expert reviews
in molecular medicine

Hepatocellular carcinoma: molecular interactions between hepatitis C virus and p53 in hepatocarcinogenesis

Mónica Anzola and Juan José Burgos

Hepatocellular carcinoma (HCC) is the most important primary hepatic cancer and is a common cancer type worldwide. Many aetiological factors have been related to HCC development, such as liver cirrhosis, hepatitis viruses and alcohol consumption. Inactivation of the *p53* tumour suppressor gene is one of the most common abnormalities in many tumours, including HCC. p53 is of crucial importance for the regulation of the cell cycle and the maintenance of genomic integrity. In HCC, hepatitis B and C virus (HBV and HCV) effect carcinogenic pathways, independently leading to anomalies in p53 function. Several authors have reported that some HCV proteins, such as the core, NS5A and NS3 proteins, interact with p53 and prevent its correct function. The mechanisms of action of these HCV proteins in relation to p53 are not completely clear, but they might cause its cytoplasmic retention or accumulation in the perinuclear region where the protein is not functional. The identification of the interactions between p53 and HCV proteins is of great importance for therapeutic strategies aimed at reducing the chronicity and/or carcinogenicity of the virus.

Hepatocellular carcinoma (HCC) is the most common primary malignant tumour of the liver, and it ranks fifth in overall frequency relative to all cancers. An estimated 372 000 new cases of HCC are diagnosed each year, constituting 4.6% of all new human cancers (6.3% in men; 2.7% in women). HCC has the fourth highest mortality rate of cancers worldwide and is responsible for an estimated one million deaths annually (Refs 1, 2). The highest incidences occur in eastern and

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Institute URL: http://www.ehu.es

Accession information: DOI: 10.1017/S1462399403006926; Vol. 5; 19 November 2003

southeastern Asia, some of the western Pacific islands and sub-Saharan Africa. Intermediate incidences are found in eastern and southern Europe, the Caribbean, Central America and western Asia. These variations between ethnic groups, sexes and geographical regions are explained by the nature, frequency and time of acquisition of the main risk factors (Ref. 3), as discussed below.

The overall survival rate for HCC is poor. Surgical resection and orthotopic liver transplantation are the only curative treatment options but are suitable for few patients. The disease often has a fulminant course (i.e. occurring suddenly, with great severity), and screening of even at-risk populations has been insufficient, so in most cases HCCs are diagnosed only at an advanced stage when surgical therapy is not possible. There is no standard treatment for patients with unresectable HCC and, when untreated, patients with inoperable HCC have a median survival of three months (Refs 4, 5, 6). Furthermore, following resection and liver transplantation, there is a high recurrence rate of HCC. However, although HCC has historically had a dismal prognosis, it is now being detected earlier as a result of improved radiological imaging and surveillance. Such screening offers the best hope for early detection, eligibility for treatment and improved survival.

Risk factors for HCC

HCC is a multistage disease whose occurrence is linked to environmental, dietary and lifestyle factors. The major risk factors include: (1) chronic infections with the hepatitis B or C virus (HBV or HCV) (discussed further below); (2) exposure to dietary aflatoxin B1, a potent mycotoxin produced by fungi in peanuts, corn and grains that is carcinogenic in humans; (3) exposure to vinyl chloride, a propellant found in aerosols; (4) haemochromatosis, a rare genetic disease that results in an over abundance of iron in tissues; and (5) alcohol consumption (Ref. 3). Many of these factors cause cirrhosis – the formation of scar tissue in the liver – which is also a major risk factor for HCC: HCC develops in more than 90% of patients with cirrhosis of different aetiologies (Ref. 7).

The increasing prevalence of HCC seems related to the widespread distribution of HCV infection, as 80% of cases arise following chronic infection caused by this agent (Ref. 8). In some regions, such as southern Africa and Qidong (China) (Ref. 9), infection with HBV and exposure to aflatoxins in the diet act synergistically to amplify risk. From a public health perspective, hepatitis virus vaccination programs and efforts both to reduce aflatoxin exposure and to attenuate the toxicological consequences of unavoidable exposure should have a major impact on the incidence of this disease (Refs 10, 11).

Hepatitis and hepatocarcinogenesis

Hepatitis is an inflammation of the liver characterised by a diffuse or focal necrosis that affects liver structure. It is mainly caused by specific hepatitis viruses, or damage following consumption of alcohol or drugs. Viral hepatitis affects several hundreds of millions of people worldwide and is a cause of considerable morbidity and mortality, both from acute infection and from chronic sequelae that include, in the case of hepatitis B, C and D, chronic active hepatitis and cirrhosis (Refs 12, 13).

So far, eight hepatotropic viruses in humans have been identified, including the hepatitis A, B, C, D, E, F and G viruses and TTV (transfusion transmitted virus). These viruses produce a wide range of hepatic pathology, from transient to chronic infections, and from subclinical to fulminant hepatic failure, cirrhosis and HCC (Ref. 14). HBV and HCV are the main causal agents of chronic hepatitis (Refs 15, 16), and approximately 5–10% of HBV and 75% of HCV cases become chronic.

Although the mechanism of HBV and HCV chronicity is uncertain, the epidemiological association of chronic HBV or HCV infection with HCC has been well established and is discussed in the following sections, particularly focusing on the molecular interactions of HCV. It has also been seen that HBV and HCV infection induces, by direct and indirect mechanisms, cellular damage that causes an increase in cellular regeneration and proliferation, which in turn increases the development of HCC (Ref. 17). For patients with chronic viral hepatitis, antiviral treatment might reduce the risk of the subsequent development of HCC, and screening for early-stage HCC might lead to the initiation of curative treatment strategies. For patients with established HCC, the presence of concurrent chronic viral hepatitis or cirrhosis might affect prognosis and survival, and could alter treatment options because of impaired hepatic function.

Accession information: DOI: 10.1017/S1462399403006926; Vol. 5; 19 November 2003

HBV infection and hepatocarcinogenesis

Epidemiological data have demonstrated that the causes of HCC vary according to the geographic region. The incidence of HCC is greater in areas where HBV infection is endemic, as in southeastern Asia and the centre and south of Africa (Ref. 18). The virus is often transmitted by a parenteral route, typically by contaminated blood or its products (Refs 19, 20, 21, 22, 23).

The effectiveness of HBV vaccination in the primary prevention of chronic HBV infection and HCC has already been demonstrated in pilot vaccination projects (Ref. 24). It has also been shown that administration of interferon α (IFN-α) is effective in the secondary prevention of HCC in patients with chronic HBV infection; it has long-term beneficial effects in terms of HBV clearance, reduction of HCC and prolonging survival (Refs 25, 26).

Two direct mechanisms of action have been described for HBV in hepatocarcinogenesis. First, following viral infection, the HBV genome might integrate in sites within the host genome that play a crucial role in the cell cycle, altering the function of these genes and thereby leading to cancer progression (Refs 27, 28, 29); second, host oncogenes might be transactivated by the HBV protein HBx or by another truncated protein derived from the pre-S2/S region of the HBV genome (both regions are commonly integrated into the host genome) (Refs 30, 31). HBV can also cause HCC by an indirect mechanism in which HCC results from chronic hepatic injury and cirrhosis caused by viral infection (Refs 32, 33, 34).

HCV infection and hepatocarcinogenesis

HCV infection has a wide spectrum of cellular tropism (e.g. dendritic cells and peripheral blood mononuclear cells) (Refs 35, 36, 37, 38, 39, 40) and clinical presentations, including asymptomatic chronic carriage, acute hepatitis, chronic hepatitis, cirrhosis, HCC and extrahepatic manifestations (Ref. 8). HCV is transmitted through blood and blood products; sexual and perinatal transmissions are less important routes.

In general, HCC develops only after two or more decades of HCV infection and the increased risk is restricted largely to patients with cirrhosis or advanced fibrosis. Factors that predispose to HCC among HCV-infected individuals include male sex, older age, HBV coinfection, heavy alcohol intake, and possibly diabetes and a transfusion-related source of HCV infection (Refs 41, 42).

Since so little is known about the biology of HCV, it is presently unclear how this RNA virus establishes a persistent infection. However, it is known that there is a very rapid turnover of plasma virus in patients, with particle half-lifes of 100–182 min (Ref. 43). Recently, it has been suggested that subversion of the humoral immune response, specifically neutralising antibody production, might allow HCV to persist (Ref. 44).

Successful antiviral therapy of patients with HCV-related cirrhosis can reduce the future risk for HCC. Nowadays, treatment of HCV infection with pegylated IFN (a complex of IFN and polyethylene glycol) and ribavirin is relatively effective (Ref. 45). New therapeutic strategies will be required in the future, the most important challenge being the development of an HCV vaccine.

HCV genome, proteins and pathogenicity

Genetic organisation and protein function

HCV belongs to the *Hepacivirinae* genus within the Flaviviridae family. HCV measures 30–60 µm and is an enveloped virus with a single-stranded, linear, positive-sense RNA genome, which is ~9.6 kb in length (Fig. 1). It contains a large open-reading frame (ORF) capable of encoding a polyprotein precursor of about 3010 amino acids. This polyprotein is post-translationally cleaved into at least ten polypeptides, including three structural proteins (core, E1 and E2) at the N-terminal end and multiple nonstructural proteins (NS2 to NS5). The 5' noncoding region precedes the large coding sequence and represents the most highly conserved sequence among the different viral isolates (Refs 46, 47, 48). A series of three short ORFs exist in this region. ORF2 and ORF3 encode peptides rich in helix-breaking amino acids; however, the function of these small ORFs is currently not understood (Refs 49, 50).

The core protein produced by cleavage at the N-terminal end of the polyprotein precusor is a nonglycosylated, basic, 19–22 kDa protein (p22) that functions as a nucleocaspsid protein (Fig. 1). Its amino acid sequence is highly conserved among different isolates of HCV. The other structural proteins thought to be formed by cleavage at the N-terminal end are E1 (gp35) and E2 (gp70) (sizes estimated from sequence data and expression in vitro), which are probably surface

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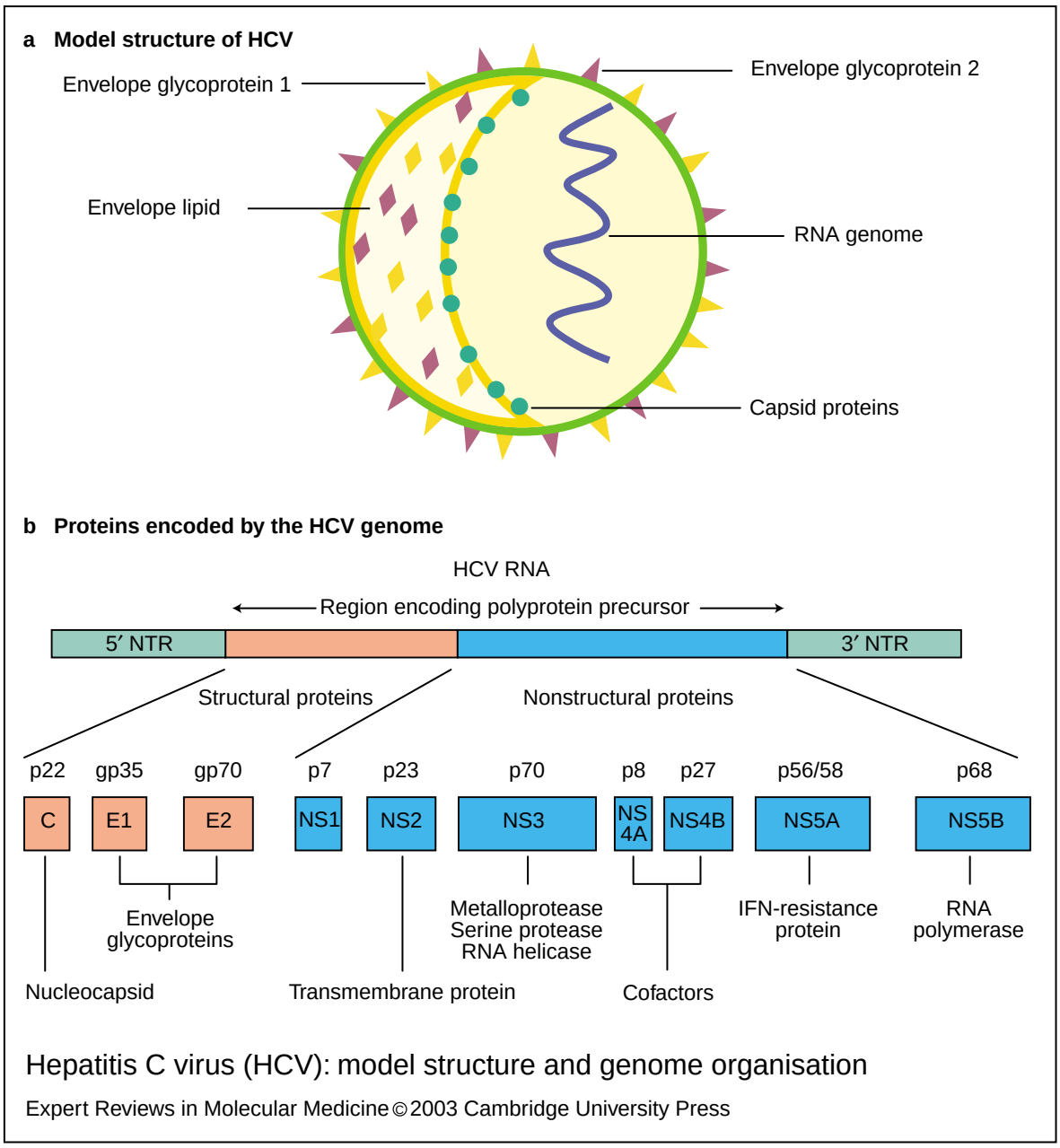

Figure 1. Hepatitis C virus (HCV): model structure and genome organisation. (a) Model structure of HCV. The left-hand side of the illustration shows the viral surface of envelope lipids and glycoproteins; the right-hand side shows the RNA genome encased by capsid proteins. (b) Proteins encoded by the HCV genome. HCV is formed by an enveloped particle harbouring a plus-strand RNA of ~9.6 kb. The genome carries a long open-reading frame (ORF) encoding a polyprotein precursor of 3010 amino acids. Translation of the HCV ORF is directed via a ~340 nucleotide long 5' nontranslated region (NTR) functioning as an internal ribosome entry site; it permits the direct binding of ribosomes in close proximity to the start codon of the ORF. The HCV polyprotein is cleaved co- and post-translationally by cellular and viral proteases into ten different products, with the structural proteins [core (C), E1 and E2] located in the N-terminal third and the nonstructural (NS2–5) replicative proteins in the remainder. Putative functions of the cleavage products are shown.

proteins of the viral envelope (Ref. 51). These glycoproteins (E1 and E2) have been studied as potential targets for viral detection and HCV vaccine development, since they contain hypervariable regions that are important as immunogenic epitopes (Refs 52, 53).

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The NS proteins NS2–5 include enzymes necessary for protein processing (proteases) and viral replication (RNA polymerase) (Fig. 1). The NS2 region is extremely hydrophobic, but its function has not been identified. NS2/NS3 polyprotein possesses an autocatalysing metalloprotease activity that cleaves the NS2/NS3 junction. The NS3 region encodes a 70 kDa protein that contains two functional domains: a viral protease involved in cleavage of the nonstructural region of the polyprotein and a helicase enzyme that is probably involved in unwinding the RNA genome for replication. The NS4 region is also extremely hydrophobic and shows 50% sequence homology among the different HCV types, but its function is unknown. The NS5B region encodes a 116 kDa RNA polymerase that replicates the RNA genome and contains the Gly-Asp-Asp motif common to viral RNA-dependent RNA polymerases (Refs 54, 55). No function has yet been attributed to NS5A. However, it has been reported to be a cytoplasmic phosphoprotein and appears to be involved in mediating the resistance of HCV to the action of IFN (Ref. 56).

Virus genotype considerations

HCV consists of a family of highly related but nevertheless distinct genotypes. Phylogenetic analysis of NS5 and E1 nucleotide sequences from samples obtained worldwide has led to the identification of six major genetic groups and 14 subgroups (Ref. 57). Genotypes 1–3 account for almost all infections in Europe, whereas genotype 4 is prevalent in Egypt and Zaire, genotype 5 in South Africa and genotype 6 in Hong Kong.

The sequence differences observed between HCV groups suggest that virus–host interactions might vary, which could result in differences in pathogenicity and the response to antiviral therapy. Several reports have suggested correlation among the various genotypes with the severity of liver disease, the outcome of IFN treatment, and the development of HCC (Refs 58, 59, 60, 61). Genotype 1, most commonly found in the USA, is less amenable to treatment than are genotypes 2 or 3. Thus, information on the genotype of the virus is important to guide treatment decisions and clinical trials. The diversity of HCV might also explain the multiple infections and coinfections with different HCV subtypes in the same individuals (Refs 62, 63). Furthermore, this heterogeneity makes the control of HCV by vaccination difficult.

In addition to the sequence diversity observed between HCV groups, there is considerable sequence heterogeneity among almost all HCV isolates in the N-terminal region of E2, implying that this region might be under strong immune selection. Indeed, sequence changes within this region might occur during the evolution of disease in individual patients and play an important role in progression to chronicity (Refs 64, 65).

Role of p53 in mechanisms of hepatocarcinogenesis

Hepatocarcinogenesis is a complex process associated with the accumulation of genetic and epigenetic changes that run through steps of initiation, promotion and progression. Molecular genetic studies have revealed that genetic alterations of proto-oncogenes and tumour suppressor genes are of great importance in human carcinogenesis (Ref. 18). Indeed, more than 20 genes involved in at least four carcinogenic pathways are implicated in the development of HCC (Refs 66, 67). These multiple genetic alterations seem to be correlated with multistep carcinogenesis and tumour progression. Activation of oncogenes of the *ras* family and others has been detected during chemically induced HCC in rodents, but there is little evidence of such activation in human tumours (Ref. 68); alterations in oncogenes have been detected in only a small proportion of HCC cases (Ref. 69). By contrast, there is evidence that tumour suppressor genes such as those encoding p53, pRb and $p16^{INKa}$ are altered in different stages of hepatocarcinogenesis and that this might directly or indirectly cause chromosomal instability, and promote cellular proliferation and neovascularisation (Refs 70, 71). Frequent loss of one allele of the *p53* tumour suppressor gene, located at chromosome 17p13.1 (Ref. 72), and mutations in the remaining allele have been reported to occur in diverse human cancer types including HCC (Refs 73, 74, 75, 76). *p53* encodes a 393 amino acid nuclear phosphoprotein, p53, that binds specific DNA sequences in the human genome (Ref. 77) (Fig. 2).

p53 function

Although it might yet be found to have other functions, the p53 protein has been shown to function as a transcriptional regulatory protein (Refs 78, 79, 80). It activates the transcription of several genes with roles in the control of the cell

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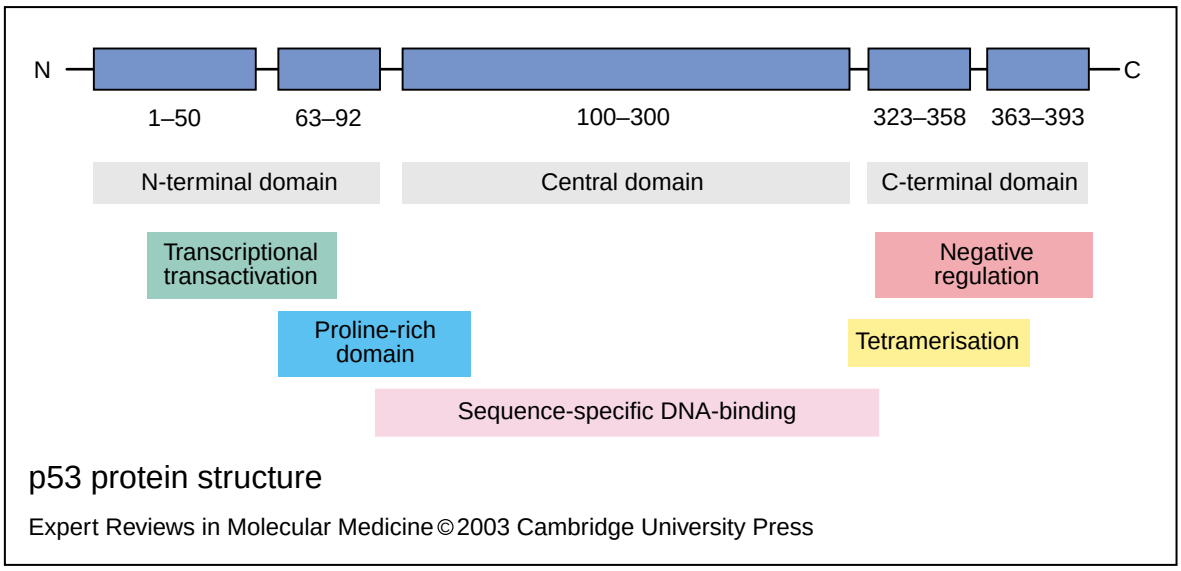

Figure 2. p53 protein structure. The 53 kDa nuclear phosphoprotein p53, of 393 amino acids, comprises several domains, including an acidic N-terminal region containing the transactivation domain, a core containing the sequence-specific DNA-binding domain and a complex C-terminal domain with multiple functions.

cycle, including GADD45 (a growth arrest, DNA-damage-inducible gene) (Ref. 81) and those encoding $p21^{WAF1/CIP1}$ (an inhibitor of cyclin-dependent kinase activity, and hereafter referred to as p21) (Ref. 82), MDM2 (a negative regulator of p53) (Ref. 83) and 14-3-3σ (a regulator of G2–M progression) (Ref. 84). It also activates various genes that probably function in apoptosis, including the gene for Bax (a pro-apoptotic, Bcl-2-related protein) and several genes encoding proteins involved in the generation of reactive oxygen species (Refs 78, 80, 85).

p53 has been implicated in the control of the cell cycle (arresting the cycle at G1 and G2 to assess genomic integrity), DNA repair and synthesis, cell differentiation, repression of transcription, genomic plasticity and apoptosis (Refs 72, 73, 86, 87, 88, 89, 90, 91). It plays a key role in the recognition and response to DNA damage. When DNA is damaged, the cell expresses a higher level of p53. This protein then blocks the G1 phase, allowing the cell to activate the DNA repair systems. When the damage is too great to be repaired, *p53* overexpression activates apoptosis, destroying the cell (Refs 86, 92, 93, 94, 95).

p53 regulation

The p53 protein is subject to tight regulation at multiple levels. Three major levels of regulation are recognised: protein stability, protein activity and subcellular distribution. Comprehensive reviews on other aspects of p53 regulation, such as p53 post-translational modifications and their effects on p53 activities have been published recently (Refs 96, 97).

The p53 protein shuttles between the cytoplasmic and nuclear compartments in a cell-cycle-dependent fashion (Refs 98, 99). The accumulation of p53 in the nucleus is crucial for its tumour suppressive activity. Prevention of nuclear accumulation provides an efficient mechanism by which tumour cells might continue to proliferate in the presence of wild-type p53. Indeed, cytoplasmic sequestration of p53 has been commonly observed in certain tumours, such as neuroblastomas, and breast and colon cancer (Refs 100, 101, 102). In at least a subset of these tumours, MDM2 is responsible for the cytoplasmic accumulation of p53 (Ref. 103), and other proteins have also been implicated in cytoplasmic retention (Refs 104, 105, 106, 107, 108). Several viral proteins also influence p53 localisation, such as human papilloma virus (HPV) E6 protein (Ref. 109), adenoviral E1B 55 kDa protein and the HBV HBx protein (Refs 110, 111, 112). In addition, defects in p53 import/export have also been reported in different tumour types (Refs 113, 114). As a shuttling protein, p53 is constantly transported through the nuclear pore complex. p53 nucleocytoplasmic transport is

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carried out by a bipartite nuclear localisation signal (NLS) located at its C-terminal domain and two nuclear export signals (NESs), located in its N- and C-terminal regions. Mutations disrupting the NLS block p53 export and prevent MDM2-mediated cytoplasmic degradation (Ref. 115).

p53 alterations and hepatocarcinogenesis

Approximately 50% of all cancers involve a defective *p53* gene, usually inactivated by a point mutation or gene deletion. These alterations are thought to prevent oligomerisation and formation of the p53 tetrameric complexes that bind to specific DNA sequences, thereby altering the physiological function of the wild-type protein (Refs 72, 86, 88, 92, 116, 117). Human cancers containing a p53 mutation are more aggressive, more apt to metastasise, and more often fatal. Thus, detection of p53 abnormalities might reveal clues about the aetiology and molecular pathogenesis of human cancer (Refs 93, 118).

p53 gene mutations and HBV DNA integration in the genome of the host are the most frequent genetic changes known in human HCC. p53 gene alterations are present in 30–60% of patients with HCC (Ref. 119), and a mutation hotspot in p53 has been described in HCC patients in areas of high aflatoxin exposure (Ref. 120). Metabolites of aflatoxin B1 promote apurinic sites and G to T mutations in chromosomal DNA, and 50% of HCC patients from high aflatoxin exposure areas were found to harbour a codon 249 G to T transversion in *p53* (Ref. 120); thus, the aflatoxin B1 that contaminates foods in endemic areas has a clear role in hepatocarcinogenesis.

Wild-type p53 is polymorphic at residue 72, where a single-base change causes a substitution of proline (Pro) for arginine (Arg) (CCC→CGC) in the transactivation domain (Ref. 121). Although the clinical significance of the variants is not known, several studies have been carried out on the frequency of these two alleles and the possible relationship with risk of cancer development. One study has shown that frequent loss of the proline allele in HCV-associated carcinogenesis of the liver might play a role in hepatocarcinogenesis. However, further studies on this matter should be carried out to clarify this point (Ref. 122). Furthermore, Okada et al. (Ref. 123) found a significant correlation between male homozygotes for p53Pro with HCV type 1b infection. Thus, there might be a relationship between this polymorphism in p53 and HCV infection.

Relationship between HCV proteins and p53 in hepatocarcinogenesis

Various HCV proteins have been reported to be involved in the process of hepatocarcinogenesis, but principal roles for three proteins has been reported – core, NS3 and NS5A proteins – through interactions with p53 in particular (see below). In addition, NS4A and NS4B inhibit p21 expression post-transcriptionally, and mediate translational inhibition and, probably, increased degradation of certain cellular proteins (Refs 124, 125, 126, 127).

Core protein

The HCV core protein is a structural viral protein that packages the viral genomic RNA. In addition to this function, the core protein can have opposing effects on cell growth – promoting both apoptosis and cell proliferation – depending on its subcellular localisation and consequent effect on the cell cycle inhibitor p21 (Refs 126, 128, 129, 130, 131, 132, 133, 134, 135) (Fig. 3).

The HCV core protein is produced as an innate form (amino acids 1–191), with a mature form (amino acids 1–173) formed by processing. In the cytoplasm, the innate form binds to the mature form to give a heteromultimer, which prevents transportation of the mature form to the nucleus. In the cytoplasm, the innate form activates p53, which in turn, as a transcription factor for p21, enhances the expression of p21. It is not clear how the innate form activates p53, but it binds to p53 and this might lead to p53 activation by stabilisation of the protein (Refs 129, 130, 136). If the level of mature core protein exceeds the binding capacity of the innate form, it enters the nucleus, where it can reduce p21 expression by a pathway independent of p53 (Refs 137, 138, 139). The p21 promoter has a core-responsive element, exactly overlapping tumour growth factor (TGF)/butyrate-responsive elements. In this case, core protein activates p21 through the element by stimulating a butyrate pathway (Ref. 128).

In addition, HCV core protein regulates p73, a member of the p53 tumour suppressor family. p73 is involved in neurogenesis and natural immune responses, and seems to be strongly involved in malignancy acquisition and maintenance (Ref. 140). The interaction between p73 and the HCV core protein results in nuclear translocation of the core protein. Furthermore, the interaction with core protein prevents p73α-, but not p73β-,

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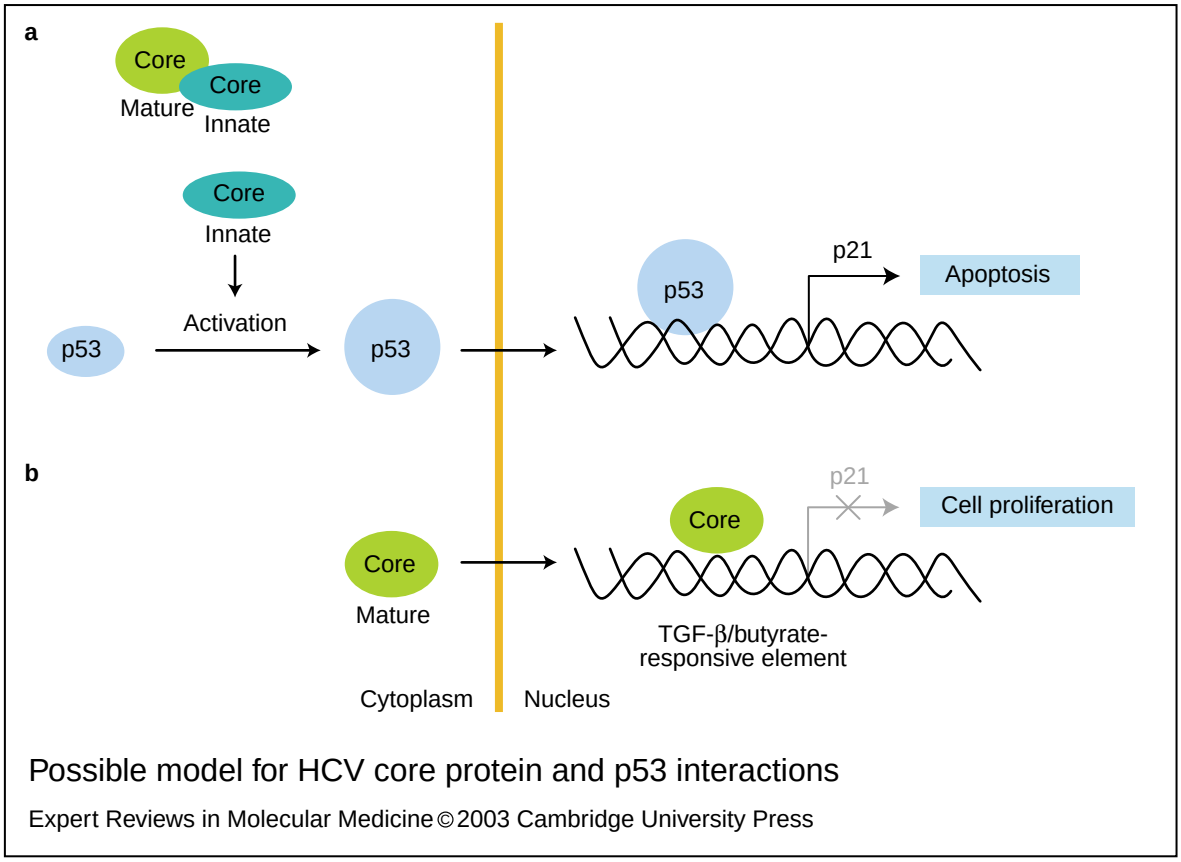

Figure 3. Possible model for HCV core protein and p53 interactions. The HCV (hepatitis C virus) core protein has two forms: a mature form and an innate one formed by processing. (a) In the cytoplasm, the innate form binds to the mature form; formation of this heteromultimer prevents transportation of the mature form to the nucleus. In the cytoplasm the innate form activates p53, which in turn, as a transcription factor for p21, enhances the expression of p21. (b) In the nucleus, the mature form reduces p21 expression by a pathway independent of p53. A core-responsive element overlaps the TGF-β/butyrate-responsive element on the p21 pathway; the core activates p21 by stimulating a butyrate pathway.

dependent cell growth arrest in a p53-dependent manner. Thus, the effect of the HCV core protein on p73 function might contribute to HCV pathogenesis (Ref. 135).

NS3 protein

The HCV NS3 protein might exert its hepatocarcinogenic effect at an early stage on host cells. It has also been postulated that it might bring about mutation of the *p53* gene and transformation of hepatocytes, but this is controversial (Refs 141, 142, 143, 144).

Wild-type p53 forms a complex with the NS3 protein (Ref. 141). A portion near the C-terminus of wild-type p53 (amino acids 301–360), which contains the oligomerisation domain, is important for this complex formation with NS3. Consistent with this finding, NS3 protein can specifically repress the promoter activity of *p21* in a dose-dependent manner by modulating the activity of p53 (Ref. 145). The effect is not cell-type specific and is synergistic with the effect of the HCV core protein.

NS5A protein

The HCV NS5A protein is a 56–58 kDa phosphoprotein. Although associated with other virus-encoded proteins as part of the viral replicase complex positioned on the cytoplasmic side of the endoplasmic reticulum, a role for NS5A in viral replication has not been defined. Post-translational modifications of NS5A include phosphorylation and potential proteolytic processing to smaller molecular weight forms able to translocate to the nucleus (Ref. 146). Truncated versions of NS5A can act

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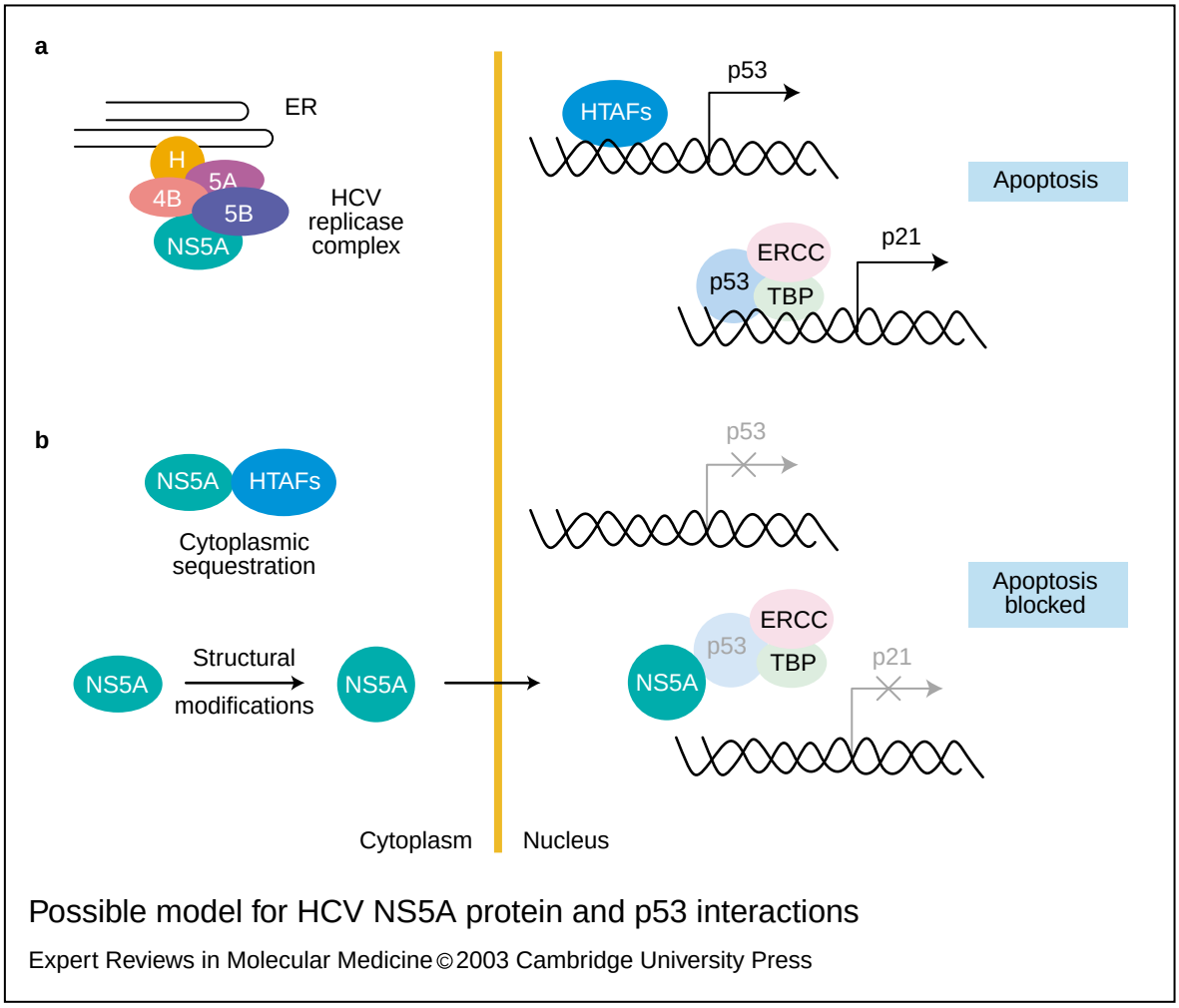

Figure 4. Possible model for HCV NS5A protein and p53 interactions. (a) Initially, NS5A protein is associated with other viral-encoded proteins as part of the viral replicase complex on the cytoplasmic side of the endoplasmic reticulum. p53 transcription mediated via hTAFs (essential coactivators of p53 transcription), and p21 expression mediated by p53 proceed as normal, allowing apoptosis. (b) During HCV (hepatitis C virus) infection, NS5A partially sequestrates hTAF(II)32 and hTAF(II)28 in the cytoplasm. In addition, post-translational modifications of NS5A give rise to smaller molecular weight forms that are able to translocate to the nucleus and interact with TATA-box-binding protein (TBP), p53 and excision repair cross complementing factor 3 (ERCC3). These interactions lead to inhibition of p53 transcription and action, and thereby inhibit apoptosis.

as transcriptional activators, whereas other recently characterised interactions of NS5A with cellular proteins – including members of the cellular signalling apparatus, transcription activation machinery and cell-cycle-regulatory kinases – indicate its pleiotropic role in HCV–host interactions (Refs 146, 147, 148, 149). Many of these interactions block the apoptotic cellular response to persistent HCV infection, which suggests a potential function of NS5A in inducing chronic liver diseases and HCC associated with HCV infection.

NS5A can suppress the binding of p53 to its specific DNA sequences by localising p53 in the perinuclear region. In this way NS5A inhibits p21 expression and apoptosis mediated by p21 (Refs 127, 150, 151, 152). NS5A also binds to TATA-box-binding protein (TBP) and p53, forming an heteromeric complex and inhibiting the binding of both p53 and TBP to their specific DNA binding sequences (Ref. 125). In addition, NS5A inhibits the formation of p53–TBP–excision repair cross complementing factor 3 (ERCC3) complex (Ref. 125). Furthermore, NS5A protein

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might inhibit p53 function by sequestering hTAF(II)32 and hTAF(II)28, which are essential coactivators of p53 (Refs 127, 153) (Fig. 4).

NS5A has recently been reported to inhibit single-strand RNA-dependent protein kinase (PKR), an IFN-induced kinase. NS5A might thus contribute to IFN resistance in HCC (Refs 150, 154).

Implications for immunohistochemistry analysis

The presence of p53 alterations has been widely evaluated by immunohistochemistry (IHC): mutant p53 has a much longer half-life than wild-type p53, which leads to accumulation of the protein in the nucleus, where it can be detected by IHC. The interactions between p53 and HCV proteins lead to nonfunctional p53 without p53 mutation and nuclear accumulation. The possible interaction between HCV proteins and p53 must therefore be taken into account in HCV-positive patients when evaluating p53 functionality, to avoid false negative results from classical analysis.

Concluding remarks and clinical implications

Chronic infection with HCV often results in cirrhosis and enhances the probability of developing HCC. Although the underlying mechanisms that lead to malignant transformation of infected cells remain unclear, it is known that the products encoded by the HCV genome interfere with and disturb intracellular signal transduction. One of the most common proteins affected by HCV proteins is the p53 tumour suppressor protein. Some HCV proteins have been shown to interact with p53, interfering with p53-dependent cell cycle control: core, NS3 and NS5A proteins bind to and modulate the activity of p53, causing the abrogation of apoptosis and uncontrolled cell proliferation. Identification of the interactions between p53 and HCV proteins and their effects on cell-cycle control should be of great importance; therapeutic strategies to inhibit protein–protein interactions might provide a first step towards reducing the chronicity and/or carcinogenicity of the virus.

Acknowledgements and funding

We thank our three anonymous peer reviewers for their helpful comments on this article. Monica Anzola is the holder of a grant from the Basque Government (BFI 02.140).

References

1 Hussain, S.A. et al. (2001) Hepatocellular carcinoma. Ann Oncol 12, 161-172, PubMed: 11300318
2 Brechot, C. et al. (2000) Molecular bases for the development of hepatitis B virus (HBV)-related hepatocellular carcinoma (HCC). Semin Cancer Biol 10, 211-231, PubMed: 10936070
3 el-Serag, H.B. (2001) Epidemiology of hepatocellular carcinoma. Clin Liver Dis 5, 87-107, vi, PubMed: 11218921
4 Dusheiko, G.M. et al. (1992) Treatment of small hepatocellular carcinomas. Lancet 340, 285-288, PubMed: 1353202
5 El-Serag, H.B. and Mason, A.C. (1999) Rising incidence of hepatocellular carcinoma in the United States. N Engl J Med 340, 745-750, PubMed: 10072408
6 Colombo, M. (1997) Treatment of hepatocellular carcinoma. J Viral Hepat 4 Suppl 1, 125-130, PubMed: 9097291
7 Akriviadis, E.A. et al. (1998) Hepatocellular carcinoma. Br J Surg 85, 1319-1331, PubMed: 9782009
8 Zoulim, F. et al. (2003) Clinical consequences of hepatitis C virus infection. Rev Med Virol 13, 57-68, PubMed: 12516062
9 Wogan, G.N. (1989) Dietary risk factors for primary hepatocellular carcinoma. Cancer Detect Prev 14, 209-213, PubMed: 2559796
10 Kensler, T.W. et al. (2002) Strategies for chemoprevention of liver cancer. Eur J Cancer Prev 11 Suppl 2, S58-64, PubMed: 12570336
11 Macdonald, G.A. (2001) Pathogenesis of hepatocellular carcinoma. Clin Liver Dis 5, 69-85, PubMed: 11218920
12 Romeo, R. and Colombo, M. (2002) The natural history of hepatocellular carcinoma. Toxicology 181-182, 39-42, PubMed: 12505282
13 Hino, O. et al. (2002) Understanding the hypercarcinogenic state in chronic hepatitis: a clue to the prevention of human hepatocellular carcinoma. J Gastroenterol 37, 883-887, PubMed: 12483242
14 Wisnom, C. and Siegel, M.A. (2003) Advances in the diagnosis and management of human viral hepatitis. Dent Clin North Am 47, 431-447, PubMed: 12848458
15 Flamm, S.L. (2003) Chronic hepatitis C virus infection. Jama 289, 2413-2417, PubMed: 12746366
16 Waris, G. (2003) Regulatory mechanisms of viral hepatitis B and C. J Biosci 28, 311-321, PubMed:

Accession information: DOI: 10.1017/S1462399403006926; Vol. 5; 19 November 2003

12734409
17 Wang, X.W. et al. (2002) Molecular pathogenesis of human hepatocellular carcinoma. Toxicology 181-182, 43-47, PubMed: 12505283
18 Dominguez-Malagon, H. and Gaytan-Graham, S. (2001) Hepatocellular carcinoma: an update. Ultrastruct Pathol 25, 497-516, PubMed: 11783914
19 Robinson, W.S. and Marion, P.L. (1988) Biological features of hepadna viruses. In Viral Hepatitis and Liver Disease (Zuckerman, A.J., ed.), pp. 449, Alan Liss, New York
20 Heathcote, J., Cameron, C.H. and Dane, D.S. (1974) Hepatitis-B antigen in saliva and semen. Lancet 1, 71-73, PubMed: 4129209
21 Alter, H.J., Holland, P.V. and Purcell, R.H. (1975) The emerging pattern of post-transfusion hepatitis. Am J Med Sci 270, 329-334, PubMed: 1235474
22 Szmuness, W. et al. (1975) On the role of sexual behavior in the spread of hepatitis B infection. Ann Intern Med 83, 489-495, PubMed: 1166979
23 Beasley, R.P. et al. (1977) The e antigen and vertical transmission of hepatitis B surface antigen. Am J Epidemiol 105, 94-98, PubMed: 835566
24 Chang, M.H. et al. (1997) Universal hepatitis B vaccination in Taiwan and the incidence of hepatocellular carcinoma in children. Taiwan Childhood Hepatoma Study Group. N Engl J Med 336, 1855-1859, PubMed: 9197213
25 Lin, S.M. et al. (1999) Long-term beneficial effect of interferon therapy in patients with chronic hepatitis B virus infection. Hepatology 29, 971-975, PubMed: 10051505
26 Ikeda, K. et al. (1998) Interferon decreases hepatocellular carcinogenesis in patients with cirrhosis caused by the hepatitis B virus: a pilot study. Cancer 82, 827-835, PubMed: 9486570
27 Favre, D., Petit, M.A. and Trepot, C. (2003) Latent hepatitis B virus (HBV) infection and HBV DNA integration is associated with further transformation of hepatoma cells in vitro. Altex 20, 131-142, PubMed: 12947486
28 Paterlini-Brechot, P. et al. (2003) Hepatitis B virus-related insertional mutagenesis occurs frequently in human liver cancers and recurrently targets human telomerase gene. Oncogene 22, 3911-3916, PubMed: 12813464
29 Ferber, M.J. et al. (2003) Integrations of the hepatitis B virus (HBV) and human papillomavirus (HPV) into the human telomerase reverse transcriptase (hTERT) gene in liver and cervical cancers. Oncogene 22, 3813-3820, PubMed: 12802289
30 Schluter, V. et al. (1994) Integrated hepatitis B virus X and 3' truncated preS/S sequences derived from human hepatomas encode functionally active transactivators. Oncogene 9, 3335-3344, PubMed: 7936659
31 Caselmann, W.H. et al. (1990) A trans-activator function is generated by integration of hepatitis B virus preS/S sequences in human hepatocellular carcinoma DNA. Proc Natl Acad Sci U S A 87, 2970-2974, PubMed: 2158099
32 Ryu, W.S. (2003) Molecular aspects of hepatitis B viral infection and the viral carcinogenesis. J Biochem Mol Biol 36, 138-143, PubMed: 12542984
33 Koike, K. (2002) Hepatocarcinogenesis in hepatitis viral infection: lessons from transgenic mouse studies. J Gastroenterol 37 Suppl 13, 55-64, PubMed: 12109667
34 Koike, K. et al. (2002) Molecular mechanism of viral hepatocarcinogenesis. Oncology 62 Suppl 1, 29-37, PubMed: 11868782
35 Zhang, Y. et al. (2002) Overexpression of cyclin A in hepatocellular carcinoma and its relationship with HBx gene integration. Zhonghua Zhong Liu Za Zhi 24, 353-355, PubMed: 12408762
36 Caselmann, W.H. (1995) Transactivation of cellular gene expression by hepatitis B viral proteins: a possible molecular mechanism of hepatocarcinogenesis. J Hepatol 22, 34-37, PubMed: 7602073
37 Crowson, A.N. et al. (2003) The dermatopathologic manifestations of hepatitis C infection: a clinical, histological, and molecular assessment of 35 cases. Hum Pathol 34, 573-579, PubMed: 12827611
38 Laporte, J. et al. (2003) Differential distribution and internal translation efficiency of hepatitis C virus quasispecies present in dendritic and liver cells. Blood 101, 52-57, PubMed: 12393733
39 Resti, M. et al. (2002) Injection drug use facilitates hepatitis C virus infection of peripheral blood mononuclear cells. Clin Infect Dis 35, 236-239, PubMed: 12115087
40 Caussin-Schwemling, C., Schmitt, C. and Stoll-Keller, F. (2001) Study of the infection of human blood derived monocyte/macrophages with hepatitis C virus in vitro. J Med Virol 65, 14-22, PubMed: 11505438
41 El-Serag, H.B. (2002) Hepatocellular carcinoma and hepatitis C in the United States. Hepatology 36, S74-83, PubMed: 12407579
42 Seeff, L.B. (2002) Natural history of chronic hepatitis C. Hepatology 36, S35-46, PubMed:

Accession information: DOI: 10.1017/S1462399403006926; Vol. 5; 19 November 2003

12407575
43 Alberti, A. and Benvegnu, L. (2003) Management of hepatitis C. J Hepatol 38 Suppl 1, S104-118, PubMed: 12591189
44 Chander, G. et al. (2002) Treatment of chronic hepatitis C: a systematic review. Hepatology 36, S135-144, PubMed: 12407587
45 Pockros, P.J. et al. (2003) The safety and tolerability of daily infergen plus ribavirin in the treatment of naiive chronic hepatitis C patients. J Viral Hepat 10, 55-60, PubMed: 12558913
46 Houghton, M. et al. (1991) Molecular biology of the hepatitis C viruses: implications for diagnosis, development and control of viral disease. Hepatology 14, 381-388, PubMed: 1650328
47 Takamizawa, A. et al. (1991) Structure and organization of the hepatitis C virus genome isolated from human carriers. J Virol 65, 1105-1113, PubMed: 1847440
48 Choo, Q.L. et al. (1989) Isolation of a cDNA clone derived from a blood-borne non-A, non-B viral hepatitis genome. Science 244, 359-362, PubMed: 2523562
49 Kato, N. et al. (1990) Molecular cloning of the human hepatitis C virus genome from Japanese patients with non-A, non-B hepatitis. Proc Natl Acad Sci U S A 87, 9524-9528, PubMed: 2175903
50 Han, J.H. et al. (1991) Characterization of the terminal regions of hepatitis C viral RNA: identification of conserved sequences in the 5' untranslated region and poly(A) tails at the 3' end. Proc Natl Acad Sci U S A 88, 1711-1715, PubMed: 1705704
51 Sobolev, B.N. et al. (2002) [Analysis of hepatitis C virus proteins based on amino acid sequence data and literature data]. Biofizika 47, 204-210, PubMed: 11969155
52 Roccasecca, R. et al. (2003) Binding of the hepatitis C virus E2 glycoprotein to CD81 is strain specific and is modulated by a complex interplay between hypervariable regions 1 and 2. J Virol 77, 1856-1867, PubMed: 12525620
53 Vizmanos, J.L. et al. (1998) Degree and distribution of variability in the 5' untranslated, E1, E2/NS1 and NS5 regions of the hepatitis C virus (HCV). J Viral Hepat 5, 227-240, PubMed: 9751009
54 Shi, S.T. and Lai, M.M. (2001) Hepatitis C viral RNA: challenges and promises. Cell Mol Life Sci 58, 1276-1295, PubMed: 11577984
55 Kato, N. (2000) Genome of human hepatitis C virus (HCV): gene organization, sequence diversity, and variation. Microb Comp Genomics 5, 129-151, PubMed: 11252351
56 De Francesco, R. et al. (2000) Biochemical and immunologic properties of the nonstructural proteins of the hepatitis C virus: implications for development of antiviral agents and vaccines. Semin Liver Dis 20, 69-83, PubMed: 10895433
57 Simmonds, P. (1995) Variability of hepatitis C virus. Hepatology 21, 570-583, PubMed: 7531173
58 Okamoto, H. et al. (1992) Genetic drift of hepatitis C virus during an 8.2-year infection in a chimpanzee: variability and stability. Virology 190, 894-899, PubMed: 1325713
59 Tran, H.T. et al. (2003) Prevalence of hepatitis virus types B through E and genotypic distribution of HBV and HCV in Ho Chi Minh City, Vietnam. Hepatol Res 26, 275-280, PubMed: 12963426
60 Zheng, X. et al. (2003) Direct comparison of hepatitis C virus genotypes tested by INNO-LiPA HCV II and TRUGENE HCV genotyping methods. J Clin Virol 28, 214-216, PubMed: 12957191
61 Craxi, A. and Licata, A. (2003) Clinical trial results of peginterferons in combination with ribavirin. Semin Liver Dis 23 Suppl 1, 35-46, PubMed: 12934167
62 Okamoto, H. et al. (1992) Typing hepatitis C virus by polymerase chain reaction with type-specific primers: application to clinical surveys and tracing infectious sources. J Gen Virol 73 (Pt 3), 673-679, PubMed: 1312125
63 Tsukiyama-Kohara, K. et al. (1991) A second group of hepatitis C viruses. Virus Genes 5, 243-254, PubMed: 1663293
64 Fan, X. et al. (1999) Comparison of genetic heterogeneity of hepatitis C viral RNA in liver tissue and serum. Am J Gastroenterol 94, 1347-1354, PubMed: 10235217
65 Kobayashi, M. et al. (1997) Antibody response to E2/NS1 hepatitis C virus protein in patients with acute hepatitis C. J Gastroenterol Hepatol 12, 73-76, PubMed: 9076628
66 Vogelstein, B. et al. (1988) Genetic alterations during colorectal-tumor development. N Engl J Med 319, 525-532, PubMed: 2841597
67 Feo, F. et al. (2000) Genetic alterations in liver carcinogenesis: implications for new preventive and therapeutic strategies. Crit Rev Oncog 11, 19-62, PubMed: 10795626
68 Buendia, M.A. (2000) Genetics of hepatocellular carcinoma. Semin Cancer Biol 10, 185-200, PubMed: 10936068

Accession information: DOI: 10.1017/S1462399403006926; Vol. 5; 19 November 2003

69 Sheu, J.C. (1997) Molecular mechanism of hepatocarcinogenesis. J Gastroenterol Hepatol 12, S309-313, PubMed: 9407351
70 Tsuda, H. et al. (1989) Low incidence of point mutation of c-Ki-ras and N-ras oncogenes in human hepatocellular carcinoma. Jpn J Cancer Res 80, 196-199, PubMed: 2542205
71 Oda, T. et al. (1992) p53 gene mutation spectrum in hepatocellular carcinoma. Cancer Res 52, 6358-6364, PubMed: 1330291
72 Vogelstein, B. and Kinzler, K.W. (1992) p53 function and dysfunction. Cell 70, 523-526, PubMed: 1505019
73 Hollstein, M. et al. (1991) p53 mutations in human cancers. Science 253, 49-53, PubMed: 1905840
74 Hsu, I.C. et al. (1991) Mutational hotspot in the p53 gene in human hepatocellular carcinomas. Nature 350, 427-428, PubMed: 1849234
75 Murakami, Y. et al. (1991) Aberrations of the tumor suppressor p53 and retinoblastoma genes in human hepatocellular carcinomas. Cancer Res 51, 5520-5525, PubMed: 1655254
76 Theobald, M. and Offringa, R. (2003) Anti-p53-directed immunotherapy of malignant disease. Exp. Rev. Mol. Med. Vol. 5, 28 March, DOI: 10.1017/S1462399403006173
77 Kern, S.E. et al. (1991) Identification of p53 as a sequence-specific DNA-binding protein. Science 252, 1708-1711, PubMed: 2047879
78 Ko, L.J. and Prives, C. (1996) p53: puzzle and paradigm. Genes Dev 10, 1054-1072, PubMed: 8654922
79 Amundson, S.A., Myers, T.G. and Fornace, A.J., Jr. (1998) Roles for p53 in growth arrest and apoptosis: putting on the brakes after genotoxic stress. Oncogene 17, 3287-3299, PubMed: 9916991
80 el-Deiry, W.S. (1998) Regulation of p53 downstream genes. Semin Cancer Biol 8, 345-357, PubMed: 10101800
81 Kastan, M.B. et al. (1992) A mammalian cell cycle checkpoint pathway utilizing p53 and GADD45 is defective in ataxia-telangiectasia. Cell 71, 587-597, PubMed: 1423616
82 el-Deiry, W.S. et al. (1993) WAF1, a potential mediator of p53 tumor suppression. Cell 75, 817-825, PubMed: 8242752
83 Juven-Gershon, T. and Oren, M. (1999) Mdm2: the ups and downs. Mol Med 5, 71-83, PubMed: 10203572
84 Hermeking, H. et al. (1997) 14-3-3 sigma is a p53-regulated inhibitor of G2/M progression. Mol Cell 1, 3-11, PubMed: 9659898
85 Polyak, K. et al. (1997) A model for p53-induced apoptosis. Nature 389, 300-305, PubMed: 9305847
86 Levine, A.J., Momand, J. and Finlay, C.A. (1991) The p53 tumour suppressor gene. Nature 351, 453-456, PubMed: 2046748
87 Oren, M. (1992) p53: the ultimate tumor suppressor gene? Faseb J 6, 3169-3176, PubMed: 1397838
88 Montenarh, M. (1992) Biochemical properties of the growth suppressor/oncoprotein p53. Oncogene 7, 1673-1680, PubMed: 1501881
89 Dutta, A. et al. (1993) Inhibition of DNA replication factor RPA by p53. Nature 365, 79-82, PubMed: 8361542
90 Levine, A.J. (1997) p53, the cellular gatekeeper for growth and division. Cell 88, 323-331, PubMed: 9039259
91 Cross, S.M. et al. (1995) A p53-dependent mouse spindle checkpoint. Science 267, 1353-1356, PubMed: 7871434
92 Harris, C.C. and Hollstein, M. (1993) Clinical implications of the p53 tumor-suppressor gene. N Engl J Med 329, 1318-1327, PubMed: 8413413
93 Greenblatt, M.S. et al. (1994) Mutations in the p53 tumor suppressor gene: clues to cancer etiology and molecular pathogenesis. Cancer Res 54, 4855-4878, PubMed: 8069852
94 Oren, M. (1994) Relationship of p53 to the control of apoptotic cell death. Semin Cancer Biol 5, 221-227, PubMed: 7948950
95 Ullrich, S.J. et al. (1992) The p53 tumor suppressor protein, a modulator of cell proliferation. J Biol Chem 267, 15259-15262, PubMed: 1639769
96 Ljungman, M. (2000) Dial 9-1-1 for p53: mechanisms of p53 activation by cellular stress. Neoplasia 2, 208-225, PubMed: 10935507
97 Appella, E. and Anderson, C.W. (2001) Post-translational modifications and activation of p53 by genotoxic stresses. Eur J Biochem 268, 2764-2772, PubMed: 11358490
98 Oyesiku, N.M. et al. (1997) Pituitary adenomas: screening for G alpha q mutations. J Clin Endocrinol Metab 82, 4184-4188, PubMed: 9398737
99 Shaulsky, G., Ben-Ze'ev, A. and Rotter, V. (1990) Subcellular distribution of the p53 protein during the cell cycle of Balb/c 3T3 cells. Oncogene 5, 1707-1711, PubMed: 2267137
100 Isaacs, J.S. et al. (1998) Differential subcellular p53 localization and function in N- and S-type neuroblastoma cell lines. Cell Growth Differ 9, 545-555, PubMed: 9690622

Accession information: DOI: 10.1017/S1462399403006926; Vol. 5; 19 November 2003

101 Lilling, G. et al. (2002) Altered subcellular localization of p53 in estrogen-dependent and estrogen-independent breast cancer cells. Cancer Invest 20, 509-517, PubMed: 12094546
102 Bosari, S. et al. (1995) bcl-2 oncoprotein in colorectal hyperplastic polyps, adenomas, and adenocarcinomas. Hum Pathol 26, 534-540, PubMed: 7750937
103 Lu, W. et al. (2000) Nuclear exclusion of p53 in a subset of tumors requires MDM2 function. Oncogene 19, 232-240, PubMed: 10645001
104 Takada, S. et al. (1997) Cytoplasmic retention of the p53 tumor suppressor gene product is observed in the hepatitis B virus X gene-transfected cells. Oncogene 15, 1895-1901, PubMed: 9365235
105 Truant, R. et al. (1995) Direct interaction of the hepatitis B virus HBx protein with p53 leads to inhibition by HBx of p53 response element-directed transactivation. J Virol 69, 1851-1859, PubMed: 7853526
106 Zhang, Y., Xiong, Y. and Yarbrough, W.G. (1998) ARF promotes MDM2 degradation and stabilizes p53: ARF-INK4a locus deletion impairs both the Rb and p53 tumor suppression pathways. Cell 92, 725-734, PubMed: 9529249
107 Weber, J.D. et al. (1999) Nucleolar Arf sequesters Mdm2 and activates p53. Nat Cell Biol 1, 20-26, PubMed: 10559859
108 Tao, W. and Levine, A.J. (1999) P19(ARF) stabilizes p53 by blocking nucleo-cytoplasmic shuttling of Mdm2. Proc Natl Acad Sci U S A 96, 6937-6941, PubMed: 10359817
109 Thomas, M., Pim, D. and Banks, L. (1999) The role of the E6-p53 interaction in the molecular pathogenesis of HPV. Oncogene 18, 7690-7700, PubMed: 10618709
110 Ahn, J.Y. et al. (2001) Transcriptional repression of p21(waf1) promoter by hepatitis B virus X protein via a p53-independent pathway. Gene 275, 163-168, PubMed: 11574165
111 Ahn, J.Y. et al. (2002) Dual effects of hepatitis B virus X protein on the regulation of cell-cycle control depending on the status of cellular p53. J Gen Virol 83, 2765-2772, PubMed: 12388812
112 Querido, E. et al. (1997) Regulation of p53 levels by the E1B 55-kilodalton protein and E4orf6 in adenovirus-infected cells. J Virol 71, 3788-3798, PubMed: 9094654
113 Fabbro, M. and Henderson, B.R. (2003) Regulation of tumor suppressors by nuclear-cytoplasmic shuttling. Exp Cell Res 282, 59-69, PubMed: 12531692
114 Inoue, T. et al. (2002) Nuclear import and export signals in control of the p53-related protein p73. J Biol Chem 277, 15053-15060, PubMed: 11847229
115 O'Keefe, K., Li, H. and Zhang, Y. (2003) Nucleocytoplasmic shuttling of p53 is essential for MDM2-mediated cytoplasmic degradation but not ubiquitination. Mol Cell Biol 23, 6396-6405, PubMed: 12944468
116 Chene, P. (2001) The role of tetramerization in p53 function. Oncogene 20, 2611-2617, PubMed: 11420672
117 Atz, J., Wagner, P. and Roemer, K. (2000) Function, oligomerization, and conformation of tumor-associated p53 proteins with mutated C-terminus. J Cell Biochem 76, 572-584, PubMed: 10653977
118 Liu, M.C. and Gelmann, E.P. (2002) P53 gene mutations: case study of a clinical marker for solid tumors. Semin Oncol 29, 246-257, PubMed: 12063677
119 Nishida, N. et al. (1993) Role and mutational heterogeneity of the p53 gene in hepatocellular carcinoma. Cancer Res 53, 368-372, PubMed: 8093350
120 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, PubMed: 8921985
121 Matlashewski, G.J. et al. (1987) Primary structure polymorphism at amino acid residue 72 of human p53. Mol Cell Biol 7, 961-963, PubMed: 3547088
122 Anzola, M. et al. (2003) Frequent loss of p53 codon 72 Pro variant in hepatitis C virus-positive carriers with hepatocellular carcinoma. Cancer Lett 193, 199-205, PubMed: 12706878
123 Okada, F. et al. (2001) A p53 polymorphism associated with increased risk of hepatitis C virus infection. Cancer Lett 172, 137-142, PubMed: 11566488
124 Florese, R.H. et al. (2002) Inhibition of protein synthesis by the nonstructural proteins NS4A and NS4B of hepatitis C virus. Virus Res 90, 119-131, PubMed: 12457968
125 Qadri, I., Iwahashi, M. and Simon, F. (2002) Hepatitis C virus NS5A protein binds TBP and p53, inhibiting their DNA binding and p53 interactions with TBP and ERCC3. Biochim Biophys Acta 1592, 193-204, PubMed: 12379483
126 Lee, M.N. et al. (2002) Hepatitis C virus core protein represses the p21 promoter through inhibition of a TGF-beta pathway. J Gen Virol 83, 2145-2151, PubMed: 12185267

Accession information: DOI: 10.1017/S1462399403006926; Vol. 5; 19 November 2003

127 Lan, K.H. et al. (2002) HCV NS5A interacts with p53 and inhibits p53-mediated apoptosis. Oncogene 21, 4801-4811, PubMed: 12101418
128 Kwun, H.J. and Jang, K.L. (2003) Dual effects of hepatitis C virus Core protein on the transcription of cyclin-dependent kinase inhibitor p21 gene. J Viral Hepat 10, 249-255, PubMed: 12823590
129 Yamanaka, T., Kodama, T. and Doi, T. (2002) Subcellular localization of HCV core protein regulates its ability for p53 activation and p21 suppression. Biochem Biophys Res Commun 294, 528-534, PubMed: 12056798
130 Yamanaka, T., Uchida, M. and Doi, T. (2002) Innate form of HCV core protein plays an important role in the localization and the function of HCV core protein. Biochem Biophys Res Commun 294, 521-527, PubMed: 12056797
131 Ray, R.B., Meyer, K. and Ray, R. (1996) Suppression of apoptotic cell death by hepatitis C virus core protein. Virology 226, 176-182, PubMed: 8955036
132 Ray, R.B. et al. (1997) Transcriptional repression of p53 promoter by hepatitis C virus core protein. J Biol Chem 272, 10983-10986, PubMed: 9110985
133 Ray, R.B. et al. (1998) Hepatitis C virus core protein represses p21WAF1/Cip1/Sid1 promoter activity. Gene 208, 331-336, PubMed: 9524287
134 Oka, K. et al. (2003) Hepatitis C virus core protein selectively inhibits synthesis and accumulation of p21/Waf1 and certain nuclear proteins. Microbiol Immunol 47, 429-438, PubMed: 12906103
135 Alisi, A. et al. (2003) Physical and functional interaction between HCV core protein and the different p73 isoforms. Oncogene 22, 2573-2580, PubMed: 12730672
136 Varaklioti, A. et al. (2002) Alternate translation occurs within the core coding region of the hepatitis C viral genome. J Biol Chem 277, 17713-17721, PubMed: 11884417
137 Harper, J.W. et al. (1993) The p21 Cdk-interacting protein Cip1 is a potent inhibitor of G1 cyclin-dependent kinases. Cell 75, 805-816, PubMed: 8242751
138 Coqueret, O. (2003) New roles for p21 and p27 cell-cycle inhibitors: a function for each cell compartment? Trends Cell Biol 13, 65-70, PubMed: 12559756
139 Zakut, R. and Givol, D. (1995) The tumor suppression function of p21Waf is contained in its N-terminal half ('half-WAF'). Oncogene 11, 393-395, PubMed: 7624153
140 Benard, J., Douc-Rasy, S. and Ahomadegbe, J.C. (2003) TP53 family members and human cancers. Hum Mutat 21, 182-191, PubMed: 12619104
141 Ishido, S. and Hotta, H. (1998) Complex formation of the nonstructural protein 3 of hepatitis C virus with the p53 tumor suppressor. FEBS Lett 438, 258-262, PubMed: 9827557
142 Muramatsu, S. et al. (1997) Nuclear localization of the NS3 protein of hepatitis C virus and factors affecting the localization. J Virol 71, 4954-4961, PubMed: 9188558
143 Ishido, S. et al. (1997) Wild-type, but not mutant-type, p53 enhances nuclear accumulation of the NS3 protein of hepatitis C virus. Biochem Biophys Res Commun 230, 431-436, PubMed: 9016797
144 Feng, D., Zheng, H. and Yan, Y. (1998) [The effect of HCV NS3 protein on expression of p53 gene protein in hepatocarcinogenesis]. Zhonghua Yi Xue Za Zhi 78, 278-280, PubMed: 10923485
145 Kwun, H.J. et al. (2001) p53-dependent transcriptional repression of p21(waf1) by hepatitis C virus NS3. J Gen Virol 82, 2235-2241, PubMed: 11514734
146 Khabar, K.S. and Polyak, S.J. (2002) Hepatitis C virus-host interactions: the NS5A protein and the interferon/chemokine systems. J Interferon Cytokine Res 22, 1005-1012, PubMed: 12433279
147 Reyes, G.R. (2002) The nonstructural NS5A protein of hepatitis C virus: an expanding, multifunctional role in enhancing hepatitis C virus pathogenesis. J Biomed Sci 9, 187-197, PubMed: 12065893
148 Giannini, C. and Brechot, C. (2003) Hepatitis C virus biology. Cell Death Differ 10 Suppl 1, S27-38, PubMed: 12655344
149 Keskinen, P., Melen, K. and Julkunen, I. (2002) Expression of HCV structural proteins impairs IFN-mediated antiviral response. Virology 299, 164-171, PubMed: 12202219
150 Ghosh, A.K. et al. (1999) Hepatitis C virus NS5A protein modulates cell cycle regulatory genes and promotes cell growth. J Gen Virol 80 (Pt 5), 1179-1183, PubMed: 10355764
151 Arima, N. et al. (2001) Modulation of cell growth by the hepatitis C virus nonstructural protein NS5A. J Biol Chem 276, 12675-12684, PubMed: 11278402
152 Majumder, M. et al. (2001) Hepatitis C virus NS5A physically associates with p53 and regulates p21/waf1 gene expression in a p53-dependent manner. J Virol 75, 1401-1407, PubMed: 11152513

Accession information: DOI: 10.1017/S1462399403006926; Vol. 5; 19 November 2003

153 Otsuka, M. et al. (2000) Hepatitis C virus core protein enhances p53 function through augmentation of DNA binding affinity and transcriptional ability. J Biol Chem 275, 34122-34130, PubMed: 10924497
154 Pawlotsky, J.M. and Germanidis, G. (1999) The non-structural 5A protein of hepatitis C virus. J Viral Hepat 6, 343-356, PubMed: 10607250

Further reading, resources and contacts

The p53 website (created by the Thierru Soussi lab) at the Institut Curie provides information on the structure, evolution and tumour association of p53:

http://p53.curie.fr/

The International Agency for Research on Cancer (IARC) TP53 database includes all TP53 gene mutations identified in human cancers and published in the peer-reviewed literature:

http://www.iarc.fr/p53/

The Human Gene Mutation Database Cardiff has a database of p53 mutations and their tumour association:

http://uwcmml1s.uwcm.ac.uk/uwcm/mg/search/120445.html

Hepatitis Central is a website on hepatitis diseases and provides information on the symptoms and treatment of each disease:

http://www.hepatitis-central.com/

Journals of the American Association for Cancer Research (AACR):

http://www.aacrjournals.org/

Features associated with this article

Figures

Figure 1. Hepatitis C virus (HCV): model structure and genome organisation.
Figure 2. p53 protein structure.
Figure 3. Possible model for HCV core protein and p53 interactions.
Figure 4. Possible model for HCV NS5A protein and p53 interactions.

Citation details for this article

Mónica Anzola and Juan José Burgos (2003) Hepatocellular carcinoma: molecular interactions between hepatitis C virus and p53 in hepatocarcinogenesis. Exp. Rev. Mol. Med. Vol. 5, 19 November, DOI: 10.1017/S1462399403006926

Accession information: DOI: 10.1017/S1462399403006926; Vol. 5; 19 November 2003