The International Journal of Biochemistry & Cell Biology xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

The International Journal of Biochemistry & Cell Biology

journal homepage: www.elsevier.com/locate/biocel

Review

Epigenetic regulation in the carcinogenesis of cholangiocarcinoma☆

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ARTICLE INFO

Article history:
Received 9 March 2015
Received in revised form 6 June 2015
Accepted 9 June 2015
Available online xxx

Keywords:
Epigenetic regulation
DNA methylation
Histone modification
microRNA
Cholangiocarcinoma

ABSTRACT

Cholangiocarcinoma (CCA) is a malignancy arising from the epithelial cells lining the biliary tract. Despite the existence of variation in incidence and etiology worldwide, its incidence is increasing globally in the past few decades. Surgery is the only curative treatment option for a minority of patients presented with early disease; while moderate effective chemotherapy remains the standard care for patients with locally advanced or metastatic diseases. In this article, we briefly review the molecular alterations that have been described in CCAs focusing on the role of epigenetic modification, including promoter methylation inactivation, histone modification and microRNA, in the carcinogenesis and progression of CCAs. This article is part of a Directed Issue entitled: Epigenetics dynamics in development and disease.

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Abbreviations: CCA, cholangiocarcinoma; OS, overall survival; miRNA, microRNA; TSG, tumor suppressor genes; ICC, intrahepatic cholangiocarcinoma; OV, *Opisthorchis viverrini*; CpG, scytosine guanine dinucleotides; DNMT, DNA methyltransferases; EZH2, zeste homolog 2; RASSF1A, Ras-association domain family protein 1A; DAPK, death-associated protein kinase; SMYD3, SET and MYND domain-containing protein 3; TMS1/ASC, target of methylation-mediated silencing/apoptosis speck like protein containing a CARD; CHFR, checkpoint with forkhead and ring finger domains; RUNX3, runt-related transcription factor 3; APC, adenomatous polyposis coli; E-cadherin, epithelial cadherin; THBS1, thrombospondin 1; RAR-β, retinoic acid receptor-β; hMLH1, human mutL homologue 1; MGMT, O6-methylguanine-DNA methyltransferease; GST, Glutathione S-transferases; FHIT, fragile histidine triad; SOCS-3, suppressor of cytokine signaling 3; IL-6, interleukin-6; PGE2, prostaglandin E2; COX-2, cyclooxygenase 2; BLU/ZMYND10, blu protein/Zinc finger MYND domain containing protein 10; HCV, hepatits C; α-KG, α-ketoglutarate; 2-HG, 2-hydroxyglutarate; MLL3, mixed-lineage leukemia 3; EMT, epithelial-mesenchymal transition.

☆ This article is part of a Directed Issue entitled: Epigenetics dynamics in development and disease.

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http://dx.doi.org/10.1016/j.biocel.2015.06.012
1357-2725/

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1. Introduction

Cholangicoarcinomas (CCA), including intrahepatic, perihilar and extrahepatic CCAs, exhibits significant variations in incidence and etiology ethnically and geographically (Sandhu et al., 2008). Curative surgical resection provides the only chance for long-term survival in a small percentage of patients. The current standard of care for patients with metastatic, recurrent or locally advanced diseases is gemcitabine plus platinum combination chemotherapy with an achievable median overall survival (OS) less than 12 months (Valle et al., 2010). Molecular targeting agents play little role in the management of this difficult disease (Chen et al., 2015), which can largely result from the poorly illustrated molecular carcinogenesis in CCAs and the all comers rather than biomarker-selected, enriched population treatment strategies of CCAs trials in the past.

With the advance of modern molecular technology, there is rapid emergence of evidence to demonstrate that the accumulation of genetic and epigenetic alternations and deregulated microRNA (miRNAs) in CCA can result in the activation of oncogenes and inactivation or loss of tumor suppressor genes (TSG) to lead to the development and progression of CCA (Andersen et al., 2012; Isomoto, 2009). Recent exome sequencing analyses have revealed novel genetic mutations involving in DNA chromatin remodeling (Jiao et al., 2013), which provides another mechanism of epigenetic regulation in CCA. In this review, we will focus on the recent advances in the knowledge of epigenetic regulation including DNA methylation, histone modification and miRNA associated with cholangiocarcinogenesis and their possible prospects of therapeutic strategies.

2. Genetic alternation in CCA

The neoplastic transformation of biliary epithelial cells and malignant progression of CCA are accompanied with complicated, genetic and epigenetic alternations. The recent whole-exome and targeted sequencing not only confirmed frequent mutations in known CCA-related genes including *TP53*, *KRAS* and *IDH1/2*, but also revealed mutations in novel chromatin remodeling-associated genes, such as *BAP1*, *ARID1A* and *PBRM1* in CCA (Table 1). In addition, those studies have also identified new, recurrent driver genetic alternations in CCA, such as *FGFR2* fusion and somatic *ARAF* mutation that are potentially actionable with available pan-FGFR inhibitors and selective RAF kinase inhibitor (Shannon and Hermiston, 2014; Sia et al., 2015).

Interestingly, there are significant differences in the frequency of mutated genes in intrahepatic cholangiocarcinoma (ICC) with or without either liver fluke (*Opisthorchis viverrini*, *OV*) or hepatitis B virus infection reflecting the impact of causal agent on cholangiocarcinogenesis. Compared to *OV*-related CCAs, mutations in *BAP1*, *IDH1* and *IDH2* were more frequently observed in non-*OV*-related CCAs (Chan-On et al., 2013), as shown in Table 1. Whereas *TP53* defectives are more likely to be detected in ICCs of HBsAg-seropositive patients; while *KRAS* mutations almost exclusively occur in ICCs of HBsAg-seronegative patients (Zou et al., 2014). These data highlight genetic differences and different carcinogenesis in CCA based on risk factors. The possible mechanisms of liver fluke infection causing CCA had been reported, including mechanical injury to the biliary epithelia, inflammation and mitogenic factors secreted by *OV* which lead the biliary epithelia cells transforming to CCA. TGF-β and EGF signaling pathways are considered to involve in the cell proliferation of stromal fibroblasts (Miwa et al., 2014). However, the possible mechanisms on hepatitis B-related ICC are limited to observational studies, which need further investigation.

Whether the genetic mutations identified currently indeed play a critical role in ICC development should be carefully considered. Driver mutations confer growth advantage on the cancer cells and are positively selected during the evolution of the cancer. On the contrary, passenger mutations do not contribute to cancer development (Stratton et al., 2009). A key mission of cancer genome analysis is to identify driver mutations and distinguish driver from passenger mutations through biological *in vivo* studies. The literature showed that *KRAS* combined *TP53* mutations could cause primary ICC (O'Dell et al., 2012), mutant *IDH* blocked hepatocyte differentiation and promoted biliary cancer (Saha et al., 2014) and aberrations in FGFR activity might participate in the development and progression of CCA (Ang, 2015). Whole-genome sequencing of CCA tumors obtained from various pathological stages will be useful for the understanding of mutation consequences of different genes and their pathogenic functions in this cancer.

3. Aberrant DNA methylation

DNA hypermethylation is a naturally reversible process that regulates the expression of cellular genes. In humans, DNA methylation is a covalent chemical modification that mostly occurs within the cytosine guanine dinucleotides (CpGs) leading to transcriptional repression of the target genes (Smiraglia et al., 2001). Aberrant promoter methylation-induced TSGs inactivation is common and contributes to the pathogenesis of majority human malignancies. Those observed in CCAs are summarized in the following sections.

3.1. Cell cycle

Downregulation of $p16^{INK4a}$ expression has been noted in 55–80% of ICCs (Tannapfel et al., 2002). Recent studies demonstrated that overexpression of histone methyltransferase enhancer of zeste homolog 2 (EZH2) is a frequent event and associated with the methylation inactivation of $p16^{IKN4a}$, $p27^{KIP1}$ and runt-related transcription factor (*RUNX3*) to promote the proliferation of CCAs (Nakagawa et al., 2014). In addition, EZH2 expression has been reported to correlate with worse clinical outcomes in both ICCs and ECCs (Nakagawa et al., 2013). Methylation inactivation of $p15^{INK4b}$, which encoding an effector of TGF-β-mediated cell cycle arrest, also occurs in roughly 50% of *OV*-related CCAs (Chinnasri et al., 2009). RAS-association domain family protein 1A (*RASSF1A*) that mediates RAS-related apoptotic pathway, has been reported to exhibited a 27–69% of promoter methylation frequency in CCAs (Yang et al., 2005). Recent study suggested a histone methyltransferase, SET and MYND domain-containing protein 3 (SMYD3) which can be regulated by hepatitis C virus (HCV) core protein may be responsible for the methylation inactivation of *RASSF1A* in HCV-related CCA (Guo et al., 2011).

Please cite this article in press as: Chiang, N.-J., et al., Epigenetic regulation in the carcinogenesis of cholangiocarcinoma. Int J Biochem Cell Biol (2015), http://dx.doi.org/10.1016/j.biocel.2015.06.012