Review Article | DOI 10.3349/ymj.2009.50.3.331 pISSN: 0513-5796, eISSN: 1976-2437 | Yonsei Med J 50(3): 331-334, 2009 | YMJ

Genetic and Epigenetic Marker-Based DNA Test of Stool Is a Promising Approach for Colorectal Cancer Screening

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Colorectal cancer (CRC) is one of the most common malignancies and leading cause of cancer-related deaths in the world.[1] However, it may be treated effectively by surgical removal of the cancerous tissue if detected at early stages. Conventional tools for screening CRC are either invasive or inaccurate. Therefore, there is an urgent need to develop a reliable screening tools for CRC to significantly reduce its morbidity. In this regard, a novel DNA markers-based detection in stool is emerging as a promising approach.

Key Words : Colorectal cancer screening, stool DNA, genetic and epigenetic alterations

Received: May 2, 2009
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INTRODUCTION

Colorectal cancer remains as one of the most common cancers worldwide.[2] Although screening can reduce cancer-related mortality, only a modest decrease in mortality has been achieved by conventional screening program. Furthermore, only less than 50% of the population has been appropriately screened. The availability of an accurate, non-invasive, cost-effective screening tool might increase the number of participants in screening program who are reluctant to undergo more invasive tests. Most of sporadic colorectal cancers develop as the result of progressive accumulation of genetic and epigenetic alterations that lead to the transformation of normal colonic epithelium to colon adenocarcinoma.[3] Given the fact that epithelial cells of the colon including tumor cells are continually exfoliated and mixed into stool,[4] genomic DNA can be isolated from these tumor cells, and alterations of DNA in colon polyps and cancer can be analyzed. (Epi)mutational profiles of each tumor cell in tumorigenesis are highly variable, therefore, identification of a specific panel of genes that are altered in the vast majority of precancerous and cancer cells is critical to increase the test sensitivity. Several types of alterations, including genetic and epigenetic, of genomic DNA in stool of colorectal cancer patients have been evaluated for potential use as biomarkers for cancer screening.

What is stool DNA testing?

To avoid misconception hereby, we define stool DNA test as a DNA-based diagnostic test for colorectal cancer whereas genetic testing as an analysis of germ-line DNA from blood lymphocytes to define a hereditary condition such as familial adenomatous polyposis or Lynch syndrome.

GENETIC ALTERATIONS

Point mutations of genes

Point mutations of following three genes that are often implicated in CRC have been targeted for stool DNA markers: 1) Adenomatous polyopsis coli (APC) residing on chromosome 5q, tumor suppressor gene. Multiple point mutations occur early in the development of an adenoma. 2) K-ras, found on chromosome 12, oncogene. Multiple point mutations of this gene frequently appear during CRC development following APC mutations. 3) TP53, tumor suppressor gene, located on chromosome 17p. Mutations at this gene are often found later in cancer progression and associated with larger adenoma with more severe grade of dysplasia.

Microsatellite instability (MI)

As consequence of mutations of mismatch repair gene products, microsatellites and repeating sequences of DNA become instable. One of microsatellites, BAT-26 which is single locus of 26 consecutive adenine nucleotides, is associated with loss of function of a mismatch gene. Thus, alteration in microsatellite on the BAT-26 locus has often been used as a maker in stool DNA test.[1]

Long -fragment human DNA: DNA integrity

Normally, genomic DNA is fragmented into short length (< 200 bp) by endonucleases during apoptosis. Longer fragment human DNAs (larger than 200 bp) exist more abundantly in stool-derived DNA samples because neoplastic cells resistant to apoptosis are exfoliated into colonic lumen. This observation suggests that DNA integrity may be used as a marker for stool DNA test.[5-7]

Evaluation of multi-target genetic markers for stool DNA test

Small scale pilot study for testing multitarget genetic markers in stool for early detection of colorectal neoplasia includes 21 mutations in APC, K-Ras and p53 as well as BAT-26 and DNA Integrity Assay (DIA) which was developed by Exact Sciences and marketed as Pre-GenPlus (version I) by Lab Corp. These approaches detected adenocarcinoma and adenoma with sensitivity ranging from 62% to 91% and from 27% to 82%, respectively. Specificity was high, ranging from 93% to 98%, for subjects without colorectal neoplasia.[7] However, in the most prominent large scale prospective screening trial, the same markers as used in version I test had a lower sensitivity of 52% for invasive and 18% for advanced cancer, although the specificity of this particular assay was 94%.[8] This lower sensitivity was attributed to unexpected low rate of positivity for DIA. Later, however, it was known that the suboptimal performance of DIA was due to DNA degradation during specimen mailing to the lab. Indeed, better sensitivity of this marker was achieved with improved condition of sample preparation.[9] According to Song et al. who estimated clinical and economic consequences of fecal DNA testing and compared them with conventional CRC screening using Markov model, the sensitivity of the panel of DNA markers in detecting adenocarcinoma and adenoma in stool needs to be increased to 65% and 40%, respectively.[12] In order for DNA testing in stool to be cost effective, the cost per test needs to be adjusted to $195. Experimental strategies that have improved analytical sensitivity of PCR to detect very low number of copies of tumor DNA in stool enhanced the performance of fecal DNA testing further. BEAMing technology identified DNA mutations in stool (92%; 23 of 25 stool DNA from CRC patients) and a digital melt curve (DMC) assay showed a high level of sensitivity in detecting individuals with colorectal neoplasms (90%; 28 of 31 tumors) and better performance in detecting those with advanced adenoma (59%; 16 of 27 advanced adenoma).[10,11] In these studies, however, they did not comment on the specificity. As a general rule, sensitivity is increased at the expense of clinical specificity. Thus, additional refinements that maximize sensitivity and specificity for both advanced adenomas and cancer are necessary before widespread clinical implementation.

Epigenetic alterations

Aberrant DNA methylation is a major epigenetic change that is early and common event in human tumorigenesis.[13-16] Methylation at a specific cytosine residue of CpG site is a key component of the epigenetic mechanism that is associated with changes in gene expression without change of DNA sequence in tumorigenesis. In CRC, several genes have been identified that are aberrantly hypermethylated while not in normal mucosa. There have been several attempts to utilize such tumor-associated DNA methylation as a biomarker for potential early detection of colon cancer.

Candidate gene approach to methylation markers for stool DNA test

Epigenetic studies have focused on analyzing single gene methylation as a candidate for a potential biomarker for stool DNA-based screening of CRC. Muller et al.[17] assessed stool DNA for SFRP2 methylation from three independent sets of patients with CRC with sensitivity of 77% to 90% and a specificity of 77%.Three different groups also reported comparable data for SFRP2 methylation in stool DNA, with a sensitivity of 87% to 94% and a specificity of 85% to 90%.[18-20] Other single methylation marker assessment such as SFRP1 gene for methylation status in stool reported a sensitivity of 89% and specificity of 86% in stool samples from 36 patients with colorectal neoplasia (7 adenoma, 29 colorectal cancer) and 17 healthy control subjects.[21] Chen et al. found that vimentin gene is transcriptionally inactive in

normal colon epithelial cells with very low level of methylation on CpG region upstream of the first exon and continuing across the first two-thirds of the first exon. This region becomes highly methylated in primary tumor of colon cancer. They detected aberrant hypermethylation in 83% (38 of 46) and 53% (57 of 107) of primary tumor tissues from two independent groups of colon cancer. However, in its recapitulation test in stool-derived DNA, they detected only 46% from third set of 94 colon cancer patients versus 10% from normal colonoscopy.[22] Itzkowitz and colleagues[9] further validated vimentin methylation as a marker for cancer detection in stool DNA under better condition of DNA preparation and obtained a remarkably improved sensitivity of 72.5% (29 of 40 patients) and a specificity of 86.9% (16 of 122 normal individuals).

Combination of vimentin methylation test plus DNA Integrity Assay (DIA)

Imperiale et al.[8] analysed the performance of combination markers for stool DNA test, namely as second generation assay, using the same markers of version I used in the previous multicenter study plus vimentin methylation as a marker. They improved the way of sample collection with new DNA stabilization buffer which was developed for preventing DNA degradation[23] and gene-based DNA capture to enhance DNA extraction from stool.[23,24] Stool samples from 40 patients with CRC and 122 subjects with normal colonoscopy were used for analysis. The sensitivity of the version I markers increased from 51.6% to 72.5% due to improved performance of DIA from 3.2% to 65%. Aberrant methylation of vimentin gene alone provided high values of sensitivity and specificity of 72.5% and 89.9%, respectively, that are very similar to those of panel of version I markers. The least complex assay, using combination of only two markers consisting of vimentin and the best marker of DIA components, yielded a maximum sensitivity of 87.5% and specificity of 82%. DNA integrity as a biomarker for CRC screening remains to be determined. It has been known that the stability of long-length genomic DNA is not constantly maintained during colon transit and fecal storage. Indeed, long-length DNAs are detected more frequently in left-side cancer than right-side one. Furthermore, long length DNA may not be specific for neoplasia of CRC, because its presence could be attributed to increased inflammation or field cancerlization characterized by enhanced proliferation and apoptosis resistance.[7,25] Notably, single marker test for vimentin methylation has replaced 23 markers test which had previously been developed by Exact-Sciences and is currently the only commercially available stool DNA test through Lab Corp in the name of ColoSure™ ($240 per test), although the performance of vimentin methylation in detection of advanced adenomas has not yet been defined.[9]

Genome-wide approach in identification of novel methylation markers

Glockner et al.[26] reported recently that hypermethylation of tissue factor pathway inhibitor 2 (*TFPI2)* gene in stool could be utilized as a marker for CRC screening. As a frequently methylated gene in human CRC, they used gene expression microarray-based strategy to identify the methylation of *TFPI2* gene. This gene was reactivated in their microarray analysis by methylation inhibiting agent, DAC, and was frequently found to be methylated in colon cancer cell lines. Many previous studies showed aberrant methylation of *TFPI2* gene in various cancers.[27-32] They detected aberrant methylation of this gene by methylation specific PCR in 94% of invasive errated adenoma (16 of 17), 100% of tubular adenomas (17 of 17), and 100% of vilous adenomas (22 of 22), all of which represent preinvasive stages, and in 99% of invasive colorectal cancer states I to IV (114 out of 115), almost all colorectal adenoma. They observed *TFPI2* methylation in stool DNA from stage I to III CRC patients with a sensitivity of 76% to 89% and a specificity of 79% to 93%. However, for adenoma, although more than 95% sensitivity was detected in primary tumor tissues, only 21% to 43% sensitivity was achieved for stool DNA samples, indicating that fewer cells are shed by adenomas.

CONCLUSION

Detection of DNA molecular markers in stool is an exciting new technology that shows increasing promise as a way to screen colorectal cancer. Detecting premalignant adenoma is the key target of any approach of screenings which aim at preventing colorectal cancer. In this point, stool DNA testing with molecular markers has shown to be superior to fecal occult blood testing for adenoma detection. However, given clinical and economical consequence compared with colonoscopy, additional refinements of panel of biomarkers that maximize sensitivity and specificity for both advanced adenomas and cancer and cost-effectiveness are necessary. There are many new approaches in the development of high-performance molecular markers that are ideal screening tools which are sensitive, specific, cost-effective, and user friendly for clinical implementation. Individuals with a positive stool DNA test are referred for colonoscopy. Ultimately, introduction of new stool-based DNA test with a panel of molecular markers is expected to encourage participation in screening program of colorectal cancer.

ACKNOWLEDGEMENTS

This study was supported by a grant (Project No. 0405-BC01-0604-0002) of Ministry for Health, Welfare and Family affairs.

REFERENCES

1. Dong SM, Traverso G, Johnson C, Geng L, Favis R, Boynton K, *et al*. Detecting colorectal cancer in stool with the use of multiple genetic targets. J Natl Cancer Inst 2001;93:858-65.
2. Parkin DM. Global cancer statistics in the year 2000. Lancet Oncol 2001;2:533-43.
3. Grady WM, Markowitz SD. Genetic and epigenetic alterations in colon cancer. Annu Rev Genomics Hum Genet 2002;3:101-28.
4. Eastwood M. Diet and intestinal disease. Compr Ther 1977;3:19-23.
5. Boynton KA, Summerhayes IC, Ahlquist DA, Shuber AP. DNA integrity as a potential marker for stool-based detection of colorectal cancer. Clin Chem 2003;49:1058-65.
6. Mak T, Lalloo F, Evans DG, Hill J. Molecular stool screening for colorectal cancer. Br J Surg 2004;91:790-800.
7. Ahlquist DA, Skoletsky JE, Boynton KA, Harrington JJ, Mahoney DW, Piercall WE, *et al*. Colorectal cancer screening by detection of altered human DNA in stool: feasibility of a multitarget assay panel. Gastroenterology 2000;119:1219-27.
8. Imperiale TF, Ransohoff DF, Itzkowitz SH, Turnbull BA, Ross ME; Colorectal Cancer Study Group. Fecal DNA versus fecal occult blood for colorectal-cancer screening in an average-risk population. N Engl J Med 2004;351:2704-14.
9. Itzkowitz SH, Jandorf L, Brand R, Rabeneck L, Schroy PC 3rd, Sontag S, *et al*. Improved fecal DNA test for colorectal cancer screening. Clin Gastroenterol Hepatol 2007;5:111-7
10. Diehl F, Schmidt K, Durkee KH, Moore KJ, Goodman SN, Shuber AP, *et al*. Analysis of mutations in DNA isolated from plasma and stool of colorectal cancer patients. Gastroenterology 2008;135:489-98.
11. Zou H, Taylor WR, Harrington JJ, Hussain FT, Cao X, Loprinzi CL, *et al*. High detection rates of colorectal neoplasia by stool DNA testing with a novel digital melt curve assay. Gastroenterology 2009;136:459-70.
12. Song K, Fendrick AM, Ladabaum U. Fecal DNA testing compared with conventional colorectal cancer screening methods: a decision analysis. Gastroenterology 2004;126:1270-9.
13. Baylin SB. Mechanisms underlying epigenetically mediated gene silencing in cancer. Semin Cancer Biol 2002;12:331-7.
14. Jones PA, Laird PW. Cancer epigenetics comes of age. Nat Genet 1999;21:163-7.
15. Laird PW. The power and the promise of DNA methylation markers. Nat Rev Cancer 2003;3:253-66.
16. Issa JP. The epigenetics of colorectal cancer. Ann N Y Acad Sci 2000;910:140-53; discussion 153-5.
17. Muller HM, Oberwalder M, Fiegl H, Morandell M, Goebel G, Zitt M, *et al*. Methylation changes in faecal DNA: a marker for colorectal cancer screening? Lancet 2004;363:1283-5.
18. Wang DR, Tang D. Hypermethylated SFRP2 gene in fecal DNA is a high potential biomarker for colorectal cancer noninvasive screening. World J Gastroenterol 2008;14:524-31.
19. Huang Z, Li L, Wang J. Hypermethylation of SFRP2 as a potential marker for stool-based detection of colorectal cancer and precancerous lesions. Dig Dis Sci 2007;52:2287-91.
20. Oberwalder M, Wonter C, Wöntner C, Fiegl H, Goebel G, Zitt M. *et al*. SFRP2 methylation in fecal DNA--a marker for colorectal polyps. Int J Colorectal Dis 2008;23:15-9.
21. Zhang W, Bauer M, Croner RS, Pelz JO, Lodygin D, Hermeking H, *et al*. DNA stool test for colorectal cancer: hypermethylation of the secreted frizzled-related protein-1 gene. Dis Colon Rectum 2007;50:1618-26; discussion 1626-7.
22. Chen WD, Han ZJ, Skoletsky J, Olson J, Sah J, Myeroff L, *et al*. Detection in fecal DNA of colon cancer-specific methylation of the nonexpressed vimentin gene. J Natl Cancer Inst 2005;97:1124-32.
23. Olson J, Whitney DH, Durkee K, Shuber AP. DNA stabilization is critical for maximizing performance of fecal DNA-based colorectal cancer tests. Diagn Mol Pathol 2005;14:183-91.
24. Whitney D, Skoletsky J, Moore K, Boynton K, Kann L, Brand R, *et al*. Enhanced retrieval of DNA from human fecal samples results in improved performance of colorectal cancer screening test. J Mol Diagn 2004;6:386-95.
25. Anti M, Armuzzi A, Morini S, Iascone E, Pignataro G, Coco C, *et al*. Severe imbalance of cell proliferation and apoptosis in the left colon and in the rectosigmoid tract in subjects with a history of large adenomas. Gut 2001;48:238-46.
26. Glöckner SC, Dhir M, Yi JM, McGarvey KE, Van Neste L, Louwagie J, *et al*. Methylation of *TFPI2* in Stool DNA: a potential novel biomarker for the detection of colorectal cancer. Cancer Res 2009;69:4691-9.
27. Wong CM, Ng YL, Lee JM, Wong CC, Cheung OF, Chan CY, *et al*. Tissue factor pathway inhibitor-2 as a frequently silenced tumor suppressor gene in hepatocellular carcinoma. Hepatology 2007; 45:1129-38.
28. Sato N, Parker AR, Fukushima N, Miyagi Y, Iacobuzio-Donahue CA, Eshleman JR, *et al*. Epigenetic inactivation of TPFI-2 as a common mechanism associated with growth and invasion of pancreatic ductal adenocarcinoma. Oncogene 2005;24:850-8.
29. Nobeyama Y, Okochi-Takada E, Furuta J, Miyagi Y, Kikuchi K, Yamamoto A, *et al*. Silencing of tissue factor pathway inhibitor-2 gene in malignant melanomas. Int J Cancer 2007;121:301-7.
30. Rollin J, Iochmann S, Bléchet C, Hubé F, Régina S, Guyétant S, *et al*. Expression and methylation status of tissue factor pathway inhibitor-2 gene in non-small-cell lung cancer. Br J Cancer 2005; 92:775-83.
31. Shames DS, Girard L, Gao B, Sato M, Lewis CM, Shivapurkar N, *et al*. A genome-wide screen for promoter methylation in lung cancer identifies novel methylation markers for multiple malignancies. PLoS Med 2006;3:e486.
32. Sova P, Feng Q, Geiss G, Wood T, Strauss R, Rudolf V, *et al*. Discovery of novel methylation biomarkers in cervical carcinoma by global demethylation and microarray analysis. Cancer Epidemiol Biomarkers Prev 2006;15:114-23.