RESEARCH ARTICLE

The first review study on association of DNA methylation with gastric cancer in Iranian population

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Abstract

Background: Gastric cancer (GC) is the second leading cause of cancer-related death worldwide. Several environmental, genetic and epigenetic factors have been suggested to have a role in GC development. Epigenetic mechanisms like histone changes and promoter hyper-methylation are now being increasingly studied. Associations between methylation of many gene promoters with the risk of gastric cancer have been investigated worldwide. Such aberrant methylation may result in silencing of specific genes related to cell cycling, cell adhesion, apoptosis and DNA repair. Thus this molecular mechanism might have a key role in proliferation and migration of cancerous cells. Materials and Methods: In this review article we included studies conducted on DNA methylation and gastric cancer in Iranian populations. Using Science direct, Pubmed/PMC, Springer, Wiley online library and SciELO databases, all published data until 31 January 2016 were gathered. We also searched Science direct data base for similar investigations around the world to make a comparison between Iran and other countries. Results: By searching these databases, we found that the association between methylation of seven gene promoters and gastric cancer had been studied in Iran until 31 January 2016. These genes were p16, hLMH1, E-cadherin, CTLA4, THRβ, mir9 and APC. Searching in science direct database also showed that 92 articles had been published around the world till January 2016. Our investigation revealed that despite the importance of GC and its high prevalence in Iran, the methylation status of only a few gene promoters has been studied so far. More studies with higher sample numbers are needed to reveal the relation of methylation status of gene promoters to gastric cancer in Iran. Conclusions: Further studies will be helpful in identifying associations of DNA methylation in candidate genes with gastric cancer risk in Iranian populations.

Keywords: Methylation - epigenetic mechansims - gastric cancer - Iran

Asian Pac J Cancer Prev, **17 (5)**, 2499-2506

Introduction

Although the incidence rate of gastric cancer (GC) has declined over the years, it is still the fourth most common malignancy and is the second leading cause of cancer-related death worldwide (Muñoz and Franceschi, 1997). About 60% of all cases occur in developing countries and the highest level of GC among these countries belongs to eastern Asia. Furthermore, the ratio of men to women is about 2:1. About 50800 new cancer cases and 35000 cancer death occur in Iran each year, and 38 % of all cancers involve gastrointestinal tract. In other words, it has been reported that the most common cancer in Iranian men and women is gastric cancer and breast cancer respectively and based on Sadjati et al. (2003), GC increased about two fold from 10 per 105 in 1972 to 26.1 in 2002. So the incidence rate of gastric cancer in Iran is above the world average figure (22.0 per 100000) , but it is still lower than China (Alireza et al., 2005). Different frequency of incidence rate for gastric cancer in worldwide can be due to diversity in the genetic polymorphisms, diet behaviors and living conditions(Kazemi et al., 2015).

GC is a multi-factorial disease and two form of this disease have been described: Cardia and non-cardia gastric cancer. Its etiology consists of environmental, genetic and epigenetic factors. Some risk factors such as age, male sex, smoking, radiation and family history are common between cardia and non–cardia GC. Obesity and gastro- esophageal reflux disease are exclusive risk factors for cardia GC while exclusive risk factors for non-cardia GC include *H.pylori* infection, poorer socio-economic condition, low consumption of fruit or high intake of salt. Chronic infection with H. Pylori has been identified as the major risk factor in gastric cancer (McNamara and El-Omar, 2008) and prevalence rates of infection vary widely between different geographical regions and ethnic groups. It is higher in developing countries due to poor socio-economic status. In Iran, Based on Pourfarzi et al (2009), diet and *H.pylori* infection were the most strong risk factors related to GC in Ardabil (Pourfarzi et al., 2009).

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Additionally , higher salt intake ,drinking strong and hot tea and infection with *H.pylori* also increase the GC risk in Ardabil .According to Nouraie et al., 2009, prevalence of *H.pylori* infection in Tehran province was 69% which is higher than developed countries and individuals 46-55 years old had the highest infection rate (79.2%) (Nouraie et al., 2009). Falsafi et al., 2005 concluded that rate of infection in Southwest was higher (70%)than the rate in northwest parts (32%) of Iran (Falsafi et al., 2005). This rate is 40% in children from Rasht, a province in northern Iran (Mansour-Ghanaei et al., 2009).

Besides, genetic damages can also result in gastric carcinoma by affecting cellular functions essential for cancer development (Hanahan and Weinberg, 2000).Two distinct mechanism have been described to cause genetic damage : microsatellite instability (MSI) and chromosomal instability (Suzuki et al., 2006). Microsatellites are short repeated sequences of DNA and MSI refers to a changing in the number of these repeats which alter the way of translating DNA to proteins. One of the underlying causation of MSI is hypermethylation of mistmatch repair gene promoters. Also , losses on chromosome 4q, 5q, 6p, 9p, 17p, and 18q or gains of chromosomes 3q, 7q, 8q, 13q,17q, and 20q which lead to oncogene activation and/ or tumor-suppressor gene inactivation, are another genetic factors of gastric cancer (Buffart et al., 2007).

Finally, Epigenetic means heritable changes in gene expression which don't come from the nucleotide sequence alterations. These processes have an important role in tumor development and three common mechanisms occur in epigenetic changes: DNA methylation, histone change and association of non-histone proteins such as Polycomb and Trithorax complexes. Methylation changes in cancer development include hypo-methylation of repetitive sequences as well as the introns de-methylation. Promoter hyper-methylation also occur in some specific genes like tumor suppressor genes and micro RNA genes .Global hypomethylation increases mutation rates and chromosomal instability, but promoter hyper-methylation results in silencing the anti cell-proliferation genes, anti-apoptosis genes, anti-angiogenesis genes, DNA repair genes, and anti- metastasis genes. In the other hands, methylation occurs via some enzymes called DNA methyltransferases (DNMTs) that add one methyl group at the 5' carbon of the cytosine ring in CpG island. In contrast to cytosine methylation in promoters, majority of CpG islands of genomic DNA are protected from methylation (Bird, 2002). The CpG island hypermethylation has been estimated to be the causation of 24-47% of gastric cancers (Lee et al., 2004). Some genes involved in signal transduction (CDH1and RUNX3) ,cell-cycle regulation(p15/INK4B,p16/INK4A, RASSF1A, CHFR, COX2), inflammatory response (DAP-K), Apoptosis (GSTP1) , DNA repairing (hMLH1 , HPP1,p14/ARF) and Angiogenesis (TIMP3), and also some genes that encode growth factors (MGMT) and Transcriptional factors (THBS1) are methylated in GC (Waki et al., 2003; Honda et al., 2004). APC is another gene that has been reported to be methylated in GC .It is not clear what molecular mechanisms are involved in silencing genes via methylation (Kang et al., 2003) . However, DNA methylation occurs along with chromatin organization, regulation of histone, acetylation and corporation of other proteins. Some proteins bind to methylated cytosine, then these parts join a complex of histone de-acetylases (Jones et al., 1998).Two or three of these complexes have been described : one involving methyl CpG-binding protein and the others involving methyl CpG-binding domain proteins MBD2 and MBD3. These complexes also have histone de-acetylases (HDAC1 and/or HDAC2) and transcriptional co-repressors. It seems that MECP2 and MBD2 repress the transcription process (Jones et al., 1998). Additionally, Mi2 protein (also known as CHD), a member of the SWI/SNF family of nuclear helicases that makes a complex with MBD3,remodeles the chromatin (Nan et al., 1998). Thus, methylation along with MECP2 and histone de-acetylases results in silencing of gene transcription in cancers. As it is concluded from information above, multiple pathways play role in DNA methylation. In normal cells, promoters are occupied by activating and repressing protein complexes, therefore expression of these genes depends on competition of transcription complexes with acetylase and de-acetylase activities(Yari et al., 2015). But in cancer cells, methylated promoters shift towards transcriptional repression via two events: aberrant methylation might exclude activating complexes with acetylase activities and MECP2 can attract the complexes containing transcriptional co-repressors and histone de-acetylases to the CpG islands. Also, methylation of specific genes, such as DNA repair genes, may lead to microsatellite instability and increases the mutation rate. Moreover, methylation also leads to spontaneous deamination, increasing of carcinogen attachment to DNA and absorption of ultraviolet by DNA, all of them increase the rate of mutations and DNA adduct formation and subsequent gene inactivation.

Some factors may induce methylation. For instance it has been suggested that Epstein-Barr virus (EBV) infection (Sudo et al., 2004), alcohol consumption (van Engeland et al., 2003) , vitamin and minor elements deficiency influence DNA methylation status.(Davis CD,2004). The aim of this review article was to investigate studies conducted on DNA methylation and Gastric cancer in Iranian population.

Materials and Methods

Study design

In this article we included studies about association between gene methylation and gastric cancer found in the databases of Science citation index, Science direct, PubMed/PMC, Springer, SCIELO and Wiley online library published up to 31 January 2016. Our keywords for searching were" methylation and gastric cancer and Iran", "methylation and gastric and Iran ", "gastric cancer and Iran".

Data extraction

From each founded study, all information such as: author, year of publication, number of cases and controls, source of control groups (study design) and methylation method was extracted. Finally, by addition of other studies

conducted in other countries published in databases, we could make a good comparison between Iran and other countries.

Results and Discussion

As it is summarized in Table 1, we found 6 articles published about association of DNA methylation with Gastric cancer. These studies had been conducted in Mashhad, Isfahan, Zahedan and Tehran provinces which are shown in Figure 1. Three out of these six articles belonged to Mashhad and two of them to Tehran. Methylation specific PCR method was used in all of the studies to detect methylation of specific gene promoters. Approximately all of the experiments were done on Gastric tissue and all of them used margin healthy tissues as control samples. Overall, following genes had been mentioned in these articles:

P16

P16 is a cell-cycle regulator which stops cells in G1-phase through inhibition of cyclin D-dependent protein kinase 4 (CDK4) and 6 (CDK6). This gene interfere with phosphorylation of the retinoblastoma protein (pRb) and transcription of proteins associated with restriction point of G1 (Sherr, 2000). It can be concluded that this gene functions as a tumor suppressor gene and it's inactivation by hypermethylation in promoter region, alters the regulatory mechanism of the cell cycle (Sato and Meltzer, 2006; Zhao et al., 2007). Besides promoter methylation, other mechanisms like homozygote deletion and point mutation are suggested for decreased p16 protein expression .For this gene, only one study was found in Mashhad province conducted on 52 cases and 52 control groups (Abbaszadegan et al., 2008). Each group consisted of tissues and sera samples gathered from 14 female and 38 male. Methylation specific PCR (MSP) and Immunohistochemical staining (IHC) were done for methylation detection. As it is summarized in Table 1, the MSP results showed that 44.23% (23 out of 52) of total pathological tissue samples were methylated in p16 promoter region (6 out of 14 females and 17 out of 38 males in details).furthermore, 55.77% (29 out of 52) of total pathological tissue samples were unmethylated (8 out of 14 females and 21 out of 38 males respectively). Also none of the control tissues were methylated (0% or 0 out of 52 control samples). Authors didn't find any correlation between p16 promoter hypermethylation and gender, smoking habit, or H pylori infection but methylation was more frequent in older patients. Besides, serum samples were gathered from case and control groups and they found that 14 out of 52 cases (26.9%) and 0 out of 52 controls (0%) had methylated status in their sera.In IHC, p16 positive and p16 negative tissues were analyzed. based on this analyzing, 3 methylated and 17 unmethylated samples were p16 positive and also 20 methylated and 12 unmethylated tissues were p16 negative. In conclusion, strong correlation was found between negative immunostaining and hypermethylation of p16 promoter region.Authors suggested that p16 is a useful serum marker for early detection of gastric cancer.

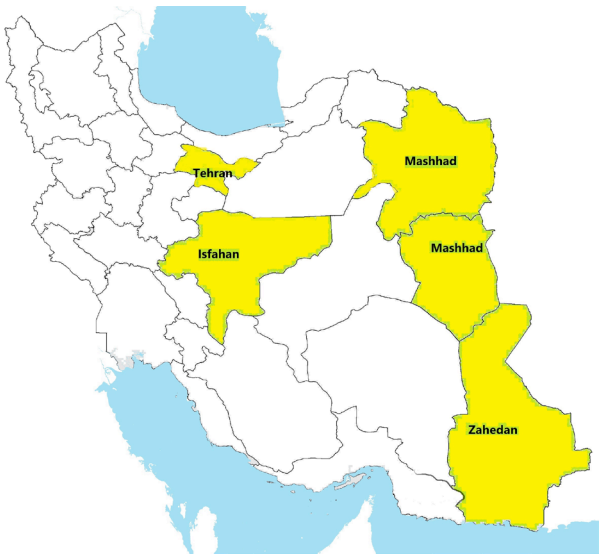

Figure 1. Geographical Distribution of the Studies on DNA Methylation among Iranian Gastric Cancer Individuals of Different Ethnicities

hLMH1

hMLH1 is a member of mismatch repair system (MMR), a system that identifies errors in base pair addition during DNA synthesis. So any aberration in this gene function results in genetic instability of genes that are associated with cell proliferation and death (Xiao et al., 2012) . In the other hand, hMLH1 is one of the caspase-3 protease substrates which lead to the production of a carboxyl terminal fragment with a pro-apoptotic role moreover; it can induce replication arrest and apoptosis via inhibition of PCNA. Finally, one of the most important mechanism which leads to gastric cancer progression is hMLH1 silencing by promoter hypermethylation (Fleisher et al., 2001) . In databases mentioned before, we found just one article which had assessed the role of hMLH1 and E-cadherin promoter hypermethylation in gastric cancer simultaneously with MSP and Real-time PCR method (results of E-cadherin assessment will be described in the next section) (Moghbeli et al., 2014). In this experiment done in Mashhad province, pathological samples were collected from gastric tissue of 16 females and 35 males. Thirteen out of these 51 (25.5 %) cases showed hypermethylation in hMLH1 promoter sequence and methylation statues was higher in males (76.9%) than females (23.1%).Also 12 out of 51 samples had hMLH1 Overexpression in Real time PCR method but 39out of 51 samples were normal/underexpressed. A significant correlation between hypermethylation of hMLH1 promoter and the level of mRNA expression was observed, in which all of the 12 overexpressed tumors had no methylated promoter sequence .The authors suggested that other mechanisms may be involved in the regulation of this gene expression except methylation, since there existed 26 cases among the 39(66.7 %) normal/underexpressed tumors without any methylation in the hMLH1 promoter. Furthermore, significant correlation was observed between the methylation status and the stage of tumor in which

10 out of 13 hMLH1 promoter hypermethylated tumors (76.9%) were in stage III of gastric cancer. Overall, their data showed that 25.5 % of the cases had hMLH1 promoter hypermethylation, indicating a significant difference with the similar studies with almost up to 73 %.

Table 1. Summary of Related Studies between DNA methylation of Candidate genes with Gastric Cancer in Iran up to 31 January 2016

Authors	Geographical region	Method	Sample type	Sample size	Gene name	Results
(Abbaszadegan et al., 2008)	Mashhad	MSP and IHC	Gastric tissue and serum	**Pathological tissue samples** :38 male and 14 female **Control tissue samples**:38 male and 14 female	*p16*	**MSP results**: Number of methylated tissue samples in patient group: 6/14 females and 17/38 males or 23/52 (44.23%) in both sexes. Number of methylated tissue samples in control group: 0/52(0%) number of methylated serum samples in patient group: 14/52 (26.9%) Number of methylated serum samples in control group: 0/52 (0%) -No correlation between p16 promoter hypermethylation and gender, smoking or H. pylori infection. -Higher frequency of methylation in older patients.
(Moghbeli et al., 2014)	Mashhad	MSP and real-time PCR	Gastric tissue	**Pathological tissue samples**: 16 females and 35 males. **Control tissue samples**: 26 females and 35 males	*hMLH1*	**MSP results**: Number of methylated samples in patient group:3/16 females and 10/35 males or 13/51 in both sexes (25.5%). **Real time PCR results**: Number of samples with overexpression of hMLH1:9/35 males and 3/16 females or 12 out of 51 cases (23.5%). Number of methylated samples in overexpressed group: 0/12 (0%) Number of unmethylated samples in normal/under expressed group: 26/39(66.7%) -No significant correlation between clinicopathological features and hMLH1 overexpression. -No difference between the tumor sizes regarding the levels of hMLH1 mRNA expression. -Lowered hMLH1 mRNA expression in all of the hypermethylated cases.
(Moghbeli et al., 2014)	Mashhad	MSP	Gastric tissue	**Pathological tissue samples** :26males and 7 females **Control tissue samples**: 26 males and 7 females	*E.cadherin*	Number of Methylated samples in patient group: 1/7 females and 11/ 26 males or 12/ 33 in both sexes (36.4%). -Significant correlations between E-cadherin promoter methylation and tumor stage and location .consistency of E-cadherin hypermethylation with other reports. -Noticeable but not significant correlation between lymph node metastasis and methylation status of Ecadherin.
(Kordi-Tamandani et al., 2014a)	Isfahan	MSP and real-time PCR	Gastric tissue	**Pathological tissue samples**: 65 males and 20 females **Control tissue samples**: 65 males and 20 females.	*CTLA4*	**MSP results**: Number of methylated samples in patient group: 15/20 females and 52/65 males or 67/85 in both sexes (78.82%) Number of methylated samples in control group (margin tissue) : 37/85 (43.53%) -Statistically significant differences between the tumor and margin-cell areas with respect to promoter methylation status.

Table 1. (Continued) Summary of Related Studies between DNA methylation of Candidate genes with Gastric Cancer in Iran up to 31 January 2016

Authors	Geographical region	Method	Sample type	Sample size	Gene name	Results
(Tamandani et al., 2015)	Zahedan	MSP and real-time PCR	Gastric tissue	**Pathological tissue samples**: 20 females and 65 males. **Control tissue samples** : 20 females and 65 males	*THRβ*	MSP results: Number of methylated samples in patient group: 15/20 females and 54/65 males or 69/85 in both sexes (81.18%) Number of methylated samples in control group: 57/85 (67.06 %) -Significant difference between tumoral and margin tissue.
(Ebrahimi-Askari et al., 2015)	Tehran	MSP	Gastric tissue	**Pathological tissue samples**: 19 males and 11 females **Control tissue samples**: 19 males and 11 females	*miR-9-1*	**MSP results**: Number of methylated mir-9-1 samples in patient group:15/19 males and 8/11 females or 23/30 in both sexes -No significant correlation between miR-9-1 methylation status and clinopathological features of tumor sample
(Alivand et al.)	Tehran	MSP	Gastric tissue and serum	**Pathological tissue samples** :36 **Control tissue samples** :36	*APC*	**MSP results**: Number of methylated samples in patient group: 21/36(58.33%) -No relation between age and DNA methylation.

E-cadherin

E-cadherin as an epithelial trans-membrane glycoprotein, mediates cell adhesion and homophilic cell– cell interaction. It also establishes cell polarity and is directly related to tumor metastasis (Wijnhoven BP , 2000). Thus reduced expression of E-cadherin allows cancer cells to dissociate from their matrix and migrate to further distances. This expression reduction is found in 51% of diffuse-type GCs. Attachment of E-cadherin to the cytoskeleton takes place via α- and β-catenin, and reduction or absence of catenin expression is identified in two thirds of GCs (Yu et al., 2000) . In conclusion, reduction of E-cadherin expression with DNA hypermethylation increases metastasis and decreases survival rate (Kawanishi et al., 2000). Article described above also had investigated the role of E-cadherin gene in gastric cancer but they did not assess all of the 51 samples (mentioned in hMLH1 section) in the case of E-cadherin promoter. As a result, 12 out of 33 samples (36.4%) consisted of 11 males and 1 female were hypermethylated in E-cadherin gene promoter (Moghbeli et al., 2014). In 11 out of these 12 samples (91.7%) metastasis to lymph node was observed. Therefore, there seems to be a noticeable but not significant correlation between E-cadherin methylation and lymph node metastasis. Besides, 21 out of 33 samples were not hypermethylated (15 males and 6 females).Again , the most hypermethylation statues was detected in males (42.3%) compared to females (14.3%). It seems better to repeat this study for E-cadherin with more sample numbers.

CTLA4

Cytotoxic T-lymphocyte-associated antigen-4 (CTLA4), is a 149-amino acid receptor belonging to the immunoglobulin superfamily and is expressed on the surface of activated CD4+ and CD8+ T cells. This antigen has a key role in cancer development and progression as increased level of CTLA4 and regulatory T cells have been observed in different types of cancer. Furthermore, a DNA vaccine against CTLA4 has been investigated to prevent tumor growth (Erfani et al., 2012) .Also, it is reported that blocking of this receptor improves survival rate in cancers. Thus, Promoter hypermethylation of the gene which encodes this antigen can be considered as a risk factor for gastric cancer (Kordi-Tamandani et al., 2014b) . As it can be interfere from Table 1, we found just one article about the association of this gene promoter hypermethylation and gastric cancer in Isfahan province. The study population was consisted of 65 males and 20 females with different stages of gastric cancer and their margin healthy gastric tissues were used as control groups. Detection methods were MSP and Real time PCR .With respect to MSP method, 67 out of 85 pathological gastric tissues were methylated in CTLA4 promoter region (15 out of 20 females and 52 out of 65 males in detail).Also in cancerous tissues , 5 out of 20 females and 13 out of 65 males (18 out of both sexes) were unmethylated. Besides , 48 out of 85 (56.47%) and 37 out of 85 margin tissues were unmethylated and methylated respectively .These results revealed that methylation of CTLA4 gene is associated with an increased risk of gastric cancer, and significantly lower expression of this gene could be observed in cancer tissues relative to their normal margins.

THRβ

Thyroid hormone receptor (TR), as a member of nuclear hormone receptor super families, is a transcription factor which originates from the alpha (TRα) and beta (TRβ) genes on chromosome 17 and 3. This receptor interacts with oncogenes, c-myc , cyclin D1 and other cell cycle regulators. Investigations have shown that TRs play role in tumor invasiveness, cell proliferation and metastasis (Aranda et al., 2009). THR suppresses the activation of the extracellular signal-regulated kinase and the phosphatidyl-inositol-3-kinase signaling pathways that are vital for cell proliferation and invasion, thus it

disrupts the mitogenic action of growth factors (Martinez-Iglesias O, 2009). TRβ have been suggested to activate apoptosis and inhibit tumor growth (Park et al., 2013). THRβ promoter hypermethylation causes different grades of GC (Cheng et al., 2010) . We found one article about aberrant methylation of this gene in Internet databases in which pathological gastric tissues of 20 females and 65 males were included and also healthy margin tissues were used as control groups. Results of MSP technique showed that there was a significant difference between cancerous and its margin healthy tissue as 15 out of 20 females and 54 out of 65 males in patient group (totally 81.18%) were positive for THRβ promoter hypermethylation .in contrast, just 67.06% of margin tissues were methylated .Also were unmethylated respectively. Authors suggested that abnormal methylation of this gene could be involved in GC development.Furthermore, real-time PCR showed that expression of THRβ was significantly decreased in tumoral tissue compared to adjacent normal area.No relation was found between methylation of this gene and patient age, gender, pathologic stage and lymph node metastasis.

Mir9 family

Mir-9 is one of the microRNAs found in mammals. Three genomic loci including 1q22 , 5q14.3 and 15q26.1 participate in miR-9-1 , miR-9-2 and miR-9-3 transcription respectively. Deregulation of expression in these genes is involved in pathogenesis of colorectal, gastric, breast and non-small cell lung cancers. It has been suggested that promoter hyper-methylation of miR-9 had role in cancer metastasis. Expression pattern of mir-9 family has shown differences in variety of tumors. For instance it is overexpressed in brain tumors, hepatocellular carcinomas , Hodgkin lymphoma (HL)and breast cancer while it has down regulation in early breast cancer and colorectal cancer via promoter methylation. Based on studies, mir-9 is a tumor suppressor factor because of its regulating effect on NF-κB1 expression (Bandres et al., 2009).About aberrant methylation of mir-9 family (including mir-9-1 , mir-9-2 and mir-9-3), one survey had been done in Tehran(Ebrahimi-Askari R.,et al , 2015).This report was consisted of 19 men and 11 women with gastric cancer.MSP showed that 15 out of 19 males and 8 out of 11 women had mir-9-1 hypermethylation. But miR- 9-2 promoter was unmethylated in both normal samples and tumors. Also, miR-9-3 showed methylation of one allele and unmethylation of the other one in all of specimens. However, miR-9-1showed methylation of at least one allele in 73.3% of normal samples and 76.6% in tumor samples, respectively .In conclusion, Methylation status of miR-9-1 CpG island was not meaningfully different between tumor and normal samples. Besides, there was no significant correlation between miR-9-1 methylation status and clinopathological features of tumor samples. Finally, the authors concluded that it seems that the exact function and regulatory mechanisms of miR-9 expressions remain to be elucidated in gastric cancer and further studies on miR-9 expression and methylation of their promoters in normal gastric and other cancer are needed.

APC

Adenomatous Polyposis Coli (APC) is a tumor suppressor gene and its mutations or loss of heterozygosity is found in nearly 60% of intestinal-type GCs. Loss of APC may be involved in the final malignant transition from high grade dysplasia to cancer. Also, methylation of APC should be considered as an early event in gastric carcinogenesis and APC methylation takes place in more than 75% of intestinal-type GCs. However, methylation of APC is not specific to gastric cancer (Waki et al., 2003). based on the study found in databases, in which 36 tissue samples were collected from patients with GC (they didn't mention men and women separately), 21(58.33%) out of 36 cases of gastric carcinoma were methylated (Alivand et al.). Also no methylation was found in control group. Comparing methylation status of cytosines in CpG of males with females, 9 males (42.86%) and 12 females (57.14%) with a mean age of 55.5 years old, showed 58.33 % hypermethylation for the APC promoter. Furthermore, 8 out of males simultaneously showed both the methylated and unmethylated promoter. Their results showed that may be the prevalence of the promoter hypermethylation is more frequent among gastric adenocarcinoma patients. They also found similar results in 6 available serum samples (they didn't emphasize on serum results due to small number of samples). Finally, it was proposed that APC hypermethylation maybe used as one of the biomarkers in gastric carcinogenesis but APC gene alone, does not seem to be a reliable marker for the different stages of cancer prognosis.

In conclusion, Gastric cancer is the second major cause of cancer-related mortality among Iranian population .Environmental, genetic and epigenetic factors have shown to play important roles in its etiology. Nowadays, methylation as an attractive and mysterious epigenetic mechanism is being studied in cancer development and metastasis.

As our investigation revealed, association of methylation status of many gene promoters with gastric cancer have been studied around the world until recently. These genes include RASSF1A, IGFBP-3, MIR-495, ZAC/PLAGL1, GDNF, RORA, MINT25, KLF7, CDH1, LINE-1, BACH2, THRβ1, MLH1, p16, E-cadherin, SOSTDC1, Mir-193a, CALCA, DAPK1, RARbeta, TIMP3, PAX6, PCDH8 , HLTF, CHRNA3, DOK1, MGMT, p14ARF, ALDH2, GNMT , MTHFR, WWOX, CDO1, VEZT, SLC6A4, miR137, ADAMTS9, MINT25, RORA, GDNF, ADAM23, PRDM5, MLF1, TFPI2, GPX3, GPX1, IGFBP6, IRF7 , DMRT1, Protocadherin10, TPEF/ HPP1, CACNA2D3, somatostatin , XAF1, ZIK1, ZNF141, KAL1, FGF14, Sox17 , FOXD3, Trefoil Factor 2 , CDX1 , Cyclooxygenase2 , DACT1 , CDKN2A , MDR1 , Dkk3 and APC. Also, the majority of these articles were published in Japan.

In comparison, just a very few genes such as P16, hMLH1, E-cadherin, CTLA4 , THRβ, miR-9 and APC have been studied in Iran till 31 January 2016. As the importance of epigenetic role in silencing some specific genes, like tumor suppressor and cell cycle regulator genes, and therefore development of gastric cancer is obvious, it seems necessary to work on other promoters

methylation with more sample numbers in Iran.

Acjknowledgement

The authors of this manuscript confirm no conflicts of interest in this review article.

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