

Reversing Hepatocellular Carcinoma Progression by Using Networked Biological Therapies

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Abstract The liver is distinguished from other tissues by (a) its detoxifying function, (b) its resistance to apoptosis, and (c) its regenerative response to damage. Hepatocellular carcinoma arises when chronic insults, such as hepatitis or iron overload, constitutively activate this regenerative program. Here, we propose that the proliferative response of the liver to damage underlies the resistance of hepatocellular carcinoma to cytotoxic therapy, and that hepatocellular carcinoma growth should therefore be more readily controlled by using a networked combination of noncytotoxic interventions to interrupt the damage-inducible regenerative pathway. To this end, hepatocellular carcinoma boasts a wealth of potential drug targets, including viral replication, the antiapoptotic immunosuppressant α -fetoprotein, hepatic iron overload, inflammatory signaling, extracellular proteases, and growth factors. By blocking these positive feedback loops in parallel, and so returning the host environment to a more normal state, epigenetic repression of tumor-suppressor gene function may be reversed and tumor dormancy restored. Noncytotoxic maneuvers that short circuit damage resistance loops may thus represent an indirect form of gene therapy meriting incorporation into hepatocellular carcinoma clinical trials.

Therapeutic progress in established hepatocellular carcinoma has largely been restricted to locoregional interventions. In the usual context of unresectable disease, response rates for ablative therapies (transcatheter arterial chemoembolization, ethanol injection, and radiofrequency ablation) have been gratifying; however, randomized proof of survival benefit remains elusive. Major improvements in the natural history of advanced hepatocellular carcinoma thus seem dependent on a quantum leap in medical therapy.

Several obstacles block this goal. First, the drug resistance of hepatocellular carcinoma means that no standard therapy exists against which to compare innovative regimens. Second, coexisting liver dysfunction all too often mandates management of “two diseases,” and reduces the number of patients eligible for trials (1). Third, the inability thus far to identify typical hepatocellular carcinoma signaling pathologies (e.g., akin to epidermal growth factor receptor mutations in non-smoking lung adenocarcinoma, or HER2 overexpression in breast cancer) challenges rational design of target-based drug trials.

The traditional “magic bullet” approach to hepatocellular carcinoma drug development is based on the assumption that cancers are irreversible states that no longer respond to discrete

signaling changes. This hypothesis has recently been challenged by the discovery that inactivation of a single oncogene can cause hepatocellular carcinoma to regress to a dormant state (2). It may thus be timely to consider a less hard-wired “reverse biology” model of hepatocellular carcinoma control in which well-characterized tumorigenic steps are targeted in a coordinated manner using a network-based approach.

Detoxifying without Dying

The liver is a defensive organ that responds to damage in a unique manner. Nonhepatic epithelial cells progress through the cell cycle (i.e., G₁-S-G₂-M) until damaged, when they either die or arrest/repair; in contrast, growth-arrested (i.e., G₀) hepatocytes proliferate in response to damage (Fig. 1; ref. 3). This antiapoptotic phenotype, which has evolved to suit the elimination of exogenous (xenobiotic) and endogenous (metabolic) toxins by the liver, is mediated by the high DNA repair capacity of the liver after up-regulation by damage.

Sublethal hepatocyte damage induces pro-inflammatory cytokine release, whereas harsher insults cause cell necrosis detectable as rupture of transaminases into the bloodstream. In contrast to the tumorolytic significance of apoptosis, necrosis drives hepatocellular carcinoma initiation and progression (4), consistent with the absence of survival benefits of local ablation. Hence, the native detoxifying capacity of the liver suggests that hepatocellular carcinoma may inactivate cytotoxins with unusual efficiency, whereas the high apoptotic threshold of the hepatocyte predicts that hepatocellular carcinoma damage will tend to cause necrogenic progression rather than remission.

Damage-Inducible Growth

The regenerative hepatic phenotype induced by wound-ing, resection, infection, or cytotoxic damage is central to the

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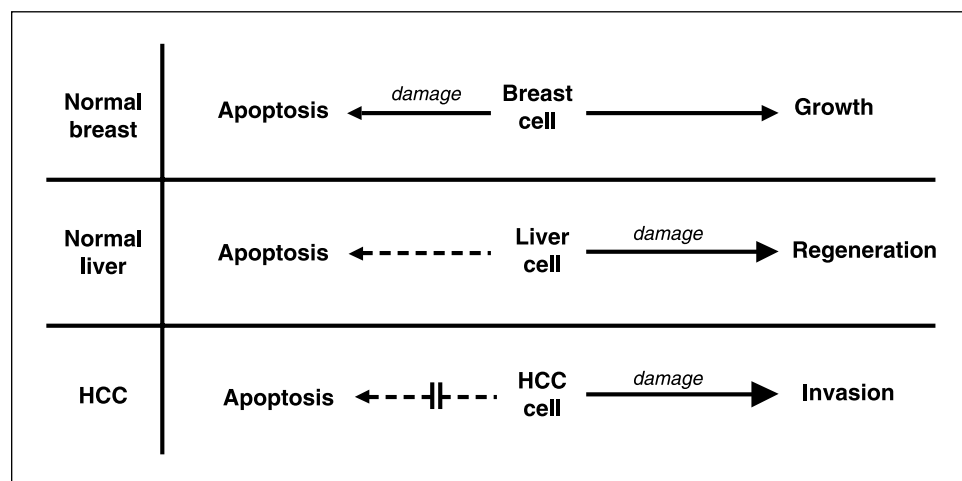


Fig. 1. Differential response of normal and malignant cell types to damage. Top, normal breast epithelial cells; middle, normal hepatocytes; bottom, hepatocellular carcinoma (HCC) cells.

hepatocellular carcinoma treatment dilemma. Cirrhosis impairs hepatic regenerativity, whereas necrotic release of hepatic survival factors drives regeneration of cirrhotic nodules (5). Treatment of hepatocellular carcinoma patients with chemotherapy may thus risk both acute hepatocellular decompensation and tumor progression. One approach to this problem involves using noncytotoxic therapies to block necrogenic pathways. This strategy is consistent with the reversible phenotype of many tumors (e.g., regression of gastric mucosal-associated lymphoid tissue lymphoma following *Helicobacter pylori* eradication, remission of EBV-associated lymphomas in response to antiviral therapy, or cytokine-driven involution of skin keratoacanthomas) and is supported by the status of hepatocellular carcinoma as the most frequent human cancer to undergo spontaneous regression (Fig. 2).

Progression of hepatocellular carcinoma involves selection for loss of proapoptotic tumor-suppressor gene function, as well as repression of DNA repair genes by mutation or promoter methylation. Because hepatitis activates the regenerative pathway, and because infection is a tumorigenic source of DNA damage, effective inhibition of viral inflammation could

help to arrest hepatocellular carcinoma suppression (6) as one component of a more broad-based therapeutic strategy.

Hit and Run, or Caught Red Handed?

Chronic hepatitis virus infection affects over half a billion individuals [70% hepatitis B virus (HBV) and 30% HCV] worldwide and accounts for over 80% of the global incidence of hepatocellular carcinoma. Vertical transmission of HBV from mother to newborn is the highest-risk mode of viral carcinogenesis, predisposing to hepatocellular carcinoma even in noncirrhotic livers of infected offspring (7). Steroid treatment increases mortality of patients with chronic HBV, yet coinfection with HIV does not increase HBV-associated hepatocellular carcinoma (8), implying that it is the immune response to chronic infection that promotes hepatocellular carcinoma progression.

If hepatocellular carcinoma progression is not in fact driven by viral infection, the tumor may be better regarded as a “hit-and-run” complication of an earlier phase of infection, reducing prospects for intervention. Against the hit-and-run model is the fact that virally induced hepatocellular carcinoma exhibits few specific molecular stigmata of an earlier pathogenic event, unlike, say, aflatoxin-induced hepatocellular carcinoma (characterized by the *TP53* codon 249 G→T transversion). If virus activity does drive tumor progression, on the other hand (i.e., the “caught-red-handed” model of viral hepatocarcinogenesis), then inhibition of HBV/HCV-dependent signaling or inflammation should slow tumor recurrence. This possibility is supported by a lower hepatocellular carcinoma frequency in HCV carriers in whom transaminitis is reduced with the use of the anticholestatic agent ursodiol (9).

Gene Methylation: The Defense of DNA

Despite many reports of HBV insertional mutagenesis in host genomes, evidence for a necessary role of HBV integration in hepatocyte transformation remains weak. A more general mode of viral carcinogenesis involves the defensive response of host cell genomes to intracellular viral DNA (10)—a response involving regional chromatin modification by cytosine methyltransferases and histone deacetylases, leading to transcriptional silencing.

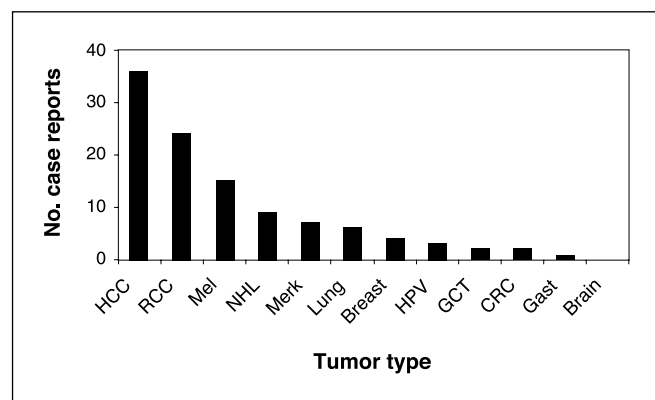


Fig. 2. Frequency of spontaneous regression. The PubMed database 1975 to 2005 was searched for titles containing the strings, (spontaneous regression) AND (cancer OR carcinoma OR malignant OR malignancy OR neoplasm OR neoplasia OR neoplastic); the number of cases involving each tumor type was recorded. HCC, hepatocellular carcinoma; RCC, renal cell carcinoma; Mel, melanoma; NHL, non-Hodgkin's lymphoma; Merk, Merkel-cell tumor; Lung, lung cancer; Breast, breast cancer; HPV, papillomavirus-related cancers; GCT, germ-cell tumor; CRC, colorectal cancer; Gast, gastric cancer; Brain, gliomas.

Both HBV- and HCV-associated hepatocellular carcinomas are more frequently associated with epigenetic gene silencing than is nonviral hepatocellular carcinoma; however, there is little difference in the patterns of gene inactivation induced by the DNA virus HBV and the nonintegrating RNA virus HCV (11), indicating that integration is not necessary to induce DNA methylation. Viral load correlates with methylation of tumor-suppressor genes such as *INK4A* (12), suggesting that effective antiviral therapies could help to restore apoptotic susceptibility (Fig. 3).

X Marks the Spot

The apoptosis-regulatory *TP53* gene lacks a promoter CpG island, making it resistant to methylation-dependent transcriptional repression. However, viruses have evolved other strategies to block the death of infected cells and thus potentiate viral chronicity. One example is the X protein of HBV (HBx), which directly inhibits p53, blocking transactivation of proapoptotic substrates (13). Conversely, HBV-inhibitory drugs, such as oltipraz, enhance death of infected cells by inducing *TP53*,

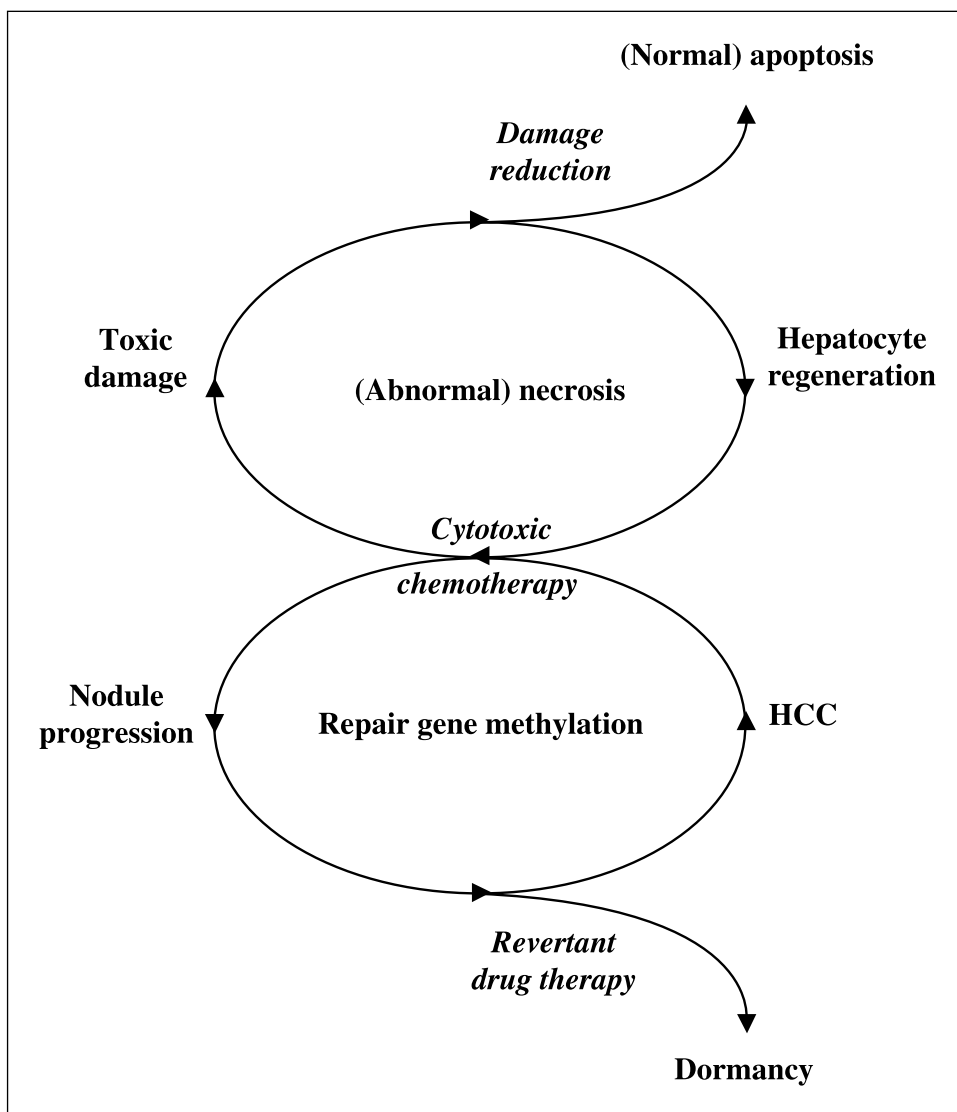
implying that HBV antivirals can improve hepatocellular carcinoma chemosensitivity by acting as an indirect form of gene therapy (14).

HBx transactivates the hepatocyte α -fetoprotein (*AFP*) gene via the same CAAT/enhancer binding protein α site that normally opposes growth by restricting developmental expression of *AFP*; CAAT/enhancer binding protein α growth control may also be subverted by phosphatidylinositol 3-kinase/Akt signaling such as is involved in liver regeneration (15). Because the CAAT/enhancer binding protein α -blocking action of HBx evolved via selection for HBV-infected hepatocyte survival, a tumor-promoting function for *AFP* is implied.

From Tumor Marker to Antitumor Target

AFP, a glycoprotein structurally related to albumin but reciprocally regulated, is expressed in pregnancy by yolk sac and fetal liver from 6 weeks of gestation; in this context, *AFP* has been linked to "fetal graft" tolerance (16). Consistent with this immunomodulatory hypothesis, transplacental passage of fetal *AFP* is associated with clinical remission of rheumatoid arthritis

Fig. 3. Schema of the rationale of revertant drug therapy. Conventional cytotoxic therapy is proposed to accelerate hepatocellular carcinoma progression by inducing necrosis and tumor suppressor gene methylation. In contrast, revertant therapy is proposed to restore apoptotic sensitivity and permit tumor cell dormancy via suppressor gene reactivation.



and multiple sclerosis; conversely, a hazard of AFP functional blockade in viral liver disease is that of immune reconstitution hepatitis (17). The *AFP* gene is reactivated in adult liver following wounding, peaking 1 to 4 days after transaminase elevation. This time course parallels hepatic regeneration, as illustrated by correlation of high AFP levels with recovery from acetaminophen-induced hepatotoxicity (18).

Although AFP has long been used by oncologists as a marker for hepatocellular carcinoma or yolk sac tumors, this so-called oncofetal antigen remains a neglected drug target despite many observations implicating it as a mediator of hepatocellular carcinoma progression: (a) AFP-dependent immunosuppression is associated with hepatocellular carcinoma recurrence (19); (b) hepatocellular carcinoma cell growth is stimulated by purified AFP (20); (c) hepatocellular carcinoma cell growth is reversed by albumin overexpression (21), suggesting that hepatocellular carcinoma progression may be slowed by reversing hypoalbuminemia; (d) AFP antibodies block onco-gene expression *in vitro* and reverse hepatocellular carcinoma drug resistance *in vivo* (22); and (e) vaccination with AFP-encoding DNA inhibits xenograft growth (23). Hepatic AFP expression can be blocked by repressing HBx-dependent *AFP* transactivation using anti-HBV drugs (24), supporting the feasibility of this treatment in hepatocellular carcinoma; in estrogen-dependent breast cancer, however, hepatocellular carcinoma-derived AFP induces tumor regression (25). This raises questions about the role of sex hormones in hepatocellular carcinoma, which is 2- to 4-fold more common in males.

The Sex Connection

Hepatocellular carcinoma risk among HBV-infected males correlates with serum testosterone levels (26). Moreover, HBV surface antigen expression is up-regulated by androgens, supporting the hypothesis that hepatocarcinogenesis may be androgen dependent. Despite positive early trials of antiandrogen therapy, however, recent studies have failed to confirm survival prolongation (27). Treating estrogen receptor-positive hepatocellular carcinoma with tamoxifen has also yielded negative results at all dosage levels (28), whereas mixed outcomes have been reported with progestins. A potential therapeutic role for estrogens in hepatocellular carcinoma has been suggested by (a) **the hepatoprotective effects of estrogens** (29); (b) the good prognostic hepatocellular carcinoma implications of wild-type estrogen receptor expression, female gender, and oral contraceptive use (30); (c) **estrogen-inducible inhibition of HBV DNA transcription** and HBV E-antigen levels (31); and (d) estrogen-dependent induction of hepatic tumor-suppressor genes, and involution of premalignant masses (32). The hypothesis that estrogens could reduce hepatocellular carcinoma risk has never been popular, however, reflecting widespread concerns over thrombogenicity, carcinogenicity, and the premorbid association with cirrhosis-associated hyperestrogenemia.

Re-regulating Receptors

Hormones are long-distance regulators of local growth factor synthesis and release. One important liver-related growth factor signaling pathway is that initiated by hepatocyte growth factor ("scatter factor") and its receptor c-Met, which is essential

for hepatic regeneration (33). A comitogenic role with hepatocyte growth factor is exerted in hepatocellular carcinoma by insulin-like growth factor-I (IGF-I; ref. 34), the receptor for which is up-regulated by chronic viral hepatitis (35). IGF-I receptor signaling in hepatocellular carcinoma directly contributes to apoptotic resistance (36), which is readily reversed *in vitro* by small-molecule IGF-I receptor signaling inhibitors (37). Upstream regulation of IGF-I synthesis and secretion occurs via the growth hormone pathway; consistent with this, blockade of this pathway by the somatostatin receptor antagonist octreotide inhibits liver regeneration in animal models (38). Few clinical hepatocellular carcinoma responses to single-agent octreotide have been reported, however, suggesting the need for a more multitargeted therapeutic approach.

The teratogenic antiemetic/leprosy drug thalidomide inhibits vascular endothelial growth factor-dependent hepatocellular carcinoma progression, and also blocks signaling via the proinflammatory Fas ligand, tumor necrosis factor- α (39). Fas activation induces release of transforming growth factor- α and thus promotes epidermal growth factor receptor-dependent hepatocyte growth, possibly accounting for the reported *in vitro* and *in vivo* efficacy of small-molecule inhibition of epidermal growth factor receptor in hepatocellular carcinoma (40). Similarly, the epithelial apoptogen and AFP repressor transforming growth factor- β 1 is stromally up-regulated in hepatitis, contributing to hepatic fibrosis (41). As fibrotic liver damage is mediated by free radicals, transforming growth factor β -induced injury is worsened by intracellular colocalization of the oxidative electron scavenger, iron.

Iron, The Forgotten Growth Factor

Although seldom noted, the gender imbalance of hepatocellular carcinoma parallels the higher lifetime iron levels of adult males relative to (menstruating) females. Iron excess is now firmly implicated in susceptibility to cancers, including hepatocellular carcinoma, even in women; the iron-transport protein transferrin likewise promotes tumor (including hepatocellular carcinoma) metastasis (42). That iron is a direct carcinogen is indicated by the elevated incidence of hepatocellular carcinoma in noncirrhotic iron-overloaded patients with idiopathic hemochromatosis (43). Moreover, although only 0.5% of Caucasians are heterozygous for the causal C282Y *HFE* gene mutation; a 25-fold higher frequency of this mutation occurs in hepatocellular carcinoma patients (44).

Juvenile hemochromatosis can occur because of loss-of-function mutations affecting the *HEPC* gene specifying the iron-regulatory peptide hepcidin. Activation of this gene by CAAT/enhancer binding protein α is blocked by HBx, leading to HBV chronicity and iron overload (45). Consistent with the antimicrobial properties of hepcidin, iron depletion protects against inflammatory damage (46), perhaps accounting for the ancient popularity of blood letting (phlebotomy, venesection). Tumors may likewise shrink after induction of iron-deficiency anemia by bleeding, whereas progression may be triggered by blood transfusions (47).

Erythropoietin: Friend or Foe?

Regenerating liver expresses the hypoxia-driven transactivator hypoxia inducible factor-1 α , a key inducer of erythropoietin.

Table 1. Promising noncytotoxic therapeutic strategies for adjunctive hepatocellular carcinoma therapy

Target	Pathways	Intervention	Antagonism	Hazards
Chronic HBV	HBV replication	Entecavir, HBIG	Steroids, Delta agent	YMDD mutation
Chronic HCV	HCV replication	IFN/ribavirin, ursodiol	Alcohol, HIV co-infection	Myelosuppression
Chronic inflammation/fibrosis	Infection/toxicity TNF- α /NF- κ B/TGF- β	Thalidomide, etanercept	Alcohol, iron overload	Hepatotoxicity
Regenerative signaling	Damage/necrosis IGF-IR/PI3K signaling	IGF-I/IGF-IR/Met inhibitors, retinoids, octreotide	Cytotoxic chemotherapy	Sepsis
HBx protein	p53 inhibition, Akt/mTOR signaling	Oltipraz, temsirolimus	Delta agent	Fulminant hepatitis
DNA methylation	Tumor-suppressor gene inactivation	Decitabine, valproate	Uncontrolled viral hepatitis	HBV integration, oncogene activation
AFP	Immunosuppression, apoptosis inhibition	AFP antibodies, albumin	Benzodiazepines	Immune restoration hepatitis
Growth factor signaling	Ras-Raf-ERK, VEGF signaling	Sorafenib, sunitinib, bevacizumab, statins	Obesity, hypercholesterolemia	Bleeding (e.g., varices), rupture, perforation
Iron overload	Free radical damage, lipid peroxidation	Chelation/phlebotomy, ascorbate	Cisplatin, alcohol	Anemia
Proteases	Extracellular proteases, trypsin	Protease inhibitors, heparanase inhibitors	Smoking	Impaired healing

Abbreviations: TNF- α , tumor necrosis factor- α ; NF- κ B, nuclear factor- κ B; ERK, extracellular-regulated kinase; mTOR, mammalian target of rapamycin (pathway); HBIG, hepatitis B immunoglobulin; PI3K, phosphatidylinositol 3-kinase; VEGF, vascular endothelial growth factor.

Clonal selection for polycythemia owing to erythropoietin overproduction by hepatocellular carcinoma could thus signify acquisition of a growth advantage via activation of the regenerative pathway. Like hypoxic stress, cytotoxic stress elevates erythropoietin levels, suggesting a further mechanism of drug resistance (48). Given the impaired survival reported in randomized studies of cancer patients (49), use of erythropoietin monotherapy in hepatocellular carcinoma seems inadvisable.

On the other hand, concurrent erythropoietin and phlebotomy/chelation efficiently redistributes iron stores and depletes free iron in both malignant and nonmalignant conditions (50). Combined iron depletion (e.g., chelation) and redistribution (erythropoietin) in hepatocellular carcinoma patients thus remains a promising antitumor manipulation. A further rationale for reversal of iron overload relates to the role of this element in metalloprotease activation.

The Promise of Proteases

Extracellular proteases cleave growth factor precursors or their binding proteins—stimulating growth factor-dependent cell motility and thus raising the therapeutic prospect of using protease inhibitors to block both preneoplastic hepatic fibrosis and hepatocellular carcinoma metastasis (51). Consistent with this possibility, the endogenous acute-phase reactant and serine protease inhibitor (serpin), α -1-antitrypsin—the genetic deficiency of which predisposes to hepatocellular carcinoma—inhibits growth of HBV-infected liver cells (52).

A complicating factor lies in the recent insight that certain proteases may be tumor suppressive (53). For example, cisplatin suppresses cell surface proteases in tumor cells (54), but it is not known whether such protease suppression helps or hinders antitumor efficacy. Cisplatin also displaces iron from transferrin, increasing iron concentrations in chemotherapy-damaged tissues while potentially activating mitogenic metalloproteases and exacerbating metallothionein-mediated drug

resistance (55). Effective anticancer exploitation of protease inhibitors may thus await greater knowledge.

Networking for Success

The advantages of pursuing a network approach to cancer therapeutics—as opposed to the one-off, target-based strategy that has dominated clinical trials to date—have become apparent with the growth of the systems biology field (56, 57). Networked therapeutic strategies focus not only on identifying discrete loci of fragility within tumor signaling pathways (58), the often-mutated *PIK3CA* gene in hepatocellular carcinoma being a relevant example (59), but also on synergistically inactivating polyvalent signaling crosstalk (60) that may otherwise create robustness (61) and drug resistance (62) within the meshwork of tissue-tumor interactions. Although immediate blockade of multiple interlocking pathways

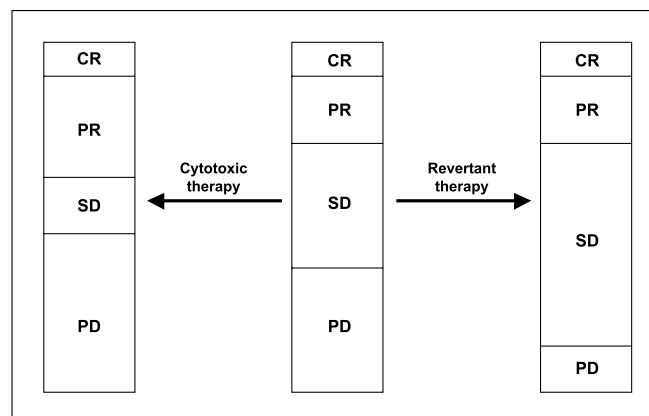


Fig. 4. Distinction between “response” (left) and “reversion” (right), modeling how cytotoxic therapies could cause both higher responses and faster progression, leading to inferior survivals, compared with revertant therapies. CR, complete remission; PR, partial remission; SD, stable disease; PD, progressive disease.

is an attractive clinical strategy that promises high response rates (63), accelerated induction of selection and resistance could limit survival improvements (64) and thus come to favor more nuanced interventions in both induction and maintenance (adjuvant) treatment phases (65).

Table 1 summarizes some of the biologically based interventions that could be used for hepatocellular carcinoma in appropriate combinations with (or without) cytotoxic therapy. Not all of these are yet practical; for example, small-molecule inhibitors of PI3KCA, Akt, Met, and IGF-I receptor are not yet licensed, nor are protease inhibitors of established relevance to hepatocellular carcinoma biology, whereas AFP antibodies have not yet been humanized for clinical trial evaluation. Nonetheless, more aggressive control of viral replication and iron overload is immediately applicable, as is vascular endothelial growth factor antibody therapy, mammalian target of rapamycin-inhibitory therapies, and semitargeted kinase inhibition using drugs such as sorafenib and sunitinib. Provided that drug companies can be persuaded to cooperate by providing free drugs for use in combination, academic units can immediately embark on an informative range of network-based biopharmaceutical control strategies.

References

- Johnson PJ, Billingham LJ. Clinical trial design in hepatocellular carcinoma. *Best Pract Res Clin Gastroenterol* 2005;19:119–27.
- Shachaf CM, Kopelman AM, Arvanitis C, et al. MYC inactivation uncovers pluripotent differentiation and tumour dormancy in hepatocellular cancer. *Nature* 2004;431:1112–7.
- Taub R. Liver regeneration: from myth to mechanism. *Nat Rev Mol Cell Biol* 2004;5:836–47.
- Newberne PM, Punyari P, de Camargo J, Suphakarn V. The role of necrosis in hepatocellular proliferation and liver tumors. *Arch Toxicol Suppl* 1987;10:54–67.
- Fausto N. Growth factors in liver development, regeneration and carcinogenesis. *Prog Growth Factor Res* 1991;3:219–34.
- Hagen TM, Huang S, Curnutte J, et al. Extensive oxidative DNA damage in hepatocytes of transgenic mice with chronic active hepatitis destined to develop hepatocellular carcinoma. *Proc Natl Acad Sci U S A* 1994;91:12808–12.
- Sezaki H, Kobayashi M, Hosaka T, et al. Hepatocellular carcinoma in noncirrhotic young adult patients with chronic hepatitis B viral infection. *J Gastroenterol* 2004;39:550–6.
- Smukler AJ, Ratner L. Hepatitis viruses and hepatocellular carcinoma in HIV-infected patients. *Curr Opin Oncol* 2002;14:538–42.
- Tarao K, Fujiyama S, Ohkawa S, et al. Ursodiol use is possibly associated with lower incidence of hepatocellular carcinoma in hepatitis C virus-associated liver cirrhosis. *Cancer Epidemiol Biomarkers Prev* 2005;14:164–9.
- Chen JY, Hsu HC, Lee CS, Chen DS, Zuckerman AJ, Harrison TJ. Detection of hepatitis B virus DNA in hepatocellular carcinoma: methylation of integrated viral DNA. *J Virol Methods* 1988;19:257–63.
- Yang B, Guo M, Herman JG, Clark DP. Aberrant promoter methylation profiles of tumor suppressor genes in hepatocellular carcinoma. *Am J Pathol* 2003;163:1101–7.
- Jicai Z, Zongtao Y, Jun L, Haiping L, Jianmin W, Lihua H. Persistent infection of hepatitis B virus is involved in high rate of p16 methylation in hepatocellular carcinoma. *Mol Carcinog* 2006;45:530–6.
- Gottlob K, Fulco M, Levrero M, Graessmann A. The hepatitis B virus HBx protein inhibits caspase 3 activity. *J Biol Chem* 1998;273:33347–53.
- Chi WJ, Doong SL, Lin-Shiau SY, Boone CW, Kelloff

- Lin JK. Oltipraz, a novel inhibitor of hepatitis B virus transcription through elevation of p53 protein. *Carcinogenesis* 1998;19:2133–8.
- Wang GL, Iakova P, Wilde M, Awad S, Timchenko NA. Liver tumors escape negative control of proliferation via PI3K/Akt-mediated block of C/EBP α growth inhibitory activity. *Genes Dev* 2004;18:912–25.
- Murgita RA, Tomasi TB. Suppression of the immune response by α -fetoprotein. *J Exp Med* 1975;141:269–86.
- Geissler M, Mohr L, Weth R, et al. Immunotherapy directed against α -fetoprotein results in autoimmune liver disease during liver regeneration in mice. *Gastroenterology* 2001;121:931–9.
- Schmidt LE, Dalhoff K. α -Fetoprotein is a predictor of outcome in acetaminophen-induced liver injury. *Hepatology* 2005;41:26–31.
- Vivarelli M, Cucchetti A, Piscaglia F, et al. Analysis of risk factors for tumor recurrence after liver transplantation for hepatocellular carcinoma: key role of immunosuppression. *Liver Transpl* 2005;11:497–503.
- Wang XW, Xu B. Stimulation of tumor-cell growth by α -fetoprotein. *Int J Cancer* 1998;75:596–9.
- Wu GX, Lin YM, Zhou TH, Gao H, Pei G. Significant down-regulation of α -albumin in human hepatoma and its implication. *Cancer Lett* 2000;160:229–36.
- Ohkawa K, Tsukada Y, Abe T, Takada K, Hibi N. Overcoming effect of antibody against rat α -fetoprotein (AFP) on the growth of daunorubicin-resistant mutant rat ascites hepatoma cell line AH66. *Int J Cancer* 1989;44:489–93.
- Hanke P, Serwe M, Dombrowski F, Sauerbruch T, Caselmann WH. DNA vaccination with AFP-encoding plasmid DNA prevents growth of subcutaneous AFP-expressing tumors and does not interfere with liver regeneration in mice. *Cancer Gene Ther* 2002;9:346–55.
- Yao FY. Dramatic reduction of the AFP level after lamivudine treatment of patients with chronic hepatitis B viral infection and cirrhosis. *J Clin Gastroenterol* 2003;36:440–2.
- Bennett JA, Zhu S, Pagano-Mirarchi A, Kellom TA, Jacobson HL. α -Fetoprotein derived from a human hepatoma prevents growth of estrogen-dependent human breast cancer xenografts. *Clin Cancer Res* 1998;4:2877–84.
- Yu MW, Yang YC, Yang SY, et al. Hormonal markers and hepatitis B virus-related hepatocellular carcinoma

risk: a nested case-control study among men. *J Natl Cancer Inst* 2001;93:1644–51.

- Pignata S, Daniele B, Gallo C, DeVivo R, Monfardini S, Perrone F. Endocrine treatment of hepatocellular carcinoma. Any evidence of benefit? *Eur J Cancer* 1998;34:25–32.
- Nowak AK, Stockler MR, Chow PK, Findlay M. Use of tamoxifen in advanced-stage hepatocellular carcinoma. A systematic review. *Cancer* 2005;103:1408–14.
- Shimizu I. Impact of oestrogens on the progression of liver disease. *Liver Int* 2003;23:63–9.
- Lam CM, Yong JL, Chan AO, et al. Better survival in female patients with hepatocellular carcinoma: oral contraceptive pills related? *J Clin Gastroenterol* 2005;39:533–9.
- Almog Y, Klein A, Adler R, Laub O, Tur-Kaspa R. Estrogen suppresses hepatitis B virus expression in male athymic mice transplanted with HBV transfected Hep G-2 cells. *Antiviral Res* 1992;19:285–93.
- Mishkin SY, Farber E, Ho RK, Mulay S, Mishkin S. Evidence for the hormone dependency of hepatic hyperplastic nodules: inhibition of malignant transformation after exogenous 17 β -estradiol and tamoxifen. *Hepatology* 1983;3:308–16.
- Borowiak M, Garratt AN, Wustefeld T, Strehle M, Trautwein C, Birschmeier C. Met provides essential signals for liver regeneration. *Proc Natl Acad Sci U S A* 2004;101:10608–13.
- Price JA, Kovach SJ, Johnson T, et al. Insulin-like growth factor I is a comitogen for hepatocyte growth factor in a rat model of hepatocellular carcinoma. *Hepatology* 2002;36:1089–97.
- Stefano JT, Correa-Giannella ML, Ribeiro CM, et al. Increased hepatic expression of insulin-like growth factor-I receptor in chronic hepatitis C. *World J Gastroenterol* 2006;12:3821–8.
- Alexia C, Fallois G, Lasfer M, Schweizer-Groyer G, Groyer A. An evaluation of the role of insulin-like growth factors (IGF) and of type-I IGF receptor signaling in hepatocarcinogenesis and in the resistance of hepatocarcinoma cells against drug-induced apoptosis. *Biochem Pharmacol* 2004;68:1003–15.
- Hopfner M, Huether A, Sutter AP, Baradari V, Schuppan D, Scherubl H. Blockade of IGF-1 receptor tyrosine kinase has antineoplastic effects in hepatocellular carcinoma cells. *Biochem Pharmacol* 2006;71:1435–48.

Conclusions

When used alone to treat hepatocellular carcinoma, conventional cytotoxic therapy causes unwanted effects, such as tissue necrosis, regenerative signaling, iron imbalance, and erythropoietin up-regulation. In contrast, certain noncytotoxic interventions are capable of blocking hepatocellular carcinoma growth, restoring tumor dormancy, and/or enhancing apoptotic sensitivity to cytotoxic drugs. From the viewpoint of clinical trial end points, it is crucial to note that reversibility in this context does not equate with response (Fig. 4). Investigator-initiated trials are now needed to test whether such networked biological strategies—whether used for induction, maintenance, chemosensitization, or chemotherapy sparing—are indeed capable of reversing hepatocellular carcinoma progression in different clinical scenarios and thus of improving patient outcomes.

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38. Pruthi RS, Farouk M, Tsai WH, Michalopoulos G, Meyers WC. The effect of octreotide on hepatic regeneration in rats. *Surgery* 1993;113:84–9.
39. Zhang ZL, Liu ZS, Sun Q. Effects of thalidomide on angiogenesis and tumor growth and metastasis of human hepatocellular carcinoma in nude mice. *World J Gastroenterol* 2005;11:216–20.
40. Matsuo M, Sakurai H, Saiki I. ZD1839, a selective EGFR tyrosine kinase inhibitor, shows antimetastatic activity using a hepatocellular carcinoma model. *Mol Cancer Ther* 2002;2:557–61.
41. Nakao K, Nakata K, Mitsuoka S, et al. Transforming growth factor β 1 differentially regulates α -fetoprotein and albumin in HuH-7 human hepatoma cells. *Biochem Biophys Res Commun* 1991;174:1294–9.
42. Cavanaugh PG, Nicolson GL. Lung-derived growth factor that stimulates the growth of lung-metastasizing tumor cells: identification as transferrin. *J Cell Biochem* 1991;47:261–71.
43. Goh J, Callagy G, McEntee G, O'Keane JC, Bomford A, Crowe J. Hepatocellular carcinoma arising in the absence of cirrhosis in genetic haemochromatosis: three case reports and review of literature. *Eur J Gastroenterol Hepatol* 1999;11:915–9.
44. Hellerbrand C, Poppl A, Hartmann A, Scholmerich J, Lock G. HFE C282Y heterozygosity in hepatocellular carcinoma: evidence for an increased prevalence. *Clin Gastroenterol Hepatol* 2003;1:279–84.
45. Courselaud B, Pigeon C, Inoue Y, et al. C/EBP α regulates hepatic transcription of hepcidin, an antimicrobial peptide and regulator of iron metabolism. *J Biol Chem* 2002;277:41163–70.
46. Kato J, Kobune M, Nakamura T, et al. Normalization of elevated hepatic 8-hydroxy-2'-deoxyguanosine levels in chronic hepatitis C patients by phlebotomy and low iron diet. *Cancer Res* 2001;61:8697–702.
47. Singh SK, Marquet RL, de Bruin RW, Hop WC, Westbroek DL, Jeekel J. Consequences of blood loss on growth of artificial metastases. *Br J Surg* 1988;75:377–9.
48. Belenkov AI, Shenouda G, Rizhevskaya E, et al. Erythropoietin induces cancer cell resistance to ionizing radiation and to cisplatin. *Mol Cancer Ther* 2004;3:1525–32.
49. Leyland-Jones B, Semiglazov V, Pawlicki M, et al. Maintaining normal hemoglobin levels with epoetin alfa in mainly nonanemic patients with metastatic breast cancer receiving first-line chemotherapy: a survival study. *J Clin Oncol* 2005;23:5960–72.
50. de la Serna J, Bornstein R, Garcia-Bueno MJ, Lahuerta-Palacios JJ. Iron depletion by phlebotomy with recombinant erythropoietin prior to allogeneic transplantation to prevent liver toxicity. *Bone Marrow Transplant* 1999;23:95–7.
51. Salvi A, Arici B, De Petro G, Barlati S. Small interfering RNA urokinase silencing inhibits invasion and migration of human hepatocellular carcinoma cells. *Mol Cancer Ther* 2004;3:671–8.
52. Propst A, Graziadei I, Herold M, Propst T, Vogel W. The acute phase protein α -1-antitrypsin inhibits transferrin uptake in PLC/PRF/5 cells and increases release of hepatitis B virus surface antigen and α -fetoprotein. *Eur J Gastroenterol Hepatol* 1998;10:497–502.
53. Overall CM, Kleifeld O. Tumour microenvironment—opinion: validating matrix metalloproteinases as drug targets and anti-targets for cancer therapy. *Nat Rev Cancer* 2006;6:227–39.
54. Steven FS, Zoeller J, Maier H, Born IA. Inhibition of a cell surface protease after cisplatin chemotherapy. *J Enzym Inhib* 1991;5:151–64.
55. Pollera CF, Ameglio F, Reina S, Nardi M, Abbolito MR, Parracino C. Changes in serum iron levels following very high-dose cisplatin. *Cancer Chemother Pharmacol* 1987;19:257–60.
56. Kitano H. Cancer robustness: tumour tactics. *Nature* 2003;426:125.
57. Hornberg JJ, Bruggeman FJ, Westerhoff HV, Lankelma J. Cancer: a systems biology disease. *BioSystems* 2006;83:81–90.
58. Zender L, Spector MS, Xue W, et al. Identification and validation of oncogenes in liver cancer using an integrative oncogenomic approach. *Cell* 2006;125:1253–67.
59. Lee JW, Soung YH, Kim SY, et al. PIK3CA gene is frequently mutated in breast carcinomas and hepatocellular carcinomas. *Oncogene* 2005;24:1477–80.
60. Altieri DC. Targeted therapy by disabling crossroad signaling networks: the survivin paradigm. *Mol Cancer Ther* 2006;5:478–82.
61. Kitano H. Cancer as a robust system: implications for anticancer therapy. *Nat Rev Cancer* 2004;4:227–35.
62. Di Nicolantonio F, Mercier SJ, Knight LA, et al. Cancer cell adaptation to chemotherapy. *BMC Cancer* 2005;5:78.
63. Keith CT, Borisy AA, Stockwell BR. Multicomponent therapeutics for networked systems. *Nat Rev Drug Discov* 2005;4:71–8.
64. Broxterman HJ, Georgopapadakou NH. Anticancer therapeutics: "Addictive" targets, multi-targeted drugs, new drug combinations. *Drug Resist Updat* 2005;8:183–97.
65. Epstein RJ. Maintenance therapy to suppress micrometastasis: the new challenge for adjuvant cancer treatment. *Clin Cancer Res* 2005;11:5337–41.