Hypermethylation of tumor suppressor genes in carcinogenesis of human gastric cardia adenocarcinoma☆

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Abstract

Genetic alterations that lead to the function loss of tumor-suppressor genes have been recognized as one of the key mechanisms to induce carcinogenesis. DNA methylation, one of epigenetic changes, seems to be one of the most important mechanisms for the increased predisposition to cancer. Methylation rich regions in CpG promoters prevent the transcription of many tumor suppressor genes (TSGs) and seem to play an important role in the pathogenesis of many human cancers, including gastric cardiac adenocarcinoma. To highlight the significance of DNA methylation in gastric cardia carcinogenesis, the accumulated data were thus summarized in this review. [Life Science Journal. 2007; 4(2): 15 – 18] (ISSN: 1097 – 8135).

Keywords: gastric cardia adenocarcinoma; tumor suppressor gene; hypermethylation

1 Introduction

Gastric cardia adenocarcinoma (GCA) is one of the most frequent digestive malignant diseases in Linzhou, Henan province, northern China. A remarkable epidemiological characteristic for GCA is the occurence together with esophageal cancer (EC) in the same high-incidence area (HIA). GCA is one of the most mortal cancers, with a low chance of cure[1]. Therefore, the current challenges in the management of GCA are to obtain a better understanding of the underlying molecular biological alterations to provide new treatment options.

Genetic and epigenetic mechanisms have been documented to be involved in the GCA carcinogenesis. Genetic alterations such as gene amplifications, loss of heterozygosity (LOH), point mutations, and chromosomal rearrangements[2–4] will change gene structures. "Epigenetic" events are the changes in gene function which cannot be explained by changes in DNA sequence, including histone acetylation, the chromatin structure and DNA methylation[5,6]. DNA methylation seems to be the most important mechanism for "epigenetic change" in human cancers[1,5].

The particular tumor suppressor genes that have been hypermethylated in tumor cells are strongly specific to the tissue of origin of the tumor. The mechanism responsible for GCA remains largely unclear. Moreover, accumulating evidence indicates that CpG island hypermethylation is an early event in cancer development and, in some cases, may precede the neoplastic process[7]. Therefore, such profiles would provide invaluable insight into mechanisms underlying the evolution of each tumor type and will provide new molecular biomarkers. This review will focus on the current understanding of DNA methylation abnormalities in GCA and discuss how this knowledge contributes to our understanding of the pathogenesis of GCA, especially on the putative tumor suppressor genes involving in apoptosis, cell adherence, DNA repair, and the cell cycle.

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2 $p14^{ARF}$ and $p16^{INK4A}$

The 9p21 chromosomal band is one of the most frequently altered genomic regions in human cancers[8]. Within a short distance of 50 kb, a gene cluster consisting of three genes, $p14^{ARF}$, p15 and $p16^{INK4A,}$ are harbored. All of which have putative tumor suppressor roles[8–10]. Inactivation of $p14^{ARF}$, p15 and $p16^{INK4A}$ genes has been observed in many types of human cancers including GCA[9–14]. A group study showed loss of heterozygosity of 9p21, which contains the $p16^{INK4A}$ tumor suppressor gene locus, is one of the most frequent genetic abnormalities in human neoplasia, including esophageal adenocarcinoma. Only a minority of Barrett's adenocarcinoma with 9p21 LOH has a somatic mutation in the remaining p16 allele, and none has been found to have homozygous deletions. From the data, 38% had p16 promoter hypermethylation and 9p21 LOH, which suggest that promoter hypermethylation with LOH is a common mechanism for inactivation of p16 in the pathogenesis of esophageal adenocarcinoma[14,15]. Complete loss of p16 (INK4A) protein expression is detectable in 45% of esophageal, cardiac and gastric adenocarcinoma and as significantly associated with hypermethylation of the p16 (INK4A) gene ($P < 0.0001$)[16–18]. The results suggest that hypermethylation of p16 (INK4A) is one of the frequent findings in esophageal, cardiac and gastric adenocarcinoma.

3 $RAR\beta_2$

The retinoic acid receptor-beta 2 ($RAR\beta_2$) gene located at 3p24 has been intensively studied in many cancers and found to have defensive function, thus making it a candidate tumor suppressor gene[19]. We found that $RAR\beta_2$ was detected in 36% (18/50) of normal esophageal tissues, and that 14 of 20 (70%) esophageal squamous cell carcinoma (ESCC) samples had hypermethylation of the $RAR\beta_2$ promoter[20]. However, the studies on the relationship between the $RAR\beta_2$ methylation and GCA have not been reported. In adenocarcinoma, methylation of the RARβ, CRBP1, and TIG1 genes was detected as 36%, 33% and 10%, respectively, comparing with 20%, 0% and 3% in corresponding non-neoplastic mucosa. DNA methylation of each gene was associated significantly with low mRNA expression of the respective gene. 57% demonstrated hypermethylation of at least 1 of the 3 genes. The results suggest that gastric carcinogenesis involves transcriptional inactivation by aberrant DNA methylation of genes related to retinoid signaling[21].

4 APC

The adenomatous polyposis coli gene (APC) located on chromosome 5q21, is a tumor suppressor gene (TSG) in the wnt signaling pathway[22]. Frequent LOH in APC gene has been observed in esophageal cancer, in contrast, the prevalence of mutations in the APC gene in esophageal cancer is low. No studies have been done on the APC methylation in GCA. But APC has been reported with a high methylation rate (93%) in Barrett's esophagus (BE) adenocarcinoma. In BE mucosa from patients who had developed to adenocarcinoma, APC shows a high rate of methylation (100%), whereas in BE mucosa from patients who had not developed to cancer, methylation was found only in 36%[23,24]. Hypermethylated APC DNA was observed in the plasma of adenocarcinoma patients (25%) and in 2 of 32 squamous carcinoma patients (6.3%). High plasma levels of methylated APC DNA were statistically significantly associated with reduced patient survival. The APC promoter region was hypermethylated in tumors of the majority of patients with primary esophageal adenocarcinoma. Hypermethylated APC gene DNA in the plasma may be a useful biomarker for esophageal adenocarcinoma patients and should be evaluated as a potential biomarker in additional tumor types[25].

5 MGMT

$O^{(6)}$-methylguanine-DNA methyltransferase (MGMT) is a gene involved in DNA repair, which is methylated in a variety of cancer types. MGMT hypermethylation in many cancers is a frequent event that is associated with loss of MGMT protein expression but not with patient's outcome[26,27]. In established cancer cell lines, MGMT expression appears to be correlated with methylation in the promoter of the gene[26,28]. A group study showed promoter methylation frequency of MGMT in Barrett's carcinomas (BC) compared with esophagogastric junction and proximal gastric adenocarcinoma (0.7 *vs.* 0.08 *vs.* 0.29, respectively), confirmed immunohistochemically by a significant loss of MGMT protein in BC ($P = 0.006$). Therefore, MGMT might become a prognosis indicator[18].

In the esophageal adenocarcinoma, hypermethylation of MGMT was found in 63.6% of cases and loss of MGMT protein expression in 49% of cases. Furthermore, MGMT hypermethylation was found in 6% of normal esophageal smooth muscle tissue, in 20% of esophageal squamous epithelium and in 62% of non-neoplastic Barrett's mucosa. In the carcinomas, hypermethylation of the MGMT gene was correlated with loss of MGMT protein expres-

sion ($P < 0.0001$) and with high tumor differentiation ($P = 0.0079$)[29].

6 E-cadherin

E-cadherin is a transmembrane glycoprotein mediates Ca^{2+}-dependent intercellular adhesion that is essential for the maintenance of normal tissue architecture[30]. Loss of E-cadherin expression occurs in a variety of human tumors and is correlated with invasion and metastasis, and activation of E-cadherin results in the growth inhibition of tumor cell lines[31]. E-cadherin can be targeted by both genetic and epigenetic means. Moreover, the hypermethylation of E-cadherin was seen frequently in most tumor types, also reduction of E-cadherin expression has been demonstrated, but mutations are only frequent in a small number of specific subtypes[31]. In patients with esophageal adenocarcinoma, E-cadherin is methylated in 84% of tumor specimens. These data suggest that epigenetic silencing via aberrant methylation of the E-cadherin promoter is a common cause of inactivation of this gene in esophageal adenocarcinoma[32]. However, the contrary for methylation of E-cadherin has been observed[18,33] . We suspect that the different results may be because of sample collecting, various types of collected samples, different methylation primes, and the different sites to be detected.

7 RASSF1A

Ras-association domain family 1 gene (RASSF1), has been identified within the region at 3p21.3. Many studies have suggested that RASSF1A is a new putative tumor suppressor gene[34–36]. RASSF1A acts as a negative effector of Ras in a pro-apoptotic signaling pathway. Interestingly, mutational inactivation of this gene is very rare (< 2%), and the main mechanism of its inactivation is through promoter methylation and LOH[36]. The RASSF1A is highly epigenetically inactivated in lung, breast, ovarian, kidney, prostate, thyroid, esophagus and several other carcinomas[34]. Data from our laboratory show that RASSF1A mRNA is presented in 41 of 42 adjacent cancer tissues, however, loss of RASSF1A mRNA expression is found in 31 cases (74%) in primary tumor ($P < 0.05$). Promoter methylation of RASSF1A occurs 64% in tumor, but with only 2% in adjacent normal tissue. All the GCA cases with promoter gene methylation show loss expression of RASSF1A mRNA, suggesting that there is close relationship between expression of RASSF1A mRNA and carcinogenesis of GCA. Loss of RASSF1A expression may be induced by methylation of the gene promoter, which is the possible molecular mechanism of GCA and might be a frequent molecular event in GCA. Thus, RASSF1A methylation could serve as a useful biomarker to screen the high-risk subjects and to make early diagnosis for GCA.

Besides the above mentioned genes, there are hypermethylation of some other genes available involving GCA, including GPX-3[37], APK FHIT[18], ESR1[33] and so on. GCA is a stepwise process of accumulation of genetic and epigenetic abnormalities. It has become clear that promoter hypermethylation of tumor suppressor gene is of such importance for this multistep process as genetic changes in GCA. The steadily growing list of genes inactivated by promoter hypermethylation in GCA provides not only new insights into the molecular basis of the diseases but also a long list of interesting candidate genes for the development of molecular markers which might contribute to the improvement of diagnosis and prognosis. In addition, the fact that methylation can be reversed *in vitro* and the effect of the demethylating agent 5-aza-2′-deoxycytidine *in vitro* raise hope for new treatment strategies for cancer patients. Furthermore, correction of aberrant DNA methylation may provide a new area in cancer prevention in people with premalignant lesions, such as Barrett's esophagus, BCH and DYS[38].

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