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Colorectal Cancer: Future Population Screening for Early Colorectal Cancer

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Colorectal cancer (CRC) is one of the most frequent cancers in Western countries. The identification of individuals at risk and the early diagnosis of CRC are of critical importance since a large proportion can be prevented or cured by surgical removal before metastasis has occurred. With increasing understanding of the genetic basis of hereditary and sporadic (non-hereditary) CRC, it becomes feasible to detect genetic alterations by molecular techniques. Familial adenomatous polyposis (FAP), hereditary nonpolyposis colorectal cancer (HNPCC), as well as early stages of spontaneous CRC, can be diagnosed by molecular characterisation of the adenomatous polyposis coli (*APC*) gene, the *RAS* oncogene and other genes in DNA from peripheral blood, stool or intestinal biopsies. With a better understanding of the genetic events leading to malignant transformation, molecular population screening should allow us to identify individuals at risk as well as patients with an early and potentially curable CRC. At present, careful patient and family history, physical examination and testing for occult blood as well as colonoscopy are still the key elements for clinical patient management. Molecular diagnosis will hopefully soon complement these analyses and should result in a reduction of morbidity and mortality from CRC.

Key words: hereditary colorectal cancer, sporadic colorectal cancer, molecular oncogenesis, molecular diagnosis, colorectal cancer prevention

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INTRODUCTION

COLORECTAL CANCER (CRC) is one of the most frequent cancers in men and women in Western countries [1]. Although most patients present with surgically resectable disease [2], approximately half of the patients will die from advanced CRC despite subsequent radiation or chemotherapy [3]. The pathogenesis of CRC involves environmental and genetic factors which may interact in the promotion of CRC development. Dietary factors, e.g. low fibre and high fat content, may promote CRC development by altering intestinal bacterial flora, slowing gastro-intestinal transit time and increasing recycling of bile acids [4, 5]. Equally important, recent studies allow an increasing understanding of the genetic basis of hereditary and sporadic (non-hereditary) CRC [6–8]. These data suggest that CRC results from the aggregate effect of multiple sequential genetic alterations (step-wise oncogenesis) which may be inherited (germ-like mutations) or acquired (somatic mutations). With the detailed molecular identification and definition of mutations predisposing to or occurring during CRC development, it is becoming feasible to detect specific genetic alterations in pre-symptomatic or asymptomatic individuals by molecular techniques. Familial adenomatous polyposis (FAP) syndrome, hereditary nonpolyposis colorectal cancer (HNPCC) syndrome as well as early stages of spontaneous CRC can be diagnosed by molecular characterisation of the adenomatous polyposis coli (*APC*) gene, the *RAS* oncogene and other genes, in DNA from peripheral blood, stool or intestinal biopsies. In the following paper, the potential and perspectives of molecular screening for CRC will be discussed.

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CONVENTIONAL CRC SCREENING

In the absence of definitive primary prevention programmes to identify individuals at risk of CRC development, conventional screening strategies are aimed at the identification of individuals at risk by careful family history, annual screening for faecal occult blood and the clinical detection and removal of lesions considered as precursors of CRC (colorectal polyps or adenomas).

Family history

Given the fact that about 10% of CRCs are inherited (approximately 1% hereditary polyposis syndromes, 5–10% hereditary non-polyposis colorectal cancer (HNPCC) syndromes), a careful family history and physical examination may identify patients at risk [8]. Screening of the index patient and potentially affected family members involves colonoscopy, upper endoscopy to detect gastroduodenal polyps in patients with colonic polyposis as well as the screening for extracolonic manifestations of these hereditary syndromes; fundoscopy to detect congenital hypertrophy of the retinal pigment epithelium in patients with adenomatous polyposis, detection of osteomas, fibromas, lipomas, desmoid tumours and neoplasms of the thyroid, adrenals, biliary tree and liver in patients with Gardner's syndrome, detection of brain tumours in patients with Turcot's syndrome, detection of mucocutaneous pigmentations in pati-

ents with Peutz–Jeghers syndrome and detection of extracolonic cancers originating in the endometrium, ovary, ureter, renal pelvis, stomach, pancreas, biliary tree, bone marrow, skin and larynx in patients with HNPCC.

Screening for faecal occult blood

Randomised trials for faecal occult blood testing have so far not provided convincing evidence for the effectiveness of this approach to reduce mortality from CRC [1]. In a recent study, however, involving 46 551 individuals, it could be demonstrated that annual (but not biannual) faecal occult blood testing and, if positive, subsequent clinical evaluation and therapy reduced mortality from CRC by 33% [9]. Whether these findings justify the recommendation of annual faecal occult blood testing in all individuals above the age of 40 years in part depends on the cost-effectiveness of this policy, where the 33% reduction of mortality from CRC has to be weighed against the costs of the increased number of diagnostic procedures.

Screening for colorectal polyps by colonoscopy

As a result of screening for faecal occult blood, many patients with colorectal polyps have been identified in recent years by flexible sigmoidoscopy or colonoscopy. It is current clinical practice to remove polyps when detected, search for additional polyps and arrange for long-term follow-up of the patients [10]. This practice is based on the concept that colorectal adenomas can progress to adenocarcinomas and that removing them will prevent CRC. A recent study indeed demonstrated that colonoscopic polypectomy prevents CRC [11]. It is, therefore, recommended that a colonoscopy should be performed every 5 years in all individuals above 50 years of age with a follow-up examination every 2 years in patients after colonoscopic polypectomy [10].

MOLECULAR CRC SCREENING

Molecular biology and recombinant DNA technology have become an integral part of research related to the diagnosis, therapy and prevention of human diseases. Apart from the better understanding of the molecular basis of inherited and malignant diseases, molecular methods allow the early detection of patients at risk for a given disease. In this context, research during the past few years on the molecular pathogenesis of CRC and its phenotypic precursor lesions (polyps, adenomas) has revealed new insights which allow one to identify individuals at risk for the development of the FAP or HNPCC syndrome as well as sporadic CRC.

Familial adenomatous polyposis (FAP)

FAP is the most common dominantly inherited adenomatous polyposis syndrome. It is characterised by the progressive development of hundreds of adenomatous colorectal polyps and the inevitable development of CRC. The inheritance of FAP was genetically linked to chromosome 5q21 [12, 13]. After the discovery of germ-like mutations of the adenomatous polyposis coli (*APC*) gene on chromosome 5 in patients with FAP [14, 15], direct genetic testing of presymptomatic individuals became feasible.

In a study of 62 unrelated patients, the presence of *APC* mutations in DNA from peripheral blood mononuclear cells was analysed in two different ways [16]:

(i) Detection of a truncated APC protein by an *in vitro* synthesised protein assay demonstrating shorter than normal *APC* gene products in 51 of the 62 patients with FAP.

(ii) Detection of the reduced expression of one allele of the *APC* gene by an allele-specific expression assay, identifying another 3 of the remaining 11 patients with FAP.

The use of these two assays in combination, therefore, identified 54/62 patients with FAP (87%), making the routine molecular diagnosis of FAP in subjects at risk feasible. While the molecular analysis of the *APC* gene remains investigational at present, its potential for prenatal genetic counselling as well as for identification and clinical management of individuals at risk are tremendous, including the pharmacological treatment of FAP patients [17], possibly before polyps appear.

Hereditary non-polyposis colorectal cancer (HNPCC)

Similar to the hereditary polyposis syndromes (familial adenomatous polyposis (FAP), Gardner's syndrome, Turcot's syndrome) and hamartomatous polyposis (Peutz–Jeghers syndrome, neuro-fibromatosis and others)), the HNPCC is an autosomal dominant disease. HNPCC predominantly affects the proximal colon and is characterised by an early age at onset (approximately 40 years), up to 100 flat adenomas and the synchronous or metachronous development of mucinous or poorly differentiated colon cancers [18]. HNPCC, therefore, is phenotypically different from FAP with respect to number and location of polyps and CRCs, respectively.

Research on the genetic basis of HNPCC revealed a new molecular mechanism of oncogenesis. Unlike FAP, no germline mutations in tumour-suppressor genes, such as *APC*, could be identified in patients with HNPCC. Instead, germ-line mutations on chromosome 2, indicative of errors in DNA replication, were discovered [19, 20]. Indeed, the concept of genetic instability due to errors of DNA replication resulting in abnormal cell growth and tumour development has been verified not only for the vast majority of CRCs in families with HNPCC [21, 22], but also in sporadic CRCs [23, 24]. The gene for HNPCC has been identified as *hMSH2*, a human homologue of the prokaryotic gene *mutS* which is involved in DNA repair [25, 26]. In addition, genes that are human homologues of the prokaryotic DNA mismatch repair gene *mutL* have been identified: on chromosome 2 and 7, *hPMS1* and *hPMS2*, respectively [27] and on chromosome 3, *hMLH1* [28, 29]. Mutations in these genes, resulting in defective DNA mismatch repair and genetic instability, are found to a variable degree in the germ-line of patients with HNPCC [27–29].

Apart from taking a careful family history to assess conformity with HNPCC criteria as well as performing colonoscopy or follow-up colonoscopy every 2 years, genetic testing to detect mutations and DNA instability is already being performed at some institutions. The discovery and characterisation of additional genetic markers of DNA instability will eventually lead to the routine testing of DNA from peripheral blood mononuclear cells to screen individuals at risk of HNPCC.

Sporadic colorectal cancer

Sporadic CRCs account for about 85% of CRCs and parallel the incidence of colorectal polyps or adenomas. The phenotypic changes associated with the development of CRCs are paralleled by genetic changes (Figure 1) and resulted in the concept that CRC is a multistep genetic disease [6]. Apart from mutations of genes involved in DNA mismatch repair [21–29], two types of oncogenic mechanisms have been defined: activation of oncogenes and inactivation of tumour suppressor genes [6]. Genetic alterations of three tumour suppressor genes have been identified in more than 70% of CRCs: the *APC* gene on

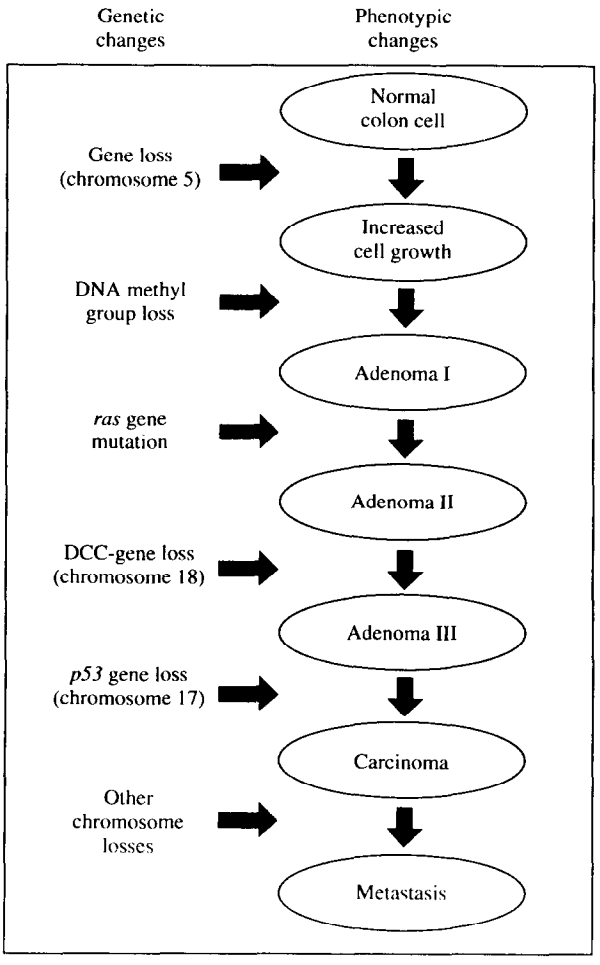

Figure 1. Model of phenotypic and genetic multistep CRC development [6].

chromosome 5 [30], the *DCC* gene on chromosome 18 [31] and the *TP53* gene chromosome 17 [32].

Among the oncogenes the most frequent mutations in CRCs are found in the *RAS* gene. Among the three *RAS* genes *H-*, *K-* and *NRAS*, the most frequently altered *RAS* gene is *KRAS*. Missense mutations are found predominantly in codons 12, 13 and 61 (Table 1). The frequency of *RAS* gene mutations correlates with the size of the lesions (adenoma < 2 cm, 14%, adenoma > 2 cm, 33%) and the degree of dysplasia (mild 0%, moderate 33%, severe or malignant 50%). *RAS* gene mutations, therefore, occur early in CRC development [33] and may be a useful genetic marker for the molecular detection of this malignancy in a potentially curable stage. Further, the presence of a codon 12 GGT to GAT mutation (Table 1) in Dukes' B or C

Table 1. Most frequent RAS *gene point mutations in CRCs*

Wild-type		Mutant	
Gly-12	GGT	GAT	Asp-12
	GGT	AGT	Ser-12
	GGT	TGT	Cys-12
	GGT	GCT	Ala-12
	GGT	GTT	Val-12
Gly-13	GGC	GAC	Asp-13
Gln-61	CAA	CAC	His-61

Table 2. Genetic screening for hereditary gastrointestinal polyposis and nonpolyposis syndromes as well as sporadic CRCs

Disease entity	Genetic screening
FAP	Analysis of *APC* gene and protein in peripheral blood mononuclear cells
HNPCC	Analysis of *hMSH2*, *hMLH1*, *hPMS1*, *hPMS2* genes in peripheral blood mononuclear cells
Sporadic CRC	*KRAS* gene mutations in DNA from stool

CRC predicts a low risk of recurrence while other *RAS* gene mutations are associated with a poor prognosis [34].

The molecular detection of *RAS* gene mutations as a screening test for early CRC in stool is based on the assumption that the mutations occur in 30–50% of growing adenomas and that epithelial cells of the gastro-intestinal tract, including cells from tumours, are constantly shed into the gut lumen. It is estimated that the colon sheds approximately 10^{10} epithelial cells daily and that a lesion of 1 cm^3 accounts for approximately 1% of these cells. Sidransky and associates developed a reliable and sensitive method for the molecular detection of specific *RAS* gene mutations in stool [35]: DNA is extracted from stool and a segment of the *KRAS* oncogene is amplified by PCR. The rare *KRAS* are then identified either by cloning of the PCR product and sequencing, or by plaque hybridisation of the clones with *RAS* mutant-specific oligodeoxynucleotide probes, or by direct dot blot hybridisation of the PCR product with *RAS* mutant-specific oligodeoxynucleotide probes. The mutations found in stool were identical to those found in the subsequently surgically removed tumours. The analyses of DNA extracted from stool detected 90% of all tumours that carried *KRAS* mutations. With respect to the study group as a whole, however, the sensitivity of this molecular screening method for the detection of early CRCs was 33% (8/24) only. Compared to the sensitivity of conventional testing for faecal occult blood [9], the molecular screening for *RAS* gene mutations, therefore, is of no routine clinical use at present.

CONCLUSION

With the better understanding of the genetic events leading to malignant transformation of colon epithelial cells, molecular population screening should allow identification of individuals at risk as well as patients with an early and potentially curable CRC. It is to be expected that the screening for a series of genetic changes (*RAS*, *APC*, *TP53*, DNA mismatch repair gene mutations and others) will increase the sensitivity of the molecular screening strategies (Table 2), resulting in the early identification, curative treatment and possibly prevention of CRCs. At present, careful patient and family history, physical examination, testing for occult blood as well as colonoscopy are still the key elements for clinical patient management. Molecular diagnosis will hopefully soon complement these analyses and should result in a reduction of morbidity and mortality from CRCs.

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