES A GAIN-OF-FUNCTION ACTIVITY SELECTS FOR P53-R249S IN HCC?

For decades it has been quite firm belief that *TP53* mutations result in loss-of-function (LOF) due to the frequently observed coincidence of mutation in one allele and loss of the WT allele, referred to as loss of heterozygosity (LOH) [92, 93], or due to mutant p53 forming a heterotetramer with WT p53, abrogating its function as transcription factor; a phenomenon known as dominant-negative (DN) effect [94-96].

However, the suspect that mutant p53 protein may also have oncogenic potential through gain-of-functions (GOFs), besides losing its WT functions, persisted [97-

99] and the frequently observed LOH itself is suggesting a biological selection process. This suspect is further fueled by the finding that patients with a germline *TP53* missense mutation have a significantly earlier cancer onset than those with *TP53* nonsense mutations [100, 101]. In support of this observation, *in vivo* experiments in mice showed that expressing mutant p53 goes together with a tumor profile that is more aggressive and metastatic than that of p53 null or p53 WT mice [102-105].

Like other p53 mutants, p53-R249S exhibits both LOF and DN effects [106, 107] so the question is, whether the high prevalence of the *TP53* R249S hotspot mutation in HCC is the result of a selection process? In other words, does the p53-R249S mutant protein provide a growth and/or survival advantage to liver cells? The likelihood of this idea is supported by a row of evidence: Ponchel *et al.* [108] showed that transfection of a *TP53* R249S mutant into p53-negative human liver cancer cells results in enhanced cell growth. Also the introduction of a murine *TP53* mutation, corresponding to human codon 249, into a murine hepatocyte cell line results in a selective growth advantage [109], which would be consistent with a GOF. Moreover, p53-R249S inhibits WT p53-mediated apoptosis [89].

However, while the hotspot mutations R175, R273 and R248 clearly display GOF in knockin mouse models [110-112], a mouse model of R246S (equivalent to human R249S) only showed LOF and DN effects [107, 113].

P53-R249S Enhances cMyc Activity

In a concise study, Liao *et al.* [41] put together a number of known cancer-related proteins in one single pathway that can justify a GOF activity of p53-R249S in HCC. The key finding was that the serine at codon 249 is a specific substrate for the CDK4/cyclin D1 complex (but not for any other cyclin/CDK complexes) during the G1 phase of the cell cycle of HCC cells. CDK4 does not bind to WT p53 but only to p53-R249S, and formation of complexes with this mutant appeared preferable to other point mutants of p53.

After phosphorylation, the phosphor-Ser249-Pro250 motif becomes a preferred target for a peptidyl-prolyl cis-trans isomerase NIMA-interacting 1 (Pin1), which binds to p53-R249S at this motif, facilitating its nuclear import. A CDK inhibitor in contrast blocked p53-R249S nuclear translocation. The nuclear-phosphorylated p53-

R249S then binds to and stabilizes c-Myc in the G1/S phase by reducing its binding to FBW7a, a major E3 ligase for degrading c-Myc [114], preventing FBW7a-mediated ubiquitination and degradation. This increases the half-life of endogenous c-Myc, and interaction with p53-R249S enhances c-Myc-dependent rDNA transcription as key for ribosomal biogenesis.

Altogether, this study demonstrates that the coordinated action of these proteins creates a CDK4/PIN1/p53-R249S/c-Myc signaling pathway that equips p53-R249S with a new GOF activity critical for HCC proliferation and growth [41].

7. IMPACT OF MUTANT P53 ON TUMORIGENESIS

Mutant p53 can as well have effects other than impairing cell cycle arrest and apoptosis. Several studies revealed that TAp63, a p53 family protein and transcription factor, interacts with mutant but not WT p53 [115, 116]. By inhibiting TAp63, mutant p53 can regulate a pro-invasive transcription program that includes regulation of the expression of a number of genes such as Dicer, DEPDC1, Cyclin G2, and Sharp1 [117, 118]. TAp63 deletion in tumor models results in an aggressive tumor profile and metastases similar to that seen in mice expressing mutant p53 [119]. However, in a mouse model of pancreatic ductal adenocarcinoma (PDAC) TAp63 deletion alone appeared less potent in inducing metastases compared to expression of mutant p53, suggesting that mutant p53 does more than inhibiting TAp63 [120].

Mutant p53 is able to downregulate Dicer expression by directly inhibiting TAp63-mediated transactivation of Dicer as well as in a TAp63-independent fashion, which is particularly interesting because Dicer depletion promotes the invasive behavior of cells. This relationship between mutant p53, TAp63, and Dicer might contribute to the metastatic function of mutant p53 [46].

Besides inhibiting TAp63, mutant p53 inhibits and interacts with other proteins including the MRE11-Rad51-NSB complex, p73, and SP-1 to induce genomic instability, chemoresistance, or proliferation [115, 120, 122].

Insulin-Like Growth Factor II is Over Expressed by p53-R249S

Human insulin-like growth factor II (IGF-II) is an important player in tumor progression, identified in

many tumors of various origins, including human HCC. In the liver, IGF-II, originally called somatomedin A, is believed to stimulate growth of hepatocytes during malignant transformation by autocrine or paracrine mechanisms [123].

Lee *et al.* [88] showed that p53-R249S significantly increases the transcription of the *IGF-II* gene, but from promoter 4, leading to accumulation of the fetal form of IGF-II, while WT p53 inhibits binding of transcription factors on the P4 promoter. According to their results, activation of IGF-II transcription by p53-R249S not only increases IGF-II production as a mitogenic growth factor in tumor development but also acts as a survival factor that helps escaping apoptosis in HBV-positive tumors by eventually selecting for neoplastic hepatocytes that have survived by synthesizing mitogenic growth factor IGF-II, during viral pathogenesis [88]. All the various proteins and pathways affected by mutant p53 are thoroughly evaluated in three recent reviews [45, 112, 124].

8. CAN P53-R249S SERVE AS BIOMARKER FOR EARLY DETECTION AND ASSESSMENT OF CLONALITY IN THE CLINICAL DIAGNOSIS OF HCC?

Surveillance, consisting of the periodic application of a diagnostic test to individuals at specific risk of developing a given disease, is a valuable measure for early detection of diseases and providing early and adequate treatment. The procedures applied in diagnosis of HCC as evaluated in the current EASL Clinical Practice Guidelines [125] provide high sensitivity and accuracy for established liver disease and cancer. However, in spite of the implementation of HCC surveillance programs in specific high-risk populations, the low sensitivity of current screening methods [126] makes early diagnosis of HCC a challenging task.

In the West, where up to 90% of HCC cases arise in a cirrhotic background [127], ultrasound is the method of choice, also because it is frequently applied to monitor other conditions beyond HCC surveillance [125]. Apart from this, α -fetoprotein (AFP) serum level is the most widely used biomarker in the screening of liver cancer, despite of its relatively low sensitivity of 40%–60% for all stages and 40%–50% for early liver cancer [128, 129]. Therefore, there is an urgent need for more sensitive biomarkers specific for the early diagnosis of HCC.

As discussed in the following, the *TP53* R249S mutation is a promising candidate as screening marker [130] in high-risk areas since it is present in approx. 50% of HCC patients from Africa and in 90% of cases from China [80, 81], two areas with the highest dietary AFB1 exposure and the highest HBV incidences worldwide [3-6]. In western countries, however, only approximately 20% of HCC cases exhibit *TP53* mutations, without specific hotspot mutations [78].

Assessment of Liquid Biopsy for Early Tumor Detection and Monitoring

Release of tumor components such as circulating-free DNA (cfDNA) into the bloodstream has been known for decades [131]. Translation of liquid biopsy from research into clinical routine has the potential to drastically reduce the need of tissue biopsies [132]. Identification of mutations that are typical for a specific tumor entity would allow early detection and also had the potential to replace tumor markers for a more reliable and specific parameter.

Szymanska *et al.* [133] assessed the aforementioned R249S mutation of *TP53* in serum from patients diagnosed with HCC from The Gambia in West Africa. Overall, they identified the R249S mutation in 35% (10/29) of tumors. The concordance of the status of codon 249 between tumor tissues and matched plasma was 88.5%. In another study, the same mutation was identified in cfDNA obtained from Egyptian patients with HCC [134].

These results suggest that the *TP53* R249S mutation may be a dependable marker for early detection of HCC in respective populations, and that cfDNA obtained from plasma is a valid source for biomarkers of tumorigenic processes.

Assessment of Clonality by the p53 Mutation Status (MO vs. IM Multifocal HCC)

HCC remains a difficult to treat tumor. Curative strategies such as orthotopic liver transplantation (OLT), liver resection (LR) and ablative techniques are only applicable in early stages and hence a minor fraction of patients [135]. Other current therapies are rather ineffective, mainly due to the large intratumoral heterogeneity in HCC [136], which is reflected in the high prevalence of multifocal HCC with an estimated 41% to 75% of patients initially diagnosed with multifocal HCCs [137]. The fact that different lesions in the same tumor may have distinctive genomic alterations, and thus respond differently to therapies

underscores the importance of this issue [138]. Identification of the clonal origin and diverse genetic defects could significantly improve tumor staging, prognostic prediction, and treatment.

Multifocality in HCC can arise from intrahepatic metastasis (IM) of the primary tumor or multicentric occurrence (MO) caused by *de novo* carcinogenesis, either synchronously or metachronously [137]. HCC with IM or MO differ in their etiology and their clinical outcomes. MO-HCC tends to be more likely caused by liver factors such as chronic hepatic inflammation and HCV infection whereas IM-HCC is more dependent on tumor factors [139] and tends to be more poorly differentiated and more aggressive [140, 141]. While MO-HCC responds well to regional therapy under the premise of adequate hepatic functional reserve, IM-HCC tends to recur early with an inferior prognosis despite aggressive treatment interventions [142, 143]. Since treatment schemes and prognosis vary between the two types, exact assessment of the clonality of individual tumors is required.

So far, all approaches to discriminate between these two types of multifocal HCC, such as evaluation of the clinicopathologic characteristics of MO and IM patients, recurrence time, LOH analysis, and the research of diagnostic and progression markers through genomic and proteomic analyses have been unsatisfactory [144]. Recently, point mutation determination and whole genome or whole exome next generation sequencing (NGS) came up as a more effective technology [145].

The fact that *TP53* mutations are present in approx. 30% of all HCC cases [14, 19, 20], makes it an interesting marker for assessment of clonality: tumor nodules bearing identical *TP53* mutation patterns are supposed to originate from the same clone. However, *TP53* mutations with a relatively low prevalence in white populations usually occur late in hepatocarcinogenesis [146], which might be the reason why some studies succeeded to identify multifocal HCC as IM-HCC by identical *TP53* point mutations after direct sequencing of exons 4 through 9 [147], while others failed [148].

A study that assessed mutations in the coding regions of *TP53* and six other previously well-characterized HCC driver genes which have been reported to be mutated in more than 10% of HCCs (*ARID1A*, *ARID2*, *AXIN1*, *CTNNB1*, *TERT*, and *UBE3C*) by targeted NGS, besides characterizing

mutations in the *TERT* promoter region by Sanger sequencing, successfully determined the relatedness in 58% of the patients analyzed with 17% exclusively IM, 33% exclusively MO, and 8% with both MO and IM [149].

These findings implicate that assessment of *TP53* mutations, especially as part of an array of genes, could make a valuable biomarker for determination of clonality in multifocal HCC.

The *TP53* R249S mutation however, although making a valuable biomarker for HCC screening in areas with high HBC prevalence and AFB1 exposure, as discussed before, may not be suitable for assessment of clonality because it is specifically induced by AFB1 [3, 82] and selected for through GOF [41] in concert with HBx [87, 88], making it possible if not likely to be also found in unrelated tumor lesions.

9. PROGNOSTIC VALUE OF *TP53* MUTATIONS IN HCC

In 2018, HCC accounted for 841,000 new cases and 782,000 deaths. Despite continuous advances in the management of chronic liver disease, in tumor detection and oncological treatment, the prognosis of HCC remains inferior in comparison to other tumor entities [1]. Several classifications of HCC were established to differentiate tumors by focusing on histological characteristics, like tumor size, staging system (TNM and BCLC staging), grading, and serum AFP levels [18, 150]. However, the prognostic factors do not fully predict individual clinical outcome which calls for better markers in order to identify patients with poor prognosis at the time of diagnosis.

Extensive studies during the last decades revealed a wealth of molecular alterations in HCC [14, 17, 18, 78]. Strikingly, patients with HCC at the same clinical stage turned out to possibly have different molecular subtypes, which correlate well with clinical features. Yet, they are not used in routine clinical practice so far.

Generally, HCC cases can be assigned to one of two main classes: the proliferation class and the nonproliferation class [151]. The proliferation class is marked by an aggressive clinical course and characterized by distinct molecular and histologic features such as high serum AFP levels, poor cell differentiation, chromosomal instability, *TP53* and *AXIN1* mutations, and activation of oncogenic pathways such as RAS-mitogen-activated protein kinase [MAPK], AKT-mammalian target of rapamycin

[mTOR], and MET, a hepatocyte growth factor receptor. Typically, patients with HBV infection belong to the proliferation class and most gene signatures associated with a poor clinical outcome are also enriched in this class. Tumors of the nonproliferation class in contrast are stable with frequent *CTNNB1* alterations, and their gene-expression pattern resembles that of normal hepatocytes [8, 152].

Especially *TP53* alterations seem to be significantly associated with an inferior prognosis as shown by the hazard ratio for overall survival (OS) and for recurrence-free survival (RFS) in tumors with mutant, upregulated p53 expression versus low/undetectable p53 [15].

A meta-analysis of 9 studies that had been conducted in four countries with a total of 1,139 patients, confirmed a statistically significant role of *TP53* mutations on OS and RFS in HCC [16]. Another study related particularly the hotspot *TP53* mutations R249S and V157F to an inferior prognosis [146], indicating that these mutations might be used as prognostic markers [153].

Assessment of concordant different gene profiles in HCC based on the *TP53* mutation status identified two genes involved in glycolysis, the hexokinase 2 (HK2) and enolase 1 (ENO1), which are significantly overexpressed in HCC with mutant p53 [13]. Kaplan-Meier plots on OS for HK2 and ENO1 revealed that high expression levels correlate with a poor outcome. Because HCC is associated with persistent inflammation, as more than 90% of cases arise in the context of hepatic injury and inflammation [154], and since p53 has the potential to modulate the inflammatory microenvironment, tumor microenvironment, and tumor suppression [155], the authors suggest that overexpression of these glycolysis genes together with mutant p53 may directly contribute to the poor prognosis [13].

Recently, a very interesting study [156] investigated how *TP53* mutations regulate the HCC immunophenotype and assessed the impact on the prognosis of HCC. Based on the assumption that *TP53* mutations are strongly related to the immune microenvironment in HCC, the authors developed an immune prognostic model (IPM) based on differential expression of immune-related genes between *TP53*^{WT} and *TP53*^{MUT} HCC samples from 865 patients in order to differentiate patients with a low or high risk of poor clinical outcome.

They found that *TP53* mutations resulted in downregulation of the immune response and identified 37 differentially expressed genes (DEGs) based on *TP53* mutation status out of 312 immune response-related genes assessed. The two genes (*TREM1* and *EXO1*) with the highest score out of these 37 DEGs were selected for the IPM. Moreover, the high-risk patient group had higher fractions of follicular helper T cells, regulatory T cells (Tregs) and macrophages and presented higher expression of CTLA-4, PD-1 and TIM-3 compared to the low-risk group. In their study, the IPM was more accurate than clinicopathological risk factors alone, and it thus may have important implications for identifying subgroups of HCC patients with low or high risk of inferior OS.

10. POSSIBLE ROLES OF P53 IN THERAPY OF HCC

HCC is a difficult to treat tumor not only because it's highly chemotherapy-resistant and the applicability of most chemotherapy regimens is severely limited by the underlying liver disease, but also because only a small fraction of patients presents with early stages of disease, where curative strategies, such as OLT, LR and ablative techniques can be employed.

OLT appears as the treatment option of choice as it prevents complications of liver disease progression, such as hepatic encephalopathy, ascites and gastrointestinal bleeding and averts HCC recurrence. However, long waiting times for donor organs limit its use. LR is an important alternative since it reduces the stress on the limited donor organ pool and does not require long waiting times with potential treatment dropout while showing only marginally inferior long-term survival rates compared to OLT.

Locoregional therapies for HCC include a variety of protocols such as transcatheter arterial chemoembolization (TACE), trans-arterial radioembolization with Yttrium-90 (Y-90), radiofrequency (RFA), microwave (MWA) and cryoablation, irreversible electroporation (IRE), percutaneous ethanol injection (PEI), high-intensity focused ultrasound (HIFU) and stereotactic body radiotherapy (SBRT). These techniques are nowadays applied for a wide range of curative and palliative indications, including their repeat application and their combination with LR [135]. The European Association for the Study of the Liver gives a concise review of all current treatment options [125].

The multi-kinase inhibitor Sorafenib (Nexavar®) which targets the Raf–MEK–ERK pathway as well as

several receptor tyrosine kinases, including vascular endothelial growth factor receptor (VEGFR) 2 and 3, platelet-derived growth factor receptor (PDGFR), Ret, FLT3, and c-Kit [157, 158] is nowadays considered as the mainstay of palliative treatment in BCLC C patients [125]. In spite of significantly increased OS and PFS under Sorafenib treatment [159, 160] the overall success is unsatisfactory and illustrates the need for further clinical strategies.

Since the most prevalent driver mutations in HCC affect the *TERT* promoter, *TP53* and the Wnt/ β -catenin signaling pathway [14, 19, 20], drug intervention at any of these targets would possibly provide new treatment options.

Induction of Senescence and Apoptosis in p53 mut HCC

In a recent publication, Wang *et al.* [161] came forth with a very interesting approach, suggesting a combination therapy of one drug that induces senescence in *TP53* mutant cells, and a second drug that selectively eliminates senescent cancer cells (senolysis). They investigated induction of senescence through inhibition of the DNA replication kinase CDC7 which is expressed at high levels in liver cancer cell lines, and pharmacological interference with downstream pathways.

XL413 turned out to be a potent inhibitor of CDC7 [162] and was most selective in inducing a senescence marker (senescence-associated β -galactosidase) in two liver cancer cell lines. Pharmacological inhibition of CDC7 through XL413 selectively induced senescence in *TP53* mutant liver cancer cell lines but not in *TP53* WT liver cancer cell lines and not in non-transformed cell lines. XL413 hence proved to be able to selectively drive liver cancer cells with mutant p53 into senescence. In-vivo experiments in xenografts indicated that CDC7 inhibition induces senescence also in-vivo.

The fact that CDC7 inhibition indeed induces senescence was underscored by the findings that XL413 withdrawal generally did not lead to re-entry into the cell cycle, that XL413 induces senescence associated heterochromatin foci (SAHFs), and that XL413 induces expression of a number of cytokines, which are described as part of the Senescence Associated Secretory Phenotype [163]. According to these findings CDC7 inhibition has the potential for a novel treatment strategy in HCC through induction of senescence.

As the second drug, that induces apoptosis in senescent HCC cells, the anti-depressant sertraline was originally identified but the concentration of sertraline needed to induce senescent-cell apoptosis precludes its clinical use [161]. However, sertraline suppresses mTOR signaling [164] and a combination of CDC7 and mTOR inhibitors resulted in dramatic tumor growth inhibition. For this reason two other mTOR inhibitors (AZD8055 and AZD2014) were identified and both induced apoptosis in XL413-treated TP53 mutant liver and lung cancer cells. A combination treatment of XL413 and AZD8055 of TP53-mutant xenografts displayed diminished proliferation and increased apoptosis [161].

This approach is very promising as it indicates a possibility to ultimately induce apoptosis selectively in TP53 mutant cells, thus specifically targeting aberrant cells while sparing cells with WT p53 function.

However, Zhu *et al.* assessed the potential of the mTOR pathway inhibitor Everolimus alone vs. placebo in course of the EVOLVE-1 randomized clinical trial but had to report that it did not meet the primary endpoint of improved survival [165]. Considering the above discussed approach, reassessment of Everolimus in combination with a CDC7 inhibitor might be a valid option.

Restoring p53-Mediated-Apoptosis by Mortalin Knockdown

Another very interesting approach to selectively induce apoptosis in HCC with mutant p53 has been described by Lu *et al.* [166], who demonstrated that interaction between mortalin and p53 suppresses apoptosis, while mortalin knockdown induces apoptosis in HCC-derived cells with mutant p53 but not in normal cells.

Upregulation of mortalin (mtHSP70, GRP75, PBP74), a member of the heat shock protein 70 family with multiple functions [167, 168] is frequently observed in cancers and apparently contributes to carcinogenesis [169-171]. It also has been identified as a marker for HCC metastasis and recurrence [170], as mortalin protein levels correlate with tumor aggressiveness [166].

Mortalin is enriched in HCC and was shown to interact with p53 in tumor, but not in normal liver tissues [166], causing cytoplasmic sequestration of WT p53, inhibiting its transcriptional activation [172-174].

In all the five HCC-derived cell lines with p53-R249S examined, mortalin silencing with mortalin shRNA restored p53-mediated-apoptosis, while cells that lacked mutant p53 did not show apoptosis subsequent to mortalin knockdown [166].

This selective effect which depends on the p53 mutation status makes targeting the mortalin-p53 interaction a promising therapeutic strategy for HCC.

E3 Ubiquitin Ligase ARF-BP1 as Potential Therapeutic Target

ARF (p14^{ARF}), an alternative transcript of the Ink4a/ARF tumor suppressor locus, binds directly to and inactivates MDM2, resulting in p53 stabilization and cell cycle arrest or apoptosis [73, 74]. ARF can also induce cell growth arrest in tumor cells that lack a functional TP53 or p21 gene and it inhibits the proliferation of cells that are null for both p53 and MDM2 [175].

In 2005, a new protein named ARF-BP1 (HECTH9, HUWE1, MULE) was purified from H1299 cells [176] and turned out to be an E3 ubiquitin ligase. Inactivation of ARF-BP1, but not MDM2 was shown to induce cell growth repression with G2/M arrest in p53 null cells in a manner reminiscent of ARF induction. In p53 WT cells, ARF-BP1 directly binds and ubiquitinates p53, and inactivation of endogenous ARF-BP1 is crucial for ARF-mediated p53 stabilization. Thus ARF-BP1 appears to be a critical mediator of both the p53-independent and p53-dependent tumor suppressor functions of ARF and may therefore serve as a potential target for therapeutic intervention in tumors regardless of the p53 status [176, 177].

ARF-BP1 was also shown to associate with and ubiquitinate MYC as one of at least three ubiquitin ligases (ARF-BP1, SKP2, and FBXO28) that are required for MYC function [178-180]. Depletion of ARF-BP1 arrested cell proliferation and inhibited expression of MYC-activated target genes in several human tumor cell lines and in mouse xenografts [178, 181]. This underscores again the potential of ARF-BP1 inhibition as a therapeutic option in HCC, especially, but not only for patients with mutant p53-R249S, which gains its function via c-myc activation [41] as discussed earlier.

P53 and Gene Therapy

The tumor suppressor p53 is impaired in approx. 50% of human cancers [35, 36] by either base substitutions resulting in missense or nonsense

mutations, or gene deletion. Since p53 is a central player in maintenance of genome integrity [34, 49, 50], and since expression of WT p53 can result in apoptosis in human tumor cells, reintroduction of WT tumor suppressor function to cells lacking the gene provides an attractive strategy to fight cancer cells from within [182].

A number of proof-of-principle studies using recombinant replication-defective adenoviral vectors (Ad-p53) for gene transfer showed that a functional p53 gene can be stably introduced into cancer cells and that overexpression of p53 in cancer cells induces growth arrest and apoptosis. Ad-p53 injections have mediated tumor regression and growth arrest as monotherapy, or in combination with radiation therapy or chemotherapy [183].

A one-year follow-up study [184] of p53 gene therapy using an Ad-p53 vector in combination with TACE (p53 treatment group) vs. TACE as monotherapy (control group) in HCC patients reported a superior outcome in the combination therapy arm with a total effective rate of 58.3% for the p53 treatment group vs. 26.5% for the control group ($P < 0.05$). Also the 3-, 6- and 12-months survival rates were significantly higher for the p53 treatment group with 89.71%, 76.13%, and 43.30%, respectively compared to the control group with 68.15%, 36.98%, and 24.02% ($P < 0.01$). Except for minor adverse effects including fever (51.5%, $P = 0.006$) and pain of muscles and joints (13.2%, $P = 0.003$), no severe vector-related complications were identified, emphasizing the safety of the vector system. The Ad-p53 gene therapy in combination with TACE therefore proved to be safe and superior to TACE monotherapy, and is indeed an interesting modality for treatment of advanced HCC.

However, the authors provided no data about the *TP53* mutation status of the patients in the study cohort. The individual p53 status with respect to whether mutant p53 or no p53 is expressed in conjunction with the respective individual outcome would be of considerable interest, having in mind the dominant-negative effect of mutant p53 on WT p53 protein [94-96]. In this light, *TP53* gene transfer would only make sense for p53 null patients. Also the 43.30% one-year survival rate in the p53 treatment group, though significantly higher than that in the control group, cannot be satisfying and calls for alternative approaches especially for patients who are expressing mutant p53.

11. DISCUSSIONS AND CONCLUSION

Mutations of the *TP53* tumor suppressor gene occur overall in approx. 30% of HCC cases [14, 20-22] with much higher frequencies in Sub-Saharan Africa and China [80, 81]. In HBV positive HCC, WT p53 can be impaired either directly through HBx [3, 87-89] or the product of a nonsense mutation of the large S gene [91]. While HCC in Western countries exhibits various *TP53* mutations [78], cases in Africa and China are marked by a specific mutation that is related to HBV infection and exposure to AFB1 [3, 82]. This mutation, which is present in approx. 50% of cases from Africa and in 90% of cases from China [80, 81], results in the substitution of a serine for an arginine in codon 249 (R249S) and mediates gain-of-function activity [89, 108, 109] which might be causative to HCC [3, 5, 78, 79] via c-myc activation [41].

By inhibiting TAp63, mutant p53 can initiate a pro-invasive transcription program [117, 118], which might be one explanation for the poor prognosis related to mutant p53 in HCC [13, 15, 16], a fact which makes evaluation of the *TP53* mutation status an important prognostic marker [153]. In conjunction with the *TP53* mutation status, the immunoprosthetic model suggested by Long *et al.* [156], has the potential to provide a sensitive framework for accurate risk stratification. Moreover, Chen *et al.* [13] identified two genes, HK2 and ENO1 which are differentially expressed between WT vs. mutant p53 and demonstrated that overexpression going together with mutant p53 is related to inferior prognosis. Since both genes are involved in glycolysis they may contribute to ongoing inflammatory processes and thereby possibly trigger cancerogenesis. If this substantiates, HK2 and ENO1 will not only make dependable prognostic markers but possibly also therapeutic targets if a specific inhibitor becomes available.

A general problem is the usual late diagnosis of HCC in spite of surveillance programs for at-risk populations. In AFB1 exposed populations, the R249S marker mutation could successfully be detected in cfDNA obtained from serum [133, 134], making this approach a good candidate for a dependable biomarker for early HCC detection. *TP53* mutations, especially in concert with other marker loci [149], may also serve as a diagnostic marker for assessment of clonality to distinguish MO from IM in multifocal HCC, which is of significance with respect to their different therapeutic approaches and clinical courses [139-143].

Mutant p53 could even be advantageous in therapy. Wang *et al.* [161] demonstrated that HCCs with mutant p53 can selectively be driven into senescence through CDC7 inhibitors, and that mTOR inhibitors have the potential to initiate apoptosis in these senescent cells. Another interesting approach is inhibition of ARF-BP1 [176], a p53 antagonist, resulting in growth repression and G2/M arrest, regardless of the p53 status [176, 177]. However, esp. carriers of the R249S mutation may benefit since depletion of ARF-BP1 inhibited expression of MYC-activated genes, possibly counteracting the p53-R249S mediated c-myc activation [178, 181]. Lu *et al.* [166] demonstrated that knockdown of the heat-shock protein mortalin in HCC-derived cell lines restores p53-mediated apoptosis selectively in cells with mutant p53.

Several proof-of-principle studies showed that restoring WT p53 activity through gene therapy is a promising scheme and might be a valuable option esp. for p53-null patients [183, 184].

The scope of this review is to collect and connect recent information around mutant p53 in HCC in order to summarize the current research status and to suggest possible approaches for surveillance, diagnosis, risk stratification and therapy.

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