

WJG 20<sup>th</sup> Anniversary Special Issues (1): Hepatocellular carcinoma**Oxidative damage in the progression of chronic liver disease to hepatocellular carcinoma: An intricate pathway**

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**Abstract**

The histo-pathologic and molecular mechanisms leading to initiation and progression of hepatocellular carcinoma (HCC) are still ill-defined; however, there is increasing evidence that the gradual accumulation of mutations, genetic and epigenetic changes which occur in preneoplastic hepatocytes results in the development of dysplastic foci, nodules, and finally, overt HCC. As well as many other neoplasias, liver cancer is considered an "inflammatory cancer", arising from a context of inflammation, and characterized by inflammation-related mechanisms that favor tumor cell survival, proliferation, and invasion. Molecular mechanisms that link inflammation

and neoplasia have been widely investigated, and it has been well established that inflammatory cells recruited at these sites with ongoing inflammatory activity release chemokines that enhance the production of reactive oxygen species. The latter, in turn, probably have a major pathogenic role in the continuum starting from hepatitis followed by chronic inflammation, and ultimately leading to cancer. The relationship amongst chronic liver injury, free radical production, and development of HCC is explored in the present review, particularly in the light of the complex network that involves oxidative DNA damage, cytokine synthesis, telomere dysfunction, and microRNA regulation.

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**Key words:** Reactive oxygen species; Viral hepatitis; Hepatocellular carcinoma; Telomere dysfunction; Cytokines; mitochondria; Antioxidant mechanisms; MicroRNA and circulating free DNA

**Core tip:** In this review, the relationship amongst chronic liver injury, free radical production, and development of hepatocellular carcinoma is explored. The review confirms the existence, in the intricate pathway involved in the progression of virus-related liver injury to cirrhosis and cancer, of a link between oxidative genomic and mitochondrial damage and telomere dysfunction. This link develops in the context of inflammatory response and induces a derangement of mechanisms controlling liver proliferation. In this scenario, mitochondria are emerging as a possible target for new treatments aimed at counteracting oxidative damage and disease progression to cancer, given their relevant role in inflammation and carcinogenesis.

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## EPIDEMIOLOGY

Hepatocellular carcinoma (HCC) is the most common primary malignancy of the liver, the fifth most common cancer in men worldwide, and the seventh most frequent in women, with over 600000-650000 new cases diagnosed annually. Hepatitis B virus (HBV) and hepatitis C virus (HCV)-related chronic liver diseases are responsible for the majority of HCC cases, making this neoplasia potentially preventable.

The distribution of HCC presents a sharp variability according to the geographic area involved. In general terms, there are three major areas with different incidence rates: Eastern Asia and sub-Saharan Africa, with incidence rates that peak at 100/100000 individuals, Mediterranean countries such as Italy, Spain, and Greece with intermediate rates (10-20 per 100000 individuals), and North and South America, with a relatively low incidence (< 5 per 100000). In regions at high incidence, the most common cause is HBV transmitted at birth, while in North America and Europe the most common etiology is HCV, with infection acquired much later in life.

Male gender is a risk factor for HCC, that is in fact more common in men than in women, partly dependent upon factors other than viral infection, such as the hormonal and immunological status, as well as alcohol consumption. The major established risk factor is cirrhosis, insofar as 80%-90% of cases of HCC occur in the setting of cirrhosis<sup>[1]</sup>.

## ETIOLOGY

A variety of factors have been associated with the development of HCC, including hepatitis viruses, heavy alcohol intake, nonalcoholic fatty liver disease, aflatoxin B1 exposure, obesity, diabetes, dietary habits, and iron accumulation<sup>[2]</sup>. The presence and importance of these factors vary according to the geographical region, thereby influencing preventive measures that may be enacted, prognosis of patients, and treatment recommendations<sup>[3]</sup>. The incidence of HCC is rising rapidly in some, but not in all, Western countries, and is declining in Europe<sup>[4]</sup> in relation to the distribution of risk factors (hepatitis C infection, alcoholism, and obesity)<sup>[5]</sup>. Recent data indeed demonstrate that HCV is now declining as a risk factor for HCC<sup>[6]</sup>. It is well established that both HBV and HCV cause malignant transformation and lead to HCC development through direct and indirect mechanisms. The former involve viral proteins and specific mutations induced by the integration of the virus into the host genome, which is particularly

true for HBV infection. Indirect mechanisms, in contrast, imply the induction of chronic inflammation by the immune response elicited. The time course of virus-related liver damage and the development of HCC is different based on the virus considered: progression of the disease to HCC in HCV-infected patients requires nearly 10 years from the establishment of cirrhosis, and approximately 30 years from the initial exposure to the virus. On the other hand, the course of HBV infection is less predictable, and HCC may thus precede the occurrence of cirrhosis<sup>[1,7]</sup>.

## INFLAMMATION AND OXIDATIVE STRESS

In the last twenty years, the main focus of our research group has been to investigate the events related to oxidative stress occurring in the natural history of viral hepatic disease, with a particular interest in the pathways of HCC development resulting from HBV- and HCV-related damage. The relationship between chronic inflammation and the risk of cancer development is well known. Virchow established more than a century ago the link between cancer and inflammation, and a bulk of evidence from published and ongoing studies contributes to elucidating the molecular mechanisms at the basis of this process. Inflammation involves macrophages, mast cells, dendritic and natural killer cells that are recruited within damaged tissues, and which release chemical mediators such as cytokines, chemokines, and reactive oxygen species (ROS)<sup>[8]</sup>. The most important ROS include free oxygen radicals like superoxide (O<sub>2</sub><sup>•-</sup>), hydroxyl radical (OH<sup>•</sup>), nitric oxide (NO<sup>•</sup>) radicals, as well as non-radical ROS like hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), organic hydroperoxides, and hypochloride. A physiologic amount of ROS plays a key role in several cellular processes including proliferation, apoptosis, cell cycle arrest, and cell senescence. Different anti-oxidant mechanisms regulate ROS production, but when generated ROS exceed the basal amount and the cellular defenses, they can damage cell macromolecules including proteins, lipids, and nucleic acids (DNA and RNA). Under these conditions, ROS might play a major role in the genesis of different chronic diseases and, particularly, in the initiation/promotion phase of carcinogenesis. In particular, ROS may stimulate the growth of malignant cells or increase the activity of carcinogenic xenobiotics by facilitating their activation to reactive compounds. Among the many ROS produced during the inflammatory process, the most damaging is OH<sup>•</sup>, which is responsible for a number of lesions. When DNA is attacked by ROS, stable covalent bonds are produced, leading to the formation of base modifications, including the formation of thymine and thymidine glycol, 5-hydroxymethyluracil, and 8-hydroxydeoxyguanosine (8-OHdG). 8-OHdG, the main ROS-induced DNA adduct, generates a point mutation in the DNA daughter strands. In fact, studies using DNA templates containing 8-OHdG indicate that the adduct accumulates and causes mispairing, thus suggesting that

this lesion is mutagenic and therefore potentially carcinogenic. Thus, 8-OHdG is used as a reliable marker not only of oxidative stress<sup>[9]</sup> but also of cancer risk.

Results in the literature suggest that both oxidative and nitrative DNA damage occur at the sites of carcinogenesis, regardless of the etiology of the disease. Inducible nitric oxide synthase (iNOS) produces NO through a reaction that converts arginine and oxygen into citrulline. NO is a major mediator of chronic inflammation and participates in the regulation of cell proliferation, survival, migration, angiogenesis, DNA repair, and drug resistance. Therefore, excessive amounts of reactive nitrogen species produced *via* iNOS in chronic inflammation may play an important role in tumorigenesis<sup>[10]</sup> and it has been suggested that selective targeting of iNOS may prove a useful therapeutic or chemopreventive measure<sup>[11,12]</sup>.

An increased production of ROS has been documented in virus-related disease, with a strong link between HCV core protein or HBV X protein on one hand, and oxidative “burst” on the other, particularly in the first phases of carcinogenesis<sup>[13]</sup>. Our own findings have suggested a progressive build-up of genomic oxidative damage which takes place not only in patients with chronic hepatitis and cirrhosis, but also in anti-HCV positive patients with persistently normal ALT levels<sup>[14]</sup>. Our data on 8-OHdG in HCV-related liver damage have been confirmed by others, such as Shimoda *et al.*<sup>[15]</sup>, who also reported increased levels of 8-OHdG in DNA extracted from liver tissues and leukocytes of individuals with chronic HCV-related liver disease<sup>[16,17]</sup>. New results on HBV-related liver damage have shown a delayed accumulation of oxidative DNA damage with respect to HCV patients, which significant increases only in the later stages of the disease, in association with a significant accumulation of fibrosis, and this is particularly true in livers of patients in whom cancer develops (unpublished data).

## OXIDATIVE STRESS AND DNA REPAIR ENZYME

Like any other DNA damage, 8-OHdG undergoes a specific repair process. A human DNA glycosylase/AP lyase encoded by the *OGG1* gene removes 8-OHdG directly from DNA and suppresses its mutagenic effect. Among the multiple *OGG1* isoforms, *OGG1*-type 1a is expressed mainly in human cells and repairs chromosomal DNA. The human *OGG1* gene maps to chromosome 3p26.2 and allelic deletions of this region frequently occur in a variety of human cancers. Inactivation of the *OGG1* gene in yeast and mice leads to high rates of spontaneous mutation in the cells. The gene is also somatically mutated in certain cancer cells, and is highly polymorphic among human populations. The repair activity of mutated and polymorphic *OGG1* protein is lower than that of the wild-type *OGG1* protein, and may consequently be involved in human carcinogenesis, though full agreement on the point has not been reached<sup>[18,19]</sup>.

We evaluated *OGG1* gene polymorphism in HBV- and HCV-related hepatitis tissue samples. No significant difference was found between 8-OHdG levels evaluated in wild type compared with heterozygous patients, nor in HCV or HBV patients with HCC, nor in chronic hepatitis and cirrhosis, therefore downsizing the relevance of *OGG1* gene polymorphism in liver disease (unpublished data).

## OXIDATIVE STRESS AND MITOCHONDRIA

In normal healthy cells, mitochondria are involved in several fundamental cellular processes, including cell proliferation, apoptosis, and intracellular calcium homeostasis. Mitochondrial dysfunction can affect a range of important cellular functions and can result in a variety of diseases<sup>[20]</sup>. Emerging evidence strongly supports a key role of mitochondria in carcinogenesis. In conditions of oxidative stress, the transcriptional and replication machinery of mitochondria is up-regulated, thus resulting in increased mitochondrial biogenesis *via* replication of the mitochondrial genome (mtDNA). In these damaged mitochondria, the electron transport chain may be blocked, resulting in an accumulation of ROS. As mitochondrial DNA is located close to the source of ROS production, DNA itself can become damaged, resulting in accumulation of deletions and mutations<sup>[21]</sup>. Increased levels of ROS also alter mitochondrial metabolism, increasing mitochondrial membrane permeability and leading to the release of pro-apoptotic factors into the cytosol. Research is now more clearly defining the molecular mechanisms and the signaling pathways involved in the process of “mitochondrial malignancy”. These pathways will become clearer as the respective roles of ROS and of cancer-related proteins such as RAS, p53 and c-myc in regulating mitochondria will be clarified<sup>[22]</sup>.

## OXIDATIVE STRESS, ANTI-OXIDANT DEFENSES AND DRUGS

Cells have developed defense mechanisms to counteract the negative effects of oxidative stress, among which redox active glutathione, thioredoxin, antioxidant enzymes (superoxide dismutase, catalase and glutathione peroxidases)<sup>[23]</sup>. These aspects are particularly relevant in view of the possible application of anti-oxidants in cancer prevention. Conventional anti-oxidants show little efficacy against oxidative stress *in vivo*, probably because they are not taken up by mitochondria. Mitochondria-targeted anti-oxidants such as Vitamin K3 or Vitamin E may be used to decrease mitochondrial oxidative damage, and drugs such as mitoquinone have been developed in this regard. This novel class of compounds combines a potent anti-oxidant, such as ubiquinone, with a lipophilic cation that causes the anti-oxidant to accumulate several hundred-fold within mitochondria. Several *in vitro* and animal studies have shown that this selective accumulation renders mitoquinone more

protective against ROS and cell death than untargeted anti-oxidants. The administration of the above compounds may constitute a novel approach to decreasing liver damage in chronic HCV infection. Potentially, anti-oxidants could be used in association with the standard interferon/ribavirin treatment in patients with chronic HCV-related hepatitis<sup>[24,25]</sup>. Yet very few studies have been published so far on the effects of anti-oxidants in patients with chronic HCV infection. Alpha tocopherol and fermented papaya both improved redox status in HCV-related cirrhosis, but only in the subgroup of vitamin E-deficient patients was normalization of transaminase levels obtained<sup>[26]</sup>. In another study, a tomato-based food supplement increased serum lycopene and carotenoids, and decreased serum hydroperoxides in patients with HCV infection, again with no effect on transaminases<sup>[27]</sup>. In yet another study, Viusid<sup>®</sup>, a nutritional supplement containing ascorbic acid, zinc and glycyrrhizin, of recognized anti-oxidant properties, achieved reduction of serum oxidative stress markers in patients with chronic HCV-related hepatitis; no effect on the evolution of liver damage was reported, however<sup>[28]</sup>. Finally, in another study, a combination of different anti-oxidants at the “appropriate” dose for twenty weeks achieved normalization of liver enzymes levels in 44% of treated patients, decreasing viral load in 25%<sup>[29]</sup>. Studies recently published by our group have demonstrated that coffee consumption induces a significant reduction of oxidative DNA damage, thus confirming that the protection exerted by coffee with respect to HCC is mediated by a reduced accumulation of oxidized bases, and consequently, of DNA mutations. The relevance of oxidative stress in carcinogenesis<sup>[30]</sup> and, particularly in patients with HCV infection<sup>[17,31]</sup>, is well known, and the association between coffee consumption and lower oxidative DNA damage has been recently observed also by Mišák *et al.*<sup>[32]</sup> in healthy volunteers. The protective effect of coffee with respect to HCC may be due to the numerous anti-oxidant compounds contained in this beverage, among which are polyphenols, or to the induction of antioxidant enzymes. This effect is exerted even at very small doses or even when coffee is consumed for a relatively short period, as in our above quoted study. Confirming what above, long-term glycyrrhizin administration reportedly reduces HCC incidence in interferon-resistant patients with chronic HCV infection<sup>[33]</sup>, as demonstrated in a cohort study, which although not randomized, included a large series of patients and produced results which are sound enough to be considered as relevant.

## OXIDATIVE STRESS, CYTOKINES AND APOPTOSIS

As has been amply discussed herein, liver injury is associated with chronic inflammation; in a local inflammatory milieu, several cell types normally residing in the liver (sinusoidal, endothelial and Kupffer cells) produce immune mediators as well as cytokines and chemokines, whose receptors are located on the cell surface of hepatocytes.

These cells also express and release interleukin (IL)-6,

an important pro-inflammatory cytokine involved in tumor cell proliferation by its role in inhibiting apoptosis through the activation of signal transducer and activator of transcription 3<sup>[34]</sup>. Tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), a pro-inflammatory immune mediator produced by Kupffer cells and other immune cells in response to tissue injury, triggers the production of other cytokines that, in turn, recruit inflammatory cells, promoting fibrogenesis and further activating the oxidative burst<sup>[35]</sup>. Amongst the myriad effects of TNF- $\alpha$  is, importantly, the activation of intracellular apoptotic and/or anti-apoptotic pathways. The role of TNF- $\alpha$  expression in HCC, however, remains controversial, with reports of expression varying from high<sup>[36]</sup> to low<sup>[37,38]</sup>. The question that ensues is henceforth to establish how persistent oxidative stress fits into this scenario. TNF- $\alpha$  also has an important role in oxidative stress induction, causing DNA damage through the formation of 8-OHdG in primary murine hepatocytes<sup>[39]</sup>. The overproduction of oxidative species linked to the over-expression of inflammatory cytokines may be responsible for inhibiting the apoptotic process, probably by activating the nuclear factor (NF)- $\kappa$ B-dependent pathway<sup>[40]</sup>. Oxidative stress is also related to the expression of proto-oncogenes, such as c-myc, which is significantly more expressed in cirrhotic than in non-cirrhotic tissues in our experience as well. This means that the progression of tissue damage from hepatitis to cirrhosis, and the related cell growth changes, may be to some degree mediated by c-myc<sup>[41]</sup>. For instance, recent studies have demonstrated that TGF- $\alpha$ /c-myc double transgenic mice exhibit enhanced cell proliferation and accumulate extensive oxidative DNA damage, a phenomenon that may account for an accelerated progression to cancer<sup>[42]</sup>. Recently, we sought the possible correlations between oxidative DNA damage and levels of pro-inflammatory cytokines, TGF- $\alpha$  and c-myc in chronic HCV-related liver damage, and a clear correlation between 8-OHdG levels and c-myc expression has been detected, confirming the relevance of oxidative DNA damage in liver carcinogenesis<sup>[17]</sup>. As far as IL-1 $\beta$  is concerned, recent reports have shown that higher levels of this cytokine are present in HCV-related liver disease with respect to other forms of liver damage<sup>[43]</sup> and that its polymorphisms are correlated with the risk of progression to HCC. IL-1 $\beta$  has been reported to trigger the inflammatory response cascade and to directly cause growth arrest and TNF- $\alpha$  expression induction<sup>[44,45]</sup>. This last effect was not confirmed in our experience, however, since no correlation emerged between IL-1 $\beta$  and TNF- $\alpha$  levels in liver tissues of patients with HCV-related liver damage<sup>[46]</sup>. On the other hand, IL-1 $\beta$  expression was higher in the later stages of HCV-related liver disease, as already demonstrated by Gramantieri *et al.*<sup>[47]</sup>.

## OXIDATIVE STRESS AND EPITHELIAL-MESENCHYMAL TRANSITION

Epithelial-mesenchymal transition (EMT) is a biologic process by which epithelial cells undergo changes that induce the development of a mesenchymal phenotype,

increasing the production of extracellular matrix proteins and the resistance to apoptotic cell death. These changes are considered as a driving force also in tumor progression since they enhance the cell migratory ability and invasiveness.

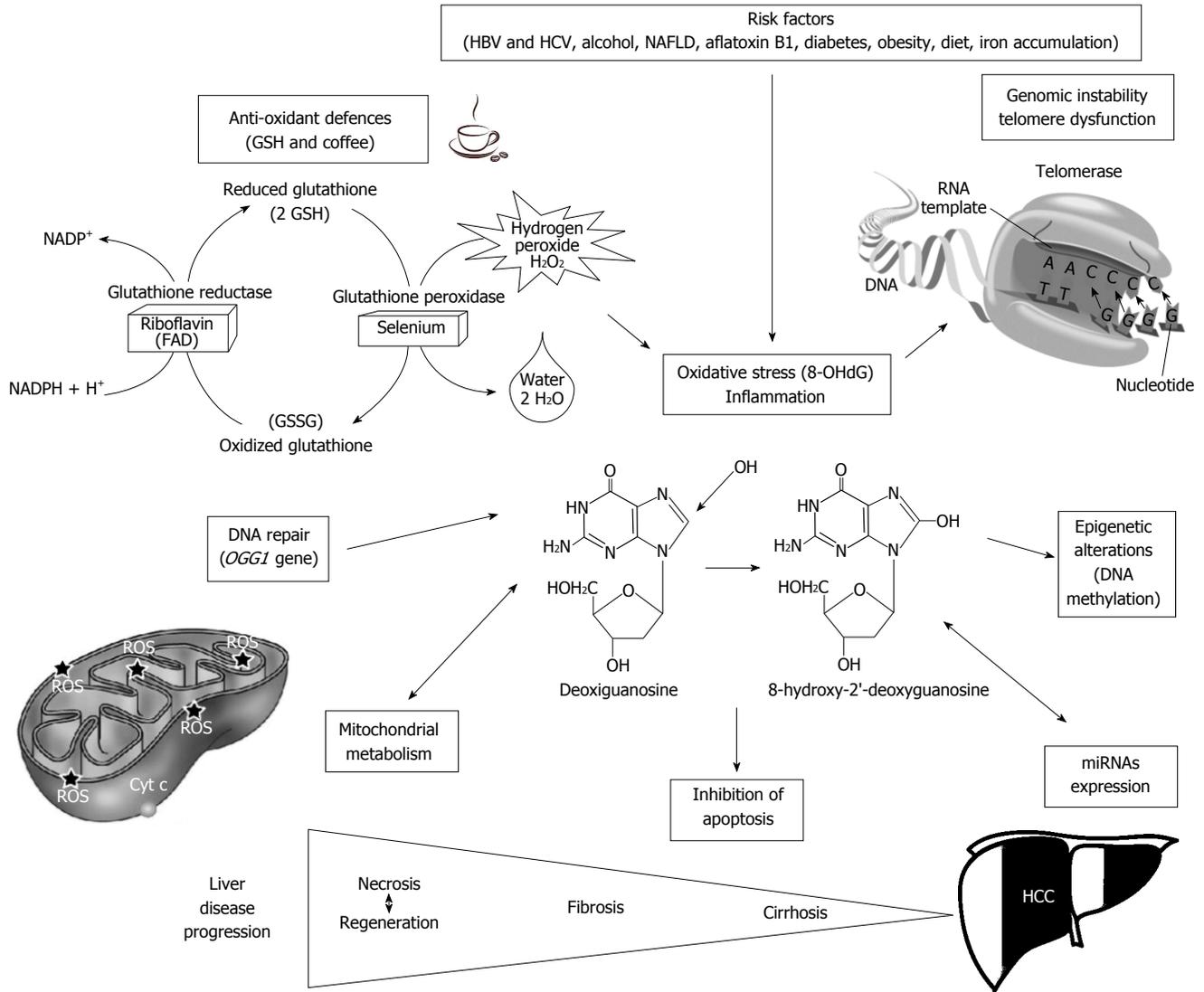
Several studies demonstrated the existence of a strong correlation between ROS production and EMT, a link that involves NF- $\kappa$ B activation in collaboration with hypoxia-related production of hypoxia-inducible factor-1 and cyclooxygenase-2<sup>[48]</sup>. HCV and HBV infections are involved in prompting EMT in the development of HCC, a process in which the progression of malignant hepatocytes depends in part on the signaling of transforming growth factor- $\beta$  (TGF- $\beta$ ), produced by stromal cells (fibroblasts, macrophages *etc.*)<sup>[23]</sup>. TGF- $\beta$  activation triggers an increase in intracellular ROS production in association with the phosphorylation of Smad2, p38 and ERK1/2<sup>[49]</sup>.

Many other factors correlated with ROS production are involved in EMT, such as mitogen-activated protein kinase activation and angiotensin II, E-cadherin and  $\alpha$ -SMA up-regulation, thus supporting a redox-mediated regulation of EMT<sup>[23]</sup>.

## OXIDATIVE STRESS AND TELOMERE DYSFUNCTION

Cells that are not able to start the process of apoptosis, in particular after DNA damage, can be more susceptible to genetic alterations and to the acquisition of immortality through the modulation of telomerase activity. Telomerase, a RNA-dependent DNA polymerase, is a complex ribonucleoprotein including two components, a catalytic subunit (TERT) and a RNA component complementary to telomeric sequences (TR). After retrotranscription of its own RNA, telomerase adds telomeric sequences to chromosomal terminal portions, thus maintaining the length of telomeres, whose main function is to stabilize the chromosomal structure, endowing this molecule with a very important role in cell proliferation, senescence, immortalization and carcinogenesis<sup>[50]</sup>. Nevertheless, telomeres shorten at each replication cycle, and lose their function after reaching a critical length (telomeric crisis). Telomere shortening may result in end-to-end fusions during the cell cycle and, consequently, somatic cells stop proliferation and enter senescence phase and apoptosis<sup>[51]</sup>. In neoplastic cells, telomeric shortening, senescence, and apoptosis are avoided by an increased telomerase function. Telomere shortening and chromosomal instability occur in the first phases of carcinogenesis, while tumor progression is linked to a telomeric preservation induced by a restarting of telomerase activity. In fact, only cells that maintain telomere length, with unlimited cell divisions and chromosomal instability, possess a higher potential of neoplastic transformation and progression to cancer. The telomeres, rich in guanines, are highly sensitive to ROS attack, in particular by hydroxyl radical. ROS interaction with telomeric sequences creates DNA adducts as 8-OHdG. Thus, oxidative stress can accelerate

telomeric shortening also because, unlike in most genomic DNA, the repair mechanisms of telomeric DNA are less efficient and telomeres more easily accumulate oxidative damage. Consequently, measuring telomere length may constitute an optimal biomarker of chronic oxidative stress<sup>[52]</sup>. Only a few studies have specifically examined the relationship between telomerase activity, telomere length and the extent of oxidative stress and consequent oxidative DNA damage in hepatocarcinogenesis. Our group has demonstrated that oxidative DNA damage interferes with telomere function, thus playing a key role in hepatic carcinogenesis (unpublished data). In fact, we confirmed the shorter telomere length in both HCV- and HBV-related HCC, possibly due to the accumulation of genetic alterations and 8-OHdG during disease progression. Alterations of the promoter may be one of the factors that control the transcriptional activity of TERT. The hypermethylation of CpG islands acts as an alternative, complementary pathway to gene mutation, and is an important mechanism involved in carcinogenesis. Both HBV and HCV induce epigenetic changes in specific genes involved in DNA repair, cell cycle control, and apoptosis signaling (RASSF1A, GSTP1, CHRNA3, and DOK1) in HCC as compared to cirrhotic or normal liver tissues<sup>[53,54]</sup>. Our data confirm changes in the methylation levels of the TERT promoter gene in the terminal stages of the disease in both HBV- and HCV-related hepatitis. Further studies are needed to better understand the mechanisms regarding genetic and epigenetic changes induced by HBV and HCV in hepatocytes. The majority of the biological properties ascribed to TERT have been limited to its effects in the nuclear genome. Several data have been published, however, demonstrating that TERT is also targeted towards mitochondria, resulting in telomerase activity in this organelle. TERT can shuttle from the nucleus to mitochondria upon oxidative challenge. Nevertheless, the exact role of mitochondrial TERT remains controversial, with some authors sustaining that it exacerbates oxidative injury while others reporting a protective effect. Recent data in literature report that conditions of chronic oxidative stress may lead to the migration of TERT subunit of telomerase to the cytosol, following the phosphorylation of tyrosine 707 by the Src kinase, thus reducing nuclear activity of the enzyme<sup>[55-58]</sup>. Our group has also observed TERT subunit translocation from nucleus to mitochondria in HCC tissue samples under oxidative stress. What the function of TERT is in mitochondria remains a matter of debate, but one of the most relevant hypothesis is that it may play a role in modulating apoptosis. In fact, Haendeler *et al.*<sup>[59]</sup> demonstrated that TERT interacts with mtDNA, improving the electron transport chain activity and protecting cells from ROS-induced oxidative damage. This mechanism would reduce the permeability of the mitochondrial membrane and would avoid the leakage of pro-apoptotic factors. The reduction of apoptotic signals in a context of chronic oxidative stress, genomic instability, and increased cell proliferation might be very crucial in terms of neoplastic transformation.



**Figure 1** Intricate pathway of molecular mechanisms involved in the progression of virus-related liver injury to cirrhosis and hepatocellular carcinoma. HBV: Hepatitis B virus; HCV: Hepatitis C virus; NAFLD: Nonalcoholic fatty liver disease; HCC: Hepatocellular carcinoma.

## OXIDATIVE STRESS AND MICRORNAS

Recently, the important role of microRNAs (miRNAs) in the different stages of chronic liver diseases in the development of HCC has been recognized<sup>[60]</sup>. MiRNAs are small (21-23 nucleotides long) noncoding RNA family involved in post-transcriptional gene regulation of their target genes. In fact, miRNAs induce translational repression *via* their binding to partially complementary sequences or mRNA degradation through their binding to perfectly complementary sequences in the 3'UTR of mRNAs<sup>[61]</sup>. Each mature miRNA potentially controls many gene targets, and each mRNA is regulated by multiple miRNAs. To date, more than 17000 distinct mature miRNA sequences have been identified from over 140 species<sup>[62]</sup>. MiRNAs are now clearly identified as key mediators of the immune system development and function, in particular for activation in response to infection during both the innate and the adaptive immune responses. At the same time, miRNAs dysregulation is a central event in

the development of a number of cancers, as it is involved in inflammation and oncogenesis. Several miRNAs, whose expression is modulated in HCC, have been identified, again also during oxidative damage (Table 1). Our research group has recently published a significant positive correlation between miRNA-92 expression, which is already linked to hepadnavirus-associated carcinogenesis, c-myc and 8-OHdG levels in HCC tissues. Besides, our data demonstrate that miRNA-199a, miRNA-199b, miRNA-195 and miRNA-122a are strongly down-regulated in the majority (55%-70%) of HCCs, while miRNA-92 and miRNA-145 show a less marked down-regulation. In contrast, miRNA-222 is up-regulated in HCC<sup>[72]</sup>.

## OXIDATIVE STRESS AND CIRCULATING FREE DNA

As previously exposed, oxidative stress is known to cause DNA damage, and cells with the greatest DNA injury die

**Table 1** MicroRNAs involved in oxidative stress in different liver diseases

miRNA	Involvement in oxidative stress liver diseases	Ref.
miR-214	Alcohol induced liver disease	[63]
miR-199a-5p	Cholestatic disease	[64]
miR-122	Hepatitis C virus/NASH	[65,66]
let-7	HCC	[67]
miR-125b	Liver inflammation	[68]
miR-199a-3p	Mitochondrial dysfunction	[69]
miR-34a/miR-93	Liver aging (rat model)	[70]
miR-196	Hepatitis C virus	[71]
miR-92	HCC	[72]

HCC: Hepatocellular carcinoma; NASH: Non-alcoholic steatohepatitis.

either by necrosis or apoptosis. Oxidized DNA released from dying cells is likely the most prominent contributor to circulating cell-free DNA (cfDNA), which is a double or single stranded extracellular DNA, released by tumor apoptotic or necrotic cells and circulating in blood as a complex with histonic proteins<sup>[73]</sup>. Low levels of cfDNA can be detected as well in healthy individuals, but higher levels characterize patients with a number of diseases including cancer<sup>[74]</sup>. Our recent study has investigated the time course of cfDNA levels in patients in different stages of liver damage, from chronic hepatitis to cirrhosis and cancer. Data have shown that cfDNA is detectable in a small share of these last patients, but in a substantially similar percentage of CIRR and HCC patients. In HCC however, cfDNA levels are, on average, two times higher than in CIRR and, by choosing the right cut-off with ROC curves, the sensitivity in diagnosis is notably high. In our experience therefore, the role of the cfDNA quantitative analysis as a valuable diagnostic test is debatable, but cfDNA levels allow for patients discrimination with more advanced stages of disease, demonstrating a prognostic relevance in patients with HCC (unpublished data).

In conclusion, we confirm the existence of a link between oxidative genomic and mitochondrial damage and telomere dysfunction in the intricate pathway (Figure 1) involved in the progression of virus-related liver injury to cirrhosis and HCC. This link develops in the context of the inflammatory response and leads to a derangement of the basic mechanisms controlling liver proliferation. Unfortunately, despite the evidence of a clear role for oxidative burst as an inducer of liver damage in patients with viral hepatitis, nor antioxidant drugs nor cocktails of vitamins or different compounds have been demonstrated to actively interfering with the development of damage. In this scenario, mitochondria are emerging as a possible target for new treatments aimed at counteracting oxidative damage and disease progression as well as cancer development, given the relevant role that these organelles play in inflammation and carcinogenesis.

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