Epigenetic Aberrations in Cholangiocarcinoma: Potential Biomarkers and Promising Target for Novel Therapeutic Strategies

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Abstract

Cholangiocarcinoma (CCA) is a notoriously lethal malignancy arising from the biliary tract epithelium. While relatively rare, incidence rates have increased markedly worldwide in the past decade. Although definite risk factors such as primary sclerosing cholangitis, liver fluke infestation, and hepatolithiasis have been well-documented, the cause of CCA remains unknown for most cases. An importance of not only genetic alterations but also epigenetic aberrations, including promoter hypermethylation and histone modifications, has been indicated for the processes of carcinogenesis and pathogenesis of CCA. This review focuses on epigenetic mechanisms involved in CCA genesis, with special emphasis on their applicability as potential biomarkers for diagnosis, prognosis and prediction as well as promising targets for novel therapeutic strategies.

Keywords: Epigenetics - methylation - histone modifications - biomarkers - cancer

Introduction

Cholangiocarcinoma (CCA) is a deadly epithelial cancer originating from the biliary tract epithelium. CCA is categorized according to its anatomical location as either intrahepatic (ICC) or extrahepatic (ECC) in which two-thirds are ECC and the remaining is ICC (Lazaridis and Gores, 2005). This fatal cancer is difficult to diagnose because of late clinical presentation and the lack of effective therapeutic modalities. Most patients are unresectable and the overall survival rate is poor with 5-year survival less than 5% which is significantly unchanged over the past three decades (Shaib and El-Serag, 2004). CCA is a relatively rare tumor, however, the incidence and mortality rate are increasing worldwide making the rising interest of this cancer (Khan et al., 2005). Several risk factors such as primary sclerosing cholangitis (PSC), liver fluke infection, and hepatolithiasis which mediate chronic inflammation are known to be associated with CCA (Sandhu et al., 2008). However, for most CCA cases the cause is unknown. It is clear that oncogene activation and tumor suppressor gene inactivation result from accumulation of genetic and epigenetic alterations leading to the development and progression of CCA (Tischoff et al., 2006; Stutes et al., 2007; Isomoto, 2009). Epigenetic refers to heritable changes in gene expression that are not due to alterations in the gene nucleotide sequence (Holliday, 1987). This review focuses on epigenetic mechanisms such as DNA methylation, histone modifications, and microRNAs associated with CCA and their applications as diagnostic, prognostic and predictive biomarkers as well as novel therapeutic targets.

DNA methylation

DNA methylation remains the widely-studied epigenetic mechanisms. It refers to a covalent addition of a methyl group to 5’ carbon position of the cytosine ring within the cytosine guanine dinucleotide (CpG) (Bird, 2002). CpG dinucleotides are found mostly in small stretches of genomic DNA called CpG islands. CpG islands are found in or near promoter regions of genes, which estimated 60% of human genes contain a CpG island. The addition of methyl groups to cytosine residues in DNA is catalyzed by DNA methyltransferases (DNMTs). DNMTs found in mammalian cells include DNMT1, DNMT3a, and DNMT3b. DNMT1 is responsible for maintenance of established patterns of DNA methylation, while DNMT3a and 3b mediate establishment of new or de novo DNA methylation patterns (Baylin, 2005). The majority of the genomic DNA is rather CpG-poor due to spontaneous deamination of methylated cytosines. Most CpG sites are heavily methylated in genomic DNA, whereas CpG islands in germ-line tissue and promoters of normal somatic cells remain unmethylated allowing gene expression to occur. DNA methylation helps to maintain transcriptional silence in nonexpressed or noncoding regions of the genome such as imprinted genes.
X-chromosome inactivation, and repetitive sequences or transposons (Reik and Lewis, 2005; Weber et al., 2007). In contrast to normal cells, promoters of tumor suppressor genes are heavily methylated in cancer cells (promoter hypermethylation) resulting in gene silencing (Herman and Baylin, 2003) (Figure 1). In addition, genomic DNA of cancer cells is less methylated (global hypomethylation) leading to increased genomic instability and reactivation of transposon elements.

**Aberrant DNA methylation in CCA**

Promoter hypermethylation of genes involved in cell cycle, apoptosis, cell adhesion, DNA repair and carcinogen/drug metabolism has been reported in CCA by several groups (Tischoff et al., 2006; Stutes et al., 2007; Sandhu et al., 2008; Hamilton, 2010; Huang et al., 2010). Aberrant methylation of p16, inhibitor of cyclin-dependent kinase at G1 stage of cell cycle, has been investigated in PSC-, liver fluke-, and hepatolithiasis-associated CCA with the frequency of 25%, 28.3%, and 100%, respectively (Ahrendt et al., 1999; Sasaki et al., 2008; Chinnasri et al., 2009). Moreover, frequencies of promoter hypermethylation of p14 and p15 in liver fluke-associated CCA reported by our group are 40.2% and 48.9%, respectively (Chinnasri et al., 2009). Human mutL homologue 1 (hMLH1) is a DNA mismatch repair gene which plays a key role in the correction of errors during DNA replication to maintain the fidelity of the genome. Limpaiboon et al. (2005) reported hypermethylation of hMLH1 in 44.6% of liver fluke-associated CCA and its correlation with the poorly differentiated CCA with vascular invasion. They also reported that hypermethylation is a major event for hMLH1 inactivation in CCA. Recently, Sriraksa et al. (2011) showed that OPCML (Opioid binding protein/cell adhesion molecule-like gene) is highly methylated (72.5%) in liver fluke-related CCA particularly in less differentiated CCA but not in normal adjacent tissue suggesting its potential use as epigenetic biomarkers for prognosis and diagnosis. Moreover, they also demonstrated that patients with methylated DcR1, the decoy receptor, had significantly longer overall survival suggesting its use as a prognostic marker of CCA. The aberrant methylation of RIZI (retinoblastoma interacting zinc finger) has been reported in liver fluke-associated CCA for 38%. RIZI is a tumor suppressor gene containing SET domain and acts as histone methyltransferase (HMT) which catalyzes methylation of histone H3K9 of target genes resulting in chromatin compaction and gene silencing (Khaenam et al., 2010). The reduction of RIZI expression contributes to increasing proliferation and migration of CCA cell line (Khaenam et al., 2012).

**Histone modifications**

Histones are basic proteins that complex with genomic DNA to form nucleosomes, the basic units of the compacted structure of chromatin. The nucleosome is made up of approximately two turns of DNA wrapped around a histone octamer composed of two subunits of each histone, H2A, H2B, H3, and H4. In between core nucleosomes, the linker histone H1 attaches and facilitates further compaction (Khorasanizadeh, 2004). The N-terminal tails of histone proteins are protruding out from the core nucleosomes. These tails serve as regulatory signatures on which covalent modifications including acetylation of lysines, methylation of lysines and arginines, phosphorylation of serines and threonines, and ubiquitination of lysines take place (Berger, 2002) (Figure 2). The pattern of histone modifications indicates the status of the chromatin locally, bromodomain and chromodomains, which act as epigenetic landmarks for a group of proteins to bind and initiate downstream biological processes such as transcriptional regulation, DNA repair, DNA replication, and chromatin remodeling (Kouzarides, 2007; Li et al., 2007; Huertas et al., 2009). Histone acetylation catalyzed by histone acetyltransferases (HATs) correlates with transcriptional activation, while acetylated histones are deacetylated by histone deacetylases (HDACs) resulting in chromatin condensation and transcriptional repression (Shukla et al., 2008). Histone methylation catalyzed by histone methyltransferases (HMTs) can serve as both transcriptional activation and repression depending on the type of amino acid and its position in the histone tail (Esteller, 2008). Proteins containing SET (SuVar39, Enhancer of Zeste, and Trithorax) domain and Polycomb-group (PcG) proteins have demonstrated to have methyltransferase capability (Lund and van Lohuizen, 2004). Histone can be mono-, di- or tri-methylated. Initial studies of histone methylation have shown that histone H3 (lysines 4, 9 and 27) and H4 (lysine 20) are frequently preferentially methylated (Grant, 2001; Taby and Issa, 2010). Methylation of histone H3K9, H3K27, and H4K20 can lead to transcriptional repression while...
MicroRNAs

MicroRNAs (miRNAs) are small noncoding RNAs (~22 nucleotides), which are transcribed by RNA polymerase II as long, capped, and polyadenylated primary miRNAs (pri-miRNAs) and subsequently processed in the nucleus by RNase III Drosha and DGR8 (DiGeorge syndrome critical region gene 8, or Pasha) into 70-100 nt hairpin-shaped RNA called precursor miRNAs (pre-miRNAs) (Bartel, 2004). These precursors are exported by an Exportin-5 to cytoplasm and cleaved by RNase III Dicer into 18-24 nt miRNA duplex. The duplex binds to a large protein complex called RISC (RNA-induced silencing complex). One strand of miRNA is degraded, while the other strand is exported by an Exportin-5 to cytoplasm and cleaved by RNase III Dicer into 18-24 nt miRNA duplex. The mature single-stranded miRNA to its target mRNAs. The mature miRNA is able to regulate gene expression in the post-transcriptional level by binding through the 3’ untranslated region (3’UTR) of target mRNA leading to mRNA degradation if perfect matching or translation inhibition when partial matching (Brennecke et al., 2005). Each miRNA is predicted to have many targets and each mRNA may be regulated by more than one miRNA (Lim et al., 2005). Approximately 30% of human gene expression is regulated by miRNAs (Lewis et al., 2005). Moreover, it has been found that several miRNAs function as oncogenes and tumor suppressor genes (Chuang and Jones, 2007). It has been shown that miR-141, miR-200b, miR-21 and let-7a are overexpressed in CCA in which their target tumor suppressor genes are CLOCK, PTPN12, PTEN and NF2, respectively (Stutes et al., 2007; Isomoto, 2009). By contrast, low expression of miR-29b and miR-370, which inhibit Mcl-1 and MAP3K8, respectively has been reported in CCA (Stutes et al., 2007). Recently, Karakatsanis et al. (2011) have demonstrated that miR-21, miR-31, and miR-223 are over-expressed but not associated with clinicopathological parameters, whereas miR-122, miR-145, miR-200c, miR-221, and miR-222 are down-regulated in ICC. However, the study of miRNAs in CCA at present remains limit.

The expression of miRNAs is controlled by several mechanisms such as the proper function of Drosha or Dicer in controlling biosynthesis of miRNA (Karube et al., 2005) and location of microRNA genes at frequently fragile sites (Calin et al., 2004), besides posttranscriptional regulation. It has been shown that DNA methylation and histone modifications control the transcription of miRNAs, on the other hand, microRNAs also regulate the expression of the epigenetic machinery (Scott et al., 2006; Han et al., 2007; Valeri et al., 2009).

Epigenetic therapy of cancer

In contrast to genetic alterations, the DNA sequence and protein product of methylated genes remain unchanged. Inhibition of epigenetically mediated suppression could therefore reactivate silenced genes and restore normal gene function. Epigenetic drugs such as demethylating agents and HDAC inhibitors exert their effects against tumors by turning on the tumor suppressor genes that are aberrantly silenced epigenetically.

The demethylating agent 5-azacitidine and its deoxy derivative, decitabine, are powerful inhibitors of DNA methylation and have been approved by the U.S. Food and Drug Administration (FDA) to treat myelodysplastic syndrome (MDS) (Yoo and Jones, 2006). However, these drugs are toxic both in vitro and in vivo, and are unstable in neutral solutions (Beisler, 1978). In contrast to other DNMT inhibitors, zebularine, a novel DNA methyltransferase inhibitor, has low toxicity in most cell line tested and is quite stable with a half-life of 510 h at pH 7.0 (Cheng et al., 2003; Marquez et al., 2005). Due to its low toxicity, it is feasible to continuously administrate effective dose of zebularine alone or in combination with other DNMT inhibitors to enhance the reexpression of epigenetically silenced genes in cancer cells (Cheng et al., 2004).

HDAC inhibitors inhibit the deacetylation of histones and weaken the histone-DNA interactions, thereby permitting a more relaxed conformation of chromatin which contributes to increasing gene transcription (Vigushin and Coombes, 2002). According to chemical structures, HDAC inhibitors can be classified into several groups, including short-chain fatty acids (such as sodium butyrate and valproic acid), hydroxamates (such as suberoylanilide hydroxamic acid, SAHA and trichostatin A, TSA), cyclic peptides (such as trapoxin A, TPX), and benzamide (such as MS-275) (Marks et al., 2004; Carew et al., 2008). These structurally distinct compounds share similar cellular function by which they all can induce growth arrest, and in cancer cells, cell death while normal cells are relatively resistant (Kelly and Marks, 2005). Among HDAC inhibitors, SAHA is the most advanced candidate as an anticancer drug. It has significant anticancer activity against many tumor types at well tolerated doses by the patients. SAHA has been
approved by FDA to treat T cell cutaneous lymphoma (Shama et al., 2010). HDAC inhibitors exert their effect by triggering both mitochondria-mediated apoptosis and caspase-independent autophagic cell death (Shao et al., 2004). As the study of epigenetics in CCA has been increasing, epigenetic drugs can be prospectively used to enhance the effectiveness of traditional chemotherapy in CCA.

Conclusion

The significant role of DNA methylation in the regulation of gene expression in both normal and cancer is well recognized. DNA methylation regulates biological processes in normal cells including transcription, X-chromosome inactivation, gene imprinting, and genome stability while DNA hypermethylation found in cancer can lead to silencing of tumor suppressor genes. Histone modifications control the accessibility of the chromatin and transcriptional activities which lead to activation or suppression. While DNA methylation and histone modifications regulate gene expression at the transcription level, microRNAs control their target gene expression posttranscriptionally. Several methylated genes show their potential as diagnostic, prognostic, and predictive biomarkers which can be detected in the serum of CCA patients. In addition, epigenetic aberrations may be applicable as a novel target for effective treatment of CCA.

References


