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Mini-review

Multiple cellular origins and molecular evolution of intrahepatic cholangiocarcinoma

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ABSTRACT

Intrahepatic cholangiocarcinoma (ICC) is an aggressive malignancy associated with unfavorable prognosis and for which no effective treatments are available. Its molecular pathogenesis is poorly understood. Genome-wide sequencing and high-throughput technologies have provided critical insights into the molecular basis of ICC while sparking a heated debate on the cellular origin. Cancer exhibits variabilities in origin, progression and cell biology. Recent evidence suggests that ICC has multiple cellular origins, including differentiated hepatocytes; intrahepatic biliary epithelial cells (IBECs)/cholangiocytes; pluripotent stem cells, such as hepatic stem/progenitor cells (HPCs) and biliary tree stem/progenitor cells (BTSCs); and peribiliary gland (PBG). However, both somatic mutagenesis and epigenomic features are highly cell type-specific. Multiple cellular origins may have profoundly different genomic landscapes and key signaling pathways, driving phenotypic variation and thereby posing significant challenges to personalized medicine in terms of achieving the optimal drug response and patient outcome. Considering this information, we have summarized the latest experimental evidence and relevant literature to provide an up-to-date view of the cellular origin of ICC, which will contribute to establishment of a hierarchical model of carcinogenesis and allow for improvement of the anatomical-based classification of ICC. These new insights have important implications for both the diagnosis and treatment of ICC patients.

Introduction

The current classification of cholangiocarcinoma (CCA) distinguishes between the intrahepatic (ICC) and extrahepatic (ECC) types, and ECC may be further divided into perihilar (also called Klatskin tumor) and distal anatomical subtypes according to the World Health Organization (WHO) and Union for International Cancer Control (UICC) classifications [1]. ICC is defined as CCA located proximally in the second-order bile ducts (proximal and distal refer to the direction of bile flow such that the intrahepatic bile ducts are proximal to the common bile duct) [2].

ICC is an extraordinarily heterogeneous malignant disease in many aspects, including its epidemiology, risk factors, morphology, pathology (including molecular pathology), modalities of growth and clinical features. Several diverse risk factors are associated with ICC, including primary sclerosing cholangitis (PSC), liver fluke infection, hepatolithiasis, and chronic hepatitis/cirrhosis related to infection with hepatitis B or C virus (HBV or HCV, respectively); however, the pathogenetic mechanisms associated with these risk factors have not yet been elucidated [3]. Geographic variations in the incidence of CCA are related to differences in risk factors.

Several classifications have been described for ICC on the basis of anatomy, the extent of disease, gross morphology, histopathology, biology and the postulated cellular origin, which may be helpful for its accurate classification. Although these classifications provide important information, they are not entirely perfect, and it is therefore necessary to further refine the classification of this disease. Tumor cell fate and pathology are strongly influenced by cellular origins; activation of the same genetic/epigenetic mutation in a different cellular compartment of a given organ may have profound implications with regard to the malignant potential [4]. Changes in oncogene and inflammatory signaling pathways, as well as genetic and epigenetic alterations and chromosome aberrations, have been shown to contribute to the development of ICC.

ICC exhibits aggressive metastatic behavior, and surgical prognosis remains poor [5]. No specific CCA markers have been identified; however, elevated CA19-9 and CEA levels are usually considered to support the diagnosis in association with clinical, radiological, and endoscopic findings [6]. A recent study has shown that N-cadherin expression is significantly increased in ICC compared with ECC and other malignancies. Its specificity for the diagnosis of ICC is 88% and reaches 98% if combined with CK7 [7].

The complexity of the cellular origins and molecular mechanisms underlying the diverse growth patterns of this malignancy

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remain of clinical concern [8]. The heterogeneity of the tumor microenvironment further increases this complexity, as the continuously changing environmental influences affect which cancer cell subpopulations are able to survive, proliferate, spread and resist therapy. These issues must be addressed in future research.

Classifications of ICC based on macroscopy, histopathology and biology

Several classifications have been proposed to aid researchers and hepato-biliary surgeons in better understanding ICC from different perspectives. The Liver Cancer Study Group of Japan has classified ICC macroscopically into the mass-forming (MF), periductal-infiltrating (PI), intraductal growth (IG) and mixed mass-forming and periductal-infiltrating (MF+PI) types [9]. ICC arising in the small bile ducts or bile ductule is characterized by the presence of a gross MF-type tumor with or without involvement of the small bile ducts, whereas ICC arising within the large second-order intrahepatic bile ducts can be of the PI, IG or mixed subtype [10].

The majority of ICCs (over 95%) are well-to-moderately differentiated adenocarcinomas with varying degrees of desmoplasia [11]. Komuta et al. have categorized ICC as muc-ICC and mixed-ICC based on the histological characteristics [12]. The former arises from columnar mucin-producing cholangiocytes lining the large bile ducts and the latter arises from cuboidal non-mucin-producing cholangiocytes lining the small bile ducts or the canal of Hering (coH). Indeed, large bile duct muc-ICC shares similarities with ECC in terms of its clinicopathological and immunohistochemical characteristics and gene expression profiles. In contrast, the small bile duct type (peripheral) or mixed-ICC has features in common with ductular type cholangiocellular carcinoma (CLC) and CK19-HCC.

Using integrative genomic analysis, researchers have identified two main biological types of ICC among 119 cases: the inflammation (38%) and proliferation types (62%) [13,14]. The inflammation type is characterized by activation of STAT3 and overexpression of CK, and the proliferation type is characterized by activation of RAS, MAPK, and c-MET and mutations in KRAS and BRAF. Furthermore, the proliferation type has several features indicative of poor prognosis and shortened survival.

All of the above-mentioned classification methods are not entirely perfect, and the clinical utility of classifications based on cell type has not yet been established. However, the classification of ICC according to cellular origin has also been supported by emerging data revealing differences in pathological and radiological findings [10,15,16].

Heterogeneity of ICC

Heterogeneity of epidemiological and risk factors

A slight male preponderance and possible differences among races in ICC are generally acknowledged. Moreover, a clear relative difference between the incidences in Eastern and Western countries has been well established [17]. More than 80% of ICC cases occur in Asia and sub-Saharan Africa, and 55% occur in mainland China [18,19]. The incidence of ICC is also high in Thailand (33.4 per 100,000 in men and 12.3 per 100,000 in women, with important differences within the country itself), and a clear association between infection with *Opisthorchis viverrini* and the development of ICC has been demonstrated in these regions [20].

Risk factors and underlying causes of ICC include chronic inflammatory biliary diseases, infectious diseases, congenital conditions, and cirrhosis. Asbestos may also be a hidden factor contributing to the increased incidence of ICC according to case-control analysis [21]. In addition, exposure to organic solvents is a potential occupational risk factor [22].

While ICC usually develops in patients with an apparently normal liver, it sometimes arises in those with past histories of biliary or hepatic diseases of various etiologies. Chronic inflammation of the bile ducts, resulting in sustained stress on biliary epithelial cells, has been reported to be at least partly responsible for cholangiocarcinogenesis, with the tendency of ICC to proliferate and spread along the affected intrahepatic bile ducts [23]. Infection with a liver fluke, particularly *Opisthorchis viverrini* and *Clonorchis sinensis*, is also a risk factor for ICC. The presence of parasites in the biliary tree leads to a chronic inflammatory response and cellular proliferation of the bile duct epithelium (adenomatous hyperplasia), resulting in an increased ICC risk [24].

PSC is a common risk factor for ICC in Western countries. Hepatolithiasis, which is fairly common in the Far East, is the primary independent risk factor for ICC; approximately 7% of hepatolithiasis patients eventually develop this disease [25]. Two case-control studies concerning risk factors for ICC in China have concluded that HBV infection is strong risk factor for the development of this disease [26,27]. Such ICCs are usually of a smaller, MF-type when clinically detectable [10]. In Japan, patients with cirrhosis due to HCV have an approximately 1000-fold higher risk of developing ICC than the general population [25].

The substantial heterogeneities of etiology and incidence suggest that ICC has different mechanisms and unique biological characteristics. We suggest that an ICC classification based on cellular origin is more in line with current knowledge regarding epidemiological and risk factors and that such a classification would resolve the controversies associated with the current anatomical-based classification of ICC.

Heterogeneity of clinical features and tumor microenvironment

R0 curative resection is one of the strongest prognostic factors affecting the long-term outcomes of ICC patients. However, only 10% to 20% of patients present with early-stage disease that is amenable to curative surgery [28]; in other words, at the time of diagnosis, approximately 70% of ICC patients have occult metastasis or advanced local disease that precludes curative resection, and the 5-year survival rate is 0–10% [29,30]. The reported 5-year postsurgical survival rate ranges from 20% to 35% [31]. The overall median survival time after surgery is 30 months, with a recurrence rate of up to 60% [32]. Compared with ECC, ICC is associated with a higher probability of achieving R0 resection, but it is more frequently associated with microvascular invasion [33]. Furthermore, the prognosis varies among individuals with similar clinical manifestations and surgical options.

The clinical features and biological behaviors of ICC differ among the different types. The MF+PI-type is significantly associated with jaundice, bile duct invasion, portal vein invasion, lymph node involvement and positive surgical margins, and patients appear to have a more unfavorable prognosis, even after R0, than those with other types of ICC [34]. The lymph node invasion rate of the IG-type is lower than those of the other types (IG: 0%; MF: 16%; MF+PI: 50%; and PI: 66%) [35]. Researchers have compared the morphological features and protein profiles of the peripheral and hilar CCA types, which differ in the incidence of perineural invasion, differentiation grades and presence of satellite nodules. Hilar CCAs are smaller in size and exhibit more extensive perineural invasion than peripheral types. Regarding protein expression, the hilar types more often exhibit increased expression of MUC5AC, Akt2, CK8 and annexin II [36]. Interestingly, VEGFA expression is upregulated in peripheral compared with hilar CCA in association with increased vascular density, and this finding has been confirmed using quantitative protein array technology. Collectively, these results suggest that anti-angiogenic therapies would be potentially beneficial for the treatment of peripheral CCA.