Hypermethylated FAM5C and MYLK in serum as diagnosis and pre-warning markers for gastric cancer

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Abstract. Most cases of gastric cancer (GC) are not diagnosed at early stage which can be curable, so it is necessary to identify effective biomarkers for its diagnosis and pre-warning. We have used methylated DNA immunoprecipitation (MeDIP) to identify genes that are frequently methylated in gastric cancer cell lines. Promoter regions hypermethylation of candidate genes were tested by methylation-specific polymerase chain reaction (MSP) in serum samples, including GC (n = 58), gastric precancerous lesions (GPL, n = 46), and normal controls (NC, n = 30). Eighty two hypermethylated genes were acquired by array analysis and 5 genes (BCAS4, CHRM2, FAM5C, PRAC and MYLK) were selected as the candidate genes. Three genes (CHRM2, FAM5C and MYLK) were further confirmed to show methylation rates increased with progression from NC to GPL, then to GC. There was obvious decrease in detection of FAM5C and MYLK hypermethylation, but not CHRM2, from preoperative to postoperative evaluation (P < 0.001). Combined detection of FAM5C and MYLK hypermethylation had a higher sensitivity in GC diagnosis (77.6%, 45/58) and pre-warning (30.4%, 14/46) than one single gene detection and also had a high specificity of 90%. The combined hypermethylated status of FAM5C and MYLK correlated with tumor size (P < 0.001), tumor invasion depth (P = 0.001) and tumor-node-metastasis (TNM) stage (P = 0.003). Hypermethylated FAM5C and MYLK can be used as potential biomarkers for diagnosis and pre-warning of GC.

Keywords: Gastric cancer, hypermethylated DNA, biomarker, diagnosis, pre-warning, FAM5C and MYLK

1. Introduction

Gastric cancer (GC) is the third most common type of cancer and the second leading cause of cancer-related death in the world [1]. Despite advances in the management of this disease, the prognosis remains dismal. GC patients have a high mortality rate, with a 5-year survival rate of approximately 20% [2]. One of the main factors limiting the survival rate is that most cases are asymptomatic until advanced stages. GC, in most instances, represents a sequential process, i.e., progressing from inflammation to intestinal metaplasia, then to gastric epithelial dysplasia, and finally, to GC [3]. Gastric precancerous lesions (GPL) which confer a high risk for GC were including chronic atrophic gastritis (CAG), intestinal metaplasia (IM) and gastric epithelial dysplasia (GED). Therefore, it is crucial to explore effective biomarkers for diagnosis and pre-warning of GC.

Epigenetic perturbation of gene regulation is pathologically important to tumorigenesis [4], and methylation of CpG island (CGIs) is among the crucial epigenetic pathways regulating gene transcription. It had
identified important correlation between methylation frequency and gastric cancers [5]. Most of the recent studies investigating methylation status of specific genes were restricted to tissues only [6–8]. Thus far, little data are available on hypermethylated DNA analyses in the sera of GC and GPL patients for diagnosis and pre-warning.

In this study, we determined the early diagnostic and pre-warning potential of DNA hypermethylation in the serum of gastric cancer patients. Five candidate genes were selected according to the results of the methylated DNA immunoprecipitation (MeDIP) assay and then were measured in the sera from patients with GC, GPL and NC. Our study showed that hypermethylated FAM5C and MYLK might be potential biomarkers for diagnosis and pre-warning of GC.

2. Materials and methods

2.1. Clinical samples and cell lines

Nine GC cell lines (SGC7901, MKN-45, and MKN-28 were obtained from Cell Bank of Chinese Academy of Sciences, AG5, NCI-N87, NCI-SNU-1, NCI-SNU-16, HTB-103, and BGC823 were obtained from ATCC) and 6 samples of normal gastric mucosa were prepared for methylated DNA immunoprecipitation (MeDIP) assay analysis. These 9 GC cell lines were conserved by Shanghai Institute of Digestive Surgery and authenticated by flow cytometry three months before experiment. Serum samples of 58 GC patients with average age of 62.0 years were diagnosed according to the WHO criteria [9]. The 46 GPL patients (3 GEDs, 20 IMs, and 23 CAGs) with average age of 60.9 years were diagnosed according to the gastroscopy exammination. NC with average age of 55.2 years were taken from 30 healthy volunteers. GC, GPL and NC patients and NC were detected without Helicobacter pylori infection. Local research ethics committees approved the collection of samples and informed consent was obtained from each patient.

2.2. Methylated DNA immunoprecipitation (MeDIP) and CpG island microarray assay

RNA-free genomic DNA extracted from 9 GC cell lines and 6 normal gastric mucosa samples were enriched with the methyl-CpG binding domain (MBD) protein by capturing mCG. MBD-enriched immunoprecipitation (IP) samples were labeled with Cy5, whereas the remaining non-enriched samples were labeled with Cy3 as control (INPUT) DNA. Then, two samples were hybridized on NimbleGen human CpG island microarray containing 300,000 probes. The two-color array was scanned and data were analyzed by the NimbleScan software. Each feature on the array had a corresponding scaled \( \log_2 \) ratio, which was the ratio of the INPUT and IP signals. From the scaled \( \log_2 \) ratio data, one-sided Kolmogorov-Smirnov (KS) test was applied to determine whether the probes were drawn from a significantly more positive distribution than those in the rest of the array. The resulting score for each probe is the \(- \log_{10} \) p-value which was called Peak. Peak data \( \geq 2 \) was considered to indicate statistical difference between INPUT and IP samples.

2.3. DNA isolation and bisulphite modification

DNA was extracted from serum samples by using QIAamp Circulating Nucleic Acid Kit (QIAGEN). The concentration of DNA was measured by spectroscopy at a wavelength of 260 nm. Sulfurization modification, which converted unmethylated cytosine to uracil and left methylated cytosine unaltered, was done with less than 2 \( \mu \)g DNA. The samples were purified by use of EpiTect Bisulfite Kit (QIAGEN). Modified DNA was stored at \(-80^\circ \)C until used.

2.4. Methylation-specific polymerase chain reaction (MSP)

Bisulfite-modified DNA was amplified using primers specific for either methylated or unmethylated promoter sequences of the target genes. The information of primers and their products is shown in Table 1. Polymerase chain reaction (PCR) was performed in 25-\( \mu \)l reaction volumes containing 2.5 \( \mu \)l of 10 \( \times \) PCR buffer (100S Tris-HCl, pH 8.3, 500 mmol/L KCl, 15 mmol/L MgCl2), 2 \( \mu \)l deoxynucleotide triphosphates (200 \( \mu \)mol/L each, final concentration), 5 pmol each primer, 1 \( \mu \)l modified DNA (10 ng), and 0.5U Taq polymerase (Hot Start Version, TaKaRa). Touch-down PCR was used for amplification; its condition was 3 min at 95\(^\circ\)C, followed by 8 cycles of denaturation at 95\(^\circ\)C for 20 s, annealing at the specific temperature for 25 s (decreased by 0.5\(^\circ\)C at each cycle), and extension at 72\(^\circ\)C for 30 s. This reaction was followed by 35 cycles of the above-mentioned program in which the annealing temperature has been decreased by 4\(^\circ\)C and a final extension step at 72\(^\circ\)C for 5 min. The PCR products were electrophoresed on 2% agarose gels. Each exper-


Table 1

| Primers Regions | Sequences | Products length(bp) |
|-----------------|-----------|--------------------|
| BCA4-M-F chr20:4844271-480 | GGGTTTATTTAGGTCGGTTTTC | 128 |
| BCA4-M-R | TAACGATCCTCGTCTTCTTC | 128 |
| BCA4-U-F | TAGGTGGAGATGTGTGTGTT | |
| BCA4-U-R | CCAAAACTAACACATTTACTTC | |
| CHRM2-M-F chr7:136204314-622 | TAGATTAGTTAAGTGTTGAGACGA | 133 |
| CHRM2-M-R | ATAAAAATACGAAAAACAGAA | 132 |
| CHRM2-U-F | TAGATTAGTTAAGTGTTGAGATGA | |
| CHRM2-U-R | TAAAAATACACAAAAACAAAAAAACAAA | |
| FAM5C-M-F chr1:18871432-341 | TTTTTTTAGGTTTTATTTTTTTGGC | 101 |
| FAM5C-M-R | TAACTTCTAATACAAAAACGTTCGA | |
| FAM5C-U-F | TAGTTTTATTTAGGTTTTTTAGTTTGGT | 110 |
| FAM5C-U-R | AAAACTCTACTTCTATACAAAAACATTC | |
| PRAC-M-F chr17:44157439-737 | AAAGGGAGGTATTAAATGGTGGATTAC | 126 |
| PRAC-M-R | ACCAAAAATATAATACACATACGAA | 126 |
| PRAC-U-F | TTGGGAGTTATAGTTGAGATATGGG | |
| PRAC-U-R | AAACCAAAAAATATAATACATACAAA | |
| MYLK-M-F chr3:125085114-300 | TAATGGGAGTTATAGGATAACGC | 134 |
| MYLK-M-R | GACCCCGGAATACCAAC | |
| MYLK-U-F | TAATAATGGGAGTTATAGGATAATGT | 134 |
| MYLK-U-R | ACAACCCCAAAAAATACACAAACC | |

The methylation frequencies in serum samples were compared using the $\chi^2$ test. ROC curve analysis was used to assess methylation detection significance for GC diagnosis. The association between clinicopathological characteristics and combined DNA hypermethylation was compared using $\chi^2$ tests. For all tests, probability values less than 0.05 were regarded as statistically significant. All datas were analyzed with SPSS 13.0 for Windows (SPSS, Chicago, IL).

3. Results

3.1. Identification of candidate hypermethylated genes

DNA samples from 9 gastric cancer cell lines and 6 normal gastric mucosas were prepared by MeDIP, and the resulting methylated and unmethylated fractions were hybridized to Nimblegen human CpG island CHIP. Eighty two hypermethylated genes in cancer cells compared with normal mucosas were acquired (Fig. 1). As our aim was to identify novel high pene-trance markers for the GC diagnosis and pre-warning, many loci previously reported as methylated in GC were not included in this study. Instead, 5 genes, BCA4 (breast carcinoma amplified sequence 4), CHRM2 (cholinergic receptor muscarinic 2), FAM5C (family with sequence similarity 5, member C), PRAC (prostate
cancer susceptibility candidate) and MYLK (myosin light chain kinase) that were not previously reported as methylated were selected for the subsequent analysis. Besides, Peak data of four genes were lower than 2 in normal controls except BCAS4. The exact peak data obtained by CHIP analysis for these 5 loci were shown in Table 2.

3.2. Identification of hypermethylated genes in a set of serum samples

To evaluate the significance of the DNA promoter region methylation for diagnosis and pre-warning of GC according to MeDIP assay results, five genes were further examined in the serum samples collected from patients with GC, GPL and NC. Methylation rates of three genes (CHRM2, FAM5C and MYLK) were confirmed to show methylation rates increased with progression from NC to GPL, then to GC (CHRM2 (6.7%, 15.2%, 31.05%), FAM5C (3.3%, 6.5%, 31.0%) and MYLK (6.7%, 28.3%, 70.7%)). However, methylation of BCAS4 and PRAC were shown to be frequently detected in NC and there was no correlation with the histological progression. The DNA methylation rates of these five genes were shown in Fig. 2A and representative examples of methylation analysis of CHRM2, FAM5C and MYLK were shown in Fig. 2B.

3.3. Alteration of DNA methylation rates in pre-and postoperative serum samples of GC patients

We next compared the DNA methylation rates of CHRM2, FAM5C and MYLK in serum collected from 58 GC patients before operation or at the 3rd day post operation. The methylation rate of FAM5C was 31.0% before operation and decreased to 3.5% post operation. Meanwhile, the methylation rate of MYLK decreased from 70.7% (preoperative) to 20.7% (postoperative). Our results showed significant decreases in the hypermethylation status of FAM5C and MYLK from the pre- to postoperative period ($P < 0.001$). However, no notable differences were detected in the hypermethylated CHRM2 between pre- and postoperative sera of GC patients. The DNA methylation rates of these three genes detected in the pre- and postoperative sera are shown in Fig. 3A, and representative examples of the methylation analysis are shown in Fig. 3B.

3.4. Combined detection analysis of FAM5C and MYLK hypermethylation

One of FAM5C and MYLK hypermethylation detected in serum samples as positive for combine detection. ROC curve analysis was used to assess DNA methylation detection significance for GC diagnosis. Area under the ROC curve of FAM5C was 0.639, MYLK was 0.820, combined FAM5C and MYLK was 0.838 (Fig. 4A). Combined detection of FAM5C and MYLK hypermethylation for GC diagnosis was more significant than one single gene. Positive rate of the combined detection in GC sera was 77.6% (45/58), which had a statistical difference in contrast to normal control (10.0%, 3/30) ($P < 0.001$) (Fig. 4B). Combined detection in GPL (30.4%, 14/46) was also had a statistical difference in contrast to normal control ($P = 0.037$) (Fig. 4B). Moreover, there was a significant decrease in the combined detection of FAM5C and MYLK hypermethylation from the pre- (77.6%, 45/58) to postoperative period (24.1%, 14/58) ($P < 0.001$) (Fig. 4C).

Table 2

| Genes     | BCAS4 | CHRM2 | FAM5C | PRAC | MYLK |
|-----------|-------|-------|-------|------|------|
| MKN-45    | 7.03  | 6.03  | 6.26  | < 2.00 | 4.04 |
| SGC-7901  | 5.85  | 4.32  | 4.36  | 3.45  | 3.6  |
| AGS       | 3.15  | 2.99  | < 2.00 | 4.47  | 3.42 |
| HTB-103   | 8.04  | 4.49  | 3.65  | 4.87  | 3.23 |
| MKN-28    | 9.12  | 3.9   | 4.72  | 4.87  | 3.38 |
| BGC823    | 7.16  | 6.48  | 8.66  | 5.39  | 3.22 |
| NCI-N87   | 7.45  | < 2.00 | < 2.00 | < 2.00 | 3.62 |
| NCI-SNU-1 | 4.12  | 2.47  | < 2.00 | < 2.00 | 2.22 |
| NCI-SNU-16| < 2.00 | < 2.00 | < 2.00 | < 2.00 | < 2.00 |
| NC1       | < 2.00 | < 2.00 | < 2.00 | < 2.00 | < 2.00 |
| NC2       | < 2.00 | < 2.00 | < 2.00 | < 2.00 | < 2.00 |
| NC3       | < 2.00 | < 2.00 | < 2.00 | < 2.00 | < 2.00 |
| NC4       | 2.04  | < 2.00 | < 2.00 | < 2.00 | < 2.00 |
| NC5       | < 2.00 | < 2.00 | < 2.00 | < 2.00 | < 2.00 |
| NC6       | < 2.00 | < 2.00 | < 2.00 | < 2.00 | < 2.00 |

NC, normal gastric mucosa.
Fig. 2. Methylation-specific polymerase chain reaction (MSP) analysis of serum samples. Methylation status of five candidate genes (BCAS4, CHRM2, FAM5C, PRAC, and MYLK) in serum samples including GC (n = 58), GPL (n = 46) and NC (n = 30) were detected by MSP. A Histogram representing methylation rates of five candidate genes in serum samples is shown. Three genes (CHRM2, FAM5C, and MYLK) were confirmed to show methylation rates increased with progression from NC to GPL, then to GC. B MSP results for three genes (CHRM2, FAM5C, and MYLK) in representative samples are shown. GC: gastric cancer, GPL: gastric precancerous lesions, NC: normal controls. WM, weight marker; M, PCR product with primers specific for methylated DNA; U, PCR product with primers specific for unmethylated DNA; CM, methylated DNA control; CU, unmethylated DNA control.

3.5. Association between clinicopathological features and combined FAM5C and MYLK methylated pattern in GC sera

The combined hypermethylated status of FAM5C and MYLK in the pretherapeutic sera of the 58 GC patients was analyzed for its association with clinicopathological parameters. No correlation between the methylated status of these two genes and age, differentiation grade and lymph node metastasis was found. However, the combined hypermethylated status of FAM5C and MYLK was shown to correlate with tumor size ($P < 0.001$), tumor invasion depth ($P = 0.001$) and TNM stage ($P = 0.003$) (Table 3).

4. Discussion

In this study, we evaluated the DNA promoter region methylation of five novel genes (BCAS4, CHRM2,
**Fig. 3.** Alteration of DNA methylation rates of **CHRM2**, **FAM5C** and **MYLK** in pre- and postoperative serum samples. Methylation status of **CHRM2**, **FAM5C** and **MYLK** in pre- and postoperative GC serum samples were detected by MSP. A Histogram representing methylation rates of three genes in pre- and postoperative serum samples is shown. There were significant decreases in the hypermethylation status of **FAM5C** and **MYLK** from the pre- to postoperative period ($P < 0.001$). B MSP results for three genes (**CHRM2**, **FAM5C** and **MYLK**) in representative samples is shown. WM, weight marker; M, PCR product with primers specific for methylated DNA; U, PCR product with primers specific for unmethylated DNA; CM, methylated DNA control; CU, unmethylated DNA control.

*FAM5C, PRAC, and MYLK* that were selected on the basis of the MeDIP assay results in NC, GPL (CAG, IM, and GED), and GC patients.

Several studies have addressed the diagnostic utility of epigenetic biomarkers in the detection of human cancer [10]. It is now necessary to develop noninvasive and affordable methods of “serologic biopsy” for the asymptomatic population. With this goal in mind, we evaluated five candidate genes (*BCAS4, CHRM2, FAM5C, PRAC, and MYLK*) in serum samples from patients with GC, GPL, and NC. Detecting aberrant DNA methylation events in patients’ sera is preferred for several reasons. First, compared with conventional serum tumor markers, such as carcinoembryonic antigen (CEA) and carbohydrate antigen (CA) 19-9, DNA methylation markers are associated with higher levels of sensitivity and specificity [11,12]. MSP assays can detect alterations of DNA methylation based on low levels of circulating tumor DNA [13]. Although Pyrosequencing results are the “golden standard” of DNA methylation, this method is very expensive and can just be fit for detection of the small samples. Second, compared with mRNA and protein, DNA is a more stable molecule. Third, in contrast with mutations, the incidence of aberrant DNA methylation is higher, with smaller specific regions of the genome effected [14], thereby making it is easier to detect methylated targets. But MSP assays results were showed by agarose electrophoresis, which was not convenient to clinical application. So we can use the Methylight method [15] to detect DNA methylation aberrant in serum samples and apply it to clinical practice.

Most previous studies focused on single gene rather than multiple genes. We found that the combined detection of hypermethylated **FAM5C** and **MYLK** achieved a more significant diagnosis for GC than one single gene.
Fig. 4. Combined detection of FAM5C and MYLK hypermethylation in GC, GPL and NC serum specimens. A ROC curve which evaluate combined detection significance for GC diagnosis. Combined detection of FAM5C and MYLK hypermethylation for GC diagnosis was more significant than one single gene. B Histogram representing the combined detection rates in serum samples of GC, GPL and NC. Combined detection of FAM5C and MYLK in GC (P < 0.001) and GPL (P = 0.037) sera were higher than in NC. C, histogram representing the combined detection rates in pre- and postoperative serum samples. There was a significant decrease in the combined detection from the pre- to postoperative period.

Table 3
Clinicopathological parameters and combined detection of FAM5C and MYLK hypermethylation in GC serum

| Characteristics          | M   | U   | \( \chi^2 \) | P   |
|--------------------------|-----|-----|-------------|-----|
| Tumor size               |     |     |             |     |
| > 5 cm                   | 15  | 12  | 1.180       | 0.352|
| \( \leq 5 \text{ cm} \)  | 20  | 8   |             |     |
| Differentiation Grade    |     |     |             |     |
| I–II                     | 20  | 8   |             |     |
| III–IV                   | 25  | 5   |             |     |
| Tumor invasion depth     |     |     |             |     |
| T1-2                     | 11  | 10  |             |     |
| T3-4                     | 34  | 3   |             |     |
| Lymph node status        |     |     |             |     |
| N0                       | 21  | 9   | 2.057       | 0.212|
| N1-3                     | 24  | 4   |             |     |
| TNM Stage                |     |     | 9.757       | 0.003|
| I–II                     | 16  | 11  |             |     |
| III–IV                   | 29  | 2   |             |     |

a: M means one gene of FAM5C and MYLK detected as methylation; b: U means both genes of FAM5C and MYLK detected as unmethylation.

Combined detection had a higher sensitivity in GC diagnosis and pre-warning than general methods [16,17]. Furthermore, the obvious decrease of combined detection of hypermethylated FAM5C and MYLK in sera of postoperative GC patients in contrast to in preoperative samples and the combined detection of FAM5C and MYLK methylation status correlates with tumor size, which can effectively reflect tumor burden status and also can help to evaluate the efficiency of operation and follow-up for tumor recurrence. The DNA methylation level can be used to discriminate tumor stages in GC [18]. Besides, we found that combined detection of FAM5C and MYLK methylation status correlates with tumor invasion depth and TNM stage. This correlation can be used for evaluation of disease condition and prognosis.

BCAS4 and PRAC play a negative regulatory role in the development of breast and prostate cancer, respectively [19,20]. Therefore, our findings might explain the high methylation rates in NC. There are currently no reports on the relationship of CHRM2 and cancer. FAM5C is identified as a novel tumor suppressor gene in tongue squamous cell carcinoma [21] and might has similar biological functions in GC. It has been found MYLK inhibitor ML-7 and ML-9 can inhibit invasion of mammary, prostate, and pancreatic cancer cells in vitro [22–24]. MYLK mRNA expression level increases obviously in non-small cell lung cancer cells and is related with recurrence and metastasis [25]. These findings suggest that MYLK may be an oncogene. It seemed to be complicated that MYLK shows DNA hypermethylation in GC. Actually, our study found that some GC highly expressed MYLK, which restricted in the stromal cells (unpublished). Up-regulated expression of MYLK in stroma cells may regulate GC cells proliferation, apoptosis, invasion and migration.

In summary, our findings suggested that combined detection of hypermethylated FAM5C and MYLK might
be a potential method for GC diagnosis and pre-warning, monitoring recurrence and auxiliary judgment of TNM stage. However, it is necessary to enlarge clinical samples and validate the potential significance for the clinical application of these findings.

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References

[1] A. Jemal, F. Bray, M.M. Center, J. Ferlay, E. Ward and D. Forman, Global cancer statistics, CA Cancer J Clin 61 (2011), 69–90.
[2] S. Nomura and M. Kaminishi, Surgical treatment of early gastric cancer, Dig Surg 24 (2007), 96–100.
[3] H. Tomita, A. H irata and Y. Yamada, Suppressing effect of global DNA hypomethylation on gastric carcinogenesis, Carcinogenesis 31 (2010), 1627–1633.
[4] M. Vauhkonen, H. Vauhkonen and P. Sipponen, Pathology and molecular biology of gastric cancer, Best Pract Res Clin Gastroenterol 20 (2006), 651–674.
[5] C.D. Joe, M.A. Kim, E.J. Jung, J. Kim and W.H. Kim, Identification of genes epigenetically silenced by CpG methylation in human gastric carcinoma, Eur J Cancer 45 (2009), 1282–1293.
[6] W. Guo, Z. Dong, Y. Guo, G. Kuang, Z. Yang and Z. Chen, Detection of promoter hypermethylation of the CpG island of E-cadherin in gastric cardiac adenocarcinoma, Eur J Med Res 14 (2009), 453–458.
[7] Y.C. Wang, Z.H. Yu, C. Liu, L.Z. Xu, W. Yu and J. Lu, Detection of RASSF1A promoter hypermethylation in serum from gastric and colorectal adenocarcinoma patients, World J Gastroenterol 14 (2008), 3074–3080.
[8] F. Ksiaa, S. Ziadi, K. Amara, S. Korbli and M. Trimeche, Biological significance of promoter hypermethylation of tumor-related genes in patients with gastric carcinoma, Clin Chim Acta 404 (2009), 128–133.
[9] J. Harder and O.G. Opitz, Gastric cancer- risk factors and medical therapy, Praxis (Bern 1994) 95 (2006), 1021–1028.
[10] T. Ushijima, Detection and interpretation of altered methylation patterns in cancer cells, Nat Rev Cancer 5 (2005), 223–231.
[11] H. Koike, D. Ichikawa, H. Ikoma, E. Otsuji, K. Kitamura and H. Yamagishi, Comparison of methylation-specific polymerase chain reaction (MSP) with reverse transcriptase-polymerase chain reaction (RT-PCR) in peripheral blood of gastric cancer patients, J Surg Oncol 87 (2004), 182–186.
[12] H. Koike, D. Ichikawa, H. Ikoma, N. Yani, D. Ikoma and E. Otsuji, Comparison of serum aberrant methylation and conventional tumor markers in gastric cancer patients, Hepatogastroenterol 52 (2005), 1293–1296.
[13] J.G. Herman, J.R. Graff, S. Mylöhänen, B.D. Nelkin and S.B. Baylin, Methylation-specific PCR: A novel PCR assay for methylation status of CpG islands, Proc Natl Acad Sci USA 93 (1996), 9821–9826.
[14] H.H. Zhou, S.Y. Yan, X.Y. Zhou, X. Du, T.M. Zhang and X. Cai, MLH1 promoter germline-methylation in selected probands of Chinese hereditary non-polyposis colorectal cancer families, World J Gastroenterol 14 (2008), 7329–7334.
[15] C.A. Eads, K.D. Danenberg, K. Kawakami, L.B. Saltz, C. Blake and D. Shibata, MethyLight: a high-throughput assay to measure DNA methylation, Nucleic Acids Res 28 (2000), E32.
[16] Y. Hao, Y. Yu, L. Wang, M. Yan, J. Ji and Y. Qu, IPO-38 is identified as a novel serum biomarker of gastric cancer based on clinical proteomics technology, J Proteome Res 7 (2008), 3668–3677.
[17] M. Carpelan-Holmström, J. Louhimo, U.H. Stenman, H. Alifthan and C. Haglund, CA, CA 19-9 and CA 72-4 improve the diagnostic accuracy in gastrointestinal cancers, Anticancer Res 22 (2002), 2311–2316.
[18] N. Oue, M. Mitani, J. Motoshita, S. Matsumura, K. Yoshida and H. Kuniyasu, Accumulation of DNA methylation is associated with tumor stage in gastric cancer, Cancer 106 (2006), 1250–1259.
[19] M. Lovf, G.O. Thomassen and A.C. Bakken, Fusion gene microarray reveals cancer type-specificity among fusion genes, Genes Chromosomes Cancer 50 (2011), 348–357.
[20] S. Edwards, C. Campbell and P. Flohr, Expression analysis on microarrays of randomly selected cDNA clones highlights HOXB13 as a marker of human prostate cancer, Br J Cancer 92 (2005), 376–381.
[21] T. Kuroiwa, N. Yamamoto, T. Onda and T. Shibahara, Expression of the FAMSC in tongue squamous cell carcinoma, Eur J Med Res 14 (2009), 453–458.
[22] L.Z. Gu, W.Y. Hu, N. Antic, R. Mehta, J.R. Turner and P. Blake and D. Shibata, MethyLight: a high-throughput assay for detection of promoter hypermethylation of the CpG island of the FAM5C in tongue squamous cell carcinoma, Eur J Med Res 14 (2009), 453–458.
[23] K. Kaneko, K. Satoh, A. Masumune, A. SATOH and T. Shimosegawa, Myosin light chain kinase inhibitors can block invasion and adhesion of human pancreatic cancer cell lines, Pancreas 24 (2002), 34–41.
[24] X. Zhou, Y. Liu, J. You, H. Zhang, X. Zhang and L. Ye, Myosin light-chain kinase contributes to the proliferation and migration of breast cancer cells through cross-talk with activated ERK1/2, Cancer Lett 270 (2008), 312–327.
[25] Y. Minamiya, T. Nakagawa, H. Saito, I. Matsuzaki, K. Taguchi and M. Ito, Increased expression of myosin light chain kinase mRNA is related to metastasis in non-small cell lung cancer, Tumour Biol 26 (2005), 153–157.