Aberrant DNA methylation profile in cholangiocarcinoma

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Abstract

Cholangiocarcinoma (CCA) is a notoriously lethal epithelial cancer originating from the biliary system. As radical resection offers a poor success rate and limited effective adjuvant modalities exist in its advanced stage, the disease leads to a fairly poor prognosis. As the incidence of CCA is increasing, although the mortality rate remains stable, and few other definite etiologies have yet to be established, renewing our knowledge of its fundamental carcinogenesis is advisable. The latest advances in molecular carcinogenesis have highlighted the roles of epigenetic perturbations and cancer-related inflammation in CCA. This review focuses on the reciprocal effects between aberrant DNA methylation and inflammatory microenvironment in CCA.

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Key words: Cholangiocarcinoma; Epigenome; DNA methylation; Cancer-related inflammation; Microenvironment

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INTRODUCTION

Cholangiocarcinoma (CCA) is the second most common epithelial cancer originating from the biliary system, accounting for 10% to 20% of primary liver cancer[1]. As the presentation of symptoms is delayed, an R0 resection (both gross and microscope negative margin) can be achieved in less than 80% of those 10% early-stage patients in whom radical surgical intervention can be applied[2,3]. Additionally, the limited adjuvant modalities available for advanced patients have failed to show substantial benefit[3]. The aforementioned factors offer CCA a fairly poor prognosis, as overall 5-year survival rates in the resectable cases are less than 40% in both extrahepatic cholangiocarcinoma (ECC) and intrahepatic cholangiocarcinoma (ICC), and the median survival time is less than 12 mo in unresectable or metastatic cases[2,3]. Incidences of CCA, especially ICC, are increasing worldwide, although mortality has remained stable during the last four decades, with the exception of the decline in gallbladder carcinoma[1,4]. To date, though some well-documented risk factors have been identified, the majority of CCA etiology has remained
unknown. Emerging advances in CCA molecular research have highlighted the roles of epigenetic perturbations and cancer-related inflammation. The aberrant DNA methylation of CCA, which regarded as one of the best-characterized, mitotically heritable and reversible epigenetic modulations, has been seen to affect multiple steps of cholangiocarcinogenesis.

Epigenetic controls of gene expression orchestrate changes of chromatin architecture tissue-specifically and dynamically without affecting gene sequences, encompassing some basic mechanisms like post-translational modification of histones, displacement of nucleosomes and DNA methylations. Broadly, it may also include RNAi and non-coding RNAs. Within the above epigenetic modulations, DNA methylation is best characterized and delineated as a post-replicative addition of a methyl (−CH3) group to the cytosine-5-carbon position, which is catalyzed by at least three DNA methyltransferases (DNMTs): DNMT1, DNMT3a and DNMT3b. Methylation occurs mainly at CpG dinucleotides, CpGpG and rarely at non-CpG dinucleotides like CpA, CpT and CpC located in the promoter or encoding regions of genes. Methylated cytosine will allow binding with methyl-CpG binding domain (MBD) proteins (MeCP1 or MeCP2) to remodel the chromatin architecture, a process that has been recognized as essential and versatile for epigenetic modification. To date, global genome hypomethylation and local tumor suppressor genes hypermethylation have been noticed in tumorigenesis. Furthermore, abundant bench and bedside evidence supports the putative association between inflammation and cancer, which potentially leads to dysregulated DNA methylation in CCA. Though clinicopathological and epidemiological differences exist between ICC and ECC, emerging evidence has revealed that both of them are closely related to chronic inflammation. This review aims to summarize current reported aberrant DNA methylation profiles of CCA and outline the involving role of cancer-related inflammation.

RECIProCAL EFFECTS BETWEEN INFLAMMATORY MICROENVIRONMENT AND CCA

Briefly, there are two pathways bridging inflammation to CCA: the extrinsic pathway (risk factors or related environmental exposures of CCA) and the intrinsic pathway (congenital or acquired genetic alterations, e.g. activation of oncogenes, inactivation of tumor suppressors, senescence-related perturbations, etc). The milieu of chronic inflammation may environmentally select the adaptive transformed cholangiocytes, thereby initiating cholangiocarcinogenesis. For instance, inactivation of 9p21 gene cluster (p16INK4a/p14ARF/p15INK4b) has been unraveled in liver fluke-related CCA and primary sclerosing cholangitis-associated CCA. Also, a high frequency of microsatellite instability (MSI) and inactivation of hMLH1 has been observed in thorotrast-related CCA. Although a recent study of thyroid carcinoma has uncovered that an early genetic event is necessary and sufficient for initiating a cancerous development by promoting an inflammatory microenvironment, similar instances of an intrinsic pathway have yet to be addressed in CCA. Instead, a mounting body of evidence of genetic alterations in CCA has indicated these pathways indirectly, such as mutations or deletions of K-ras, p53, p16INK4a, p15INK4b, p14ARF or Smad4; loss of heterozygosity (LOH) of adenomatous polyposis coli gene (APC) or allelic losses on 3p13-p21 and 8q22-23.

INFLAMMATION-RELATED EPIGENETIC PERTURBATIONS IN CCA

Some key intrinsic factors can mediate inflammation-related gene regulation in CCA, including transcription factors [signal transducer and activator of transcription 3 (STAT3), etc], cytokines (IL-6, TNF-α, etc), growth factors, nitric oxide, reactive nitrogen oxide species (RNS) and bile acids. Among the aforementioned mediators, IL-6 plays a crucial role in many cancers, especially in epithelial cancer. Upregulation of IL-6 in carcinogenesis is triggered by an autocrine or paracrine loop, or even by an intrinsic somatic mutation of epidermal growth factor receptor (EGFR). It is also well documented that in chronic cholangiopathies or biliary infection, the level of IL-6 is increased in bile. Briefly, in CCA, the negative feedback of the IL-6 pathway is deficient and replaced by an unlimited autocrine loop owing to aberrant epigenetic silence of suppressors of cytokine signaling 3 (SOCS-3), which is mediated by the IL-6/STAT3 pathway to maintain hypermethylation of the gene promoter. Aberrant activated IL-6 in CCA leads to carcinogenesis promotion, through mechanisms such as up-regulating anti-apoptotic myeloid cell leukemia-1 (Mcl-1) mediated by phosphorylated STAT3; up-regulating EGFR expression through decreasing its promoter methylation level mediated by undefined mechanisms, and activating telomerase. In breast cancer, overloaded IL-6 also enhances expression of stem cell survival regulator Notch-3 and activates the hypoxia-resistant gene carboxic anhydrase IX (CA-Ix) through the Notch-3/Jagged-1 pathway. However, effect of IL-6 such as these on cancer stem/progenitor cells in CCA has yet to be clearly defined. Moreover, IL-6 had been shown to affect microRNA profiles of CCA. Overloaded IL-6 also increased let-7a expression via an undefined mechanism, resulting in suppression of neurofibromatosis 2 (NF2) and a subsequent increase of phosphorylated STAT3. Recently, Meng and colleagues have revealed that overload of IL-6 in CCA can up-regulate expressions of two DNA methyltransferases, DNMT1 and HASJ4442, leading to hypermethylation of the CpG island where the mir-370 encoding gene was
embedded. Consequently, oncogenic mitogen-activated protein kinase kinase kinase 8 (MAP3K8) suppressing effect of miR-370 was abrogated in CCA\(^{[43]}\). (The role of IL-6 signaling in CCA inflammation-related epigenetic regulation is summarized in Figure 1).

Infiltrated leukocytes also fuel the inflammation-related epigenetic perturbations in CCA. The mechanisms of leukocyte recruitment and homing in cholangiopathies, like primary biliary cirrhosis (PBC), primary sclerosing cholangitis (PSC) and chronic viral hepatitis C, have long been investigated. Briefly, chemokines CCL21, CCL28, CX3CL1, CXCL9 and CXCL10 mediate the recruitment of leukocytes into the portal tract and subsequently CX3CL1, CXCL12, CXCL16 and CCL28 retain these inflammatory cells in the bile duct to serve their under-determined roles\(^{[44,45]}\). Little research has been conducted regarding the immunosuppressive milieu of CCA-related inflammation. Recently, the roles of PD-L1 (also termed B7-H1 or CD274, an inhibitor of activated effector T cells)\(^{[46]}\) and Foxp3+ regulatory T cells\(^{[47]}\) in ICC have been explored. Meanwhile, Opisthorchis viverrini infection has been shown to upregulate inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) expression in rodent macrophages through the Toll-like receptor 2/NF-κB pathway\(^{[48]}\). However, epigenetic modulations on immunity against CCA have yet to be unraveled. Actually, in the Foxp3+ regulatory T cells of the mouse colitis model, the immunosuppressive effect related to Foxp3 was mediated by its co-repressors, Eos and C-terminal binding protein-1 (CtBP1), by promoting hypermethylation of IL-2 gene promoter, trimethylation of histone 3 lysine 4 and acetylation of histone 3 and histone 4\(^{[49]}\). Furthermore, both of the antigen-presentation molecule major histocompatibility complex class 2 (MHC-II) and its co-activator, class II transactivator (CIITA) can be epigenetically modulated in cancer\(^{[50]}\). Briefly, in renal carcinoma and pancreatic carcinoma, dysregulated IL-10 and Decoy receptor 3 (DcR3) can suppress CIITA expression respectively in immune cells through epigenetic modifications on CIITA gene promoter\(^{[51-52]}\).

Besides leukocytes, much research has also highlighted the pivotal roles of other stroma constituents in the progression of chronic cholangiopathies, encompassing fibroblasts, hepatic stellate cells (HSC), extracellular matrix (ECM), etc\(^{[53-57]}\). Recently stromal effect on parenchymal epithelial-to-mesenchymal transition (EMT) has also been noticed in cholangiopathies\(^{[58-60]}\). However, in CCA, less evidence involving the contribution of remodeled stroma to the epigenetic perturbations than in other cancers could be obtained. Briefly, cancer-associated fibroblasts (CAFs) have been proven to exert their epigenetic modulations on neighboring immortalized human breast epithelial cells MCF10A by direct cell-cell contact, simultaneously activating Akt1 and suppressing Akt1 repressor, inositol polyphosphate-4-phosphatase type II (INPP4B), subsequently leading to de novo promoter hypermethylation of the tumor suppressor gene Cystatin M (CST6)\(^{[61]}\). Furthermore, mechanical forces of the remodeled ECM or tissue architecture
may also exert potential modulated effects on epigenetic perturbations of cholangiocarcinogenesis. In this context, some details about dynamic biochemical pathways and mechanotransduction, termed “mechanoreciprocity”, have been explained in other cancers, like breast cancer [61-64]. Effects of ECM on the cellular epigenome are increasingly being deciphered [62-64]. Briefly, the ECM exert their cis- and trans-regulations on transcription via specific membrane receptors such as integrin, and various intracellular molecules like focal adhesion kinase (FAK), RhoGTPases, ATP-dependent chromatin remodeling complexes (SWI/SNF, ISWI, CHD, INO80 and SWR1), which couple extracellular signals to the cytoskeleton and chromatin and mainly mediate by targeting the ECM-responsive elements (EREs) in the genome as well as transcription factors [62-63]. Moreover, it is increasingly accepted that ECM-mediated mechanotransduction can “prepare” chromatin structures to receive specific biochemical signals and can control common sets of genes in cells possessing similar morphology. Subsequently, defined biochemical signaling networks permit further tissue-specific transcription in differentiation-specific genes [62-63]. One typical example of this intricate reciprocity is nuclear lamina-associated transcriptional silencing [63]. The nuclear lamina is made of intermediate filament proteins like lamins A/C and B, which can bind smaller nesprins that belong to the inner nuclear membrane proteins. Smaller nesprins can shuttle between the outer and inner nuclear membrane, while the biggest nesprins are anchored in the outer nuclear membrane where they can extend into the cytoplasm and bind actin microfilaments and intermediate filaments [63]. Moreover, associations of negative transcription factors with components of the nuclear lamina have also been explained. For instance, the lamin B receptor can bind histone 3 methylated lysine 9 and heterochromatin component HP1 [64], while lamin A/C and lamin B can bind MOK2 and Oct-1, respectively [65,68]. Recently, a genome-wide high-resolution mapping of lamin B1-associated DNA domains further implied this putative association between remodeled ECM and epigenetic control, revealing that lamin B1-associated DNA domains contain H3K27me3, the insulator protein CTCF or methylated CpG islands [69].

ABERRANT DNA METHYLATION PROFILES VARYING WITH SPECIFIC RISK FACTORS OF CCA

As the inflammatory microenvironment may vary with diverse cholangiopathies, disease-specific DNA methylation profiles or epigenome can reasonably be expected. Some of the aberrantly methylated genes reported in currently available literature are summarized in Table 1 [70-74], according to respective specific risk factors of CCA.

CONCLUSION

From the above Table 1, more details about aberrant DNA methylation profiles in CCA remain to be unraveled. To date, although the mechanism of demethylation and the candidate enzymes exhibiting direct demethylase activity and related cofactors are not yet firmly established in mammalian cells, recent trends in research about DNA demethylation have revealed the putative role of the BER/NER pathways and the association with DNMT1 [75,76]. Moreover, repair-mediated DNA demethylation of Oct-4 gene promoter by Gadd45a has been observed [77]. Recently, Gadd45a has also been shown to connect neuronal circuit activity with DNA demethylation in mature neurons for extrinsic modulation of adult neurogenesis [78]. Further progresses in this field will help to facilitate our understanding about mechanisms of aberrant DNA methylation in CCA further.

In conclusion, chronic inflammation of cholangiopathies predisposes individuals to CCA. Since alterations of the cellular epigenome usually precede morphologic changes and genetic alterations, identification of related aberrant DNA methylation profiles according to specific inflammation milieu may serve as a reasonable early diagnostic marker and an intervention target for CCA.

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