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Review

Molecular biomarkers of colorectal carcinogenesis and their role in surveillance and early intervention

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Abstract

Modern medicine is increasingly focused towards population surveillance for disease, coupled with the implementation of preventative measures applied to 'at-risk' patients. Surveillance in colorectal cancer is limited by the cost and risk of endoscopy. Trials of putative chemopreventive agents in colorectal cancer are hampered by difficulties in following up large cohorts of patients over long periods of time to ascertain the clinical effect. Research into possible pathways of colorectal carcinogenesis has revealed a range of biological intermediates which could be used in surveillance, the identification of high risk populations and early diagnosis of cancer. The aim of this paper was to review the possible role of biomarkers in surveillance and the timing of intervention. A literature review using both Medline and Web of Science was performed from 1995 onwards using keywords: **biomarkers, colorectal cancer, carcinogenesis, chemoprevention, surveillance** and **screening**. Research has identified many potential biomarkers, such as cyclooxygenase-2 (COX-2), oxidative DNA adducts and glutathione S-transferase (GST) polymorphisms, which could be applied in a clinical setting to screen for and detect colorectal cancer. Molecular biomarkers, such as COX-2, oxidative DNA adducts and GST polymorphisms offer new prospects in the detection of early colorectal cancer, surveillance of high-risk populations and prediction of the clinical effectiveness of chemopreventive drugs. Their role could be extended into surgical surveillance for potentially operable disease and post-operative follow-up for disease recurrence. Research should be directed at assessing complementary biomarkers to increase clinical effectiveness in determining management options for patients.
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Keywords: Biomarkers; Colorectal cancer; Carcinogenesis; Chemoprevention; Surveillance; Screening

1. Introduction

Colorectal cancer is the second commonest cause of cancer death in developed countries [1]. An individual's lifetime risk of developing colorectal cancer is estimated at 5% and individuals with first-degree relatives diagnosed with colon cancer or adenomatous polyps, have a risk 2–3 times greater than this [2]. In the US National Polyp Study, 1418 patients were followed up for approximately 6 years after colonoscopy [3]. All polyps were removed and as a result, only five colorectal cancers were diagnosed on 5-year follow-up, corresponding with a reduction in colorectal cancer incidence of 76–90% when compared with the control group. Further evidence accrued since then suggests that surveillance of patients who have polyps, can improve survival from colorectal adenocarcinomas [4–6]. An important benefit of polyp surveillance is that polyps that do escape detection and progress to cancer, are diagnosed far earlier with a subsequent favourable impact on survival following resection surgery. Polyps can be used as markers of a population who have an above average risk of developing colorectal cancer and also as an intervention point to prevent cancer from occurring. Endoscopic screening of the general population might be beneficial, and it has therefore been advocated. Nevertheless, large-scale endoscopic screening is a contentious issue, because the cost is probably beyond the capabilities of contemporary health services whilst the benefit might be only modest. Furthermore, not all colorectal cancers arise from polyps and the polyp to carcinoma sequence is variable and probably takes many years. In addition, an invasive procedure is required to both diagnose and remove them. Therefore, alternative markers are urgently needed, the detection of which is less invasive

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and cheaper to perform than polyp screening, whilst offering equal accuracy and reproducibility.

In principle, colorectal cancer prevention can be targeted at the molecular level by interfering with carcinogenesis via alteration of behaviour, diet or by using chemical agents. Interference with carcinogenesis using chemicals is known as chemoprevention. Many putative chemopreventive substances have been identified and they can be broadly classified as 'cancer-blocking' or 'cancer-suppressing' agents according to the site along the carcinogenesis pathway at which they exert their action [7,8]. Chemoprevention requires individuals to take a compound for prolonged periods of time, possibly for life. Furthermore, a chemopreventive agent must be of low cost, high efficacy, low toxicity and display little interaction with other medications. Before large scale studies on chemopreventive agents are undertaken, smaller pilot studies are needed in which intermediate end-points are assessed, to explore whether the tested agent has a significant impact on tumour initiation, promotion and/or progression. Intermediate markers of early carcinogenesis are needed to assess any clinical effect of these tested drugs. In this review, the role of biomarkers is discussed with reference to (i) identification of populations at-risk of colorectal cancer, (ii) early diagnosis of colorectal cancer and (iii) colorectal cancer chemoprevention. Examples of pertinent molecular markers are described, and problems are addressed which may be encountered in the development of these biomarkers into clinically valid tests.

2. Properties of biomarkers of carcinogenesis

Biomarkers of carcinogenesis are quantifiable molecules involved in physiological or pathological events which occur between exposure to exogenous or endogenous carcinogens and the subsequent development of cancer [9]. Biomarkers could be the consequence of a continuous process such as an increased cell mass or cell type, or of a discrete event, such as a genetic mutation [10,11]. Biomarkers of early carcinogenesis provide a means of diagnosing very early changes associated with cancer development before actual tumours or even polyps are present. In contrast to histological biomarkers, such as polyp formation or the presence of high-grade dysplasia, molecular biomarkers can often be assayed in surrogate tissue such as blood or faeces, which obviates the need for tissue biopsies. Alterations of molecular biomarkers are often detectable much earlier than histological changes, and assays of molecular biomarkers are frequently quantitative, reducing inter-observer variation.

A molecular biomarker must exhibit properties intimately related to the particular role which it plays in the development of the specific disease it is intended to indicate. For example, markers germane to the *APC* tumour suppressor gene relate particularly to colorectal carcinogenesis. The expression of a biomarker which is to be used in surveillance or early diagnosis of cancer should correlate with early stage of the disease, i.e. it should be differentially expressed in normal, premalignant and malignant tissue. Few biomarkers currently under assessment show such a differential expression pattern. Cytokines such as interleukin-2 and interleukin-10 constitute a suitable example because their levels may be related to colorectal tumours (see below). The correlation must be reproducible between individuals, and the biomarker should be detectable sufficiently early in the carcinogenic cascade to enable intervention to have a favourable impact on mortality [12,13].

For the purposes of chemoprevention intervention studies, levels of biomarkers must also be modifiable by the particular agent tested [13]. Furthermore, modification of biomarker expression should correlate with a reduction in tumour incidence. Other necessary qualities in biomarkers include their presence in tissues or excreta that are easily collected and available for repeated assays, such as blood, serum, urine or faeces. This point is exemplified by glutathione S-transferase (GST) enzymes, which can be measured in blood leucocytes. Desirable properties of biomarkers are summarised in Fig. 1.

2.1. Risk biomarkers

Colorectal cancer risk biomarkers are used to identify individuals who are at a high risk of developing colorectal cancer. Given the molecular complexity underlying colorectal carcinogenesis, several potential biomarkers have been proposed. Among them is the GST family, or gene mutations such as the *APC* gene defect. Most risk biomarkers are, in essence, indicators of exposure to endogenous or exogenous carcinogens, e.g. DNA adducts with reactive chemicals such as reactive oxygen species, and the genetic mutations which they may eventually engender, such as mutated *APC*. There are considerable difficulties in assessing how predictably a putative risk biomarker manifests itself in the phenotype of the individual, i.e. how its alteration correlates with cancer risk. The multi-step nature of carcinogenesis and the many different pathways which feed into it, make identification of single biomarkers, which accurately determine risk, difficult. This difficulty can be overcome by choosing biomarkers which are pivotal to the carcinogenesis cascade. Thus, the ability of a biomarker to predict risk reflects in part its relative importance in the development of a cancer. For example, *APC* mutation is a key event in the malignant progression of colorectal cells. Therefore, the presence of a germ-line *APC* gene mutation is a strong predictor of an individual's

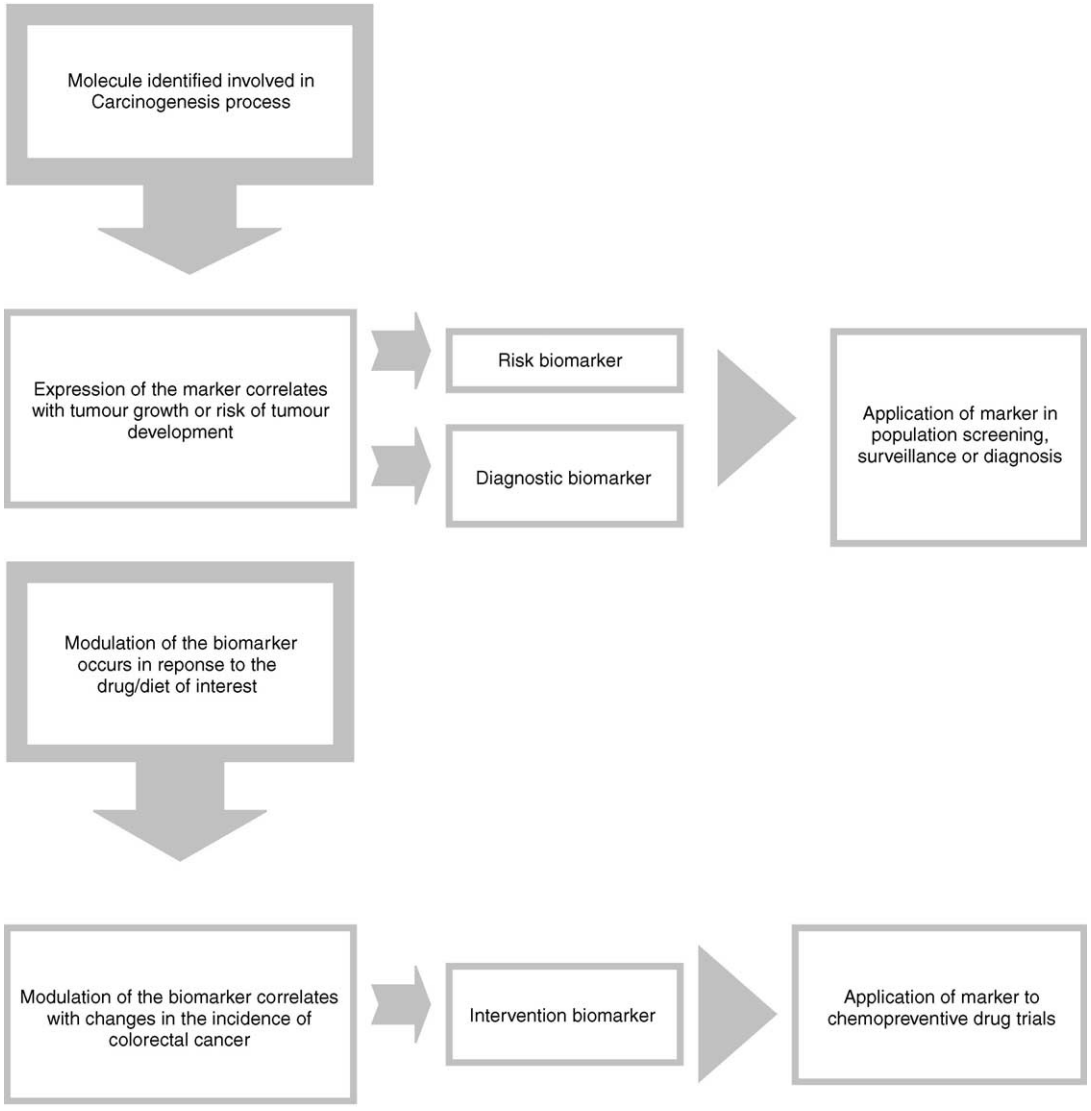

Fig. 1. Flow diagram summarising the steps to identifying biomarkers and the properties exhibited by biomarkers relating to their clinical application.

risk of developing colorectal cancer. However, germ-line mutations, such as those characterising the familial adenomatous polyposis (FAP) syndrome, account for only a small proportion of colorectal cancers, and thus their suitability to be used as a screening tool is limited. Markers with lower specificity and higher sensitivity are required for cost-effective population screening. The relative risk prediction on the basis of enzyme polymorphisms, illustrated by that characterising the glutathione-s-tranferase (GST) family [14], is lower than that associated with germ-line genetic defects, but it involves a larger proportion of the population [14]. Identifying groups with an above-average risk, who can then be targeted for more invasive investigations, is perhaps a feasible strategy for the use of risk biomarkers. Alternatively, a similar effect may be achieved by combining combinations of biomarkers, each of which contributes to overall risk. In the following selections, a selection of important risk biomarkers are described.

2.2. Cytochrome P450 enzymes

Cytochrome P450 (CYP) enzymes belong to a multigene superfamily and catalyse the metabolism of both xenobiotics and endobiotics [15]. Genetic polymorphisms of several classes of CYPs exist. Polymorphisms in CYP1A1, CYP1A2, CYP2C9M, CYP2D6 and CYPE1, result in altered expression of the active enzyme [16]. The frequency of certain genotypes, such as those linked with the CYP2C9*1 and CYP2E1*2 genes are significantly raised in individuals with a history of colorectal carcinoma [17,18]. These polymorphisms convey a small individual risk of cancer, but when applied to large groups of individuals, they could be used to identify a population of individuals who would benefit from regular endoscopic screening. Furthermore, their assessment could be combined with that of other polymorphic enzymes such as the GSTs (see below).

2.3. Glutathione S-transferases

GSTs are a family of phase II detoxification enzymes, which catalyse the conjugation of xenobiotics with glutathione [19]. In humans, GSTs can be divided into isoenzyme classes α, μ, π and θ. Each class consists of several isoenzymes with partially overlapping substrate selectivity [20]. In humans, the GSTμ phenotype is associated with three alleles existing at the loci GSTM1*A, GSTM1*B and GSTM1*0U. GSTμ enzymes are more effective at detoxifying potential cytotoxic and genotoxic epoxides than other GSTs [21]. Patients with the GSTM1* genotype have a non-functioning allele, and therefore the isoenzyme has a decreased ability to deactivate xenobiotics. GSTθ enzymes also exhibit this polymorphism with a null genotype being present [22] (Fig. 2). Both null phenotypes

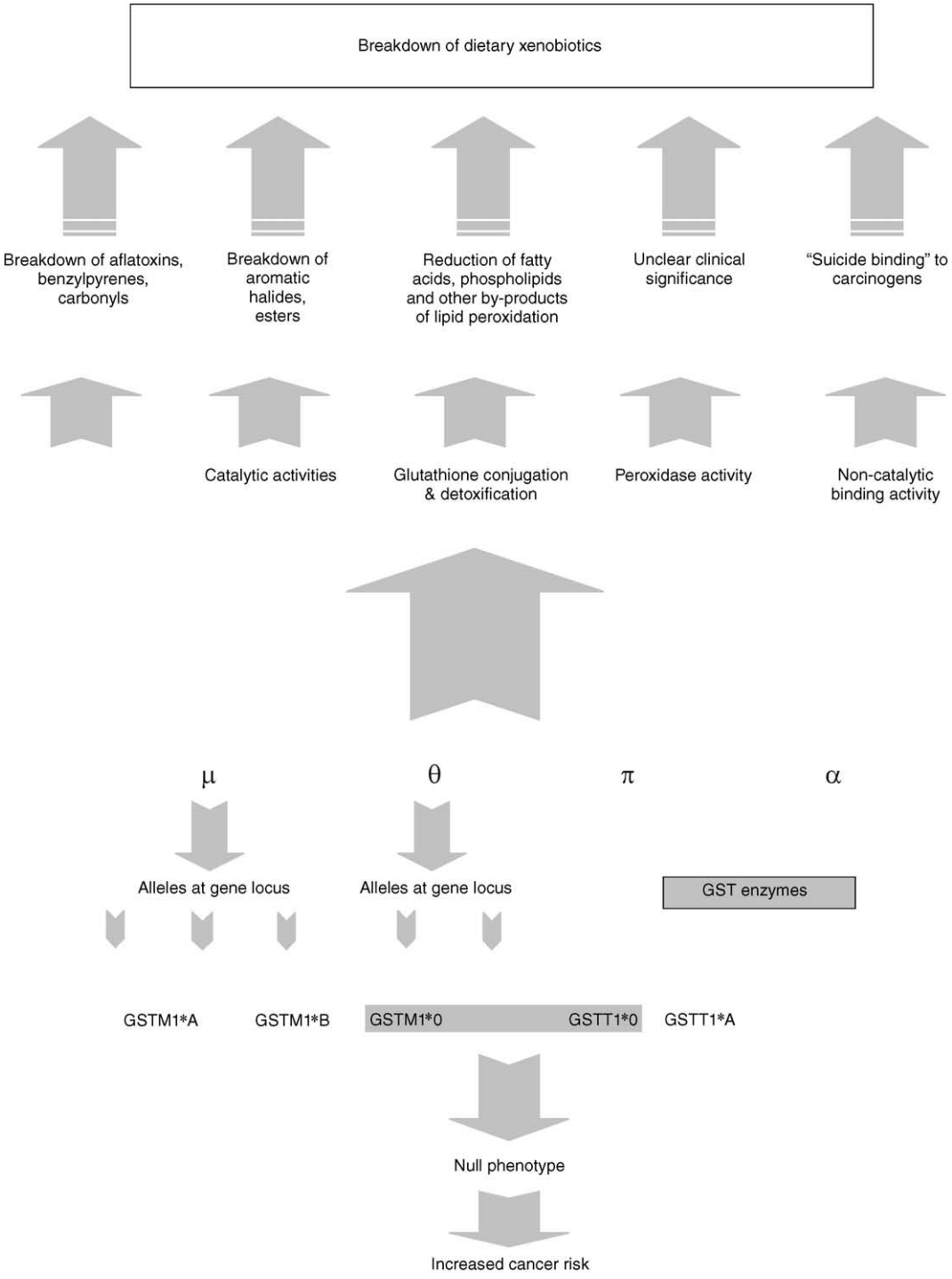

Fig. 2. GST polymorphisms and carcinogen detoxification: upward facing arrows display the role of GST enzymes in carcinogen detoxification, downward facing arrows show the polymorphisms found and their effect on cancer risk.

have been investigated in terms of correlation with an increased predisposition to colorectal cancer. They were found to be more common in healthy individuals who later developed colorectal and gastric cancer [23]. Up to 40% of Caucasians exhibit these null phenotypes, and these individuals could have a three-fold risk of developing adenocarcinomas of the stomach or colon when compared with individuals with normal enzyme expression [24]. Clearly, the ability to detect approximately half of the population group which is characterised by an increased risk of colorectal cancer would be of immense clinical benefit. GST polymorphisms can be detected reliably in lymphocytes [25,26], enabling screening or monitoring of patients via a blood test. This screening strategy would render large-scale population screening economically feasible. The polymerase chain reaction (PCR) is used to amplify DNA recovered from blood to allow gel electrophoresis of the sample. Alternatively testing can focus on events 'downstream' of the genotype using immunosorbent-linked enzyme assays. Recently, it has been suggested that the genotype may not necessarily reflect the GST phenotype when the two were compared [27]. Hence, testing for enzyme function may prove to be more reliable as a screening test.

2.4. DNA mismatch repair genes

Up to 15–20% of colorectal cancers are thought to be familial [28], and identifying this group of patients would enable clinicians to implement regular surveillance to detect early cancers. Clinical criteria to detect these patients, such as the Amsterdam criteria I and II, rely on a family history of cancer and age at presentation [29]. These clinical criteria lack accuracy, as they miss up to 30% of patients with defects in mismatch repair (MMR) genes. Attempts to increase sensitivity, for example those involving the Bethesda criteria, improve the detection rate, but are less specific, with a specificity index of $<25\%$ [28]. In clinical practice, many patients are identified as having a family history of colorectal malignancies only after several generations of family members have died early deaths secondary to cancer. Molecular assays which detect mutations in one or more of the five mismatch repair genes, *hMLH1*, *hMSH2*, *hPMS1*, *hPMS2*, *GTBP*/*hMSH6* permit a more accurate detection of these individuals [30]. Such germ-line mutations can be assayed in DNA extracted from blood monocytes, allowing an easy and cheap collection method in relatives of patients who have been diagnosed with colorectal tumours [31].

3. Biomarkers used in early diagnosis

Risk biomarkers indicate a risk above that of the general population, but they do not predict early disease in any given individual [14]. Biomarkers which predict individual risk accurately could be used to diagnose early cancer in individuals without clinical evidence of colorectal cancer. The discovery of a biomarker with a 100% capability of predicting cancer is highly unlikely, however certain biomarkers are currently under investigation for their usefulness in the clinic to aid the diagnosis of suspected colorectal cancer.

3.1. Serum testing for genetic defects

The *APC* gene, which is responsible for FAP, was localised to chromosome *5q21* in the late 1980s [32]. Testing DNA from refined phlebotomy samples can identify the mutation in 60–80% of individuals with this defect [33]. Germ line mutations involving the *APC* gene account for just 1% of all colorectal cancer cases, the remainder being non-inherited idiopathic APC mutations, rather than an inherited defect [34]. Attempts have been made to assay *APC* gene defects in sporadic colorectal carcinoma cases. In one study involving patients with a histological diagnosis of colorectal carcinoma, the *APC* gene defect was found in the serum of 20 out of a cohort of 25 patients [35]. Serum levels of the oncogene *K-RAS* have also been shown to be elevated in patients with histological disease [36]. These tests rely on detecting tumour cell DNA in the serum, rather than germ-line mutations, and hence a patient must have a significant haematogenous dissemination of tumour before these serum assays can pick up the mutated *APC* and *K-RAS* genes. It therefore seems doubtful that this detection method can reliably pick up early pre-invasive colorectal carcinoma.

Testing for genetic mutations such as APC and mismatch repair genes is difficult, because the genes are large and the mutations scattered throughout [37,38]. Furthermore, many of these mutations may be small deletions or insertions which could be missed by techniques such as direct gene sequencing. This fact may account for the large variation in pick-up rates of APC mutations in patients with FAP, which varies from 30 to 80% [29,38]. Moreover, direct gene testing is labour-intensive and therefore not applicable to large-scale screening of populations. APC gene mutations are characterised by a truncated protein, and this can be exploited by using PCR to isolate specific gene segments, and hence proteins can be produced from the amplified gene segments using *in vitro* transcription and translation reactions [38]. Truncated proteins can be analysed quickly using gel electrophoresis making this method an attractive alternative to direct gene testing. Alternatively, PCR amplification has been used followed by mass spectrometry which allows the identification of nucleic acid fragments corresponding to genotypes or mutations [39–41]. This combination of methods offers the advantage of being fast and selective for clinical APC genotyping.

Protein truncation testing has not been found to be effective for mismatch repair mutations, because the mutations do not always result in truncated protein products [37]. Little is known about the biochemical defects associated with non-truncating mutations rendering functional analysis of MMR mutations difficult [42]. Phenotypic changes associated with MMR mutations such as micro-satellite instability are likely to miss up to 10% of hereditary non-polyposis colorectal cancer (HNPCC) associated cancers [37]. An alternative method is to examine the protein expression of MMR genes using immunohistochemistry. Although normal expression of protein correlates well with the absence of micro-satellite instability [43], there may be no protein expression in cases without a germline defect leading to false-positive errors. Immunohistochemistry may be able to distinguish between *hMSH2* and *hMLH1* mutations and thus narrow down the search for the genetic defect with direct gene testing [37].

The examples of APC genes, MMR genes and GST polymorphisms highlight the difficulty of translating a laboratory based assay into a clinically reproducible, efficient and fast diagnostic test. Frequently, functional assays relating to the phenotype coded by the genes in question, may be more reliable and accurate than attempting to directly detect the genetic mutation.

The immune response to a progressing tumour could be a more accurate detection method than serum testing for genetic mutations. Because an immune response is a systemic event serum assays of biomarkers can be made. The serum levels of cytokines, such as interleukins 2 and 10, differed significantly between normal patients, patients with adenomas and patients with malignancy, in that they were higher in relation to the degree of malignancy [44]. This difference could be exploited in the surveillance of high-risk patients, obviating the need for regular endoscopic investigation. Cytokine determination might be employed in the post-operative surveillance of patients who have had resection of their primary tumours. Cytokine analysis has the disadvantage of lacking specificity, as inflammatory conditions or other tumours can give rise to increased levels. Antibodies against genetic mutations generated by the immune system are potentially more specific in their application. Circulating antibody levels against the *TP53* gene have been shown to correlate with *TP*53 mutations in colorectal tissue [45]. Such a correlation could not only be used to make early diagnoses, it could help detect recurrence in the follow-up of patients with colorectal cancer.

3.2. Mass spectrometry and CDNA microarray as novel tools

Advances in mass spectrometry and DNA microarray offer new possibilities for the identification of biomarkers. Mass spectrometry is currently being evaluated in the search for proteins which are over-expressed during carcinogenesis. This technique is capable of pinpointing specific proteins, as exemplified by the calcium-binding protein calgranulin, which was found to be increased by mass spectrometry in preneoplastic lesions of colonic mucosa and inflammatory diseases of the bowel [46]. Such proteins can be investigated further to ascertain their role in cancer development, with the view to use them eventually as targets for intervention with chemopreventive, chemotherapeutic or immunotherapeutic intent. Characterisation of plasma membrane proteins has already been undertaken in colorectal cancer cell lines in an attempt to create a reference database [47,48]. Another use of mass spectrometry entails the adoption of a 'shotgun' approach, whereby the pattern of protein expression is recorded, rather than specific markers individually [49]. Protein 'finger-printing' can be exploited even if the exact nature and function of each finger-printed protein is unknown. Mass spectrometric analysis of urine samples has shown to be a fast and reproducible tool to detect changes in levels of methylated purine bases differentiating between patients with and without tumours [50,51]. This application could conceivably lend itself to serum and stool analysis of individuals suspected of having occult primary or recurrent colorectal neoplasms. Indeed, reports on mass spectrometry of serum samples suggest that sensitive and specific identification is possible in more than 80% of patients with prostate cancer [52,53].

cDNA microarray analysis enables the identification of up- or down regulated genes in tissues. A large number of different genes can be studied, and those associated with tumour development can be identified. As with mass spectrometry, specific genes can be studied, but the technique lends itself particularly to profiling clusters of genes which differ between normal, premalignant and malignant tissues. Genes which have been found to be frequently up-regulated in malignant tissues are those coding for proteins involved in metabolism, transcription and translation [54]. In one study, fewer than 50% of the genes found to be up-regulated in tumour tissue were commonly known genes [55]. The profile of up regulated genes has been able to distinguish between normal and cancerous colorectal tissue [54], and even to accurately predict histological features of colorectal tumours [56]. Microarray analysis has also revealed between 40 and 200 genes involved in the regulation of cell-to-cell adhesion and cytoskeleton remodelling, which are important processes in colorectal metastatic lesions [57,58], offering a potential role in identifying occult haematogenous dissemination. Genetic profiling of colorectal tumours offers the opportunity for a molecular classification ultimately perhaps proffering suggestions for highly individualised therapy tailored to the specific tumour.

A disadvantage of both mass spectrometry of proteins and microarray analysis of genes is the large amount of data generated by these assays. Complex computer-based algorithms are needed to allow efficient analysis of the data and to translate it into a practical diagnostic tool.

3.3. Stool testing for genetic defects

Colorectal cancers shed tumour cells in the faeces even in the early stages of development. As a result, there has been considerable interest in detection of the *K-RAS* proto-oncogene in stool samples [59,60]. This test can be conducted in a similar manner to faecal occult blood testing with a possible advantage of increased specificity. No trials comparing the two methods have been conducted as yet. Due to the small yields of DNA from early cancers, it has been technically difficult to obtain reproducible yields of DNA in the stool. This methodological problem is likely to confound the clinical application of stool testing for *K-RAS* mutations [59]. Another disadvantage of this method is that individuals may exhibit *K-RAS* mutations in the stool in the absence of colorectal malignancy [60], leading to a high false-positive rate and subjecting healthy individuals to unwarranted anxiety and needless investigations. Combining *K-RAS mutations* analysis with other genetic mutations occurring 'down stream' in the carcinogenesis cascade, such as *TP53* or *BAT-26*, has improved the specificity of stool DNA analysis, enabling identification of 71% of all patients with colorectal carcinomas [61]. Analysis of *K-RAS* mutations in bile has also been explored as a means of detecting occult hepatic metastases, but thus far with limited success [62]. In contrast, exploration of *K-RAS* mutations in liver tissue has proved successful in the identification of hepatic micro-metastases at the time of surgery [63]. Alterations in *K*-RAS and *TP*53 have been shown to correlate with the aggressiveness of colorectal tumours [64]. It is conceivable that expression of these genes may be useful in giving prognostic information regarding colorectal tumours, and thus they may be used alongside pathological grading systems such as the Dukes or TNM criteria to predict 5-year survival in patients post-surgery.

4. Biomarkers in chemoprevention trials

A major obstacle in the design of chemoprevention trials is the lack of a suitable endpoint that correlates with delay or prevention of carcinogenesis. The most obvious end-point would be the incidence of primary tumours. Large-scale drug trials of chemopreventive agents involve large cohorts of individuals who receive the trial agent over many years, such trials are thus very expensive. If an agent proves to be ineffective, individuals do not benefit, if an agent possesses unexpected toxicity, harm can be done. Therefore, small phase I, pilot trials are needed to select which compounds could conceivably be taken forward to larger scale phase II/III drug trials. The duration of such pilot trials should be short, and they necessitate rapid and sensitive endpoints to permit assessment of biological activity as 'surrogate' for clinical efficacy. Biomarkers of early carcinogenesis can be used as such indicators, and if levels of biomarkers in humans are altered indicating efficacy and if the agent does not cause undue side-effects, it should be considered for advancement into phase II and III trials. As in the case of the biomarkers discussed in the context of early diagnosis and surveillance, assaying such markers in accessible tissues other than the target tissue is advantageous, as it allows frequent and easy sampling. Examples of markers which are potentially useful in chemoprevention studies are DNA alterations (such as oxidative or carcinogenic DNA adducts), differences in polyamine metabolism (such as ornithine decarboxylase), cytokine levels (such as prostaglandins PGE2 or regulatory enzymes such as COX-2) and markers of cell cycle events (such as DNA strand breaks). Below two such biomarkers are described in detail.

4.1. Oxidised DNA adducts

Endogenous oxidants, products of cell metabolism, directly or indirectly damage DNA by the generation of adducts. Examples are the pyrimidopurinone adduct of deoxyguanosine (M_1G) and 8-oxo-deoxyguanosine [65–67]. M_1G is formed from malondialdehyde (MDA) which is a by-product of lipid peroxidation and prostaglandin synthesis. MDA reacts with DNA to form up to six different adducts, of which M_1G is the predominant species and the most carcinogenic molecule [65]. 8-Oxo-deoxyguanosine is formed by the reaction of oxygen radical or singlet oxygen with DNA. In DNA it induces G→T transversions, which are often seen in oncogenes and tumour suppressor genes [68]. Oxidative DNA adducts in general have been shown to be increased during all stages of colorectal carcinogenesis [69–71]. Levels of such adducts can be utilised to predict the risk of malignancy [72,73], and as intermediate endpoints in chemoprevention intervention trials. For example, in a murine model of inherited colorectal cancer, a decrease in M_1G levels has been found in polyps following a course of the chemopreventive agent; curcumin. This decrease in M_1G correlated with a decrease in polyp formation [74]. All cells in the body accumulate oxidative DNA adducts, accompanied by an increase in genetic mutations and therefore an increased risk of carcinogenesis. Suppressing DNA adduct formation inhibits tumorigenesis at the early stages. DNA adducts might be important in colorectal carcinogenesis, because there is no clear link between exposure to exogenous

carcinogens and susceptibility to disease, unlike in the case of, for example, smoking and lung cancer. It is possible that oxidative DNA damage contributes significantly to the accumulation of genetic damage in colorectal cells, when compared with other solid gastrointestinal tumours. An accumulation of harmful genetic changes eventually leads to uncontrolled cell replication and hence cancer development. Lipid peroxidation contributes to oxidative DNA damage. One source of lipid peroxides is the activity of cyclooxygenases (COX) [65]. The isoenzyme COX-2 plays a role in tumorigenesis [75] and the formation of DNA adducts as products of COX-induced lipid peroxidation may be an important factor in its role in cancer promotion.

4.2. COX-2 expression

COX is an enzyme which catalyses the metabolism of arachidonic acid to prostaglandins (Fig. 3). Two COX isoforms have been identified. COX-1 is constitutively expressed in all tissues, and the prostaglandins the formation of which it catalyses, are involved in many regulatory functions [75]. COX-2 is induced by inflammatory cytokines, tumour promoters and oncogenes [75]. Elevated levels of COX-2 have been found in 85% of colorectal tumours [76], raising the possibility that it plays an important role in colorectal carcinogenesis. This notion is further supported by evidence that COX-2 inhibitors can delay or prevent the development of colorectal adenocarcinoma in rodent models of carcinogenesis [77–79]. Prostaglandin levels as products of COX-2 may be a suitable intervention end-point biomarker, because levels are modulated by some chemopreventive drugs such as the non-steroidal anti-inflammatory agent (NSAID) naproxen and sulindac and the COX-2-specific inhibitor celecoxib. Such modulation has been shown to correlate with a decreased incidence of adenoma formation in patients with FAP [77–79]. The notion that FAP patients may derive long-term benefit from treatment with NSAIDs has recently been challenged. In a randomised double-blind placebo-controlled trial of sulindac in genotypically-positive FAP patients who had not yet developed adenomas, sulindac had only a short-lived effect on polyp formation with any effect lost after 9 months of treatment [80]. Significantly, this lack of long-term efficacy was nevertheless accompanied by reduced prostaglandin levels in the treated group. This finding hints at the possibility that long-term sulindac therapy can select for non-COX-2-dependent or NSAID-resistant tumours. Thus, there is the real possibility that chemopreventive strategies may impose selective pressure which engenders tumours refractory to intervention.

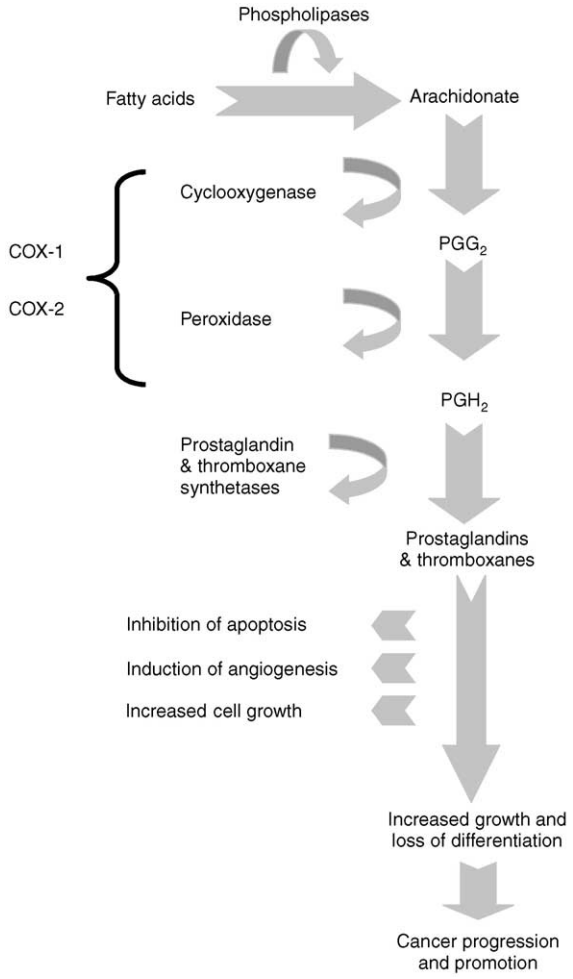

Fig. 3. Figure showing the role of cyclooxygenase-2 in colorectal carcinogenesis. Arrows show the relationship between COX-1 and COX-2 enzyme and their involvement in tumour progression via the prostaglandin synthesis pathway.

Other potential biomarkers for selecting chemopreventive agents are phase II drug-metabolising enzymes such as the GSTs. It is arguable that agents which up-regulate their concentration might reduce an individual's risk of colorectal cancer by increasing metabolism of putative dietary carcinogens, such as heterocyclic amines common in barbecued or roasted meats. NSAIDs such as celecoxib, have been shown not only to reduce COX-2 levels [77–79], but also to up regulate GST levels in the rat colon [81]. The multiple mechanisms by which NSAIDs might achieve their action is typical of many chemopreventive agents. It highlights the importance of studying combinations of biomarkers together rather than focusing on single ones individually. Thus, full assessment of chemopreventive compounds should involve exploration of their effect on several biomarkers involved in colorectal carcinogenesis.

5. Biomarkers in tumour staging

β-catenin is a multifunctional protein involved in a variety of cellular processes including cell–cell adhesion

and intracellular signalling [82]. Normally very low levels of β-catenin exist in the nucleus and cytoplasm of cells, but in the presence of APC mutations, the ability of the cell to downregulate β-catenin is lost, secondary to resistance to proteasome-mediated destruction [82]. Alternatively, direct mutations of the β-catenin gene leading to β-catenin overexpression can also be found in some colorectal cancers [83]. The corollary of either event appears to be cytoplasmic and nuclear accumulation of β-catenin. Once in the nucleus, β-catenin interacts with Tcf transcription factors leading to activation of targets genes such as *c-myc*, *c-jun*, *cyclin D1*, and *Wisp*, ultimately eliciting uncontrolled cellular proliferation [84] (Fig. 4). β-catenin could prove to be a useful biomarker in detecting early colorectal cancer and also in determining its aggressive potential, as a significant correlation has been found between the levels of cytoplasmic β-catenin and ability of colorectal tumours to invade blood vessels [85]. This relationship between cytoplasmic β-catenin accumulation and metastatic potential has been observed also in cancers other than colorectal cancer, such as hepatocellular carcinomas and pancreatic carcinomas [86–88].

Molecules involved in cell-to-cell adhesion have also been shown to correlate with tumour aggressiveness and hence with prognosis. Other proteins, such as CD44, have been found to give prognostic information regarding tumour grade and, more importantly, long-term survival and recurrence-free survival in a cohort of 194 colorectal cancer patients [89].

COX-2 may also provide prognostic information regarding colorectal tumours. Levels of COX-2 in colorectal tumour tissue can predict tumour invasiveness, likelihood of vascular invasion, probability of liver metastases and likelihood of tumour reccurrence [90,91]. Therefore, patients with a high expression of COX-2 in tumour tissue could be candidates for further therapy even in the absence of radiologically-determined metastases.

Survivin is a recently described member of the family of inhibitors of apoptosis proteins (IAP). Its expression

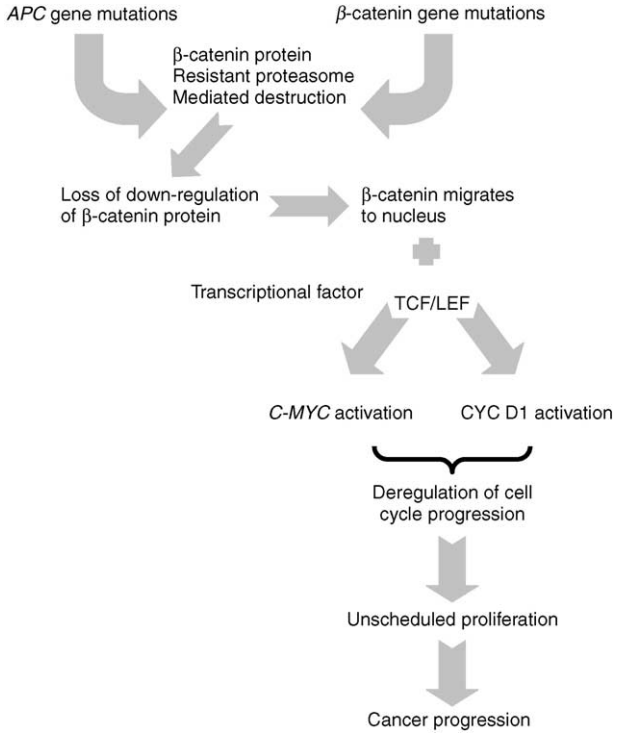

Fig. 4. β-Catenin overexpression and colorectal cancer. Arrows display mutations leading to direct and indirect loss of down regulation of β-catenin expression and its subsequent accumulation in the cytoplasm and nucleus leading to uncontrolled cell proliferation.

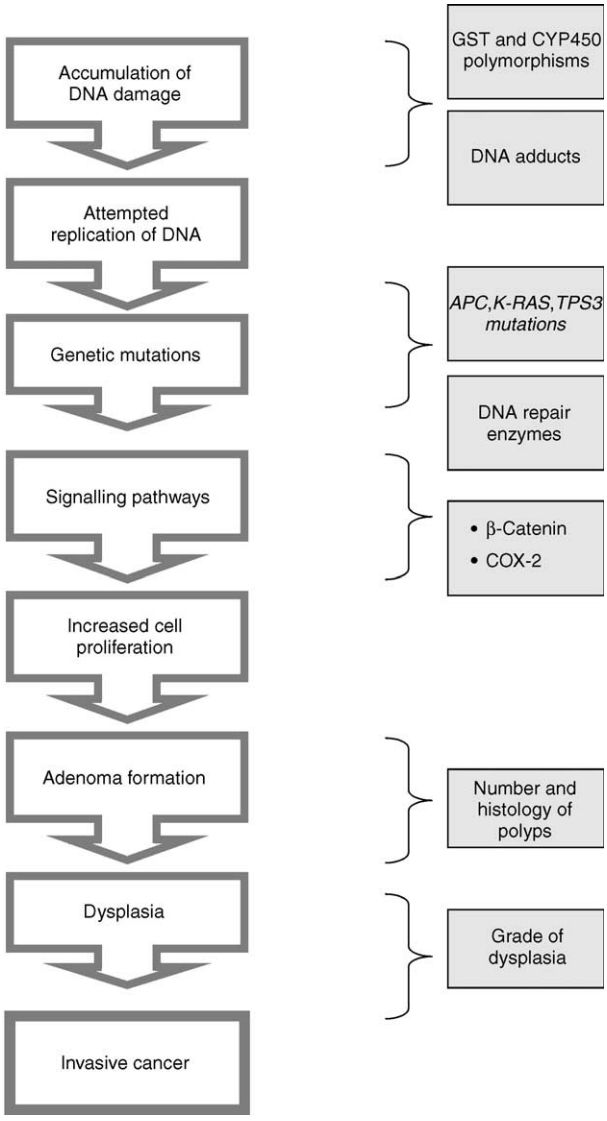

Fig. 5. Outline of carcinogenesis in colorectal cancer and potential points for biomarker recruitment. The carcinogenic pathway is detailed on the left and biomarker recruitment is shown in the shaded boxes. Box arrows show the generic pathway leading to colorectal cancer. Shaded boxes on right show potential biomarkers of early carcinogenesis and their interaction with the carcinogenic pathway.

has been shown to identify patients with stage II colorectal cancer who are at risk of recurrent disease [92]. Other modulators of apoptosis, such as the apoptosis-regulatory protein BAX have also been linked with prognosis [93]. Patients with metastatic colorectal cancer who were BAX-negative had a 75% better 2-year survival chance than those who tested BAX-positive [94]. Impaired apoptosis is associated with a higher rate of tumour progression and metastatic potential due to increased survival of tumour cells. Whilst such 'molecular staging' of colorectal tumours is unlikely to replace the present classification, they may provide valuable additive information postoperatively.

Survivin, like COX-2, is not detectable in normal tissue [95], hence chemopreventive agents which solely influence survivin levels could hold important benefits. Chemoprevention will ultimately involve individuals who consume the putative chemopreventive agent for a lifetime. When consumed over a long time, chemopreventive agents which target a wide range of different cell signalling pathways may have unforeseen effects which could cause harm, or even increase the risk of cancer at certain sites. In the α-tocopherol β-carotene prevention study [96], it was found that the incidence of lung cancer was increased in patients who continued to smoke at least 20 cigarettes daily and amongst those in the highest quartile of alcohol consumption. Chemopreventive agents with a very specific site of action might therefore be clinically more desirable than those which are poly-mechanistic.

6. Conclusions

It is important to realise that in the clinical management of bowel cancer not all individuals who would be identified as at-risk by the methods described above progress to colorectal cancer. Identifying individuals as having an above-average risk of cancer could have dramatic consequences on their psychological morbidity and may even cause practical problems such as those pertaining to life insurance and sickness cover. It is well recognised in breast and cervical cancer surveillance programmes that individuals experience high levels of anxiety when they are told that they are at an increased risk of cancer. The limitations of the use of biomarkers must be appreciated by the clinician before embarking on testing large cohorts of the general population. However, in spite of these caveats several important conclusions can be drawn from the above discussion.

Early detection of colorectal cancer is essential to improve long-term survival rates following surgery. Biomarkers of early carcinogenesis offer the possibility of identifying high-risk individuals who may then benefit from surveillance methods such as regular colonoscopic assessment. Biomarkers might also be used in diagnosing early disease. Their ability to be assayed in samples other than colorectal mucosa renders them potentially useful as a screening tool for the general population. Biomarkers are pivotal in chemoprevention to measure the ability of a putative chemopreventive agent to retard carcinogenesis. More work is needed to evaluate combinations of different biomarkers with the aim to improve their reliability and sensitivity. It is an ancient adage that "prevention is better than cure". Biomarkers may well provide us with the resource to apply this dictum to the management of colorectal cancer (Fig. 5).

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References

1. Boring CC, Squires TS, Tong MS. Cancer statistics. *CA Cancer J Clin* 1994, **44**, 7–26.
2. Rudy DR, Zdon MJ. Update on colorectal cancer. *AM Fam Phys* 2000, **61**, 1759–1769.
3. Winawer SJ, Zauber AG, Ho MN. Prevention of colorectal cancer by colonoscopic polypectomy. *N Eng J Med* 1993, **329**, 1977–1981.
4. Bond JH. Colorectal cancer update. Prevention, screening, treatment and surveillance for high risk groups. *Med Clin North Am* 2000, **84**, 1163–1182.
5. Syngal S, Weeks JC, Schrag D, Garcer JE, Kuntz KM. Benefits of colonoscopic surveillance and prophylactic colectomy in patients with hereditary nonpolyposis colorectal cancer mutations. *Am J Gastroenterol* 1998, **93**, 911–915.
6. Schapiro M. Cancer surveillance colonoscopy in ulcerative colitis: sense or nonsense? *Schw Med Woon* 1996, **126**, s5–s8.
7. Manson MM, Gescher A, Hudson EA, Plummer SM, Squires MS, Prigent SA. Blocking and suppressing mechanism of chemoprevention by dietary constituents. *Toxicol Lett* 2000, **112–113**, 499–505.
8. Morse MA, Stoner GD. Cancer chemoprevention: principles and prospects. *Carcinogenesis* 1993, **14**, 1737–1746.
9. Kellof GJ, Boone CW, Steele VW, Crowell JA, Lubet R, Sigman CC. Progress in cancer chemoprevention: perspectives on agent selection and short-term clinical trials. *Cancer Res* 1994, **54s**, 2015s–2024s.
10. Toby M. Polyps as biomarkers for colorectal neoplasia. *Frontiers in Bioscience* 1999, **4**, D329–D338.
11. Srnivas PR, Barnett SK, Srivastava S. Trends in biomarker research for cancer detection. *Lancet Onc* 2001, **2**, 698–703.
12. Groopman J, Kensler T. The light at the end of the tunnel for chemical-specific biomarkers: daylight or headlight? *Carcinogenesis* 1999, **20**, 1–11.
13. Einspahr JG, Alberts DS, Gapstur SM, Bostick RM, Emerson SS, Gerner EW. Surrogate end-point biomarkers as measures of colon cancer risk and their use in cancer chemoprevention trials. *Cancer Epidemiol Biomarkers Prev* 1997, **6**, 37–48.
14. Meyskens FL. Cancer population genetics and tumour prevention: an unfulfilled paradigm. *Eur J Cancer* 2000, **36**, 1189–1192.

15. Katiyar SK, Matsui MS, Mukhatar H. Ultraviolet-B exposure of human skin induces cytochromes P450 1A1 and 1B1. *J Investigative Dermatol* 2000, **114**, 328–333.
16. Ozawa S. Genetic polymorphisms in xenobiotics metabolising enzymes as a determinant of susceptibility to environmental mutagens and carcinogens in humans. *J Pharmaceut Soc Jpn* 1997, **117**, 895–909.
17. Martinez C, Garcia-Martin E, Ldero JM, *et al*. Association of CYP2C9 genotypes leading to high enzyme activity and colorectal cancer risk. *Carcinogenesis* 2001, **22**, 1323–1326.
18. Kiss I, Sandor J, Pajkos G, Bogner B, Hegedus G, Ember I. Colorectal cancer risk in relation to genetic polymorphism of cytochrome P450 1A1, 2E1 and glutathione S-transferase M1 enzymes. *Anticancer Res* 2000, **20**, 519–522.
19. Szarka CE, Pfeiffer GR, Hum ST, *et al*. Glutathione S-transferase activity and glutathione S-transferase μ expression in subjects with risk for colorectal cancer. *Canc Res* 1995, **55**, 2789–2793.
20. Pemble S, Schroeder KR, Spencer SR, *et al*. Human glutathione S-transferase theta (GSTT1): cDNA cloning and the characterisation of a genetic polymorphism. *Biochem J* 1994, **300**, 271–276.
21. Strange RC, Matharoo B, Faulder GC, *et al*. The human glutathione S-transferases: a case-control study of the incidence of GST1 0 phenotypes in patients with adenocarcinoma. *Carcinogenesis* 1991, **12**, 25–28.
22. Saadat I, Saadat M. Glutathione S-transferase M1 and T1 null genotypes and the risk of gastric and colorectal cancer. *Canc Lett* 2001, **169**, 21–26.
23. Chenevix-Trench X, Young J, Coggan M, Board. Glutathione S-transferase M1 and T1 polymorphism: susceptibility to colon cancer and age of onset. Carcinogen 1995; 16: 1655–1677.
24. Van Lieshout EM, Tiemessen DM, Roelofs HM, Peters WH. Non-steroidal anti-inflammatory drugs enhance glutathione S-transferase that levels in rat colon. *Biochem Biophys Acta* 1998, **1381**, 305–311.
25. Fujita T, Matsui M, Takaku K, Uetaka H, Ichikawa W, Taketo MM, Sugihara K. Size and invasion dependent increase in cyclooxygenase-2 levels in human colorectal carcinomas. *Can Res* 1998, **58**, 4823–4826.
26. Hiu HJ, Taylor J, Linko P, Lucier GW, Thompson CL. Glutathione S-transferase μ in human lymphocyte and liver: role in modulating formation of carcinogen-derived DNA adducts. *Carcinogen* 1991, **12**, 2269–2275.
27. Barker HJ, Alpert LC, Compton CC, Malsen A, Kirby GM. Loss of glutathione S-transferase (GST) phenotype in colorectal adenocarcinomas from patients with a GSTM1 genotype. *Canc Lett* 2002, **177**, 65–74.
28. Lynch HT, de la Chapelle A. Genetic susceptibility to non-polyposis colorectal cancer. *J Med Genetics* 1999, **36**, 801–818.
29. Syngal S, Fox EA, Eng C, Kolodner RD, Garber JE. Sensitivity and specificity of clinical criteria for hereditary non-polyposis colorectal cancer associated mutations in MSH2 and MLH1. *J Med Genetics* 2000, **37**, 641–645.
30. Wahlberg S, Liu T, Lindblom P, Lindblom A. Various mutation screening techniques in the DNA mismatch repair genes hMSH2 and hMLH1. *Genetic Testing* 1999, **3**, 259–264.
31. de Leon MP, Pedroni M, Benatti P, *et al*. Hereditary colorectal cancer in general population: from cancer registration to molecular diagnosis. *Gut* 1999, **45**, 32–38.
32. Bodmer WF, Bailey CJ, Bodmer J, *et al*. Localization of the gene for familial adenomatous polyposis on chromosome 5. *Nature* 1987, **328**, 614–616.
33. Warren H, Fell J. Screening for familial adenomatous polyposis. *Arch Dis Childhood* 2001, **84**, 377–380.
34. Midgley R, Kerr D. Colorectal cancer. *Lancet* 1999, **353**, 391–399.
35. Lauschke H, Caspari R, Friedl W, *et al*. Detection of APC and k-ras mutations in the serum of patients with colorectal cancer. *Can Detect Preven* 2001, **25**, 55–61.
36. Kopreski MS, Benko FA, Borys DJ, Khan A, McGarrity TJ, Gocke CD. Somatic mutation screening: identification of individuals harbouring K-ras mutations with the use of plasma DNA. *J Natl Can Inst* 2000, **92**, 918–923.
37. Schoen R. Families at risk for colorectal cancer: risk assessment and genetic testing. *J Gastroenterol* 2000, **31**, 114–120.
38. Powell SM, Peterson GM, Krush AJ, *et al*. Molecular diagnosis of familial adenomatous polyposis. *N Engl J Med* 1993, **329**, 1982–1987.
39. Bonk T, Humeny A, Sutter C, Gebert J, von Knebel Doebertiz M, Becker CM. Molecular diagnosis of familial adenomatous polyposis (FAP): genotyping of adenomatous polyposis coli (APC) alleles by MALDI-TOF mass spectrometry. *Clin Biochem* 2002, **35**, 87–92.
40. Laken SJ, Jackson PE, Kinzler KW, *et al*. Genotyping by mass spectrometric analysis of short DNA fragments. *Nature Biotech* 1998, **16**, 1352–1356.
41. Naito Y, Ishikwawa K, Koga Y, Tsuneyosi T, Terunuma H, Arakawa R. Molecular mass measurement of polymerase chain reaction products amplified from human blood DNA by electro spray ionization mass spectrometry. *Rap Comm Mass Spectr* 1995, **9**, 1484–1486.
42. Nystrom-Lahti M, Perrera C, Raschle M, *et al*. Functional analysis of MLH1 mutations linked to hereditary nonpolyposis colon cancer. *Genes Chromosomes Cancer* 2002, **33**, 160–167.
43. Thibodeau SN, French AJ, Roche PC. Altered expression of *hMSH2* and *hMLH1* in tumours with microsatellite instability and genetic alterations in mismatch repair genes. *Cancer Res* 1996, **56**, 4836–4840.
44. Berghella AM, Pellegrini P, Del Beato T, Adorno D, Casciani CU. Il-10 and sIL-2R serum levels as possible peripheral blood prognostic in the passage from adenoma to colorectal cancer. *Can Bio Radiopharm* 1997, **12**, 265–272.
45. Hammel P, Boissier B, Chaumette MT, Piedbois P, Rotman N, Kouyoumjian JC, Lubin R, Delchier JC, Soussi T. Detection and monitoring of serum p53 antibodies in patients with colorectal cancer. *Gut* 1997, **40**, 256–361.
46. Stulik J, Kovarova H, Macela A, Bures J, Jandik P, Langr F, Otto A, Thiede B, Jungblut P. Over expression of calcium-binding protein calgranulin B in colonic mucosal diseases. *Clinica Chemica Acta* 1997, **265**, 41–55.
47. Simpson RJ, Connolly LM, Eddes JS, Pereira JJ, Moritz RL, Reid GE. Proteomic analysis of the human colon carcinoma cell line (LIM): development of a membrane protein database. *Electrophoresis* 2000, **1215**, 21 1707-1732.
48. Ji H, Whitehead RH, Reid GE, Moritz RL, Ward LD, Simpson RJ. Two-dimensional electrophoretic analysis of proteins expressed by normal and cancerous human crypts: application of mass spectrometry to peptide-mass finger printing. *Electrophoresis* 1994, **15**, 391–405.
49. McDonald WH, Yates III JR. Shotgun proteomics and biomarker discovery. *Dis Markers* 2002, **18**, 99–105.
50. Procelli B, Pagani R, Lorenzini L, De Martino A, Catinella S, Traldi P. Different mass spectrometric approaches in the identification of endogenous methylate purine bases in urine extracts. *Rapid Comm Mass Spectromet* 1994, **8**, 443–450.
51. Lorenzini L, De Martino A, Testi W, *et al*. Pattern of methylated purine bases in urine of cancer patients. Analysis by mass spectrometry. *Adv Exp Med Biol* 1994, **370**, 269–273.
52. Qu Y, Adam BL, Yasui Y, *et al*. Boosted decision tree analysis of surface-enhanced laser desorption/ionization mass spectral serum profiles discriminates prostate cancer from noncancer patients. *Clinical Chemistry* 2002, **48**, 1835–1843.
53. Adam BL, Qu Y, Davis JW, *et al*. Serum protein fingerprinting coupled with a pattern-matching algorithm distinguishes prostate cancer from benign prostate hyperplasia and healthy men. *Cancer Res* 2002, **62**, 3609–3614.

54. Birkenkamp-Demtroder K, Christensen LL, Olesen SH, *et al*. Gene expression in colorectal cancer. *Cancer Res* 2002, **62**, 4352–4363.
55. Ichikawa Y, Ishikeawa T, Takahashi S, *et al*. Identification of genes regulating colorectal carcinogenesis by using the algorithm for diagnosing malignant state method. *Bioch Biophys Res Comm* 2002, **296**, 497–506.
56. Lin YM, Furukawa Y, Tsunoda T, Yue CT, Yang KC, Nakamura Y. Molecular diagnosis of colorectal tumours by expression profiles of 50 genes expressed differentially in adenomas and carcinomas. *Oncogene* 2002, **21**, 4120–4128.
57. Yanagawa R, Furukawa Y, Tsunoda T, *et al*. Genome wide screening of genes showing altered expression in liver metastases of human colorectal cancers by cDNA microarray. *Neoplasia* 2002, **3**, 395–401.
58. Hegde P, Qi R, Gaspard R, *et al*. Identification of tumour markers in models of human colorectal cancer using a 19,200-element of complementary DNA microarray. *Cancer Res* 2001, **61**, 7792–7797.
59. Lev Z, Kislitsin D, Rennert G, Lerner A. Utilisation of K-ras mutations identified in stool DNA for the early detection of colorectal cancer. *J Cell Biochem* 2000, **34**, 35–38.
60. Doolittle BR, Emanuel J, Tuttle C, Costa J. Detection of the mutated K-Ras biomarker in colorectal carcinoma. *Exp Mol Path* 2001, **70**, 289–301.
61. Dong SM, Traverso G, Johnson C, *et al*. Detecting colorectal cancer in stool with the use of multiple genetic targets. *J Nat Can Inst* 2001, **93**, 858–865.
62. de Kok JB, Winkles DW, Ruers TJ. Clinical value of bile for the detection of mutant *K-ras* from colorectal liver metastases. *Anticancer Res* 2000, **2**, 2469–2472.
63. Schimanski CC, Linnemann U, Berger MR. Sensitive detection of K-ras mutations augments diagnosis of colorectal cancer metastases in the liver. *Can Res* 1999, **59**, 5169–5175.
64. Tortola S, Marcello E, Gonzalez I, *et al*. p53 and K-ras gene mutations correlate with tumour aggressiveness but are not of routine prognostic value in colorectal cancer. *J Clin Onc* 1999, **17**, 1375–1381.
65. Ames BN. Endogenous DNA damage as related to cancer and ageing. *Mutat Res* 1989, **214**, 41–46.
66. Esterbauer J, Eckle P, Ortner A. Possible mutagens derived from lipids and lipid precursors. *Mutat Res Rev Gen Toxicol* 1990, **238**, 223–233.
67. Marnett LJ. Lipid peroxidation—DNA damage by malondialdehyde. *Mutat Res* 1999, **424**, 83–95.
68. Hussain SP, Harris CC. Molecular epidemiology of human cancer: contribution of mutation spectra studies of tumour suppressor genes. *Cancer Res* 1998, **58**, 4023–4037.
69. Hendrickerse CW, Kelly RW, Radley S, Donovan IA, Keighley B, Neoptoloemos JP. *Br J Surg* 1994, **81**, 1219–1223.
70. Scmid K, Nair J, Winde G, Velic I, Bartcsh H. Increased levels of DNA adducts in colonic polyps of patients with FAP. *Int J Cancer* 2000, **87**, 1–3.
71. Kawanishi M, Enya T, Suzuki H, Takebe H, Matsui S, Yagi T. Post labelling analysis of DNA adducts formed in human hepatoma cells treated with 3-nitrobenzene. *Mutat Res* 2000, **470**, 133–139.
72. Otteneder M, Lutz WK. Correlation of DNA adduct levels with tumour incidence: carcinogenic potency of DNA adducts. *Mutat Res* 1999, **424**, 237–244.
73. Tan W, Lin DX, Xiao Y, Kadlubar FF, Chen JS. Chemoprevention of 2-amino-1-methyl-6-phenylimidazo[4,5-b] pyridine-induced carcinogen-DNA adducts by Chinese cabbage in rats. *World J Gastroenterol* 1999, **5**, 138–142.
74. Perkins S, Sharma RA, Vershoyle R, Lawrence MJ, Steward WP, Gescher A. Biomarkers of the chemopreventive efficacy of curcumin in the min/+ mouse. *Proceedings AACR* 2002, **43**, 126.
75. Prescott SM, Fitzpatrick FA. Cyclooxygenase-2 and carcinogenesis. *Biochemica and Biophysica Acta* 2000, **1470**, M69–M78.
76. Williams C, Moss M, DuBois R. The role of cyclooxygenases in inflammation, cancer and development. *Oncogene* 1999, **18**, 7908–7916.
77. Goldman AP, Williams CS, Sheng H, *et al*. Meloxicam inhibits the growth of colorectal cancer cells. *Carcinogenesis* 1998, **19**, 2195–2199.
78. Taketo MM. Cyclooxygenase-2 inhibitors in tumourogenesis. *J Natl Cancer Inst* 1998, **90**, 1609–1620.
79. Bertagnolli MM. Cyclooxygenase-2 as a target for prevention of colorectal cancer. *Curr Onc Rep* 1999, **1**, 173–178.
80. Giardiello FM, Vincet WY, Hylind LM, *et al*. *N Engl J Med* 2002, **346**, 1054–1059.
81. Hayes JD, Pulford DJ. The glutathione S-transferase supergene family: regulation of GST and the contribution of the isoenzymes to cancer chemoprotection and drug resistance. *Crit Rev Biochem Mol Biol* 1995, **30**, 445–600.
82. Wageneer R, Crawford H, Matrisian L. Stabilized β-catenin immortalises colonic epithelial cells. *Can Res* 2001, **61**, 2097–2103.
83. Sparks AB, Morin PJ, Vogelstein B, Kinzler KW. Mutational analysis of the APC, β-catenin, Tcf pathway in colorectal cancer. *Can Res* 1998, **58**, 1130–1134.
84. Chen R, McCormick F. Selective targeting to the hyperactive β-catenin/T-cell factor pathway in colon cancer cells. *Can Res* 4445, **200**, 61 -4449.
85. Maruyama K, Ochiai A, Akimoto S, *et al*. Cytoplasmic β-catenin accumulation as a predictor of haematogenous metastasis in human colorectal cancer. *Oncology* 2000, **59**, 302–309.
86. Calvisi DF, Factor WM, Loi R, Thorgeirsson SS. Activation of β-catenin during hepato-carcinogenesis in transgenic mouse models: relationship to phenotype and tumour grade. *Can Res* 2001, **61**, 2085–2091.
87. Qiao QL, Ramadani M, Gansuage S, Gansuage F, Leder G, Beger HG. Reduced membranous and ectopic expression of β-catenin correlate with cyclin D1 over expression and poor prognosis in pancreatic cancer. *Int J Can* 2001, **95**, 194–197.
88. Cui J, Zhou XD, Liu YK, Tang ZY, Zile MH. Abnormal β-catenin gene expression with invasiveness of primary hepatocellular carcinoma in China. *W J Gastroenterol* 2001, **7**, 542–546.
89. Ropponen KM, Eskelinen MJ, Lipponen PK, Alhava E, Kosma VM. Expression of CD44 and variant proteins in human colorectal cancer and its relevance for prognosis. *Scand J Gastroenterol* 1998, **33**, 301–309.
90. Wei SJ, Liu JM, Hsiao M, Kou-Lin J, Yang WK. Tumour invasiveness and liver metastasis of colon cancer cells correlated with cyclooxygenase-2 expression and was inhibited by a COX-2 inhibitor. *Intl J Can* 2001, **91**, 894–899.
91. Hull MA, Fenwick SW, Chapple KS, Scott NM, Toogood GJ, Lodge JP. Cyclooxygenase-2 expression in colorectal cancer liver metastases. *Clin Exp Metastases* 2000, **18**, 21–27.
92. Sarela A, Scott N, Ramsdale J, Markham AF, Guillou PJ. Immunohistochemical detection of the anti-apoptosis protein, survivin, predicts survival after curative resection of stage II colorectal carcinomas. *Ann Surg Oncol* 2001, **8**, 305–310.
93. Grizzle WE, Manne U, Weiss HL, Jhala N, Talley L. Molecular staging of colorectal cancer in African-American and Caucasian patients sing phenotypic expression of p53, Bcl-2, MUC-1 and p27. *Int J Cancer* 2002, **97**, 403–409.
94. Paradise A, Simone G, Lena MD, Leone B, Vallejo C, Lavaca J, Dellpasqua S, Daidone MG, Costa A. Expression of apoptosis-related biomarkers and clinical outcome in patients with advanced colorectal cancer. *Br J Cancer* 2001, **84**, 651–658.
95. Boulomwini JK. Novel anticancer drug discovery. *Curr Opin Chem Biol* 1999, **3**, 500–509.
96. Omenn GS, Goodman GE, Thornquist MD, Balmes J, Cullen MR, Glass A. Risk factors for lung cancer and for intervention effects in CARET, the beta-carotene and retinal efficacy trial. *J Natl Cancer Institute* 1996, **88**, 1550–1559.