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Hepatic stem cells and transforming growth factor β in hepatocellular carcinoma

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Abstract

Hepatocellular carcinoma (HCC) is one of the most common and lethal cancers worldwide. It arises from modulation of multiple genes by mutations, epigenetic regulation, noncoding RNAs and translational modifications of encoded proteins. Although >40% of HCCs are clonal and thought to arise from cancer stem cells (CSCs), the precise identification and mechanisms of CSC formation remain poorly understood. A functional role of transforming growth factor (TGF)- β signalling in liver and intestinal stem cell niches has been demonstrated through mouse genetics. These studies demonstrate that loss of TGF- β signalling yields a phenotype similar to a human CSC disorder, Beckwith–Wiedemann syndrome. Insights into this powerful pathway will be vital for developing new therapeutics in cancer. Current clinical approaches are aimed at establishing novel cancer drugs that target activated pathways when the TGF- β tumour suppressor pathway is lost, and TGF- β itself could potentially be targeted in metastases. Studies delineating key functional pathways in HCC and CSC formation could be important in preventing this disease and could lead to simple treatment strategies; for example, use of vitamin D might be effective when the TGF- β pathway is lost or when *wnt* signalling is activated.

Introduction

Hepatocellular carcinoma (HCC) is the fifth and seventh most common cancer in men and women, respectively, and the third most common cause of cancer-related mortality worldwide.¹ According to WHO projections, the global burden of HCC is expected to rise, and by 2030 it is predicted to account for the second highest increase in cancer-related death rates.² HCV infection is responsible for the increasing incidence of HCC in the USA,³ and HBV infection is the leading cause of HCC worldwide. A WHO estimate suggests that 2 billion people worldwide are infected with HBV and approximately 200 million are infected with HCV. According to the different rates of progression to chronic disease (5–10% for

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HBV and 50–80% for HCV), 350 million people are chronically infected with HBV and 170 million with HCV. About 30–40% of chronically infected patients develop long-term complications, such as cirrhosis and eventually HCC. In the USA, where nearly 4 million people have been infected, HCV is the most common cause of liver disease and HCC, and is the leading indication for liver transplantation.⁴ Preventing infection is potentially the most beneficial strategy because of the high rate of mortality related to late-stage disease. However, although hepatitis vaccine approaches have great promise—particularly for the prevention of HBV—vaccination will not, of course, eliminate HCC. New therapeutic strategies are needed.

Current evidence indicates that during hepatocarcinogenesis, two main pathogenic mechanisms prevail: firstly, cirrhosis associated with hepatic regeneration after tissue damage caused by viral infection, exposure to toxins (for example, alcohol or aflatoxin) or metabolic influences; and secondly, the occurrence of mutations in single or multiple oncogenes or tumour suppressor genes. A number of major pathways are implicated in HCC (Box 1),⁵ although the molecular mechanisms have not been fully elucidated.

Box 1

Pathways in HCC initiation and progression⁴

- RAF–MKK1–MAPK3
- PI3K–AKT1–mTOR
- WNT–catenin β -1
- IGF-I
- Hepatocyte growth factor–c-MET
- Growth-factor-regulated angiogenic signalling

Abbreviations: HCC, hepatocellular carcinoma; IGF-I, insulin-like growth factor I; mTOR, mammalian target of rapamycin.

TGF- β is an important modulator of a broad spectrum of cellular processes, including growth, differentiation, wound repair and apoptosis.^{6,7} The TGF- β pathway might converge with other pathways within the nucleus and stimulate transcription of fibrosis-related genes.^{8–10} Additional evidence suggests that retinoic acid regulates TGF- β in the liver.¹¹ Thus, apoptosis induced by TGF- β could explain the anticarcinogenic action of retinoic acid analogues such as vitamin A, α -carotene and β -carotene in liver cancer. The *TGFB* gene is located on chromosome 19q13. The TGF- β promoter SNP (Cys509Thr) might be associated with HCC risk and outcomes (L. Mishra *et al.*, unpublished data). Studying the effect of genetic variations on the risk of HCC could lead to the identification of patients at high risk of HCC or prediction of HCC prognosis.

The existence of cancer stem cells (CSCs) was first proposed >40 years ago, but they have only been identified in haematological malignancies and in solid tumours of the prostate, breast, brain, colon and liver over the past decade.^{12–16} In liver tissue, the continuous proliferation of stem cells has a vital role in tissue regeneration.^{17–20} However, the occurrence of mutations in these renewing tissues is likely to lead to an expansion of altered stem cells, perpetuation of these mutations, development of further mutations and tumour progression. The fact that around 40% of HCCs are clonal suggests that these tumours originate from stem-cell-like precursors. Understanding the key functional pathways that regulate these CSCs could lead to strategies to suppress oncogenesis.^{21,22} Indeed, some

clonal HCCs could arise not only from stem cells but also from hepatocytes, which have an unusual capacity for uncontrolled proliferation *in vivo* and tend to express genes associated with stem cells.²³ Lineage tracing studies of cells expressing SOX9, FOXL1, GDF-15 (also known as MIC-1), CD133 and EpCAM confirm the presence of adult liver progenitors lining the biliary epithelium, and 4% of those progenitors differentiate into hepatocytes and biliary cells.^{24–27} Lineage conversion of fibroblasts into hepatocytes can occur in cell lines that co-express GATA-4, HNF-3 γ and HNF-1 α , but that do not express CDKN2A.^{21,28–30} Putative CSCs are considered to be characterized by expression of CD133, CD44, EpCAM and ALDH, and are modulated by microRNAs such as miR-181b. This modulation is driven by β -catenin and results in the inhibition of CDX-2 and GATA-6, thereby preventing hepatocyte differentiation.^{31–37} Notably, a functional role for TGF- β signalling in intestinal and liver stem cell niches has been demonstrated. In CSCs, embryonic stem cell proteins, such as Oct-4 and NANOG, combined with activated Toll-like receptor 4 (TLR4) signalling can drive self renewal and lead to HCC in the absence of TGF- β signalling. Suppression of HCC growth can also occur through another TGF- β pathway connected complex—the COP9 signalosome complex subunit 5 (SGN5).³⁸ Through functional genetics studies, key roles of the TGF- β signalling pathway have been elucidated in both suppression of HCC as well as endoderm proliferation; this pathway has dual roles, contributing to tumour suppression and facilitating proliferation from a stem (or progenitor) stage (Box 2, Figure 1).^{6,39}

Box 2

TGF- β signalling in HCC progression and suppression

Tumour suppressive effects

- Inhibits proliferation by increasing tumour suppressing genes (e.g. *p15*, *p21*) and decreasing oncogenes
- Induces differentiation, senescence and apoptosis
- Suppresses mitogens from stromal cells
- Inhibits inflammatory cytokine production from lymphocytes and macrophages
- Suppresses CSCs in liver

Tumour promoter effects

- Promotes growth and migration of HCC cells
- Induces epithelial–mesenchymal transition
- Amends expression of antiapoptotic genes
- Increases evasion of immune surveillance
- Augments microenvironment-modifying proteases and cytokines
- Promotes invasion and angiogenesis by inducing expression of genes such as *MMP2*, *MMP9* and *CTGF*

Abbreviations: CSC, cancer stem cell; HCC, hepatocellular carcinoma.

This Review explores the roles of TGF- β in tumour suppression, progression and other functions. Molecular and genomic studies provide insights into the involvement of this cytokine and its signalling pathways, which can be exploited for diagnostic and therapeutic benefit. Strategies include chemotherapy, genetic manipulations and targeting inflammatory pathways. Understanding the molecular pathogenesis of HCC will provide a framework for

future investigation. We highlight studies crucial for translational work that could effectively alter the course of this lethal cancer.

Role of TGF- β in HCC

Tumour suppressor function of TGF- β

Novel mouse models of HCC with crosstalk between major tumour suppressor pathways (such as p53), TGF- β signalling and protumorigenic pathways (such as the platelet-derived growth factor [PDGF] pathway), should reveal important clues for managing this cancer.^{40–42} Hereditary cancers can also provide key insights into understanding the somatic mutations that are present in sporadic cancers and can help to identify the vital cell signalling pathways involved.^{6,7,12,43,44} Beckwith–Wiedemann syndrome (BWS) is a hereditary condition that is considered to have a stem cell origin and is associated with an 800-fold increased risk of cancers—including HCC. *Sptnb*^{+/-} mice that develop HCC have phenotypic similarities to humans with BWS.⁴² Loss of TGF- β signalling could provide important clues to both BWS and sporadic cancers, such as HCC. Moreover, mouse models of TGF- β signalling could be important models for human HCC. These studies could, therefore, lead to further insights into defects generated by dysfunctional stem cells and identification of new treatment strategies and functional markers for the detection of these lethal cancers that otherwise cannot be detected at an early stage (Figure 2).⁴²

Spectrin and Praja1 in TGF- β signalling

The TGF- β family includes activins and inhibins, as well as bone morphogenetic proteins (BMPs).^{45–47} The TGF- β pathway orchestrates an intricate signalling network that is crucial to determine cell fate, differentiation, proliferation, motility, adhesion and apoptosis, leading not only to tumour suppression but also to progression during late stages of disease. TGF- β binding activates type II (TGFR-2) and then type I (TGFR-1) receptors that in turn activate the eight ubiquitously expressed SMAD homologues (Figure 3).^{48–50} Activation of these proteins results in transcriptional regulation and arrest of the cell cycle at G1–S through suppression of *MYC*, cyclin dependent kinases and induction of *CDKN1A* (also known as *p21*), *CDKN2B* (also known as *p15*) and *Serpine-1* (also known as *PAI-1*).^{45,48–51} Tissue-specific modulation of SMAD homologue function occurs through adaptors (including β -II spectrin, filamin or the zinc finger FYVE domain-containing protein 9 [ZFYVE9]), E3 ligases (such as Smurfs, Nedd1 and Praja1) as well as by functional interactions within multiple signal transduction pathways.^{45,46,52–54} Adaptor proteins, such as β -II spectrin and ZFYVE9, have critical roles in mediating access to the receptors and SMAD homologue activation at the cell membrane, which controls the complex functions of TGF- β .^{53,54} Praja1 might polyubiquitinate SMAD3 and β -II spectrin, which targets them for proteosomal degradation.⁵³ SMAD3 has also been reported to undergo proteosomal degradation by interacting with RBX1–SCF(BTRC) complex in a ligand dependent manner, leading to SMAD3 polyubiquitination.⁵⁵ A number of epithelial cancers from the mesoendoderm are associated with TGF- β –BMP pathway inactivation, which could regulate progenitor cell fate.^{51,56} Molecular profiles of each stage of tumour development, from metaplasia to dysplasia to carcinoma, have not been determined. Studies support a key role for TGF- β signalling in suppressing these tumours.^{6,7} Our research group has found that deletion of β -II spectrin results in sizeable and spontaneous formation of HCCs, with exon 15 mutations found in 11% of these tumours. About 70% of *Sptbn1*^{+/-} and *Sptbn1*^{+/-} *Smad3*^{+/-} mice develop visceromegaly and multiple cancers including HCC, which is a model of BWS and human HCC (Figure 4).^{42,58,59} Our preliminary studies demonstrate that loss of β -II spectrin through ubiquitination by Praja1 could have an important role in the development of liver and gastrointestinal tumours (Figure 3).

Loss of β -II spectrin is associated with faster entry into the S phase of the cell cycle and with HCC formation.^{12,58} These observations led us to further investigate the role of β -II spectrin in cell cycle progression *in vivo*. Gene chip-array analysis revealed markedly raised *Tp53* (also known as *p53*) expression levels. Yet inhibition of *Tp53* did not alter the defects and, paradoxically, a marked decrease in number of tumours was observed in the *Tp53*-*Sptnb1* double heterozygotes.⁵⁹ These findings are supported by the decrease in tumour formation in *TP53* null mice after crossbreeding with *Tgfr2* knockouts that lack TGF- β type 2 receptor,⁶⁰ although the molecular basis of this decrease is not clear.

Exploration of the functional pathways involved in the development of CSCs from normal stem cells is crucial not only for understanding HCC tumour biology but also for developing specific therapies that effectively target these cells in patients. E3 ligases and poly-ADP-ribose polymerases (PARPs) are dramatically overexpressed in HCC with inactivation of TGF- β signalling, making them attractive targets for new therapeutics.⁶¹ These findings have led to the use of small-molecule compounds that target the intracellular and nuclear oncogenic pathways that are specifically and highly activated in the absence of the TGF- β signalling pathway. Such therapeutics are aimed at E3 ligases, PARP inhibition and HDAC inhibition (Figure 2). Several of these agents are now in clinical trials including: E3 ligase inhibitors cis-imidazole⁶² and tryptamine,^{63–65} which target MDM2 (a well-characterized E3 ligase) to inhibit its interactions with p53; a number of PARP inhibitors, including benzamide (in phase III trials for treating glioma and ovarian cancer); and tricyclic indole (in phase II trials for the treatment of breast and ovarian cancer).⁶⁶

TGF- β signalling and DNA repair

TGF- β is an extracellular sensor of damage caused by injury from ultraviolet and ionizing radiation.^{67,68} Keratinocytes cultured from *Tgfb1*^{-/-} mice have marked genomic instability that could accelerate tumour progression.⁶⁹ Studies in *Smad4* conditional knockout mice that develop head and neck cancers have demonstrated a key role for this gene as a guardian of the genome through regulation of the Fanconi anaemia–breast cancer 1 (*Fanc*-*Brca1*) DNA repair pathway.⁷⁰ Interestingly, the development of HCCs in *Sptbn2* heterozygote mutants established β -II spectrin as a functional tumour suppressor.^{12,42,58} Spectrins associate with Fanconi proteins groups G and D, as well as with DNA interstrand crosslinks.^{71,72} In addition, SMADs have been identified as regulators of Fanconi genes.⁷³ β -II spectrin involvement in SMAD3 and SMAD4 localization and their subsequent activation might promote TGF- β tumour suppressor function. These studies together suggest that TGF- β is a potential guardian of genomic stability through modulation of the *Fanc* pathway at interstrand crosslinks, although the precise mechanisms remain to be elucidated.

A well-known mechanism by which the TGF- β pathway induces antiproliferative responses is through arrest of cell cycle progression during G1 by inhibiting c-Myc and CDK1.^{6,7,74,75} Interestingly, β -II spectrin deficiency results in p53-independent, dysfunctional hepatocyte cell cycle progression and delayed liver regeneration 48 h after partial hepatectomy. Additionally, increased levels of p53 (fourfold) and p21 (200-fold) persist and correlate with increased expression levels of the DNA damage markers pChk2 and γ H2A.x.⁵⁹ Inhibition of *TP53* did not correct the defects.⁵⁹ β -II spectrin loss might increase susceptibility to DNA damage, impair cell cycle progression and ultimately lead to HCC, independently of p53. Escape from the suppressive role of TGF- β is observed in many tumours, potentially driving uncontrolled cell proliferation. The precise mechanisms of key adaptors, such as β -II spectrin, in controlling such proliferation remain to be fully elucidated.

Histone acetylation transferases (HATs) and histone deacetylases (HDACs) control the acetylation status of histones and modulate the recruitment of the chromatin remodelling complex to permit or block DNA repair.⁷⁶ In general, targeting HDACs is expected to

increase the expression of genes that are important for apoptosis, cell cycle arrest and differentiation, as well as inhibit the pathways that contribute to DNA damage response. For example, inhibition of survivin and aurora A, cleavage of caspases and increased levels of apoptotic gene products and acetylated-histone H4 are observed in cancer cells treated with HDAC inhibitors.^{77,78} Interestingly, HDAC inhibitors are also known to exert antitumour activity against HCC in preclinical models.^{79,80}

KEAP1–NRF2 pathway and TGF- β signalling

NRF2 is a transcription factor that binds to antioxidant response element (ARE) in the promoter region of target genes to induce their expression. NRF2 induces the expression of many cytoprotective genes, such as those that encode phase II detoxifying and antioxidant enzymes (for example, heme oxygenase-1 [HO-1]), which are critical in protecting cells against toxic or carcinogenic exposure;⁸¹ depletion of NRF2 has been collectively demonstrated to increase chemical-induced carcinogenesis^{82–84} and inflammation-induced tumorigenesis.^{85–87} Low levels of NRF2 and reduced expression of its target gene have been observed in skin and prostate tumours,^{88,89} and loss of NRF2 expression is related to the progression of human HCC.⁹⁰ Kelch-like ECH-associated protein 1 (KEAP1) is a substrate adaptor protein for an E3 ubiquitin ligase complex dependent on CUL-3–RBX1,⁹¹ and the substrate for the KEAP1–CUL3 ubiquitin E3 ligase complex is NRF2, which it polyubiquitinates for proteosomal degradation in order to prevent excess phase II detoxifying and antioxidant enzyme production in the absence of cell stress.^{92–94} Inhibition of NRF2 synthesis by short hairpin RNA or overexpression of KEAP1 increases tumour cell migration and plasticity through upregulated SMAD homologue malignant signalling pathways.⁹⁰ These data, together, suggest that NRF2 has a tumour suppressive function, but can be regulated by KEAP1.

TGF- β induces NRF2-mediated HO-1 synthesis and NRF2–ARE signalling,⁹⁵ and investigators found that TGF- β failed to induce HO-1 expression in NRF2-deficient cells, which demonstrates another tumour suppressive function of this cytokine.⁹⁵ Therefore, preventing KEAP1, and similarly Praja1, from polyubiquitinating target proteins could be important in the development of therapeutic strategies against liver cancers (Figure 3). RTA 402, a novel triterpenoid molecule that is now in human clinical trials, inhibits KEAP1 and Praja1, thereby exerting antitumour activity.⁹⁶

Tumorigenic role of TGF- β in HCC

More than 50,000 publications exist relating to TGF- β , partly because of its multiple and often paradoxical functions. *Tgfb1* mouse knockout studies showed that TGF- β can suppress tumour formation.^{6,7,45} However, persistently high levels of TGF- β promote malignancies and metastases.⁹⁷ Analysis of human patients with HCC further demonstrated that increased expression of TGF- β correlates with a decrease in response to effective therapy for HCC, suggesting that TGF- β could have a role as a prognostic marker in HCC.^{98,99} Levels of TGF- β also correlate with the degree of metastasis; patients with aggressive metastatic HCC have higher expression levels of TGF- β compared with patients who have nonmetastatic HCC.¹⁰⁰ Loss of *Smad4* acts as a switch to convert TGF- β from a tumour suppressor to tumour promoter. Reinstating *Smad4* in *Smad4*^{−/−} cells restores TGF- β tumour suppressor activity.¹⁰¹ TGF- β has protumorigenic activity through facilitation of epithelial to mesenchymal transition by inducing the expression of *SNAIL* transcription and inhibiting E-cadherin, also at the transcriptional level.¹⁰⁰ Epithelial to mesenchymal transition is the process by which epithelial cells lose cell–cell contact and acquire the properties of fibroblasts, inducing cell migration, which is observed in metastases.¹⁰² TGF- β increases migration of SMMC-7721 cells (human HCC cells with low differentiation) by inducing epithelial to mesenchymal transition.¹⁰³ An initial step in HBV-associated HCC progression

is epithelial to mesenchymal transition.¹⁰⁴ Furthermore, hepatitis B virus protein X (HBx) induces TGF- β -driven linker phosphorylation of SMAD3 through the JNK–MAPK pathway, ultimately promoting growth of HCC cells.¹⁰⁵

TGF- β also increases expression of antiapoptotic genes.¹⁰⁶ TGF- β induces apoptosis in HCC cell lines by inhibiting protein kinase CKII (previously known as casein kinase II), thereby stabilizing NF κ B inhibitor- α (I κ B- α) and leading to inhibition of antiapoptotic factor NF α B.¹⁰⁷ TGF- β has also been shown to induce the expression of hepatic microRNAs such as microRNA-181b (miR-181b), which increases matrix metalloproteinase (MMP2 and MMP9) activity, thereby promoting growth, migration and invasion of HCC cells. Consequently, depletion of miR-181b inhibits HCC-cell-mediated tumour growth in a xenograft mouse model.¹⁰⁸ TGF- β also induces the expression of connective tissue growth factor (CTGF), which promotes invasion and angiogenesis.¹⁰⁹ Inhibiting the TGF- β pathway by using TGF- β inhibitor LY2109761 inhibits CTGF production and tumour growth in HCC.¹¹⁰ Despite these interesting advances, the mechanisms responsible for the dichotomic effect of TGF- β have not been fully elucidated (Box 2).

Potential therapeutic targets

Vitamin D and treatment of HCC

Vitamin D has been observed to reduce fibrosis, and patients with cirrhosis often have a deficiency of this vitamin. Proliferation of HCC cells is suppressed by vitamin D, and an analogue (EB 1089) tested in a trial of patients with advanced HCC yielded some promising results, although the studies were terminated because the dose needed to induce a response was toxic.^{111,112} Although the signalling pathways that are affected by vitamin D analogues in dysplastic hepatic lesions or tumours have not yet been identified, vitamin D and synthetic analogues are potent cytostatic or apoptotic agents against most vitamin D3 receptor (VDR)-expressing malignant cells.^{113,114} Vitamin D can suppress β -catenin in colon and breast cancer cells, and loss of TGF- β signalling in HCC stem cells is associated with increased *wnt*/ β -catenin signalling. A potential target for inhibition of HCC by vitamin D, therefore, is the β -catenin pathway.^{115,116} Another promising vitamin D target in HCC is insulin-like growth factor I (IGF-I) signalling. This growth factor is profoundly increased in HCC, and one function of VDR is the transcriptional upregulation of several IGF-binding proteins known to sequester the action of IGF-I, particularly IGF-binding protein 3.^{117,118} TGF- β and IGF-I signalling is found to be dysregulated in type 2 diabetes and cancer,^{119–121} and metformin, a drug used to reduce insulin, has been associated with a decrease in cancer incidence by stimulating the tumour suppressor LKB1.^{122,123} Inhibitors like metformin have potential use for prevention of HCC and require further investigation.

p38–MAPK inhibitors

A number of pathways are activated when TGF- β signalling is lost. One of the most prominent is the p38–MAPK (also known as MAPK 14) signalling pathway. Cirrhosis progresses through a sequence of fibrotic and inflammatory responses and is a major risk factor for HCC. This process activates TLR4 on macrophages and other hepatic cell types, leading to production of inflammatory cytokines and activation of p38–MAPK, both of which contribute to activation of the NF κ B signalling cascade.¹²⁴ Liver fibrosis results from increased sensitivity of hepatic stellate cells to PDGF and TGF- β , leading to increased proliferation of these cells and production of an extracellular matrix of nonhepatic cells through activation of p38–MAPK signalling. In chronic liver disease, therefore, p38–MAPK is an excellent molecular target for inhibition of both fibrotic and inflammatory processes that increase the risk of HCC.¹²⁵ Indeed, in 2007 a selective p38 inhibitor was shown to inhibit cirrhosis in a rodent model,¹²⁶ and several p38–MAPK inhibitors are currently being

tested in clinical trials for treatment of chronic inflammatory diseases, such as rheumatoid arthritis.

Tyrosine kinase inhibition

Few effective HCC therapies exist, whether chemotherapy or biologic agents, and they only have a narrow therapeutic index (only effective in a limited dose range). Translational progress in HCC has been extremely slow and sorafenib is one of the few innovations, which only increases survival by 4 months.¹²⁷ miR122 has been shown to inhibit tumorigenic properties of HCC cells and sensitizes them to sorafenib.¹²⁸ The high morbidity and mortality rates associated with this cancer highlight the urgent need to identify safe and effective cancer prevention strategies for clinical use.

Conclusions

Information from genomic studies can provide strategies to improve HCC management. The strong tumour suppressor role of the TGF- β signalling pathway has already been demonstrated through functional genetics studies in mice that have the TGF- β signalling pathway knocked out. Genome-wide association studies could be important to identify subgroups of patients at high risk of developing HCC, although few have shown true associations. Various strategies are expected to yield innovative and powerful new therapeutic approaches to combat HCC, such as improving early identification and prevention through genomic approaches, expanding experimental therapeutics that target E3 ligases, improving DNA repair mechanisms, and using functional genetics to identify key pathways involved in suppressing and generating HCC.

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Key points

- Hepatocellular carcinoma (HCC) is a common and lethal cancer; its incidence is expected to increase over the next 20 years
- Worldwide, HCC is the fifth most commonly occurring cancer and the third most common cause of cancer-related deaths
- Around 40% of liver cancers are clonal and could arise from cancer stem cells (CSCs)
- Multiple pathways, including the transforming growth factor (TGF)- β tumour suppressor pathway, are involved in the generation of CSCs
- Genetic studies demonstrate the protective role of the TGF- β pathway, and focused research is giving unique insight into new treatment strategies
- Future therapies that target CSCs are likely to be beneficial

Review criteria

A literature search was carried out in PubMed for papers published from 1985 to 2012. The search terms used were “HCC”, “TGF- β ”, “PARP inhibitors” and “p38” inhibitors. Other reference sources included the reference lists of papers selected from the PubMed search, relevant national and international meetings, online materials, and specific searches of reports of colleagues in gastroenterology. The search was restricted to English language papers.

Figure 1.

Effect of loss of TGF- β signalling in the development of hepatocellular carcinoma. Induction of cellular changes through loss of TGF- β , such as SMAD linker phosphorylation and loss of β -II spectrin and SMAD proteins, can lead to development of hepatocellular carcinoma.

Figure 2.

Developing treatment strategies in HCC from bench to bedside. Loss of TGF- β signalling results in HCC. Approaches to identifying appropriate targets and the pathways activated when TGF- β signalling is lost (for example, IL-6–STAT3) have yielded few promising leads for drug development in HCC. Vitamin D, Keap1 inhibitor RTA 402, PARP and HDAC inhibitors are currently in clinical trials. Identification of biomarkers based on genome-wide association studies has been another avenue of research and has revealed molecules that could in future be monitored during the progression of HCC. Abbreviations: GWAS, genome-wide association studies; HCC, hepatocellular carcinoma; HDAC, histone deacetylase; PARP, and poly-ADP-ribose polymerase; SNP, single nucleotide polymorphism; TGF- β , transforming growth factor β ; TGFR-2, TGF- β receptor 2.

Figure 3.

The TGF- β signalling pathway. This pathway is initiated by a TGF- β signal, which activates TGFR-2 that in turn activates TGFR-1. The adaptor protein β -II spectrin is phosphorylated by TGFR-1 and recruits SMAD proteins to the receptor. The tumour suppressor SMAD4, becomes associated with SMAD homologues after they are phosphorylated, and a complex, made up of SMAD3, SMAD4, and phosphorylated β -II spectrin, is created to maintain signalling that fosters the normal growth of cells. The RBX1-SCF(BTRC) complex regulates the proteosomal degradation of SMAD3 by polyubiquitination. Praja1 is responsible for the proteosomal degradation of β -II spectrin. This occurs after specific genes, including *CDKN1A* (also known as *p21*), *CDKN2B* (also known as *p15*), *PAI1*, and *CDH1* have been activated by SMAD complexes. In HCC, as in almost all gastrointestinal cancers, the TGF- β pathway is inactivated. NRF2 induces the expression of cytoprotective genes and is inhibited by KEAP1, which is associated with an increase in tumour cell migration. KEAP1 forms a complex with CUL3-RBX1, which then polyubiquitinates NRF2, thereby targeting it for proteosomal degradation. The novel triterpenoid RTA 402 (which is now in clinical trials for the treatment of HCC) inhibits KEAP1 and Praja1 and thereby exerts its antitumour activity. Abbreviations: HCC, hepatocellular carcinoma; SBE, SMAD binding element; TGF- β , transforming growth factor β ; TGFR, TGF- β receptor.

Figure 4.

TGF- β pathway proteins β -II spectrin and SMAD3 are involved in hepatocarcinogenesis. **a** | Schematic diagram of liver cancer development showing a role for the lack of TGF- β signalling in different stages during hepatocarcinogenesis. **b** | Liver samples from wild-type and **c** | $\beta 2SP^{+/-}$ mice treated with alcohol were stained with Masson trichrome. Significant steatosis (arrowhead) and fibrosis (arrow) are seen in the $\beta 2SP^{+/-}$ mice. **d** | HCC in $\beta 2SP^{+/-}$ and **e** | $\beta 2SP^{+/-}Smad3^{+/-}$ mice. These mice exhibit an increased prevalence of HCC (arrows) and gastrointestinal cancers and share many phenotypic characteristics observed in patients with Beckwith–Wiedemann syndrome, a hereditary cancer stem cell syndrome. Abbreviations: HCC, hepatocellular carcinoma; TGF- β , transforming growth factor β . Part d reproduced with permission from Nature Publishing Group © Katuri, V. *et al. Oncogene* **25**, 1871–1886 (2006).