Molecular Pathogenesis of Cholangiocarcinoma

Sumera Rizvi Gregory J. Gores

Division of Gastroenterology and Hepatology, Mayo Clinic, Rochester, Minn., USA

Key Words

Cancer-associated fibroblast · Cholangiocarcinoma · Inflammation · Tumor microenvironment

Abstract

It has become increasingly apparent of late that inflammation plays an integral role in a spectrum of malignancies including cholangiocarcinoma (CCA). Primary sclerosing cholangitis with chronic inflammation is the most common risk factor for CCA in the Western world. Recent work has highlighted that inflammatory pathways are essential in carcinogenesis and tissue invasion and migration. Inflammation advances carcinogenesis by induction of DNA damage, evasion of apoptosis, promotion of cell proliferation, and neoangiogenesis. CCA is characterized by the presence of a desmoplastic stroma consisting of cancer-associated fibroblasts, tumor-associated macrophages, and tumor-infiltrating lymphocytes. This rich inflammatory milieu is vital to the cancer ecosystem, and targeting its components represents an attractive therapeutic option.

© 2014 S. Karger AG, Basel

Inflammation and Biliary Tract Carcinogenesis

In 1863, Virchow generated the hypothesis that there was a link between inflammation and cancer [1]. Indeed, he stated that ‘lymphoreticular infiltration’ of cancer re-

flected the origin of the cancer at sites of inflammation [1]. We now know that cholangiocarcinoma (CCA) is a prototype of cancers associated with inflammation. However, the association between inflammation and malignancy is not unique to CCA in the gastrointestinal tract. For example, chronic esophagitis results in the development of the metaplastic epithelium characteristic of Barrett’s esophagus, a premalignant lesion for adenocarcinoma of the esophagus [2]; chronic pancreatitis is a risk factor for developing pancreatic cancer [3]; chronic gastritis from *Helicobacter pylori* is a well-established risk factor for adenocarcinoma of the stomach [4], and ulcerative colitis of the colon places patients at high risk for the development of colon cancer [5]. Thus, chronic inflammation in the gastrointestinal tract can certainly predispose to the development of adenocarcinoma. Several modern studies have now resurfaced and demonstrated the relationship between inflammation and biliary tract cancer [6–11]. Primary sclerosing cholangitis (PSC) with chronic inflammation of the biliary tree is the most common predisposing condition for CCA in the Western world. Patients with PSC are at extremely high risk for developing this devastating malignancy with a lifetime risk of approximately 5–10% [12].

Recent work by Llovet and colleagues [13] utilized integrative molecular analysis of intrahepatic CCA (iCCA) to examine the pathogenesis of this disease. Two biological classes of iCCA were identified: the inflam-

Fig. 1. Role of inflammatory cells in cancer. Inflammatory cells are associated with oxidative stress which can lead to genetic mutations. They also generate cytokines which foster survival by evasion of apoptosis and increase in cell proliferation. Soluble factors, such as VEGF, produced by inflammatory cells promote angiogenesis. Inflammatory cells also play a role in tissue remodeling, migration, and invasion.

mation class is characterized by activation of inflammatory pathways and the proliferation class is characterized by activation of oncogenic signaling pathways such as mitogen-activated protein kinase and KRAS. Thus, their molecular analysis highlighted the relationship between inflammation and the development of CCA. The genes involved in the inflammation subtype are often related to cytokines including interleukin (IL)-6. These cancers had chromosomal instability and were reasonably well-differentiated.

The relationship between inflammation and biliary tract cancer can be viewed as an inverse of the concept introduced in the seminal review by Hanahan and Weinberg [14] in 2000. They described the following six essential alterations or hallmarks of cancer: self-sufficiency in growth signals, insensitivity to anti-growth signals, evasion of apoptosis, limitless replicative potential, sustained angiogenesis, and tissue invasion and metastasis [14]. One could look at the inverse of this and propose that inflammatory cells are responsible for all of these cancer hallmarks. Inflammatory cells are associated with oxidative stress which can lead to genetic mutations, produce soluble factors such as vascular endothelial growth factor (VEGF) which can promote angiogenesis, and generate cytokines which can aid in evasion of apoptosis and promotion of cell proliferation (fig. 1) [6, 7, 15]. Inflammation is paramount in tissue remodeling and it is not surprising that inflammatory cells play a major role in tissue migration and invasion.

Inflammation-Associated DNA Damage in PSC and CCA

Several years ago, we investigated the relationship between inducible nitric oxide synthase (iNOS) expression, PSC, and CCA [6]. iNOS is activated by inflammatory cytokines and generates nitric oxide, leading to nitrosative stress [6]. We demonstrated that iNOS is expressed in PSC and CCA but not in normal biliary epithelium [6]. Expression of iNOS by CCA infers that it continues to express this inflammatory gene product to enhance cancer progression. Oxidative and nitrosative stress can induce DNA damage by producing oxidative DNA lesions and inhibition of DNA repair enzymes. Indeed, we were able to show that there was an increase in 8-oxo-deoxyguanosine, the most abundant oxidative DNA lesion, in PSC and CCA compared to normal tissue [16]. In additional studies, we demonstrated that nitric oxide inhibited DNA base repair resulting in accumulation of 8-oxo-deoxyguanosine [16]. Thus, this is a great example of the relationship between oxidative stress and DNA damage promoting carcinogenesis.

Another mechanism by which the oxidative milieu may promote carcinogenesis is through the generation of oxysterols. Oxysterols are cholesterol oxidation products which have been identified in human bile [17]. An increase in oxysterols in bile from patients with biliary tract inflammation was demonstrated by Haigh and Lee [18] several years ago. Oxysterols can activate the hedgehog signaling pathway, which has been implicated in a variety

of gastrointestinal cancers [19–21]. Recent experimental data has demonstrated that oxysterols are endogenous ligands for the extracellular domain of smoothened, a key molecule in hedgehog signaling [22]. The smoothened inhibitor, vismodegib, decreased cancer progression in an animal model of biliary tract cancer, further supporting the role of hedgehog signaling in cholangiocarcinogenesis [44].

Epigenetic Alterations in CCA

The epigenetic alterations in biliary tract cancer have recently been reviewed by Andersen and Thorgeirsson [23]. They highlighted that several epigenetic changes occur in CCA by promoter hypermethylation, a mechanism of gene silencing. P16, a tumor suppressor gene, is frequently silenced in CCA [23]. Several point mutations occurring in the promoter region of p16 have been identified in patients with PSC-associated CCA [24].

More recently, further information has been obtained linking oncogenes to methylation changes in the human genome. Isocitrate dehydrogenase (IDH)1 and IDH2 are metabolic enzymes, and mutations in genes encoding *IDH1* and *IDH2* have been demonstrated in 10–23% of CCA patients in several recent studies [25–27]. *IDH1* and *IDH2* mutations have been associated with epigenetic changes resulting in hypermethylation of several different genes [26]. These data are quite intriguing as they suggest a potential target for the treatment of biliary tract cancer. Mutant *IDH1* and *IDH2* result in overproduction of 2-hydroxyglutarate, which has potential as a biomarker for these mutations [28]. One can envision an era of identifying the genetic mutation, demonstrating an increase in 2-hydroxyglutarate in the tissue or bile, and using this as a biomarker to monitor therapy. Specific targeted inhibitors of IDH mutations have been developed and tested in animal models where they promote tumor differentiation and inhibit growth [29, 30]. This would be one strategy for the treatment of these biliary tract cancers.

Cytokine-Induced Inhibition of Cell Death

One of the key cytokines generated in inflammation is IL-6, which is elevated in the serum of patients with biliary tract cancer [31]. Enhanced IL-6 expression has been demonstrated in the tumor stroma of patients with CCA [32]. We now have models implicating IL-6 in carcino-

genesis of breast and lung tissue [33]. IL-6 inhibits cell death by activating the transcription factor signal transducer and activator of transcription 3 (STAT3), which in turn can upregulate survival factors such as myeloid cell leukemia sequence 1 (Mcl-1) and Bcl-x_L [7, 34]. Suppressor of cytokine signaling 3 (SOCS3), an endogenous feedback inhibitor of IL-6, is epigenetically silenced via methylation of its promoter in CCA [35]. Treatment with demethylating agents restored IL-6 induction of SOCS3 [35]. This gives rise to the interesting notion that the use of demethylating agents is one way to inhibit the procarcinogenic effects of IL-6.

Recent data have highlighted a critical role for NOTCH signaling in the formation of biliary tract cancers [36]. The NOTCH signaling pathway plays an essential role in biliary tract development. Indeed, patients with mutations of the NOTCH endogenous ligand, jagged 1, develop Alagille syndrome [37]. NOTCH expression is enhanced in PSC and in patients with CCA, and can be induced by iNOS [38].

Experimental Model Demonstrating the Relationship between Biliary Tract Inflammation and Carcinogenesis

We have recently developed an interesting animal model of biliary tract cancer by introducing the oncogenes myristoylated AKT and Yes-associated protein (YAPS127A) into the biliary epithelium using a transposon system. Cancer development does not occur unless biliary tract inflammation is promoted via systemic administration of the cytokine IL-33.

Cancer-Associated Fibroblasts and Growth Factor Receptor Signaling

Kalluri and Zeisberg [39] highlighted the role of cancer-associated fibroblast in carcinogenesis in 2006. A variety of ligands are generated by cancer-associated fibroblasts including hepatocyte growth factor, VEGF, and platelet-derived growth factors [40]. Intriguing data now suggests that many human CCAs have targetable fibroblast growth factor receptor (FGFR) gene fusion products [41, 42]. These are fusion products with FGFR2, and have been described not only in CCA but also in breast cancer, thyroid cancer, and prostate cancer [41]. These fusion genes can be identified by fluorescent in-situ hybridization probes, thereby providing an approach to identify

Fig. 2. Therapeutic deletion of cancer-associated fibroblasts (CAFs) in CCA. In the course of tumorigenesis, stromal fibroblasts acquire a modified phenotype and become activated or primed for

apoptosis. Navitoclax, a BH3 mimetic, causes apoptotic cell death in activated CAFs [43]. This targeted deletion of CAFs leads to secondary cancer cell apoptosis and reduction in tumor size.

them at the cellular level [42]. This exciting development awaits further knowledge in regards to the incidence, prevalence, and response to therapy in cancers with these fusion products.

Finally, we want to note that cancer-associated fibroblasts are primed for apoptosis, and can be targeted with BH3 mimetics such as navitoclax [43]. In animal models, induction of apoptosis in cancer-associated fibroblasts results in loss of tumor growth and enhanced animal survival (fig. 2) [43].

In summary, cancer can be viewed as an ecosystem with a rich tumor stroma characterized by an inflammatory milieu. This stroma consists of tumor-associated macrophages, antigen-presenting cells, tumor-infiltrating lymphocytes, cancer-associated fibroblasts, and neo-angiogenesis. Like any ecosystem which depends upon a

broad network for support, targeting the supporting structures can lead to tumor inhibition.

Acknowledgements

The authors would like to thank Ms. Courtney Hoover for excellent secretarial support. This work was supported by National Institutes of Health grants DK59427 (G.J.G.) and T32 DK007198 (S.R.), and the Mayo Foundation.

Disclosure Statement

The authors have no conflicts of interest to disclose.

References

- Balkwill F, Mantovani A: Inflammation and cancer: back to Virchow? *Lancet* 2001;357:539–545.
- Spechler SJ: Barrett esophagus and risk of esophageal cancer: a clinical review. *JAMA* 2013;310:627–636.
- Yadav D, Lowenfels AB: The epidemiology of pancreatitis and pancreatic cancer. *Gastroenterology* 2013;144:1252–1261.
- Wilson KT, Crabtree JE: Immunology of *Helicobacter pylori*: insights into the failure of the immune response and perspectives on vaccine studies. *Gastroenterology* 2007;133:288–308.
- Ullman TA, Itzkowitz SH: Intestinal inflammation and cancer. *Gastroenterology* 2011;140:1807–1816.
- Jaiswal M, LaRusso NF, Burgart LJ, Gores GJ: Inflammatory cytokines induce DNA damage and inhibit DNA repair in cholangiocarcinoma cells by a nitric oxide-dependent mechanism. *Cancer Res* 2000;60:184–190.
- Kobayashi S, Werneburg NW, Bronk SF, Kaufmann SH, Gores GJ: Interleukin-6 contributes to Mcl-1 up-regulation and trail resistance via an Akt-signaling pathway in cholangiocarcinoma cells. *Gastroenterology* 2005;128:2054–2065.
- Yoon JH, Gwak GY, Lee HS, Bronk SF, Werneburg NW, Gores GJ: Enhanced epidermal growth factor receptor activation in human cholangiocarcinoma cells. *J Hepatol* 2004;41:808–814.
- Lai GH, Radaeva S, Nakamura T, Sirica AE: Unique epithelial cell production of hepatocyte growth factor/scatter factor by putative precancerous intestinal metaplasias and associated 'intestinal-type' biliary cancer chemically induced in rat liver. *Hepatology* 2000;31:1257–1265.
- Yoon JH, Higuchi H, Werneburg NW, Kaufmann SH, Gores GJ: Bile acids induce cyclooxygenase-2 expression via the epidermal growth factor receptor in a human cholangiocarcinoma cell line. *Gastroenterology* 2002;122:985–993.
- Yoon JH, Canbay AE, Werneburg NW, Lee SP, Gores GJ: Oxysterols induce cyclooxygenase-2 expression in cholangiocytes: implications for biliary tract carcinogenesis. *Hepatology* 2004;39:732–738.
- Razumilava N, Gores GJ: Classification, diagnosis, and management of cholangiocarcinoma. *Clin Gastroenterol Hepatol* 2013;11:13–21.e11; quiz e13–e14.
- Sia D, Hoshida Y, Villanueva A, Roayaie S, Ferrer J, Tabak B, Peix J, Sole M, Tovar V, Alsinet C, Cornella H, Klotzle B, Fan JB, Cotsoglou C, Thung SN, Fuster J, Waxman S, Garcia-Valdecasas JC, Bruix J, Schwartz ME, Beroukhi R, Mazzaferro V, Llovet JM: Integrative molecular analysis of intrahepatic cholangiocarcinoma reveals two classes that have different outcomes. *Gastroenterology* 2013;144:829–840.
- Hanahan D, Weinberg RA: The hallmarks of cancer. *Cell* 2000;100:57–70.
- Sia D, Tovar V, Moeini A, Llovet JM: Intrahepatic cholangiocarcinoma: pathogenesis and rationale for molecular therapies. *Oncogene* 2013;32:4861–4870.
- Jaiswal M, LaRusso NF, Shapiro RA, Billiar TR, Gores GJ: Nitric oxide-mediated inhibition of DNA repair potentiates oxidative DNA damage in cholangiocytes. *Gastroenterology* 2001;120:190–199.
- Kuvar R: Mechanisms of oxysterol-induced disease: insights from the biliary system. *Clin Lipidol* 2012;7:537–548.
- Haigh WG, Lee SP: Identification of oxysterols in human bile and pigment gallstones. *Gastroenterology* 2001;121:118–123.
- Nachtergaele S, Mydock LK, Krishnan K, Rammohan J, Schlesinger PH, Covey DF, Rohatgi R: Oxysterols are allosteric activators of the oncoprotein smoothened. *Nat Chem Biol* 2012;8:211–220.
- Corcoran RB, Scott MP: Oxysterols stimulate sonic hedgehog signal transduction and proliferation of medulloblastoma cells. *Proc Natl Acad Sci USA* 2006;103:8408–8413.
- Dwyer JR, Sever N, Carlson M, Nelson SF, Beachy PA, Parhami F: Oxysterols are novel activators of the hedgehog signaling pathway in pluripotent mesenchymal cells. *J Biol Chem* 2007;282:8959–8968.
- Nedelcu D, Liu J, Xu Y, Jao C, Salic A: Oxysterol binding to the extracellular domain of smoothened in hedgehog signaling. *Nat Chem Biol* 2013;9:557–564.
- Andersen JB, Thorgerirsson SS: Genetic profiling of intrahepatic cholangiocarcinoma. *Curr Opin Gastroenterol* 2012;28:266–272.
- Ahrendt SA, Eisenberger CF, Yip L, Rashid A, Chow JT, Pitt HA, Sidransky D: Chromosome 9p21 loss and p16 inactivation in primary sclerosing cholangitis-associated cholangiocarcinoma. *J Surg Res* 1999;84:88–93.
- Kipp BR, Voss JS, Kerr SE, Barr Fritcher EG, Graham RP, Zhang L, Highsmith WE, Zhang J, Roberts LR, Gores GJ, Halling KC: Isocitrate dehydrogenase 1 and 2 mutations in cholangiocarcinoma. *Hum Pathol* 2012;43:1552–1558.
- Wang P, Dong Q, Zhang C, Kuan PF, Liu Y, Jeck WR, Andersen JB, Jiang W, Savich GL, Tan TX, Auman JT, Hoskins JM, Misher AD, Moser CD, Yourstone SM, Kim JW, Cibulskis K, Getz G, Hunt HV, Thorgerirsson SS, Roberts LR, Ye D, Guan KL, Xiong Y, Qin LX, Chiang DY: Mutations in isocitrate dehydrogenase 1 and 2 occur frequently in intrahepatic cholangiocarcinomas and share hypermethylation targets with glioblastomas. *Oncogene* 2013;32:3091–3100.
- Borger DR, Tanabe KK, Fan KC, Lopez HU, Fantin VR, Straley KS, Schenkein DP, Hezel AF, Ancukiewicz M, Liebman HM, Kwak EL, Clark JW, Ryan DP, Deshpande V, Dias-Santagata D, Ellisen LW, Zhu AX, Iafrate AJ: Frequent mutation of isocitrate dehydrogenase (IDH)1 and IDH2 in cholangiocarcinoma identified through broad-based tumor genotyping. *Oncologist* 2012;17:72–79.
- Reitman ZJ, Parsons DW, Yan H: IDH1 and IDH2: Not your typical oncogenes. *Cancer Cell* 2010;17:215–216.
- Rohle D, Popovici-Muller J, Palaskas N, Turcan S, Grommes C, Campos C, Tsoi J, Clark O, Oldrini B, Komisopoulou E, Kunii K, Pedraza A, Schalm S, Silverman L, Miller A, Wang F, Yang H, Chen Y, Kernysky A, Rosenblum MK, Liu W, Biller SA, Su SM, Brennan CW, Chan TA, Graeber TG, Yen KE, Mellinghoff IK: An inhibitor of mutant IDH1 delays growth and promotes differentiation of glioma cells. *Science* 2013;340:626–630.
- Wang F, Travins J, DeLaBarre B, Penard-Lacronique V, Schalm S, Hansen E, Straley K, Kernysky A, Liu W, Gliser C, Yang H, Gross S, Artin E, Saada V, Mylonas E, Quivoron C, Popovici-Muller J, Saunders JO, Salituro FG, Yan S, Murray S, Wei W, Gao Y, Dang L, Dorsch M, Agresta S, Schenkein DP, Biller SA, Su SM, de Botton S, Yen KE: Targeted inhibition of mutant IDH2 in leukemia cells induces cellular differentiation. *Science* 2013;340:622–626.
- Cheon YK, Cho YD, Moon JH, Jang JY, Kim YS, Lee MS, Lee JS, Shim CS: Diagnostic utility of interleukin-6 (IL-6) for primary bile duct cancer and changes in serum IL-6 levels following photodynamic therapy. *Am J Gastroenterol* 2007;102:2164–2170.
- Andersen JB, Spee B, Blechacz BR, Avital I, Komuta M, Barbour A, Conner EA, Gillen MC, Roskams T, Roberts LR, Factor VM, Thorgerirsson SS: Genomic and genetic characterization of cholangiocarcinoma identifies therapeutic targets for tyrosine kinase inhibitors. *Gastroenterology* 2012;142:1021–1031.e1015.
- Schafer ZT, Brugge JS: IL-6 involvement in epithelial cancers. *J Clin Invest* 2007;117:3660–3663.
- Bollrath J, Phesse TJ, von Burstin VA, Putoczki T, Bennecke M, Bateman T, Nebelsiek T, Lundgren-May T, Canli O, Schwitalla S, Matthews V, Schmid RM, Kirchner T, Arkan MC, Ernst M, Greten FR: Gp130-mediated STAT3 activation in enterocytes regulates cell survival and cell-cycle progression during colitis-associated tumorigenesis. *Cancer Cell* 2009;15:91–102.
- Isomoto H, Mott JL, Kobayashi S, Werneburg NW, Bronk SF, Haan S, Gores GJ: Sustained IL-6/STAT-3 signaling in cholangiocarcinoma cells due to SOCS-3 epigenetic silencing. *Gastroenterology* 2007;132:384–396.

- 36 Zender S, Nickeleit I, Wuestefeld T, Sorensen I, Dauch D, Bozko P, El-Khatib M, Geffers R, Bektas H, Manns MP, Gossler A, Wilkens L, Plentz R, Zender L, Malek NP: A critical role for NOTCH signaling in the formation of cholangiocellular carcinomas. *Cancer Cell* 2013;23:784–795.
- 37 Hofmann JJ, Zovein AC, Koh H, Radtke F, Weinmaster G, Iruela-Arispe ML: Jagged1 in the portal vein mesenchyme regulates intrahepatic bile duct development: Insights into Alagille syndrome. *Development* 2010;137:4061–4072.
- 38 Ishimura N, Bronk SF, Gores GJ: Inducible nitric oxide synthase up-regulates NOTCH-1 in mouse cholangiocytes: implications for carcinogenesis. *Gastroenterology* 2005;128:1354–1368.
- 39 Kalluri R, Zeisberg M: Fibroblasts in cancer. *Nature Reviews Cancer* 2006;6:392–401.
- 40 Sirica AE: The role of cancer-associated myofibroblasts in intrahepatic cholangiocarcinoma. *Nat Rev Gastroenterol Hepatol* 2012;9:44–54.
- 41 Wu YM, Su F, Kalyana-Sundaram S, Khazanov N, Ateeq B, Cao X, Lonigro RJ, Vats P, Wang R, Lin SF, Cheng AJ, Kunju LP, Siddiqui J, Tomlins SA, Wyngaard P, Sadis S, Roychowdhury S, Hussain MH, Feng FY, Zaluski MM, Talpaz M, Pienta KJ, Rhodes DR, Robinson DR, Chinnaiyan AM: Identification of targetable FGFR gene fusions in diverse cancers. *Cancer Discov* 2013;3:636–647.
- 42 Arai Y, Totoki Y, Hosoda F, Shiota T, Hama N, Nakamura H, Ojima H, Furuta K, Shimada K, Okusaka T, Kosuge A, Shibata T: FGFR2 tyrosine kinase fusions define a unique molecular subtype of cholangiocarcinoma. *Hepatology* 2013, Epub ahead of print.
- 43 Mertens JC, Fingas CD, Christensen JD, Smoot RL, Bronk SF, Werneburg NW, Gustafson MP, Dietz AB, Roberts LR, Sirica AE, Gores GJ: Therapeutic effects of deleting cancer-associated fibroblasts in cholangiocarcinoma. *Cancer Res* 2013;73:897–907.
- 44 Razumilava N, Gradilone SA, Smoot RL, Mertens JC, Bronk SF, Sirica AE, Gores GJ: Non-canonical Hedgehog signaling contributes to chemotaxis in cholangiocarcinoma. *J Hepatol* 2014;60:599–605.