ELSEVIER

Critical Reviews in Oncology/Hematology 60 (2006) 9–18

Critical Reviews in Oncology Hematology

www.elsevier.com/locate/critrevonc

Genetic and epigenetic biomarkers in cancer diagnosis and identifying high risk populations

Mukesh Verma [a,*], Upender Manne [b]

[a] *Analytic Epidemiology Research Branch, Epidemiology and Genetics Research Program, Division of Cancer Control and Population Sciences, National Cancer Institute, Bethesda, MD 20892, United States*

[b] *Department of Pathology, University of Alabama at Birmingham, Birmingham, AL 35294, United Kingdom*

Accepted 20 April 2006

Contents

1. Background and Introduction 10
2. Genetic markers in cancer diagnosis and risk assessment 10
 2.1. Nucleotide excision repair 10
 2.2. Long DNA 10
 2.3. Loss of heterozygosity 10
 2.4. Microsatellite instability 11
 2.5. Mitochondrial DNA 11
 2.6. Circulating DNA 11
 2.7. Examples 11
  2.7.1. Brain cancer 11
  2.7.2. Colorectal cancer 11
  2.7.3. Esophageal cancer 11
  2.7.4. Gastric cancer 12
  2.7.5. Kidney cancer 12
  2.7.6. Acute myeloid leukemia (AML) and childhood acute lymphocytic leukemia (ALL) 12
  2.7.7. Liver cancer 12
  2.7.8. Lung cancer 12
  2.7.9. Ovarian cancer 12
  2.7.10. Pancreatic cancer 12
  2.7.11. Prostate cancer 12
  2.7.12. Thyroid cancer 13
3. Epigenetic markers in cancer diagnosis and risk assessment 13
 3.1. Examples 13
  3.1.1. Bladder cancer 13
  3.1.2. Brain cancer 13
  3.1.3. Breast cancer 13
  3.1.4. Cervical cancer 13
  3.1.5. Colorectal cancer 14

Abbreviations: A, astrocytomas; AA, anaplastic astrocytomas; CRC, colorectal cancer; DAPK, Death-associated protein-kinase; FAP, familial adenomatous polyposis; GB, glioblastomas; HMRD, hereditary mismatch repair deficiency; HNPCC, hereditary non-polyposis colorectal cancer; LOH, loss of heterozygosity; LOI, loss of imprinting; MSI, microsatellite instability; MMR, mismatch repair; NER, nucleotide excision repair; XP, xeroderma pigmentosum

* Corresponding author at: Analytic Epidemiology Research Branch, Epidemiology and Genetics Research Program, Division of Cancer Control and Population Sciences, National Cancer Institute, Rm. 5104, 6130 Executive Boulevard, Rockville, MD 20852, United States. Tel.: +1 301 594 7344; fax: +1 301 402 4279.

E-mail address: vermam@mail.nih.gov (M. Verma).

1040-8428/$ – see front matter © 2006 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.critrevonc.2006.04.002

3.1.6. Endometrial cancer ... 14
3.1.7. Liver cancer ... 14
3.1.8. Lung cancer ... 14
3.1.9. Oral cancer ... 14
3.1.10. Prostate cancer ... 14
4. Challenges and potential solutions ... 15
5. Concluding remarks ... 15
Reviewers ... 15
References ... 15
Biography ... 17

Abstract

Biomarkers present the normal and/or disease state in humans. Genetic and epigenetic biomarkers assessed in easily accessible biological materials are useful in diagnosis, early onset or risk of developing cancer or to predict the treatment efficacy or clinical outcome of different human malignancies. Moreover, some of these markers are expressed during early stages of the tumor development and hence provide an opportunity to develop intervention and treatment strategies. Attempts are being made to validate cancer biomarkers in non-invasively collected samples. Multiplexing of clinically validated markers is still a challenge. Once validated, these markers can be utilized in clinical settings and to identify high risk populations. In this review, the current status of the clinical genetic and epigenetic biomarkers and their implication in cancer diagnosis and risk assessment are discussed.
© 2006 Elsevier Ireland Ltd. All rights reserved.

Keywords: Biomarker; Epigenetics; Risk Assessment; Screening

1. Background and Introduction

Biomarkers are used for risk assessment, prevention, early detection, diagnosis, treatment, prognosis and recurrence of different diseases including cancer [1]. Among all types of markers, several genetic and epigenetic markers have been very well characterized and utilized in clinical studies. We have discussed both markers in different tumor types and compared their strengths and weaknesses.

Epidemiological studies based on biomarkers, behavior, and lifestyle, are crucial to predict and identify high risk populations. Different populations are susceptible to different cancers. For example, there has been a dramatic change in the epidemiology of upper gastrointestinal cancer in the Western World in general and United States in particular, over past two decades [2]. However, a substantial decline in the gastric cancer and increase in mortality from esophageal cancer has been observed. Esophageal adenocarcinoma is more common than esophageal squamous carcinoma in the US. Identification of cancer early in population studies gives an opportunity for intervention and in this whole process genetic and epigenetic biomarkers play a major role.

In the following two sections we have discussed about the current status of genetic and epigenetic markers in cancer diagnosis and risk prediction and establish that both markers provide complementary information.

2. Genetic markers in cancer diagnosis and risk assessment

Cancer is a genetic, epigenetic and cytogenetic disease. A number of DNA based markers have been identified for cancer detection. Some of these markers are described below.

2.1. Nucleotide excision repair

These markers have been used in identifying high risk population by examining the association between DNA repair capacity phenotype and genetic polymorphisms of the nucleotide excision repair (NER) genes and risk of tobacco-related cancers, including cancers of the lung, head and neck, prostate, bladder, breast, and esophagus [3]. DNA repair plays a central role in maintaining genomic stability. Some patients with xeroderma pigmentosum (XP) are highly susceptible to ultraviolet light (UV)-induced melanoma and nonmelanoma skin cancers. It is the consensus among scientists that DNA repair capacity may contribute to genetic susceptibility leading to cancer development in the general population.

2.2. Long DNA

DNA seems to be stable in stool and available in sufficient quantity to utilize PCR based methods for detection of mutations in colorectal cancer (CRC). The challenge in this technique is the potential of degradation of DNA and the presence of inhibitors of the polymerase enzyme [4–6]. Unless the DNA degradation problem is resolved, it is less likely that long DNA is used in clinic as a marker.

2.3. Loss of heterozygosity

Loss of heterozygosity (LOH) may contribute to cancer development. In gastric cancer, LOH and mutation in *PTEN*

are common features and have been utilized as a marker [7]. The disadvantage of using this marker is its low specificity in different tumor types.

2.4. Microsatellite instability

Microsatellite instability (MSI) is the phenotypic hallmark of a deficient DNA mismatch repair system, observed in 10–20% of sporadic colorectal cancers (CRC). In general, five dinucleotide MSI are sufficient to identify high risk CRC population but among Asian population two MSI are sufficient for screening [8]. In some CRC patients, combination of LOH and MSI markers give better results than MSI alone during the progression of the disease [9]. Since 90% of CRC cases occur in people who are above median familial/genetic risk, it makes sense to use genetics to prevent colorectal cancer. Two rare inherited syndromes, familial adenomatous polyposis (FAP) and hereditary non-polyposis colorectal cancer (HNPCC) have been identified. FAP appears to be mostly due to mutations in the *APC* gene, and HNPCC occurs due to mutations in mismatch repair (MMR) genes, so it would be better named as hereditary mismatch repair deficiency syndrome (HMRDS). MSI has been reported in other tumor types also and it is wise to utilize this marker as a complementary marker along with information from other markers used for diagnosis and prognosis.

2.5. Mitochondrial DNA

D loop or displacement loop of the mitochondria carries mutations in a variety of cancers, such as breast, esophagus, head and neck, lung, and colon [10–12]. However, it has not yet been established whether mitochondrial DNA contribute to the genomic instability of the nuclear DNA. Considering the small size of the mitochondrial genome (16.5 kb) and the available high-throughput sequencing technologies, mitochondrial DNA provides a good source of mutation detection. A mitochip containing key mutations of different tumor types has recently been developed and most of the steps involved in mutation detection have been automated.

2.6. Circulating DNA

Tumors release a substantial amount of genomic DNA into the systemic circulation, probably through cellular necrosis and apoptosis. The plasma DNA ranges from 10–30 ng/ml in healthy control subjects to 30–120 ng/ml in some cancer patients. This DNA contains the genetic and epigenetic alterations that are specific to the primary tumor. These alterations include microsatellite alterations, mutations, and aberrant patterns of methylation. Examples of such alterations have been reported for cancer of lung, liver, prostate, head and neck, gastric, and glioma [13–17]. Since the circulating DNA is derived from the tumor cells, it cannot be used as an early cancer detection marker. However, this DNA is suitable for disease diagnosis and treatment follow up. The challenge in the field is to identify the anatomic-origin of the DNA which warrants further investigation.

2.7. Examples

In the following section, we have described tumor specific genetic markers and their strength and weakness when utilized for disease detection.

2.7.1. Brain cancer

In astrocytoma, chromosome 22 accumulates abnormal changes including MSI, which can be detected by using more than 20 markers indicating allelic imbalance. In one study, tile path array covering 10 Mb of the chromosome was analyzed for astrocytic tumors-diffuse astrocytomas (A), anaplastic astrocytomas (AA), and glioblastomas (GB) and results indicated that a combination of deletion, alteration of copy number and reduplication occurred [18]. For example, in glioblastoma, deletion of *DEPDC5/KIAA0645, YWHAH, C22ORF24/HSN44A4A* genes was observed. In another report, meningioma showed abnormalities in chromosome 22 [19]. Abnormalities included deletion and amplification of certain regions [18]. In oligodendroglial tumors, deletions on the short arm of chromosome 1 (1p) and the long arm of chromosome 19 (19q) have been used as diagnostic markers. Based on above observations simple diagnostic assays have been developed [20].

2.7.2. Colorectal cancer

Colorectal Cancer is the third most common malignancy worldwide. In 2000, there were 945,000 new cases and 492,000 deaths caused by this cancer [21]. Identification of genetic abnormalities led to the development of a stepwise mutation accumulating model starting from adenoma and ending in carcinoma. For screening of populations, mutations in genes *K-ras*, *p*53, *APC* have been used. For these studies the DNA was isolated from blood cells or stool. Mutations in *K-ras* are located in codon 12 and 13 only. *K-ras* is involved in signal transduction pathway and stimulate cell proliferation. *p*53 is involved in DNA damage, DNA repair and cell death. Mutations in *p*53 lead to genomic instability and malignant progression. Mutations in *APC* are considered "gatekeeper" mutations which initiate the carcinogenesis process [22,23]. Mutated *APC* cannot degrade and inactivate betacatenin in the WNT signaling pathway. For early detection of CRC, *APC* mutations are used [24]. In contrast to the *K-ras* where mutations are localized, mutation in *APC* can occur at almost any site in the first 1600 codons of the gene.

2.7.3. Esophageal cancer

A number of markers have been identified for adenocarcinoma of the esophageal cancer but very few have been reported for squamous carcinoma. Barrett's esophagus represents the precursor of the esophageal cancer [25]. There is mounting evidence that there is an underlying genetic susceptibility to Barrett's esophagus and esophageal adeno-

carcinoma [26]. However, this is likely to be the result of multiple low penetrance susceptibility genes which have yet to be identified. The presence of aneuploidy, loss of heterozygosity of *p*53 and *cyclin D*1 overexpression are indicators of the onset of esophageal carcinoma [25,27]. At present time the mortality from esophageal carcinoma has exceeded 80% in five years (http://www.cancer.gov). A radical approach is necessary to prevent cancer development in high risk individuals with precancerous lesions.

2.7.4. Gastric cancer

Polymorphism in selected genes, LOH and *PTEN* mutations have been detected in gastric cancer [7,28,29]. *Helicobacter pylori* infection is a known risk factor of gastric carcinogenesis [30]. *H. pylori* infection induces genomic instability and inflammation in gastric cancer patients [31]. Serum levels of *p*53, *c-met*, and *APC* reflects progression of the disease [32]. Since *H. pylori* is associated with gastric cancer, it was an obvious thought to develop a vaccine against this bacterium or use antibiotic to kill *H. pylori*. Due to the reasons not understood to date, removal of *H. pylori* was found to be associated with development of the esophageal cancer. Certainly, future research in this direction will shed light on this complex problem.

2.7.5. Kidney cancer

Not many markers have been studied in kidney cancer. However, kallikrein 1 has shown promising results in kidney cancer detection [33,34]. Kallikreins are present on the walls of blood vessels of kidney and abnormal expression of the gene results in cancer development. In few studies microsatellite instability has also been reported [35]. Benign mixed epithelial and stromal tumor of the kidney (MEST) is a new and rare entity. These tumors are composed of two components: a stromal and an epithelial one. Clinical outcome is usually good; no specific cytogenetic alterations have been described up till now.

2.7.6. Acute myeloid leukemia (AML) and childhood acute lymphocytic leukemia (ALL)

Translocation markers on selected chromosomes have been reported for ALL and AML [36]. The samples are collected from neonatal blood spots. Suggestions have been made that the childhood cancer starts before birth and exogenous factors, such as environment, infectious agents, and life-style of parents contribute to the development of cancer. Polymorphism in *NR3C1* has also been reported [37].

2.7.7. Liver cancer

Major etiologic factors associated with human hepatocellular carcinomas (HCCs) include infection with hepatitis C (HCV) and hepatitis B virus (HBV), excess alcohol intake and aflatoxin B-1 exposure. In a recent study, chromosomal alteration was observed in liver cancer, specifically deletion of 4q, 16q, 13q, and 8p positively correlated with hepatitis B virus etiology whereas loss of 8p correlated with the cancer in hepatitis negative cases [38]. Nakajima et al. reported genomic instability due to centrosome duplication, thus affecting the cell cycle check point regulation, and mutation in *p*53 [39]. Due to centrosome aberration and a defective checkpoint system, liver cells have the potential for genetic instability and aggressive behavior. These effects were observed irrespective of the tumor size or stage.

2.7.8. Lung cancer

Smoking is the causative factor in lung cancer. Lung cancer is divided in two main histological groups, 80% of them are non-small cell lung carcinomas (NSCLCs) and the remaining are small cell lung carcinomas (SCLCs). A number of chromosomal deletions are reported in these two types of cancers (NSCLCs: 3p, 6q, 8p, 9p, 9q, 13q, 17p, 18q, 19p, 21q, 22q; SCLCs: 3p, 4p, 4q, 5q, 8p, 10q, 13q, 17p) [40,41]. Specifically, the frequency of LOH at 3p, 9p, 13q, and 17p was related to proliferative activity in smokers with Stage I NSCLC [42,43]. Mutation in several genes (such as *EGFR, K-ras*, *p53*, *erbB2*) and polymorphism in *CYP1A1, GSTM1* and *GSTT1* have also been reported [44,45]. Mitochondrial genomic instability is also a diagnostic marker for lung cancer when this marker is used in combination with other lung markers [46,47]. Information about the smoking history of the participants in the study is also available.

2.7.9. Ovarian cancer

Epithelial ovarian cancer (EOC) is the leading cause of death from gynecological malignancies in the United States. *BRCA* mutations predispose a person's risk to develop breast and ovarian cancer. So far no reliable genetic marker has been identified which can be used to detect this cancer in early stages of cancer development. Microarray technologies have indicated the role of the *BRCA*1, *BRCA*2, *p*53, *Her*2*/neu, PIK*3*CA*, *AKT*2, *K-ras, c-myc, p*53, *p*16, and *p*27 in ovarian cancer [48]. In small number of plasma DNA samples, mutation of *p*53 has been reported [49]. Attempts are being made to identify proteomic or glycomic markers of ovarian cancer but success is awaited.

2.7.10. Pancreatic cancer

Pancreatic cancer is the fourth leading cause of cancer-related deaths in the United States. The incidence rate and mortality is almost the same for this cancer. The most frequently used markers are *K-ras* mutation and telomerase although these markers are not sufficient to detect the disease in early stages and more research is needed in this area [50]. To identify high risk population, patient related information is very useful. If a person has long-standing diabetic, history of alcohol uptake, has *K-ras* mutation, and shows hypermethylation of *p*16, chances are the person is at high risk of developing pancreatic cancer.

2.7.11. Prostate cancer

Allelic imbalance, loss of 8p, mutations in androgen and progesterone receptors, and loss of heterozygosity have been

observed in prostate cancer [51–56]. Similar to other epithelial cancers, telomerase activity is reduced in this cancer. Current research focus in several labs is on the identification of genetic polymorphism in genes involved in hormone-regulated pathways in cancer [56,57]. Presently, men with a family history of prostate cancer can be provided with little advice in terms of preventive action. It is likely that one or more genetic mutations associated with a high risk for prostate cancer will be identified in the near future. The etiology of prostate cancer remains obscure. Racial differences in genetic polymorphisms that have a role in biosynthesis and metabolism of androgen may partly account for racial differences in prostate cancer risk. Phylogenetic models for prostate cancer should be developed incorporating multiple loci from individual genes. This will maximize the chance of identifying individuals with high risk genotypes resulting in better intervention strategies.

2.7.12. Thyroid cancer

CA125 marker, detected in cells isolated from circulating blood can distinguish malignant from benign follicular thyroid tumors [58]. Another marker, *HBME*-1, which is normally utilized for the diagnosis of papillary carcinoma, can also be used for follicular carcinoma diagnosis using fine-needle aspiration biopsy samples [10].

In general, the advantage of using genetic markers, as opposed to RNA or proteomic markers, is that the starting material, DNA, is very stable and since most of the detection technologies are PCR based, only very small amount of material is needed for the assay.

3. Epigenetic markers in cancer diagnosis and risk assessment

Epigenetics of human cancer has become an area of emerging research due to growing understanding of specific epigenetic pathways, identification of epigenetic markers, and rapid development of detection technologies. Since only 15% cancers are familial, it is logical to understand epigentics which covers the remaining cancers. Changes in the status of DNA methylation and chromatin modifications are among the most common molecular alterations in human neoplasia. The CpG islands of many genes, which are mostly unmethylated in normal tissue, are methylated to varying degrees in human cancers, thus representing tumor specific alterations in age-matched population [59]. DNA methylation markers are used in cancer diagnostics for both disease classification and disease detection. As a classification tool, CpG island hypermethylation is generally analyzed on sufficient quantities of primary tissues such as surgically resected tumor sample. DNA methylation status of individual gene can also be used for diagnosis and risk assessment [60–62]. Abnormal methylation can predispose cells into precancerous stage through inactivation of tumor suppressor genes and cell cycle regulatory genes by hypermethylation and reactivation of oncogenes by hypomethylation within the promoter region.

Some regions of a gene are referred to “core regions” for methylation although methylation has been observed in “non-core regions” also [63]. If methylation of non-core region leads to core region, especially in tumor suppressor genes, this could be used as a tumor marker for diagnosis and/or risk assessment to identify high risk population. Some groups are intensely investigating the role of methylation in non-promoter regions. The advantage of utilizing methylation markers in screening high risk population is that methylation changes can be detected in exfoliated cells from buccal cells (oral cancer), urine (bladder cancer), pancreatic juice (pancreatic cancer), sputum (lung cancer), and in free DNA from the plasma to detect different cancers [13,64]. Screening stool for colorectal cancer DNA markers has a potential to be used in clinic [5,6]

3.1. Examples

A brief description of epigenetic markers in different tumor types is described below.

3.1.1. Bladder cancer

Death-associated protein-kinase (*DAPK*) is associated with early recurrence of bladder cancer as detected by methylation analysis [65]. Statistical analyses showed a significant association between *DAPK* promoter methylation and higher pathological stage, but not with tumor size or nuclear grade. There is a need to identify more markers for this cancer type. Comparing MSI results with methylation profiles of specific genes in the samples collected from a nested case-control study would be useful in establishing the importance of epigenetic markers in clinic.

3.1.2. Brain cancer

The most studied brain tumor epigenetic markers are abnormal methylation of tumor suppressor genes *CDKN2A, CDKN2B* and *p14ARF* [66]. Imprinting of the human neuronatin (*NNAT*) gene, located on 20q11.2-q12, a region exhibiting loss of heterozygosity in acute myeloid leukemia and myelodysplastic/myeloproliferative disease, has also been used in diagnosis [67].

3.1.3. Breast cancer

When serum DNA of breast cancer patients was analyzed for methylation analysis, hypermethylation of *ESR1, APC, HSD17B4, H1C1*, and *RASSF1A* genes was observed [68]. Nevertheless, further studies, including larger sets of patients and more diversified tumors, as well as benign lesions, are needed to validate these results.

3.1.4. Cervical cancer

Hypermethylation of three genes, *MYOD1, CDH1*, and *CDH13,* was observed in sera of cervical cancer patients as

determined by MethyLight technology of methylation analysis [68]. These investigators used cervical cancer markers in prognosis of the disease. Presence of human papilloma virus (HPV) genome is a risk factor for cervical cancer. Methylation of HPV16 and HPV18 specific genes has also been reported [69–71]. Preliminary reports indicate hypermethylation of *SPARC, TFPI2, RRAD, SFRP1, MT1G*, and *NMES1* genes in samples isolated from cervical cancer patients.

3.1.5. Colorectal cancer

In CRC and gastric cancer methylation markers have been detected during very early stages. Loss of imprinting (LOI) has been observed in peripheral leukocytes and has been used in predicting CRC [72]. The DNA repair gene O6-methylguanine-DNA methyltransferase (*MGMT*) is frequently methylated in colorectal cancer [73]. Some colorectal cancers arise from a '*field defect*' defined by epigenetic inactivation of *MGMT*. Detection of this abnormality was reported to be a useful tool in risk assessment for colorectal cancer [74]. Based on Restriction Landmark Genomic Analysis (RLGS) analysis, tumor suppressor genes, *SLC5A8* and *SFRP1*, were identified from small cell colorectal cancer patient samples. These genes silence WNT signaling pathway [75]. *MLH1* also gets hypermethylated in CRC [76].

3.1.6. Endometrial cancer

DNA isolated from vaginal secretion collected from tampons for aberrant methylation of five genes (*CDH13, HSPA2, MLH1, RASSF1A*, and *SOCS2*) was examined using MethyLight in patients with endometrial cancer [77]. Involvement of *hMLH1, p16(INK4a)* and *PTEN* in the malignant transformation of endometriosis have also been proposed in contribution of endometrial cancer [78]. Since collection of samples was achieved by non-invasive techniques, these tests have potential to be applied in clinic.

3.1.7. Liver cancer

Analysis of hepatocellular carcinoma by RLGS led to the identification of *COSC1*, a tumor suppressor gene, associated with JAK-STAT signaling pathway [79]. Methylation of *p16, RASSF1a, CASP8*, and *CDH13* has been observed in liver cancer [80]. Other potential markers of the liver cancer are *DCL1, MAGE-A1* and *MAGE-A3*.

3.1.8. Lung cancer

Based on RLGS analysis, a tumor suppressor gene, *BMP3B*, was identified from small cell lung cancer patient samples. Other genes which frequently show hypermethylation in lung cancer include *p16, DAPK, FHIT*, *MGMT, CDKN2A*, and *RARbeta* [60,76]. There was a strong correlation between the expression of *FHIT* at the transcript and protein level.

3.1.9. Oral cancer

In a recent study, oral cancer was diagnosed using methylation of a number of genes including *p16(INK4a), p14(ARF), RB1, p21(Waf1), p27(Kip1), PTEN, p73, 0(6)-MGMT*, and *GST-P* [81]. Data on patient age, sex, tobacco use, alcohol consumption, lesion site, degree of tumor differentiation, tumor size, presence of lymph node metastasis, and clinical stage was also available for these patients. Overall, gene methylation was detected in about 50% of samples and was closely correlated with tobacco use and/or alcohol consumption. Of the genes investigated, *p16(INK4a), p14(ARF), 0(6)-MGMT, RB1, PTEN,* and *p27(Kip1)* were found to be methylated, but methylation of *p21(Waf1), p73*, and *GST-P* was not detected. Methylation frequencies were much higher for each gene when computed among informative cases only. The most significant outcome of this investigation was the concurrent promotor hypermethylation of *p16(INK4a)* and *p14(ARF)* which correlated significantly with tumor size, lymph node metastasis, and stage III/IV advanced OSCC.

3.1.10. Prostate cancer

A number of genes get inactivated in prostate cancer due to hypermethylation. These genes include 14-3-3 *sigma factor, laminin*-5 and *GSTP1* [82,83] Since *GSTP1* gene does not have mutations on specific sites and polymorphism in this gene has not been reported in prostate cancer, gene inactivation of *GSTP1* by hypermethylation seems to be the primary mechanism which contributes to the development of prostate cancer. Another potential marker is *PDLIM4* which could be used as a sensitive molecular tool in detection of prostate tumorigenesis.

Additional Comments Some genes, such as *BRCA1* and *APC*, have multiple promoters. Methylation of one promoter does not seem to be sufficient to silence these genes in breast cancer [63]. In thyroid cancer, thyroid-stimulating hormone receptor (*TSHR*) expression is frequently silenced in epithelial thyroid cancers associated with decreased or absent TSH-promoted iodine uptake. This occurs due to hypermethylation of the *TSHR* and the GA-binding protein, a transcription factor, binds the unmethylated *TSHR* promoter in FRTL-5 cells (cell lines derived from thyroid) but does not bind to the methylated promoter in FRT cells. Attempts are being made to utilize level of methylation of different genes to correlate with the stage of the disease.

Generally, tissues and serum are used as samples to isolate DNA and measure methylation levels of specific genes. In recent years, a number of other novel sources of DNA have been successfully tested, for example, nipple aspirate fluid, breast fine-needle washings, pancreatic juice, bronchial brush samples, buccal cells, needle biopsies, prostate fluid or ejaculate, lymph nodes, bronchialveolar lavage, exfoliated cells from bladder and cervix, urine or urine sediments, peritoneal fluid, stool and vagina tampons. Some attempts also have been made to isolate DNA from paraffin blocks with limited success.

Since one gene can be regulated genetically as well as epigenetically simultaneously, it is important to understand the context in which a gene is used for diagnostic purposes and for understanding the mechanism of carcinogenesis. DNA based

biomarkers can be easily transferred from a research laboratory setting into routine diagnostics in a clinic due to the stable and amplifiable nature of DNA. Furthermore, methylation analysis is attractive since positive signals can be detected that are independent of the expression levels of genes.

Diagnosic care must be taken into account regarding the information associated with the sample, such as patient's lifestyle, exposure and genetic history, consumption of alcohol, presence of diseases other than cancer.

4. Challenges and potential solutions

Challenges in the cancer diagnostic markers can be divided into two broad categories: biological and technical. Biological challenges relate to the nature of carcinogenesis and the sample which is used for DNA analysis (either for genetic or epigenetic markers). Technological challenges include the ability to collect quality material and analyze to get high sensitivity and specificity of biomarkers. We have explained both types of challenges by giving several examples in the following section.

Although it is evident that DNA circulates freely in the blood stream of healthy controls or even cancer patients, the source (origin) of this DNA remains enigmatic. It is possible that lymphocytes and other nucleated cells are the source of this circulating DNA in normal person but DNA may be released from the tumor cells of a cancer patient. One such example exists for lung cancer where the levels of plasma DNA are 4–6 times higher in cancer patients than in normal cells [84–86]. However, hard core evidence does not exist showing that the source of DNA is lung in case of lung cancer patient. Investigations on circulating DNA pave the way for the development of a tumor specific biomarker that could be critical as a serial biomarker and may also be important diagnostically.

In methylation analysis by microarray approach, selection of the primers should be done very carefully. A key in such analysis is quantitation to best utilize these markers in clinic. Furthermore, combining genetic, epigenetic, and proteomic markers for diagnosis of specific tumor types is still a challenge. Care should be taken in such approaches to measure sensitivity and specificity. Multiplexing needs improvement too.

Prenatal diagnosis in selected cancers can be achieved by direct DNA testing if the population at risk has a limited number of relatively common mutations, or if the gene being tested is small. The use of polymorphic markers located very close to a given gene location can identify patients and carriers indirectly and may be used for early diagnosis in pregnancy. The challenge is in efficient utilization of human genome sequence information and high-throughput technologies.

Most of the samples for genotyping studies are in the form of serum or plasma stored 20–30 years ago for epidemiological studies. Although serum and plasma are not a good source of DNA, compared to tissue or cells, whole genome amplification (WGA) can be achieved using the latest technology in the genotyping field [87]. The amplified DNA is good for mutation detection and SNP identification [88,89]. The only problem with this method is that it is expensive due to high price of the enzymes used for amplification [90].

When researching markers of cancer for clinical use, it is important that biomedical pathways and their alterations are measured in the same tumor populations.

5. Concluding remarks

Current genetic and epigenetic markers offer unprecedented sensitivity to detect cancer even during the preinvasive stage. The high sensitivity of these tests, however, is associated with low specificity. Better selection of individuals at highest risk of cancer using biomarkers in easily collectable samples, such as sputum, blood, or exhaled breath, as well as a better understanding of genetic susceptibility, may improve their positive predictive values, minimize unnecessary downstream investigations or treatment, as well as reduce screening costs.

Reviewers (MA 758)

Frank L. Meyskens Jr., M.D., F.A.C.P., Professor of Medicine and Biological Chemistry, Director, Chao Family, Comprehensive Cancer Center, Senior Associate Dean of Health Sciences, College of Health Sciences, University of California Irvine, 101 The City Drive South, Rt. 81, Building 23, Room 4O6, Orange, CA 92868-3298, USA.

Deepak Kumar, Ph.D., Assistant Professor, Department of Biological and Environmental Sciences, University of the District of Columbia, Building 44, Room 312, 4200 Connecticut Avenue NW, Washington, DC 20008, USA.

Takao Takahashi, M.D., Ph.D., Department of Oncologic Surgery, Gifu University, Graduate School of Medicine, 1-1 Yanagido, Gifu 501-1194, Japan.

References

[1] Shih IM, Zhou W, Goodman SN, Lengauer C, Kinzler KW, Vogelstein B. Evidence that genetic instability occurs at an early stage of colorectal tumorigenesis. Cancer Res 2001;61(3):818–22.
[2] Landis SH, Murray T, Bolden S, Wingo PA. Cancer Statistics. CA J Clin 1999;49(1):8–31.
[3] Neumann AS, Sturgis EM, Wei Q. Nucleotide excision repair as a marker for susceptibility to tobacco-related cancers: a review of molecular epidemiological studies. Mol Carcinog 2005;42(2):65–92.
[4] Winawer SJ. Screening of colorectal cancer: progress and problems. Recent Results Cancer Res 2005;166:231–44.
[5] Osborn NK, Ahlquist DA. Stool screening for colorectal cancer: molecular approaches. Gastroenterology 2005;128(1):192–206.
[6] Ahlquist DA. Colorectal cancer prevention and detection. Introduction. Gastroenterol Clin North Am 2002;31(4):xi–ii.
[7] Li YL, Tian Z, Wu DY, Fu BY, Xin Y. Loss of heterozygosity on 10q23 3 and mutation of tumor suppressor gene PTEN in gastric cancer and precancerous lesions. World J Gastroenterol 2005;11(2):285–8.

[8] Salto-Tellez M, Tan SY, Chiu LL, Koay ES. Dinucleotide microsatellite repeats are essential for the diagnosis of microsatellite instability in colorectal cancer in Asian patients. World J Gastroenterol 2005;11(18):2781–3.
[9] Chang SC, Lin JK, Lin TC, Liang WY. Loss of heterozygosity: an independent prognostic factor of colorectal cancer. World J Gastroenterol 2005;11(6):778–84.
[10] Ito Y, Yoshida H, Tomoda C, et al. HBME-1 expression in follicular tumor of the thyroid: an investigation of whether it can be used as a marker to diagnose follicular carcinoma. Anticancer Res 2005;25(1A):179–82.
[11] Kumimoto H, Yamane Y, Nishimoto Y, et al. Frequent somatic mutations of mitochondrial DNA in esophageal squamous cell carcinoma. Int J Cancer 2004;108(2):228–31.
[12] Jeronimo C, Nomoto S, Caballero OL, et al. Mitochondrial mutations in early stage prostate cancer and bodily fluids. Oncogene 2001;20(37):5195–8.
[13] Esteller M, Sanchez-Cespedes M, Rosell R, Sidransky D, Baylin SB, Herman JG. Detection of aberrant promoter hypermethylation of tumor suppressor genes in serum DNA from non-small cell lung cancer patients. Cancer Res 1999;59(1):67–70.
[14] Goessl C, Muller M, Straub B, Miller K. DNA alterations in body fluids as molecular tumor markers for urological malignancies. Eur Urol 2002;41(6):668–76.
[15] Lee TL, Leung WK, Chan MW, et al. Detection of gene promoter hypermethylation in the tumor and serum of patients with gastric carcinoma. Clin Cancer Res 2002;8(6):1761–6.
[16] Sanchez-Cespedes M, Esteller M, Wu L, et al. Gene promoter hypermethylation in tumors and serum of head and neck cancer patients. Cancer Res 2000;60(4):892–5.
[17] Usadel H, Brabender J, Danenberg KD, et al. Quantitative adenomatous polyposis coli promoter methylation analysis in tumor tissue, serum, and plasma DNA of patients with lung cancer. Cancer Res 2002;62(2):371–5.
[18] Seng TJ, Ichimura K, Liu L, Tingby O, Pearson DM, Collins VP. Complex chromosome 22 rearrangements in astrocytic tumors identified using microsatellite and chromosome 22 tile path array analysis. Gene Chromosome Cancer 2005;43(2):181–93.
[19] Wada K, Maruno M, Suzuki T, et al. Chromosomal and genetic aberrations differ with meningioma subtype. Brain Tumor Pathol 2004;21(3):127–33.
[20] Hartmann C, Mueller W, Lass U, Kamel-Reid S, von Deimling A. Molecular genetic analysis of oligodendroglial tumors. J Neuropathol Exp Neurol 2005;64(1):4–10.
[21] Davies RJ, Miller R, Coleman N. Colorectal cancer screening: prospects for molecular stool analysis. Nat Rev Cancer 2005;5(3):199–209.
[22] Wang Z, Cummins JM, Shen D, et al. Three classes of genes mutated in colorectal cancers with chromosomal instability. Cancer Res 2004;64(9):2998–3001.
[23] Arends JW. Molecular interactions in the Vogelstein model of colorectal carcinoma. J Pathol 2000;190(4):412–6.
[24] He TC, Sparks AB, Rago C, et al. Identification of c-MYC as a target of the APC pathway. Science 1998;281(5382):1509–12.
[25] Fitzgerald RC. Genetics and prevention of oesophageal adenocarcinoma. Recent Results Cancer Res 2005;166:35–46.
[26] Metzger R, Schneider PM, Warnecke-Eberz U, Brabender J, Holscher AH. Molecular biology of esophageal cancer. Onkologie 2004;27(2):200–6.
[27] Wang QH, Zhang PH, Li G, Cong J, Hu N. [Loss of heterozygosity in esophageal squamous cell carcinoma and its precursor lesion]. Zhonghua Bing Li Xue Za Zhi 2004;33(4):346–9.
[28] Garza-Gonzalez E, Bosques-Padilla FJ, El-Omar E, et al. Role of the polymorphic IL-1B IL-1RN and TNF-A genes in distal gastric cancer in Mexico. Int J Cancer 2005;114(2):237–41.
[29] Dai YC, Ho CL, Tsai YC, et al. Allelic loss of 14q32 in the pathogenesis of gastrointestinal and ampullary malignancies: mapping of the target region to a 17 cM interval. J Cancer Res Clin Oncol 2005;131(2):94–100.
[30] Gologan A, Graham DY, Sepulveda AR. Molecular markers in *Helicobacter pylori*-associated gastric carcinogenesis. Clin Lab Med 2005;25(1):197–222.
[31] Perez RO, Jacob CE, D'Ottaviano FL, et al. Microsatellite instability in solitary and sporadic gastric cancer. Rev Hosp Clin Fac Med Sao Paulo 2004;59(5):279–85.
[32] Wang JY, Hsieh JS, Chen CC, et al. Alterations of APC, c-met, and p53 genes in tumor tissue and serum of patients with gastric cancers. J Surg Res 2004;120(2):242–8.
[33] Yousef GM, Obiezu CV, Luo LY, et al. Human tissue kallikreins: from gene structure to function and clinical applications. Adv Clin Chem 2005;39:11–79.
[34] Obiezu CV, Diamandis EP. Human tissue kallikrein gene family: applications in cancer. Cancer Lett 2005;224(1):1–22.
[35] Hoefling C, Schmidt H, Meinhardt M, et al. Comparative evaluation of microsatellite marker AP-PCR and CGH studies in primary renal cell carcinoma. Int J Mol Med 2004;13(6):835–42.
[36] Smith MT, McHale CM, Wiemels JL, et al. Molecular biomarkers for the study of childhood leukemia. Toxicol Appl Pharmacol 2005;206(2):237–45.
[37] Fleury I, Primeau M, Doreau A, et al. Polymorphisms in genes involved in the corticosteroid response and the outcome of childhood acute lymphoblastic leukemia. Am J Pharmacogenomics 2004;4(5):331–41.
[38] Moinzadeh P, Breuhahn K, Stutzer H, Schirmacher P. Chromosome alterations in human hepatocellular carcinomas correlate with aetiology and histological grade – results of an explorative CGH meta-analysis. Br J Cancer 2005;92(5):935–41.
[39] Nakajima T, Moriguchi M, Mitsumoto Y, et al. Centrosome aberration accompanied with p53 mutation can induce genetic instability in hepatocellular carcinoma. Mod Pathol 2004;17(6):722–7.
[40] Mao L. Molecular abnormalities in lung carcinogenesis and their potential clinical implications. Lung Cancer 2001;34(Suppl. 2):S27–34.
[41] Sugio K, Kishimoto Y, Virmani AK, Hung JY, Gazdar AF. K-ras mutations are a relatively late event in the pathogenesis of lung carcinomas. Cancer Res 1994;54(22):5811–5.
[42] Haga Y, Hiroshima K, Iyoda A, et al. Frequency of loss of heterozygosity at 3 p, 9 p, 13 q, and 17 p is related to proliferative activity in smokers with stage I non-small cell lung cancer. Thorac Cardiovasc Surg 2005;53(2):114–7.
[43] Pan H, Califano J, Ponte JF, et al. Loss of heterozygosity patterns provide fingerprints for genetic heterogeneity in multistep cancer progression of tobacco smoke-induced non-small cell lung cancer. Cancer Res 2005;65(5):1664–9.
[44] Sonobe M, Manabe T, Wada H, Tanaka F. Mutations in the epidermal growth factor receptor gene are linked to smoking-independent, lung adenocarcinoma. Br J Cancer 2005;93(3):355–63.
[45] Vineis P, Veglia F, Anttila S, et al. CYP1A1 GSTM1 and GSTT1 polymorphisms and lung cancer: a pooled analysis of gene-gene interactions. Biomarkers 2004;9(3):298–305.
[46] Lee HC, Yin PH, Lin JC, et al. Mitochondrial genome instability and mtDNA depletion in human cancers. Ann N Y Acad Sci 2005;1042:109–22.
[47] Jakupciak JP, Wang W, Markowitz ME, et al. Mitochondrial DNA as a cancer biomarker. J Mol Diagn 2005;7(2):258–67.
[48] Tammela J, Odunsi K. Gene expression and prognostic significance in ovarian cancer. Minerva Ginecol 2004;56(6):495–502.
[49] Otsuka J, Okuda T, Sekizawa A, et al. Detection of p53 mutations in the plasma DNA of patients with ovarian cancer. Int J Gynecol Cancer 2004;14(3):459–64.
[50] Verma M. Pancreatic cancer epidemiology. Technol Cancer Res Treat 2005;4(3):295–301.
[51] Linja MJ, Visakorpi T. Alterations of androgen receptor in prostate cancer. J Steroid Biochem Mol Biol 2004;92(4):255–64.

[52] Harkonen P, Kyllonen AP, Nordling S, Vihko P. Loss of heterozygosity in chromosomal region 16q24 3 associated with progression of prostate cancer. Prostate 2005;62(3):267–74.

[53] Mattos Dos Santos R, Aparecida Rainho C, Carlos Souza Trindade J, Carlos Souza Trindade Filho J, Lauro Viana De Camargo J, Regina Rogatto S. Genotyping of AR and PSA polymorphisms in a patient with Klinefelter syndrome, non-Hodgkin lymphoma, and adenocarcinoma of the prostate. Cancer Genet Cytogenet 2004;153(2):165–9.

[54] Heinlein CA, Chang C. Androgen receptor in prostate cancer. Endocr Rev 2004;25(2):276–308.

[55] Boyle P, Severi G, Giles GG. The epidemiology of prostate cancer. Urol Clin North Am 2003;30(2):209–17.

[56] Haiman CA, Brown M, Hankinson SE, et al. The androgen receptor CAG repeat polymorphism and risk of breast cancer in the Nurses' Health Study. Cancer Res 2002;62(4):1045–9.

[57] Li W, Cavasotto CN, Cardozo T, et al. Androgen receptor mutations identified in prostate cancer and androgen insensitivity syndrome display aberrant ART-27 coactivator function. Mol Endocrinol 2005;19(9):2273–82.

[58] Sato T, Harao M, Nakano S, Jotsuka T, Suda N, Yamashita J. Circulating tumor cells detected by reverse transcription-polymerase chain reaction for carcinoembryonic antigen mRNA: distinguishing follicular thyroid carcinoma from adenoma. Surgery 2005;137(5):552–8.

[59] Jones PA. Overview of cancer epigenetics. Semin Hematol 2005;42(3 Suppl. 2):S3–8.

[60] Toyooka S, Toyooka KO, Maruyama R, et al. DNA methylation profiles of lung tumors. Mol Cancer Ther 2001;1(1):61–7.

[61] Lowenfels AB, Maisonneuve P. Epidemiology and risk factors for pancreatic cancer. Best Pract Res Clin Gastroenterol 2006;20(2):197–209.

[62] Feinberg AP. The epigenetics of cancer etiology. Semin Cancer Biol 2004;14(6):427–32.

[63] Ushijima T. Detection and interpretation of altered methylation patterns in cancer cells. Nat Rev Cancer 2005;5(3):223–31.

[64] Kawakami T, Okamoto K, Ogawa O, Okada Y. XIST unmethylated DNA fragments in male-derived plasma as a tumour marker for testicular cancer. Lancet 2004;363(9402):40–2.

[65] Tada Y, Wada M, Taguchi K, et al. The association of death-associated protein kinase hypermethylation with early recurrence in superficial bladder cancers. Cancer Res 2002;62(14):4048–53.

[66] Rousseau E, Ruchoux MM, Scaravilli F, et al. CDKN2A CDKN2B and p14ARF are frequently and differentially methylated in ependymal tumours. Neuropathol Appl Neurobiol 2003;29(6):574–83.

[67] Kuerbitz SJ, Pahys J, Wilson A, Compitello N, Gray TA. Hypermethylation of the imprinted NNAT locus occurs frequently in pediatric acute leukemia. Carcinogenesis 2002;23(4):559–64.

[68] Muller HM, Fiegl H, Widschwendter A, Widschwendter M. Prognostic DNA methylation marker in serum of cancer patients. Ann N Y Acad Sci 2004;1022:44–9.

[69] Kalantari M, Calleja-Macias IE, Tewari D, et al. Conserved methylation patterns of human papillomavirus type 16 DNA in asymptomatic infection and cervical neoplasia. J Virol 2004;78(23):12762–72.

[70] Jakubickova L, Barathova M, Pastorekova S, Pastorek J, Gibadulinova A. Expression of S100P gene in cervical carcinoma cells is independent of E7 human papillomavirus oncogene. Acta Virol 2005;49(2):133–7.

[71] Wiley DJ, Huh J, Rao JY, et al. Methylation of human papillomavirus genomes in cells of anal epithelia of HIV-infected men. J Acquir Immune Defic Syndr 2005;39(2):143–51.

[72] Cui H, Cruz-Correa M, Giardiello FM, et al. Loss of IGF2 imprinting: a potential marker of colorectal cancer risk. Science 2003;299(5613):1753–5.

[73] Pegg AE. Repair of O(6)-alkylguanine by alkyltransferases. Mutat Res 2000;462(2–3):83–100.

[74] Shen L, Kondo Y, Rosner GL, et al. MGMT promoter methylation and field defect in sporadic colorectal cancer. J Natl Cancer Inst 2005;97(18):1330–8.

[75] Thorstensen L, Lind GE, Lovig T, et al. Genetic and epigenetic changes of components affecting the WNT pathway in colorectal carcinomas stratified by microsatellite instability. Neoplasia 2005;7(2):99–108.

[76] Takahashi T, Shigematsu H, Shivapurkar N, et al. Aberrant promoter methylation of multiple genes during multistep pathogenesis of colorectal cancers. Int J Cancer 2006;118(4):924–31.

[77] Fiegl H, Gattringer C, Widschwendter A, et al. Methylated DNA collected by tampons–a new tool to detect endometrial cancer. Cancer Epidemiol Biomarkers Prev 2004;13(5):882–8.

[78] Martini M, Ciccarone M, Garganese G, et al. Possible involvement of hMLH1, p16(INK4a) and PTEN in the malignant transformation of endometriosis. Int J Cancer 2002;102(4):398–406.

[79] Yoshikawa H, Matsubara K, Qian GS, et al. SOCS-1, a negative regulator of the JAK/STAT pathway, is silenced by methylation in human hepatocellular carcinoma and shows growth-suppression activity. Nat Genet 2001;28(1):29–35.

[80] Lofton-Day C, Lesche R. DNA methylation markers in patients with gastrointestinal cancers. Current understanding, potential applications for disease management and development of diagnostic tools. Dig Dis 2003;21(4):299–308.

[81] Ishida E, Nakamura M, Ikuta M, et al. Promotor hypermethylation of p14(ARF) is a key alteration for progression of oral squamous cell carcinoma. Oral Oncol 2005;41(6):614–22.

[82] Li LC, Carroll PR, Dahiya R. Epigenetic changes in prostate cancer: implication for diagnosis and treatment. J Natl Cancer Inst 2005;97(2):103–15.

[83] Henrique R, Jeronimo C, Hoque MO, et al. Frequent 14-3-3 sigma promoter methylation in benign and malignant prostate lesions. DNA Cell Biol 2005;24(4):264–9.

[84] Bremnes RM, Sirera R, Camps C. Circulating tumour-derived DNA and RNA markers in blood: a tool for early detection, diagnostics, and follow-up? Lung Cancer 2005;49(1):1–12.

[85] Garcia-Olmo DC, Gutierrez-Gonzalez L, Ruiz-Piqueras R, Picazo MG, Garcia-Olmo D. Detection of circulating tumor cells and of tumor DNA in plasma during tumor progression in rats. Cancer Lett 2005;217(1):115–23.

[86] Xie GS, Hou AR, Li LY, Gao YN, Cheng SJ. Quantification of plasma DNA as a screening tool for lung cancer. Chin Med J (Engl) 2004;117(10):1485–8.

[87] Gillespie KM, Valovin SJ, Saunby J, et al. HLA class II typing of whole genome amplified mouth swab DNA. Tissue Antigens 2000;56(6):530–8.

[88] Adkins KK, Strom DA, Jacobson TE, Seemann CR, O'Brien DP, Heath EM. Utilizing genomic DNA purified from clotted blood samples for single nucleotide polymorphism genotyping. Arch Pathol Lab Med 2002;126(3):266–70.

[89] Sjoholm MI, Hoffmann G, Lindgren S, Dillner J, Carlson J. Comparison of archival plasma and formalin-fixed paraffin-embedded tissue for genotyping in hepatocellular carcinoma. Cancer Epidemiol Biomarkers Prev 2005;14(1):251–5.

[90] Sun G, Kaushal R, Pal P, et al. Whole-genome amplification: relative efficiencies of the current methods. Leg Med (Tokyo) 2005;7(5):279–86.

Biography

Mukesh Verma is the Acting Branch Chief and a Program Director in the Analytic Epidemiology Research Branch (AERB), Epidemiology and Genetics Research Program (EGRP) of the Division of Cancer Control and Population Sciences (DCCPS) at the National Cancer Institute (NCI). Before coming to the DCCPS, he was a Program Director in the Division of Cancer Prevention (DCP), NCI, provid-

ing direction in the areas of biomarkers, early detection, risk assessment and prevention of cancer, and cancers associated with infectious agents. Dr. Verma holds a M.Sc. from Pantnagar University, Ph.D. in the field of host-virus interaction from Banaras Hindu University, and did postdoctoral research at George Washington University. He also was a faculty member at Georgetown University where he pioneered research in the field of tracheobronchial mucin, which is a glycoprotein that is abnormally expressed in lung cancer. He has published 91 research articles in major peer-reviewed journals and has edited two books, *Molecular Signatures of Infectious Agents in Cancer and Epigenetics and Cancer Prevention*. Dr. Verma also serves on the Editorial Board of *Applied Genomics and Proteomics*.