Published in final edited form as:

Curr Mol Med. 2010 March ; 10(2): 123–132.

DNA Methylation Based Biomarkers in Non-Invasive Cancer Screening

N. Shivapurkar^{*,1,2} and A.F. Gazdar^{1,2}

¹Hamon Center for Therapeutic Oncology Research, University of Texas Southwestern Medical Center, Dallas, TX 75390, USA

²Departments of Pathology, University of Texas Southwestern Medical Center, Dallas, TX 75390, USA

Abstract

DNA methylation plays a critical role in the regulation of gene expression, differentiation and in the development of cancer and other diseases. Hypermethylation of CpG islands located in the promoter regions of tumor suppressor genes is now firmly established as the most frequent mechanism for gene inactivation in cancers. Feasibility of using DNA methylation based biomarkers for early detection of cancer has been shown. Potential of using DNA methylation for prediction of therapeutic outcome and patient survival has also been shown. DNA originated from cancer cells has been routinely detected in clinical specimens (ex. Plasma/serum, sputum, urine etc.) from cancer patients. Presence of methylated DNA sequences in clinical specimens and potential of using them as biomarkers have been recognized. Novel methylation based biomarkers that can be used in clinical specimens, obtained non-invasively from cancer patients, offer significant practical advantages. More resources need to be committed to this area of biomarker research. Thus, we review recent findings on DNA methylation based cancer biomarkers with particular focus on these applicable to the clinical specimens obtained non-invasively from cancer patients.

Keywords

DNAmethylation; cancer; promoter methylation; hypermethylation; hypomethylation; non-invasive biomarker; body-fluids

INTRODUCTION

In biology, the term “genetics” refers to heritable changes in phenotype or gene expression caused by changes in the underlying DNA sequence [1]. Classic genetics alone can not explain the diversity of phenotypes within a population. In biology, the term epigenetics refers to heritable changes in phenotype or gene expression caused by mechanisms other than alteration of the underlying DNA sequence [2]. These changes may remain through cell divisions for the remainder of the cell's life and may also last for multiple generations. However, there is no change in the underlying DNA sequence of the organism [2], instead, non-genetic factors cause the organism's genes to behave (or “express themselves”) differently [3]. While the field of cancer genetics has enjoyed a great deal of attention

among cancer researchers in the last few decades, the appreciation of cancer epigenetics is more recent, owing to the fact that epigenetic mechanisms have emerged as key mechanisms in cancer development. All critical changes in cancer cells, such as silencing of tumor-suppressor genes, activation of oncogenes and defects in DNA repair are caused not only by genetics but also by epigenetic mechanisms.

There are two primary and interconnected epigenetic mechanisms-DNA methylation and covalent modification of histones. This enzymatic addition of a methyl group at the 5-position of the cytosine in a CpG (cytosine-guanine) dinucleotide is a normal process within cells. In cancer, despite a global hypomethylation, one observes hypermethylation in regions of the genome described as CpG islands [4]. These islands are present in almost half of all genes and are frequently promoter-associated [5]. With the exception of imprinted loci and tissue-specific genes, gene-proximal CpG islands are normally unmethylated; in some cases these CpG islands acquire dense methylation during cellular transformation, which correlates with silencing of expression of the associated gene [6, 7]. CpG islands were characterized originally as conserved genomic regions where the GC content is >50% and the CpG dinucleotide is relatively enriched with an observed vs. expected ratio of >0.6, over a distance of at least 200 base pairs [8]. The hypermethylation recruits histone deacetylase at the promoter region resulting into transcriptional silencing of the gene while histone acetylase into reactivation of the gene [9–11]. Tumor-specific changes in DNA methylation affect chromatin structure and alter gene expression patterns through successive cell divisions. During cancer development, two distinct changes in DNA methylation occur: genome-wide hypomethylation and locus-specific gain or loss of cytosine methylation in promoter-associated CpG islands [12–14]. Genome-wide hypomethylation influences genome stability, causes loss of imprinting, and may result in the induction of ectopic onco-fetal gene expression [3, 15, 16]. It is likely that genome-wide changes in methylation substantially alter overall chromatin architecture, chromosome segregation in mitosis, and ultimately cell ploidy, all of which augment cellular transformation. Extensive evidence, linking DNA methylation to oncogenesis led to overwhelming interest in exploring DNA methylation as cancer biomarker.

DNA Methylation as Biomarker in Cancer

The experimental evidence suggests that promoter methylation contributes to gene silencing [6, 7]. Since, many of the tumor suppressor genes were found to be specifically methylated and silenced in cancers, detection of DNA methylation was explored for its potential as a sensitive and specific biomarker assay for cancer screening in high risk population [17–21]. As the downstream products of the gene transcription (RNA) and the phenotypic determinants (proteins), both RNA and protein are the most intensively evaluated biomarkers for cancer detection and molecular classification [22, 23]. Biochemically, both RNA and protein are less stable than their DNA counterparts, making their full recovery from the body fluids difficult, if not impossible. Other negative aspects related to the quantitative nature of both biomarkers by current assays are the quality of data that is heavily affected by the cellular heterogeneity of the cancer tissues, which varies from one sample to another. The expression from non-cancerous cells is a likely source of the poor reproducibility of studies that examine same type of cancer [24, 25]. Moreover, expression of both molecules in cancer tissues is also influenced by other non-cancerous variable, which are not registered in the clinical study. For instance, expression of the genes in cancer lesion may also fluctuate with circadian rhythm [26]. Other physiological factors that influence the gene transcription in peripheral organs include fluctuating hormone levels, which may also change the RNAs and protein levels in the cancer tissues significantly. An advantage of DNA methylation over protein-based markers is that it is readily amplifiable and easily detectable using PCR-based approaches. Additionally, contrary to cancer-specific

mutations, which could occur anywhere in a gene, cancer-specific DNA hypermethylation occurs in defined regions, usually in or near the promoter of genes. Thus, it is easy to devise targeted probes to measure this molecular alteration. Conveniently, these probes can be readily combined into panels, which is important because no single molecular alteration involved in cancer can be expected to be present in every cancer case. Assembly of a complementary panel of DNA methylation probes would therefore increase sensitivity [27, 28]. Finally, it has been demonstrated that methylated DNA can be isolated from body fluids derived from cancer patients, making it well suited for non-invasive detection [29–32].

The methylation-specific PCR (MSP) analysis [33] represents the most important technological advance in DNA methylation study. It takes advantage of the selective converting power of bisulfite for unmethylated cytosines to uracil, but not methylated cytosines. MSP allows the detection of one hypermethylated allele in presence of as many as 10^4 unmethylated alleles, and vice versa. In other words, it is possible to detect a single tumor cell among 10^4 normal cells as long the tumor cell contains a DNA methylation state distinguished from normal cells. Using the real time PCR version of MSP (Methyl light), the sensitivity of the DNA methylation analysis is further improved by approximately two orders of magnitude [34]. Other approaches to quantify the DNA methylation state of the cellular DNA after bisulfite-conversion include the mass spectrometric platform by Sequenom [35] and beads-array format by Illumina [36]. However, a broader application of these two platforms in the clinic sample analyses has been hampered by the requirement for the expensive specialized facilities and the fact that only a few targets can be analyzed using available application kits. Given its quantitative nature, MSP suits well for the purpose of early diagnosis of cancer (*via* detection of the cancer-associated DNA methylation state) in body fluids where the target molecule (DNA, RNA and proteins) are derived from normal cells rather than from tumor cells which takes up only a minor proportion. Compared to genetic analyses of SNP, LOH and mutations, analysis of DNA methylation often exhibits several advantages, including great stability and tolerance of heterogeneity of samples, etc. In contrast, genetic biomarkers are often masked by normal counterparts in the non-cancerous cells, which are unavoidably present in clinical samples [37]. The reliable detection of altered methylation patterns of cancer cell can be easily achieved *via* non-invasive approach has been well received and highlighted by the published results in various types of cancer.

Considerable amounts of work have been done which shows numerous methylated genes are useful as prognostic or diagnostic biomarkers based on analysis of cancer biopsies [38, 39]. However, only a fraction of such biomarkers appear to be of use when cancer cells in the body fluid (ex. Plasma/serum, sputum, urine, peritoneal fluid etc) were analyzed for methylation. Since, such approach is clinically more useful, the purpose of the review is to focus on DNA methylation based biomarkers that have been used in clinical samples obtained non-invasively from cancer patients.

DNA Methylation as Non-Invasive Biomarker for Detection of Cancer: Promises and Challenges

Presence of cancer cell DNA in clinical specimens containing exfoliated tumor cells has offered opportunity to use non-invasively obtained clinical samples for methylation analysis [23]. Thus, methylation-based biomarkers based on analysis of exfoliated cancer cells have shown great promise in early detection and prognosis of cancer. The examples of clinical samples readily available for detection for DNA methylation in cancer cells are sputum for lung cancer [29–31], urine sediments for bladder cancer and prostate cancer [40, 41], stool for colorectal cancer [42] and plasma/serum DNA for all types of cancers [43]. Dramatic progress has been made over last few years in identifying genes that are found methylated in

cancer tissue but not in corresponding non-cancer tissues [18, 19]. A number of the genome-wide approaches to the DNA methylation biomarkers to various types of cancer have been developed, producing a decent list of candidate targets. However, only limited progress has been made in identifying methylation markers that could be used in reliable detection and prognosis of cancers based on analysis of exfoliated cancer cells. Thus, there is an urgent need to step up efforts for identify methylation markers that may be used in clinical samples obtained non-invasively from cancer patients or high risk populations. Although such investigations offer enormous promises, they are not without significant challenges. It is anticipated that more research emphasis and resources will be directed to this avenue of biomarker research.

The feasibility of using methylation of a particular gene as a biomarker is initially explored by analysis of the DNA from cancer tissues biopsies and corresponding non-cancerous tissue. Genes that are found to be methylated at higher frequency/higher prevalence in cancer tissue and at very lower frequency/lower prevalence in normal tissue may have potential as biomarkers. However, studies conducted by our laboratory and other laboratories suggest that the promise of using such markers in blood, sputum etc will depend on biochemical composition of these body fluids. For examples, genes that are specifically methylated in cancer cells but not in the normal epithelial cells and fibroblasts might be excellent biomarkers in biopsies but may not be as promising in blood or sputum analysis, if they are found methylated in normal lymphocytes [30]. Additionally, there could be enzymatic degradation of DNA in body fluids such as sputum that can affect the integrity of the DNA sequences differently in different genes [30, 31]. These might be some of the possibilities, which can complicate application of DNA methylation based biomarkers in body fluids. Thus, it is important to evaluate candidate methylation based biomarkers using biological samples with similar biochemical composition. This has been a major challenge and the primary reason why out of large number of promising DNA methylation based biomarkers, only a relatively small number of biomarkers have shown significant promise in clinical application.

Table 1 summarizes a list of studies that have used aberrant methylation of a gene or a panel of genes based on the analysis of body fluids from cancer patients for non-invasive detection of multiple types of cancers. Lung cancer is the number one cancer killer in the United States [44]. Early detection of lung cancer could reduce lung cancer mortality. There are multiple reports that have tested methylation of *P16INK4A* alone or with a panel of other genes in plasma or serum of lung cancer patients as biomarkers [45–54]. It is important to note that most of these studies showed moderate concordance in terms of presence of methylation of genes between tumor tissue and plasma/serum of the cancer patients. In general, none these studies reported methylation frequencies of 25% or higher for any one of the multiple markers tested in plasma/serum. Notably, none of these studies reported false positive methylation in plasma/serum from non-cancer patients, with any of the genes tested. In certain studies [55–57] *RASSF1A*, *APC* and *DAPK1* were reported to be methylated in plasma/serum at a frequencies of higher than 30%. However, substantially lower frequencies were reported in other studies. This was attributed to differences in primer design and/or to differences in the methodologies used. Besides, plasma or serum, sputum from cancer patients has also been used as potential medium for non-invasive detection of lung cancer. Studies by Belinsky *et al.* [29] have tested methylation of multiple genes in sputum samples from cancer and non-cancer patients. Based on their studies, methylation of *P16INK4A* in sputum DNA may have significant potential as a biomarker of lung cancer risk. Recent studies from our laboratory identified a methylated panel of genes that appear to be more promising than those previously reported as potential lung cancer biomarkers based on analyses of biopsies as well as sputum samples from cancer patients [30, 31]. These novel

DNA methylation based biomarkers should be tested in plasma/serum to determine their potential as lung cancer biomarkers.

Table 1 further summarizes a list of studies that have used aberrant methylation of a gene or a panel of genes in detection of breast cancer based on analysis of body fluids from breast cancer patients [58–63]. There was significant variation in results from different studies, even with the same markers. These variations in results were attributed to differences in stage and grade of the cases analyzed. However, as a general observation, *RASSF1A* appeared to show significant promise as a biomarker of breast cancer when serum/plasma samples were used for analysis. Recent studies from our laboratory identified a methylated panel of genes showing characteristics useful for detection of breast cancer cells in body fluids from cancer patients [31, 64].

Table 1 further summarizes a list of studies that have used aberrant methylation of a gene or a panel of genes in detection of multiple cancer types such as prostate ovarian cancer, hepatocellular cancer, gastric cancer, colorectal cancer, bladder cancer, pancreatic cancer, esophageal cancer, cervical cancer and hematologic cancers. Prostate cancer is one of the most common male malignancies in the industrialized countries [65]. There has been great deal of interest in early detection of prostate cancer. Prostatic intraepithelial neoplasia (PIN) is considered to be possible precursors to prostate cancers. Multiple genes have been found to be methylated in prostatic tumors and PINs and thus they may be good candidates for early detection of prostatic cancer in high-risk populations [66]. *GSTPI* was found to be commonly methylated in prostatic cancers and plasma of cancer patients [41, 67–69]. *GSTPI* methylation was also found in ejaculates (41). There is need for investigations of additional specific and sensitive biomarkers that can be used in non-invasive detection of prostate cancer. The highly lethal nature of ovarian cancer is related to the absence of symptoms in the majority of women with early stages of the disease [70]. Early detection of ovarian cancer is critical to increase the possibility of favorable prognosis. Thus, better biomarkers for ovarian cancer may be helpful in early detection and treatment of ovarian cancer. Ibanez *et al.* [71] have reported application of a methylation panel of six genes (*BRCA1*, *RASSF1A*, *APC*, *p14ARF*, *p16INK4A*, *DAPK1*) in peritoneal fluid washings from cancer patients. Most importantly, none of the genes were observed to be methylated in peritoneal fluid washings from non-cancer patients. Similarly, as reported in the same study, detection of tumor cell-specific *BRCA1* and *RASSF1A* hypermethylation in serum, plasma and peritoneal fluid from early stage ovarian cancer patient should enhance early detection of ovarian cancer. Muller *et al.* [72] have reported application of a methylation panel of 15 genes in peritoneal fluid washings from ovarian cancer patients. Using a high through put assay Wei *et al.* [73] have identified number of highly prognostic DNA methylation biomarkers. Such biomarkers need to be tested in serum, plasma and peritoneal fluid from cancer patients to identify those with great clinical application.

Hepatocellular cancer is one of the most common malignancies worldwide (74). In hepatocellular cancer the usual outcome is poor, because only 10 – 20% of hepatocellular carcinomas can be removed completely using surgery. If the cancer cannot be completely removed, the disease is usually deadly within 3 to 6 months. Table 1 further summarizes a list of studies that describe genes that might be useful as biomarkers in hepatocellular cancer. Besides promoter methylation of *p16INK4A* and *CDKN2B* not many other markers have been reported to be useful in detection of hepatocellular cancer based on plasma analysis [75–78]. Specifically, *p16INK4A* methylation appears to be at very high frequency >50% in plasma of cancer patients with no false positive in plasma samples from non-hepatocellular cancer patients. *RASSF1A* promoter hypermethylation was detected in 93% of HCC tissues. Of the paired plasma from the HCC patients, aberrant methylation was detected in 43% of the patients. No *RASSF1A* methylation was detected in the plasma in the

absence of methylation in the corresponding tumor. The presence of *RASSF1A* promoter hypermethylation in plasma DNA was found to associate with HCC size. Thus *RASSF1A* promoter methylation in plasma should be evaluated as a screening tool and/or prognosticator of HCC patients.

Gastric cancer is the fourth most common cancer worldwide [79]. It is a disease with a high death rate (700,000 per year) making it the second most common cause of cancer death worldwide after lung cancer. It represents roughly 2% (25,500 cases) of all new cancer cases yearly in the United States but the incidence is slowly rising. *P16INK4A* along with *CDKN2B*, *CDH1* and *DAPK1* were found to be useful makers based on serum analysis in gastric cancer patients [80–83]. Colorectal cancer includes cancerous growths in the colon, rectum and appendix. With 655,000 deaths worldwide per year, it is the third most common form of cancer and the second leading cause of cancer-related death in the Western world [84]. Methylation of *P16INK4A* along with that of *CDH1*, *DAPK* and *hMLH1* has been reported in plasma samples from colorectal cancer patients [85–90]. Although, frequency of methylation of these genes in plasma samples of primary cancer patients did not appear to be high, it is substantially higher in recurrent cancers. The feasibility of amplification of ethylated DNA from stool samples of patients with CRC has been reported. In a previous study [91] *SFRP2*, *SFRP5*, and *PGR* genes were found to be methylated differentially in the stool of patients with CRC. Of these markers, *SFRP2* was found to be the most sensitive fecal methylation marker, detecting 77%–90% of CRCs. However, specificity of *SFRP2* methylation was quite poor, at 77%. Lenhard *et al.* [92] studied the potential of *HIC1* gene promoter methylation as a stool-based DNA marker. They showed that *HIC1* promoter methylation can be detected frequently and with high specificity in stool samples from patients with CRCs. The combination of *HIC1* methylation analysis with fecal occult blood test allowed for the detection of two thirds of CRCs. Assay of methylated DNA markers in stool is a promising approach for colorectal cancer (CRC) screening. A method to capture and enrich hypermethylated CpG islands from stool using Methyl-binding domain (MBD) protein was recently reported [93]. With MBD enrichment, methylated vimentin was detected in stools enriched with 10 ng of cancer cell DNA in stool from CRC patients. In stools from healthy individuals methylated vimentin was not detected, even with MBD enrichment.

Bladder cancer is currently the second leading cause of genitourinary cancer mortality after prostate cancer [84]. Prognosis for non-invasive cancer is very good but that for invasive cancer very poor. Thus development of reliable prognostic and diagnostic markers to improve strategies for disease management for patients with bladder cancer is crucial. There is a clear need of noninvasive procedures for detection of bladder cancer, whether at initial diagnosis or during follow-up. Multiple genes have been found to be ethylated in bladder cancer with high specificity and sensitivity. Methylation of *p14ARF*, *P16INK4A* has been reported in bladder cancers as well as plasma from the cancer patients [94, 95]. Application of using DNA methylation markers in urine may have significant potential in initial diagnosis or during follow-up to surgery or treatment. However, even though some promising methylation markers showed excellent tumor specificity in bladder cancer biopsies, unexpectedly they showed very poor tumor specificity in urine analysis [96, 97]. Thus, development of novel methylation based markers with high sensitivity as well as specificity for bladder cancer, based on urine analysis are needed that urine can also be used in urine samples.

Head and neck cancer refers to a group of biologically similar cancers originating from the upper aerodigestive tract and are very common in United States [84]. Recent studies by Wong *et al.* 2003 [78] have shown that methylation of *p16INK4A* and *CDKN2B* in plasma might be potential useful biomarkers in screening high-risk populations for early HNSCC

and monitoring their to response to treatment. Although, significant amounts of work has been done in identifying genes that are methylated in head and neck cancer specimens, only few reports exist that describe application of these methylation based biomarkers to body fluids from cancer patients.

Pancreatic cancer is the fifth leading cause of cancer-related death, more than 31,000 deaths are anticipated in 2004 [98, 99]. Since pancreatic cancer is typically detected late in its development, early detection markers are needed for disease diagnosis, risk assessment, and treatment follow up. Hypermethylation of *16INK4A* and *ppENK* genes was detected in 60% and 80% of the plasma samples taken from patients whose tumors harbored the same methylation, respectively. No methylation was detected in the plasma sample of the patients whose corresponding tumor DNA had no methylation in the *p16INK4A* and *ppENK* promoter, with 100% specificity of methylation detection using plasma DNA [100]. Pancreatic juice has been found to be suitable for early detection of pancreatic cancer. A recent study [101] has reported, methylation of *p14ARF* and *p16INK4a* was reported in the pancreatic fluid of ~50% of cancer patients but undetected in pancreatic fluid of chronic pancreatitis patients. In another study Methylation of six genes (*Cyclin D2*, *FOXO1*, *NPTX2*, *ppENK*, *p16INK4A* and *TFPI2*) were reported in >80% cancer patients but <10% in pancreatic fluid of chronic pancreatitis patients. In this study based on qMSP it was clearly possible to separate cancer patients from non-cancer patients.

Esophageal cancer ranks sixth in incidence among cancers worldwide, with 400,000 new cases being diagnosed per year [102]. This malignancy exists in two principal forms, each possessing distinct pathologic characteristics: Esophageal cell carcinoma, which occurs at high frequencies in many developing countries, especially in Asia; and esophageal adenocarcinoma, which is more prevalent in Western countries, with a rapid rate of increase in recent years. Although significant advances have been made in the treatment of esophageal cancers, these aggressive malignancies commonly present as locally advanced disease, with a very poor prognosis. Using qMSP Jin *et al.* [103] reported *TAC-1* promoter methylation in plasma samples from >50% of the esophageal cancer patients but at significantly less frequency and levels in plasma samples from non-cancer patients. In another studies promoter methylation of *APC* and *p16 INK4A* in plasma was reported to have potential as biomarker of esophageal cancer [104, 105].

Cancer of the uterine cervix is an important cause of death in women worldwide [106, 107]. New molecular and biochemical approaches for the recognition and treatment of high-risk patients are needed to improve survival and avoid over treatment of low-risk patients. Aberrant methylation of CpG islands within the promoter regions of several genes such as *p16INK4A*, *DAPK*, *MGMT*, *CDH-1* and *RAR-β* has been identified in cervical cancer (106). Recently, Widshwendel 2004 identified five additional genes, namely *CALCA*, *hTERT*, *MYOD1*, *PGR* and *TIMP3*, as being methylated significantly more frequently in cervical cancer than in normal cervical tissue. On the basis of these observations, we examined the methylation status of *CALCA*, *hTERT*, *MYOD1*, *PGR*, and *TIMP3* genes in serum samples of cervical cancer patients and compared it with clinicopathological characteristics and outcome of the disease. Promotor CpG island methylation of *DAPK1*, *p16INK4A*, and *MGMT* was detectable, respectively, in 60%, 28%, and 18% of cases of cervical tumor DNA; and in 40%, 10%, and 8% of cases of patients' plasma DNA [107, 108]. At least one of the three genes was found to be methylated in 75% tumors and 55% plasma samples. The methylation pattern in primary tumor and plasma was found to be concordant in ~50% of patients with matched tissue and plasma samples.

In the area of methylation biomarkers much less work has been done with hematologic cancers compared to that with solid tumors. In one study, 39 of 43 blood samples (91%)

sequentially collected from 12 patients with AML, ALL, or ABL showed *CDKN2B* methylation status in excellent concordance with morphologic disease stage. Early detection of *CDKN2B* methylation at apparent remission or its acquisition during follow-up may prove valuable for predicting relapse. Overall survival of patients with *CDKN2B* methylation was notably shortened among 38 adults with AML and 12 adults with ALL. Aberrant *p15* methylation may have important prognostic implications for clinical monitoring and risk assessment [109]. Non-Hodgkin's lymphoma (NHL) is a group of malignancies with heterogeneous genetic and epigenetic alterations. Discovery of molecular markers that better define NHL should improve diagnosis, prognosis and understanding of the biology. Shi *et al.* [110] developed a CpG island DNA microarray for discovery of aberrant methylation targets in cancer, and applied this method to examine NHL cell lines and primary tumors. This methylation profiling revealed differential patterns in six cell lines originating from different subtypes of NHL. The investigators further identified 30 hypermethylated genes in these cell lines and independently confirmed 10 of them. Methylation of six of these genes was then further examined in 75 primary NHL specimens composed of four subtypes representing different stages of maturation. Each gene (*DLC-1*, *PCDHGB7*, *CYP27B1*, *EFNA5*, *CCND1* and *RAR-β*) was frequently hypermethylated in these NHLs (87, 78, 61, 53, 40 and 38%, respectively), but not in benign follicular hyperplasia. Although some genes such as *DLC-1* and *PCDHGB7* were methylated in the vast majority of NHLs, others were differentially methylated in specific subtypes. The methylation of the candidate tumor suppressor gene *DLC-1* was detected in a high proportion of primary tumor and plasma DNA samples by using quantitative methylation-specific PCR analysis. This promoter hypermethylation inversely correlated with *DLC-1* gene expression in primary NHL samples (110). Deligezer *et al.* [111] investigated methylation of *p16INK4A* gene in plasma DNA of lymphoma patients by the methylation-sensitive restriction enzyme-related PCR. *p16INK4A* methylation was found to occur in 73% of patients but in none of the healthy controls. Nucleosomal DNA fragmentation was detectable in 81% of patients. In 67% of patients, copresence of both parameters was observed. Presence of both parameters was associated with the stage of disease which was more pronounced for nucleosomal DNA fragmentation. The results suggest that presence of methylated and apoptotic DNA in plasma of patients with lymphoproliferative diseases is a frequent event and may be used as a marker for early diagnosis and during the follow-up of the disease.

There is enormous promise in identifying biomarkers that can be reliably used using DNA from body-fluids of cancer patients. Usually, most of DNA methylation based biomarkers are identified for their suitability in body fluids, through actually testing them in similar clinical samples. Future studies should include attempts to integrate the methylation data for a gene in cancer cells and also in types of normal cells which are normal constituents of a certain type of body fluid. Also studies should include attempts to extrapolate bioinformatics features to predicting potential as biomarker in certain type of body-fluid. Thus future challenge should be to use all such information and develop algorithm to come up with non-invasive biomarkers. Future challenge should be to develop criteria or algorithms predicting whether a particular biomarker may be used with a particular type of clinical sample.

ABBREVIATIONS

MSP	methylation specific PCR
qMSP	quantitative methylation specific PCR

REFERENCES

1. Schwartz DW, McCormick KM. *J. Cardiovasc. Nurs.* 1999; 13:1–18. [PubMed: 10386268]
2. Bird A. *Nature.* 2007; 447:396–398. [PubMed: 17522671]
3. Jaenisch R, Bird A. *Nat. Genet.* 2003; 33:245–254. [PubMed: 12610534]
4. Takai D, Jones PA. *In. Silico. Biol.* 2003; 3:235–240. [PubMed: 12954087]
5. Suzuki MM, Kerr AR, De Sousa D, Bird A. *Genome Res.* 2007; 17:625–631. [PubMed: 17420183]
6. Holm TM, Jackson-Grusby L, Brambrink T, Yamada Y, Rideout WM 3rd, Jaenisch R. *Cancer Cell.* 2005; 8:275–285. [PubMed: 16226703]
7. Merlo A, Herman JG, Mao L, Lee DJ, Gabrielson E, Burger PC, Baylin SB, Sidransky D. *Nat. Med.* 1995; 1:686–692. [PubMed: 7585152]
8. Gardiner-Garden M, Frommer M. *J. Mol. Biol.* 1987; 196:261–282. [PubMed: 3656447]
9. Daujat S, Bauer UM, Shah V, Turner B, Berger S, Kouzarides T. *Curr. Biol.* 2002; 12:2090–2097. [PubMed: 12498683]
10. Herman JG, Baylin SB. *N. EnglJ. Med.* 2003; 349:2042–2054.
11. Ringrose L, Ehret H, Paro R. *Mol. Cell.* 2004; 16:641–653. [PubMed: 15546623]
12. Feinberg AP, Ohlsson R, Henikoff S. *Nat. Rev. Genet.* 2006; 7:21–33. [PubMed: 16369569]
13. Baylin SB, Ohm JE. *Nat. Rev. Cancer.* 2006; 6:107–116. [PubMed: 16491070]
14. Jenkins PJ, Besser MJ. *Clin. Endocrinol. Metab.* 2001; 86:2935–2941.
15. Bernstein BE, Mikkelsen TS, Xie X, Kamal M, Huebert DJ, Cuff J, Fry B, Meissner A, Wernig M, Plath K, Jaenisch R, Wagschal A, Feil R, Schreiber SL, Lander ES. *Cell.* 2006; 125:315–326. [PubMed: 16630819]
16. Hong SJ, Kim YH, Choi YD, Min KO, Choi SW, Rhyu MG. *J. Korean. Med. Sci.* 2005; 20:790–805. [PubMed: 16224153]
17. Jones PA, Baylin SB. *Nat. Rev. Genet.* 2002; 3:415–428. [PubMed: 12042769]
18. Shames DS, Minna JD, Gazdar AF. *Curr. Mol. Med.* 2007; 7:85–102. [PubMed: 17311535]
19. Shames DS, Girard L, Gao B, Sato M, Lewis CM, Shivapurkar N, Jiang A, Perou CM, Kim YH, Pollack JR, Fong KM, Lam CL, Wong M, Shyr Y, Nanda R, Olopade OI, Gerald W, Euhus DM, Shay JW, Gazdar AF, Minna JD. *PLoS Med.* 2006; 3:e486. [PubMed: 17194187]
20. Verma M, Manne U. *Crit. Rev. Oncol. Hematol.* 2006; 60:9–18. [PubMed: 16829121]
21. Mulero-Navarro S, Esteller M. *Crit. Rev. Oncol. Hematol.* 2008; 68:1–11. [PubMed: 18430583]
22. Levenson VV. *Biochim. Biophys. Acta.* 2007; 1770:847–856. [PubMed: 17368950]
23. Fleischhacker M, Schmidt B. *Biochim. Biophys. Acta.* 2007; 1775:181–232. [PubMed: 17137717]
24. Walker MS, Hughes TA. *Int. J. Mol. Med.* 2008; 21:13–17. [PubMed: 18097611]
25. Shi L, Perkins RG, Fang H, Tong W. *Curr. Opin. Biotechnol.* 2008; 19:10–18. [PubMed: 18155896]
26. Reinke H, Saini C, Fleury-Olela F, Dibner C, Benjamin IJ, Schibler U. *Genes Dev.* 2008; 22:331–345. [PubMed: 18245447]
27. Tsou JA, Galler JS, Siegmund KD, Laird PW, Turla S, Cozen W, Hagen JA, Koss MN, Laird-Offringa IA. *Mol. Cancer.* 2007; 6:70. [PubMed: 17967182]
28. Anglim PP, Galler JS, Koss MN, Hagen JA, Turla S, Campan M, Weisenberger DJ, Laird PW, Siegmund KD, Laird-Offringa IA. *Mol. Cancer.* 2008; 7:62. [PubMed: 18616821]
29. Belinsky SA, Klinge DM, Dekker JD, Smith MW, Bocklage TJ, Gilliland FD, Crowell RE, Karp DD, Stidley CA, Picchi MA. *Clin. Cancer Res.* 2005; 11:6505–6511. [PubMed: 16166426]
30. Shivapurkar N, Stastny V, Suzuki M, Wistuba II, Li L, Zheng Y, Feng Z, Hol B, Prinsen C, Thunnissen FB, Gazdar AF. *Cancer Lett.* 2007; 247:56–71. [PubMed: 16644104]
31. Shivapurkar N, Stastny V, Xie Y, Prinsen C, Frenkel E, Czerniak B, Thunnissen FB, Minna JD, Gazdar AF. *Cancer Epidemiol. Biomarkers. Prev.* 2008; 17:995–1000. [PubMed: 18398044]
32. Hoque MO, Feng Q, Toure P, Dem A, Critchlow CW, Hawes SE, Wood T, Jeronimo C, Rosenbaum E, Stern J, Yu M, Trink B, Kiviat NB, Sidransky D. *J. Clin. Oncol.* 2006; 24:4262–4269. [PubMed: 16908936]

33. Herman JG, Graff JR, Myohanen S, Nelkin BD, Baylin SB. Proc. Natl. Acad. Sci. U.S.A. 1996; 93:9821–9826. [PubMed: 8790415]
34. Eads CA, Danenberg KD, Kawakami K, Saltz LB, Blake C, Shibata D, Danenberg PV, Laird PW. Nucleic Acids Res. 2000; 28:E32. [PubMed: 10734209]
35. Ehrlich M, Nelson MR, Stanssens P, Zabeau M, Liloglou T, Xinarianos G, Cantor CR, Field JK, van den Boom D. Proc. Natl. Acad. Sci. U. S. A. 2005; 102:15785–15790. [PubMed: 16243968]
36. Bibikova M, Lin Z, Zhou L, Chudin E, Garcia EW, Wu B, Doucet D, Thomas NJ, Wang Y, Vollmer E, Goldmann T, Seifart C, Jiang W, Barker DL, Chee MS, Floros J, Fan JB. Genome Res. 2006; 16:383–393. [PubMed: 16449502]
37. Kolble K, Ullrich OM, Pidde H, Barthel B, Diermann J, Rudolph B, Dietel M, Schlag PM, Scherneck S. Lab. Invest. 1999; 79:1145–1150. [PubMed: 10496533]
38. Costello JF, Fruhwald MC, Smiraglia DJ, Rush LJ, Robertson GP, Gao X, Wright FA, Feramisco JD, Peltomaki P, Lang JC, Schuller DE, Yu L, Bloomfield CD, Caligiuri MA, Yates A, Nishikawa R, Su Huang H, Petrelli NJ, Zhang X, O'Dorisio MS, Held WA, Cavenee WK, Plass C. Nat. Genet. 2000; 24:132–138. [PubMed: 10655057]
39. Esteller M, Corn PG, Baylin SB, Herman JG. Cancer Res. 2001; 61:3225–3229. [PubMed: 11309270]
40. Chan MW, Chan LW, Tang NL, Tong JH, Lo KW, Lee TL, Cheung HY, Wong WS, Chan PS, Lai FM, To KF. Clin. Cancer Res. 2002; 8:464–470. [PubMed: 11839665]
41. Goessl C, Muller M, Straub B, Miller K. Eur. Urol. 2002; 41:668–676. [PubMed: 12074786]
42. Jubb AM, Quirke P, Oates AJ. Ann. N Y Acad Sci. 2003; 983:251–267. [PubMed: 12724230]
43. Wong IH, Lo YM, Johnson PJ. Ann. N Y Acad Sci. 2001; 945:36–50. [PubMed: 11708493]
44. Jemal A, Siegel R, Ward E, Murray T, Xu J, Thun MJ. Cancer statistics. CA. Cancer. J. Clin. 2007; 57:43–66. [PubMed: 17237035]
45. An Q, Liu Y, Gao Y, Huang J, Fong X, Li L, Zhang D, Cheng S. Cancer Lett. 2002; 188:109–114. [PubMed: 12406555]
46. Bearzatto A, Conte D, Frattini M, Zaffaroni N, Andriani F, Balestra D, Tavecchio L, Daidone MG, Sozzi G. Clin. Cancer Res. 2002; 8:3782–3787. [PubMed: 12473590]
47. Esteller M, Sanchez-Cespedes M, Rosell R, Sidransky D, Baylin SB, Herman JG. Cancer Res. 1999; 59:67–70. [PubMed: 9892187]
48. Fujiwara K, Fujimoto N, Tabata M, Nishii K, Matsuo K, Hotta K, Kozuki T, Aoe M, Kiura K, Ueoka H, Tanimoto M. Clin. Cancer Re. 2005; 11:1219–1225.
49. Ng CS, Zhang J, Wan S, Lee TW, Arifi AA, Mok T, Lo DY, Yim AP. J. Surg. Oncol. 2002; 79:101–106. [PubMed: 11815997]
50. Ramirez JL, Sarries C, de Castro PL, Roig B, Queralt C, Escuin D, de Aguirre I, Sanchez JM, Manzano JL, Margeli M, Sanchez JJ, Astudillo J, Taron M, Rosell R. Cancer Lett. 2003; 193:207–216. [PubMed: 12706879]
51. Zemaitis M, Rieger N, Fischer JR, Sakalauskas R, Malakauskas K, Lahm H. Medicina (Kaunas). 2005; 41:123–131. [PubMed: 15758578]
52. Suga Y, Miyajima K, Oikawa T, Maeda J, Usuda J, Kajiwaru N, Ohira T, Uchida O, Tsuboi M, Hirano T, Kato H, Ikeda N. Oncol. Rep. 2008; 20:1137–1142. [PubMed: 18949413]
53. Tan SH, Ida H, Lau QC, Goh BC, Chieng WS, Loh M, Ito Y. Oncol. Rep. 2007; 18:1225–1230. [PubMed: 17914577]
54. Hsu HS, Chen TP, Hung CH, Wen CK, Lin RK, Lee HC, Wang YC. Cancer. 2007; 110:2019–2026. [PubMed: 17876837]
55. Ramirez JL, Taron M, Balana C, Sarries C, Mendez P, de Aguirre I, Nunez L, Roig B, Queralt C, Botia M, Rosell R. Rocz. Akad. Med. Bialymst. 2003; 48:34–41. [PubMed: 14737938]
56. Rykova EY, Skvortsova TE, Laktionov PP, Tamkovich SN, Bryzgunova OE, Starikov AV, Kuznetsova NP, Kolomiets SA, Sevostianova NV, Vlassov VV. Nucleosides Nucleotides Nucleic Acids. 2004; 23:855–859. [PubMed: 15560072]
57. Usadel H, Brabender J, Danenberg KD, Jeronimo C, Harden S, Engles J, Danenberg PV, Yang S, Sidransky D. Cancer Res. 2002; 62:371–375. [PubMed: 11809682]

58. Dulaimi E, Hillinck J, Ibanez de Caceres I, Al-Saleem T, Cairns P. Clin. Cancer Res. 2004; 10:6189–6193. [PubMed: 15448006]
59. Fiegl H, Millinger S, Mueller-Holzner E, Marth C, Ensinger C, Berger A, Klocker H, Goebel G, Widschwendter M. Cancer Res. 2005; 65:1141–1145. [PubMed: 15734995]
60. Muller HM, Widschwendter A, Fiegl H, Ivarsson L, Goebel G, Perkmann E, Marth C, Widschwendter M. Cancer Res. 2003; 63:7641–7645. [PubMed: 14633683]
61. Hu XC, Wong IH, Chow LW. Oncol. Rep. 2003; 10:1811–1815. [PubMed: 14534701]
62. Silva JM, Dominguez G, Villanueva MJ, Gonzalez R, Garcia JM, Corbacho C, Provencio M, Espana P, Bonilla F. Br. J. Cancer. 1999; 80:1262–1264. [PubMed: 10376981]
63. Silva JM, Silva J, Sanchez A, Garcia JM, Dominguez G, Provencio M, Sanfrutos L, Jareno E, Colas A, Espana P, Bonilla F. Clin. Cancer Res. 2002; 8:3761–3766. [PubMed: 12473587]
64. Shivapurkar N, Stastny V, Okumura N, Girard L, Xie Y, Prinsen C, Thunnissen FB, Wistuba II, Czerniak B, Frenkel E, Roth JA, Liloglou T, Xinarianos G, Field JK, Minna JD, Gazdar AF. Cancer Res. 2008; 68:7448–7456. [PubMed: 18794132]
65. Seidman H, Mushinski MH, Gelb SK, Silverberg E. CA. Cancer. J. Clin. 1985; 35:36–56. [PubMed: 3917841]
66. Reynolds MA, Kastury K, Groskopf J, Schalken JA, Rittenhouse H. Cancer Lett. 2007; 249:5–13. [PubMed: 17303324]
67. Jeronimo C, Usadel H, Henrique R, Silva C, Oliveira J, Lopes C, Sidransky D. Urology. 2002; 60:1131–1135. [PubMed: 12475696]
68. Vis AN, Oomen M, Schroder FH, van der Kwast TH. Mol. Urol. 2001; 5:199–203. [PubMed: 11790283]
69. Chuang CK, Chu DC, Tzou RD, Liou SI, Chia JH, Sun CF. Cancer Detect. Prev. 2007; 31:59–63. [PubMed: 17291695]
70. Vergote I, De Brabanter J, Fyles A, Bertelsen K, Einhorn N, Sevelde P, Gore ME, Kaern J, Verrelst H, Sjøvall K, Timmerman D, Vandewalle J, Van Gramberen M, Trope CG. Lancet. 2001; 357:176–182. [PubMed: 11213094]
71. Ibanez de Caceres I, Battagli C, Esteller M, Herman JG, Dulaimi E, Edelson MI, Bergman C, Ehya H, Eisenberg BL, Cairns P. Cancer Res. 2004; 64:6476–6481. [PubMed: 15374957]
72. Muller HM, Millinger S, Fiegl H, Goebel G, Ivarsson L, Widschwendter A, Muller-Holzner E, Marth C, Widschwendter M. Clin. Chem. 2004; 50:2171–2173. [PubMed: 15502091]
73. Wei SH, Balch C, Paik HH, Kim YS, Baldwin RL, Liyanarachchi S, Li L, Wang Z, Wan JC, Davuluri RV, Karlan BY, Gifford G, Brown R, Kim S, Huang TH, Nephew KP. Clin. Cancer Res. 2006; 12:2788–2794. [PubMed: 16675572]
74. Chen CJ, Yu MW, Liaw YF. J. Gastroenterol. Hepatol. 1997; 12:S294–S308. [PubMed: 9407350]
75. Chu HJ, Heo J, Seo SB, Kim GH, Kang DH, Song GA, Cho M, Yang US. J. Korean. Med. Sci. 2004; 19:83–86. [PubMed: 14966347]
76. Wong IH, Lo YM, Yeo W, Lau WY, Johnson PJ. Clin. Cancer Res. 2000; 6:3516–3521. [PubMed: 10999738]
77. Wong IH, Lo YM, Zhang J, Liew CT, Ng MH, Wong N, Lai PB, Lau WY, Hjelm NM, Johnson PJ. Cancer Res. 1999; 59:71–73. [PubMed: 9892188]
78. Wong TS, Man MW, Lam AK, Wei WI, Kwong YL, Yuen AP. Eur. J. Cancer. 2003; 39:1881–1887. [PubMed: 12932666]
79. Parkin DM. Oncogene. 2004; 23:6329–6340. [PubMed: 15322508]
80. Koike H, Ichikawa D, Ikoma H, Otsuji E, Kitamura K, Yamagishi H. J. Surg. Oncol. 2004; 87:182–186. [PubMed: 15334633]
81. Kanyama Y, Hibi K, Nakayama H, Kodera Y, Ito K, Akiyama S, Nakao A. Cancer Sci. 2003; 94:418–420. [PubMed: 12824886]
82. Ichikawa D, Koike H, Ikoma H, Ikoma D, Tani N, Otsuji E, Kitamura K, Yamagishi H. Anticancer Res. 2004; 24:2477–2481. [PubMed: 15330201]
83. Lee TL, Leung WK, Chan MW, Ng EK, Tong JH, Lo KW, Chung SC, Sung JJ, To KF. Clin. Cancer Res. 2002; 8:1761–1766. [PubMed: 12060614]

84. Jemal A, Siegel R, Ward E, Murray T, Xu J, Smigal C, Thun MJ. CA. Cancer. J. Clin. 2006; 56:106–130. [PubMed: 16514137]
85. Grady WM, Rajput A, Lutterbaugh JD, Markowitz SD. Cancer Res. 2001; 61:900–902. [PubMed: 11221878]
86. Lecomte T, Berger A, Zinzindohoue F, Micard S, Landi B, Blons H, Beaune P, Cugnenc PH, Laurent-Puig P. Int. J. Cancer. 2002; 100:542–548. [PubMed: 12124803]
87. Nakayama H, Hibi K, Taguchi M, Takase T, Yamazaki T, Kasai Y, Ito K, Akiyama S, Nakao A. Cancer Lett. 2002; 188:115–119. [PubMed: 12406556]
88. Nakayama H, Hibi K, Takase T, Yamazaki T, Kasai Y, Ito K, Akiyama S, Nakao A. Int. J. Cancer. 2003; 105:491–493. [PubMed: 12712439]
89. Yamaguchi S, Asao T, Nakamura J, Ide M, Kuwano H. Cancer Lett. 2003; 194:99–105. [PubMed: 12706863]
90. Zou HZ, Yu BM, Wang ZW, Sun JY, Cang H, Gao F, Li DH, Zhao R, Feng GG, Yi J. Clin. Cancer Res. 2002; 8:188–191. [PubMed: 11801557]
91. Muller HM, Oberwalder M, Fiegl H, Morandell M, Goebel G, Zitt M, Muhlthaler M, Ofner D, Margreiter R, Widschwendter M. Lancet. 2004; 363:1283–1285. [PubMed: 15094274]
92. Lenhard K, Bommer GT, Asutay S, Schauer R, Brabletz T, Goke B, Lamerz R, Kolligs FT. Clin. Gastroenterol. Hepatol. 2005; 3:142–149. [PubMed: 15704048]
93. Zou H, Harrington J, Rego RL, Ahlquist DA. Clin. Chem. 2007; 53:1646–1651. [PubMed: 17712002]
94. Dominguez G, Carballido J, Silva J, Silva JM, Garcia JM, Menendez J, Provencio M, Espana P, Bonilla F. Clin. Cancer Res. 2002; 8:980–985. [PubMed: 11948103]
95. Valenzuela MT, Galisteo R, Zuluaga A, Villalobos M, Nunez MI, Oliver FJ, Ruiz de Almodovar JM. Eur. Urol. 2002; 42:622–628. [PubMed: 12477660]
96. Hoque MO, Begum S, Topaloglu O, Jeronimo C, Mambo E, Westra WH, Califano JA, Sidransky D. Cancer Res. 2004; 64:5511–5517. [PubMed: 15289362]
97. Friedrich MG, Weisenberger DJ, Cheng JC, Chandrasoma S, Siegmund KD, Gonzalgo ML, Toma MI, Huland H, Yoo C, Tsai YC, Nichols PW, Bochner BH, Jones PA, Liang G. Clin. Cancer Res. 2004; 10:7457–7465. [PubMed: 15569975]
98. Safioleas MC, Moulakakis KG. Hepatogastroenterology. 51:862–868. [PubMed: 15143935]
99. Skinner HG, Michaud DS, Giovannucci EL, Rimm EB, Stampfer MJ, Willett WC, Colditz GA, Fuchs CS. Am. J. Epidemiol. 2004; 160:248–258. [PubMed: 15257998]
100. Jiao L, Zhu J, Hassan MM, Evans DB, Abbruzzese JL, Li D. Pancreas. 2007; 34:55–62. [PubMed: 17198183]
101. Matsubayashi H, Canto M, Sato N, Klein A, Abe T, Yamashita K, Yeo CJ, Kalloo A, Hruban R, Goggins M. Cancer Res. 2006; 66:1208–1217. [PubMed: 16424060]
102. Jemal A, Murray T, Ward E, Samuels A, Tiwari RC, Ghafoor A, Feuer EJ, Thun MJ. CA. Cancer J. Clin. 2005; 55:10–30. [PubMed: 15661684]
103. Jin Z, Olaru A, Yang J, Sato F, Cheng Y, Kan T, Mori Y, Mantzur C, Paun B, Hamilton JP, Ito T, Wang S, David S, Agarwal R, Beer DG, Abraham JM, Meltzer SJ. Clin. Cancer Res. 2007; 13:6293–6300. [PubMed: 17975140]
104. Kawakami K, Brabender J, Lord RV, Groshen S, Greenwald BD, Krasna MJ, Yin J, Fleisher AS, Abraham JM, Beer DG, Sidransky D, Huss HT, Demeester TR, Eads C, Laird PW, Ilson DH, Kelsen DP, Harpole D, Moore MB, Danenberg KD, Danenberg PV, Meltzer SJ. J. Natl. Cancer Inst. 2000; 92:1805–1811. [PubMed: 11078757]
105. Hibi K, Taguchi M, Nakayama H, Takase T, Kasai Y, Ito K, Akiyama S, Nakao A. Clin. Cancer Res. 2001; 7:3135–3138. [PubMed: 11595706]
106. Widschwendter A, Ivarsson L, Blassnig A, Muller HM, Fiegl H, Wiedemair A, Muller-Holzner E, Goebel G, Marth C, Widschwendter M. Int. J. Cancer. 2004; 109:163–166. [PubMed: 14750164]
107. Widschwendter A, Muller HM, Fiegl H, Ivarsson L, Wiedemair A, Muller-Holzner E, Goebel G, Marth C, Widschwendter M. Clin. Cancer Res. 2004; 10:565–571. [PubMed: 14760078]
108. Yang HJ, Liu VW, Wang Y, Chan KY, Tsang PC, Khoo US, Cheung AN, Ngan HY. Gynecol. Oncol. 2004; 93:435–440. [PubMed: 15099958]

109. Wong IH, Ng MH, Huang DP, Lee JC. *Blood*. 2000; 95:1942–1949. [PubMed: 10706859]
110. Shi H, Guo J, Duff DJ, Rahmatpanah F, Chitima-Matsiga R, Al-Kuhlani M, Taylor KH, Sjahputera O, Andreski M, Wooldridge JE, Caldwell CW. *Carcinogenesis*. 2007; 28:60–70. [PubMed: 16774933]
111. Deligezer U, Yaman F, Erten N, Dalay N. *Clin. Chim. Acta*. 2003; 335:89–94. [PubMed: 12927689]

Table 1

Presence of Methylated DNA Sequences in Body Fluids from Cancer Patients

Cancer type	Genes Analyzed	Detection	Type of Biological samples analyzed	References
Lung cancer	p16INK4A	MSP	Tumor and plasma	[45]
Lung cancer	p16INK4A	MSP	Tumor and plasma	[46]
Lung cancer	p16INK4A, MGMT, RASSF1A	MSP	Tumor and plasma and sputum	[29]
Lung cancer	p16INK4A, DAPK11, GSTP1, MGMT	MSP	Tumor and plasma	[47]
Lung cancer	p16INK4A, DAPK11, GSTP1, RASSF1A, RAR- β	MSP	Tumor and plasma	[48]
Lung cancer	p16INK4A, DAPK11, TMS1, RASSF1A	MSP	Tumor and plasma	[48, 49]
Lung cancer	APC, RASSF1A	MSP	Plasma	[56]
Lung cancer	APC	MSP	Plasma	[57]
Lung cancer	p16INK4A, DAPK11, APC, RASSF1A, CDH1, MGMT, RAR- β , FHIT, p14ARF	MSP	Tumor and Plasma	[51]
Lung cancer	p16INK4A, ESR1	qMSP	Plasma	[52]
Lung cancer	RUNX3, p16INK4A, RASSF1A, CDH1	MSP	Serum	[53]
Lung cancer	BLU, CDH13, FHIT, p16INK4A, RAR- β	MSP	Tumor and Plasma	[54]
Lung cancer	MGMT, p16INK4A, RASSF1A, DAPK11 and RAR- β	MSP	Serum	[48]
Lung cancer	p16INK4A, HS3ST2, APC, RASSF1A	MSP	Sputum	[30]
Lung cancer	TCF21, RASSF1A	MSP	Sputum	[31]
Lung cancer	CYGB	MSP	Sputum	[64]
Breast cancer	p16INK4A	MSP	Tumor and Plasma	[62, 63]
Breast cancer	RASSF1A	MSP	Plasma	[59]
Breast cancer	p16INK4A, CDH1	MSP	Tumor and Plasma	[61]
Breast cancer	RASSF1A, APC, DAPK11	MSP	Tumor and Plasma	[58]
Breast cancer	HIC1, RASSF1A, RAR- β , CDH1, CDH13, ESR1	MSP	Plasma	[60]
Breast cancer	APC, RASSF1A, CDH1, CDH13, RAR- β	MSP	Plasma	[56]
Breast cancer	APC, RASSF1A, GSTP1, RAR- β	MSP	Plasma	[32]
Prostate cancer	GSTP1	MSP	Tumor and Plasma	[41]
Prostate cancer	GSTP1	MSP	Tumor and Plasma	[67]
Prostate cancer	GSTP1	qMSP	Tumor and Plasma	[69]
Prostate cancer	CD44	MSP	Tumor and Plasma	[68]
Ovarian cancer	CDH1, CDH13, APC, TIMP3, GSTP1, PPP1R13B, HSPA2, HSD17B4, ESR1	MSP	peritoneal fluid	[72]
NKX2-1, GSTM3, CYP1B, BRCA1, MYOD1, SOCS1				
Ovarian cancer	RASSF1A, APC, BCRA1, p14ARFARF, P16INK4A, DAPK1	MSP	Tumor and Plasma, peritoneal fluid	[71]
Hepatocellular cancer	P16 INK4A	MSP	Tumor and Plasma	[75]
Hepatocellular cancer	P16 INK4A	MSP	Tumor and Plasma	[76]
Hepatocellular cancer	P16 INK4A	MSP	Tumor and Plasma	[77]
Hepatocellular cancer	P16 INK4A	MSP	Tumor and Plasma	[78]

Cancer type	Genes Analyzed	Detection	Type of Biological samples analyzed	References
Hepatocellular cancer	P16 INK4A, CDKN2B	MSP	Tumor and Plasma	[78]
Gastric cancer	P16 INK4A, CDH1	MSP	Tumor and Plasma	[82]
Gastric cancer	P16 INK4A, CDKN2B, DAPK1, GSTP1	MSP	Tumor and Plasma	[83]
Gastric cancer	P16 INK4A, CDH1, RAR- β	MSP	Plasma	[81]
Colorectal cancer	DAPK1	MSP	Tumor and Plasma	[89]
Colorectal cancer	P16 INK4A	MSP	Tumor and Plasma	[87]
Colorectal cancer	P16 INK4A	MSP	Tumor and Plasma	[88]
Colorectal cancer	P16 INK4A	MSP	Tumor and Plasma	[88]
Colorectal cancer	MLH1	MSP	Tumor and Plasma	[85]
Colorectal cancer	SFRP2, SFRP5, PGR	MSP	Stool	[91]
Colorectal cancer	HIC1	MSP	Stool	[92]
Colorectal cancer	vimentin	MSP	Stool	[93]
Bladder cancer	p14ARFARF, p16INK4A	MSP	Tumor and Plasma	[94]
Bladder cancer	p16INK4A	MSP	Tumor and Plasma	[95]
Bladder cancer	CDH1, APC, MGMT, RASSF1A, GSTP1, P16INK4A, RAR- β , ARF	qMSP	Tumor and Urine	[96]
Bladder cancer	DAPK1, BCL2, TERT, EDNRB, RASSF1A, and TNFRSF25	qMSP	Tumor and Urine	[97]
Head and neck cancer	p16INK4A, CDKN2B	MSP	Plasma	[78]
pancreatic cancer	P16 INK4A, ppENK	MSP	Tumor and plasma	[100]
pancreatic cancer	CCND2, FOXE1, NPTX2, ppENK, p16INK4A, TFP12	qMSP	Tumor and pancreatic juice	[101]
Esophageal cancer	APC	MSP	Tumor and Plasma	[104]
Esophageal cancer	TAC-1	MSP	Tumor and Plasma	[103]
Esophageal cancer	p16INK4A	MSP	Tumor and Serum	[105]
Cervical cancer	CDH1, CDH13	MSP	Plasma	[106]
Cervical cancer	hTERT, MYOD1, PGR, TIMP3, CALCA	MSP	Tumor and Plasma	[107, 108]
AML and ALL	CDKN2B, p16INK4A	MSP	Tumor and Plasma	[109]
NHL	DLC1, PCDHGB7, CYP27B1, EFMA5	MSP	Tumor and Plasma	[110]
HL, NHL	p16INK4A	MSP	Plasma	[111]