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Position Paper

Methylated genes as new cancer biomarkers

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ABSTRACT

Aberrant hypermethylation of promoter regions in specific genes is a key event in the formation and progression of cancer. In at least some situations, these aberrant alterations occur early in the formation of malignancy and appear to be tumour specific. Multiple reports have suggested that measurement of the methylation status of the promoter regions of specific genes can aid early detection of cancer, determine prognosis and predict therapy responses. Promising DNA methylation biomarkers include the use of methylated *GSTP1* for aiding the early diagnosis of prostate cancer, methylated *PITX2* for predicting outcome in lymph node-negative breast cancer patients and methylated *MGMT* in predicting benefit from alkylating agents in patients with glioblastomas. However, prior to clinical utilisation, these findings require validation in prospective clinical studies. Furthermore, assays for measuring gene methylation need to be standardised, simplified and evaluated in external quality assurance programmes. It is concluded that methylated genes have the potential to provide a new generation of cancer biomarkers.

1. Introduction

Tumour biomarkers are potentially useful in the identification of individuals at increased risk of developing cancer, in screening for early malignancies and in aiding cancer diagnoses. Following a diagnosis of cancer, biomarkers may be used for determining prognosis, predicting therapeutic response, surveillance following curative surgery for cancer and monitoring therapy (for review, see Refs. [1,2]). Currently used tumour markers are mostly proteins that are measured in either serum or plasma (e.g. by sandwich-type immunoassay) or in tumour tissue (e.g. by ELISA or immunohistochemistry).[1,2]

The primary defect in cancer resides in genomic DNA. Molecular alterations in DNA that contribute to cancer

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include mutation, amplification, translocation, loss of heterozygosity, microsatellite instability and aberrant gene methylation.[3,4] Specific genes with such abnormalities have been suggested as potential new tumour biomarkers.[5,6]

Compared with other molecular structures such as mRNA, miRNA and certain proteins, the use of DNA for the measurement of tumour marker has a number of attractive features.[7] Firstly, DNA molecules are very stable and in contrast to mRNA and many proteins, can survive harsh conditions for long periods of time. Most importantly, relatively intact DNA can be isolated from formalin-fixed, paraffin-embedded tissue. Secondly, unlike proteins, nucleic acid can be amplified by PCR and related techniques, thus allowing measurements on small amounts of test sample. The aim of this article is to review the use of one form of DNA alteration in cancer, i.e. aberrant gene promoter methylation, for the detection and management of patients with cancer. Firstly, however, a brief introduction to DNA methylation is presented.

2. DNA methylation

DNA methylation involves the substation of a hydrogen ion with a methyl group at the carbon 5 position of cytosine (C) residues, using S-adenosylmethionine as the donor molecule (for review, see Refs. [8,9]). In mammalian cells, methylation is mostly restricted to C residues that proceed guanine (G) residues, i.e. CpG dinucleotides. In general, the CpG dinucleotide is underrepresented in the mammalian genome but it can be found at relatively high frequency in short genomic sequences, known as CpG islands.

CpG islands range in size from 0.5 to 5 kb, and have a G:C content of at least 55% and an CpG to GpC frequency of at least 0.65.[10] CpG islands are associated with approximately 50% of mammalian genes and are mostly located in the promoter and first exon regions of the gene, although occasionally they are also found towards the 3′ end.[8] CpG islands are mostly unmethylated in normal adult healthy tissues, but can be methylated to varying extents in cancer.[11] Methylation of CpG islands in gene promoter regions is generally associated with gene silencing due to the abrogation of gene transcription.[8,9]

Genomic scanning of 98 different primary tumours showed that on average, there were approximately 600 aberrantly methylated genes per tumour.[12] Indeed, genes implicated in most of the steps in tumourigenesis and tumour progression can be silenced by DNA promoter methylation.[12,13] These genes include not only those encoding proteins, but also those coding for microRNAs.[8,9]

The genes undergoing methylation during the early phases of tumourigenesis are potential markers for identifying individuals at increased risk of developing malignancy or for aiding the diagnosis of early malignancy, while those genes undergoing methylation during progression of malignancy are potential prognostic markers. In addition, measurement of the methylation status of genes involved in drug sensitivity and/or resistance may yield therapy predictive information.

3. Advantages of using methylated genes as tumour biomarkers

Blood levels of most currently used protein biomarkers are rarely increased in the early stage malignancy.[1] Consequently, most existing blood protein biomarkers are of little value in either screening or aiding the early diagnosis of cancer. On the other hand, aberrant methylation of the promoter regions of multiple genes is now known to exist in both early and advanced cancers (Table 1). Release of cells or free DNA containing these aberrantly methylated genes into surrounding luminal fluids or blood might thus permit the early detection of cancer or the identification of individuals at high risk of developing cancer. Some of the advantages of using methylated genes as cancer markers are now briefly discussed.

3.1. *Serum concentrations of methylated genes display a high specificity for malignancy*

In contrast to the existing biomarkers, methylated genes appear to have superior specificity for cancer. In a review of the early literature, Laird[10] identified 599 hypermethylated CpG islands in serum or plasma from 325 independent control subjects. Remarkably, all were found to be negative for the DNA methylation biomarkers investigated, yielding an overall specificity of 100%. Lower specificity, however, was found when DNA methylation analysis was carried out on the relevant luminal fluids from these subjects.[10] As pointed out by

Table 1 – Methylated genes detected in preneoplastic lesions that have a high predisposition of progressing to invasive malignancy. HGPIN, high-grade prostate intraepithelial neoplasia; DCIS, ductal carcinoma in situ; CIN, cervical intraepithelial neoplasia.

Methylated gene	Preneoplastic lesion	Cancer type	Refs.
p16^{INK4A}	Barrett's esophagus	Oesophageal cancer	[14,15]
p16^{INK4A}	*In situ* squamous cell carcinoma of lung	Squamous cell lung cancer	[16,17]
p16^{INK4A}	*In situ* squamous cell carcinoma of cervix	Squamous cell carcinoma of cervix	[18]
p16^{INK4A}, *p14*^{ARF}, *MGMT*, *APC*	Colorectal adenoma	Colorectal cancer	[19,20]
hMLH1	Ulcerative colitis	Colorectal cancer	[21]
hMLH1	Endometrial hyperplasia	Endometrial cancer	[22]
GSTP1	HGPIN	Prostate cancer	[23,24]
APC, *DAPK*, *MGMT*	Cirrhotic liver	Hepatocellular cancer	[25]
14-3-3 sigma, *RASSF1*, *APC*, *DAPK*	Atypical hyperplasia, DCIS	Breast cancer	[26–28]
DAPK1, *RARB*, *TWIST1*	CIN-3	Cervical cancer	[29]