Current Insights on Cholangiocarcinoma Research: a Brief Review

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Abstract

Cholangiocarcinoma (CCA) is a progressively fatal disease which generally occurs due to malignant transformation of hepatic biliary cholangiocytes. The incidence of CCA has been increasing worldwide and there is an urgent requirement for effective diagnosis and treatment strategies against this devastating disease. Different factors including liver-fluke infestation, viral hepatitis, exogenous nitrosamine-mediated DNA damage, and chronic inflammation have been linked to CCA genesis. However, the risk factors and underlying complex mechanisms leading to development of CCA are not sufficiently understood to devise an effective targeted treatment therapy. In this review, we summarize currently known epidemiological and pathological aspects of the disease and briefly describe various potential biomarkers and experimental anticancer phytochemicals related to CCA research. In addition, we also sum up recent findings that link chronic inflammation of hepatic biliary cholangiocytes with CCA. The collective information concisely presented in this article would provide useful insights into the current understanding of this cancer.

Keywords: Cholangiocarcinoma - biomarkers - Opisthorchis viverrini - inflammation

Introduction

Cholangiocarcinoma (CCA) basically refers to the cancer arising from malignant transformation of cholangiocytes, the epithelial cells lining the hepatic biliary tree. The CCA accounts for almost 15% of all hepatobiliary malignancies and are difficult to diagnose with dismal prognosis. The disease is progressively fatal and less than 5% of patients suffering from advanced stages of CCA survive for up to five years (Oh et al., 2005; Ustundag and Bayraktar, 2008). CCA is lethal unless the malignancy is diagnosed at its early stages and surgically removed. However, lack of single effective early diagnostic procedure and complex nature of the disease presents a great challenge for timely detection and treatment of CCA (Blechacz et al., 2011). Recent years have seen a surge in research related to the CCA-associated inflammation and immunological aspects of the disease to identify and correlate specific host immune response against CCA (Razumilava and Gores, 2012). In addition, the increasing incidents of CCA and absence of proper prophylaxis strategy has led to an urgent demand for identification and development of biomarkers that might hold clinical potential for timely diagnosis of the disease (Ruys et al., 2013).

In this review, we briefly discuss about the epidemiological and immunological aspects of CCA and the disease-associated pathogens. Moreover, we also summarize current trends in biomarker and anticancer research associated with diagnosis and potential therapeutic intervention of this life-threatening disease.

Epidemiology and Classification of Cholangiocarcinoma

CCA is comparatively rare form of carcinoma and constitutes less than 1% of overall human malignancies. Nonetheless, the disease is reportedly a leading form of liver-associated cancer after hepatocellular carcinoma (HCC) in several continents including parts of Europe and America (Parkin et al., 1993; Ustundag and Bayraktar, 2008). In USA alone, the incidence of CCA is 1-2 cases per 100,000 which equates to 3,500 new cases each year (Anderson et al., 2004). In disease incidence is also high in parts of Asia including northeastern Thailand and Laos (Sripa et al., 2009). Separate study indicates that the affected CCA cases are relatively more in males on their 60s-70s as compared to the females of similar age group (Shaib and El-Sерag, 2004). However, it is questionable that weather observed prevalence of CCA is merely due to actual increase in CCA incidence or better diagnostic options available today. The CCA can be anatomically categorized as perihilar CCA (pCCA), distal CCA, and intrahepatic CCA (iCCA) depending upon the site of malignancy (Marrero, 2014). The pCCAs can arise from anywhere between the second-order biliary ducts and...
Cholangiocarcinoma and Inflammation

Chronic inflammation has been attributed to initiate, assist or induce tumor growth in several diseases which in turn can presumably result in cancer (Takeuchi and Akira, 2010). Aberrant production of interleukin (IL)-6, a pro-inflammatory cytokine, has been correlated with the predisposition of chronic biliary tract inflammation to CCA (Johnson et al., 2012). Although the actual mechanism is barely understood, it seemingly implicates the role of IL-6 for promoter methylation and expression of growth regulator genes that potentially contribute to carcinogenesis (Webhe et al., 2006). In particular, the involvement of IL-6 in tumor progression has been associated with modulation of expression of selected microRNAs which are responsible for post-transcriptional regulation of messenger RNA (mRNA) involved in synthesis of DNA methyltransferase, a crucial enzyme required for DNA methylation (Fabbri et al., 2007; Braconi et al., 2010). Moreover, IL-6 was attributed to promote survival of malignant cholangiocyte xenografts via an autocrine-paracrine mechanism in athymic mice (Braconi et al., 2010). Evidences from few investigation also suggest that during certain helminthic infection, the host immune system can exhibit IL-6 and metastasis-associated protein 1 (MAP-1)-regulated chronic inflammatory response that may contribute to development of CCA (Sripa et al., 2009; Nair et al., 2011). Likely, in parts of Thailand where CCA incidence is more frequent, the IL-6 receptor polymorphism has been frequently observed in Opisthorchiasis-linked CAA patients (Prayong et al., 2014).

The Nuclear factor kappa-B (NF-kB) is a major transcription factor associated with a wide range of cellular responses including inflammation, cell survival, and proliferation (Takeuchi and Akira, 2010). Results from in vitro studies conducted in CCA cell line suggested that diethyldithiocarbamate, a major metabolite of disulfiram, suppressed the metastatic activity of the cancer cells via inhibiting NF-κB pathway (Srikoon et al., 2013). Since this transcription factor is also an essential regulator of gene responsible for encoding pro-inflammatory cytokines such as IL-6, IL-12, and MAP-1, the anti-inflammatory drugs targeting NF-κB signaling pathway might hold wide-range therapeutic potential for CCA research (Karim, 2006). Activation-induced cytidine deaminase (AID) belongs to the DNA/RNA editing enzyme family and can be induced by pro-inflammatory cytokines in cholangiocytes. The aberrant production of AID has been previously reported to assist development of CCA via its mutagenic activity (Komori et al., 2008). In addition, studies on CCA-associated patients with a history of chronic inflammation were reported to exhibit abnormally high levels of AID along with germinal center-associated nuclear protein that might be related to DNA damage potentially leading to hepatobiliary malignancy (Chan-On et al., 2009). Over expression of inducible nitric oxide synthase due to prolonged inflammatory response and subsequent inhibition of DNA repair mechanism by its product, nitric oxide, has been reported to promote or assist CCA progression (Jaiswal et al., 2000). In addition, the CCA-associated tumor cells were reported to exhibit aberrant levels of 8-nitroguanine, usually formed as a result of nitrative DNA damage, along with increased formation of DNA lesions (Thanan et al., 2013). Thus, it clearly indicates the critical role played by inflammatory immune response that can potentially lead to CCA.

Pathogens Associated with Cholangiocarcinoma

Epidemiological and experimental evidences suggest that some helminthic, bacterial and viral pathogen may act as carcinogenic agents in the development of CCA. In particular, patients suffering from infestation of certain types of liver flukes namely, Opisthorchis viverrini and Clonorchis sinensis are presumably more vulnerable to CCA (Boonyanugomol et al., 2012). The O. viverrini infestation is prevalent in parts of Thailand, Cambodia, and Laos correlating with the high incidents of CCA in these countries as compared to other parts of the world (Parkin, 2006; Sripa and Pairojkul, 2008). These parasites survive within bile duct and are known to produce multiple excretory proteins including superoxide dismutase, venom allergen-like proteins, and granulin-like growth factor that can potentially contribute to malignant transformation of cholangiocytes (Young et al., 2014). Likely, the gastrointestinal tract-associated bacterium Helicobacter pylori is considered as a significant risk factor in development of CCA (Kawainshi et al. 2006; Matsumoto et al., 2007; Xiao et al., 2014). It has been suggested that the cag pathogenicity island found in H. pylori which were previously known to be involved in pathogenesis of gastric epithelial cells have now been linked to the development of biliary carcinoma (Matsumoto et al.,
Biomarkers in Cholangiocarcinoma

Patients with early stages of CCA are often asymptomatic or display non-specific symptoms such as cachexia, abdominal pain, and fatigue (Blechacz et al., 2011). Patients are often diagnosed with CCA only during the advanced stages of carcinoma where surgical procedures become inapplicable for curing the disease (Sriwanitchrak et al., 2011). Absence of effective early diagnostic techniques and limited therapeutic options make it difficult to prevent CCA-associated mortalities. Thus, there is an urgent need for identification of biomarkers to assist effective diagnose of CCA during early stage of the disease. A few serum-derived biomarkers including carcinoembryonic antigen and CA19-9 have shown some promising signs for its application in timely diagnosis of CCA (Patel et al., 2000; Marrero, 2014). However, the markers suffer from low sensitivity and cannot discriminate between malignant and benign form of biliary cholangitis (Sriwanitchrak et al., 2011). In particular, CA19-9 is suggested to have sensitivity of up to 80% in diagnosing perihilar CCA and distal CCA (Tao et al., 2010). Moreover, in patients suffering from PSC, a combined approach involving measurement of serum CA19-9 level and cytopathological studies of hepatic tissues were used as a strong predictive test for CCA development (Barr et al., 2013). Likely, the cytopathological techniques such as fluorescence in situ hybridization (FISH) in patients with PSC has identified polysomy in chromosome 3 and 7 as a significant risk-factor for increased predisposition to iCCA. Currently, FISH assay are being recommended to PSC patients as a predicting test to access their genetic susceptibility towards CCA genesis (Navaneethan et al., 2013). The cytokeratins (CK) represents an essential group of proteins that contribute to cytoskeleton of various cell types. Over 20 different types of CKs have been identified and CK-19, specifically expressed in bile duct epithelia, is known to be associated with CCA (Stroescu et al., 2006). The CK-19 is considered as a biomarker specific to iCCA that can be used to distinguish intrahepatic cholangiocarcinoma from hepatocellular carcinoma (Tao et al., 2010; Marrero, 2014). In addition, high levels of CK-19 were also detected as a prominent sign of liver fluke-related CCA (Thanan et al., 2013). However, for clinical application, this biomarker requires further validation and currently there exists no precise detection protocol to identify and correlate aberrant levels of CK-19 during early stages of iCCA. Separate studies on inflammation-mediated CCA involving stem/progenitor cell markers (CD133 and Oct3/4) suggested that the aberrant expression of CD133 and/or Oct3/4 correlates with increased formation of DNA lesions in CCA patients (Thanan et al., 2013). Further studies of these proteins in relation to CCA might hold diagnostic potentials for identification and development of biomarkers based on immunological approach. The hepatoma-derived growth factor (HDGF) is a mobility promoting growth factor and is known to be implicated in promoting metastatic activities of several types of tumor. Recently, the HDGF profiling of CCA-associated patients suggested that HDGF might hold diagnostic potentials as a non-invasive biomarker for detecting extrahepatic CCA and also appears to be a suitable prognostic factor after curative resection of extrahepatic CCA (Han et al., 2013). Likely, multiple signaling proteins and associated factors such as facin, Akt, TROP2, and HSP27 are currently being considered for their potential diagnostic use in CCA research (Ruys et al., 2013). In addition to intracellular molecules, study of bile for its chemical composition and quantitative evaluation of proteins including SSP411, pancreatic esterases and amylases have also been investigated for identification of potential biomarkers with promising results (Alvaro 2009; Shen et al., 2012). List of few selected biomarkers used in some CCA-associated research is provided in this article (Table 1). Hence, identification and establishment of a highly sensitive and robust early diagnostic procedure based on such biomarkers would definitely be a major positive step in CCA research.

Anticancer Drug Research on Cholangiocarcinoma

Plant-derived chemicals have been used throughout the history of medicine for development of various anticancer and antiinflammatory drugs. In this context, multiple researches have been conducted to identify phytochemicals with potential anticancer properties against CCA. Experiments utilizing in-vitro and nude mouse CCA-xenograft models have suggested that purified curcumin from Curcuma longa Linn., crude ethanolic extract from Zingiber officinale Roscoe, and Atractylodes lancea (thung) possess significant antitumor activity against CCA (Plengsuriyakarn et al., 2012 a & b). In particular, the anticancer effects of A. lancea were comparable to 5-flourouracil and were most effective for reduction of tumor mass, inhibition of lung metastasis and prolongation of survival time in mouse CCA-xenograft models (Plengsuriyakarn et al., 2012a). In addition, a similar research has previously indicated that the crude ethanolic extracts of A. lancea have been reported to exhibit selective and strong anti-cancer properties
against in vitro cultured CL-6 cells with values for half maximal inhibitory concentration (IC\textsubscript{50}) less than 30 ug/ml (Mahavorasirikul et al., 2010). Hence, further in-depth mechanistic studies on known pure active components of \textit{A. lancea} such as: beta-eudesmol, atractylone, and hinesol may hold promising therapeutic potentials for treatment of CCA (Mahavorasirikul et al., 2010; Koonrungsesomboon et al., 2014). The \textit{in vitro} studies of ethanolic extracts from other Thai medicinal plants including \textit{Kaempferia galanga} and traditionally used Thai folklore recipe (Pra-Sa-Prao-Yha) have also displayed significant cytotoxic activities against the CCA CL-6 cells (Mahavorasirikul et al., 2010). Recent evidence indicates that capsaicin, an active component found in fruits of plant belonging to
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Table 1. List of a Few Promising Biomarkers as Indicated in Some Studies Associated with Cholangiocarcinoma Research

| Biomarkers                                      | Description                                           | Reference                  |
|------------------------------------------------|-------------------------------------------------------|----------------------------|
| Carbohydrate antigen 19-9 (CA19-9)             | Serum marker commonly used in clinical practice       | Patel et al. 2000          |
| Carcinoembryonic antigen                       | Serum marker associated with hepatic cancer           | Patel et al. 2000          |
| Cytokeratin-19 (CK-19)                         | Protein associated with cytoskeleton                  | Stroescu et al. 2006       |
| Fascin                                          | Actin cross-linking protein monomer                    | Ruys et al. 2014           |
| Hepatoma-derived growth factor (HDGF) CD133 and Oct3/4 | Factor associated with cell mobility and adhesion      | Han et al. 2013            |
| Mucins                                         | Surface receptor and transcription factor, respectively| Thana et al. 2013          |
| Metalloproteinase-7                            | Heavily glycosylated protein produced by epithelial cells | Ruys et al. 2014           |
| Spermatogenesis associated 20 (SSP411)         | Associated with breakdown of extracellular matrix and also promote metastasis of tumor cells | Lee et al. 2014            |
|                                                 | Protein found in serum and also associated with hepatic abnormalities | Shen et al. 2012           |

个股属Capsicum，其抑制性作用影响于细胞移动性和侵袭。该抗癌作用很大程度上归因于它在抑制癌症中抑制了转录情况，从而减少细胞骨架中的金属蛋白酶7。细胞的金属蛋白酶7导致了细胞外基质的降解，从而促进细胞移动性。CD133和Oct3/4是细胞表面的受体和转录因子，Thana et al. 2013。

Genital growth factor (HDGF)在人类恶性细胞中具有重要作用。Thana et al. 2013。

Mucins是与细胞膜和细胞骨架相关的蛋白质，Thana et al. 2013。

Spermatogenesis associated 20 (SSP411)存在于血清中，并且与肝细胞癌的预后有关，Shen et al. 2012。

Conclusions

CCA is progressively fatal form of hepatobiliary cancer and extremely difficult to diagnose at its early stages. Multiple risk factors and their complex interactions are involved during development of this cancer which remains a topic of extensive research. Epidemiological evidence indicates that the global incidence of CCA is increasing and bears a very dismal prognosis among patients with progressive stage of the disease. Currently, apart from timely detection and immediate surgical resection of the tumor there is no effective treatment strategy to cure CCA. Thus, investigating the role of pathogens-host interaction, host immune response, potential risk factors, and biomarkers associated with CCA is essential in devising effective diagnostic and therapeutic strategies against this disease (Figure 1). In addition, continuous focus should also be given to exploration of natural as well as synthetic anticancer compounds utilizing both in vivo and in vitro experimentation approaches in CCA research. Taken together, all currently available knowledge on this devastating disease must be seriously considered for further research on diagnosis and targeted therapies for treating CCA.

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