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Review

Does oxidative stress participate in the development of hepatocellular carcinoma?

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Key words: oxidative stress, HCC, ROS, hepatitis virus, alcohol, NASH

Introduction

Hepatocellular carcinoma (HCC) ranks among the most common cancers in the world, and it is one of the leading causes of cancer death in Japan. Chronic hepatitis and liver cirrhosis associated with either hepatitis B virus (HBV) or hepatitis C virus (HCV) infection represent major risk factors for HCC development, being implicated in more than 80% of HCC cases worldwide.[1] Alcohol is also a strong primary cause when HCC develops in patients who are heavy alcoholics, most of whom have alcohol-induced cirrhosis. In addition, alcohol is a cofactor when associated with other causative factors including HCV, HBV, and diabetes mellitus.

On the other hand, oxidative stress can occur through overproduction of reactive oxygen species (ROS) or reactive nitrogen species (RNS) through either endogenous or exogenous insults, and is recognized to play an important role in the initiation and promotion of the events of carcinogenesis.[2,3] In this context, oxidative stress has emerged as a key player in the pathogenesis of chronic liver diseases and precancerous lesions, induced by HBV or HCV infection, because polymorphonuclear neutrophils (PMNs) in an inflamed liver are a major source of ROS.[4] Moreover, nonparenchymal cells, including Kupffer cells and macrophages, which release cytokines, are another cause of ROS induction in hepatocytes.[5] In addition, virus proteins may also generate oxidative stress. On the other hand, alcohol liver disease is associated with significant oxidative stress as well as iron accumulation.[6] The combined prooxidant potentials of ethanol and iron are at least additive and possibly synergistic with regard to induction of oxidative stress and antioxidant depletion in hepatocytes. Lately, calorie-enriched diets and lack of exercise are causing a worldwide surge in obesity, insulin resistance, and lipid accumulation in the liver (hepatic steatosis), which can lead to steatohepatitis. Evidence is accumulating that mitochondrial dysfunction plays a crucial role in the progression of nonalcoholic steatohepatitis (NASH).[7] Generation of ROS as well as RNS, accompanied by lipid peroxidation, further impair mitochondrial function.

From these points of view, oxidative stress plays a central role in the pathogenesis and progression of liver diseases. Important to carcinogenesis, unregulated or prolonged ROS production has been linked to mutation as well as to modification of gene expression. This review focuses on the mechanisms of ROS production and of ROS-induced cellular damage and modification of gene expression. Finally, this review discusses how ROS generated in chronic liver diseases participate in hepatocarcinogenesis.

Generation of ROS

ROS encompass a variety of partially reduced metabolites of oxygen (i.e., superoxide anions, hydrogen peroxide, and hydroxyl radicals), which possess higher reactivities than molecular oxygen and are generated endogenously as a consequence of normal cell functions or derived from external sources (Fig. 1).

Endogenous sources of ROS

Potential endogenous sources include oxidative phosphorylation, p450 metabolism, peroxisomes, and inflammatory cell activation.

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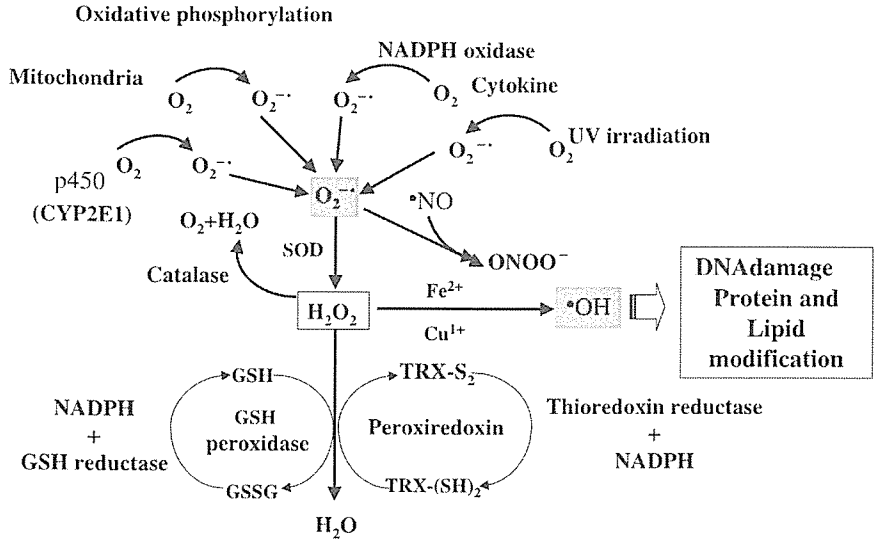

Fig. 1. Reactive oxygen species (ROS) and antioxidant defense mechanisms. ROS encompass a variety of partially reduced metabolites of oxygen possessing higher reactivities than molecular oxygen, and are generated endogenously as a consequence of normal cell functions or derived from external sources. A number of antioxidant defense systems have evolved to combat the accumulation of ROS. These include enzymatic and nonenzymatic molecules. *CYP2E1*, cytochrome p450 2E1; *SOD*, superoxide dismutase; *GSH*, reduced glutathione; *GSSG*, oxidized glutathione

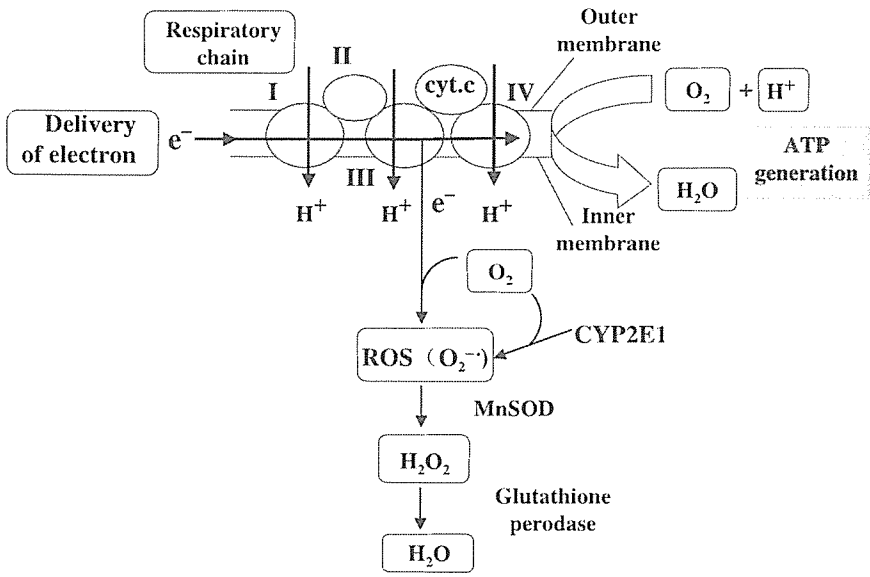

Fig. 2. Oxidative phosphorylation accompanied by ROS generation. During mitochondrial oxygen metabolism, most electrons provided to the respiratory chain migrate all the way along the respiratory chain and finally reach cytochrome c oxidase (complex IV). However, a fraction of these electrons can directly react with molecular oxygen to form ROS, primarily $O_2^{-\cdot}$. $O_2^{-\cdot}$ is, in turn, dismutated by mitochondrial MnSOD into H_2O_2, which is subsequently detoxified into H_2O by the mitochondrial glutathione peroxidase. *MnSOD*, manganese superoxide dismutase

During mitochondrial oxygen metabolism, most electrons provided to the respiratory chain migrate all the way along the respiratory chain, and finally reach cytochrome c oxidase (complex IV), where they safely combine with oxygen and protons to form H_2O. However, at several upstream sites along the respiratory chain, a fraction of these electrons can react directly with molecular oxygen to form ROS, primarily the superoxide anion ($O_2^{-\cdot}$). In other words, the majority of the oxygen is reduced to H_2O, but the approximately 4%–5% converted to $O_2^{-\cdot}$. $O_2^{-\cdot}$ is, in turn, dismutated by mitochondrial manganese superoxide dismutase (MnSOD) into hydrogen peroxide (H_2O_2), which is subsequently detoxified into H_2O by mitochondrial glutathione peroxidase[8] (Fig. 2). Glutathione peroxidase plays an important role in H_2O_2 detoxication, because mitochondria in hepatocytes do not have catalase. Of note, glutathione peroxidase needs an adequate amount of reduced glutathione (GSH) in order to detoxify H_2O_2. The depletion of mitochondrial GSH below a critical level may, therefore, lead to mitochondrial dysfunction and cell death.[9] In this way, even with healthy mitochondria, the respiratory chain generates ROS. Most ROS are detoxified into H_2O, and only a small amount of residual ROS persists. In contrast, damaged mitochondria generate larger amounts of ROS, which can alter mitochondria as well as other cellular components.

It should be emphasized that in the presence of reduced metal ions, especially iron and copper, H_2O_2 is subsequently converted through Fenton reactions ($Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + {}^{\bullet}OH$) or a Harber-Weiss reaction

Table 1. Reactions involved in oxidant generation by neutrophils

Reactions	Products
NADPH oxidase $2O_2 + NADPH = 2O_2^{-\cdot} + NADP^+ + H^+$	$O_2^{-\cdot}$
SOD $2O_2^{-\cdot} + 2H^+ = O_2 + H_2O_2$	H_2O_2
MPO $Cl^- + H_2O_2 = HOCl + {}^{\cdot}OH$	·OH
Nitric oxide synthetase L-arg + O_2 + NADPH = ·NO + L-citrulline + $NADP^+$	·NO

SOD, superoxide dismutase: MPO, myeloperoxidase

into a hydroxyl radical (·OH). The hydroxyl radical (·OH) is highly reactive and can interact with nucleic acids, lipids, and proteins.[10]

In addition, metabolic activation and production of ROS by cytochrome p450 has been described.[11] ROS generation may vary considerably depending on the forms of p450: cytochrome p450 2E1 (CYP2E1) exhibits a higher rate of oxidative activity than the other forms of p450.[12] CYP2E1 is an important source of ROS in hepatocytes, not only in the cytosol and endoplasmic reticulum but also in mitochondria. CYP2E1 is involved in the microsomal ethanol oxidizing system (MEOS) and in the oxygenation of substrates such as ethanol, and can generate a prolonged burst of ROS at the site of substrate oxidation. Although most ethanol is oxidized by alcohol dehydrogenase (ADH), CYP2E1 assumes a more important role in ethanol oxidation at elevated levels of ethanol or after chronic consumption of ethanol. Moreover, CYP2E1 has the ability to metabolize and activate many other toxicologically substrates, including acetaminophen and *N*-nitrosodimethylamine, to more toxic products.[13] Thus, mitochondrial CYP2E1 can produce ROS and induce lipid peroxidation.

Production of ROS derived from peroxisomes also has been proposed. Compounds such as peroxisome proliferators are potent inducers of p450 4A and induce formation of peroxisomes, accompanied by an increase in H_2O_2 production. Consequently, H_2O_2 escapes from peroxisomes and shifts the cellular redox balance toward an oxidative state.[14]

On the other hand, neutrophils and nonparenchymal cells are additional endogenous sources of ROS. An association between chronic inflammation and the development of cancer has been recognized for a long time.[4] Inflammatory neutrophils are a major source of oxidants in an inflamed liver, and release of ROS from these cells provides a plausible mechanism by which chronic hepatitis and HCC development might be related. In the course of their defense activities, neutrophils produce a vast amount of oxidants. The whole spectrum of oxidants generated by neutrophils is due to the actions of four different enzymes (Table 1). Among these enzymes, NADPH oxidase initiates oxidant generation. Superoxide anion ($O_2^{-\cdot}$), generated by NADPH oxidase, is a substrate for the enzyme superoxide dismutase (SOD), which catalyzes the formation of H_2O_2 from $O_2^{-\cdot}$ (Fig. 1). H_2O_2 is relatively stable and capable of diffusing and penetrating the cellular membrane, which provides to neutrophils the possibility of action at a distance. However, most H_2O_2 is consumed by myeloperoxidase (MPO). MPO is the most abundant enzyme in neutrophils and catalyzes the conversion of H_2O_2 into HOCl. In addition, neutrophils are also able to produce RNS, the production of which is facilitated by inducible nitric oxide synthase (iNOS), which catalyzes the production of ·NO from oxygen, L-arginine, and NADPH. The ability of neutrophils to yield ·NO is much less than that of macrophages. In any case, the four enzymes in neutrophils generate four types of oxidants: $O_2^{-\cdot}$, H_2O_2, HOCl, and ·NO. These products constantly interact with one another, causing the formation of a myriad of oxidants, among which ·OH is the most DNA-reactive compound.[5] Furthermore, the release of biologically active molecules, such as tumor necrosis factor (TNF)-α and ROS, from activated Kupffer cells has been implicated in hepatocarcinogenesis.

In this regard, activation of Kupffer cells directly or indirectly by toxic agents results in the release of an array of inflammatory mediators, growth factors, and ROS. The activation of Kupffer cells appears to modu-

late acute hepatocyte injury as well as chronic liver responses, including hepatocarcinogenesis.[15]

Exogenous sources of ROS

ROS can be generated exogenously. Environmental agents, including xenobiotics, radiation, metal ions, and some peroxisome-proliferating compounds, are among the classes of compounds that have been shown to induce ROS and damage in vitro and in vivo. 2-butoxyethanol is an example of a chemical that produces ROS indirectly, resulting in liver cancer in mice.[16]

Antioxidant defense mechanisms

A number of antioxidant defense systems have evolved to combat the accumulation of ROS (Fig. 1). These include enzymatic molecules (e.g., SOD, catalase, and glutathione peroxidase), and nonenzymatic molecules (e.g., glutathione, vitamins C and E, coenzyme Q, flavonoids). SODs are localized to the cytosol and mitochondria, and reduce $O_2^{-\cdot}$ to H_2O_2 and H_2O. Glutathione peroxidases, which are also localized to the cytosol and mitochondria, remove the majority of H_2O_2,[17] while catalase, located in peroxisomes, contributes to the removal of yet more H_2O_2.

Among the molecules constituting antioxidant defense systems, GSH redox cycle and redox-sensitive proteins, including glutathione and thioredoxin, are of primary importance. Of note, the intracellular redox state is determined by the relative ratio of the reduced and oxidized forms of each redox pair. In this regard, glutathione is the most important nonprotein thiol to the overall redox balance, because the intracellular concentration of glutathione is 500- to 1000-fold higher than the other redox-regulatory molecules. Glutathione is present in reduced (GSH) and oxidized (GSSG) forms. The concentration of GSH is 10- to 100-fold higher; therefore, GSH prevails over GSSG. An increase in the GSSG level can arise from the breakdown of H_2O_2 by GSH peroxidase. Because the concentration of GSSG is relatively low, the oxidation of a limited amount of GSH to GSSG can dramatically change this ratio, affecting the cellular redox state. GSSG can be reduced to GSH by NADPH-dependent glutathione reductase. Thus, the GSH/GSSG ratio is strictly regulated, and can be maintained even in the face of oxidative stress by increasing GSH reductase activity or elimination of GSSG.[18] As an antioxidant, glutathione metabolizes ROS primarily by serving as a cofactor for GSH-dependent enzymes such as GSH peroxidase, and also modulates the activity of thiol-dependent enzymes that contain cysteine residues sensitive to redox change.[19]

The cellular redox state is regulated by cellular thiols, including GSH and thioredoxin (TRX)-1. There is considerable evidence that the TRX system is as important as the GSH system in cellular redox regulation against oxidative stress. TRX, a family of small proteins that contain a conserved redox active center, is known to protect cells against ROS.[20] TRX-1 has a variety of biological activities, including the scavenging of ROS and the regulation of redox-sensitive molecules such as NFκB. Recent studies indicate that TRX-1 is induced to protect host cells from various types of stresses, including ROS, viral infection, and ischemic insult.[21] Moreover, serum TRX-1 levels are recognized as an oxidative stress marker.[22]

Oxidative stress and cellular responses

ROS generation often exceeds a cell's antioxidant capacity, leading to a condition called oxidative stress. Oxidative stress can occur through overproduction of ROS and RNS through endogenous and exogenous insults, and interacts with a wide range of intracellular molecules, eliciting cytostatic/cytotoxic damage to cellular DNA, proteins, and lipids. Consequently, oxidative stress is implicated in a wide variety of disease processes, including atherosclerosis, diabetes mellitus, and pulmonary fibrosis, and is considered to be a major factor of aging.

Under normal metabolic processes, it is estimated that as many as 10000 oxidative hits take place per cell per day. Oxidative hits would increase substantially following bursts of cellular metabolism, inflammation, or depletion of cellular antioxidants.[23]

Nuclear DNA damage

Among many forms of ROS, $^{\cdot}OH$, in particular has been shown to generate a number of oxidized DNA lesions. Because the migration of $^{\cdot}OH$ is limited, it reacts rapidly with cellular components. H_2O_2, a precursor to $^{\cdot}OH$, is less reactive but more readily diffusible and thus more likely to be involved in the formation of oxidized bases. Recent attention has been focused on reactive oxygen products formed in DNA, with particular emphasis on the formation and repair of 8-hydroxy deoxyguanosine (8-OHdG), one of the important lesions in base mispairing. There are two mechanisms of the formation of 8-OHdG in cellular DNA: direct interaction of ROS with guanine at the C8 position, and guanine oxidization in the nucleotide pool during DNA replication.[24–26] In normal cells, it has been estimated that around 200 8-OHdG genomic DNA lesions are formed per cell per day by the two mechanisms described above. These oxidative DNA lesions result in site-specific

mutagenesis and produce G to T transversions that are widely found in mutated oncogenes and tumor suppressor genes.[27] In addition, during DNA replication, 8-OHdG in the nucleotide pool can be incorporated into DNA, resulting in A:T to C:G transversions. Further support for the involvement of 8-OHdG in carcinogenesis comes from studies showing that 8-OHdG produces dose-related increases in cellular transformation, which can be prevented by antioxidants.[28] Conversely, DNA repair enzymes and a repair system exist and function to remove altered bases produced by oxidative stress. 8-OHdG lesions in cellular DNA are repaired by the action of formamidopyrimidine-DNA-glycosylase (FPG), a product of the human *MMH* gene.[29] In nuclear DNA, around 90% of oxidized bases are repaired by single-nucleotide repair mechanisms, and the remaining 10% by long-patch base excision repair, indicating that single-nucleotide repair is the primary pathway for the repair of 8-OHdG.

DNA methylation

DNA methylation is an important regulator of gene expression, decreased methylation being associated with increased gene expression. In this context, many cancer cells have been shown to exhibit global hypomethylation of DNA compared with control cells. In particular, hypomethylation of tumor-promoting genes has been proposed as a possible mechanism for cancer development. Oncogenes can become hypomethylated and their expression amplified. In contrast, the promoters of some tumor suppressor genes are methylated, resulting in their inactivation.[30,31] Hypermethylation of genes may inhibit transcription of tumor suppressor genes. Although mutation in the coding region of genes has been considered the major mechanism of inactivation of the tumor suppressor genes, aberrant DNA methylation of the CpG islands in the promoter region has recently emerged as an alternative mechanism for the silencing of tumor suppressor genes, and may be one of the earliest events in the neoplastic transformation of cells.[32,33]

Among the agents and situations that can alter methylation status, ROS are most potent and can modify DNA methylation. In particular, oxidative DNA damage elicited by ROS can result in decreased DNA methylation.[34] In this regard, the formation of 8-OHdG in DNA can lead to hypomethylation, because the presence of 8-OHdG in CpG islands inhibits the methylation of adjacent C residues by methyltransferase. Additionally, 8-OHdG formation can interfere with the normal function of DNA methyltransferase and alter DNA methylation.

With regard to HCC development, hypermethylation of CpG islands in the $p16^{INK4}$ promoter region has been detected in not only HCC but also liver cirrhosis and chronic hepatitis.[35] In addition, hypermethylation has also been reported on CpG islands in the 5′ noncoding region of the silencing of the suppressor cytokine signaling-1 (*SOCS-1*) gene.[36] The precise mechanisms leading to hypermethylation of CpG islands are not known. However, in nickel carcinogenesis, hypermethylation of $p16^{INK4a}$ is apparently induced by ROS-mediated mitogen-activated protein (MAP) kinase activation.[37] Although it has been reported that levels of methyltransferase mRNA are increased in liver tissue in chronic hepatitis and liver cirrhosis compared with in normal liver tissue,[38] several lines of evidence suggest that there is no correlation between DNA methyltransferase activity and aberrant methylation.[39] Other mechanisms such as loss of protection against de novo methylation may account for aberrant methylation of tumor suppressor genes.[33]

Target genes exhibiting a significantly higher frequency of changes in DNA methylation in tumor tissues than in the neighboring noncancerous tissues have been reported to represent a late phase of carcinogenesis, with early-phase specific changes occurring at the same frequency in both cancerous and noncancerous tissues.[40] In any case, hypomethylation of tumor promoting genes (oncogenes) and hypermethylation of tumor suppressor genes contribute to hepatocarcinogenesis, and their detection is promising in the follow-up of patients at a high risk of developing HCC.

Mitochondrial DNA damage

Mitochondrial DNA (mtDNA) is a circular double-stranded molecule located in the mitochondrial matrix, and it is extremely sensitive to oxidative stress owing to (1) its proximity to the inner membrane, where ROS is mainly generated; (2) the absence of protective histone; and (3) incomplete repair mechanisms in the mitochondria. These findings may account for the increased frequency of mtDNA mutations seen in tumor cells. Indeed, the mutation rate in mtDNA is at least two orders of magnitude higher than that in nuclear DNA. Mutations in mtDNA encoding oxidative phosphorylation would disturb the respiratory chain associated with an increase in ROS, and affect cellular ATP levels, resulting in the prevention of cell cycle progression. Furthermore, it has been shown that fragments of mtDNA are inserted into nuclear DNA, which has been suggested as a mechanism for activation of oncogenes.[41] In this regard, mtDNA mutations have been identified in a variety of tumors.

Thus, mtDNA damage may constitute one step in carcinogenesis via mitochondria-derived ROS and the insertion of mitochondrial genes into nuclear DNA.

Lipid peroxidation

Free radical-mediated damage to cellular membranes results in lipid peroxidation, a process that generates a variety of DNA-reactive aldehydes, such as 4-hydroxy-2-nonenal (4HNE), and malondialdehyde (MDA). These products have cellular half-lives upward of 2–3 min and therefore are capable of diffusing from their site of production to more distant sites within the cell to interact with DNA or proteins. In particular, 4HNE can react with DNA, yielding etheno (epsilon)-modified DNA bases.[42] Hepatic etheno-adduct levels are significantly higher in patients with alcohol-related hepatitis, fatty liver, fibrosis, or cirrhosis than in those with asymptomatic livers, indicating that etheno-adducts may serve as potential markers for assessing progression of inflammatory cancer-prone diseases.

In this way, the diffusible and electrophilic characteristics of lipid peroxidative products may contribute much to carcinogenesis.

Signal transduction pathways

At the cellular level, oxidative stress elicits a variety of cellular responses, ranging from proliferation to growth arrest, senescence, and cell death. Effects within cells appear to be cell-specific and dependent on the form and intercellular concentration of ROS.

ROS function to induce cell proliferation during the tumor progression stage of carcinogenesis. Both H_2O_2 and $O_2^{-\cdot}$ induce mitogenesis and cell proliferation in several cell lines. In addition, a reduction in cellular oxidants by antioxidants inhibits cell proliferation. In contrast, high concentrations of ROS trigger apoptotic signaling pathways, resulting in cell death.[43] Whatever effect is observed, it largely reflects the balance among a variety of intracellular stress signalings activated in response to the oxidative stress.[44]

Mitogen-activated protein kinases (MAPKs) comprise a large number of serine/threonine kinases involved in regulating a wide array of cellular processes, including proliferation, differentiation, and apoptosis. On the basis of structural differences, they are divided into three multimember subfamilies: extracellular signal-regulated kinases (ERK), c-Jun N-terminal kinases (JNK), and p38kinases (p38MAPK). The latter two are categorized as stress-activated protein kinases (SAPKs).

The ERK pathway is most linked to the regulation of cell proliferation, while the SAPKs (JNK and p38MAPK) pathways are more strongly tied to stress. It is clear that oxidative stress leads to substantial activation of ERK. Two possible mechanisms have been proposed for this effect. One is that oxidative stress may mimic the effects of the ligand–receptor interaction through the modification of cysteine residues on the receptor.[45] The other is that oxidative stress may inactivate GSH-sensitive phosphatases necessary for dephosphorylation of the receptor.[46] However, other experiments have indicated that ERK activation can contribute to apoptosis in response to oxidative stress. What determines whether ERK will act in a proapoptotic or antiapoptotic fashion remains to be clarified, but the kinetics and durations of its activation may be important factors. Specifically, if activation of ERK occurs rapidly and is more transient, ERK activity may enhance survival, whereas if activation tends to be delayed and sustained, ERK activity may induce apoptosis.

The SAPK (JNK and p38MAPK) pathways are noted for their activation by a wide range of stresses. For oxidative stress-induced activation of these pathways, change in the cellular redox state seems to be a key factor. Under normal conditions, the redox regulatory protein TRX has been shown to bind and inhibit apoptosis signal-regulating kinase (ASK1), a MAPK kinase kinase (MAPKKK) involved in both JNK and p38MAPK activation.[47] However, oxidative stress causes dissociation of the TRX-ASK1 complex, leading to activation of JNK and p38MAPK. As is the case with TRX, under nonstress conditions, GST binds to JNK and inhibits its activity, but this interaction is disrupted by oxidative stress.[48]

Thus, oxidative stress may act at multiple levels in the SAPK pathways to regulate their activities. The influence of JNK activation on cell survival following oxidative stress is complex and controversial. Many studies have shown that JNK activation is correlated with cell death or apoptosis. The role of p38MAPK is also controversial. Previous studies have yielded evidence for proapoptotic[49] as well as antiapoptotic[50] activity. These signaling pathways exert their phenotypic influences through modulation of transcriptional factor activities.

Gene expression

The most significant effects of oxidative stress on signaling pathways have been observed in the MAPK/AP-1 and NFκB pathways.[51] Activation of these transcriptional factors is involved in both cell proliferation and apoptosis. The cellular redox state appears to influence the selective activation of these transcriptional factors and therefore, may help explain the observation that either cell death or cell proliferation may result from exposure to oxidative stress.

AP-1 is a collection of dimeric basic region leucine zipper (bZIP) proteins, including the Jun (c-JunM, JunB, JunD), Fos (FosB, Fra-1, Fra-2), and ATF families.[52] A common effect of AP-1 activation is an increase in cell proliferation. One of the genes regulated by AP-1

is *cyclinD1*, which supports the inference that AP-1 promotes entry into the cell division cycle.[53] AP-1 proteins also function as positive or negative regulators of apoptosis. Whether AP-1 induces or inhibits apoptosis is dependent on the balance between proapoptotic and antiapoptotic target genes, which varies from one cell type to another, and the durations of stimuli. Finally, AP-1 proteins participate in oncogenic transformation through interaction with activated oncogenes.[54] Among these oncogenes, *c-Jun* has been the most studied in this regard. Just as with its upstream regulator JNK, both pro- and antiapoptotic functions have been ascribed to c-Jun. Like JNK, c-Jun functions in a manner that is cell-type specific, agent specific, or both.

The NFκB family of transcriptional factors is composed of homodimers or heterodimers of Rel proteins consisting of p50 (NFκB1), p52 (NFκB2), and so on.[55] The predominant mechanism by which NFκB is activated by various stimuli is through the phosphorylation of IκB. IκB is an inhibitory protein that under normal conditions binds to NFκB, preventing its access to DNA. However, the phosphorylation of IκB results in its ubiquitination and degradation, freeing NFκB to translocate to the nucleus and activate transcription. A number of different kinases, including Iκ-kinase (IKK), and NFκB-inducing kinase (NIK), have been reported to phosphorylate IκB.[56] Many of these kinases offer obvious points for cross-talk with the signaling pathways known to be activated by ROS. Virtually, every step of the NFκB signaling cascade consists of redox-sensitive proteins whose activities are modulated upon changes in ROS.[57] In addition, NFκB needs to be in reduced form to exhibit DNA-binding activity. Ergo, reducing agents enhance DNA activity of NFκB, while oxidizing agents inhibit this activity. Activation of NFκB has been considered to be linked to carcinogenesis, because NFκB regulates several genes involved in cell transformation, proliferation, angiogenesis, and cell survival.[58] In this context, a large number of NFκB target genes have antiapoptotic functions. These include those coding for TNF-α, TNF receptor-associated factor1 (TRAF1), TRAFS, and cellular inhibitors of apoptosis proteins (CIAPs).[59] NFκB is also involved in regulating the expression of *Bcl-Xl*, an antiapoptotic member of the Bcl-2 family. Accordingly, NFκB expression has been shown to exert protective effects under various conditions. Carcinogens and tumor promoters, including UV radiation, phorbol esters, alcohol, and benzo(a)pyrene, are among the external stimuli that activate NFκB.

The tumor suppressor protein p53 exerts its action in response to oxidative stress. Indeed, many chemotherapeutic agents generate ROS, leading to activation of p53. Activation of p53 by oxidative stress can result in either growth arrest or apoptosis. What determines this decision is unclear, but a number of factors are involved, including the cell type and the magnitude and severity of the damage. Oxidative stress contributes to p53 activation in many ways; both JNK and p38MAPK can phosphorylate p53, and both have been implicated in regulating p53 expression levels through stabilization of the p53 protein. Downstream targets of p53 activation have been identified. Genes linked to growth arrest include *p21/Waf1*, *GADD45*, and *14-3-3*, which are important in mediating G2/M arrest,[60] while genes linked to apoptosis include *Bax*, a proapoptotic Bcl-2 family member, and *Fas*. On the other hand, elimination of p53 function enhances survival, even if the cells are exposed to oxidative stress. This is likely to be an important factor contributing to the chemotherapeutic resistance of cancer in which the *p53* gene is mutated. In this regard, allelic loss on chromosome 17p is among the most common genetic abnormalities associated with the genesis of many cancers, including HCCs. Loss of p53 function occurs mainly through allelic loss at chromosome17p13, where the *p53* gene is located.[61] Loss of heterozygosity at chromosome 17p13 has been reported in 25%–60% of HCCs, and the worldwide prevalence of *p53* mutation is around 28%.[62] These findings indicate that preserved p53 function is a requisite for a chemotherapeutic approach against HCC.

Of note, p53 activation itself results in the generation of ROS. Although the exact mechanism whereby p53 activation leads to increase in oxidative stress remains unclear, it is possible that p53 activation modulates expression of the genes involved in regulating the cellular redox state.[63] p53 activation can also interfere with survival signals to render cells permissive to apoptosis; p53 represses expression of *Bcl-2*, an antiapoptotic gene of the Bcl-2 family, and increases expression of *Bax*, a proapoptotic gene of the Bcl-2 family.[64] Thus, an important consequence of ROS-induced p53 activation is a further increase in the cellular level of oxidative stress.

Through regulation of transcriptional factors and disruption of signal pathways, ROS are involved in the maintenance of concerted networks of gene expression that may correlate with neoplastic development.

Viral infection-associated oxidative stress and hepatocarcinogenesis

HCV infection-related oxidative stress

HCV infection frequently leads to severe liver diseases, including liver cirrhosis and HCC. Although the precise pathogenesis of chronic liver disease remains obscure, oxidative stress has been focused on as a central player in the progression of many pathological conditions in HCV-related liver diseases. Indeed, HCV infection is

characterized by increased markers of oxidative stress: lipid peroxidation and oxidative DNA damage are enhanced in serum and liver specimens of patients with HCV infection.[65,66] The increased oxidative stress in HCV infection may be explained by chronic inflammation, and the continued generation of ROS and RNS in the liver may be accounted for by NAD(P)H oxidase, especially Nox-2 in PMNs and Kupffer cells in the liver.[67] It has become evident that structural and nonstructural proteins of HCV are involved in the generation of ROS in an infected liver. For instance, NS3 protein of HCV has been found to activate Nox-2 proteins of Kupffer cells to induce apoptosis of T cells, NK cells, and NKT cells.[68] Nox2 protein increases generation of ROS and other reactive species, which can exert oxidative stress on nearby cells. Furthermore, HCV can directly induce oxidative stress in hepatocytes. HCV core protein has been associated with increased ROS, decreased intracellular and/or mitochondrial GSH content, and increased levels of lipid peroxidation products, leading to hepatocarcinogenesis.[69] A recent study further showed increased oxidation of mitochondrial GSH and decreased NADPH content in liver mitochondria from transgenic mice expressing the HCV structural proteins, including core protein. Furthermore, there was reduced activity of the electron transport complex I and increased generation of ROS from complex I substrates. Incubation of control mitochondria in vivo with recombinant core protein also causes oxidation of GSH, complex I inhibition, and increased ROS production. In addition, HCV core protein enhances mitochondrial Ca^{2+} uptake, resulting in inhibition of electron transport accompanied by ROS production at complex I.[70] These findings are consistent with the mitochondrial abnormalities found in vivo in the core-expressing animal model as well as in patients with HCV infection.[71]

It has been shown that HCV also induces ER (endoplasmic reticulum) stress.[72,73] For instance, HCV NS5A protein perturbs the host redox state. NS5A induces an accumulation of misfolded proteins and generation of ER stress with the subsequent release of Ca^{2+} from the ER, followed by mitochondrial Ca^{2+} uptake and the generation of ROS in the mitochondria. ER stress is a homeostatic mechanism that regulates cellular metabolism and protein synthesis in response to perturbations in protein folding and biosynthesis.[74] It has been hypothesized that persistent ER stress induction may result in intracellular and extracellular accumulation of DNA-damaging factors that could predispose a cell to mutagenesis.

It should be noted that the cellular redox environment is tightly regulated by antioxidant/reductants as well as antioxidant enzymes. During oxidative stress, many of the antioxidant enzymes are upregulated in response to oxidative stress. It is interesting to note that core protein, but not NS5A, decreases the GSH content. Similarly, no compensatory induction of heme oxygenase 1 (HO-1) or catalase was detected, despite ROS production in the cells expressing core protein. In contrast, NS5A can increase MnSOD, HO-1, and GSH.[75] These findings indicate that HCV may not only increase ROS production but also modulate antioxidant genes.

Recent and striking evidence of the causal role of HCV in HCC development derives from studies using transgenic mice. Transgenic mouse expressing HCV core protein shows an increased accumulation of ROS, which correlates with HCC development,[76] and transient expression of NS5A alters intracellular calcium levels, leading to oxidative stress and activity of STAT3 and NFκB.[77] Oxidative DNA damage increases chromosomal aberrations associated with cell transformation, which may account, in part, for the evidence implicating oxidative stress in the development of HCV-associated HCC.[78]

As described before, ROS may promote pathogenesis through cell signaling pathways. NS5A-induced oxidative stress has been revealed to activate NFκB, leading to further activation of the *COX-2* gene.[79] Activation of COX-2, in turn, would result in increased PGE2 production, which can inhibit apoptosis of the tumor cells, induce proliferation, and promote metastasis.[80] Oxidative stress also activates the MAPKs, which have profound effects on cell growth and may promote transformation. Indeed, through ROS generation, HCV infection induces activation of ERK, a conventional MAPK, in human HCC tissue.[81]

On the other hand, iron overload, which is often observed in HCV-infected hepatocytes, may also participate in liver injury.[82] Moreover, iron overload induces mitochondrial injury and increases the risk of HCC development in transgenic mice expressing the HCV polyprotein.[83] In contrast, iron reduction therapy by repeated phlebotomy improves hepatocyte injury in patients with HCV infection.[84]

Furthermore, steatosis is another common feature in HCV-infected hepatocytes.[71] In this regard, high β oxidation rates increase electron delivery to the mitochondrial respiratory chain, leading to an imbalance between a high input and a restricted flow of electrons. Consequently, overproduction of complex I and III in the respiratory chain may lead to their reaction with oxygen to form ROS. In this way, steatosis in hepatocytes of patients with HCV infection may contribute, in part, to hepatocarcinogenesis, through mitochondrial dysfunction and ROS generation.

Taken together, these facts suggest that ROS production associated with HCV infection may cooperate with other factors and promote hepatocarcinogenesis.

HBV-related oxidative stress

HBV is one of the causative agents of acute and chronic hepatitis, cirrhosis, and HCC. Transgenic mice expressing the HBV large envelope protein or surface antigen (HBsAg), display the generation of oxidative stress and DNA damage, leading to development of HCCs.[85,86] In addition, HBV X (HBx) protein has drawn considerable attention owing to its role in viral replication and the generation of HCC. Although the oncogenic property of HBx remains controversial, it has been established that HBx protein contributes to HCC development, in conjunction with genotoxic stresses and/or oncogene activation.[87,88] On the other hand, HBx protein binds to a voltage-dependent anion channel (VDAC3) and alters the mitochondrial transmembrane potential, leading to enhanced ROS generation. Consequently, the association of HBx protein with mitochondria induces the activation of transcriptional factors, including STAT 3 and NFκB, which is prevented by antioxidants or overexpression of MnSOD.[89] These observations indicate that HBx protein participates in the development of HCC via ROS generation.

Alcohol-associated oxidative stress and HCCs

There is compelling evidence that chronic alcohol consumption increases the risk of developing HCC. However, the exact role of alcohol in the development of HCC, compared with chronic HBV or HCV infection, is still incompletely defined, since ethanol itself is not a carcinogen.[90] Chronic alcohol consumption leads to multiple biochemical changes that could directly initiate or potentiate liver cancer. Involvement of ROS generated by ethanol consumption is the focus of this section.

Nongenotoxic mechanisms

Evidence has accumulated that acetaldehyde, the main metabolite of ethanol, is predominantly responsible for alcohol-associated carcinogenesis, because acetaldehyde is carcinogenic and mutagenic, binds to DNA or protein, destroys folate, and results in secondary hyperregeneration. In this context, it has become evident that the binding to DNA to form stable adducts represents one mechanism whereby acetaldehyde can trigger the occurrence of replication errors or mutations in oncogenes or tumor suppressor genes.[91] Furthermore, acetaldehyde inhibits methylguanyltransferase, an enzyme important for the repair of adducts by alkylating agents.[92] Moreover, acetaldehyde elicits overproduction of ROS through aldehyde metabolism in mitochondria, which induces hepatocyte apoptosis,[93] and increases ROS formation in hepatic stellate cells, leading to activation of stellate cells and promotion of fibrosis.[94]

Both generation and degradation of acetaldehyde is modulated by polymorphisms or mutations of the genes responsible for the enzyme involved. In Japan and other Asian countries, a high percentage of individuals carry a mutation of the aldehyde dehydrogenase 2 (*ALDH2*) gene. In humans, there are at least four classes of ALDH isozymes, and mitochondrial class 2 ALDH (ALDH2) is primarily responsible for acetaldehyde oxidation. The gene encoding ALDH2 enzyme is polymorphic with two distinct alleles: *ALDH2*1* and *ALDH2*2*. *ALDH2*2* results from a single point mutation in chromosome 6 coding the normal *ALDH2*1*. While individuals homozygous for the mutated *ALDH2*2* are completely devoid of ALDH2 activity, heterozygous individuals show 30%–50% of ALDH2 activity. Blood acetaldehyde levels of *ALDH2*2* homozygous individuals are 6–20 times higher than those of *ALDH2*1* individuals. In this regard, Japanese studies have shown that the odds ratio for HCC is higher in patients with HCV infection and alcohol consumption who lack aldehyde dehydrogenase activity (*ALDH2*2* homozygous) than in patients with HCV infection and alcohol consumption who have aldehyde dehydrogenase activity (*ALDH2*1* homozygous or *ALDH2*1/2* heterozygous). In addition to mutation of the *ALDH2* gene, polymorphisms of alcohol dehydrogenase (ADH) 1B may also modulate acetaldehyde levels; the *ADH1B*2* allele encodes for an enzyme that is around 40 times more active than the enzyme encoded by the *ADH1B*1* allele. In Japan and other Asian countries, *ADH1B*2* allele frequency is high, leading to generation of high amounts of acetaldehyde.[95]

Thus, polymorphisms or mutations of the genes responsible for the enzyme involved in acetaldehyde metabolism may modulate the role of acetaldehyde in hepatocarcinogenesis.[96]

On the other hand, CYP2E1 is involved in the MEOS and is induced by heavy ethanol use. The concentration of CYP2E1 in the liver can be correlated with the generation of α-hydroxyethyl radicals and with lipid peroxidation. Induction of CYP2E1 may contribute to the development of HCC by two distinct mechanisms. First, CYP2E1 generates ROS, leading to apoptosis or DNA damage and cancer initiation.[97] Second, CYP2E1 oxidizes xenobiotics, including the procarcinogens (nitrosamines, aflatoxin, vinylchloride, etc), causing them to become carcinogens.[98] The *CYP2E1* gene is polymorphic in the promoter region, and the relatively rare *c2* allele is associated with increased *CYP2E1* gene expression. Increased frequency of the *c2* allele has been reported in HCC patients who have a history of alcohol intake, whereas the frequency of the *c2* allele

in HCC patients who do not drink is similar to that of controls.[99]

In this way, genetic differences, especially in the aldehyde dehydrogenase and *CYP2E1* genes, may increase the risk of the HCC development in the people who drink alcohol.

Alcoholic liver disease is associated with significant oxidative stress as well as the hepatic accumulation of iron, which also initiates oxidative stress. It is widely recognized that hepatic iron overload develops in a significant portion of individuals who consume alcohol on a chronic basis. The role of iron, alone or in combination with ethanol, has been difficult to establish, because this transition metal is among the most abundant elements and is essential for healthy individuals. It has been established that iron augments ROS-induced damage by catalyzing the formation of hydroxyl radicals. On the other hand, production of ethanol-derived α-hydroxyethyl radical ($CHOHCH_3$) has been observed in the livers of animals chronically treated with ethanol.[100] This radical has been proposed to mediate its cytotoxic effects through adduct formation with cellular proteins. Therefore, the unregulated and sustained generation of such free hydroxyl radicals and ethanol-derived α-hydroxyethyl radicals undoubtedly contributes to the cocarcinogenic actions of ethanol and iron. On the other hand, Kupffer cells play an important role in cellular responses associated with inflammation and cell death. Of particular importance is the observation that elevated nonheme iron concentrations in Kupffer cells isolated from alcohol-treated rats are associated with increased NFκB binding as well as increased mRNA for TNF-α.[101] This observation has significant implications with regard to the role of iron as a potential signaling molecule in modulating hepatic TNF-α. In this regard, TNF-α is released by Kupffer cells stimulated by lipopolysaccharide (LPS). In addition to its proinflammatory property, initiation of TNF-α-mediated hepatotoxicity has been linked to the action of LPS[102] and oxidative stress by ethanol.[103] Thus, accumulation of iron in Kupffer cells is a key event for activation or sensitization of these cells to stimuli such as LPS or other extracellular effectors.

Alteration of DNA methylation

ROS are most important and can modify DNA methylation, and, in particular, oxidative DNA damage can result in decreased DNA methylation.[104] Thus, hypomethylation is a plausible consequence of the metabolic alterations associated with ethanol consumption. The generation of ROS by ethanol consumption diminishes the activity of methyladenosyltransferase II in chronic alcohol cirrhosis, accompanied by decreased levels of *S*-adenosylmethionine, the methyl donor for DNA methylation. In addition, ethanol increases homocysteine levels, followed by an increase in *S*-adenosylhomocysteine levels.[105] Chronic ethanol consumption also decreases glutathione levels in the liver, and enhances the susceptibility of the liver to alcohol-related oxidative stress. Furthermore, chronic ethanol consumption interacts with the absorption and subsequent metabolism of vitamin B, which is involved in hepatic transmethylation reactions, resulting in impaired methyl group synthesis and transfer.[105] To date, it is evident that dietary depletion of methionine, choline, and folate leads to DNA hypomethylation, in particular, hypomethylation of oncogenes, including *c-Ha-ras*, *c-Ki-ras*, and *c-fos*, and to DNA strand breaks, resulting in an increase in the incidence of HCC.[106]

Thus, several mechanisms, including ROS generation and dietary depletion, have been proposed by which ethanol can interact with DNA methylation and thereby enhance carcinogenesis.

Nonalcoholic fatty liver disease-associated oxidative stress and HCCs

Nonalcoholic fatty liver disease (NAFLD) is now recognized as one of the most common causes of liver disease in the United States and other countries. NAFLD ranges from simple fatty liver to nonalcoholic steatohepatitis (NASH), which may lead to cryptogenic cirrhosis and, in some cases, to HCC. The marked apoptosis rate in patients with NASH requires a compensatory increase in the cell proliferation rate of progenitor cells to maintain liver mass.[107] Concomitantly, both ROS and lipid peroxidation products damage DNA. The combination of DNA damage and increased cell proliferation causes gene mutations. As these mutations accumulate over the years, accompanied by constant apoptotic pressure, cells that resist apoptosis or escape the control of the cell cycle may be selected for, finally allowing the development of HCC. However, although some HCC patients exhibit NASH, whether HCC is part of the natural history of NASH has been controversial. In this regard, no case of HCC was found during 5 years of follow-up in one prospective study, in striking contrast to 1%–3% per year with hepatitis C infection.[108] It needs to be clarified whether metabolic determinants, such as steatosis, insulin resistance, and glucose intolerance, predispose a person to liver cancer, allowing the determination as to whether fibrotic NASH is a cause of HCC, or whether the metabolic factors serve as epigenetic determinants of hepatocarcinogenesis initiated by other causative factors.

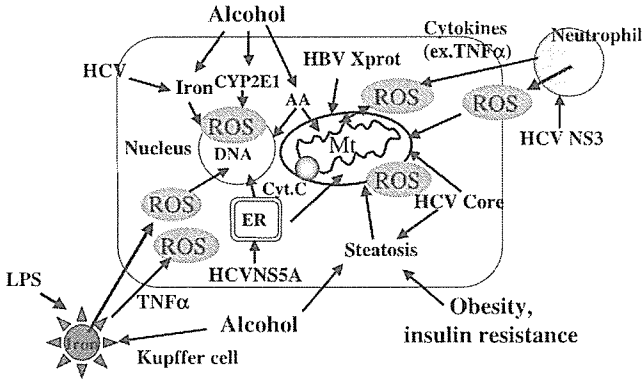

Fig. 3. A variety of etiologies enhance generation of ROS in hepatocytes. Overproduction of ROS through either endogenous or exogenous insults, is recognized to play an important role in the initiation and promotion events of hepatocarcinogenesis. See details in the text. *Mt*, mitochondria; *AA*, acetaldehyde; *LPS*, lipopolysaccharide; *ER*, endoplasmic reticulum; *Cyt. C*, cytochrome C

Conclusion

As described above, ROS in hepatocytes are generated endogenously through a variety of processes, and also can be derived from exogenous sources (Fig. 3).

A number of defense systems have evolved to combat accumulation of ROS. However, when these defense mechanisms are exhausted or overrun, the cellular redox potential shifts toward oxidative stress, in turn increasing the potential for damage to cellular nucleic acids, lipids, or protein. Although these events may be derived by different mechanisms, a commonality is the involvement of ROS in the development of HCC. In particular, unrepaired damage to DNA may result in mutations, provided that cell replication ensues prior to repair of modified bases. In addition to oxidative nuclear DNA damage, formation of mitochondrial DNA damage and mutation and alteration of mitochondrial genomic function have been revealed to contribute much to the process of carcinogenesis. At least three distinct stages of carcinogenesis, initiation, promotion and progression, have been identified. Aside from a role of oxidative stress in the induction of mutation, it is apparent that ROS and the cellular redox state mediate cell signaling pathways that are involved in cell growth and survival, leading to promotion and progression.

Therefore, oxidative stress is involved in all stages of carcinogenesis. As long as HCC develops mostly from chronic liver diseases, antioxidant therapeutic strategy and/or anti-inflammatory treatment might be required to prevent the development of hepatocarcinogenesis.

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バリアンス解析からみた肝切除クリニカルパスの適応

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原　著

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はじめに：クリニカルパスの視点で術後バリアンスが多いと考えられる肝切除術周術期管理において，当院では肝機能/肝切除量に応じた2種類の術後の輸液指示の異なるクリニカルパス（以下：パス）を作成し導入してきた経緯から，このパスのバリアンスやアウトカムを解析し，問題点およびその適応について検討した．対象と方法：2004年1月から12月までに当院で行われた肝切除例120例（胃癌および結腸/直腸癌同時肝切除症例を除く）のうち，パス適応となった115例を解析の対象とし，術後のバリアンス発生とアウトカム，術前肝機能・手術侵襲因子との相関関係を解析した．結果：パスが完遂された症例は115例中92例（80%）であった．術後平均在院日数はバリアンス発生のなかった全症例で平均9.0日であった．バリアンス発生に寄与する臨床的因子は疾患内訳，術式，手術時間，出血量，輸血の有無であった．また，術後2日目の経口摂取不良もバリアンス発生に関連していた．考察：2種類のパスのうち高度肝機能不良，大量肝切除症例への適応を想定して作られたパスは使用頻度も完遂率も低かった．そうした症例はパス適応外とするか，適応とするならば指標としては手術侵襲，特に手術時間5時間以内の症例が対象となるのではないかと考えられた．予定手術時間が5時間以上，かつ他の解析で求められた手術侵襲因子のカットオフ値も越えることが予想されるような場合はパスの適応外と考えられた．

緒　言

近年，消化器外科領域においてもクリニカルパス（以下，パス）の普及は目覚しく[1,2]，治療の安全性の向上のみならず医療経済効果に貢献することが上げられている[3)~6)]．また，パス作成がこれまでの治療や検査そしてその経過を見直すきっかけとなり，不要と考えられる薬剤投与や検査の削減が実現され治療効率の向上も達成されている．しかし，手術侵襲が大きかったり，多彩な病態を呈する患者，腫瘍を有する疾患などにおける周術期ではバリアンスが多いためパス導入は遅れている[7)~9)]．

我々は，複雑な患者背景，肝機能低下を有し，術式・手術侵襲の差のある肝切除術に対し2003年から1年の試行期間を経て2004年より正式に医療用，患者用パスを作成・導入し，現在に至っている．今回，このパスのバリアンスやアウトカム解析を行い，肝切除術におけるパスの問題点およびその適応について検討した．

対象と方法

国立がんセンター東病院にて2004年1月から12月までに行われた肝切除症例120例（胃癌/結腸・直腸癌同時肝切除症例を除く）のうち，パスの適応とし115例を検討対象とした．胆道再建を要する4例と術前よりネフローゼを合併した症例1例はパスの適応外とした．経口摂取を肝切除術翌日より開始している．胆道再建（消化管再建）を要する肝切除症例については経口摂取開始を遅らせるためパス適応外とした．

肝切除パスの導入に際し工夫点として，複雑な患者背景や肝機能，また手術術式や手術侵襲の程

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Table 1　Differences between CP type A and CP type B

	CP type A	CP type B
Indication		
Liver function (Liver damage) or liver resection	A/B or 2 ≦ Segment	B/C or 2 ≧ Segment
Parenteral nutrition	Common parenteral fluids are administered through a peripheral venous catheter	Hyper alimentation is administered through a central venous catheter and fresh frozen plasma
Hospital discharge	POD 8 ～ 10	POD 10 ～ 12

Table 2　Patient characteristics in each CP

	CP type A	CP type B	*P* value
No. of patients	100	15	
Age (years)	64 (37—87)	63 (52—78)	*NS**
Sex (M/F)	73/26	12/3	*NS*§
Disease			
Hepatocellular carcinoma	56	12	*NS*§
Liver metastases of colorectal/gastric carcinoma and others	43	3	
Liver resection			
Partial resection	79	2	
Minor (1 ≧ Segment)	11	2	*< 0.0001*§
Major (2 ≦ Segment)	9	11	

Values represent (mean (range), NS. not significant)
* : Statistical significance between groups was analyzed by Mann-Whitney's U test
§ : Statistical significance between groups was analyzed by Chi-square test

度の違いを一つのパスにまとめることは困難と考えられたため，肝機能・肝切除量に着目し周術期管理を分けて術後輸液内容の異なる2種類のパスを作成した(医療用，患者用を分けると計4種類)．肝機能が肝障害度AもしくはBの症例または2区域切除以下の肝切除量を予定する症例のパス（以下，肝切除Aのパス）と肝障害度BもしくはCの症例または2区域切除以上の大量肝切除を予定する症例のパス（以下，肝切除Bのパス）を作成した．主な相違点はTable 1に示すように肝切除Aのパス (CP type A) では末梢血管確保のみによる通常の1号，3号液を中心とした組成の輸液指示を，肝切除Bのパス (CP type B) では術前より中心静脈ラインを確保し術後の輸液内容は新鮮凍結血漿投与も予定にいれた糖やナトリウム，カリウムなどの組成を計算した輸液内容でパスを作成した．また，肝切除Bのパスを選択する際は高度肝機能不良あるいは大量肝切除といった大きな手術侵襲が加わり，回復期間を要すると思われたため，予定退院日を肝切除Aで術後8～10日目，肝切除Bで術後10～12日目と違いをもたせて設定した．肝切除パスAとBの大きな相違はこの2点でその他ドレーン抜去は術後3～5日目，経口摂取は術翌日昼より全粥（朝から水分可）を開始，離床・歩行開始も術翌日からとAB同じ設定とした．各症例に対しどちらのパスを採用するかは明確な基準を設けず各担当医が決めることとした．患者の体格指数 (body mass index；BMI) の違いや術直後の尿量，経口摂取の増減に応じての輸液の追加，減量はパス逸脱とせず，アウトカムの一つである予定退院日を守れないような合併症が生じた場合，バリアンス発生，パス逸脱とした．また，こうした起こりうる術後合併症については術前より文書を用いて説明し，実際に合併症が起こ

Table 3 Preoperative liver function and surgical stress in each CP

	CP type A (n = 100)	CP type B (n = 15)	*P* value
Preoperative liver function			
Total bilirubin (mg/dl)	0.9 (0.3—1.7)	0.9 (0.4—1.5)	*NS* *
Albumin (g/dl)	3.9 (3.1—4.8)	3.7 (3.2—4.1)	*NS* *
Prothrombin time (PT) (%)	79.6 (39.4—109.8)	75.9 (58.3—91.0)	*NS* *
ICG R15 (%)	13.5 (3.3—36.3)	14.4 (7.0—30.9)	*NS* *
Platelet ($\times 10^4/\mu l$)	17.7 (4.5—35.6)	19.1 (7.0—42.2)	*NS* *
Hemoglobin (g/dl)	13.3 (9.0—15.8)	13.2 (11.3—15.2)	*NS* *
Surgical stress			
Operative time (min.)	201 (55—515)	318 (160—558)	*< 0.0001* *
Hepatic ischemic time (min.)	68 (15—176)	87 (25—148)	*0.02* *
Blood loss (ml)	919 (10—7,270)	3,245 (605—14,399)	*< 0.0001* *
Resected liver weight (g)	216 (5—1,780)	779 (190—2,000)	*< 0.0001* *

Values represent (mean (range), NS : not significant)
* : statistical significance between groups was analyzed by Mann-Whitney's U test

Table 4 Variance in each CP

	CP type A (n = 100)	CP type B (n = 15)	*P* value
Variances (total)	16 (16%)	7 (47%)	*0.0112*
Postoperative complications	14 (14%)	7 (47%)	
Bile leakage	5	1	
Poor oral intake	2	2	
Pulmonary infarction	0	1	
Wound infection	1	2	
Intestinal tract injury	3	1	
Cholangitis	2	0	
Prolonged jaundice	1	0	
Change of operative procedure	2 (2%)	0	

Data are shown as numbers of patients (percentage) and analyzed by chi-square test.

りバリアンスが発生した際にはそれに対する必要な処置（例えば絶飲食など）を患者に説明し，パス逸脱とした．

なお，各解析結果は連続変数に関しては平均値（範囲）で表記，統計学的検定はMann-Whitney's U検定にて比較検定および単回帰分析を，名義変数に関してはカイ2乗検定により比較検定を行った．また術後バリアンス発生に寄与する独立因子検索にはロジスティック回帰分析を用いた．いずれも$P<0.05$をもって有意差ありと判定した．

結　果

パス適応となった115例のうち100例に肝切除Aが，15例に肝切除Bのパスが用いられた．肝切除AとBにおける患者背景（年齢・性別）および疾患内訳に有意差を認めなかったが，肝切除術式として肝切除A群に肝部分切除が，肝切除B群に2区域以上の肝切除が多く行われている傾向が認められた(Table 2)．同様に手術侵襲因子の比較検討でも手術時間，肝阻血時間，出血量，肝切除重量のすべてにおいて肝切除B群で有意差が認められた(Table 3)．一方で術前の血液・生化学検査

Table 5　Outcomes in cases without variances

	CP type A (n = 84)	CP type B (n = 8)	P value
Removal drains (POD)	3.7	4.5	*NS*
Postoperative hospital stay (days)	8.9	10	*0.0035*
Oral intake			
POD1 diet (%)	43.7	38.1	*NS*
POD2 diet (%)	69.4	70.0	*NS*

Data are analyzed by Mann-Whitney's U test.

Table 6　Clinical factors associated with failure of CP type A

Clinical factors	P value
Age	*NS*
Sex	*NS*
Disease	*0.0108*
Operative procedure	*0.0381*
Preoperative liver function	
Total bilirubin	*NS*
Albumin	*NS*
Prothrombin time	*NS*
ICG R15	*NS*
Platelet	*NS*
Hemoglobin	*NS*
Surgical stress	
Operative time	*< 0.0001*
Hepatic ischemic time	*NS*
Blood loss	*0.0025*
Resected liver weight	*NS*
Blood transfusion	*< 0.0001*
Oral intake	
POD1 diet	*NS*
POD2 diet	*0.0167*

*：Statistical significance between CP type A with/without variances groups was analyzed by Logistic regression. NS：not signiticaut

結果については，肝切除ＡとＢにおいて各数値間に有意差を認めなかった．

バリアンスが発生することなくパスが完遂された症例は肝切除ＡとＢ群合わせて115例中92例(80%)であった．それぞれでみるとバリアンスが発生した症例は肝切除Ａ群で16例(16%)，肝切除Ｂ群で7例(47%)で，肝切除Ｂ群においてパス完遂率が低かった(Table 4)．術後合併症については胆汁漏，経口摂取不良，肺梗塞，創感染，術中腸管損傷による術後絶飲食，胆管炎による発熱，遷延性の黄疸などが認められた(Table 4)．術後合併症以外でパス逸脱の原因として術式の変更が2例あった．1例は転移性肝癌症例で開腹時に腹膜播種を認め試験開腹のみとなり，もう1例は同じく転移性肝癌症例で胃への直接浸潤があり胃全摘を併施した症例であった．

バリアンスのなかった症例におけるパスのアウトカム解析においてはTable 5に示すように肝切除ＡとＢ群それぞれにおいてドレーン抜去は術後平均3.7日目と4.5日目で有意差はなかった．ドレーン抜去と同様，術後の経口摂取量は術後1日目と2日目ともに肝切除ＡとＢにおいて有意差を認めなかった．術後平均在院日数についてはパス間で設定が異なるため，8.9日と10日で有意差を認めた．術後在院日数に関してはバリアンス発生のなかった症例では肝切除ＡとＢ群合わせても平均9.0日で，バリアンスの発生したパス逸脱症例も含めたすべての症例では平均11.7日であった．術後第1，2日目の経口摂取量はバリアンスの発生しなかった症例を対象とすると手術侵襲程度に差があっても同程度摂取していた．

パス逸脱率が高く，有効性の低かった肝切除Ｂ群を除いた肝切除Ａ群の症例群において検討した結果，バリアンス発生に寄与する臨床的因子は術前としては疾患内訳と術式で，手術侵襲因子としては手術時間，出血量，輸血の有無であった．また，術後の経口摂取量において術後2日目の摂取不良はバリアンス発生に寄与していた（Table 6)．さらにこの術後2日目の経口摂取量と背景因