Introduction

Since its discovery in the early 1980s, TGF-β signaling has been increasingly recognized as a key driver in cancer (1, 2). Unlike its tumor suppressor function in normal tissue, TGF-β activation causes tumor promotion in cancer tissue. The switch from tumor suppression to promotion is not well understood, but intrinsic and extrinsic factors seem to play important roles. The loss of cell polarity, acquisition of motile properties, and a mesenchymal phenotype during epithelial–mesenchymal transition (EMT) are considered crucial intrinsic changes of the tumor cells (3). These tumor promotion changes mediated by TGF-β signaling are also accompanied by extrinsic factors originating from the tumor microenvironment, such as angiogenesis, inflammation, and fibroblast activation. In addition to changes in the tumor tissue, alterations in the TGF-β signaling pathway can also contribute to tumor growth. The TGF-β signaling occurs via a canonical and a noncanonical pathway. The canonical TGF-β signaling pathway is activated when one of the three ligands (TGF-β1, TGF-β2, TGF-β3) binds to the TGF-β receptor I (TGF-βRI), heterodimerizes with the TGF-β-receptor II (TGF-βRII or ALK5), and transphosphorylates the kinase domain of both receptors. This phosphorylation step leads to a recruitment and phosphorylation of SMAD2 and SMAD3. After this phosphorylation, a SMAD signaling cascade is initiated and it results in nuclear translocation and gene transcription for a wide range of tumor-promoting mediators (4). In some tumors (including hepatocellular carcinoma), SMAD4 is mutated, which alters the TGF-β signaling pathway, rendering the pathway inactive. The less-known noncanonical activation pathway is associated with several intracellular phosphorylation of proteins, such as jun N-terminal kinase (JNK), p38 MAPK, ERK, or MEKK. For example, the TGF-β inhibitor LY2109761, a surrogate compound of LY2157299 monohydrate in hepatocellular carcinoma. Preliminary results from a phase II clinical trial have shown improved clinical outcome and also changes consistent with a reduction of EMT. Cancer Res; 74(7): 1890–4. ©2014 AACR.
orchestrates the homeostasis of microenvironment components and tumor cells by balancing the activity of inflammatory cells or fibroblast with tumor cell growth or progression. For example, in treatment-resistant viral hepatitis, TGF-β and IL-10 secretions are increased and associated with increased T regulatory cells; these have a local immunosuppressive effect and consequently contribute to a persistent viral infection. With continued inflammation of the liver, reversible and irreversible remodeling are often observed, including fibrosis and early cirrhosis (6). This remodeling is characterized by accumulation of extracellular matrix (ECM) proteins. The rich ECM deposition is driven in large part by activation of TGF-β; if not stopped, this leads to cirrhosis and eventually to liver failure. The accumulation of ECM proteins leading to fibrosis is still a reversible step. This phase would be particularly important for therapeutic interventions aimed at blocking the progression of liver disease, thus preventing hepatocellular carcinoma occurrence. Unfortunately, such therapies are not yet available. As cirrhotic process progresses inside the liver, dysplastic nodules with initial chromosomal changes are often observed. These dysplastic nodules can progress to the early stages of hepatocellular carcinoma. In the liver tissue, cancer cells grow embedded in an environment enriched with ECM and mediated by TGF-β and connective tissue growth factor (CTGF; refs. 7, 8). This tight interaction between the tumor and the surrounding cirrhotic liver is perhaps the most remarkable hallmark of hepatocellular carcinoma (Fig. 1).

**TGF-β Signaling in Hepatocellular Carcinoma**

A seminal gene expression study by Coulouarn and colleagues demonstrated in a mouse model and later also in human tissue (9), that there are two different TGF-β...
signaling responses, one called an "early" and another termed "late" TGF-β signaling response. Intriguingly, the early response pattern is associated with longer and the late response pattern with shorter survival. It is possible that the "early response" pattern reflects the physiologic inflammatory response, while the "late response" pattern is associated with a long-term TGF-β activation similar to the one described in colorectal cancer (10). The role of TGF-β is also recognized in a molecular classification of hepatocellular carcinoma, where the Wnt signaling pathway is regulated by TGF-β. In one subgroup of hepatocellular carcinoma tumors, TGF-β signaling appears to control tumor cell growth; in a second subgroup, it is associated with larger tumors, low α-fetoprotein (AFP) expression and poor prognosis (11). The identification of such a subgroup may represent a first attempt to identify patients with hepatocellular carcinoma likely to respond to TGF-β signaling inhibition. Because of the differences between transcriptome-based studies, a review of such data is being conducted to obtain a common classification and to better understand the pathophysiologic pathways driving hepatocellular carcinoma. Upon review of these transcriptome-based reports, TGF-β signaling may be associated with expression of AFP and EpCAM (12). On the basis of this concomitant expression, TGF-β signaling may be present in 25% of all early hepatocellular carcinoma. It is important to remember that transcriptome-based evaluations are derived from surgical specimen after local resections and are not from advanced hepatocellular carcinoma. This has implications when interpreting the effect of TGF-β signaling in later stages of hepatocellular carcinoma or blocking this pathway with pharmacologic agents in more advanced hepatocellular carcinoma stages.

The extrinsic effect of TGF-β signaling is a result of tumor cell growth embedded in an ECM-enriched environment. This condition is characterized by secretion of TGF-β and other associated factors, such as CTGF, which affects cancer-associated fibroblasts (7, 8). In addition to activation of fibroblasts, TGF-β signaling has been associated with T regulatory cells, for example by activation of chemokines (such as CCL22) or by immune presentation of AFP (13, 14). More recently, TGF-β signaling has also been associated with tumor-initiating cells (15). Ongoing stimulation of liver progenitor cells by TGF-β leads to their transformation into tumor-initiating cells as shown by TGF-β-induced changes in CD90 and CD133 expression (15).

The intrinsic effects of TGF-β signaling are mostly observed in highly invasive tumor conditions. TGF-β signaling is associated with loss of cell polarity, acquisition of cellular motility, and increased tumor invasion (5, 16–22). A main change in tumor cells is related to EMT as measured by E-cadherin expression, a marker of EMT. In the presence of TGF-β, E-cadherin is shed from tumor cells and renders tumor cells more invasive and migratory. In association with other components of the tissue microenvironment such as laminin-5 (Ln-5) or CD44, TGF-β induces the transcription factor Snail, another component of EMT; this induction is also associated with poor prognosis (19, 23). Taken together, it appears that TGF-β signaling causes EMT and renders tumors more invasive.

Barriers for Developing TGF-β Inhibitors in Hepatocellular Carcinoma

One of the main challenges for developing an effective therapy against hepatocellular carcinoma is related to the underlying cirrhosis, which is not only associated with a reduced liver function, but also provides the support for tumor cell growth. Because of this unique microenvironment, new drugs should not only treat the tumor cells, but also prevent new tumoral lesions. Hence, the ideal drug should have a low toxicity profile, antitumor activity, and chemopreventive activity. Such a drug may have activity in later lines of treatment, but also in the adjuvant setting together with other ablative therapies.

For example, multikinase inhibitors have toxicity profiles that limit their use to later lines of treatment. Sunitinib was discontinued because of serious adverse events; other antiangiogenic kinase inhibitors carried a high risk of bleeding in patients with compromised coagulation. With the exception of sorafenib that has been used in conjunction with transarterial chemoembolization (24), few kinase inhibitors have been evaluated in adjuvant treatment regimens primarily because of their toxicity profile.

The antitumor activity of the ideal drug candidate would also have to show a relatively immediate antitumor effect. Sorafenib blocks multiple signaling pathways and has not only an antiangiogenic effect, but also induces tumor cell apoptosis (25). However, several attempts to develop similarly active agents have thus far failed.

Finally, the ideal drug candidate would need to block processes associated with liver cirrhosis. By targeting this microenvironment signal, it is possible to inhibit the stimulus for tumor cell growth and thus prevent new tumor lesion. This aspect of drug development may have been overlooked during recent years; only recently have agents demonstrated this activity.

Unlike previously investigated agents, the inhibition of TGF-β signaling affects hepatocellular carcinoma in different stages of development and progression and may offer a wider therapeutic application. This is due to the presence of TGF-β signaling throughout the development of hepatocellular carcinoma. However, a modulator of microenvironment may not have an immediate antitumor effect as observed for chemotherapeutic treatments. Rather, the impact on the molecular and cellular tumor biology may require longer treatment times to "restore" the host tissue to a more physiologic phenotype.

Blocking TGF-β in Hepatocellular Carcinoma Using a Specific TGF-β Inhibitor

Given the hypothesized activity of TGF-β signaling in hepatocellular carcinoma, small-molecule inhibitors targeting the TGF-βRII serine/threonine kinase have been developed, including LY2157299 monohydrate (LY2157299; ref. 16). LY2157299 is now in clinical evaluation and has shown antitumor effects in patients with glioblastoma and hepatocellular carcinoma (26). Studying the antitumor activity in vitro and in vivo remains a challenge for LY2157299. Hence, LY2157299 and the surrogate compound LY2109761 have been used in some select in vitro
LY2157299 blocks directly, LY2157299 blocks VEGF production with better biologic growth and progression by inhibiting neoangiogenesis. Indi-
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reaction. This reduces tumor growth in experimental models CTGF production with the consequent reduction of stromal
E-cadherin tissue expression and reducing the secretion of E-
cadherin in the supernatant. LY2157299 seems to also block the
CTGF production with the consequent reduction of stromal
reaction. This reduces tumor growth in experimental models and will have a beneficial impact on the cirrhotic process
(17, 20). LY2157299 also reduces hepatocellular carcinoma growth and progression by inhibiting neoangiogenesis. Indirect-
ly, LY2157299 blocks VEGF production with better biologic activity than the antiangiogenic drug bevacizumab. Moreover,
LY2157299 blocks β1-integrin activation in cancer cells and consequently blocks intravasation of hepatocellular carcinoma
cells into the blood vessels (18, 21).

Overall, the encouraging antitumor activities observed with LY2157299 formed the rationale for investigating LY2157299 in a phase II clinical trial of patients who either failed previous sorafenib treatment or were ineligible to receive sorafenib (NCT01246986, http://clinicaltrials.gov). On the basis of the
previous observations that high AFP levels correlate with high TGF-β1 levels (27), a study in patients with elevated AFP was
initiated to investigate the activity of a TGF-β inhibitor in patients with hepatocellular carcinoma, where EMT is a key
driver. In these patients, LY2157299 reduced not only TGF-β1, but also E-cadherin in plasma of patients (28). This reduction is
consistent with the in vitro studies, where LY2157299 also reduced the secretion of E-cadherin while inhibiting the
TGF-β signaling (17). So far, no other drug treatment has been
associated with changes in plasma TGF-β1 and E-cadherin levels. This is remarkable because biomarker responses are
rarely observed in hepatocellular carcinoma treatments. In approximately 23% of patients treated with LY2157299, serum
AFP levels were reduced by more than 20% compared with baseline. These patients had a benefit assessed by time-to-
tumor progression (18.6 weeks) and also by overall survival (93.1 weeks) compared with patients who had no AFP
responses (29). Although AFP is a tumor marker of hepatocellular carcinoma, the responses in patients with elevated AFP
may perhaps mean more than just an overall tumor response. Currently, the relationship of AFP to E-cadherin is being
investigated to better understand the significance of AFP responses.

The use of LY2157299 to block TGF-β signaling appears to modulate EMT and has a clinically meaningful benefit to
patients. The measurement of E-cadherin and TGF-β1 levels may also open new opportunities in novel drug development
approaches; ongoing clinical studies will determine whether LY2157299 will provide a new treatment option for patients.
Meanwhile, it is gratifying that some of the in vitro observations have resulted in clinical observations.

Disclosure of Potential Conflicts of Interest
M. Lahm is a medical fellow and has ownership interest (including patents) in Eli Lilly and Company. No potential conflicts of interest were disclosed by the other authors.

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Transforming Growth Factor-β as a Therapeutic Target in Hepatocellular Carcinoma

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