

Stool DNA analysis detects premorphological colorectal neoplasia: a case report

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We found, in an asymptomatic patient with familial occurrence of malignancy, that mutations in the oncogene *Kras* could be detected in stool 18 months before a premalignant polyp was detected and removed endoscopically. Colorectal cancers usually develop from benign adenomas in a lengthy period of 5–10 years. During this period, several major biochemical pathways are involved, each characterized by one or several genetic alterations. Our patient did not present any signs or symptoms of colorectal disease during his two visits to the endoscopist. This case report shows that the use of genetic markers in stool testing has the potential to detect colon cancer in its very early stages when treatment is simple and

Introduction

Colorectal cancer (CRC) affects about 6% of the adult population and is the second leading cause of cancer-related deaths in United States and other developed countries [1]. On the basis of the evidence from epidemiological and pathological studies, most sporadic CRCs are thought to develop from benign adenomas, which accumulate various genetic abnormalities in a step-wise manner known as the adenoma–carcinoma sequence [2–4]. The time period required for the development of malignancy from adenomas is estimated to span a period of at least 5–10 years [5]. At present, no way exists to distinguish which adenomas will become malignant, although severe dysplasia, increasing patient age, increasing size of adenoma and histological subtype are all factors that are clearly associated with subsequent malignant progression [6]. An unsolved medical challenge is that the early changes of this malignant development are not manifest by any symptoms or signs that can easily be detected by physicians. Advances in our understanding of the molecular pathology in the adenoma–carcinoma sequence have led to the development of diagnostic and screening tools using colonocyte DNA in human stool [7]. Mutations in the oncogene *Kras*, and the tumour-suppressor genes *p53* and *APC*, are among the commonly used markers for early detection of CRC in fecal samples [8]. Stool testing has several important advantages over other diagnostic and screening methods, with promising performance characteristics and good patient acceptance [7,8]. We report a case where mutation in *Kras* was detected in stool DNA 18 months before the polyp was identified endoscopically.

Case report

A 61-year-old man with a 10-year history of hypertension and a family history of malignancy (prostate and breast

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cancer) had his annual doctor's visit in November 2004. Medical examination was normal, he had a slightly elevated level of lactate dehydrogenase and a fecal DNA test (Genefec; NorDiag ASA, Bergen, Norway) analyzing *Kras* mutations was positive. He was subsequently admitted for colonoscopy that was described as completely normal in all sections from the ileo-cecal valve to rectum. As mutations in *Kras* detected in fecal DNA can also be associated with malignancies in pancreas or lung (aerodigestive cancers) [7], CT scan of abdomen and chest radiograph were performed without pathological findings.

According to the guidelines of the producer of the Genefec test, another fecal test and colonoscopy was performed 18 months later. The patient did not present any bowel or other symptoms at this follow-up. The DNA test was again positive for the same *Kras* mutation. A second colonoscopy now identified a broad-based polyp close to the ileocecal valve that was removed endoscopically (Fig. 1). Pathological examination identified a hyperplastic polyp with serrated epithelium, classified as advanced neoplasia. Two months after removal of the serrated polyp, the fecal DNA test showed normal *Kras* DNA.

Discussion

It is generally accepted that CRC develops by genetic alterations, and traditionally two major pathways has been described: the chromosomal instability pathway (adenoma–carcinoma sequence) and the microsatellite instability pathway [3,9,10]. Recent genetics research has also identified other major routes for malignant progression, including the transforming growth factor pathway,

Fig. 1



Endoscopic appearance of the polyp located close to the ileocecal valve.

the serrated and the epigenetic pathways [11]. Genetic changes that are involved in the various pathways have been extensively studied and classified [11]. Whereas tumours showing chromosomal instability show high rates of *Kras*, *p53* and *APC* mutations, serrated adenomas and serrated hyperplastic polyps display a high rate of microsatellite instability and low rates of *Kras*, *p53* and *APC* mutations [12,13]. Recent evidence indicates that a serrated histological subtype is one of the major risk factors associated with malignant transformation [6].

Although *Kras* mutations are infrequent in polyps transformed through the serrated pathway [12,13], it was a mutation in this gene that led to the endoscopic detection of a polyp in our patient. DNA sequencing showed a mutation in *Kras* (GGT→AGT, base 1 mutation in exon 12, glycine→serine) that has only infrequently been described in human tumours [14]. As there are so few observations of *Kras* changes in serrated polyps, we do not know if this type of mutation could be particularly linked to this kind of morphological change. As expected from earlier observations, analyzing exons 5–8 of *p53* from the stool in our patient did not show any mutations.

Our case report also supports the general view that colorectal carcinogenesis spans a period of many years [5]. The disappearance of the mutation after removal of the polyp strongly indicates that this polyp was the single source for the genetic change. The likelihood of this polyp developing into a malignancy would be rather high on the basis of recent observations for serrated polyps [6].

Our patient was tested for fecal DNA mutations because of a family history of multiple cancers. He did not present clinical signs or symptoms at the time of examination or at the time of polyp removal. Compared to Faecal Occult Blood Tests, the major challenge for faecal molecular tests is that they so far are dependent on a number of different markers to meet sensitivity and specificity criteria, increasing the cost [15,16]. Nevertheless, as pointed out in a number of recent studies, and supported by this case report, the use of assays with molecular markers in stool represents a new, noninvasive and promising approach for both early diagnosis and screening of CRC [7,8,15]. Early detection of CRC or premalignant lesions of the large bowel are associated with both reduced treatment cost and, more importantly, with a marked increase in survival [17,18].

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