Proliferative drive and liver carcinogenesis: Too much of a good thing?
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Abstract
There have been innumerable studies published in the attempt to identify gene expression signatures in hepatocellular carcinoma (HCC). When all the regulators and targets of the differentially expressed genes are analyzed from larger studies, the most striking theme is upregulation of mitosis-promoting and cell proliferation genes in HCC compared with ‘liver-specific gene clusters’ in non-tumorous tissue. A major limitation of expression profiling is that it only provides a ‘snapshot’ of what is an evolving process and thus cannot distinguish the differences in gene expression that are primary effectors of dysregulated growth from those that represent downstream consequences. The development of HCC in a chronically diseased liver, often referred to as hepatocarcinogenesis, is a multistep process characterized by the progressive accumulation and interplay of genetic alterations causing aberrant growth, malignant transformation of liver parenchymal cells, followed by vascular invasion and metastasis. This review will discuss HCC precursor lesions, draw on the ‘proliferation cluster’ genes highlighted from HCC expression profiling studies, relate them to a selection of regulatory networks important in liver regeneration, cell cycle control and their potential significance in the pathogenesis of HCC or primary liver cancer.

Key words
Cyclins, dysplastic foci, hepatocellular carcinoma, hepatitis B, hepatitis C, interleukin 6, liver regeneration, p53.

Wrath of Zeus: Prometheus and hepatocyte proliferation
The Greek myth of Prometheus has had generations of poets, playwrights, authors and medical students enthralled. The logic of this myth presupposes the knowledge that ancient Greeks must have had of the liver’s marvelous capacity to regenerate. Prometheus was punished by Zeus when he stole fire from the gods and gave it to mankind. His wrath took the form of torturing Prometheus by continual aquiline partial hepatectomy (PH). The reason that the liver was selected for injury rather than another visceral organ was to avoid killing Prometheus, maximize inflicted pain and to prolong the punishment indefinitely. It took centuries before Higgins and Anderson published their landmark observations in 1931, on the restorative powers of the liver after partial two-thirds surgical resection.1 It was not for another decade that Bucher began pioneering research into defining the morphological, physiological, biochemical and cellular kinetics in regenerating liver.2 The PH model has been used extensively to elucidate the mechanisms responsible for hepatocyte proliferation and is the best in vivo model of synchronous cell cycle progression in mammals.1,2

There have been innumerable studies published in the past decade which have attempted to identify gene expression signatures in hepatocellular carcinoma (HCC). These results are remarkable for their highly heterogeneous genetic alteration profiles which make it difficult to define a characteristic molecular signature for these tumors.3–8 When all the regulators and targets of the differentially expressed genes are pooled and analyzed from larger studies, the most striking theme is upregulation of mitosis-promoting and cell proliferation genes in HCC compared with ‘liver-specific gene clusters’ in non-tumorous tissue. A major limitation of expression profiling is that it only provides a ‘snapshot’ of what is an evolving process and thus cannot distinguish the differences in gene expression that are primary effectors of dysregulated growth from those that represent downstream consequences. The development of HCC in a chronically diseased liver, often referred to as hepatocarcinogenesis, is a multistep process characterized by the progressive accumulation and interplay of genetic alterations causing aberrant growth, malignant transformation of liver parenchymal cells, followed by vascular invasion and metastasis. This review will discuss HCC precursor lesions, draw on the ‘proliferation cluster’ genes highlighted from HCC expression profiling studies, relate them to a selection of regulatory networks important in liver regeneration, cell cycle control and their potential significance in the pathogenesis of HCC or primary liver cancer.
The prevalence of SCD is higher in cirrhotic liver with HCC than those without. SCD is a highly proliferative lesion that bears morphological resemblance to HCC, and there is a histological continuum between SCD and HCC. Foci of SCD also contain chromosomal losses and gains that are present in adjacent HCC, but not in the surrounding cirrhotic parenchyma; in this context, it is suggestive that SCD foci in cirrhotic liver are possible early precursor lesions of HCC. However, it is also known that only a small minority of SCD will eventually evolve into HCC, either directly or through the formation of dysplastic nodules (DN), and that LCD foci are more prevalent in cirrhotic livers with HCC than without. These LCD contain abnormal and increased DNA content in hepatocytes which supports the notion that such foci may also be direct precursor lesions of HCC. Conversely, the features of LCD such as the normal nucleocytoplasmic ratio, low proliferative index, high apoptotic activity, absence of a histological continuum with HCC and lack of genetic aberrations present in adjacent cancer tissue argue against this hypothesis. Instead, it has been recently suggested that foci of LCD arise from hepatocytes that are driven towards replicative senescence by persistent hepatocellular necroinflammation and regeneration. Genetic alterations in such terminally differentiated, senescent and polyploid hepatocytes may adversely impact on normal cell division. Thus, they may lead to the formation of ‘end-stage’ LCD foci with abnormal or increased DNA content.

### Dysplastic nodules

The development of HCC in a cirrhotic liver is often preceded and accompanied by macroscopically recognizable nodular lesions showing mild-to-moderate atypical morphological features. Some of these nodules may contain malignant foci referred to as ‘nodule-in-nodule’. Such histological observations suggest a role for DN as immediate precursors of HCC. DN are classified as low grade (LDG) or high grade (HGD) by morphological criteria. Immunohistochemical, molecular and clinical studies have demonstrated loss of heterozygosity at multiple loci, while chromosomal abnormalities gradually anticipate from regenerative nodule to LDG, LDG to HGD and from HGD to HCC. Of note, HGD cells are frequently endowed with vascular and metastatic profiles that partially resemble those of their adjacent HCC.

### Regulatory pathways in normal liver regeneration

Under normal physiological conditions, hepatocyte turnover is very low with a half-life estimated at 6 months. However, adult liver cells retain the remarkable capacity to proliferate in response to injury or to the loss of liver mass. Progenitor cells (also referred to as oval cells) do not play a major role in this growth response but instead, existing ‘resting’ hepatocytes re-enter the cell cycle and divide; these hepatocytes replicate once or twice during the period of mass restoration before returning to a state of quiescence.

The earliest activating signals after PH emanate from Kupffer and endothelial cells in the liver. Cytokines and growth factors, such as tumor necrosis factor-α (TNF-α), interleukin (IL)-6, hepatocyte growth factor (HGF) and transforming growth factor-α, are defined as a collection of hepatocytes characterized by increased DNA content. This cellular accumulation is suggestive of HCC and reflects a histological continuum with HCC and lack of genetic aberrations present in adjacent cancer tissue. Immunohistochemical, molecular and clinical studies have demonstrated loss of heterozygosity at multiple loci, while chromosomal abnormalities gradually anticipate from regenerative nodule to LDG, LDG to HGD and from HGD to HCC. Of note, HGD cells are frequently endowed with vascular and metastatic profiles that partially resemble those of their adjacent HCC.
(TGF-α), are rapidly secreted.\textsuperscript{70,71} These molecules then activate several transcription factors including nuclear factor κ-B (NF-κB), signal transducer and activator of transcription 3 (STAT3) and C/EPBβ through post-translational modifications.\textsuperscript{71,72} Activation of such transcription factors triggers immediate-early growth genes that include \textit{myc}, \textit{fos} and \textit{jun} as well as mitogen-activated protein kinases, such as extracellular regulated signal kinase (ERK) and c-Jun N-terminal kinase (JNK). JNK is also activated by epidermal growth factor (EGF) and TGF-α post-PH, while cyclin D1, the rate-limiting step of the cell cycle in transition past the G1 restriction point (discussed later), is a key downstream transcriptional target of JNK.\textsuperscript{71,72}

There are two discrete phases of liver regeneration, a priming phase in which quiescent hepatocytes are induced to enter the cell cycle following TNF and IL-6 stimulation, followed by a second phase in which hepatocytes become responsive to growth factors and progress through the G1 stage of the cell cycle.\textsuperscript{72} As cells pass through G1, they reach the restriction point, defined as the critical point at which hepatocytes no longer depend on mitogenic stimulation in order to proceed to mitosis.\textsuperscript{72}

**Figure 1** Pathogenic mechanisms in hepatocarcinogenesis. AFB1, aflatoxin B1; ETOH, alcohol; HCC, hepatocellular carcinoma; HBV, hepatitis B virus; HCV, hepatitis C virus; ROS, reactive oxygen species.

**Priming phase**

**Interleukin-6**

Several clinical studies have reported higher levels of serum IL-6 in patients with HCC at the time of diagnosis; however, it is not clear from these single time point observations whether this cytokine drives hepatocarcinogenesis, or if the high IL-6 levels are simply a reflection of the highly proliferative nature of these cancers or a recruited inflammatory response. A recent nested case–control study of Hong Kong Chinese patients with chronic hepatitis B, revealed that increased serum IL-6 levels pre-dated the development of HCC; the sensitivity, specificity and positive predictive values were approximately 70%.\textsuperscript{10} Combination of IL-6 and α-fetoprotein (AFP; a widely used HCC tumor marker) improved the sensitivity in diagnosing HCC, as well as predicting future liver cancer development in a surveillance program.\textsuperscript{10}

Increased IL-6 production has also been implicated in the pathogenesis of HCC in one animal model.\textsuperscript{11} Mice subjected to the alkylating carcinogen diethylnitrosamine produced high levels of
IL-6 in response to this form of injury whilst lack of this cytokine in IL-6 null mice abrogated HCC development.\textsuperscript{13} The recent observations of IL-6 involvement in stem cells and hepatocarcinogenesis have also challenged the classical paradigm in which abnormal hepatocyte proliferation induces carcinogenesis (Fig. 1). An emerging alternative view is one where liver stem cell populations give rise to tumor cells.\textsuperscript{73} Within the regenerating human liver, there are small clusters of stem cells in the frequency of approximately 1:10 000. In experiments using \textit{tibh} (IL-6 regulatory protein) and embryonic liver fodrin (ELF)-deficient mice, IL-6/STAT3 signaling was noted to be impaired.\textsuperscript{73} In this setting, liver stem cells expressing embryonic markers octamer 4 and Nanog were observed to give rise to HCC; the evolution of liver cancer from stem cells in these mice required the critical interplay of IL-6 and TGF-\textbeta.\textsuperscript{73}

G1/S restriction point

Signaling pathways that link growth and metabolic signals to regulation of the restriction point in hepatocytes have been targets of active investigation as this checkpoint is frequently disrupted in HCC.

E2F proteins

E2F proteins are central mediators of G1/S progression and consist of a family of transcription factors with both pro-proliferative and inhibitory members. Proliferation-associated genes are normally transcriptionally repressed in quiescent cells and during the early G1 phase, as a result of binding of an antiproliferative member of the E2F family (E2F4, E2F5), a ‘pocket’ protein (pRb, p130) and chromatin remodeling proteins.\textsuperscript{74} During G1/S transition, cyclin D1 and cyclin-dependent kinases (CDK4 and 6, cyclin E as well as CDK2 dimers, sequentially phosphorylate the pocket proteins leading to the dissolution of the inhibitory complex.\textsuperscript{74,75} Relief of this repression enables the transcription of E2F target genes. Interestingly, amongst the first genes to be activated are those encoding E2F proteins; these in turn regulate specific target genes including c-myc, cyclin D1 and survivin.\textsuperscript{79} In normal cells, \(\beta\)-catenin associates with E-cadherin at adherens junctions of the cell membrane, linking this protein to the actin cytoskeleton.\textsuperscript{80,81} The signaling cascade is normally initiated extracellularly when enough Wnt ligands accumulate to outnumber Wnt antagonists, thereby stimulating the relay of message through Frizzled (FZD), a transmembrane receptor.\textsuperscript{81,82} FZD then signals to \(\beta\)-catenin to escape its association with E-cadherin. Once activated, cytoplasmic elements of the Wnt pathway prevent \(\beta\)-catenin from being phosphorylated by a degradation complex made up of a serine-threonine kinase, GSK3B and protein scaffolds, AXIN and adenomatous polyposis coli (APC).\textsuperscript{18,20} Normally, when the Wnt pathway is not activated, cytoplasmic \(\beta\)-catenin is marked for proteosomal degradation, phosphorylation and ubiquitination.\textsuperscript{18,82} However, mutations in these proteins may allow \(\beta\)-catenin to escape into the nucleus to promote transcription of its target genes in a constitutive manner.\textsuperscript{18,82}

Cyclin D1

The earliest cyclin–CDK complex activated during G1 phase progression is cyclin D1-CDK4/6. As mentioned already, cell cycle progression through the G1/S phase transition is dependent on the induction of cyclin D1. Overexpression of cyclin D in hepatocytes induces replication.\textsuperscript{14} In the regenerating liver, cyclin D1 plays a critical role as an integrator of growth, cytokine and metabolic signals which usher hepatocytes through the G1/S restriction point.\textsuperscript{70,71} Multiple signaling pathways activate cyclin D1, including JNK, HGF, TNF and IL-6.\textsuperscript{72-78} Cyclin D1 expression is increased in HCC and correlates with advanced tumor stage and progression.\textsuperscript{15,16} Transient overexpression of cyclin D1 in mouse hepatocytes causes chromosomal instability with centrosome amplification and mitotic spindle abnormalities in liver cells and this may explain the association between cyclin D1 and aggressive growth characteristics.\textsuperscript{17} In contrast to the regulatory mechanisms responsible for cyclin D1 induction after PH, exaggerated expression of cyclin D1 in human and murine models of HCC is thought to arise from amplification of this gene leading to chromosomal aberrations.\textsuperscript{15-17} These findings imply that the overexpression of cyclin D1 is likely to be a late event in liver carcinogenesis rather than in tumor initiation.

Wnt and \(\beta\)-catenin

The Wnt pathway has a fundamental role in embryogenesis with signaling effects on proliferation and apoptosis in developing cells.\textsuperscript{18} Its proper function is so indispensable that cells bear multiple fail-safe mechanisms, components of which have been highly conserved throughout evolution.\textsuperscript{79} The ‘canonical Wnt pathway’ describes a cascade of events beginning with the translocation of \(\beta\)-catenin from the cell membrane into the nucleus, where \(\beta\)-catenin then acts as a co-activator of the TCF/LEF family of transcription factors; these in turn regulate specific target genes including c-myc, cyclin D1 and survivin.\textsuperscript{79}

In normal cells, \(\beta\)-catenin associates with E-cadherin at adherens junctions of the cell membrane, linking this protein to the actin cytoskeleton.\textsuperscript{30,81} The signaling cascade is normally initiated extracellularly when enough Wnt ligands accumulate to outnumber Wnt antagonists, thereby stimulating the relay of message through Frizzled (FZD), a transmembrane receptor.\textsuperscript{81,82} FZD then signals to \(\beta\)-catenin to escape its association with E-cadherin. Once activated, cytoplasmic elements of the Wnt pathway prevent \(\beta\)-catenin from being phosphorylated by a degradation complex made up of a serine-threonine kinase, GSK3B and protein scaffolds, AXIN and adenomatous polyposis coli (APC).\textsuperscript{18,20} Normally, when the Wnt pathway is not activated, cytoplasmic \(\beta\)-catenin is marked for proteosomal degradation, phosphorylation and ubiquitination.\textsuperscript{18,82} However, mutations in these proteins may allow \(\beta\)-catenin to escape into the nucleus to promote transcription of its target genes in a constitutive manner.\textsuperscript{18,82}

Nuclear localization of \(\beta\)-catenin is considered a harbinger of its oncogenicity.\textsuperscript{32} Most mutations in the Wnt pathway leading to cancer either inactivate the function of APC or stabilize \(\beta\)-catenin by altering its binding site to the protein scaffold, therefore allowing it to escape destruction by the proteosome.\textsuperscript{82,83} Although APC gene mutations are substantially detected in colon cancer (90% contain APC mutations), they are rare in HCC.\textsuperscript{18,19,83} In contrast, Wnt signaling intermediates have been shown to be upregulated in HCC, with more than 10 studies demonstrating \(\beta\)-catenin mutations.\textsuperscript{18-20} Others have also reported that preneoplastic lesions with \(\beta\)-catenin activation have a higher risk of malignant transformation than their counterparts, with \(\beta\)-catenin mutation rates ranging 0–45% in some tumors.\textsuperscript{18,19,21,22} Mutations of \(\beta\)-catenin described in HCC are located in exon 3 of the CTNNB1 gene, which is the phosphorylation site for GSK3B. AXIN1 and AXIN2 mutations have also been described in human HCC, in addition to extracellular inhibitors of Wnt signaling.\textsuperscript{23,24}
The Wnt receptor, FZD-7 has also been found to be overexpressed in up to 90% of HCC.25 Twenty to 40% of human HCC bear abnormal cytoplasmic and nuclear accumulation of β-catenin by immunohistochemical staining.84,85 However, not all studies show a correlation between elevated nuclear β-catenin and expression of its transcriptional targets; this implies that the expression of these target genes is likely to be regulated by alternative signaling mechanisms. While most of the preceding mutations discussed have not been detected in allelotype analysis, it is salient to note that deletions in the AXIN1 locus (16p) have been described in HCC, and that the β-catenin locus (3p) itself is not a usual chromosomal aberration reported in this cancer.86,87

**Finding Hedgehog**

The Hedgehog signaling pathway consists of a complex suite of molecules which regulate cell differentiation, regeneration and stem cell biology; the elements of this pathway also play important roles in the development and homeostasis of gut tissue.88 There are three potential ligands described: Indian, Sonic and Desert Hedgehog. Sonic is the predominant isoform in the liver. In the absence of ligand activation, the receptor Patched exerts an inhibitory effect on Smoothened (Smo), a transmembrane homolog of G-protein coupled receptors, which in combination with Cos-2, impairs nuclear translocation of Gli.88 Like β-catenin, after ligand stimulation, Gli accumulates in the nucleus and induces transcription of genes related to cell cycle and growth including insulin-like growth factor-2 (IGF-2), cyclins B1, D1, E and β-catenin, as well as negative regulators such as Patched and Hh binding protein (Hip1).48 Studies have identified a possible role for this pathway in HCC with expression of Sonic in up to 60% of human HCC samples, and concomitant downregulation of Gli-related target genes after specific blockade of this pathway.96,97 Moreover, tumororigenic activation of Smo can mediate overexpression of c-myc, a gene which is well known to play an important pathogenic role in liver carcinogenesis.98

**pRb**

Mutations in the pocket protein pRb have been frequently found in human HCC. pRb may participate in long-term silencing of proliferative genes in those cells that are terminally quiescent.24,29 Because hepatocytes retain the capacity to rapidly re-enter the cell cycle in response to liver injury, it is likely that the related pRb family members, p130 and p107, are responsible for repression of cell cycle-associated genes.15 The observation that pRb was maintained in HCC where cyclin D1 was amplified, supports the hypothesis that inactivation of other pocket proteins may be responsible for deregulation of growth in some liver tumors.13,17,29

**G2/M checkpoint regulation**

Understanding the mechanisms by which G2/M checkpoint control are executed are highly relevant to the pathogenesis of HCC, a cancer in which chromosomal instability is a near-universal finding. Defects in mitotic checkpoint regulation can lead to aneuploidy in daughter cells resulting in cells escaping cell cycle control. Alterations in genes involved in mitotic assembly have been highlighted in HCC by microarray expression analyses as well as experimental models.

**Foxm1b**

Among several genes that control the G2/M transition, one is FOXM1B, a member of the forkhead transcription factor family.30 Gene array analyses in human HCC have found Foxm1b to be highly expressed in some tumors.3 Interestingly, Foxm1b is not normally expressed in quiescent liver.71,72 In contrast to other hepatic transcription factors whose expression increases in early G1 post-PH, Foxm1b is induced late in G1, near the G1/S phase transition. In aged mice, forced expression of Foxm1b restores hepatocyte proliferation to levels found in young adults.30 Also, young mice null for hepatocyte Foxm1b display decreased DNA synthesis and mitosis, associated with sustained expression of p21, and reduced cdc25A and cdc25B phosphatases.30,31 Identification of p21, cdc25A and cdc25B as Foxm1b targets indicates that Foxm1b is an important regulator of both G1/S and G2/M phase transitions.30,32 Interestingly, the transcriptional activity of Foxm1b requires docking with CDK2-cyclin E/A (S phase) or cdk1-cyclin B (G2 phase) complexes.28–32 In a separate study, others have shown that Foxm1b-deficient mice are resistant to diethylnitosamine-induced HCC.31 This refractoriness is associated with increased expression of cdc25B and sustained nuclear localization of p27, another CDK inhibitor.33 The investigators also showed that Foxm1b was a novel inhibitory target of p19, a G1/S regulator of p53 stability, which, as discussed later, is frequently disrupted in HCC.

**Mitosis regulators**

Microarray analyses have also identified large clusters of genes controlling mitotic spindle assembly and checkpoint control in human HCC. These genes include those induced in the S phase such as bul1b, Aurora kinases and survivin.34–37 The coordinate regulation at the mRNA level of a large number of genes important for G2/M phase progression strongly supports the idea that transcriptional activation plays a key role in this phase of the hepatocyte cell cycle.

Defects in chromosome segregation during mitosis result in aneuploidy, a common cytogenetic abnormality detected in cancer cells including HCC.34,35,39 Many genes have been linked to the complex series of events that ensure accurate chromosome segregation. Not surprisingly, mutations in these genes can lead to carcinogenesis. Aurora A kinase is one such factor involved in chromosome segregation during mitosis.36,37 This gene is overexpressed in human HCC as well as in a diethylnitosamine-induced mouse model of accelerated liver carcinogenesis, in which animals are null for the non-homologous end joining DNA repair pathway, Ku70 (see section ‘p53 tumor suppressor’).38

**Halting the hepatocytes’ proliferative response**

The signals that terminate the proliferative response in hepatocytes are still not known. However, the phenomena of return to consistent liver to body mass ratio after PH supports the existence of
‘stop’ signals to prevent hepatocytes from replicating continually when the liver reaches sufficient functional capacity. In this molecular ‘yin and yang’ of the hepatocyte, proteins that inhibit or retard cell cycle progression include p53, TGF-β, p21, p27 and p18; they act possibly as safeguards to limit the extent of regeneration after injury. Forced overexpression of these proteins has been shown to inhibit hepatocyte proliferation in several in vitro and in vivo models.30–32

p53 tumor suppressor

It is widely accepted that p53 functional deficiency participates in HCC development.39 However, whether its mutation contributes to the initiation and/or the progression of liver carcinogenesis remains the subject of fervent pursuit. Functional inactivation of p53 by hepatitis B × protein (HBx) has been described in HCC arising in a HBx transgenic mouse model, inferring an important role for this tumor suppressor in hepatocarcinogenesis.39 Further, mutations in p53 were detected only in larger HCC, indicating that full genetic inactivation of p53 was associated with advanced to late-stage disease. In humans, HBV- and HCV-related HCC have shown greater frequency of p53 mutations in advanced tumors (40%) than in regenerative nodules (< 10%).40–43 Although such trends are indicative of a likely role for loss of p53 in tumor progression, they do not exclude the possibilities that mutant p53 in regenerative nodules are those that have ‘initiated’ the carcinogenic process, or that rare p53 mutant cells are common in such nodules, but not easily detected by conventional sequencing approaches.42,43

It is also worth considering p53 in relation to aflatoxin B1 (AFB1). Notably, patients from areas of high AFB1 exposure display frequent p53 mutations in their HCC, whilst individuals from low AFB1 endemic regions show p53 mutations only in the later stages of HCC.40–42,44 From these observations, it is plausible that p53 mutations may operate in either HCC initiation or progression, depending on the context. In the setting of AFB1, the p53 mutation may serve to drive initiation in cooperation with other events. Interestingly, HBV envelope protein (hepatitis B surface antigen; HBsAg) transgenic mice have a HCC-prone phenotype that is accelerated and made fully penetrant when combined with AFB1 and the p53ser246 allele (which corresponds to the p53 stop codon 249 mutation of AFB1 related HCC).40,90

Other etiological factors that ultimately lead to cirrhosis may also provoke oxidative stress, regeneration and telomere erosion in the hepatocyte milieu; here, the loss of p53 may play a more prominent role in HCC progression by facilitating continued proliferative potential in the face of activated DNA damage signaling and genomic instability. Our group has shown that a defect in the Ku70 gene (mentioned earlier as a DNA repair pathway) causes multiple chromosomal abnormalities and accelerates liver carcinogenesis in mice injected with diethylnitrosamine.38 In those animals, the molecular pathogenesis of HCC development lies in cell cycle checkpoint failure caused by loss of p53 through proteasomal degradation.38 HCC in Ku70-deficient mice display high expression of Aurora A kinase, phospho-ATM, phospho-mdm2 (Ser166), as well as high ubiquitination activity, all features that resemble human HCC.38 Along these lines, other investigators have shown that p53 heterozygosity (through germ line mutations) may also enable HCC progression in mice with shortened telomeres suggestive of the notion that p53 cooperates with telomere-induced chromosomal instability in liver carcinogenesis.91–93

Transforming growth factor-β

Transforming growth factor-β mediates growth arrest in epithelial and other cell types through several regulatory pathways.70–72,45 These include transcriptional activation of the CDK inhibitors p15 and p21, and transcriptional repression of c-myc, CDK4 and cdc25A expression.72,45 In normal cultured hepatocytes, the addition of TGF-β inhibits hepatocyte proliferation, whilst infusion of this signaling protein delays DNA synthesis in rats after PH. However, studies in rodent HCC models have yielded conflicting data regarding the role of TGF-β in hepatocarcinogenesis.39 The multiplicity of cell types that express TGF-β and its receptors in the liver, effects on the microenvironment and the ability of TGF-β to regulate other processes in addition to proliferation, apoptosis, angiogenesis, tumor invasiveness and immune surveillance, add to the complexity of dissecting the role of TGF-β in liver carcinogenesis. In clinical samples, TGF-β and TGF-β II receptors have been found to be downregulated in early HCC compared to surrounding liver, while the expression of TGF-β receptors inversely correlated with tumor size and proliferative index.46–48

Concluding remarks and future opportunities

The pathogenic mechanisms underlying HCC still remain elusive. What we do know is that this cancer is driven by diverse etiologies ranging from hepatotoxins, viruses and metabolic disorders. Also, the available data on HCC represent a very heterogeneous collection of tumor subtypes from specific causal factors driving distinct or common genetic and genomic events during tumor development. As is the case for other common solid tumors, the multitude of genomic profiles of HCC, sometimes conflicting, with a multitude of amplifications and deletions only further muddies the quest to elucidate the fundamentals underlying hepatocarcinogenesis. Using human tissues, one can usually only establish a ‘snapshot in time’ of a complex series of events, many of which are late changes in a neoplastic phenotype that favor disease progression, rather than being pivotal to its initiation.

What then of the future? The author believes an emphasis should be placed on the development of new mouse models which recapitulate the key features found in human HCC, including chromosomal instability, high proliferative activity, cell cycle and tumor suppressor dysfunction in the setting of a tumor environment such as fibrosis and regenerative nodules that mimics the setting of pre-neoplastic human chronic liver diseases. Ultimately, such models would serve as valuable tools for gene identification, validation as well as intervention and drug development. Beyond viral-associated inflammation, there still exists a poor understanding of the role of the cirrhotic microenvironment and what constituents in such a milieu predicate the development of liver cancer. Major therapeutic advances may not necessarily come from new therapies or agents for advanced HCC, but the prevention of cancer by the elimination of virus-related hepatocyte necroinflammation,
and the detection of early tumors for surgical resection, thereby allowing regeneration of normal liver from remaining ‘quiescent’ hepatocytes. Thus, yet again, Viva Prometheus!

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