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A Murine Monoclonal Antibody to Human Breast Cancer Cells Associated with DNA Ploidy Status

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A murine monoclonal antibody, 5D10, raised against the human breast cancer cell line MCF7 reacted preferably with mammary carcinomas and weakly with normal epithelial cells. The antigens recognised by the antibody had molecular weights of about 28 and 90 kD. The reactivity of the antibody to human breast carcinomas correlated with the DNA ploidy status of the tumour cells. Upon analysis of 54 breast carcinoma specimens, the percentage of antibody positive cells was significantly higher in tumours with an aneuploid stemline than in those with a diploid DNA content ($P < 0.001$). This antibody therefore could be a useful tool in evaluating the prognosis of breast carcinomas.
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INTRODUCTION

THE MAJOR goal for making monoclonal antibodies to breast cancer cells has been the development of specific probes to differentiate among the breast tumours, to identify markers that correlate with disease progress and to resolve important therapeutic dilemmas [1]. DNA ploidy status may be a useful prognostic factor in breast cancer [2–10].

Previously we reported a panel of murine monoclonal antibodies raised against human breast cancer cell line MCF7 [11]. The antibodies were highly specific for epithelial cells. Breast tumour cells were often stained cytoplasmically, while in normal and benign breast tissues the staining was concentrated at luminal sites and in the secretory products. One of the antibodies, 5D10, was useful in the evaluation of invasiveness *in vitro* of MCF7 cells [12]. We have examined the antibodies further on postoperative specimens from breast cancer patients to evaluate their value for diagnosis and prognosis. The nuclear DNA content of these specimens was also analysed.

MATERIALS AND METHODS

Immunoblotting. MCF7 cells were extracted by Triton-X-100 (0.5%), acetone or Nonidet-P40 (1%) and electrophoresed in a vertical 10% polyacrylamide slab gel. After electrophoresis, the separated constituents were transfered electrophoretically to nitrocellulose membranes in a trans-blot cell (Pharmacia) for 2 h at 180 mA at room temperature. The membranes were then incubated with 5D10, biotinylated-rabbit anti-mouse Ig and alkaline phosphatase conjugated avidin–biotin complex. The alkaline phosphatase reaction was developed with naphthol AS-MX phosphatase and fast red as substrate.

Indirect immunocytochemical assay on frozen sections. Indirect peroxidase staining was done on formalin or acetone fixed frozen sections. After washing with phosphate-buffered saline (PBS), the slides were incubated consecutively with normal rabbit serum (Dakopatts, Prosan, Belgium) for 20 min, 5D10 2 μg/ml for 30 min, rabbit anti-mouse Ig (Dakopatts) as a bridging antibody for 30 min and finally a peroxidase conjugated mouse anti-peroxidase complex (Dakopatts) for 30 min, all at room temperature. Each step was followed by a brief wash with PBS. The enzymatic reaction was done with a mixture of 0.5 mg/ml diaminobenzidine (Janssen Chimica) and 0.03% hydrogen peroxidase for 5 min. The slides were washed with running tap water for 5 min and counterstained with methyl green. Mouse immunoglobulin was substituted for primary antibody as a control for non-specific binding. The immune stainings were evaluated with a quantitative image analysis system (CAS 200, Becton Dickinson). The antibody reactivity was expressed as a percentage of positive stained cells in total measured cells.

DNA ploidy analysis. The air-dried cytological preparations of the breast tumour tissues were primarily stained with May–Grünwald–Giemsa (MGG) and examined microscopically to confirm the diagnosis. The MGG stain was then removed in absolute methanol for 60 min, refixed with 10% neutral buffered formalin (Sigma) for 30 min and hydrolysed for 60 min in 5 mol HCl [13]. DNA Feulgen staining was done with the CAS quantitative DNA staining kit (CAS Inc, Lombard, Illinois) according to the manufacturer's instructions. One calibrator slide was included for each batch of stainings to calibrate the normal DNA content and to control the staining quality. The nuclear DNA staining results were analysed on the CAS 200 with CAS quantitative DNA program. The program was initialised by counting 20–150 cells on the calibrator slide. At least 20 normal cells within the specimen were used as internal controls. The slides in which no normal diploid cells were present were discarded. The optical density of the Feulgen stained nuclear DNA was measured for at least 100 single tumour cells per slide and the results are presented in histograms and DNA indices.

Histogram analysis of DNA content. Histograms were analysed without knowing the results of the immunochemical assay with 5D10. The histograms were grouped as diploid (DNA index 1.1 or less), "tetraploid" (near-tetraploid with DNA index 1.8–1.9, and tetraploid with DNA index 2.0), and "other DNA-aneuploid" (DNA indices 1.2–1.7, and 2.1 or more), as proposed by Baildam *et al.* [14].

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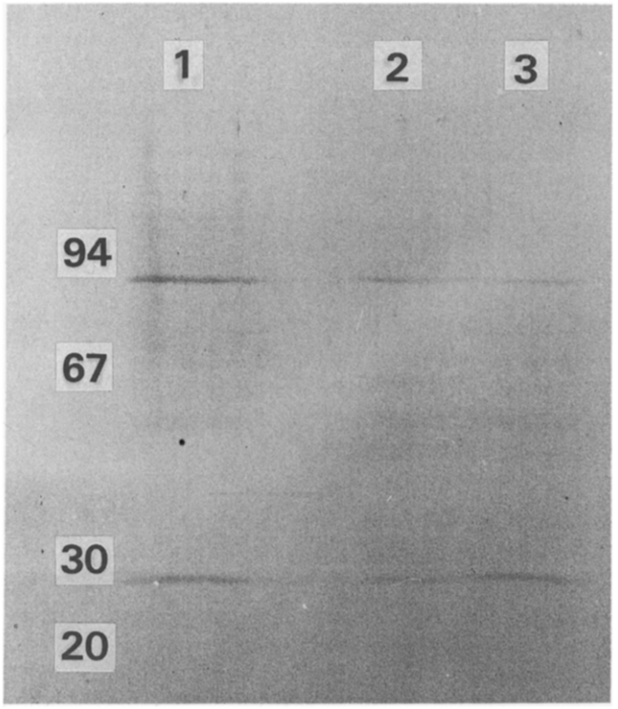

Fig. 1. Immunoblotting of antigens extracted from MCF7 cells with Triton-X-100 (track 1), acetone (track 2) and Nonidet-P40 (track 3). Molecular weight markers (kD) are shown on left.

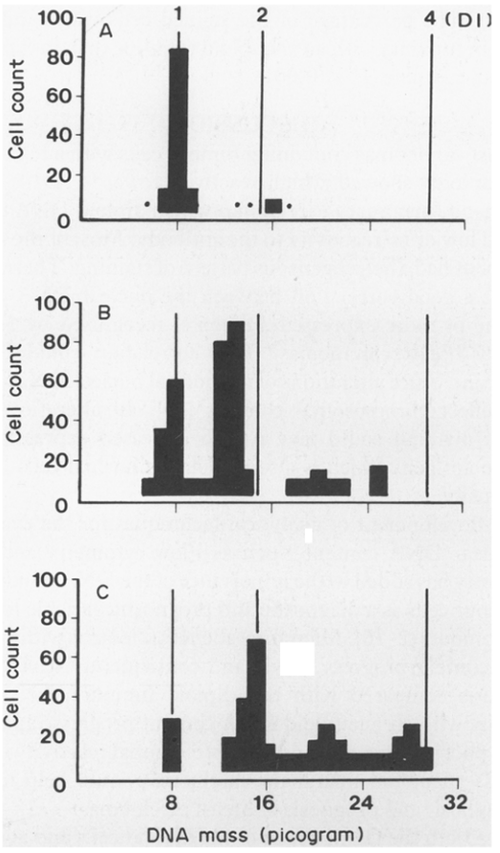

Fig. 2. DNA histograms (A) of diploid cells, DNA index (DI) 1.0; Only a few proliferating cells are seen in S and G2/M phases. (B) shows aneuploid cells with the main cell population (G0/G1) at DI of 1.53 and more proliferating cells in S and G2/M phases; first peak of cells in histogram with DI 1.0 represents normal cells in specimen. (C) shows tetraploid DNA cells with most cells at DI 2.0, and many cells in proliferation stage.

RESULTS

Characterisation of MAb 5D10

Positive staining was mainly confined to epithelial cells and their related adenocarcinomas. Not all cells were stained and the staining intensity itself was heterogeneous in the positively stained cells. The antigens recognised by the antibody had molecular weights of about 28 and 90 kD (Fig. 1).

DNA content analysis

DNA histograms were plotted for the imprints of 54 breast tumour specimens. Of these tumours 19 had a diploid DNA content, 16 were "tetraploid" and 19 were "other DNA-aneuploid" (Fig. 2). The frequency of "tetraploid DNA" and "other DNA aneuploid" tumours showed no significant differences from the data obtained by others [14].

Immunocytochemical reactions of 5D10

Most of the breast carcinomas with non-diploid DNA content were stained by 5D10 to a variable degree and most of the staining was confined to the cytoplasm and occasionally to the perimembrane. Of the 19 diploid specimens, 15 were either unstained or stained weakly. However, the percentages of the stained cells in these weakly stained specimens were less than 10%. Among 35 non-diploid tumours, 25 were stained by the antibody. When tumours with "tetraploid" DNA content were not considered, all of the "other DNA-aneuploid" tumours were positively stained and the percentage of stained cells in each specimen was more than 10%. In tumours with "tetraploid" DNA content, some specimens were positively stained by the antibody and some were not. Statistical analysis revealed significant differences among the three groups tested ($P < 0.01$)

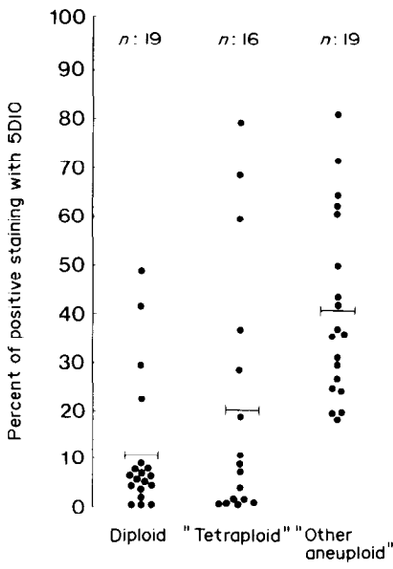

Fig. 3. Relations between 5D10 staining of breast cancer specimens and DNA ploidy status. • = an individual specimen and — = mean staining percentage.

(Fig. 3). The percentage of the stained cells was significantly higher in tumours with an aneuploid stemline than in those with a diploid stemline ($P < 0.001$).

DISCUSSION

Breast carcinomas containing tumour cells with a high degree of aneuploidy showed a high reactivity towards 5D10. On the other hand, mammary carcinomas with a diploid DNA content showed low or no reactivity to the antibody. Most of the tumour specimens had a heterogeneous pattern of staining. These results suggest a good correlation between the nuclear DNA content and one or more expressed antigen(s) recognised by 5D10 in primary breast carcinomas. This correlation could be very significant. Since alterations of the normal nuclear DNA content may reflect chromosomal changes [15], an alteration of the genetic material could give rise to increased expression of a specific antigen, which is also present on normal cells but at a low density.

The development of analytical techniques for the evaluation of nuclear DNA content, such as Flow cytometry and image cytometry has added to the importance of the DNA ploidy status of tumour cells as a diagnostic and prognostic variable for many solid tumours [2–16]. Mammary adenocarcinomas with a diploid DNA content progress slowly and consequently have a better prognosis compared with non-diploid tumours. In contrast, tumours with an aneuploid DNA content progress rapidly and have a poor prognosis [3, 4]. The preferential reactivity of 5D10 towards aneuploid mammary carcinomas could help to refine the diagnosis and prognosis of breast carcinomas.

Since both the G2/M phase of the diploid cells and aneuploid cells can give tetraploid patterns, it is often difficult to distinguish these two populations by cytometry. Antibody reactive with aneuploid cells, such as 5D10, could thus be used to distinguish a real aneuploid stemline with tetraploid pattern from a G2/M phase of diploid cells. Tetraploid mammary tumours have attracted much interest because some of these tumours are responsive to endocrine therapy while tumours with diploid or true aneuploid DNA content showed no response [9, 14, 17, 18].

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