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(54) Title: EPIGENETIC CHANGE IN SELECTED GENES AND CANCER

(57) Abstract: A method of detecting a predisposition to, or the incidence of, cancer in a sample comprises detecting an epigenetic change in at least one gene selected from an NDRG4/NDRG2 subfamily gene, GATA4, OSMR, GATA5, SFRPl, ADAM23, JPH3, SFRP2, APC, MGMT, TFPI2, BNIP3, FOXEl, SYNEl, S0X17, PHACTR3 and JAM3, wherein detection of the epigenetic change is indicative of a predisposition to, or the incidence of, cancer. Also described are pharmacogenetic methods for determining suitable treatment regimens for cancer and methods for treating cancer patients, based around selection of the patients according to the methods of the invention. The present invention is also concerned with improved methods of collecting, processing and analyzing samples, in particular body fluid samples. These methods may be useful in diagnosing, staging or otherwise characterizing various diseases. The invention also relates to methods for identifying, diagnosing, staging or otherwise characterizing cancers, in particular gastrointestinal cancers such as colorectal cancers, gastric cancers and oesophageal cancers. The methods of the invention relate, inter alia, to isolating and analyzing the human DNA component from faecal samples and blood-based samples.

WO 2008/084219 A1

EPIGENETIC CHANGE IN SELECTED GENES AND CANCER

FIELD OF THE INVENTION

The present invention relates to methods and kits for identifying and diagnosing cancer which include detecting an epigenetic change, such as a change in the methylation status, or the expression levels, or a combination thereof of any one or more of a number of genes. Also described are pharmacogenetic methods for determining suitable treatment regimens for cancer and methods for treating cancer patients, based around selection of the patients according to the methods of the invention. The present invention is also concerned with improved methods of collecting, processing and analyzing samples, in particular body fluid samples. More particularly, the invention relates to methods for identifying epigenetic changes in body fluid samples. These methods may be useful in diagnosing, staging or otherwise characterizing various diseases. The invention also relates to methods for identifying, diagnosing, staging or otherwise characterizing cancers, in particular gastrointestinal cancers such as colorectal cancers, gastric cancers and oesophageal cancers. The methods of the invention relate, *inter alia*, to isolating and analyzing the human DNA component from faecal samples and blood-based samples.

BACKGROUND OF THE INVENTION

In their earliest stages most cancers are clinically silent. Patient diagnosis typically involves invasive procedures that frequently lack sensitivity and accuracy. Highly reliable, non-invasive screening methods would permit easier patient screening, diagnosis and prognostic evaluation.

Tumour derived markers are biological substances that are usually produced by malignant tumours. Ideally a tumour derived marker should be tumour-specific, provide an indication of tumour burden and should be produced in sufficient amounts to allow the detection of minimal disease. Most tumour derived markers used in clinical practice are tumour antigens, enzymes, hormones, receptors and growth factors that are detected by biochemical assays. The detection of DNA alterations such as mutations, deletions and epigenetic modifications (Baylin et al., 2000) provide another means for identifying cancers.

An epigenetic modification can be described as a stable alteration in gene expression potential that takes place during development and cell proliferation, mediated by mechanisms other than alterations in the primary nucleotide sequence of a gene. It is now general knowledge that both genetic and epigenetic alterations can lead to gene silencing and cellular dysfunction. Synergy between these two processes drives tumor progression and malignancy. Three related mechanisms that cause alteration in gene expression are recognised: DNA methylation, histone code changes and RNA interference.

DNA hypermethylation is an epigenetic modification whereby the gene activity is controlled by adding methyl groups (CH_3) to specific cytosines of the DNA. In particular, methylation occurs in the cytosine of the CpG dinucleotides (CpG islands) which are concentrated in the promoter regions and introns in human genes (P.A. Jones et al., 2002; P.W. Laird et al., 2003). Methylation is associated with gene

silencing. DNA hypermethylation is found to be involved in a variety of cancers including lung, breast, ovarian, kidney, cervical, prostate and also colorectal cancer. Methylation patterns of DNA from cancer cells are significantly different from those of normal cells. Therefore, detection of methylation patterns in appropriately selected genes of cancer cells can lead to discrimination of cancer cells from normal cells, thereby providing an approach to early detection of cancer.

DNA tumour markers, in particular DNA methylation markers, offer certain advantages when compared to other biochemical markers. An important advantage is that DNA alterations often precede apparent malignant changes and thus may be of use in early diagnosis of cancer. Since DNA is much more stable and, unlike protein, can be amplified by powerful amplification-based techniques for increased sensitivity, it offers applicability for situations where sensitive detection is necessary, such as when tumour DNA is scarce or diluted by an excess of normal DNA (Sidransky et al.,1997). Bodily fluids provide a cost-effective and early non-invasive procedure for cancer detection. In this context, faecal-based cancer testing has been one area of investigation.

Human colorectal cancer has provided a good model for investigating whether DNA cancer markers can be adopted as an optimal faecal-based diagnostic screening test. Central to faecal-based colorectal cancer testing has been the identification of specific and sensitive cancer derived markers.

The N-Myc downstream-regulated gene (NDRG) family comprises four family members: NDRG1 (NDRG-family member 1), NDRG2 (NDRG-family member 2), NDRG3 (NDRG-family member 3) and NDRG4 (NDRG-family member 4). The human NDRG1 and NDRG3 belong to one subfamily, and NDRG2 and NDRG4 to another. At amino acid (aa) level, the four members share 53-65% identity. The four proteins contain an alpha/beta hydrolase fold as in human lysosomal acid lipase but are suggested to display different specific functions in distinct tissues.

NDRG1 codes for a cytoplasmic protein believed to be involved in stress responses, hormone responses, cell growth, and cell differentiation. NDRG1 has been demonstrated to be upregulated during cell differentiation, repressed by N-myc and c-myc in embryonic cells, and suppressed in several tumor cells (Qu X *et al.*,2002; Guan *et al.*, 2000).

NDRG3 is believed to play a role in spermatogenesis since it is highly expressed in testis, prostate and ovary (Zhao W *et al.*, 2001). Its involvement in brain cancer development has also been suggested (Qu X *et al. 2002*).

NDRG2 codes for a cytoplasmic protein that seems to be involved in neurite outgrowth and in glioblastoma carcinogenesis (Deng Y *et al., 2003)*. It is upregulated at both the RNA and protein levels in Alzheimer's disease brains (Mitchelmore C *et al., 2004)*, and has also been suggested to play an important role in the development of brain cancer (Qu X *et al. 2002*), pancreatic cancer and liver cancer (Hu XL *et al.*, 2004).

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