Case report

Flow-cytometric DNA analysis as a means for early detection of malignancy in patients with chronic ulcerative colitis

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SUMMARY A new approach to the problem of monitoring patients with chronic ulcerative colitis is presented and discussed in connection with a case report. When annual colonoscopies are performed, biopsies are taken for histopathological examination and DNA measurements are made using flow-cytometric techniques (FCM). Using the latter approach, gross chromosomal aberrations indicating malignant transformation in a cell population may be detected. In a 46 year old man with a long history of ulcerative colitis, an area with slight mucosal dysplasia at light microscopy was accompanied by two aneuploid cell lines – that is, colonic mucosa cells with an abnormal amount of DNA in the nuclei. An operation one year later revealed a 5×2 mm large adenocarcinoma in the corresponding area of the colon. We suggest that flow-cytometric techniques can be used as a complement to already practised methods for monitoring the colorectal mucosa in colitic patients for the early detection of malignancy.

Patients with chronic ulcerative colitis run an increased risk of developing colorectal cancer. Proctocolectomy has therefore been widely recommended after the disease has been present for 10 years. For obvious reasons, it would be highly desirable to have methods which could more accurately guide the selection of patients for operation. The introduction of colonoscopy during the last decade has enabled direct examination of the colon and the taking of biopsies for histopathological evaluation. The finding of severe dysplasia in a biopsy has been considered to indicate a high risk of developing malignancy.^{1,2} It can be difficult, however, to distinguish between severe and lighter forms of dysplasia, especially in the presence of inflammation. Furthermore, it has been reported that adenocarcinoma may develop without previous findings of dysplasia in the biopsies, and also that severe dysplasia is not always found again on repeated investigation, indicating either very local or reversible changes.³⁻⁵ For the early detection of

malignancy, additional methods for routine supervision of the patient would therefore be of great value.

In a previous investigation⁶ using flow-cytometric techniques we have shown that colorectal adenocarcinomas display gross chromosomal changes in the mucosal cell nuclei, detected by the presence of aneuploid cell lines in 80–90% of the cancers. In connection with routine colonoscopies of patients with ulcerative colitis we have therefore, in addition to the biopsies taken for histopathological evaluation, also taken biopsies for measurement of the DNA content of the colorectal mucosal cell nuclei. The aim is to detect aneuploid cell populations which may serve as an early indicator of malignant transformation. We present here results obtained from the first patient in a prospective investigation of 200 patients with chronic ulcerative colitis, in which we have had the opportunity of following up the discovery of aneuploid cell populations during a later operation.

Methods

At colonoscopy nine biopsies were taken at equal

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distances from the colorectum. Each biopsy was divided in two. One half was fixed in 10% neutral formalin for histopathological examination and the other half was put in saline for flow-cytometric DNA measurement, the technique having been described in detail elsewhere.^{7,8} Briefly, the cell material was pressed through a nylon grid and the resulting cell suspension fixed in 96% ethanol. The fixed cells were washed in buffer solution with RNase in order to remove the RNA. Suspensions of single cell nuclei were obtained by pepsin treatment. After washing in the buffer the cell nuclei were stained using ethidium bromide in Tris EDTA buffer with the molarity of 385 mOsm. The DNA content of the cell nuclei was analysed using the rapid flow cytofluorometer ICP 11 (Phywe, W Germany). The output was sorted with a 256 multichannel analyser. The DNA values of the analysed cells were calculated in relation to the DNA content of normal human lymphocytes. Figure 1 shows examples of DNA histograms, both of diploid and aneuploid pattern, found in the colon mucosa of the present patient (see further case report).

CASE REPORT

This male patient was born in 1934, and in 1949 he suffered his first attack of ulcerative colitis to a rather severe degree. The initial attack and frequent relapses during the following years all subsided upon conservative treatment.

Until 1968 barium enemas showed only left sided

colonic involvement. A stricture of the ascending colon was then found and a right sided hemicolectomy was performed. The specimen displayed ulcerative colitis with chronic inflammation and fibrosis. No dysplastic changes or malignancy were observed.

The first colonoscopy carried out in 1975 revealed colitic engagement of all the remaining colorectum. Colonoscopic biopsies showed no dysplastic changes. Further colonoscopies were refused by the patient until October 1980. On this occasion the gross appearance of the mucosa was normal. In nine biopsies taken at approximately equal distances from the colorectum, the two most proximal ones showed slight dysplasia, the third one showed chronic inflammation, and the six most distal biopsies showed normal mucosa. DNA analysis was performed with the biopsies pooled in three samples, each one containing three consecutive biopsies. In the sample of the three proximal biopsies (I, Fig. 2), there were two aneuploid cell populations at a relative DNA value of 4.2c and 5.4c (diploid=2.0c), respectively, while in the two distal samples (II and III, Fig. 2) only diploid DNA values were found (Fig. 1a). The results of a barium enema performed in March 1981 were normal. Because of a long history of disease it was, however, decided to operate. The patient refused proctocolectomy and a colectomy with ileorectal anastomosis was performed in November 1981. In the proximal part of the colon resectate, corresponding to sample I above (Fig. 2), one of the two aneuploid cell lines

Fig. 1 (a, b) Histograms from DNA analysis of two biopsy fractions collected at colonoscopy. (a) A diploid cell population and (b) a diploid population mixed with two aneuploid populations at relative DNA values 4.2c and 5.4c, respectively. (c, d) DNA histograms from two analysed biopsies taken at operation. Two aneuploid cell populations at relative DNA values 2.2c (c) and 4.0c (d), respectively, are shown. The diploid point on the abscissa is in all histograms established by analysis of normal human lymphocytes.

Fig. 2 Distribution within the colon of nine biopsies taken at operation. Ca=cancer. I, II, and III show approximately the regions corresponding to the analysed biopsy samples I-III taken at colonoscopy one year before operation.

found at colonoscopy (4.2c, Table) was found. Furthermore, two additional aneuploid cell lines, at 1.8c and 2.2c (Fig. 2, Table) were found in the same region. In the middle and distal parts of the colon (corresponding approximately to samples II and III at the colonoscopy), where one year earlier the biopsies had been only diploid, two aneuploid cell populations (at 4.0c and 3.8c, Table) were found. In addition a 5×2 mm tumour was found approximately 2 cm from the site of the ileocolonic anastomosis (Fig. 2). Histopathology revealed the tumour to be a moderately differentiated adenocarcinoma. Flow-cytometric techniques showed diploid and near diploid DNA patterns. In the biopsies with aneuploid cell lines histopathology

showed atrophy, inflammation, or slight dysplasia (Table).

Discussion

In the majority of cases colorectal adenocarcinomas, like solid tumours in other tissues,⁹ show gross chromosomal changes. Furthermore, aneuploid DNA patterns have been shown exclusively in the presence of malignancy. In the follow up of patients with chronic ulcerative colitis, we have in a number of cases with long duration of disease noted widespread aneuploidy in biopsies taken at colonoscopy.¹⁰ With the present case, which is the first operated patient in which aneuploidy was found at colonoscopy, we wish to show the possible value of using flow-cytometric techniques in addition to histopathology for monitoring patients with chronic ulcerative colitis. Thus, at operation a small adenocarcinoma was found in the region where aneuploidy had been observed when the colonoscopy had been performed one year before operation. The adenocarcinoma was near diploid. Grossly aneuploid cell populations were found in the same region and also, in contrast with the findings when the colonoscopy was performed, in the middle and distal parts of the colon, which may indicate a progression of the malignant transformation during the time period between colonoscopy and operation. The existence of an aneuploid DNA pattern reflecting grossly chromosomal aberrations must be interpreted as a certain manifestation of malignancy in the mucosa. Diploid and near diploid DNA patterns do not, however, exclude the presence of malignancy, as shown by cytogenetic studies.⁹ The coexistence of malignant cells with near diploid DNA content together with grossly aneuploid cell populations in the same tumour have been previously shown.^{6,9,11} It can also be assumed that in the presence of grossly aneuploid cell populations there may also exist near diploid malignant cell populations. This assumption is in the present case supported by the finding of a tumour with near diploid DNA content in close vicinity to mucosa cells with gross aneuploid DNA pattern. By

Table DNA pattern and histopathology in nine biopsies taken at operation

	Biopsy no								
	1	2	3	4	5	6	7	8	9
Relative DNA value	1.8c	2.0c	2.0c	1.8c	2.2c	4.2c	4.0c	2.0c	3.8c
Histopathology	Ca	Ca	Dy	In	Dy	Dy	Dy	At	At

Ca=cancer, Dy=slight dysplasia, In=inflammation, At=atrophy (see also Fig. 2).

chance either a near diploid or a grossly aneuploid cell population may thus give rise to a tumour as defined by histopathology. It is notable that in the present case the finding of aneuploidy was at most reflected in slight dysplasia in the histopathological evaluation of the samples from colonoscopy as well as from the operation. Consequently aneuploidy may precede certain histopathological signs of malignancy. The present case shows the possible value of studying the nuclei DNA content of mucosa cells in patients with chronic ulcerative colitis for the early detection of developing malignancy. The prognostic significance of the existence of aneuploid cell populations must, however, be evaluated by a thorough clinical and histopathological follow up of a large number of patients.

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