Epigenetics in diagnosis of colorectal cancer

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ABSTRACT

Colorectal cancer (CRC) is a third most common epithelial carcinoma. CRC is known to develop from the early precancerous lesion to full blown malignancy via definite phases due to cumulative mutations and aberrant methylation of number of genes. The use of serum biomarkers that is non-invasive to discriminate cancer patients from healthy persons will prove to be an important tool to improve the early diagnosis of CRC. This will serve as the boon to the clinical management of the disease.

Keywords: Colorectal Cancer; Epigenetics; Hypermethylation; Biomarkers

INTRODUCTION

Colorectal cancer (CRC) is a multifactorial disease that arises due to the cumulative accumulation of genetics as well as epigenetic alterations in a number of onco-, tumor suppressor-, mismatch repair-, cell cycle- genes in colon mucosa cells [1, 2]. All of these alterations aggregate to drive the critical pathways of CRC initiation and progression along a multistep tumorigenesis process, known as the adenoma-carcinoma sequence or vogelgram [3, 4]. Since CRC has been defined as the heterogenous malignancy, it has several different subtypes, each of which are characterized by distinct genetic, cytogenetic and epigenetic alterations [1, 2, 5, 6]. A number of specific phenotypes of CRC have been identified on the basis of molecular profiles as given by Vogelstein et al., [3] and defined as the genetic instability model of colorectal carcinogenesis. Two major mechanisms of genomic instability in CRC have been given for the evolution of normal mucosa to adenoma and carcinoma namely: chromosomal instability (CIN), microsatellite instability (MSI) [5, 7]. However, a decade of elucidating the alternative mechanism of colorectal carcinogenesis since the discovery of high frequency of aberrant DNA methylation in CpG islands of number of genes by
Toyota et al. [8, 9] and a recent identification of a number of genes that are more frequently methylated in CRC by Lao and Grady [10], epigenetics has been identified as one of the important pathways for CRC carcinogenesis [11] to explain the transformation of the normal mucosa into the malignant one [12]. Hence, CpG island methylator phenotype (CIMP) has been added as the third mechanism for driving the colorectal carcinogenesis [1, 5, 6, 13, 14]. In this minireview, we are aiming to understand the diagnostic value of the methylated genes in serving as biomarkers for early as well as advanced stage CRC detection.

**DNA METHYLATION IN DIAGNOSTICS**

As DNA methylation plays a significant role in CRC initiation it helps in identifying the strong biomarkers (as methylated DNA) for the early detection of CRC. Many studies have identified a number of genes which serve as the potential biomarkers for the CRC [15-20, see Table 1]. The reported sensitivities for blood and stool based CRC DNA methylation biomarkers range in between 90-95% with a specificity ranges of 85-94% [21]. Among the most potential diagnostic biomarkers for CRC, the tumor specific M2 isoform of pyruvate kinase (PKM2) and tissue inhibitor of matrix metalloproteinase 1 (TIMP1), vimentin (VIM) and septin 9 (SEPT9) take a lead in being the most extensively investigated ones [17]. PKM2 has been shown to have a relatively high sensitivity for CRC diagnosis, with sensitivity of over 90% and TIMP1 of 63% in stool for CRC [22, 23]. SEPT9 has been reported to have higher sensitivity (80%-90%) and specificity (80%) [24-26] while as VIM has a sensitivity ranging from 38–88 % [18]. Both SEPT9 and VIM are available in commercially available kits and are widely used for the detection of CRC [18, 24, 27]. Warren et al., [25] had also identified SEPT9 as the specific blood based biomarker for the detection of CRC with the overall sensitivity of 90%. Church et al., [28] also reported the similar observations for the accuracy of circulating methylated SEPT9 DNA to detect CRC, with the sensitivity of 48.2% and 91.5% specificity. A recent study by Carmona et al., [16] observed the potentiality of five selected genes VIM, SEPT9, angiotensin II receptor, type 1 (AGTR1), wingless-type MMTV integration site family member 2 (WNT2), to serve as the biomarkers for non-invasive early detection of colorectal cancer using stool DNA (sDNA). In this study three of five selected genes i.e., AGTR1, WNT2 and slit homolog 2 (SLIT2) were validated in stool DNA of affected patients with a detection sensitivity of 78% [95% confidence interval (CI), 56%–89%]; while as, DNA methylation of VIM and SEPT9 was evaluated in a subset of stool samples yielding sensitivities of 55% and 20%, respectively. Thus, indicating that sDNA test achieved greater sensitivity than SEPT9. A recent study by Ahlquist et al., [26] reported a DNA stool test that detects methylated Bone morphogenic protein 3 (BMP3), NDRG family member 4 (NDRG4), VIM and tissue factor pathway inhibitor 2 (TFPI2), mutant KRAS, the actin beta (ACTB) gene and the quantity of hemoglobin. Lee et al., [29] identified another set of biomarkers i.e., O-6-methylguanine-DNA methyltransferase (MGMT), Ras association (RalGDS/AF-6) domain family member 2 (RASSF2A), and Wnt inhibitory factor 1 (Wif-
I) genes for the early detection of CRC. Another study by Wasserkort et al., [30] also corroborated that SEPT9 is an aberrantly hypermethylated in one of several CpG islands in adenoma and CRC specimens reflect the cellular progression towards malignancy in colon mucosa.

Table 1: Various methylated genes used as potential biomarkers for the detection of CRC

| Study             | Genes methylated in CRC | Biomarker in     |
|-------------------|-------------------------|-----------------|
| Carmona et al16   | VIM, SEPT9, AGTR1, WNT2 | Stool           |
| Fung et al17      | PKM2, VIM, TIMP1, SEPT9 | Serum/Plasma    |
| Kim et al.19      | ADHFE1, BOLL, SLC6A15, ADAMTS5, TFPI2, EYA4, NPY | Tissue         |
| Ahlquist et al10  | BMP3, NDRG4, VIM, TFPI | Stool           |
| Lee et al10       | MGMT, RASSF2A, Wf-1     | Plasma          |
| Wasserkort et al  | SEPT9                   | Tissue          |
| Imperiale et al.11| BMP3, NDRG4             | Stool           |
| Melotte et al.32  | NDRG4                   | Stool           |
| Silva et al.98    | RUNX3, PCDH1, SFRP5, IGF, Hnflb | Tissue         |
| Ogino et al.71    | RUNX3, CACNA1G, IGF2, MLH1 | Tissue         |
| Wallner et al.38  | HLF, HPP1/TPEF, hMLH   | Serum           |
| Philipp et al.19  | HLF, HPP1               | Serum           |
| Tanzer et al.77   | ALX4, SEPT9             | Serum           |
| Lofton-Day et     | SEPT9, TMEFF2, NGFR    | Plasma          |
| Védéled et al.52  | DLCK1                   | Tissue          |
| Mitchell et al.99 | SOX21, SLC6A1, NPY, GRASP, SBSS1A1, ZSCAN18 | Stool         |
| Mitchell et al.50 | BCAT1, COLAA2, DLX5, FGF5, FOX1, FOXI2, GRASP, IKZF1, IRF4 | Blood         |
| Roperch et al.41  | NPY, PENC, Wf-1         | Tissue and serum|
| Ahn et al.57      | WNT5A, SFRP1, SFRP2, hMLH1, p16, p14, MINT1, MINT2, MINT3 | Tissue         |
| Lind et al.46     | CNP1, FBNI, INA, SNCA, MA1, SPG20 | Tissue         |

In this year’s pioneer study by Imperiale et al., [31] a noninvasive, multitarget stool DNA test was compared with fecal immunochemical test (FIT) for the detection of colorectal cancer. It was observed by them, that multitarget stool DNA testing detected significantly more cancers than did FIT but had more false positive results, with sensitivity for detecting CRC was 92.3% using stool DNA testing and 73.8% with FIT (P = 0.002). The multitarget stool DNA test consists of molecular assays for aberrantly methylated BMP3 and NDRG4 promoter regions, mutant KRAS, and β-actin (a reference gene for human DNA quantity), as well as an immunochemical assay for human hemoglobin. The study by Melotte et al., [32] had the similar results with another gene namely NDRG4. They found that a significant promoter hypermethylation of NDRG4 promoter in CRC tissue when compared to normal colon tissue, and hence identified NDRG4 as a potential CRC biomarker in stool. Furthermore a number of studies identified a Kunitz-type serine proteinase inhibitor, namely TFPI2, as a potential sDNA marker as well as a prognostic marker for CRC [33-35].

In another study by Silva et al. [36], a group of five genes i.e., runt-related transcription factor 3 (RUNX3), protocadherin 10 (PCDH10), secreted frizzled-related protein 5 (SFRP5), insulin-like growth factor 2 (IGF2) and hepatocyte nuclear factor 1β (Hnf1b) were found to be having highest percentage of methylation within their
promoter regions and consequently with highest repression of gene expression in CRC patients. Hence, they were identified to be, therefore, the most promising biomarkers for the diagnosis of CRC. Ogino et al., [37] had previously identified the panel of eight genes: \textit{RUNX3}, calcium channel, voltage-dependent, T type, alpha 1G subunit (\textit{CACNA1G}), \textit{IGF2}, mutL homolog 1 (\textit{MLH1}), neurogenin 1 (\textit{NEUROG1}), cellular retinoic acid binding protein 1 (\textit{CRABP1}), suppressor of cytokine signaling 1 (\textit{SOCS1}), and cyclin-dependent kinase inhibitor 2A (\textit{CDKN2A}) out of which at least four (\textit{RUNX3}, \textit{CACNA1G}, \textit{IGF2}, and \textit{MLH1}) were identified to serve as a sensitive and specific marker panel for CIMP-high.

Wallner et al., [38] in their multivariate analysis identified three methylation markers: helicase-like transcription factor (\textit{HLTF}), hyperpigmentation, progressive 1/transmembrane protein containing epidermal growth factor and follistatin domain (\textit{HPP1}/\textit{TPEF}), and \textit{hMLH} in serum of colorectal cancer patients to be independently associated with poor outcome and a relative risk of death. Hence, these genes were identified as pre-therapeutic predictor of the outcome of disease. A separate study by Philipp et al., [39] reported that the methylation of \textit{HLTF} and \textit{HPP1} DNA in serum was significantly associated with tumor size, stage, grade and metastatic disease and hence were identified as independent prognostic factors in metastasized CRC. Tanzer et al., [40] observed that serum methylated DNA from advanced precancerous colorectal lesions can be detected using a panel of two DNA methylation markers, aristless-like homeobox 4 (\textit{ALX4}) and \textit{SEPT9}. They observed a significantly higher frequency of \textit{ALX4} and \textit{SEPT9} methylated DNA in plasma from patients with polyps as well as colorectal adenomas versus healthy controls. Both these markers had a sensitivity and specificity of 71\% and 95\%, respectively, for the detection of advanced precancerous colorectal lesions. Another pioneer study on the blood-based detection of methylated DNA by Lofton-Day et al., [41] identified three genes: \textit{SEPT9}, transmembrane protein with EGF-like and two follistatin-like domains 2 (\textit{TMEFF2}) and nerve growth factor receptor (\textit{NGFR}), to serve as sensitive biomarkers for CRC. \textit{SEPT9} methylation was detected in 69\% of plasma samples from CRC patients, while as \textit{TMEFF2} and \textit{NGFR} methylation status were 65\% and 51\% respectively.

Another study by Vedeled et al., [42] identified a new gene double cortin-like kinase 1 (\textit{DLCK1}) promoter hypermethylation as a promising novel epigenetic biomarker for early detection of CRC. They observed a significant negative correlation between \textit{DCLK1} methylation pattern and expression in 74 cancer cell lines derived from 15 different tissues. The gene expression also showed a direct correlation with epigenetic drug induced silencing which increased significantly after drug treatment of initially methylated cancer cell lines. However the testing being invasive has certain limitation in putting it into practice. Mitchell et al., [43] identified a panel of 23 genes that show elevated DNA methylation in >50\% of CRC tissue in comparison to non-cancerous tissue. These 23 genes consisted of collagen type 1 alpha 1 (\textit{COL1A2}), collagen type IV alpha 1 (\textit{COL4A1}) Collagen type IV alpha 2 (\textit{COL4A2}), distal-less homeobox 5 (\textit{DLX5}), EGF-like repeats and discoidin I-like domains 3 (\textit{EDIL3}), EGF containing fibulin-like extracellular matrix protein 2 (\textit{EFEMP}), Fibrillin 1 (\textit{FBN1}), fibroblast growth factor 5 (\textit{FGF5}), forkhead box B1 (\textit{FOXB1}), forkhead box D2 (\textit{FOXD2}),
forkhead box F1 (FOXF1), general receptor for phosphoinositides 1 (GRASP), iroquois-related homeobox 1 (IRX1), meis homeobox 1 (MEIS1), matrix metallopeptidase 2 (MMP2), neuropeptide Y gene (NPY), pancreatic and duodenal homeobox 1 (PDX1), protein phosphatase 1 regulatory inhibitor subunit 14A (PPP1R14A), syndecan 2 (SDC2), Sry-related HMG box 21 (SOX21), sushi domain containing 5 (SUSD5), transcription factor 21 (TCF21) and Zinc Finger Protein 471 (ZNF471). Out of 23, six genes (SOX21, solute carrier family 6 member 15 (SLC6A15), NPY, GRASP, sialyltransferase 8 (alpha-N-acetylneuraminate: alpha-2,8-sialyltransferase, GD3 synthase A1 (ST8SIA1) and zinc finger & SCAN domain containing 18 (ZSCAN18) show very low methylation in non-cancerous colorectal tissue and hence were identified as candidate biomarkers for stool-based assays, while as 11 genes (branched chain amino-acid transaminase 1 (BCAT1), COL4A2, DLX5, FGF5, FOXF1, FOXI2, GRASP, ikaros family zinc finger protein1 (IKZF1), interferon regulatory factor 4 (IRF4), SDC2 and SOX21) have very low methylation in peripheral blood DNA and hence were suitable for blood-based diagnostic markers as these 11 genes were found to be hypermethylated in at least 70% of cancerous tissues. Roperch et al., [44] observed that NPY, proenkephalin (PENK), and Wnt inhibitory factor 1 (WIFI) can be used as combined epigenetic markers for the diagnosis of CRC, both in tissue and serum. Ahn et al., [45] identified wingless-type MMTV integration site family, member 5A (WNT5A), secreted frizzled-related protein 1 (SFRP1), secreted frizzled-related protein 2 (SFRP2), human mutL homolog 1 (hMLH1), p16, p14, methylated in tumor (MINT1, MINT2, and MINT31) to be good prognostic markers of CIMP+ CRC. Lind et al., [46] identified six different highly sensitive and specific biomarkers CNIP1, FBN1, internexin neuronal intermediate filament protein, alpha (INA), synuclein alpha SNCA, myelin and lymphocyte protein gene (MAL), and SPG20, all of which displayed significantly higher methylation pattern in both adenomas and carcinomas. In addition co-methylation of all six genes was detected in 99% of CRC samples and in 90% of adenomas.

Kim et al., [19] have also identified ten gene biomarkers: alcohol dehydrogenase, iron containing 1 (ADHFE1), Boule-like RNA-binding protein (BOLL), SLC6A15, ADAM metallopeptidase with thrombospondin type 1 motif 5 (ADAMTS5), TFP12, eyes absent homolog 4 (EYA4), NPY, twist family BHLH transcription factor 1 (TWIST1), laminin, alpha 1 (LAMA1), and growth arrest-specific 7 (GAS7) and two genes: maelstrom spermatogenic transposon silencer (MAEL), SFT2 domain containing 3 (SFT2D3) showing hypomethylation in CRC tissues. The study analyzed the methylation profile of 27,578 CpG sites spanning more than 14,000 genes in CRC.

In conclusion, the focus of this article was on the use of various methylated genes to be used as the diagnostic markers for the early detection of CRC for the better clinical management of the disease. Currently, fecal occult blood test (FOBT) is the only screening modality used for the detection of CRC together with CEA to monitor the therapy in advanced CRC. A number of companies working in junction with FDA are now focusing to utilize the sensitivity of many methylation sensitive genes like SEPT9, TIMP-1, NGFR to be used as early markers with a simple blood testing kits available over the counter for clinical use [47, 48].
Conflict of Interest: The authors declare that they have no competing interest.

REFERENCES

1. Migliore L, Igheli F, Spisni R, Coppede F. Genetics, Cytogenetics and Epigenetics of colorectal cancer. J Biomed Biotech 2011; 792362. doi:10.1155/2011/792362.
2. Grady WM, Ulrich CM. DNA alkylation and DNA methylation: cooperating mechanisms driving the formation of colorectal adenomas and adenocarcinomas? Gut 2007;56:318-320.
3. Vogelstein B, Fearon ER, Hamilton SR, Kern SE, Preisinger AC, Leppert M, Nakamura Y, White R, Smits AM, Bos JL. Genetic alterations during colorectal-tumor development. N Engl J Med 1988;319:525-532.
4. Fearon ER and Vogelstein B. A genetic model for colorectal tumorigenesis. Cell 1990;61:759-767.
5. Cunningham D, Atkin W, Lenz HJ, Lynch HT, Minsky B, Nordlinger B, Starling N. Colorectal cancer. Lancet 2010;375:1030-1047.
6. Perea J, Lomas M, Hidalgo M. Molecular basis of colorectal cancer: Towards an individualized management? Rev Esp Enférn Dig 2011;103:29-35.
7. Sameer AS. Colorectal cancer: molecular mutations and polymorphisms. Front Oncol 2013;3:114.
8. Toyota M, Ahuja N, Ohe-Toyota M, Herman JG, Baylin SB, Issa JP. CpG island methylator phenotype in colorectal cancer. Proc Natl Acad Sci USA 1999;96:8681-8686.
9. Toyota M, Ho C, Ahuja N, Jair KW, Li Q, Ohe-Toyota M, Baylin SB, Issa JP. Identification of differentially methylated methylated sequences in colorectal cancer by methylated CpG island amplification. Cancer Res 1999;59:2307-2312.
10. Lao VV, Grady MW. Epigenetics and colorectal cancer. Nat Rev Gastroenterol Hepatol 2011;8:686-700.
11. Wong JJL, Hawkins NJ, Ward RL. Colorectal cancer: a model for epigenetic tumorigenesis. Gut 2007;56:140-148.
12. Jass JR, Iino H, Ruszkiewicz A, Painter D, Solomon MJ, Koorey DJ, Cohn D, Furlong KL, Walsh MD, Palazzo J, Edmonston TB, Fishel R, Young J, Leggett BA. Neoplastic progression occurs through mutator pathways in hyperplastic polyposis of the colorectum. Gut 2000;47:43-49.
13. Miglieli F, Migliore L. Epigenetics of colorectal cancer. Clin Genet 2012;81:312–318.
14. Snover DC. Update on serrated pathway to colorectal carcinoma. Hum Pathol 2011; 42:1-10.
15. Vaiopoulos AG, Athanasoula KC, Papavassiliou AG. Epigenetic modifications in colorectal cancer: Molecular insights and therapeutic challenges. Biochim Biophys Acta 2014;1842:971-980.
16. Carmona FJ, Azuara D, Berenguer-Llergo A, Fernández AF, Biondo S, de Oca J, Rodriguez-Moranta F, Salazar R, Villanueva A, Fraga MF, Guardiola J, Capellá G.
Esteller M, Moreno V. DNA methylation biomarkers for noninvasive diagnosis of colorectal cancer. Cancer Prev Res 2013;6:656-665.

17. Fung KYC, Nice E, Priebe I, Belobrjadic D, Phatak A, Purins L, Tabor B, Pompeia C, Lockett T, Adams TE, Burgess A, Cosgrove L. Colorectal cancer biomarkers: To be or not to be? Cautionary tales from a road well travelled. World J Gastroenterol 2014;20:888-898.

18. Gyparaki MT, Basdra EK, Papavassiliou AG. DNA methylation biomarkers as diagnostic and prognostic tools in colorectal cancer. J Mol Med 2013;91:1249-1256.

19. Kim YH, Lee HC, Kim SY, Yeom YI, Ryu KJ, Min BH, Kim DH, Son HJ, Rhee PL, Kim JJ, Rhee JC, Kim HC, Chun HK, Grady WM, Kim YS. Epigenomic analysis of aberrantly methylated genes in colorectal cancer identifies genes commonly affected by epigenetic alterations. Ann Surg Oncol 2011;18:2338-2347.

20. Levin B. Molecular screening testing for colorectal cancer. Clin Cancer Res 2006;12:5014-5017.

21. Lange CP, Laird PW. Clinical applications of DNA methylation biomarkers in colorectal cancer. Epigenomics 2013;5:105-108.

22. Hardt PD, Toepler M, Ngoumou B, Rupp J, Kloer HU. Measurement of fecal pyruvate kinase type M2 (tumor M2-PK) concentrations in patients with gastric cancer, colorectal cancer, colorectal adenomas and controls. Anticancer Res 2003;23:851-853.

23. Holten-Andersen MN, Christensen IJ, Nielsen HJ, Stephens RW, Jensen V, Nielsen OH, Sørensen S, Overgaard J, Lilja H, Harris A, Murphy G, Brünner N. Total levels of tissue inhibitor of metalloproteinases 1 in plasma yield high diagnostic sensitivity and specificity in patients with colon cancer. Clin Cancer Res 2002;8:156-164.

24. Tóth K, Sipos F, Kalmár A, Patai AV, Wichmann B, Stoehr R, Golcher H, Schellerer V, Tulassay Z, Molnár B. Detection of methylated SEPT9 in plasma is a reliable screening method for both left- and right-sided colon cancers. PLoS One 2012;7:e46000.

25. Warren JD, Xiong W, Bunker AM, Vaughn CP, Furtado LV, Roberts WL, Fang JC, Samowitz WS, Heichken KA. Septin 9 methylated DNA is a sensitive and specific blood test for colorectal cancer. BMC Med 2011;9:133.

26. Ahlquist DA, Taylor WR, Mahoney DW, Zou H, Domanico M, Thibodeau SN, Boardman LA, Berger BM, Lindgard GP. The stool DNA test is more accurate than the plasma septin 9 test in detecting colorectal neoplasia. Clin Gastroenterol Hepatol 2012;10:272-277.

27. Ned RM, Melillo S, Marrone M. Fecal DNA testing for colorectal cancer screening: the ColoSure™ test. PLoS Curr 2011;3:RRN1220.

28. Church TR, Wandell M, Lofton-Day C, Mongin SJ, Burger M, Payne SR, Castaños-Vélez E, Blumenstein BA, Rösch T, Osborn N, Snover D, Day RW, Ransohoff DF. Prospective evaluation of methylated SEPT9 in plasma for detection of asymptomatic colorectal cancer. Gut 2014;63:317-325.

29. Lee BB, Lee EJ, Jung EH, Chun HK, Chang DK, Song SY, Park J, Kim DH. Aberrant methylation of APC, MGMT, RASSF2A, and Wif1 genes in plasma as a
biomarker for early detection of colorectal cancer. Clin Cancer Res 2009;15:6185-6191

30. Wasserkort R, Kalmar A, Vacez G, Spisak S, Krispin M, Toth K, Tulassay Z, Slodziewski AZ, Molnar B. Aberrant septin 9 DNA methylation in colorectal cancer is restricted to a single CpG island. BMC Cancer 2013;13:398.

31. Imperiale TF, Ransohoff DF, Itzkowitz SH, Levin TR, Lavin P, Lidgard GP, Ahlquist DA, Berger BM. Multitarget Stool DNA Testing for Colorectal-Cancer Screening. N Engl J Med 2014;370:1287-1297.

32. Melotte V, Lentjes MHFM, van den Bosch SM, Hellenbrekers DMEI, de Hoon JPJ, Wouters KAD, Daenen KJL, Partoun- Hendriks IEJM, Stresses F, Louwagie J, Smits KM, Weijenberg MP, Sanduleanu S, Khalid-de Bakker CA, Oort FA, Meijer GA, Jonkers DM, Herman JG, de Bruïne AP, van Engeland M. N-Myc downstream-regulated gene 4 (NDRG4): a candidate tumor suppressor gene and potential biomarker for colorectal cancer. J Natl Cancer Inst 2009;101:916-927.

33. Glöckner SC, Dhir M, Yi JM, McGarvey KE, Van Neste L, Louwagie J, Chan TA, Kleeberger W, de Bruïne AP, Smits KM, Khalid-de Bakker CA, Jonkers DM, Stockbrügger RW, Meijer GA, Oort FA, Iacobuzio-Donahue C, Bierau K, Herman JG, Baylin SB, van Engeland M, Schuebel KE, Ahuja N. Methylation of TFPI2 in stool DNA: a potential novel biomarker for the detection of colorectal cancer. Cancer Res 2009;69:4691-4699.

34. Hibi K, Goto T, Kitamura YH, Yokomizo K, Sakuraba K, Shirahata A, Mizukami H, Saito M, Ishibashi K, Kigawa G, Nemoto H, Sanada Y. Methylation of TFPI2 gene is frequently detected in advanced well differentiated colorectal cancer. Anticancer Res 2010;30:1205-1207.

35. Hibi K, Goto T, Shirahata A, Saito M, Kigawa G, Nemoto H, Sanada Y. Detection of TFPI2 methylation in the serum of colorectal cancer patients. Cancer Lett 2011;311:96-100.

36. Silva TD, Vidigal VM, Felipe AV, DE Lima JM, Neto RA, Saad SS, Forones NM. DNA methylation as an epigenetic biomarker in colorectal cancer. Oncol Lett 2013;6:1687-1692.

37. Ogino S, Kawasaki T, Kirkner GJ, Kraft P, Loda M, Fuchs CS. Evaluation of markers for CpG island methylator phenotype (CIMP) in colorectal cancer by a large population-based sample. J Mol Diagn 2007;9:305-314.

38. Wallner M, Herbst A, Behrens A, Crispin A, Stieber P, Göke B, Lamerz R, Kolligs FT. Methylation of serum DNA is an independent prognostic marker in colorectal cancer. Clin Cancer Res 2006;12:7347-7352.

39. Philipp AB, Stieber P, Nagel D, Neumann J, Spelsberg F, Jung A, Lamerz R, Herbst A, Kolligs FT. Prognostic role of methylated free circulating DNA in colorectal cancer. Int J Cancer 2012;131:2308-2319.

40. Tanzer M, Balluff B, Distler J, Hale K, Leodolter A, Röcken C, Molnar B, Schmid R, Loffon-Day C, Schuster T, Ebert MP. Performance of epigenetic markers SEPT9 and ALX4 in plasma for detection of colorectal precancerous lesions. PLoS One 2010;5:e9061.
41. Lofton-Day C, Model F, Devos T, Tetzner R, Distler J, Schuster M, Song X, Lesche R, Liebenberg V, Ebert M, Molnar B, Grützmann R, Pilarsky C, Sledziewski A. DNA methylation biomarkers for blood-based colorectal cancer screening. Clin Chem 2008;54:414-423.

42. Vedeld HM, Skotheim RI, Lothe RA, Lind GE. The recently suggested intestinal cancer stem cell marker DCLK1 is an epigenetic biomarker for colorectal cancer. Epigenetics 2014;9:346-350.

43. Mitchell SM, Ross JP, Drew HR, Ho T, Brown GS, Saunders NF, Duesing KR, Buckley MJ, Dunne R, Beetsen I, Rand KN, McEvoy A, Thomas ML, Baker RT, Wattchow DA, Young GP, Lockett TJ, Pedersen SK, Lapointe LC, Molloy PL. A panel of genes methylated with high frequency in colorectal cancer. BMC Cancer 2014;14:54.

44. Roperch JP, Incitti R, Forbin S, Bard F, Mansour H, Mesli F, Baumgaertner I, Brunetti F, Sobhani I. Aberrant methylation of NPY, PENK, and WIF1 as a promising marker for blood-based diagnosis of colorectal cancer. BMC Cancer 2013;13:566.

45. Ahn JB, Chung WB, Maeda O, Shin SJ, Kim HS, Chung HC, Kim NK, Issa JP. DNA methylation predicts recurrence from resected stage III proximal colon cancer. Cancer 2011;117:1847-185.

46. Lind GE, Danielsen SA, Ahlquist T, Merok MA, Andresen K, Skotheim RI, Hektoen M, Rognum TO, Meling GI, Hoff G, Brethauer M, Thuis-Evensen E, Nesbakken A, Lothe RA. Identification of an epigenetic biomarker panel with high sensitivity and specificity for colorectal cancer and adenomas. Mol Cancer 2011;10:85-99.

47. Warton K, Samimi G. Methylation of cell-free circulating DNA in the diagnosis of cancer. Front Mol Biosci 2015;2:13.

48. Tanaka T, Tanaka M, Tanaka T, Ishigamori R. Biomarkers for colorectal cancer. Int J Mol Sci 2010;11:3209-3225.