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Tumour Marker Acquisition in the Polyp-to-Cancer Sequence in the Colon*

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Abstract—*Sequential studies of 6 tumour markers in an adenomatous polyp which eventually metastesized as adenocarcinoma are described. The findings suggest that with the development of malignancy, clones of cells with an increasing number of markers are favoured for continued growth and that this probably represents progessive gene derepression.*

INTRODUCTION

OVER the years evidence has accumulated which points to the fact that carcinoma of the large bowel arises in adenomatous polyps. It has also previously been shown that there are similarities between adenomatous polyps and colon cancer in their expression of tumour markers [1]. What is not known is if or how a given tumour changes its neoantigen profile during its development. The opportunity to make observations relating to this was provided by a patient with a large rectal, predominantly villous tumour who refused treatment by resection. The lesion was removed piecemeal with biopsy forceps through a sigmoidoscope on 25 separate occasions over a period of 6 yr until it eventually metastasized as a mucinous adenocarcinoma.

MATERIALS AND METHODS

The first specimen consisted of three large tissue fragments of a villous tumour each measuring approximately 2.5 cm in diameter. Thereafter the 24 subsequent specimens, removed over a 6-yr period, varied greatly in size (1–7 mm) and number (1–15). All the tissues were embedded *in toto*. The final specimen was an abdomino-perineal resection of an invasive moderately differentiated mucinous adenocarcinoma with metastases in the regional nodes.

All the tissues were collected in 4% phosphate-buffered formaldehyde, pH 7.0. The blocks were processed through graded alcohol into xylol and then embedded in paraffin wax (melting point 52°C) in a vacuum-embedding vessel.

Serial sections were cut on a rotary microtome at a setting of 5 μm, placed individually on glass slides, dewaxed in xylol then washed in graded alcohols. Finally, they were placed for 30 min in 0.2 M phosphate-buffered saline, pH 7.14.

On one set of sections routine hematoxylin–eosin (H and E) staining was performed.

Other sections were used in immunohistochemical preparations employing rabbit antibodies against carcino-embryonic antigen (CEA), alpha-fetoprotein (AFP), colon-specific antigen (CSA), pregnancy-specific beta glycoprotein (SPI), human placental lactogen (HPL) and placental alkaline phosphatase (PLAP). All of these substances, except for CSA, are present in foetal-derived tissue. The antibodies, with the exception of CSA, were available in the laboratory and had previously been used in serum immunoassays and found to be specific. The PAP method as modified by Burns [2] was used.

Before use the rabbit antisera to CEA and AFP which are available as Ig fractions (DAKO immunoglobulins) were absorbed 3 times with a suspension of fresh adult human liver and blood cells to remove non-specific cross-reacting antigens. Antibodies to SPI and HPL were obtained from Behringwerke AG. Before use the whole rabbit serum was separated on an ion exchange column and the resulting immunoglobulin fraction was then absorbed as above. The antibody to CSA (high-molecular-weight fraction) was prepared in rabbits according to the method of Goldenberg *et al.* [3] except that a mixture of extracts from 3 primary colon

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carcinomas was used as the source material. The immunoglobulin fraction of the antiserum was absorbed repeatedly (up to 5 times) with extracts of normal human mucosa from cases of diverticular disease, until the antibody gave negative results in the peroxidase–antiperoxidase (PAP) method with normal mucosa.

The resulting antibody gave very weak or negative results with colon carcinoma tissue when diluted beyond 1 in 250 and it showed no cross-reactivity with CEA in an Ouchterlony plate.

Each antibody concentration was determined using positive control material from human foetus or placenta processed in the same manner as the test material. The concentrations finally used were the lowest that gave positive results in the control tissues without significant background staining: for CEA, 1/100; CSA, 1/200; AFP, 1/100; SPI, 1/100; HPL, 1/100; and PLAP, 1/50.

Negative controls were sections of macroscopically and microscopically normal colon removed for diverticular disease.

Positive controls for CEA and AFP were sections of human foetus between 12 and 30 mm long; for SPI, PLAP and HPL sections of fresh human placenta was used.

As a control for the blocking reagent, diaminobenzidine alone was used to detect endogenous peroxidase not blocked by the peroxide–methanol procedure. Results were always negative.

The PAP reagent plus diaminobenzidine were used to detect heterophile antibodies in the swine anti-rabbit antisera with activity against human antigens or non-specific protein binding. The rabbit anti-human component antibody was preincubated with antigen (CEA, CSA and SPI). The results were negative on known positive controls. Pure extracts of SPI and HPL were available in limited amounts. After absorption the antibodies gave negative (SPI), or in the case of HPL greatly diminished, staining in the PAP method using normal placenta as substrate. Thereafter the antibody activity against SPI and HPL was tested by prior incubation with suspensions of fresh placental tissue, which eliminated all activity.

Interpretation of immunohistochemical staining

Within each test section either a positive or a negative result was recorded. In some sections recorded as positive only part of the tumour tissue showed staining (this was sometimes as little as 10%), but in other sections all the tumour tissue was positive. No attempt was made to quantify accurately the percentage of positive cells or to assess the intensity of staining as the relative avidity and protein concentration of the antibodies used was not known.

Prior trypsin treatment of processed sections can unmask some antigens. Negative examples for each antibody were treated with trypsin (Sigma) for 30 min at 37°C. No extra positive results ensued, although similar treatment of known positives tended to enhance the intensity of the staining reaction.

RESULTS

The pattern of staining of positive cells was the same, i.e. sometimes granular or vesicular in the cytoplasm, with some tumour cells also showing a positive lumenal border where they formed acinar structures (Fig. 1). Positive extracellular material was often also seen lying in tissue spaces. Initially (Table 1) the positivity for all the

TABLE 1

	1976	1977	1978	1979	1980	1982
CEA	⊕⊕⊕	⊕⊕⊕⊕⊕	⊕⊕⊕	⊕⊕⊕	⊕⊕⊕⊕⊕✚	✚
CSA	⊕⊕⊕	⊕⊕⊕⊕⊕	⊕⊕⊕	⊕⊕⊕	⊕⊕⊕✚✚✚	✚
PLAP	⊕⊕⊕	⊕⊕⊕⊕⊕	⊕⊕⊕	⊕⊕⊕	⊕⊕⊕⊕⊕✚	✚
SPI	− ⊕ −	− − − − −	− − −	⊕⊕⊕	⊕⊕⊕⊕⊕⊕	✚
HPL	− − −	− − − − −	− − −	− − −	− − − − − −	−
AFP	− − −	− − − − −	− − −	− − −	− − − − − −	−

⊕ - Only a percentage of the tumour positive

✚ - All tumour tissue positive

− - No staining

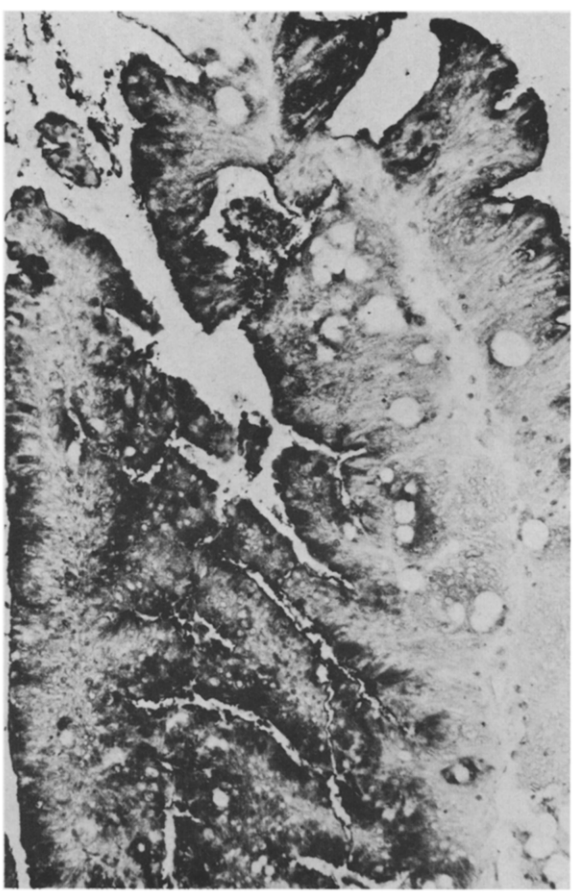

Fig. 1. *Positively stained granular and vesicular SPI (black) within cytoplasm and lying free in extracellular spaces. (PAP with haematoxylin counterstain,* ×340.)

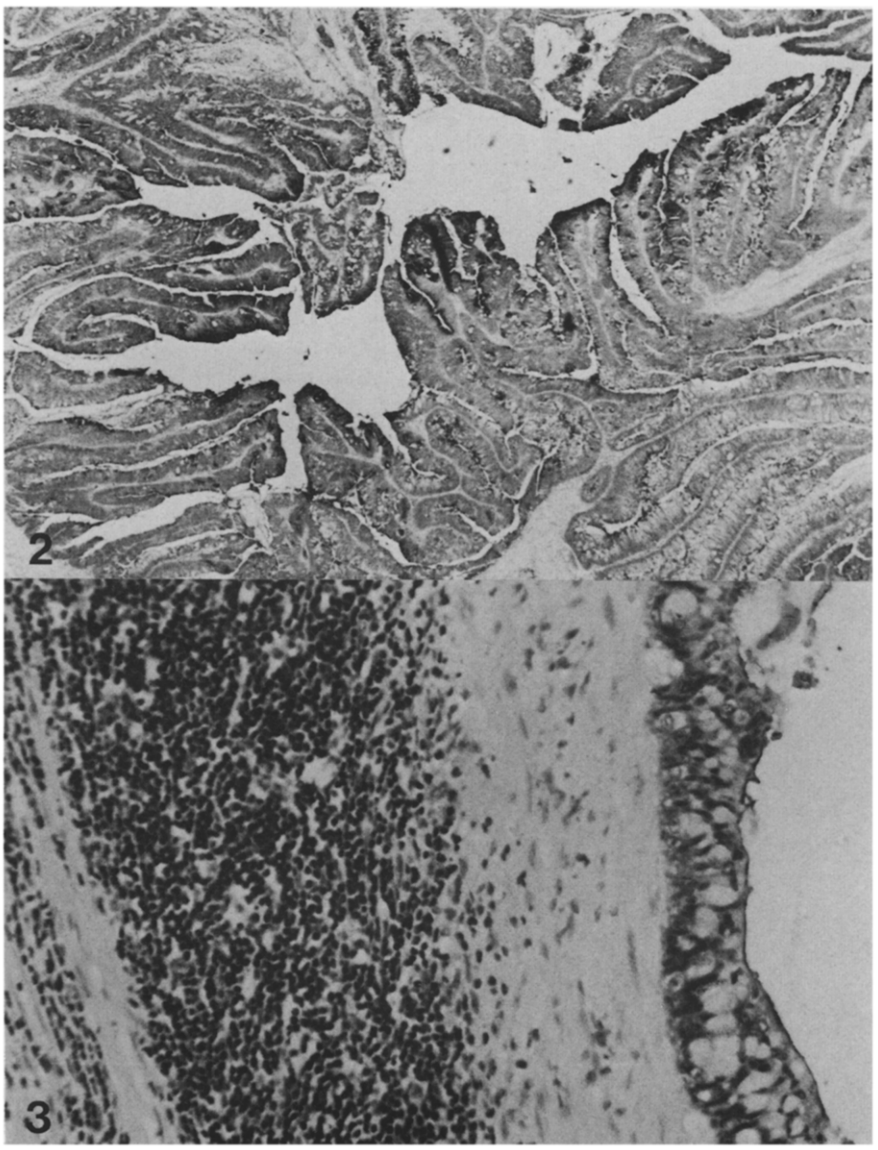

Fig. 2. Area of tumour showing both positive and negative CSA staining. (PAP with haematoxylin counterstain, ×340.)

Fig. 3. Metastatic tumour in a lymph-node positive for PLAP. Note the desmoplastic reaction. (PAP with haematoxylin counterstain, ×212.)

antigens was patchy in distribution (Fig. 2), whereas there was a tendency with time for all the tumour tissue to be positive and this was so in both tumour and lymph-node secondaries in the resection specimen (Fig. 3). The tumour was predominently villous in type with a minor variable tubular component which was largely absent in tissues from the end of 1980. It was also noted that the tumour became more obviously mucinous with time.

DISCUSSION

Adenomatous polyps of the colon are relatively common in comparison with adenocarcinoma, and the polyp-to-cancer sequence only occurs in a small minority during the life of patients who harbour such lesions. With the advent of colonoscopy, there is a theoretical possibility of eradicating adenocarcinoma of the colon as a disease of significant proportions. It has been shown, for example, that periodic sigmoidoscopy and polyp removal will reduce the anticipated incidence of rectosigmoid carcinoma by 85% [4].

A dilemma concerning the detection of adenomatous polyps, their management when discovered and the follow-up of the patients in whom polyps have been removed is rapidly developing. The essential difference between an adenomatous polyp and an adenocarcinoma is the latter's capacity to invade. There is, however, some evidence that the degree of dysplasia in an adenomatous polyp at the time of its removal is associated with an increased risk of subsequent colo-rectal carcinoma [5]. Dysplasia as subjectively assessed is probably the morphological counterpart of genetic derepression which occurs in the malignant process. Manifestation of this derepression is the acquisition of the capacity of synthesis by cancer cells of new macromolecules, present on the cell membrane, within the cytoplasm or secreted, which can be detected using suitably prepared antibodies or histochemical techniques. The sequential tissues in this case afforded the opportunity of investigating whether with the evolution of the malignant process there was an increase of progressive gene derepression. This seemed possible for it has already been shown [1] when comparing a series of adenomatous polyps with adenocarcinomas that whereas a similar range of markers was present in both, carcinomas consistently had larger numbers of different antigens.

In the tumour examined in this study, CEA, CSA and PLAP were demonstrated in a patchy distribution in all tumour tissue between 1976 and 1980. SPI was present in a very small area of tumour tissue removed in 1976 but only reappeared in 1979. At the end of 1980 CEA, CSA and PLAP were present throughout the tumour and in the resection specimen SPI had the same distribution. The reappearance of SPI is of some interest, the inference being that with the development of malignancy, the cells with increased gene derepression manifested by acquisition of an increasing number of antigens were being favoured for continued growth. Presumably the clone of cells positive for all four antigens at the primary excision were removed *in toto* but cells with a similar antigen profile re-emerged, forming only part of the tumour in the last few years but the whole of the tumour at the time of the abdomino-perineal resection. Those cells with a fewer number of antigens probably belonged to a cell line which finally died out. Implicit in this argument is that an increased marker acquisition is related to the capacity to metastasize. There is every possibility, therefore, that further studies using a larger number of markers would delineate those adenomatous polyps of most malignant potential with greater accuracy than a subjective grading of dysplasia. This has important practical implications in the management of patients with colo-rectal adenomatous polyps.

REFERENCES

1. SKINNER JM, WHITEHEAD R. Tumour-associated antigens in polyps and carcinoma of the human large bowel. *Cancer* 1981, **47**, 1241–1245.
2. BURNS J. Background staining and sensitivity of the unlabelled antibody enzyme (P.A.P.) method. Comparison with the peroxidase labelled antibody sandwich method using formalin fixed paraffin embedded material. *Histochemistry* 1975, **43**, 291–294.
3. GOLDENBERG DM, PANT KD, DAHLMAN HL. Antigens associated with normal and malignant gastrointestinal tissues. *Cancer Res* 1976, **36**, 3455–3463.
4. GILBERTSEN VA. Proctosigmoidoscopy and polypectomy in reducing the incidence of rectal cancer. *Cancer* 1974, **34**, 936–939.
5. KONISHI F, MORSON BC. Pathology of colorectal adenomas: a colonoscopic survey. *J Clin Pathol* 1982, **35**, 830–841.