INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print) 2222-5234 (Online)
http://www.innspub.net
Vol. 4, No. 10, p. 13-21, 2014

RESEARCH PAPER OPEN ACCESS

Methylation status of *CDH1* promoter and E-cadherin expression in colorectal cancer

Jamshid Mehrzad[1*], Mahdieh Mohammaditabr[2], Zohreh Ebrahimi[3]

[1]*Department of Biochemistry, Neyshabur Branch, Islamic Azad University, Neyshabur, Iran*

[2]*Department of Statistic and Mathematics, Neyshabur Branch, Islamic Azad University, Neyshabur, Iran*

[3]*Department of Biochemistry, Neyshabur Branch, Islamic Azad University, Neyshabur, Iran*

Key words: *CDH1*, methylation status, mRNA expression, colorectal cancer, MS PCR, q-PCR.

http://dx.doi.org/10.12692/ijb/4.10.13-21 Article published on May 18, 2014

Abstract

The E-cadherin plays a crucial role in epithelial cell cell adhesion and in the maintenance of a tissue. In colorectal cancer (CRC), changes in E-cadherin expression have been correlated with histopathology and differentiation, but results are still inconsistent. Further studies should aid the development of novel strategies for both prevention and treatment of cancer. Aberrant methylation in promoter regions of the genes might lead to reduce gene expression and result in cancer. Thus in the present study for better understanding role of the E-cadherin in CRC, methylation status of the *CDH1* promoter region was determined by methylation specific PCR following bisulphite modification, and compared with mRNA E-cadherin expression by qPCR. 43 of 70 cancers (61.4%) were aberrant methylated in the *CDH1* promoter region and this correlated with reduced E-cadherin expression ($p<0.05$). Generally, our study demonstrated aberrant methylation of *CDH1* can significantly reduce its expression among tumor tissues in CRC, and contribute to tumor staging, tumor differentiation and progression or prognosis.

* **Corresponding Author:** Jamshid Mehrzad ✉ mehrzadjam@yahoo.com

Introduction

Colorectal cancer (CRC) arises as a result of the accumulation of genetic and epigenetic changes in epithelial cells during neoplastic transformation. CRC is the third most common cancer in men and the second in women. CRC rates are about 2 to 5 times higher in the developed countries in comparison to the developing countries which may be attributable to a range of variations in a disparate set of risk factors and diagnostic practices (Notani, 2001; Jemal *et al.*, 2011). In USA, data from migration population studies revealed that some ethnic groups are showing increased CRC incidence rate while they are migrating from low-risk to high-risk areas (Grulich *et al.*, 1995; Lee *et al.*, 2007; Flood *et al.*, 2000). Despite genetic variation, epidemiological data strongly suggest a role of environmental and lifestyle factors deeply contributing to the etiology of CRC. Lifestyle related predisposing modifiable risk factors for CRC include physical inactivity, overweight and obesity, red and processed meat consumption, smoking and excessive alcohol consumption (Jemal *et al.*, 2011). It is well accepted that environmental and dietary factors greatly influence epigenetic events including DNA hypermetylation. DNA cytosine methylation has been widely studied, with investigations often focusing on the methylation level of CpG dinucleotides in promoter regions that usually have higher concentrations of CpGs, known as CpG islands (Jones and Baylin, 2002). The promoter hypermethylation is one of the mechanisms leading to gene silencing by either physically inhibiting the binding of transcription factors, or by recruiting proteins that have transcription repressive properties in the cancer pathogenesis (Miyamoto *et al.*, 2005; Radhakrishnan *et al.*, 2011).

CRC spreads locally by direct invasion through the bowel wall into adjacent tructures and by metastasis, through lymphatic and venous channels, to regional lymph nodes and distant organs (Linn and Giaccone, 1995). For this process to occur, malignant cells initially must detach from the primary tumor mass and become motile (Ruoslahti and Giancotti, 1989) The ability of malignant cells to modulate their intercellular cohesiveness is considered to be an early and pivotal event in tumor progression (Jouanneau, 1991). E-Cadherin (ECD), encoded by the *CDH1* gene which is located on chromosome 16q22, is a transmembrane glycoprotein confined to epithelial cells and is mainly responsible for adherence junctions between them (Takeichi, 1991). Loss of ECD expression leads to the dissociation of cells from coherent tissues (Pignatelli *et al.*, 1992) and several studies have shown a causal relationship between down-regulation of ECD expression in tumor cells and the acquisition of an invasive phenotype (Frixen *et al.*, 1991; Sommers *et al.*, 1991; Vleminckx *et al.*, 1991).

Many studies conducted on cancer-associated genes including cyclin-dependent kinase inhibitor 2A (*P16*), E-cadherin (*CDH1*), O^6-methyl guanine methyl transferase (*MGMT*) and Estrogen Receptor-α (*ESR-α*) genes, have been found to harbor hypermethylated regulatory sequences that lead to gene silencing in CRC (Mehrzad *et al.*, 2014; Yu *et al.*, 2004; Wani *et al.*, 2013). However, numbers of studies to evaluate methylation profile of *CDH1* in promoter region is low in CRC and further investigation is necessary. The identification of biomarkers for early diagnosis or as therapeutic target in CRC treatment is urgently needed.

Here we used methylation-specific polymerase chain reaction (PCR) (MS PCR) to investigate *CDH1* methylation to distinguish methylated from unmethylated cytosines. MS PCR is very sensitive to detect one methylated cytosine in 1000, and the primers used are highly specific and cost effective (Herman *et al.*, 1996). Also we used real -time quantitative PCR, that it is a very precision method, to assay *CDH1* mRNA expression. The aim of the present study is to evaluate the effect of methylation status on *CDH1* mRNA expression and correlation between these cases and CRC risk.

Materials and methods

Patient population and tissue samples

Seventy cases with histologically confirmed sporadic

colorectal cancer were examined in this study. All patients underwent colorectal resection primarily at Neyshabur Medical Science Faculty Hospital (Neyshabur, Iran) from January 2008 to February 2013. No patient had received preoperative treatment in the form of radiation or chemotherapy. Patients who had other malignant disease except CRC were excluded. This study was approved by the IAU-Neyshabur and Neyshabur Medical science Faculty. All the tissue samples were collected within 10 minutes of resection. Tumor samples were divided into two parts; one part was sent for histopathological diagnosis and staging and the other half was stored as formalin fixed paraffin embedded (FFPE) as well as adjacent normal samples (normal non tumorous healthy tissue at >2 cm distance from the tumor) (Li *et al*., 2012). Histopathological grades and clinical staging were evaluated according to the standard criteria (Edge *et al*., 2010). Only histopathologically confirmed cases were included for further molecular analysis.

DNA and RNA extraction from tissue samples

DNA was extracted three times from seventy paired normal background and tumor FFPE tissue specimens with the AccuPrep Genomic DNA Extraction Kit (BioNEER, Cat.NO.: K-3032, Korea).

The most important consideration in generating useful data with real-time q-PCR is the quality of the isolated RNA. RNA isolated 5 times with the Jena Bioscience kit (Cat.-No. PP-210xs, Germany).

Before all extractions FFPE tissue specimens was deparaffinized by xylen. Quantity/quality was checked spectrophotometrically/1% agarose gel electrophoresis and stored for further use at -20°C.

Bisulfite modification and methylation- specific polymerase chain reaction (MS PCR)

Aberrant DNA methylation in the CpG islands in the promoter region of the *CDH1* in 70 patients of CRC was determined by chemical modification of genomic DNA with sodium bisulfite followed by MS PCR. Genomic DNA was treated with sodium bisulfate as reported by Herman *et al* (1996). Briefly, 2 µg of genomic DNA was denatured in 50 µl NaOH (final concentration, 0.2 M) for 10-15 min at 50 °C. Thirty microliters of 10 mM hydroquinone (Fluka) and 520 µl 3 M sodium bisulfite (Fluka) at pH 5, freshly both prepared, were added and mixed, and the samples were incubated at 50 °C for 16 h. The unmethylated cytosine was converted to thymine, whereas methylated cytosine remains unchanged.

The primer sequence of *CDH1* was based on the previous report and is listed in Table 1 (Rasti *et al*., 2009). Bisulfite-modified DNA was denatured in a total volume of 25 µl containing 1 µl of each primer, 2 µl of deoxynucleotide triphosphate, 2.5 µl of 10× PCR buffer, and 0.4 µl of hot-taq polymerase at 95^{oC} for 10 minutes. This was followed by 45 cycles of 95^{oC} for 35 seconds, incubation at the primer specific annealing temperature for 45 seconds, and 72^{oC} for 45 seconds. Samples were finally incubated at 72^{oC} for 10 minutes. Annealing temperature was 57^{oC} and 53^{oc} for methylated and unmethylated primers, respectively. Blood Sample of normal human DNA was used as the control for unmethylated alleles. In vitro methylated DNA from placenta with SssI methyltransferase was used as a positive control for methylated reaction. Distilled water was used as a negative control.

PCR products were electrophoresed on 2% agarose gels and visualized by ethidium bromide staining. Samples were scored as methylated when there was a clearly visible band on the gel with the methylation-specific primers. The sizes of PCR product in this study were 116 bp for methylation and 97 bp for unmethylation respectively. All samples were examined by one experimenter who was unaware of the patient's clinical features.

Real-time quantitative PCR (qPCR)

Two-step procedure was conducted in this study. cDNA was prepared according to manufacturer's instructions of kit (AccuPower RoketScript RT PerMix) from BioNEER company of korea. q PCR was performed in triplicates with the Applied Biosystems step one Real-Time PCR System, using

SYBR® Green PCR Master Mix (Part number 4309155) in comparative Ct method {ΔCt = Ct(main target cDNA) – Ct (refrence cDNA) and ΔΔCt = ΔCt (test cDNA) - ΔCt (calibrator cDNA) }. The primers were used for CDH1 and GAPDH are shown in table 2 based on other references **(**Kroepil *et al*., 2012; Caradec *et al*., 2010**)**. qPCR was performed in a total reaction volume of 25 µl containing 12.5 µl, 1.25 µl (30 pmol) primermix and 11.25 µl cDNA, or water as control. Thermal cycling conditions included 2 min at 50uC to allow for cleavage of cDNA double-strands and 10 min at 95uC to activate the Taq polymerase, followed by 45 cycles at 95°C for 15 sec and 60°C for 1 minute. Relative mRNA expression was calculated with the Relative Quantification Software using comparative Ct. GAPDH gene is a housekeeping gene and this gene has previously been shown to be appropriate for normalization in human tumor and normal colorectal tissue (Caradec *et al*., 2010)

Statistical analysis

Statistical analyses were performed with SPSS statistical software, version 20.0 for Windows (SPSS IBM, IL, USA). Pearson chi-square test was performed to analyze the distribution of hypermethylation in test groups compared with control. Statistical significance was defined as $P < 0.05$.

Results

Patients and tumor characteristics

A total of 140 consecutive surgically resected FFPE tissue specimens comprising of 70 tumor tissues and 70 adjacent normal control regions of primary sporadic CRC were collected for analysis in the present study. Their ages ranged from 35 to 87 years (median, 66 years). Patients included 47 men (67.1%) and 23 women (32.9%). Of the 70 cases analyzed, tumor was located in the colon, rectum and rectosigmoid regions in 55.7%, 31.4% and 12.9% cases, respectively. Histological examination was done in all of the cases to evaluate tumor histotype. Details of each patient related to their demographic profile, clinical characteristics and methylation status of CDH1 in tissues have been shown in table 2.

Table 1. Primer sequences used in methylation-specific PCR.

Gene	Profile	Sequence
CDH1	Unmetylated	5'-TAATTTTAGGTTAGAGGGTTATTGT-3' (sense)
		5'-CACAACCAATCAACAACACA-3' (antisense)
	Metylated	5'-TTAGGTTAGAGGGTTATCGCGT-3' (sense)
		5'-TAACTAAAAATTCACCTACCGAC-3' (antisense)

Methylation status of CDH1

Methylation in CDH1 promoter was detected in 61.4% of tumor tissues and %8.6 in adjacent normal control regions. Then aberrant methylation status significantly correlates with occurrence of CRC (p-value 0.000).

A significant number of cases with methylated CDH1 gene, 62.8%, had tumor stage III (p-value 0.005), significant cases, 58.1%, had poorly differentiated grade (p-value 0.002) and not significant cases had in rectum (p-value 0.468). As it is shown in table 3, the correlation of methylation status with gender is significant and with age is not significant (p-values 0.011, 0.270, respectively).

Expression analysis of CDH1 by qPCR

We performed a quantitative -PCR assay to analyze the mRNA expression of *CDH1* in FFPE tissue samples of colorectal cancer and normal adjacent tissue. We could detect *CDH1* mRNA expression in normal tissue than tumor is 15.3- fold. Therefore, *CDH1* expression was significantly reduced in colorectal cancer compared to normal adjacent tissue. As expected, expression of *CDH1* mRNA was clearly decreased in colorectal cancer stage III compared to the expression in normal adjacent tissue. Also, the amount of CDH1 mRNA differs significantly between tumor stages and grades.

Discussion

Colorectal cancer (CRC) is one of the major diseases, which its main reason is not determined. However, we know aberrant methylation of some genes, such as *P16* and *MGMT* involve in CRC. But, hypermethylation of other important genes, as *CDH1,* that can be a reason for reduction of gene expression has not been highly described in human colorectal cancer. On the other hand, we know that down-regulation of E-cadherin expression correlates with a strong invasive potential, resulting in poor prognosis in human carcinomas. Thus, in CRC, some studies mentioned that loss of E-cadherin function also permits or accelerates invasion and is associated with a more malignant phenotype and poor differentiation. Then, to better understanding the role of the epigenetic events in the etiology of CRC among Iranian patients, we examined the methylation status of *CDH1* in sporadic CRC and their association with distinctive pathological characteristics.

Table 2. Primer sequences used in qPCR.

Gene	Sequence
CDH1	5′- GAA CAG CAC GTA CAC AGC CCT -3′ (sense)
	5′- -GCA GAA CTG TCC CTG TCC CAG -3′ (antisense)
GAPDH	5′-GCTCTCTCCTCCTGTTCG-3′ (sense)
	5′-ACGACCAAATCCGTTGACTC-3′ (antisense)

Table 3. Demographic and clinical characteristics of 70 CRC tissues.

Patient characteristic	No. of samples (%)	CDH1 methylatation status (tumor tissue)		P value Pearson Chi - Square
		No. of M (%)	No. of UM (%)	
Total	70 (100)	43 (61.4)	27(38.6)	
Gender				
Male	47 (67.1)	24	23	0.011
Female	23 (32.9)	19	4	
Age				
≤50	28 (40)	15	13	0.270
50 <	42 (60)	28	14	
Tumor location				
Colon	39(55.7%)	12	6	0.033
Rectum	22(31.4%)	16	18	
Rectosigmoid	9(12.9%)	15	3	
Tumor Stage				
I	17(24.3%)	5	12	0.005
II	11(15.7%)	6	5	
III	37(52.9%)	27	10	
IV	5 (7.1%)	5	0	
Tumor grade#				
WD	17(24.3%)	4	13	0.002
MD	10(14.3%)	6	4	
PD	33(47.1%)	25	8	
U	10(14.3%)	8	2	

UM, unmethylated; M, methylated;

#WD, well differentiated; MD, moderately differentiated; PD, poorly differentiated; U, undifferentiated.

p value <0.05 was taken as significant.

In the present study MS PCR was used to assay methyaltion status of *CDH1*. We detected aberrant methylation in the CDH1 promoter region in a significant proportion of colorectal cancers (61.4%, P value 0.000). In another study, Hypermethylation of the *CDH1* promoter has been detected in 46% of samples (Wheeler *et al*., 2001). Yoshiura *et al* (1995) studied a panel of human carcinoma cell lines. However, only one cell line (SW1116) originated from the colon and was found to be unmethylated in the

CDH1 promoter and to express E-cadherin mRNA. Hypermethylation of the promoter region of *CDH1* is seen in more than 80% of papillary thyroid carcinomas, which is associated with reduced E-cadherin expression (Graff *et al.*, 1998). Also, other studies have shown that hypermethylation of *CDH1* in the promoter region in prostate, breast, and hepatocellular carcinoma is associated with reduced protein expression (Graff *et al.*, 1995; Hiraguri *et al.*, 1998; Kanai *et al.*, 1997).

Our results suggest that hypermethylation of the promoter region of *CDH1* in colorectal cancers is associated with a reduction in E-cadherin expression. This is similar to work using paraffin sections of thyroid and hepatocellular carcinomas where *CDH1* promoter methylation is associated with a reduced intensity or heterogeneous pattern of E-cadherin expression (Graff *et al.*, 1995; Graff *et al.*, 1998). Almost similar this study, Kroepil *et al* (2012) performed a quantitative RT-PCR assay to analyze the mRNA expression of CDH1 (E-cadherin) and a few another genes in FFPE tissue samples of colorectal adenomas (n = 41), colorectal cancer (n = 10) and normal colon mucosa (n = 10). CDH1 mRNA expression was detected in 39 of 41 (95%) colorectal adenoma samples, in all 10 (100%) colorectal cancer samples and in all 10 (100%) normal colonic mucosa samples. As expected, expression of CDH1 mRNA was clearly decreased in colorectal cancer compared to the expression in normal colonic mucosa.

As mentioned above, our work shows a significant correlation between aberrant methylation and reduced expression of *CDH1*. But reduced expression of CDH1 in some tumors lacking methylation suggested that not only the methylation but also genetic alterations are responsible. These other genetic alterations could possibility be mutation or polymorphism in the promoter or coding region of *E-cadherin* gene which could reduce the expression of CDH1 mRNA in tumors lacking methylation. The illustration of the relationship between CDH1 expression and *CDH1* gene methylation in primary tumors requires further studies on a large number of samples and this may certainly help us to better understand the role of methylation of adhesion molecule genes in carcinogenesis.

Based on the hypothesis that selective adhesion molecule interactions can be involved in metastasis formation, studies carried out on human cancers have shown the existence of a negative correlation between invasive capacity and cadherin expression (Mialhe *et al.*, 1997, Moll *et al.*, 1993). Like our results, some studies have shown that loss of expression of E-cadherin is associated with tumor staging, grade, invasion and metastasis, and is therefore suggested (Kwak *et al.*, 2007, Ghadimi *et al.,* 1993) as a potential prognostic factor in colorectal cancer. However, some studies failed to demonstrate any correlation between reduced expression of E-cadherin with conventional staging, tumor differentiation, invasive metastatic potential or prognosis (Van der Wurff *et al.,* 1992, Kinsella *et al.,* 1993; Kitadai *et al.*, 1996; Nigam *et al.*, 1993).

Also, the present study determined there was aberrant methylation in the adjoining tissues, thus suggests that normal tissues may contaminant with tumor tissue. This is the first study in the Iranian population in which *CDH1* gene has been analyzed at both epigenetic and expression level in CRC in relation to clinicopathological features and prognosis and confined to North East Iran. We need more data from the other parts of the country to validate our findings.

In conclusion, our study demonstrated that aberrant methylation *CDH1* can reduce its expression among tumor tissues in CRC, and contribute to tumor staging, tumor differentiation and progression or prognosis.

References

Caradec J, Sirab N, Revaud D, Keumeugni C, Loric S. 2010. Is GAPDH a relevant housekeeping gene for normalization in colorectal cancer experiments? British Journal of Cancer **103,** 1475 – 1476.
http://dx.doi.org/10.1038/sj.bjc.6605851

Edge SB, Byrd DR, Compton CC, Fritz AG, Greene FL. 2010. Cancer Staging Handbook: From the AJCC Cancer Staging Manual.: Springer Publishing Company, 173–206.

Frixen UH, Behrens J, Sachs M, Eberle G, Voss B, Warda A, Lochner D, Birchmeier W. 1991. E-cadherinmediated cell-cell adhesion prevents invasiveness of human carcinoma cells. Journal of Cell Biology **113,** 173-185.

Ghadimi BM, Behrens J, Hoffmann I, Haensch W, Birchmeier W, Schlag PM. 1999. Immunohistological analysis of E-cadherin, alpha-, beta- and gamma-catenin expression in colorectal cancer: implications for cell adhesion and signaling. European Journal Cancer **35,** 60-65.

Graff JR, Greenberg VE, Herman JG. 1998. Distinct patterns of E-cadherin CpG island methylation in papillary, follicular, Hurthle's cell, and poorly differentiated human thyroid carcinoma. Cancer Research **58,** 2063–6.

Graff JR, Herman JG, Lapidus RG. 1995. E-cadherin expression is silenced by DNA hypermethylation in human breast and prostate carcinoma. Cancer Research **55,** 5195–9.

Herman JG, Graff JR, Myohanen S. 1996. Methylation-specific PCR: a novel PCR assay for methylation status of CpG islands. Proceedings of the National Academy Sciences USA **93,** 9821-6.

Hiraguri S, Godfrey T, Nakamura H. 1998. Mechanisms of inactivation of E-cadherin in breast cancer cell lines. Cancer Research **58,** 1972–7.

Jemal A, Bray F, Center MM, Ferlay J, Ward E. 2011. Global cancer statistics. CA Cancer Journal of Clinicians **61,** 69–90. doi: 10.3322/caac.20107. Epub 2011 Feb 4.

Jones PA, Baylin SB. 2002. The fundamental role of epigenetic events in cancer. Nature Reviews Genetics **3,** 415-428.

Jouanneau J, Tucker GC, Boyer B, Valles AM, Thiery JP. 1991. Epithelial cell plasticity in neoplasia. Cancer Cells **12,** 525-529.

Kanai Y, Ushijima S, Hui A. 1997. The E-cadhrin gene is silenced by CpG methylation in human hepatocellular carcinoma. International Journal of Cancer **71,** 355–9. http://dx.doi.org/10.1002/(SICI)10970215(19970502)71:33.3.CO;2-4

Kinsella AR, Green B, Lepts GC, Hill CL, Bowie G, Taylor BA. 1993. The role of the cell-cell adhesion molecule E-cadherin in large bowel tumour cell invasion and metastasis. British Journal of Cancer **67,** 904-909.

Kitadai Y, Ellis LM, Tucker SL, Greene GF, Bucana CD, Cleary KR, Takahashi Y, Tahara E, Fidler IJ. 1996. Multiparametric in situ mRNA hybridization analysis to predict disease recurrence in patients with colon carcinoma. American Journal of Pathology **149,** 1541-1551.

Kroepil F, Fluegen G, Totikov ZE, Baldus S, Vay C, Schauer MA, Topp S, Schulte am Esch JT, Knoefel WH, Stoecklein N. 2012. Down-Regulation of CDH1 Is Associated with Expression of SNAI1 in Colorectal Adenomas. PLOS ONE **7(9),** e46665. http://dx.doi.org/10.1371/journal.pone.0046665.Epub 2012 Sep 28.

Kwak JM, Min BW, Lee JH, Choi JS, Lee SI, Park SS, Kim J, Um JW, Kim SH, Moon HY. 2007. The prognostic significance of E-cadherin and liver intestine-cadherin expression in colorectal cancer. Diseases of the *Colon* **&** *Rectum* **50,** 1873-1880.

Li Z, Fang ZY, Ding Y, Yao WT, Yang Y. 2012. Amplifications of NCOA3 gene in colorectal cancers in a Chinese population. World Journal of

Gastroenterology **18,** 855–860. http://dx.doi.org/10.3748/wjg.v18.i8.855.

Linn SC, Giaccone G. 1995. MDR1/P glycoprotein expression in colorectal cancer. European Journal of Cancer **31 A,** 1291-1294.

Mehrzad J, Hashemi M, Jamshidi M, Motavalizadekakhky A, Saeidi J, Mohammaditabr M, Khafi AS, Taheri-MB. 2014. Hypermethylation status of Promoter induced silencing of $P^{16/INK4a}$ in colorectal cancer in Iranian patients. Journal of Biodiversity and Environmental Sciences **4(2),** 94-103.

Mialhe A, Louis J, Montlevier S, Peoch M, Pasquier D, Bosson JL, Rambeaud JJ, Seigneurin D. 1997. Expression of E-cadherin and alpha-, beta- and gamma-catenins in human bladder carcinomas: are they good prognostic factors? Invasion Metastasis **17(3),** 124-137. http://dx.doi.org/10.1309/DKCLN4K2RK0V87YG

Miyamoto K, Ushijima T. 2005. Diagnostic and Therapeutic Applications of Epigenetics. Japanese Journal of Clinical Oncology **35,** 293-301. http://dx.doi.org/10.1093/jjco/hyi088

Moll R, Mitze M, Frixen UH, Birchmeier W. 1993. Differential loss of E-cadherin expression in infiltrating ductal and lobular breast carcinomas. American Journal of Pathology **143(6),** 1731-1742.

Nigam AK, Savage FJ, Boulos PB, Stamp GW, Liu D, Pignatelli M. 1993. Loss of cell–cell and cell–matrix adhesion molecules in colorectal cancer. British Journal of Cancer **68,** 507-514.

Notani PN .2001. Global variation in cancer incidence and mortality. Current Science **81,** 465–474.

Pignatelli M, Liu D, Nasim MM, Stamp GW, Hirano S, Takeichi M. 1992. Morphoregulatory activities of E cadherin and beta 1 integrins in colorectal tumour cells. British Journal of Cancer **66,** 629-634, 1992.

Radhakrishnan R, Kabekkodu S, Satyamoorthy K. 2011. DNA hypermethylation as an epigenetic mark for oral cancer diagnosis. Journal of Oral Pathology & Medicine **40,** 665-676. http://dx.doi.org/10.1111/j.1600-0714.2011.01055.x

Rasti M, Entezam M, Monabati A. 2009. Hypermethylation of E-Cadherin and Estrogen Receptor-a Gene Promoter and Its Association with Clinicopathological Features of Breast Cancer in Iranian Patients. International Journal of Molecular Sciences. **34(3),** 50-56.

Ruoslahti E, Giancotti FG. 1989. Integrins and tumor cell dissemination.
Cancer Cells **1,** 119-126.

Sommers CL, Thompson EW, Torri JA, Kemler R, Gelmain EP, Byers SP. 1991. Cell adhesion molecule uvomorulin expression in human breast cancer cell lines. Relationship to morphology and invasive capacities. Cell Growth Differentiation **2,** 365-372.

Takeichi M. 1991. Cadherin cell adhesion receptors as a morphogenetic regulator. science **251,** 1451-1455.

Van der Wurff AA, ten Kate J, van der Linden EP, Dinjens WN, Arends JW, Bosman FT. 1992. L-CAM expression in normal, premalignant, and malignant colon mucosa. Journal of Pathology **168(3),** 287-291.

Vleminckx K, Vakaet L, Mareel M, Fiers W, Van Roy F. 1991. Genetic manipulation of E-cadherin expression by epithelial tumor cells reveals an invasion suppressor role. Cell **66,** 107-119. http://dx.doi.org/10.1016/0092-8674(91)90143-M

Wani HA, Beigh MA, Amin S, Bhat AA, Bhat S, Khan H, Mattoo AA, Showkat M, Masood A, Majid S. 2013. Methylation profile of promoter

region of p16 gene in colorectal cancer patients of Kashmir valley. Journal of Biological Regulation Homeostais Agents **27(2),** 297-307.

Wheeler JMD, Kim HC, Efstathiou JA, Ilyas M, McC Mortensen NJ, Bodmer WF. 2001. Hypermethylation of the promoter region of the E-cadherin gene (CDH1) in sporadic and ulcerative colitis associated colorectal cancer. Gut **48**, 367–371. http://dx.doi.org/10.1136/gut.48.3.367

Yoshiura K, Kanai Y, Ochiai A. 1995. Silencing of the E-cadherin invasion-suppressor gene by CpG methylation in human carcinomas. Proceedings of the National Academy of Sciences USA **92,** 7416–19.

Yu J, Zhang HY, Sun MH, Gu J, Du X, Shi DR, Wang P, Yang ZH, Zhu JD. 2004. Methylation profile of the promoter CpG islands of 31 genes that may contribute to colorectal carcinogenesis. World Journal of Gastroenterology **10(23),** 3441-54**.**