Molecular Pathogenesis of Cholangiocarcinoma

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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.

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 reflected 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-
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-oxodeoxyguanosine [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 by promoter hypermethylation, a mechanism identified in patients with PSC-associated CCA 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 carcinogenesis 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-xL [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 oncoprotein 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...
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.

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Disclosure Statement

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