

Review

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‘Liver let die’: oxidative DNA damage and hepatotropic viruses

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Chronic infections by the hepatotropic viruses hepatitis B virus (HBV) and hepatitis C virus (HCV) are major risk factors for the development of hepatocellular carcinoma (HCC). It is estimated that more than 700 000 individuals per year die from HCC, and around 80 % of HCC is attributable to HBV or HCV infection. Despite the clear clinical importance of virus-associated HCC, the underlying molecular mechanisms remain largely elusive. Oxidative stress, in particular DNA lesions associated with oxidative damage, play a major contributory role in carcinogenesis, and are strongly linked to the development of many cancers, including HCC. A large body of evidence demonstrates that both HBV and HCV induce hepatic oxidative stress, with increased oxidative DNA damage being observed both in infected individuals and in murine models of infection. Here, we review the impact of HBV and HCV on the incidence and repair of oxidative DNA damage. We begin by giving a brief overview of oxidative stress and the repair of DNA lesions induced by oxidative stress. We then review in detail the evidence surrounding the mechanisms by which both viruses stimulate oxidative stress, before focusing on how the viral proteins themselves may perturb the cellular response to oxidative DNA damage, impacting upon genome stability and thus hepatocarcinogenesis.

Introduction

Hepatocellular carcinoma (HCC) is an increasing global health problem, accounting for more than 90 % of primary liver tumours. Worldwide, HCC is the third cause of cancer-related death, responsible for 700 000 deaths per year (Ferlay *et al.*, 2010), and the sixth most common cancer. Chronic infections with hepatitis B virus (HBV) and hepatitis C virus (HCV) are major risk factors for the onset and progression of HCC (El-Serag, 2002; Simonetti *et al.*, 1992). Globally, up to 80 % of HCC is attributable to HBV or HCV infection (Perz *et al.*, 2006). The underlying mechanisms remain unclear, although an increasing body of evidence suggests that the viral proteins themselves may contribute directly to tumorigenesis.

Timely and precise repair of DNA lesions is crucial for the maintenance of genome stability. Since genomic instability is a characteristic of all tumours, DNA damage recognition and repair processes represent an important barrier to the initiation and progression of tumorigenesis. Cells in multicellular organisms are continuously exposed to DNA damage arising from a variety of endogenous and exogenous sources. These include reactive oxygen species (ROS), UV light, background radiation and environmental mutagens. In particular, oxidative stress and elevated ROS levels are linked with number of human diseases. ROS that accumulate as a result of oxidative

stress can directly react with DNA molecules to produce a variety of oxidative DNA lesions, whose repair by components of the base excision repair (BER) and nucleotide excision repair (NER) pathways is crucial in maintaining genome stability.

Oxidative DNA damage may play a pivotal role in hepatocarcinogenesis associated with chronic viral infection. A growing number of publications have concluded that chronic HBV and HCV infection correlates with an increased incidence of oxidative DNA damage (Bolukbas *et al.*, 2005; Demirdag *et al.*, 2003; Farinati *et al.*, 2007; Fujita *et al.*, 2008; Machida *et al.*, 2006; Nishina *et al.*, 2008). Whilst it is clear that ROS are continuously generated during chronic inflammation over the course of chronic viral infection, increasing evidence suggests that the viral proteins of HBV and HCV may themselves contribute to a state of chronic oxidative stress in infected hepatocytes.

In this review, we will summarize the current knowledge surrounding oxidative stress and DNA damage during hepatotropic viral infection. We will compare and contrast the mechanisms by which the proteins encoded by HBV and HCV induce oxidative stress, before providing an overview of the impact of these viral proteins on oxidative DNA damage and repair, and briefly examining the ultimate consequences on the development of HCC.

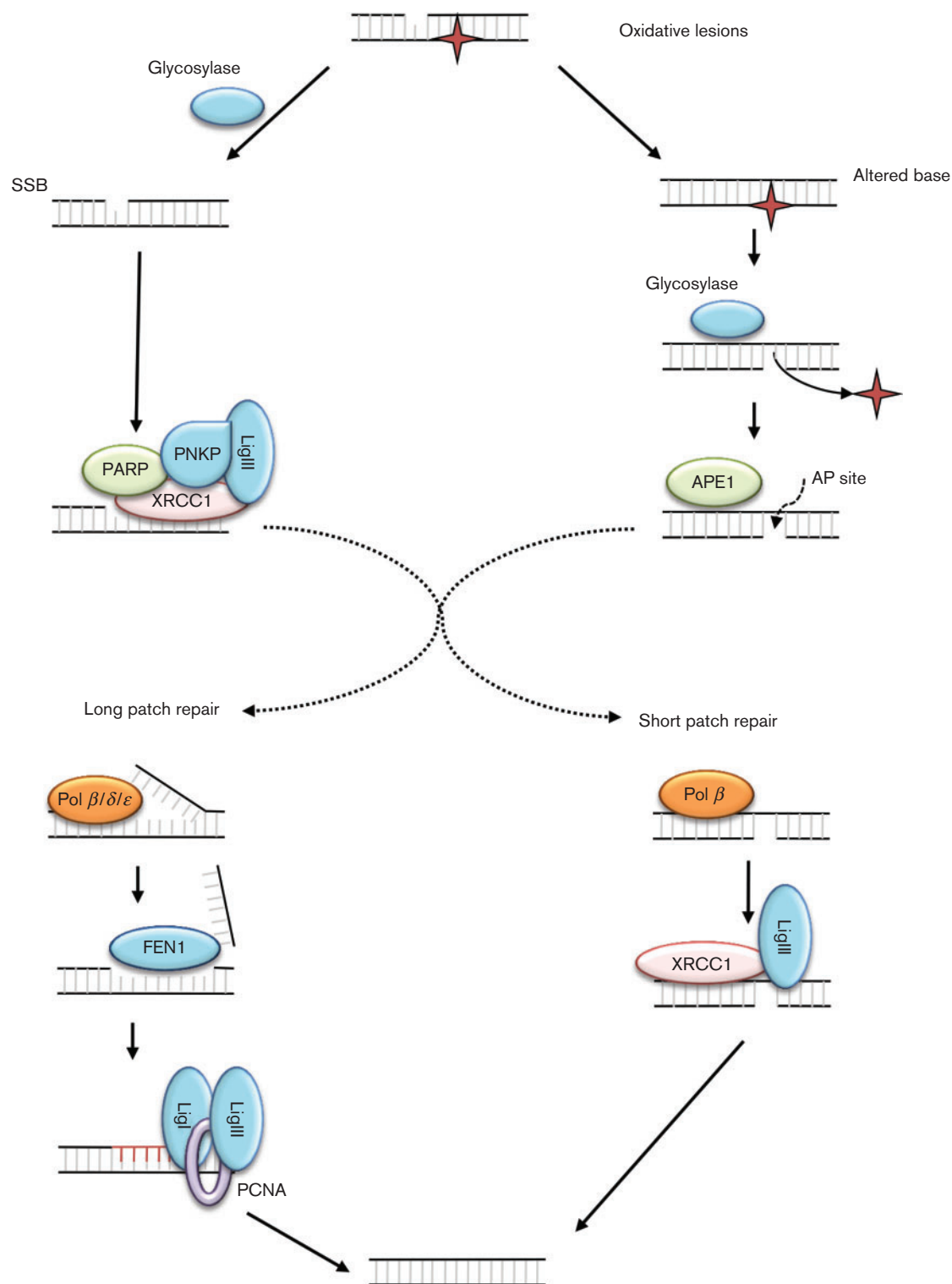


Fig. 1. Repair of ROS-induced DNA lesions. The majority of oxidative lesions are repaired by the BER pathway. Oxidative damage is recognized by a glycosylase (Ogg1 or Neil1 in the case of 8-OHdG), which removes the altered base. Depending upon the glycosylase involved, this will result in either an SSB or an abasic (AP) site. The latter is then processed by the APE1 endonuclease, giving an SSB. These breaks are then repaired by two subpathways of the BER pathway: short patch (bottom right) or long patch (bottom left). During short patch BER, DNA polymerase (Pol) β fills the gap with a single nucleotide, and the nicked DNA is ligated by XRCC1 and LigIII. In the alternative long patch repair, SSB repair involves the replacement of 2–12 nt. This is dependent upon synthesis of a new 2–12 bp fragment by either Pol β , Pol δ or Pol ϵ , and the concomitant displacement

of a 5' DNA flap. This flap is then removed by the enzyme Flap endonuclease 1 (FEN1). Ligation of the nick involves PCNA, LigI and LigIII. The choice between short patch repair and long patch repair (dotted arrows) is yet to be understood, and is complicated by the high degree of redundancy between the two pathways.

Oxidative stress

ROS may arise by exposure to exogenous agents such as ionizing radiation or drugs, or may be generated from endogenous sources such as metabolism, apoptosis or inflammation. Oxidative stress represents a shifting of the balance between oxidants (such as ROS) and the cellular antioxidant response, leading to potential cellular damage and contributing to disease. Cells are able to withstand a relatively low level of oxidative damage. However, sustained

oxidative stress, arising through the increased presence of radicals and ROS, or by a lack of antioxidant capacity within the cell, will engender increased damage to lipids, proteins and DNA. A detailed description of the repair of oxidative DNA damage is covered in a subsequent section. ROS are primarily generated in the mitochondria as by-products of cellular metabolism, through electron leakage from the mitochondrial electron transport chain. However, they also play a key role as second messengers in cellular signalling.

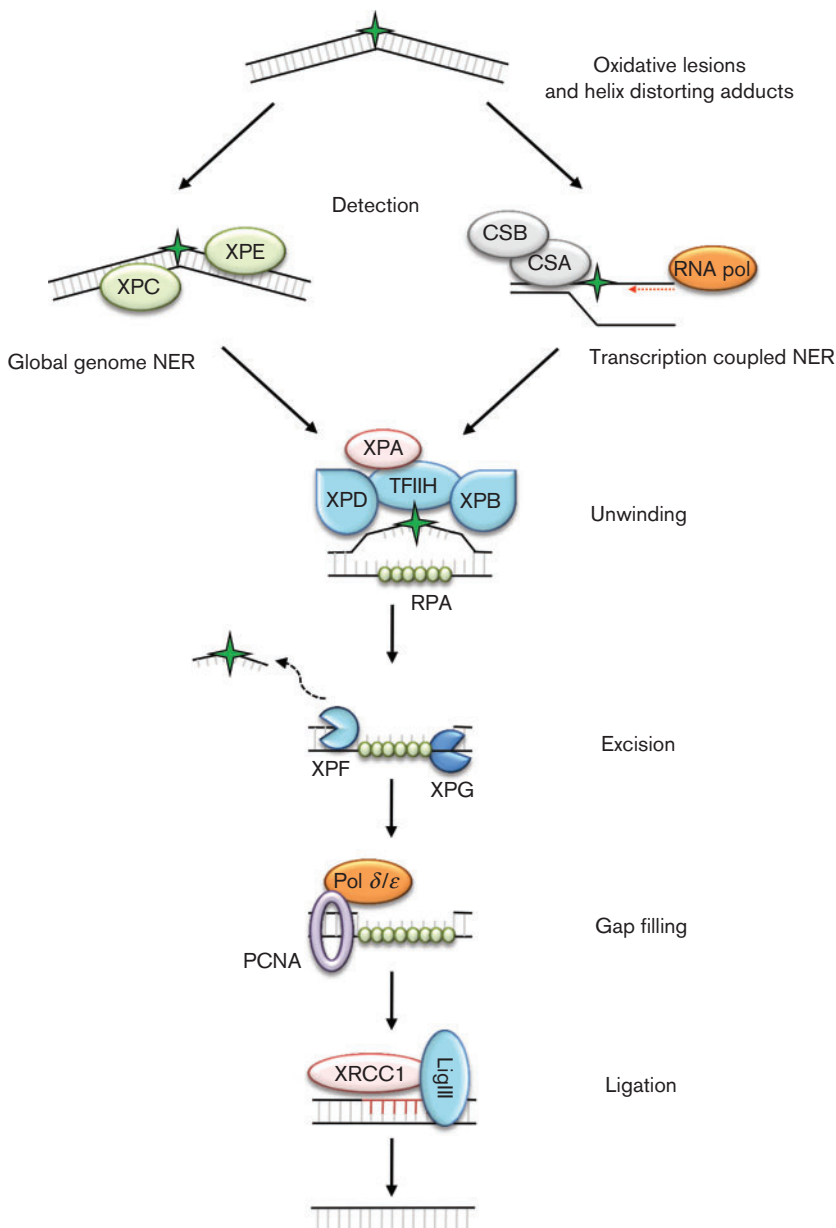


Fig. 2. Alternative repair of oxidative damage by NER. An unknown proportion of oxidative lesions may be repaired by NER. Two NER subpathways exist, which differ in their ability to detect helix-distorting regions arising anywhere within the genome (global genome NER; left), or those lesions which arise in the transcribed strands of expressed genes (transcription coupled NER; right). In both cases, repair is effected through the actions of detector proteins (XPC and XPE or CSA and CSB) which recruit TFIIH and its components to the site of damage. TFIIH, XPB and XPD act in conjunction with XPF and XPG to unwind the DNA surrounding the lesion and to excise one strand of the unwound bubble. The final stages of repair involve gap filling by Pol δ or Pol ϵ , and DNA ligation involving LigIII and XRCC1.

ROS-induced damage may influence pathway signalling, gene expression, cell cycle, metabolism and apoptosis. Oxidative stress may also activate oncogenic signalling pathways, ultimately contributing to cellular transformation (Hussain *et al.*, 2003).

Viral infection and oxidative stress: non-specific inflammation

Although this review focuses on how the HBV and HCV viral proteins induce oxidative stress, it is clear that chronic infection by either virus triggers a non-specific immune-mediated inflammation (hepatitis), which is innately linked to oxidative stress. During acute liver injury and hepatic inflammation, ROS are generated by both neutrophils and Kupffer cells (reviewed by Jaeschke, 2011), as the principal toxic mediators to induce cell death. Since these cells exist in close proximity to hepatocytes, some ROS (i.e. H_2O_2) are able to diffuse into hepatocytes, although the plasma membrane represents a barrier to the free diffusion of O_2^- . Membrane superoxide dismutases are able to convert these O_2^- anions to H_2O_2 in the extracellular space, molecules of which are then able to cross membranes and elicit intracellular signalling (Fisher, 2009). Thus, these ROS will enhance and amplify the intracellular effects engendered by the viral proteins themselves, and will also affect neighbouring (uninfected) hepatocytes (Jaeschke, 2011), although they may also serve to activate intracellular antioxidant defences. Moreover, mitochondrial ROS are able to drive proinflammatory cytokine production (Naik & Dixit, 2011), further exacerbating both inflammation and the production of ROS.

Oxidative DNA damage and repair

ROS may directly interact with DNA to induce oxidative DNA damage. Oxidative DNA lesions may comprise abasic sites, deaminated or adducted bases and single-stranded DNA breaks (SSBs). These lesions include thymine and thymidine glycol, 5-hydroxymethyluracil and 8-hydroxy-deoxyguanosine (8-OHdG). The last represents the most widely studied of oxidative DNA lesions, and is used as a robust marker of oxidative DNA damage. The accumulation of oxidative DNA lesions is considered mutagenic and a significant contributory factor to human disease (reviewed by Sedelnikova *et al.*, 2010).

The majority of oxidative DNA lesions are repaired by components of the BER pathway, and to a lesser extent by NER. The rate of transient oxidative DNA damage is typically balanced by its rate of repair, but chronic oxidative stress may result in permanent genetic damage. Since there are multiple, overlapping pathways for the repair of oxidative DNA damage, perturbation of one component of these pathways is expected to slow but not abolish repair.

BER (Fig. 1) is initiated by a DNA glycosylase that recognizes and removes the damaged base, generating either an SSB or

an abasic (AP) site which requires further processing by an AP endonuclease (APE1). The resultant SSB may be repaired by two alternative pathways: short patch BER (Fig. 1, bottom right), involving Pol β and the DNA ligase III/XRCC1 complex, which inserts a single nucleotide; or long patch BER (Fig. 1, bottom left), which replaces 2–12 nt and is dependent upon proliferating cell nuclear antigen (PCNA) and Flap endonuclease 1 (FEN1), and may involve several polymerases (Pol β , δ and ϵ). The factors governing the choice of long or short patch BER remain unclear.

The NER pathway (Fig. 2), which is generally associated with repair of bulky, helix-distorting adducts, is also able to repair oxidative DNA lesions, albeit to a lesser extent. Detection of bulky lesions, whether in actively transcribed regions (Fig. 2, right panel) or not (Fig. 2, left panel), leads to recruitment of the TFIIH complex to sites of damage. Members of the TFIIH complex facilitates helix unwinding and excision of the strand containing the adduct, and repair is completed by several proteins which complete gap-filling/ligation. NER is capable of removing all the oxidative lesions induced by ROS (Kuraoka *et al.*, 2000; Reardon *et al.*, 1997), as NER initiating factors (CSA, CSB, XPC and XPE) rapidly bind to oxidative lesions (Menoni *et al.*, 2012). However, the overall contribution of NER to the repair of oxidative lesions remains to be determined, and it is likely that NER acts as a backup pathway to BER. Interestingly, oxidative stress generated by activated neutrophils, and potentially by Kupffer cells, results in a reduction in NER capacity, suggesting that inflammation may non-specifically reduce NER efficiency (Güngör *et al.*, 2007).

In some circumstances, oxidative DNA damage may result in double strand break (DSB) formation, especially if lesions are clustered together on opposite strands. Although DSBs will arise at a lower frequency than SSBs, their rapid repair is crucial to maintaining genetic stability. Repair of DSBs involves either recombination using homologous sequences from undamaged sister chromatids (homologous recombination; HR), or non-homologous end-joining (NHEJ), which can result in small deletions. For a comprehensive view on DSB repair, the reader is directed to an excellent recent review by Chapman *et al.* (2012).

In addition, a growing body of literature suggests that cellular tumour suppressors, including those involved in repair of other types of DNA lesions, may play a vital role in regulating oxidative stress. p53 is activated in response to oxidative stress and is thought to play an antioxidant role in such circumstances. Regulation of both pro- and antioxidant genes by p53 is thought to be crucial in regulating the cellular response and cell fate to acute oxidative stress (reviewed by Vurusaner *et al.*, 2012). Moreover, the breast cancer susceptibility genes BRCA1 and BRCA2, involved in DNA repair and cell cycle checkpoint progression, also play caretaker roles against oxidative stress. In particular, in response to oxidative

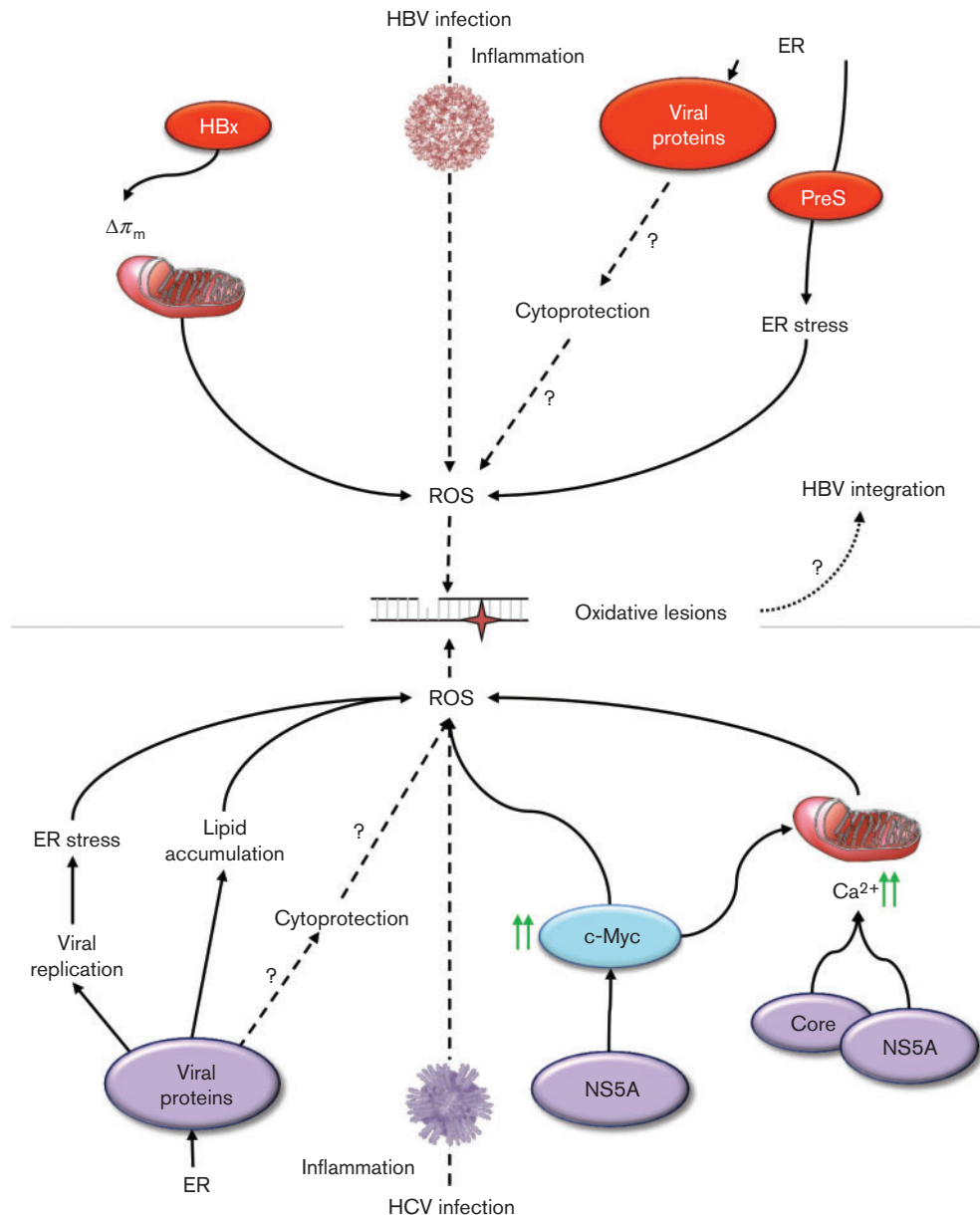


Fig. 3. Sources of oxidative stress induced by the HBV (upper panel) and HCV (lower panel) viral proteins. As a result of the innate immune response, chronic infection with HBV or HCV will induce chronic inflammation, eliciting ROS production and creating oxidative DNA lesions. Both viruses and their proteins may cause ER stress or induce lipid accumulation, which in turn leads to oxidative stress and oxidative DNA lesions. In addition, HBx and HCV core and NS5A stimulate ROS by perturbing mitochondrial function. HCV NS5A also stimulates c-Myc transcription, leading to perturbation of cytochrome function and thus mitochondrial ROS, so directly increasing oxidative stress. In the case of HBV, the resultant oxidative DNA lesions may contribute to HBV genome integration if left unrepaired. The impact of either virus on the cellular detoxification of ROS remains unclear (denoted by ?).

stress, BRCA1 upregulates the expression of numerous antioxidant genes, e.g. glutathione-S-transferase, alcohol dehydrogenases (Bae *et al.*, 2004). BRCA1 overexpression protects against oxidizing agents, maintaining the cellular redox balance in the face of oxidative stress. Similarly, BRCA1 may enhance the activity of the antioxidant

transcription factor Nrf2 (Ishikawa *et al.*, 2005). In addition to its well-characterized role in the repair of DSBs by promoting HR, BRCA2 is also critical for the repair of oxidative lesions. Specifically, BRCA2 (as well as BRCA1) is required for the transcription coupled repair of 8-OHdG lesions, crucial for the prevention of transcription

stalling and subsequent mutagenesis (Bae *et al.*, 2004; Le Page *et al.*, 2000).

Thus, members of multiple, overlapping and functionally diverse pathways (namely BER, NER and DSB repair) are required for the effective repair of oxidative DNA lesions resulting from oxidative stress.

HBV, oxidative stress and DNA damage

HBV infection and oxidative stress

HBV is one of several closely related DNA viruses within the family *Hepadnaviridae*. The viral genome encodes a small number of gene products: a reverse transcriptase and DNA polymerase (Pol); a capsid protein (core); envelope proteins L, M and S; and a multifunctional protein (X) involved in replication, oncogenesis and a myriad of other cellular metabolic dysfunctions. Replication of HBV is complex and proceeds via the reverse transcription of genome-length RNA; thus, HBV is classified as a dsDNA retrovirus (group VII).

Chronic HBV infection is a major aetiological factor for HCC: the risk of HCC development in chronic HBV carriers is more than 100-fold greater than in uninfected individuals (Ito *et al.*, 2010). The vast majority of new cases of HBV-associated HCC occur in developing countries, especially sub-Saharan Africa and South East Asia. Although HCC generally occurs in cirrhotic livers, HBV is also able to transform hepatocytes in the absence of chronic inflammation and cirrhosis (Br  chot, 2004). During chronic infection, fragments of HBV DNA may integrate into the host genome, preferentially into chromosome 17, creating mutations. These integrated fragments often encode the X protein of HBV (HBx) or truncated PreS proteins, the integration of which often correlates with hepatocarcinogenesis (Ding *et al.*, 2012).

Several groups have shown that HBV infection is associated with oxidative stress in chronically infected individuals (Bolukbas *et al.*, 2005; Demirdag *et al.*, 2003). Both lipid peroxidation and oxidative DNA damage, markers of oxidative stress, are elevated in patients infected with HBV. Indeed, patients with chronic HBV infection exhibit increased 8-OHdG accumulation (Fujita *et al.*, 2008). Furthermore, *in vitro* HBV replication in hepatoma cell lines (specifically HepAD38 cells) induces oxidative stress (Severi *et al.*, 2006).

A direct role for HBV-encoded proteins in oxidative stress

In addition to non-specific oxidative stress generated by local inflammation in response to viral infection, increasing evidence suggests that the HBV proteins directly regulate cellular ROS production (Fig. 3, upper panel) and deleteriously alter intracellular antioxidant defences in HBV-infected cells, causing apoptosis and extensive liver damage and thus engendering accelerated hepatocellular renewal.

However, the consequences of these interactions and their impact are yet to be fully understood.

Over a decade ago, HBx was shown to localize to mitochondria, decreasing mitochondrial membrane potential, and increasing both cytochrome *c* release and apoptosis (Takada *et al.*, 1999). Moreover, transgenic mice expressing the HBV proteins (including HBx) also display elevated hepatic oxidative stress levels compared non-transgenic controls, with a concurrent increase in oxidative DNA damage (Hagen *et al.*, 1994). These findings were extended to demonstrate that ROS scavengers were able to inhibit HBx-mediated mitochondrial membrane depolarization and subsequent apoptosis (Shirakata & Koike, 2003). In agreement, Lee *et al.* (2004) demonstrated that HBx alters mitochondrial membrane potential, perturbs mitochondrial electron transport, affects hepatocyte metabolism and increases cellular ROS production. HBx has accordingly been reported to sensitize cells to apoptosis induced by oxidative stress, mainly through loss of the anti-apoptotic protein Mcl-1 (Hu *et al.*, 2011). In general agreement, HBx was shown to induce apoptosis through regulation of Bcl-XL and Bax (Kim *et al.*, 2008; Miao *et al.*, 2006). It is therefore clear that expression of HBx stimulates intracellular ROS production and impacts upon apoptosis.

The HBV surface antigen (HBsAg) and associated PreS region have also been associated with oxidative stress. Expression of truncated mutants of the HBV PreS/S polypeptide in hepatoma (Huh7) cells induced production of ROS via endoplasmic reticulum (ER) stress pathways, resulting in oxidative DNA damage (Hsieh *et al.*, 2004; Wang *et al.*, 2005), although these results might be strongly influenced by non-specific ER stress triggered by high levels of HBV protein expression. Hsieh *et al.* (2004) also showed that transgenic mice expressing these mutants exhibited elevated oxidative DNA damage and upregulated expression of Ogg1, the DNA glycosylase mainly responsible for the repair of 8-OHdG lesions. Such oxidative damage may play an important role in hepatocarcinogenesis associated with HBV infections, illustrated by elevated nodular proliferation and increased tumour development in mice expressing PreS mutants (Wang *et al.*, 2005). In contradiction, examination of a small cohort of patients (*n*=38) failed to reveal a link between PreS mutation and increased oxidative stress in HBV-infected patients, although HBV infection was again linked to elevated levels of 8-OHdG and Ogg1 expression (Gwak *et al.*, 2008).

Given these data, one would expect that intracellular antioxidant defences would be activated in the presence of the HBV proteins. Accordingly, HBV upregulates the expression of cytoprotective genes containing antioxidant response elements (AREs), both *in vitro* and in HBV-infected liver tissues (Schaedler *et al.*, 2010). However, Schaedler *et al.* demonstrated that this upregulation was independent of ROS, and, in contrast to other studies, suggested that this upregulation might confer survival benefits upon HBV-infected cells, allowing them to survive

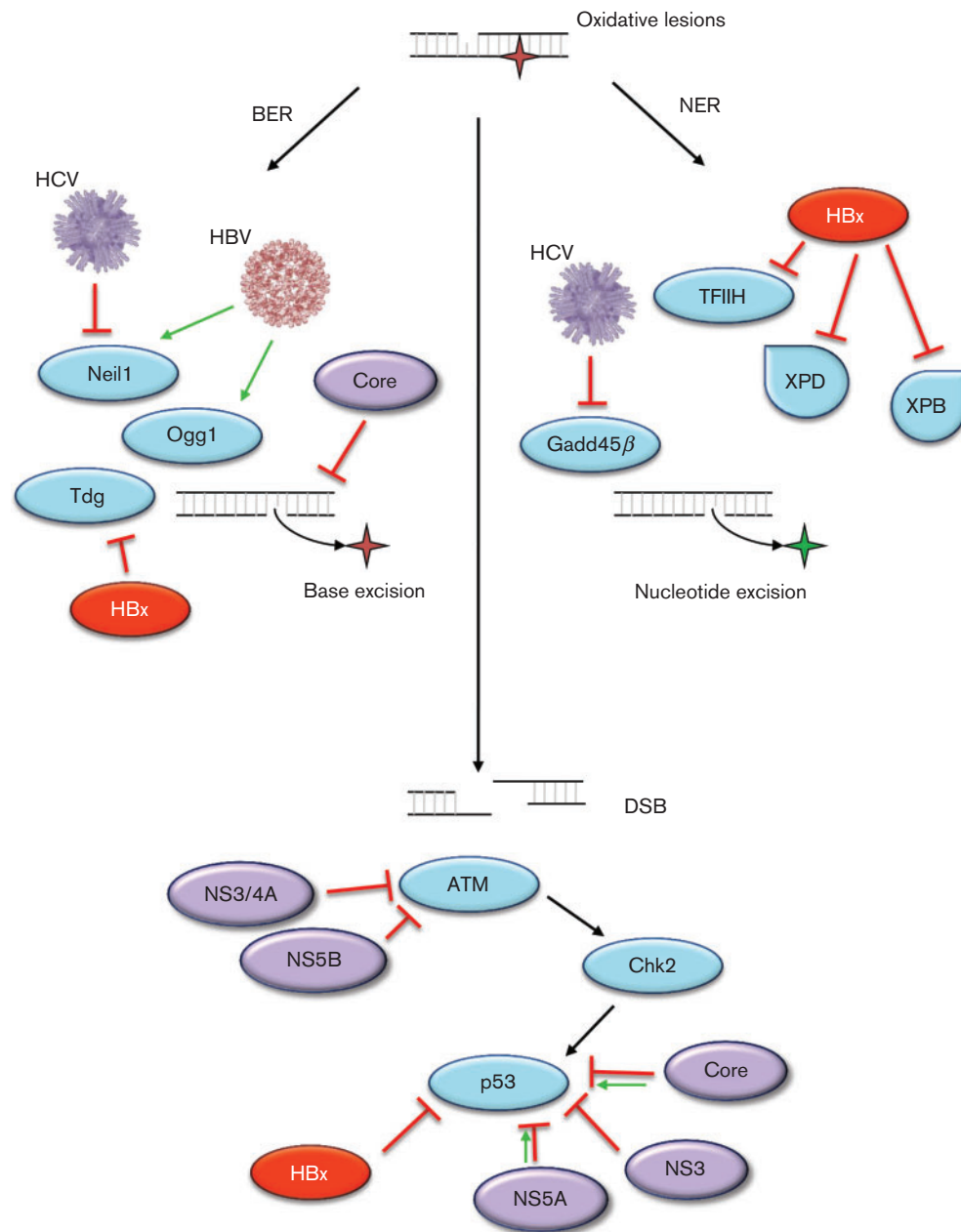


Fig. 4. Interactions between HBV and HCV proteins and the cellular actors of oxidative DNA repair. Upper panel: HBV infection upregulates the activity of the BER glycosylases Neil1 and Ogg1 (green arrows), probably as a consequence of increased oxidative stress. In contrast, the HCV proteins inhibit the activity of the Neil1 glycosylase, and HCV core decreases the repair of oxidative DNA lesions (red lines). HBx also inhibits the activity of a third glycosylase, Tdg, although the impact of Tdg on oxidative DNA lesions is unclear. HBx also inhibits NER by reducing expression of the NER proteins XPD and XPB, as well as inhibiting transcription coupled NER. In addition, the HCV proteins also seem to inhibit NER, perhaps as a consequence of reduced Gadd45 β expression. Lower panel: A subset of oxidative lesions are converted into DSBs. HCV non-structural proteins NS3, NS4A and NS5B are reported to interact with the DSB sensor ATM and inhibit its function. Both HBx and the HCV core, NS3 and NS5A proteins also inhibit p53 (red lines), although there are suggestions that core and NS5A may also stimulate p53 activity (green arrows).

the sustained oxidative stress found in the infected liver. The discrepancy between Schaedler's findings and other published data described above, emphasizes the difficulty

of studying changes in the redox balance occurring during a natural HBV infection and questions the relevance of using HBV models usually devoid of an immune response.

These studies demonstrate that HBV infection induces extensive oxidative stress and activates intracellular oxidative repair pathways, and suggest that HBx expression (and perhaps that of PreS) is, in part, responsible. However, certain aspects require further clarification, and may be addressed by controlling the level of HBV protein expression to resemble more closely that observed in infected individuals and by validating the results in the context of the whole HBV genome. Although antioxidant treatments seem a promising avenue of therapeutic research for HBV infection, their efficacy is as yet unknown.

HBV and oxidative DNA damage

As detailed above, HBV infection, HBx and PreS induce oxidative stress, which culminates in increased hepatic oxidative DNA damage, with increased levels of 8-OHdG found in HBV-infected patients, in transgenic mice expressing either PreS mutants or HBx, and in hepatoma cells expressing HBx (Fujita *et al.*, 2008; Gwak *et al.*, 2008; Hagen *et al.*, 1994). A number of studies have suggested that the HBV-encoded proteins, in addition to their role in inducing oxidative stress, may also inhibit cellular DNA repair pathways. Despite the conflicting nature of some of these reports, we attempt to summarize the current state of knowledge below.

HBV and the repair of oxidative DNA lesions. A growing body of literature suggests that the HBV proteins may alter the repair of DNA lesions, including oxidative DNA damage (Fig. 4). There are, however, conflicting accounts of the effect of HBV on the BER pathway. Gwak *et al.* (2008) demonstrated that expression of Ogg1 was increased in hepatic tissues from HBV-infected patients, regardless of whether they originated from tumoral or non-tumoral regions. In agreement, expression of PreS mutants induced Ogg1 expression *in vitro* and in murine models (Hsieh *et al.*, 2004). These studies suggest that infected cells respond to HBV-induced oxidative stress by upregulating cellular glycosylases involved in BER, activating DNA repair (Fig. 4, left panel). However, recent studies have demonstrated that HBx inhibits BER initiated by thymidine glycosylase (Tdg) (van de Klundert *et al.*, 2012). Whilst this study examined the effects of HBx on BER induced by Tdg *in vitro*, the effect of HBx on accumulation of 8-OHdG remains unstudied, as Tdg acts on G/T mismatches and not on 8-OHdG.

A considerable number of studies report that HBV inhibits NER (Fig. 4, right panel). This has in large part been attributed to the HBx protein. Cells expressing HBx render hepatoma cells more sensitive to UV-C, and HBx inhibits global NER in a host-cell reactivation assay (Jia *et al.*, 1999; Lee *et al.*, 2005; Mathonnet *et al.*, 2004). Moreover, HBx inhibits the expression of the TFIIH subunits XPB and XPD, and interacts with and inhibits the function of TFIIH, leading to increased UV-C sensitivity of cells expressing

HBx (Jaitovich-Groisman *et al.*, 2001; Qadri *et al.*, 2011). In addition to inhibiting global NER, HBx may also impede NER within transcriptionally active genes (transcription coupled NER) (Mathonnet *et al.*, 2004). However, since the impact of NER on repair of oxidative DNA damage is unclear (as mentioned above), the impact of HBx-induced NER inhibition upon oxidative damage during HBV infection remains to be studied.

As outlined previously, the p53 tumour suppressor is also involved in the response to oxidative DNA damage. Intriguingly, HBV has been shown to perturb p53 function (Fig. 4, lower panel). The interaction between HBx and p53 is well established (Chung *et al.*, 2003; Lin *et al.*, 1997; Wang *et al.*, 1994; Yun *et al.*, 2000), and it seems that this alters the binding of p53 to p53-responsive elements, resulting in aberrant gene expression (Chan *et al.*, 2013). Given these data, one may imagine that interactions between HBx and p53 would negatively impact the repair of oxidative DNA lesions.

In addition to the specific mechanisms detailed above, the frequent random integration of HBV DNA into genes encoding DNA repair and checkpoint proteins (e.g. Wrn, hTERT, Rad17; Toh *et al.*, 2013) may also serve to perturb the cellular response to DNA damage. Furthermore, oxidative stress may increase the rate of HBV fragment integration *in vitro* (Dandri *et al.*, 2002). In agreement with this latter remark, this study also suggests that the SSB repair factor PARP-1 may play a protective role against HBV DNA integration, presumably by rapidly repairing breaks which would otherwise favour integration of exogenous DNA.

From these studies, it is apparent that HBV proteins (notably HBx) interact with components of both BER and NER pathways, implying that the function of these pathways in the repair of oxidative lesions may be perturbed during HBV infection, ultimately contributing to the elevated levels of 8-OHdG observed in HBV-infected patients.

HCV, oxidative stress and DNA damage

HCV infection and oxidative stress

HCV is a member of the family *Flaviviridae* of enveloped, positive ssRNA viruses. The positive-sense RNA genome acts as a template for viral genome replication and is also translated into a polyprotein which is cleaved by both host and virally encoded proteases to generate 10 proteins: the capsid (core) protein; envelope glycoproteins E1 and E2; the p7 ion channel; and six non-structural proteins (NS2–NS5B).

Chronic infection by HCV is a major risk factor for the onset and progression of HCC (El-Serag, 2002; Saito *et al.*, 1990). Chronic HCV infection is responsible for approximately a third of HCCs, and has become the principal cause of HCC in most industrialized areas. Cirrhosis appears to be an important non-specific determinant of HCC occurrence in HCV-infected patients, and very few

cases of HCC without cirrhosis have been reported in these individuals (Simonetti *et al.*, 1992).

Oxidative stress and elevated ROS production are frequently observed during chronic HCV infection. As with HBV, they are thought to play a central role in HCV-associated HCC. Elevated levels of oxidative DNA damage (namely 8-OHdG), 4-hydroxynoneal and increased lipid peroxidation have been observed in HCV-infected patients (Farinati *et al.*, 2007; Kato *et al.*, 2001; Konishi *et al.*, 2006; Mahmood *et al.*, 2004; Shackel *et al.*, 2002). HCV infection has been associated with an almost fourfold increase in 8-OHdG levels compared with uninfected controls, and HCV infection induces higher levels of oxidative stress than does HBV (Farinati *et al.*, 2007). In line with this, antioxidant therapies are able to alleviate to some extent the level of hepatic damage in chronic HCV infection, although there is no clear evidence that antioxidants alone are useful therapeutic agents in these patients (reviewed by Singal *et al.*, 2011).

HCV proteins directly induce oxidative stress

A considerable body of experimental evidence demonstrates a direct role for the HCV proteins in inducing oxidative stress (Fig. 3, lower panel), from a variety of different models (reviewed by Ivanov *et al.*, 2013; Simula & De Re, 2010). Indeed, FL-N/35 mice transgenic for the entire ORF of HCV (thus expressing the entire complement of viral proteins) exhibit elevated ROS levels (Higgs *et al.*, 2012; Nishina *et al.*, 2008), and expression of the HCV polyprotein induced ROS production (Piccoli *et al.*, 2007). Expression of several individual HCV proteins has also been linked with overproduction of ROS, as detailed below. Although a causative role of HCV-associated oxidative stress in the development of HCC in murine models has yet to be shown, several studies suggest that oxidative stress induced by the HCV proteins may trigger genomic instability, eventually leading to HCC.

HCV core and oxidative stress

Although core (and other HCV proteins) primarily localizes to the ER, it also associates with mitochondria (Korenaga *et al.*, 2005). Several studies have demonstrated that expression of the HCV core protein induces oxidative stress in a variety of experimental systems. Mice transgenic for core, or for core, E1 and E2, demonstrate increased oxidative stress and enhanced ROS production (reviewed by Wang & Weinman, 2006, 2013). Moriya *et al.* (2001) showed that core induced a shift in the hepatic oxidant/antioxidant state, leading to mitochondrial damage and possibly contributing to the onset of HCC in their core-transgenic mice. Korenaga *et al.* (2005) demonstrated that core protein associates with mitochondria and remains associated with the mitochondrial outer membrane in core-E1-E2 transgenic mice, leading to a disruption of mitochondrial electron transport complex 1, the generation of ROS and oxidation of the glutathione pool. Thus it seems

that expression of the HCV core protein provokes mitochondrial dysfunction, leading to oxidative stress, coupled with activation of components of the intracellular superoxide scavenging system, including catalase and glutathione (Koike, 2007).

Similar results have been obtained from study of HCV-infected cell cultures, or of tumour cells expressing core. Expression of core under the control of a tetracycline-regulated promoter induced oxidative stress and lipid peroxidation in HeLa and Huh7 cells (Okuda *et al.*, 2002). Such expression efficiently induced a cellular antioxidant response and increased expression of antioxidant genes (Li *et al.*, 2002). Expression of core (as well as E1 and NS3) in Huh7 cells leads to an increase in ROS and a decrease in mitochondrial permeability (Machida *et al.*, 2006; Pal *et al.*, 2010). Overexpression of core in Huh7 hepatoma cells also increased mitochondrial Ca^{2+} uptake, perhaps explaining the increased cytochrome *c* release by mitochondria in response to Ca^{2+} in the presence of core (Li *et al.*, 2002). Similar observations have also been made *in vivo* in core-transgenic mice (Korenaga *et al.*, 2005). It is therefore clear that increased mitochondrial uptake of Ca^{2+} induced by HCV core stimulates ROS production, leading to the modification of electron transport components and inducing cellular oxidative stress.

The HCV non-structural proteins and oxidative stress

Numerous studies have linked the HCV non-structural proteins, especially NS3 and NS5A, to oxidative stress. The vast majority of these have involved expression of a single viral protein in isolation, although expression of the non-structural HCV proteins also induces ROS (Boudreau *et al.*, 2009; Rivas-Estilla *et al.*, 2012). Expression of the NS3 protease enhances ROS production in hepatoma cells (Machida *et al.*, 2006; Pal *et al.*, 2010). Similarly, NS5A expression in hepatoma cells induces mitochondrial ROS production, Ca^{2+} release and activates downstream kinases (Gong *et al.*, 2001; Machida *et al.*, 2006; Pal *et al.*, 2010). Overproduction of ROS has also been observed in NS5A-transgenic mice (Wang *et al.*, 2009).

Expression of both NS4B and NS5A also induce ER stress (Gong *et al.*, 2001; Li *et al.*, 2009). In agreement, Asselah *et al.* (2010) reported that ER stress markers were activated in biopsies from HCV-infected patients. However, transgenic mice expressing low levels of the HCV proteins do not exhibit ER stress (Lerat *et al.*, 2009). Taken together, these data suggest that ROS production through HCV-induced ER stress might be linked to HCV infection rather than HCV protein expression (Asselah *et al.*, 2010).

Recently, we demonstrated both in HCV patients' biopsies and in the FL-N/35 mouse lineage (transgenic for the entire ORF of an HCV genotype 1b isolate and expressing the full repertoire of HCV proteins at a low level in the liver), that hepatic c-Myc expression is elevated, increasing ROS production, and that NS5A is involved in this process

(Higgs *et al.*, 2012). It is well established that expression of the proto-oncogene c-Myc can induce ROS production (Dang *et al.*, 2005; Graves *et al.*, 2009; Karlsson *et al.*, 2003; Ray *et al.*, 2006; Vafa *et al.*, 2002). We demonstrated that the increased ROS production induced by c-Myc is, at least in part, associated with transcriptional deregulation of cytochrome P2C9 (CYP2C9), a component of the mitochondrial respiratory chain cytochrome P450 (CYP450).

Limited evidence also suggests that the HCV non-structural proteins, together with core, repress hepcidin expression in a ROS-dependent manner, altering iron metabolism (Miura *et al.*, 2008). Importantly, NS5A-induced ROS production may also impact on glucose production, since an NS5A-dependent decrease in the phosphorylation of the transcription factor Foxo1 and subsequently increased glucose production was decreased by *N*-acetyl cysteine (Deng *et al.*, 2011). It is probable, therefore, that oxidative stress induced by HCV impacts on several other HCV-associated pathologies, including diabetes.

HCV and ROS detoxification

Since oxidative stress is a hallmark of HCV-infected cells, the excess ROS produced are clearly inefficiently detoxified. In an attempt to examine the impact of HCV on ROS detoxification pathways, several publications have examined the impact of core and the other HCV proteins on the Nuclear factor-erythroid 2-related factor 2 (Nrf2) pathway, which is of crucial importance in the regulation of intracellular oxidation. When associated with small proteins (sMaf), Nrf2 positively regulates the transcription of genes containing AREs in their promoters. (Hirotsu *et al.*, 2012). Recently, Carvajal-Yepes *et al.* (2011) showed that, in hepatoma cells harbouring JFH1 replicons, HCV core triggers the delocalization of sMaf proteins from the nucleus to the ER where they bind HCV NS3. This ultimately restrains Nrf2 from entering into the nucleus and thereby inhibits the induction of Nrf2/ARE-regulated genes, thus resulting in lower expression of cytoprotective genes. In contradiction, other published results showed that the overexpression of core, NS3 and NS5A enhances Nrf2 expression (Ivanov *et al.*, 2011), and that the Nrf2/ARE antioxidant pathway is activated in cells infected with HCV *in vitro*, providing an anti-apoptotic protection mechanism (Burdette *et al.*, 2010). Further study is therefore necessary to determine how HCV impacts on the Nrf2/ARE pathway.

Collectively, these studies clearly demonstrate that several of the HCV proteins (notably core, NS3 and NS5A) induce oxidative stress through a variety of pathways, thus increasing the likelihood of oxidative DNA damage. ROS-induced apoptosis and oxidative DNA damage may both contribute to carcinogenesis, by on the one hand increasing compensatory hepatocellular proliferation to create a mitogenic and mutagenic environment, whilst on the other hand inducing further heritable genetic damage.

HCV and oxidative DNA damage

As described above, the role of HCV and the viral proteins in inducing oxidative stress is well established, and results in the increased 8-OHdG levels found both in HCV-infected patients and in HCV-infected cells *in vitro*. We have also observed increased levels of ssDNA damage in mice transgenic for the entire complement of HCV proteins (Higgs *et al.*, 2012). In a manner similar to HBx, several studies have indicated that, in addition to their role in inducing oxidative stress, HCV core, NS3 and NS5A may also have deleterious consequences on the repair of oxidative lesions (amongst other types of DNA damage) (Fig. 4).

HCV and the repair of oxidative DNA lesions. There is a relative paucity of studies examining the impact of HCV on the DNA damage response. Moreover, given the importance of HCV in inducing oxidative DNA damage, there are surprisingly few reports concerning HCV and BER. HCV core has been suggested to inhibit the DNA glycosylase activity responsible for excision of 8-OHdG, although the mechanisms remain unclear, since core fails to interact with or perturb the expression of the BER components (Machida *et al.*, 2010a). Pal *et al.* (2010) found that expression of the Neil1 DNA glycosylase (which has marginal activity towards 8-OHdG) was perturbed in HCV-infected cell cultures and biopsies from HCV-infected patients, with a concomitant reduction in Neil1-specific glycosylase activity and increased 8-OHdG levels. Together, these two reports provide the only preliminary evidence that HCV may have deleterious consequences on BER.

In a similar manner, there are limited reports that HCV deregulates NER. We have shown that hepatocytes of mice expressing the HCV proteins exhibit reduced NER repair by means of a plasmid reactivation assay (Higgs *et al.*, 2010). Although unconfirmed, it is possible that the reduced expression of Gadd45 β , a p53-response gene linked to NER, plays a contributory role. Intriguingly, these FL-N/35 mice also develop hepatic steatosis (as do many HCV-infected individuals), which, since NER is diminished in steatotic livers (Schults *et al.*, 2012), may suggest that HCV-induced steatosis contributes to the observed decrease in NER.

The ataxia telangiectasia mutated (ATM) kinase plays a key role in the cellular response to DSBs and also an important role in the response to oxidative DNA damage (reviewed by Chen *et al.*, 2012). ATM is necessary for repair of ROS-induced DSBs in non-replicating cells (Guo *et al.*, 2010; Woodbine *et al.*, 2011). Previous reports have suggested that HCV interacts with ATM, and perturbs its function (Ariumi *et al.*, 2008; Machida *et al.*, 2010b). Thus, it is tempting to speculate that HCV may also disrupt the repair of DSBs arising from both clustered oxidative lesions as well as exogenous sources.

A substantial body of evidence demonstrates that various HCV proteins alter p53 signalling and function *in vitro*

(Alisi *et al.*, 2003; Deng *et al.*, 2006; Kao *et al.*, 2004; Kwun & Jang, 2003; Lan *et al.*, 2002; Smirnova *et al.*, 2006; Yamanaka *et al.*, 2002). From these studies, it is apparent that core, NS3 and NS5A interact with p53, and this interaction seems to inhibit p53 activity, although the reported consequences are sometimes contradictory. However, these findings are yet to be repeated *in vivo* in the various transgenic or humanized murine models currently available, and these observations may be a consequence of the use of hepatoma cells and/or of overexpressed proteins in the majority of these studies. However, it is clear that the impact of the HCV proteins on p53 function, especially during DNA repair, is worth further investigation.

From these data, it is apparent that HCV proteins interact with several DNA repair factors and may therefore contribute to the elevated levels of 8-OHdG and SSBs observed in HCV-infected individuals by negatively regulating the repair of oxidative DNA lesions. Clearly future work must focus on whether HCV perturbs these processes and on the precise mechanisms involved.

Conclusions and future perspectives

The causative link between HBV and HCV infection and oxidative DNA damage is well established. Although a number of mechanistic insights concerning the ability of virus-infected cells to repair such damage have been provided from *in vitro* and *in vivo* models, it is still not possible to present a final picture of the effect of the HBV or HCV viral proteins on such repair processes. Therefore, at present it is probably easier to list the areas surrounding these viruses and DNA damage which remain to be studied, rather than those that have been studied.

Clearly, further detailed studies need to be carried out on the ability of cells expressing the viral HBV or HCV proteins to repair oxidative DNA lesions, since sustained oxidative damage probably plays a contributory role in the development of virus-associated HCC. Given the importance of both viruses in inducing oxidative DNA damage, there is currently a relative lack of literature on the impact of the viral proteins on BER. On the other hand, despite a number of publications describing the ability of both viruses to perturb NER, it is unclear whether this impacts significantly upon oxidative repair. The eventual impact on virally associated pathogenesis, including HCC, must also be studied, although this work is hampered by the lack of current suitable *in vivo* models for both HBV and HCV (reviewed by Lerat *et al.*, 2011). Increased future understanding of how these viruses regulate host cellular DNA repair pathways in response to oxidative stress will be invaluable in the development of new strategies for the treatment and prevention of chronic liver diseases.

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References

- Alisi, A., Giambartolomei, S., Cupelli, F., Merlo, P., Fontemaggi, G., Spaziani, A. & Balsano, C. (2003). Physical and functional interaction between HCV core protein and the different p73 isoforms. *Oncogene* **22**, 2573–2580.
- Ariumi, Y., Kuroki, M., Dansako, H., Abe, K., Ikeda, M., Wakita, T. & Kato, N. (2008). The DNA damage sensors ataxia-telangiectasia mutated kinase and checkpoint kinase 2 are required for hepatitis C virus RNA replication. *J Virol* **82**, 9639–9646.
- Asselah, T., Bièche, I., Mansouri, A., Laurendeau, I., Cazals-Hatem, D., Feldmann, G., Bedossa, P., Paradis, V., Martinot-Peignoux, M. & other authors (2010). In vivo hepatic endoplasmic reticulum stress in patients with chronic hepatitis C. *J Pathol* **221**, 264–274.
- Bae, I., Fan, S., Meng, Q., Rih, J. K., Kim, H. J., Kang, H. J., Xu, J., Goldberg, I. D., Jaiswal, A. K. & Rosen, E. M. (2004). BRCA1 induces antioxidant gene expression and resistance to oxidative stress. *Cancer Res* **64**, 7893–7909.
- Bolukbas, C., Bolukbas, F. F., Horoz, M., Aslan, M., Celik, H. & Erel, O. (2005). Increased oxidative stress associated with the severity of the liver disease in various forms of hepatitis B virus infection. *BMC Infect Dis* **5**, 95.
- Boudreau, H. E., Emerson, S. U., Korzeniowska, A., Jendrysik, M. A. & Leto, T. L. (2009). Hepatitis C virus (HCV) proteins induce NADPH oxidase 4 expression in a transforming growth factor beta-dependent manner: a new contributor to HCV-induced oxidative stress. *J Virol* **83**, 12934–12946.
- Bréchet, C. (2004). Pathogenesis of hepatitis B virus-related hepatocellular carcinoma: old and new paradigms. *Gastroenterology* **127** (Suppl 1), S56–S61.
- Burdette, D., Olivarez, M. & Waris, G. (2010). Activation of transcription factor Nrf2 by hepatitis C virus induces the cell-survival pathway. *J Gen Virol* **91**, 681–690.
- Carvajal-Yepes, M., Himmelsbach, K., Schaedler, S., Ploen, D., Krause, J., Ludwig, L., Weiss, T., Klingel, K. & Hildt, E. (2011). Hepatitis C virus impairs the induction of cytoprotective Nrf2 target genes by delocalization of small Maf proteins. *J Biol Chem* **286**, 8941–8951.
- Chan, C., Wang, Y., Chow, P. K., Chung, A. Y., Ooi, L. L. & Lee, C. G. (2013). Altered binding site selection of p53 transcriptional cassettes by hepatitis B virus X protein. *Mol Cell Biol* **33**, 485–497.
- Chapman, J. R., Taylor, M. R. & Boulton, S. J. (2012). Playing the end game: DNA double-strand break repair pathway choice. *Mol Cell* **47**, 497–510.
- Chen, B. P., Li, M. & Asaithamby, A. (2012). New insights into the roles of ATM and DNA-PKcs in the cellular response to oxidative stress. *Cancer Lett* **327**, 103–110.
- Chung, T. W., Lee, Y. C., Ko, J. H. & Kim, C. H. (2003). Hepatitis B virus X protein modulates the expression of PTEN by inhibiting the function of p53, a transcriptional activator in liver cells. *Cancer Res* **63**, 3453–3458.
- Dandri, M., Burda, M. R., Bürkle, A., Zuckerman, D. M., Will, H., Rogler, C. E., Greten, H. & Petersen, J. (2002). Increase in de novo HBV DNA integrations in response to oxidative DNA damage or inhibition of poly(ADP-ribosylation). *Hepatology* **35**, 217–223.
- Dang, C. V., Li, F. & Lee, L. A. (2005). Could MYC induction of mitochondrial biogenesis be linked to ROS production and genomic instability? *Cell Cycle* **4**, 1465–1466.

- Demirdag, K., Yilmaz, S., Ozdarendeli, A., Ozden, M., Kalkan, A. & Kilic, S. S. (2003). Levels of plasma malondialdehyde and erythrocyte antioxidant enzyme activities in patients with chronic hepatitis B. *Hepatogastroenterology* **50**, 766–770.
- Deng, L., Nagano-Fujii, M., Tanaka, M., Nomura-Takigawa, Y., Ikeda, M., Kato, N., Sada, K. & Hotta, H. (2006). NS3 protein of Hepatitis C virus associates with the tumour suppressor p53 and inhibits its function in an NS3 sequence-dependent manner. *J Gen Virol* **87**, 1703–1713.
- Deng, L., Shoji, I., Ogawa, W., Kaneda, S., Soga, T., Jiang, D. P., Ide, Y. H. & Hotta, H. (2011). Hepatitis C virus infection promotes hepatic gluconeogenesis through an NS5A-mediated, FoxO1-dependent pathway. *J Virol* **85**, 8556–8568.
- Ding, D., Lou, X., Hua, D., Yu, W., Li, L., Wang, J., Gao, F., Zhao, N., Ren, G. & other authors (2012). Recurrent targeted genes of hepatitis B virus in the liver cancer genomes identified by a next-generation sequencing-based approach. *PLoS Genet* **8**, e1003065.
- El-Serag, H. B. (2002). Hepatocellular carcinoma and hepatitis C in the United States. *Hepatology* **36** (Suppl 1), S74–S83.
- Farinati, F., Cardin, R., Bortolami, M., Burra, P., Russo, F. P., Rugge, M., Guido, M., Sergio, A. & Naccarato, R. (2007). Hepatitis C virus: from oxygen free radicals to hepatocellular carcinoma. *J Viral Hepat* **14**, 821–829.
- Ferlay, J., Shin, H. R., Bray, F., Forman, D., Mathers, C. & Parkin, D. M. (2010). *GLOBOCAN 2008 v2.0, Cancer Incidence and Mortality Worldwide: IARC CancerBase No. 10*. Lyon, France: International Agency for Research on Cancer.
- Fisher, A. B. (2009). Redox signaling across cell membranes. *Antioxid Redox Signal* **11**, 1349–1356.
- Fujita, N., Sugimoto, R., Ma, N., Tanaka, H., Iwasa, M., Kobayashi, Y., Kawanishi, S., Watanabe, S., Kaito, M. & Takei, Y. (2008). Comparison of hepatic oxidative DNA damage in patients with chronic hepatitis B and C. *J Viral Hepat* **15**, 498–507.
- Gong, G., Waris, G., Tanveer, R. & Siddiqui, A. (2001). Human hepatitis C virus NS5A protein alters intracellular calcium levels, induces oxidative stress, and activates STAT-3 and NF-kappa B. *Proc Natl Acad Sci U S A* **98**, 9599–9604.
- Graves, J. A., Metukuri, M., Scott, D., Rothermund, K. & Prochownik, E. V. (2009). Regulation of reactive oxygen species homeostasis by peroxiredoxins and c-Myc. *J Biol Chem* **284**, 6520–6529.
- Güngör, N., Godschalk, R. W., Pachen, D. M., Van Schooten, F. J. & Knaapen, A. M. (2007). Activated neutrophils inhibit nucleotide excision repair in human pulmonary epithelial cells: role of myeloperoxidase. *FASEB J* **21**, 2359–2367.
- Guo, Z., Deshpande, R. & Paull, T. T. (2010). ATM activation in the presence of oxidative stress. *Cell Cycle* **9**, 4805–4811.
- Gwak, G. Y., Lee, D. H., Moon, T. G., Choi, M. S., Lee, J. H., Koh, K. C., Paik, S. W., Park, C. K., Joh, J. W. & Yoo, B. C. (2008). The correlation of hepatitis B virus PreS mutation with cellular oxidative DNA damage in hepatocellular carcinoma. *Hepatogastroenterology* **55**, 2028–2032.
- Hagen, T. M., Huang, S., Curnutte, J., Fowler, P., Martinez, V., Wehr, C. M., Ames, B. N. & Chisari, F. V. (1994). Extensive oxidative DNA damage in hepatocytes of transgenic mice with chronic active hepatitis destined to develop hepatocellular carcinoma. *Proc Natl Acad Sci U S A* **91**, 12808–12812.
- Higgs, M. R., Lerat, H. & Pawlotsky, J. M. (2010). Downregulation of Gadd45beta expression by hepatitis C virus leads to defective cell cycle arrest. *Cancer Res* **70**, 4901–4911.
- Higgs, M. R., Lerat, H. & Pawlotsky, J. M. (2012). Hepatitis C virus-induced activation of beta-catenin promotes c-Myc expression and a cascade of pro-carcinogenic events. *Oncogene* **32**, 4683–4693.
- Hirotsu, Y., Katsuoka, F., Funayama, R., Nagashima, T., Nishida, Y., Nakayama, K., Engel, J. D. & Yamamoto, M. (2012). Nrf2-MafG heterodimers contribute globally to antioxidant and metabolic networks. *Nucleic Acids Res* **40**, 10228–10239.
- Hsieh, Y. H., Su, I. J., Wang, H. C., Chang, W. W., Lei, H. Y., Lai, M. D., Chang, W. T. & Huang, W. (2004). Pre-S mutant surface antigens in chronic hepatitis B virus infection induce oxidative stress and DNA damage. *Carcinogenesis* **25**, 2023–2032.
- Hu, L., Chen, L., Yang, G., Li, L., Sun, H., Chang, Y., Tu, Q., Wu, M. & Wang, H. (2011). HBx sensitizes cells to oxidative stress-induced apoptosis by accelerating the loss of Mcl-1 protein via caspase-3 cascade. *Mol Cancer* **10**, 43.
- Hussain, S. P., Hofseth, L. J. & Harris, C. C. (2003). Radical causes of cancer. *Nat Rev Cancer* **3**, 276–285.
- Ishikawa, M., Numazawa, S. & Yoshida, T. (2005). Redox regulation of the transcriptional repressor Bach1. *Free Radic Biol Med* **38**, 1344–1352.
- Ito, K., Arai, M., Imazeki, F., Yonemitsu, Y., Bekku, D., Kanda, T., Fujiwara, K., Fukai, K., Sato, K. & other authors (2010). Risk of hepatocellular carcinoma in patients with chronic hepatitis B virus infection. *Scand J Gastroenterol* **45**, 243–249.
- Ivanov, A. V., Smirnova, O. A., Ivanova, O. N., Masalova, O. V., Kochetkov, S. N. & Isagulians, M. G. (2011). Hepatitis C virus proteins activate NRF2/ARE pathway by distinct ROS-dependent and independent mechanisms in HUH7 cells. *PLoS One* **6**, e24957.
- Ivanov, A. V., Bartosch, B., Smirnova, O. A., Isagulians, M. G. & Kochetkov, S. N. (2013). HCV and oxidative stress in the liver. *Viruses* **5**, 439–469.
- Jaeschke, H. (2011). Reactive oxygen and mechanisms of inflammatory liver injury: Present concepts. *J Gastroenterol Hepatol* **26** (Suppl 1), 173–179.
- Jaitovich-Groisman, I., Benlimame, N., Slagle, B. L., Perez, M. H., Alpert, L., Song, D. J., Fotouhi-Ardakani, N., Galipeau, J. & Alaoui-Jamali, M. A. (2001). Transcriptional regulation of the TFIIH transcription repair components XPB and XPD by the hepatitis B virus x protein in liver cells and transgenic liver tissue. *J Biol Chem* **276**, 14124–14132.
- Jia, L., Wang, X. W. & Harris, C. C. (1999). Hepatitis B virus X protein inhibits nucleotide excision repair. *Int J Cancer* **80**, 875–879.
- Kao, C.-F., Chen, S.-Y., Chen, J.-Y. & Wu Lee, Y.-H. (2004). Modulation of p53 transcription regulatory activity and post-translational modification by hepatitis C virus core protein. *Oncogene* **23**, 2472–2483.
- Karlsson, A., Deb-Basu, D., Cherry, A., Turner, S., Ford, J. & Felsher, D. W. (2003). Defective double-strand DNA break repair and chromosomal translocations by MYC overexpression. *Proc Natl Acad Sci U S A* **100**, 9974–9979.
- Kato, J., Kobune, M., Nakamura, T., Kuroiwa, G., Takada, K., Takimoto, R., Sato, Y., Fujikawa, K., Takahashi, M. & other authors (2001). Normalization of elevated hepatic 8-hydroxy-2'-deoxyguanosine levels in chronic hepatitis C patients by phlebotomy and low iron diet. *Cancer Res* **61**, 8697–8702.
- Kim, H. J., Kim, S. Y., Kim, J., Lee, H., Choi, M., Kim, J. K. & Ahn, J. K. (2008). Hepatitis B virus X protein induces apoptosis by enhancing translocation of Bax to mitochondria. *IUBMB Life* **60**, 473–480.
- Koike, K. (2007). Hepatitis C virus contributes to hepatocarcinogenesis by modulating metabolic and intracellular signaling pathways. *J Gastroenterol Hepatol* **22** (Suppl 1), S108–S111.
- Konishi, M., Iwasa, M., Araki, J., Kobayashi, Y., Katsuki, A., Sumida, Y., Nakagawa, N., Kojima, Y., Watanabe, S. & other authors (2006). Increased lipid peroxidation in patients with non-alcoholic fatty liver disease and chronic hepatitis C as measured by the plasma level of 8-isoprostane. *J Gastroenterol Hepatol* **21**, 1821–1825.

- Korenaga, M., Wang, T., Li, Y., Showalter, L. A., Chan, T., Sun, J. & Weinman, S. A. (2005). Hepatitis C virus core protein inhibits mitochondrial electron transport and increases reactive oxygen species (ROS) production. *J Biol Chem* **280**, 37481–37488.
- Kuraoka, I., Bender, C., Romieu, A., Cadet, J., Wood, R. D. & Lindahl, T. (2000). Removal of oxygen free-radical-induced 5',8-purine cyclodeoxynucleosides from DNA by the nucleotide excision-repair pathway in human cells. *Proc Natl Acad Sci U S A* **97**, 3832–3837.
- Kwun, H. J. & 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.
- Lan, K.-H., Sheu, M.-L., Hwang, S.-J., Yen, S.-H., Chen, S.-Y., Wu, J.-C., Wang, Y.-J., Kato, N., Omata, M. & other authors (2002). HCV NS5A interacts with p53 and inhibits p53-mediated apoptosis. *Oncogene* **21**, 4801–4811.
- Le Page, F., Randrianarison, V., Marot, D., Cabannes, J., Perricaudet, M., Feunteun, J. & Sarasin, A. (2000). BRCA1 and BRCA2 are necessary for the transcription-coupled repair of the oxidative 8-oxoguanine lesion in human cells. *Cancer Res* **60**, 5548–5552.
- Lee, Y. I., Hwang, J. M., Im, J. H., Lee, Y. I., Kim, N. S., Kim, D. G., Yu, D. Y., Moon, H. B. & Park, S. K. (2004). Human hepatitis B virus-X protein alters mitochondrial function and physiology in human liver cells. *J Biol Chem* **279**, 15460–15471.
- Lee, A. T., Ren, J., Wong, E. T., Ban, K. H., Lee, L. A. & Lee, C. G. (2005). The hepatitis B virus X protein sensitizes HepG2 cells to UV light-induced DNA damage. *J Biol Chem* **280**, 33525–33535.
- Lerat, H., Kammoun, H. L., Hainault, I., Mèroux, E., Higgs, M. R., Callens, C., Lemon, S. M., Foulfelle, F. & Pawlotsky, J. M. (2009). Hepatitis C virus proteins induce lipogenesis and defective triglyceride secretion in transgenic mice. *J Biol Chem* **284**, 33466–33474.
- Lerat, H., Higgs, M. & Pawlotsky, J. M. (2011). Animal models in the study of hepatitis C virus-associated liver pathologies. *Expert Rev Gastroenterol Hepatol* **5**, 341–352.
- Li, K., Prow, T., Lemon, S. M. & Beard, M. R. (2002). Cellular response to conditional expression of hepatitis C virus core protein in Huh7 cultured human hepatoma cells. *Hepatology* **35**, 1237–1246.
- Li, S., Ye, L., Yu, X., Xu, B., Li, K., Zhu, X., Liu, H., Wu, X. & Kong, L. (2009). Hepatitis C virus NS4B induces unfolded protein response and endoplasmic reticulum overload response-dependent NF-kappaB activation. *Virology* **391**, 257–264.
- Lin, Y., Nomura, T., Yamashita, T., Dorjsuren, D., Tang, H. & Murakami, S. (1997). The transactivation and p53-interacting functions of hepatitis B virus X protein are mutually interfering but distinct. *Cancer Res* **57**, 5137–5142.
- Machida, K., Cheng, K. T.-H., Lai, C.-K., Jeng, K.-S., Sung, V. M.-H. & Lai, M. M. C. (2006). Hepatitis C virus triggers mitochondrial permeability transition with production of reactive oxygen species, leading to DNA damage and STAT3 activation. *J Virol* **80**, 7199–7207.
- Machida, K., Tsukamoto, H., Liu, J. C., Han, Y. P., Govindarajan, S., Lai, M. M., Akira, S. & Ou, J. H. (2010a). c-Jun mediates hepatitis C virus hepatocarcinogenesis through signal transducer and activator of transcription 3 and nitric oxide-dependent impairment of oxidative DNA repair. *Hepatology* **52**, 480–492.
- Machida, K., McNamara, G., Cheng, K. T., Huang, J., Wang, C. H., Comai, L., Ou, J. H. & Lai, M. M. (2010b). Hepatitis C virus inhibits DNA damage repair through reactive oxygen and nitrogen species and by interfering with the ATM-NBS1/Mre11/Rad50 DNA repair pathway in monocytes and hepatocytes. *J Immunol* **185**, 6985–6998.
- Mahmood, S., Kawanaka, M., Kamei, A., Izumi, A., Nakata, K., Niyama, G., Ikeda, H., Hanano, S., Suehiro, M. & other authors (2004). Immunohistochemical evaluation of oxidative stress markers in chronic hepatitis C. *Antioxid Redox Signal* **6**, 19–24.
- Mathonnet, G., Lachance, S., Alaoui-Jamali, M. & Drobetsky, E. A. (2004). Expression of hepatitis B virus X oncoprotein inhibits transcription-coupled nucleotide excision repair in human cells. *Mutat Res* **554**, 305–318.
- Menoni, H., Hoeijmakers, J. H. & Vermeulen, W. (2012). Nucleotide excision repair-initiating proteins bind to oxidative DNA lesions in vivo. *J Cell Biol* **199**, 1037–1046.
- Miao, J., Chen, G. G., Chun, S. Y. & Lai, P. P. (2006). Hepatitis B virus X protein induces apoptosis in hepatoma cells through inhibiting Bcl-xL expression. *Cancer Lett* **236**, 115–124.
- Miura, K., Taura, K., Kodama, Y., Schnabl, B. & Brenner, D. A. (2008). Hepatitis C virus-induced oxidative stress suppresses hepcidin expression through increased histone deacetylase activity. *Hepatology* **48**, 1420–1429.
- Moriya, K., Nakagawa, K., Santa, T., Shintani, Y., Fujie, H., Miyoshi, H., Tsutsumi, T., Miyazawa, T., Ishibashi, K. & other authors (2001). Oxidative stress in the absence of inflammation in a mouse model for hepatitis C virus-associated hepatocarcinogenesis. *Cancer Res* **61**, 4365–4370.
- Naik, E. & Dixit, V. M. (2011). Mitochondrial reactive oxygen species drive proinflammatory cytokine production. *J Exp Med* **208**, 417–420.
- Nishina, S., Hino, K., Korenaga, M., Vecchi, C., Pietrangelo, A., Mizukami, Y., Furutani, T., Sakai, A., Okuda, M. & other authors (2008). Hepatitis C virus-induced reactive oxygen species raise hepatic iron level in mice by reducing hepcidin transcription. *Gastroenterology* **134**, 226–238.
- Okuda, M., Li, K., Beard, M. R., Showalter, L. A., Scholle, F., Lemon, S. M. & Weinman, S. A. (2002). Mitochondrial injury, oxidative stress, and antioxidant gene expression are induced by hepatitis C virus core protein. *Gastroenterology* **122**, 366–375.
- Pal, S., Polyak, S. J., Bano, N., Qiu, W. C., Carithers, R. L., Shuhart, M., Gretch, D. R. & Das, A. (2010). Hepatitis C virus induces oxidative stress, DNA damage and modulates the DNA repair enzyme NEIL1. *J Gastroenterol Hepatol* **25**, 627–634.
- Perz, J. F., Armstrong, G. L., Farrington, L. A., Hutin, Y. J. & Bell, B. P. (2006). The contributions of hepatitis B virus and hepatitis C virus infections to cirrhosis and primary liver cancer worldwide. *J Hepatol* **45**, 529–538.
- Piccoli, C., Scrima, R., Quarato, G., D'Aprile, A., Ripoli, M., Lecce, L., Boffoli, D., Moradpour, D. & Capitanio, N. (2007). Hepatitis C virus protein expression causes calcium-mediated mitochondrial bioenergetic dysfunction and nitro-oxidative stress. *Hepatology* **46**, 58–65.
- Qadri, I., Fatima, K. & Abdel-Hafiz, H. (2011). Hepatitis B virus X protein impedes the DNA repair via its association with transcription factor, TFIIH. *BMC Microbiol* **11**, 48.
- Ray, S., Atkuri, K. R., Deb-Basu, D., Adler, A. S., Chang, H. Y., Herzenberg, L. A. & Felsher, D. W. (2006). MYC can induce DNA breaks in vivo and in vitro independent of reactive oxygen species. *Cancer Res* **66**, 6598–6605.
- Reardon, J. T., Bessho, T., Kung, H. C., Bolton, P. H. & Sancar, A. (1997). In vitro repair of oxidative DNA damage by human nucleotide excision repair system: possible explanation for neurodegeneration in xeroderma pigmentosum patients. *Proc Natl Acad Sci U S A* **94**, 9463–9468.
- Rivas-Estilla, A. M., Bryan-Marrugo, O. L., Trujillo-Murillo, K., Pérez-Ibave, D., Charles-Niño, C., Pedroza-Roldan, C., Ríos-Ibarra, C., Ramirez-Valles, E., Ortiz-López, R. & other authors (2012). Cu/Zn superoxide dismutase (SOD1) induction is implicated in the anti-oxidative and antiviral activity of acetylsalicylic acid in HCV-expressing cells. *Am J Physiol Gastrointest Liver Physiol* **302**, G1264–G1273.
- Saito, I., Miyamura, T., Ohbayashi, A., Harada, H., Katayama, T., Kikuchi, S., Watanabe, Y., Koi, S., Onji, M. & Ohta, Y. (1990).

Hepatitis C virus infection is associated with the development of hepatocellular carcinoma. *Proc Natl Acad Sci U S A* **87**, 6547–6549.

Schaedler, S., Krause, J., Himmelsbach, K., Carvajal-Yepes, M., Lieder, F., Klingel, K., Nassal, M., Weiss, T. S., Werner, S. & Hildt, E. (2010). Hepatitis B virus induces expression of antioxidant response element-regulated genes by activation of Nrf2. *J Biol Chem* **285**, 41074–41086.

Schults, M. A., Nagle, P. W., Rensen, S. S., Godschalk, R. W., Munnia, A., Peluso, M., Claessen, S. M., Greve, J. W., Driessen, A. & other authors (2012). Decreased nucleotide excision repair in steatotic livers associates with myeloperoxidase-immunoreactivity. *Mutat Res* **736**, 75–81.

Sedelnikova, O. A., Redon, C. E., Dickey, J. S., Nakamura, A. J., Georgakilas, A. G. & Bonner, W. M. (2010). Role of oxidatively induced DNA lesions in human pathogenesis. *Mutat Res* **704**, 152–159.

Severi, T., Ying, C., Vermeesch, J. R., Cassiman, D., Cnops, L., Verslype, C., Fevery, J., Arckens, L., Neyts, J. & van Pelt, J. F. (2006). Hepatitis B virus replication causes oxidative stress in HepAD38 liver cells. *Mol Cell Biochem* **290**, 79–85.

Shackel, N. A., McGuinness, P. H., Abbott, C. A., Gorrell, M. D. & McCaughan, G. W. (2002). Insights into the pathobiology of hepatitis C virus-associated cirrhosis: analysis of intrahepatic differential gene expression. *Am J Pathol* **160**, 641–654.

Shirakata, Y. & Koike, K. (2003). Hepatitis B virus X protein induces cell death by causing loss of mitochondrial membrane potential. *J Biol Chem* **278**, 22071–22078.

Simonetti, R. G., Cammà, C., Fiorello, F., Cottone, M., Rapicetta, M., Marino, L., Fiorentino, G., Craxi, A., Ciccaglione, A. & other authors (1992). Hepatitis C virus infection as a risk factor for hepatocellular carcinoma in patients with cirrhosis. A case-control study. *Ann Intern Med* **116**, 97–102.

Simula, M. P. & De Re, V. (2010). Hepatitis C virus-induced oxidative stress and mitochondrial dysfunction: a focus on recent advances in proteomics. *Proteomics Clin Appl* **4**, 782–793.

Singal, A. K., Jampana, S. C. & Weinman, S. A. (2011). Antioxidants as therapeutic agents for liver disease. *Liver Int* **31**, 1432–1448.

Smirnova, I. S., Aksenov, N. D., Vonsky, M. S. & Isaguliant, M. G. (2006). Different transformation pathways of murine fibroblast NIH 3T3 cells by hepatitis C virus core and NS3 proteins. *Cell Biol Int* **30**, 915–919.

Takada, S., Shirakata, Y., Kaneniwa, N. & Koike, K. (1999). Association of hepatitis B virus X protein with mitochondria causes mitochondrial aggregation at the nuclear periphery, leading to cell death. *Oncogene* **18**, 6965–6973.

Toh, S. T., Jin, Y., Liu, L., Wang, J., Babrzadeh, F., Gharizadeh, B., Ronaghi, M., Toh, H. C., Chow, P. K. & other authors (2013). Deep sequencing of the hepatitis B virus in hepatocellular carcinoma patients reveals enriched integration events, structural alterations and sequence variations. *Carcinogenesis* **34**, 787–798.

Vafa, O., Wade, M., Kern, S., Beeche, M., Pandita, T. K., Hampton, G. M. & Wahl, G. M. (2002). c-Myc can induce DNA damage, increase reactive oxygen species, and mitigate p53 function: a mechanism for oncogene-induced genetic instability. *Mol Cell* **9**, 1031–1044.

van de Klundert, M. A., van Hemert, F. J., Zaaier, H. L. & Kootstra, N. A. (2012). The hepatitis B virus x protein inhibits thymine DNA glycosylase initiated base excision repair. *PLoS ONE* **7**, e48940.

Vurusaner, B., Poli, G. & Basaga, H. (2012). Tumor suppressor genes and ROS: complex networks of interactions. *Free Radic Biol Med* **52**, 7–18.

Wang, T. & Weinman, S. A. (2006). Causes and consequences of mitochondrial reactive oxygen species generation in hepatitis C. *J Gastroenterol Hepatol* **21** (Suppl 3), S34–S37.

Wang, T. & Weinman, S. A. (2013). Interactions between hepatitis C virus and mitochondria: impact on pathogenesis and innate immunity. *Curr Pathobiol Rep* **1**, 179–187.

Wang, X. W., Forrester, K., Yeh, H., Feitelson, M. A., Gu, J. R. & Harris, C. C. (1994). Hepatitis B virus X protein inhibits p53 sequence-specific DNA binding, transcriptional activity, and association with transcription factor ERCC3. *Proc Natl Acad Sci U S A* **91**, 2230–2234.

Wang, H. C., Chang, W. T., Chang, W. W., Wu, H. C., Huang, W., Lei, H. Y., Lai, M. D., Fausto, N. & Su, I. J. (2005). Hepatitis B virus pre-S2 mutant upregulates cyclin A expression and induces nodular proliferation of hepatocytes. *Hepatology* **41**, 761–770.

Wang, A. G., Lee, D. S., Moon, H. B., Kim, J. M., Cho, K. H., Choi, S. H., Ha, H. L., Han, Y. H., Kim, D. G. & other authors (2009). Non-structural 5A protein of hepatitis C virus induces a range of liver pathology in transgenic mice. *J Pathol* **219**, 253–262.

Woodbine, L., Brunton, H., Goodarzi, A. A., Shibata, A. & Jeggo, P. A. (2011). Endogenously induced DNA double strand breaks arise in heterochromatic DNA regions and require ataxia telangiectasia mutated and Artemis for their repair. *Nucleic Acids Res* **39**, 6986–6997.

Yamanaka, T., Kodama, T. & 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.

Yun, C., Lee, J. H., Park, H., Jin, Y. M., Park, S., Park, K. & Cho, H. (2000). Chemotherapeutic drug, adriamycin, restores the function of p53 protein in hepatitis B virus X (HBx) protein-expressing liver cells. *Oncogene* **19**, 5163–5172.