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Role of desmoplasia in cholangiocarcinoma and hepatocellular carcinoma

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COMMENTARY ON:

Hepatic myofibroblasts promote the progression of human cholangiocarcinoma through activation of epidermal growth factor receptor. Clapéron A, Mergey M, Aoudjehane L, Ho-Bouldoires TH, Wendum D, Prignon A, Merabtene F, Firrincieli D, Desbois-Mouthon C, Scatton O, Conti F, Housset C, Fouassier L. Hepatology. 2013 Dec;58(6):2001–2011. Copyright © 2013. Reprinted by permission from the American Association for the Study of Liver Diseases.

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Abstract: *Intrahepatic cholangiocarcinoma (CCA) is characterized by an abundant desmoplastic environment. Poor prognosis of CCA has been associated with the presence of alpha-smooth muscle actin (α-SMA)-positive myofibroblasts (MFs) in the stroma and with the sustained activation of the epidermal growth factor receptor (EGFR) in tumor cells. Among EGFR ligands, heparin-binding epidermal growth factor (HB-EGF) has emerged as a paracrine factor that contributes to intercellular communications between MFs and tumor cells in several cancers. This study was designed to test whether hepatic MFs contributed to CCA progression through EGFR signaling. The interplay between CCA cells and hepatic MFs was examined first in vivo, using subcutaneous xenografts into immunocompromised mice. In these experiments, cotransplantation of CCA cells with human liver myofibroblasts (HLMFs) increased tumor incidence, size, and metastatic dissemination of tumors. These effects were abolished by gefitinib, an EGFR tyrosine kinase inhibitor. Immunohistochemical analyses of human CCA tissues showed that stromal MFs expressed HB-EGF, whereas EGFR was detected in cancer cells. In vitro, HLMFs produced HB-EGF and their conditioned media induced EGFR activation and promoted disruption of adherens junctions, migratory and invasive properties in CCA cells. These effects were abolished in the presence of gefitinib or HB-EGF-neutralizing antibody. We also showed that CCA cells produced transforming growth factor beta 1, which, in turn, induced HB-EGF expression in HLMFs.*

CONCLUSION: *A reciprocal cross-talk between CCA cells and myofibroblasts through the HB-EGF/EGFR axis contributes to CCA progression.*

Cholangiocarcinoma (CCA) and hepatocellular carcinoma (HCC) are the two most common primary liver cancers worldwide [1] and are malignancies that arise from epithelial cells that share an early common developmental program. The risk of developing CCA has been linked to liver flukes and primary sclerosing cholangitis (PSC), although most cases are thought to be sporadic with chronic inflammation as a likely risk factor [2]. Cirrhosis is the primary risk factor for HCC, and results from a chronic inflammatory fibrotic milieu. This 'microenvironment' can be the consequence of diverse diseases including viral hepatitis, chronic alcoholism, metabolic syndrome, and inherited genetic mutations. Rather than occurring in a background of cirrhosis, CCA is often associated with desmoplasia, the deposition of fibrotic or connective tissue, which is frequently regarded as a response to malignant cholangiocytes. However, carcinogenesis research has illustrated the importance of the stroma or tumor microenvironment in tumor progression resulting in re-evaluation of impact of the "desmoplastic response" associated with CCA.

In other solid tumors (e.g., pancreatic, prostrate or breast carcinomas), the term "desmoplastic response" refers to the abnormal activity of an epithelial neoplasia, wherein the tumor stimulates stromal fibroblasts to produce and deposit copious amounts of collagens and extracellular matrix (ECM), which surround the tumor. The desmoplastic stroma adjacent to these cancers commonly includes activated alpha smooth muscle actin (αSMA)-positive fibroblasts, which produce fibrillar collagens, fibronectins, proteoglycans, and tenascin C, in collaboration with other liver cell types. Thus formation of stroma in CCA appears as a secondary insult in response to malignant growth. Conversely, in hepatocellular carcinogenesis, fibrosis and cirrhosis are thought to generate a microenvironment that promotes tumorigenesis. Therapeutic implications for causal *vs.* consequential role(s) of fibrous tissue and collagenous stroma in liver tumors are obvious. Surgical resection offers the only curative therapy for either CCA or HCC, however few patients are cured of CCA

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Abbreviations: CCA, Cholangiocarcinoma; HCC, Hepatocellular carcinoma; CAF, cancer-associated fibroblasts; HSCs, hepatic stellate cells; PSC, primary sclerosing cholangitis; αSMA, smooth muscle alpha actin; ECM, extracellular matrix; TKI, tyrosine kinase inhibitor; EGF, epidermal growth factor; EGFR, EGF receptor; HB-EGF, heparin-binding growth factor.

or HCC because of late detection of the tumor or high surgical risk. Hence primary research goals for either liver malignancy are to identify risk factors that enable earlier detection, and define the molecular and cellular programs involved in their respective pathogenesis to develop effective treatments.

The contribution of the stromal microenvironment to liver carcinogenesis has been more fully appreciated in the last decade (Fig. 1). In HCC molecular profiling studies from tumor and adjacent cirrhotic liver indicate that the gene signature of the adjacent non-tumor tissue contains critical molecular information on recurrence and prognosis [3], indicating that molecular prognostic determinants depend on both tumor and non-tumor tissue. Analogous studies with CCA and adjacent liver have not yet been performed, although CCA-genetic signatures have been identified [4,5]. Recent studies in CCA development support the notion that cancer-associated fibroblasts (CAFs) promote tumor progression [6]. While many different cell types influence the tumor microenvironment, CAFs are fundamentally important by virtue of their ability to overproduce ECM proteins and alter the cellular architecture [7]. Yet the precise mechanisms by which CAFs facilitate tumor progression and its molecular evolution are not well understood. In particular, determining the cellular origin(s) of CAFs and whether CAFs have different functions when they participate in the desmoplastic response in CCA compared to cirrhosis and subsequent HCCs will facilitate novel therapeutic approaches. PSC may offer a unique vantage point for CAF-pathogenesis studies. The sequential pathogenesis in PSC, an autoimmune disease, begins with inflammation, bile duct collapse and subsequent concentric fibrosis. As CAFs are present in advanced PSC, they may be a consequence of inflammation and BEC cell death [8]. Recent cell fate tracing studies indicate that HSCs are the primary fibroblastic cell type in fibrosis that give rise to myofibroblasts regardless of mouse injury or surgical models used [9]. Extension of cell fate mapping studies to animal models of CCA or HCC with desmoplastic or fibrotic pathogenesis will be a next logical step.

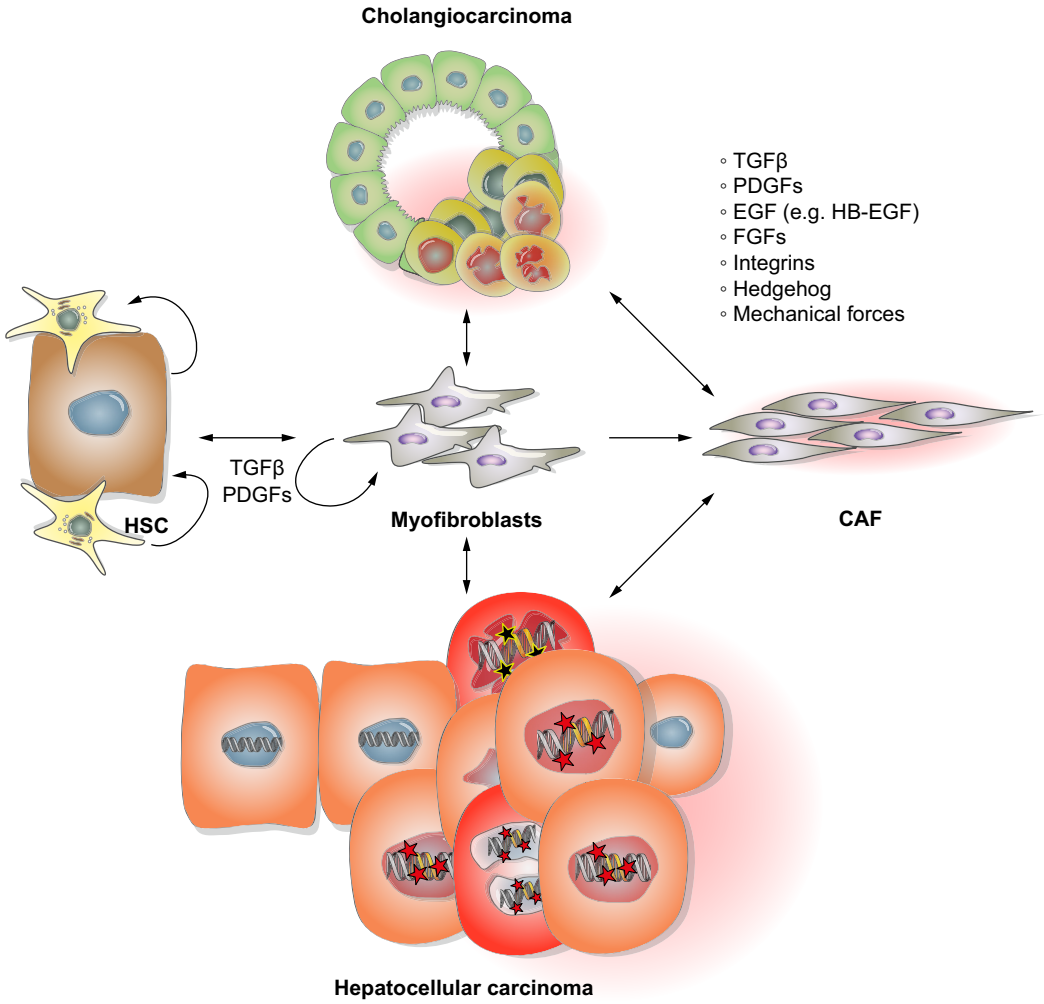

Fig. 1. Possible signaling interactions between myofibroblasts (MFB) and cancer-associated fibroblasts (CAF), and with cholangiocarcinoma (CCA) and hepatocellular carcinoma (HCC). A hypothetical continuum is shown for the evolution of hepatic stellate cells (HSC) and myofibroblasts to CAF. TGFβ and PDGF ligands are primary cytokines that activate quiescent HSCs, and perpetuate MFB and CAF phenotypes. Multiple paracrine signaling pathways exist between CCA and HCC and CAFs, which propagate CAF phenotype and promote tumorigenesis.

A recent study furthers our mechanistic understanding of the contribution of CAFs through the use of an admixture xenographic mouse model of CCA [10]. Clapéron and co-workers used a combination of *in vivo* and *in vitro* approaches to show that human hepatic myofibroblasts promote the growth of human CCAs. Human intrahepatic fibroblasts were isolated from livers from patients undergoing resection for metastases or benign tumors, allowed to expand *in vitro* (termed human liver myofibroblasts, HLMF) and then co-injected with human CCA cell lines into immune-compromised mice. HLMFs stimulated CCA growth *in vivo* and Gefitinib, a tyrosine kinase inhibitor (TKI) specific for EGFR1, blunted xenograph growth illustrating that EGFR signaling pathways contribute to CCA progression in this model. The authors further demonstrated that heparin-binding EGF (HB-EGF), one of the seven EGF ligands, was produced by HLMF and promoted CCA growth.

The role of EGFR in carcinogenesis is well established [11,12], yet challenges remain in developing selective therapies [13]. Genetic alterations in the EGFR ligand-receptor family have been identified in CCA, including amplifications, deletions, and point mutations [4,5] with *ErbB1* being the primary focus of prior investigations. The study by Clapéron and co-workers indicates yet another mechanism by which EGFR pathways appears to contribute to tumor progression, a mechanism whereby paracrine signaling is perturbed. As with any ligand-receptor family, subtle changes in cellular patterns of ligand or receptor expression manifest in exaggerated cell growth or migration. In the Clapéron study, HB-EGF expression was elevated in CCA specimens, in the xenographic tumors, and in cultures of HLMF implicating CAFs as a potential source of this EGF ligand. *In vitro* studies demonstrate that HB-EGF binds to ErbB4/ErbB2 receptor heterodimers in addition to ErbB1/ErbB2 dimers, albeit with a lower affinity [14] expanding possible EGFR pathways that could contribute to CCA progression.

The admixture xenographic CCA model described by Clapéron has a number of experimental advantages and therapeutic entrées. It would be interesting to create a variety of cell mixtures using different sources of human CAFs/HLMFs isolated from different etiologies or uninjured liver (e.g., quiescent HSCs). Will CCA cell lines evoke a more dramatic 'desmoplastic response' from freshly isolated HSCs *in vivo*? Evidence from gene profiling studies of isolated HSCs in human [15] and mice [16] suggest that plastic-activation of HSCs does not fully recapitulate their *in vivo* activation profiles. Thus use of '*in vivo* activated MFBs' will likely produce different results than plastic-activated MFBs. It would be also be interesting to determine whether CAFs execute their tumor-promoting effect when their numbers were less than CCA tumor cells, which may tease apart autocrine or juxtacrine signaling mechanisms from paracrine mechanisms. Therapeutically, CCA admixture xenographs could be used for testing combination therapies with cytostatic TKIs and targeted monoclonal antibodies to the various EFGR receptor isoforms.

As with any study using preclinical models, there are limitations. In particular biliary architecture impacts the development of CCA, and intrahepatic *vs.* extrahepatic CCA origin may be important to delineate treatment [6,17]. A particular challenge in CCA models is mimicking the *in vivo* topographical variation observed in this malignancy. The microenvironmental mechanical forces resulting from excess collagen and ECM deposition continually and reciprocally alter MFB and epithelial cell phenotypes. How can the dynamically changing cellular architecture and mechanical forces be modeled *in vivo* [7]? Nevertheless, the role of CAFs in CCA and HCC progression will offer great insight into the pathogenesis of these tumors.

Conflict of interest

The authors declared that they do not have anything to disclose regarding funding or conflict of interest with respect to this manuscript.

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