Demonstration of progesterone receptors in breast carcinoma by immunohistological methods

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Summary

Immunohistological methods for the demonstration of progesterone receptors were applied to routinely processed paraffin embedded sections of breast carcinoma using Abbott's PgR-IcA monoclonal antibody. 36 cases of breast carcinoma were studied and their subtypes and histological grades recorded. Different immunohistological methods were tried initially on ten cases. The best results were obtained with the avidin biotin complex (ABC) method with no prior digestion by trypsin and with imidazole added to the final diaminobenzidine (DAB) developing solution. This method was therefore used in studying the rest of the breast carcinomas. A simple semi-quantitative scoring system was used to assess the staining results. The less differentiated grade III tumours showed significantly lower levels of progesterone receptors ($p < 0.001$) than grade II tumours. Medullary carcinomas had very low levels and mucoid carcinomas had the highest levels of progesterone receptors.

Key words: Breast carcinoma, progesterone receptor demonstration, relationship to grade of carcinoma.

Introduction

Progesterone receptor (PgR) assay was introduced into the analyses of breast cancer specimens to enhance the predictive value of oestrogen receptor (ER) determinations (1). PgR production was thought to be an oestrogen-dependent phenomenon (2) and its presence was believed to be indicative of a functional ER system. If these assumptions be correct, the presence of both these receptor proteins should have a greater predictive value of the clinical endocrine response as well as of disease-free survival. It has been shown that breast cancers that are both ER positive and PgR positive are more likely to be hormone sensitive than those that are positive for ER alone (3). There is a difference of opinion regarding the value of PgR as a predictor of survival. However some find it to be predictive (4, 5).

The production of monoclonal anti-PgR antibody (PgR-IcA) has enabled the development of immunocytochemical methods for studying breast cancers, using different techniques (7, 8, 10, 11).

In order to find a technique that would clearly demonstrate the receptors, several immunohistological techniques have been tried out on routinely processed paraffin wax sections. An attempt has also been made to ascertain the relationship, if any, between the grade of breast carcinoma and the incidence of progesterone receptors.

Materials and methods

The study was carried out on 36 cases of breast carcinoma. A representative section stained with haematoxylin and eosin was chosen from each case and a histological grading given. Six new sections, 5 microns thick, were cut from each formalin-fixed paraffin wax embedded block, and kept overnight in an incubator at 37C°. Biochemically proven -ve PgR and +ve PgR breast carcinoma tissue fixed and embedded in paraffin wax was obtained from Charing Cross and Westminster Medical School, London, England. They were used as -ve and +ve controls, respectively. In a preliminary study, sections from 10 cases of invasive duct carcinoma were investigated. The sections were dewaxed by placing the slides in two changes of xylene for 2 minutes each and then hydrated through graded alcohols. From each block, 2

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sections were pretreated with 20% solution of trypsin for 30 min, another 2 sections for 15 min and the other 2 were not trypsinized. The -ve and +ve controls were also treated similarly.

Endogenous peroxide activity was blocked by applying 3% hydrogen peroxide in methanol for 30 min. After rinsing for 5 min in each of three changes of phosphate buffered saline (PBS), pH 7.0, the sections were covered with one in ten dilution of goat serum for 30 minutes. This was then tipped off and the sections incubated with 3-5 drops of PgR-IcA monoclonal antibody for ten hours at 24°C. Sections were rinsed in three changes of PBS for five minutes each.

Thereafter sections were covered for 1 hour by biotinilated anti-rat IgC (Dakopatts) diluted in 1/100, in PBS. Sections were then rinsed in PBS as before and incubated for 2 hours with avidin-biotin complex (Dakopatts). After rinsing in PBS, the sites of peroxidase activity were visualised by incubating the sections in 0.05% diaminobenzidine, DAB (Sigma) and 0.01% hydrogen peroxide, with and without adding 50% solution of imidazole, for 5-15 minutes. Sections were then washed with tap water and counterstained with haematoxylin, dehydrated in alcohol and xylene, and mounted with DPX. The sections with no pretreatment with trypsin and with imidazole added to the final DAB solution gave the best results in +ve control. This method was therefore applied to the rest of the 36 breast carcinoma tissue. The staining results were assessed semi-quantitatively according to the percentage of cells stained and the intensity of the staining. A scale of 1-3 was used for each of these components. The resulting two figures were multiplied by each other and the final result was expressed as follows:

negative (- no staining)
weakly positive (+ score 1-3);
moderately positive (++ score 4-6); and
strongly positive (+++ score 6-9). Scoring was carried out independently by two observers and the differences between the two observers were resolved after re-examining under the multihead microscope.

Results

Out of 36 breast carcinomas there were 28 invasive duct carcinomas, 4 mucoid carcinomas, 2 medullary carcinomas and 2 duct carcinomas *in situ*. Out of 28 invasive duct carcinomas, 22 were grade II and 6 were grade III carcinomas.

By immunohistological methods the deep brown positive staining for progesterone receptors was mostly seen in the nuclei of tumour cells (Fig. 1). Some sections revealed very minimal light brown coloured staining in the background which was ignored. The extra nuclear staining did not interfere with the interpretation of the results, which were assessed on the basis of the nuclear staining of neoplastic cells only.

The intensity of the colour was greater in sections treated with DAB and imidazole than in the sections treated with DAB alone. Therefore positive staining was interpreted only in sections treated with DAB and imidazole.

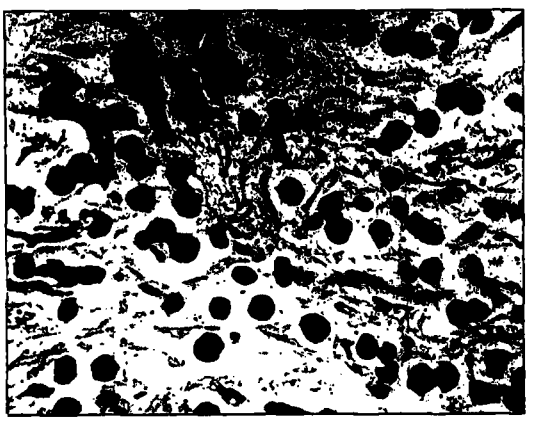

Fig. 1. Invasive duct carcinoma stained with immunohistochemical methods. Progesterone receptor positive cells show staining of the nuclei (x 400)

Strongly positive staining artefacts at the periphery of the section were not interpreted when counting positively stained cells. The invasive part of the tumour was more positive staining than the centre of the tumour which showed a more desmoplastic reaction. A few cells from the adjacent non-neoplastic breast lobules also showed positive staining.

The detailed immunohistological results are shown with their histological grade or subtype, in Table 1. The size was recorded only in 31 tumours. In both medullary carcinomas the

staining was very poor and the score was 1 in both cases.

There was also one case of grade III invasive duct carcinoma having very low receptors and the score was 1. In the other 5 invasive duct carcinomas of grade III, the score was between 2 and 4. The grade II invasive duct carcinomas had scores varying between 2 and 9. All 4 mucoid carcinomas had strongly +ve receptors (Fig. 2) and the scores were between 6 and 9. Two cases of duct carcinoma *in situ* had scores 4 and 6 (Fig. 3).

Discussion

Immunohistochemical determination of oestrogen and progesterone receptors in tissue sections using commercially available monoclonal antibodies has become a promising alternative to the cytosol steroid binding assay (6, 7, 8). The steroid receptor state of breast tumour is also very important in the management and planning of treatment of patients.

It is also preferable to have a method that can be used with paraffin wax sections, because frozen sections are seldom used routinely in some centres since the advent of fine needle aspiration cytology with limited surgical intervention. Paraffin wax sections also have the advantage of better quality and availability for retrospective studies. In the present study the method that gave the maximum intensity of positive staining was established by studying ten cases along with a positive control, namely the avidin biotin complex method on sections with no prior trypsinization and with imidazole added to the final DAB developing solution. The rest of the sections were studied using this method.

In this study +ve results of immunological staining has been compared with the grade of the tumour. The mucoid carcinomas and the

Table 1. Details of immunological results – final score

Mucoid carcinoma	Medullary carcinoma	Duct ca *in situ*	Invasive duct Grade II	Invasive duct Grade III
9	1	4	2	4
6	1	6	6	1
9			4	2
9			9	2
			6	2
			6	2
			4	
			4	
			6	
			9	
			6	
			4	
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			6	

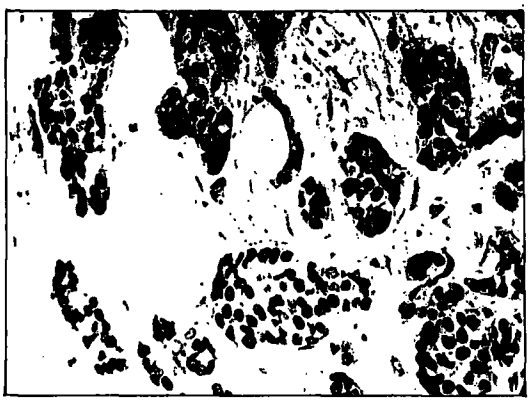

Fig. 2. Mucoid carcinoma stained with immunohistochemical methods. Progesterone receptor positive cell clusters show staining of the nuclei (x 400)

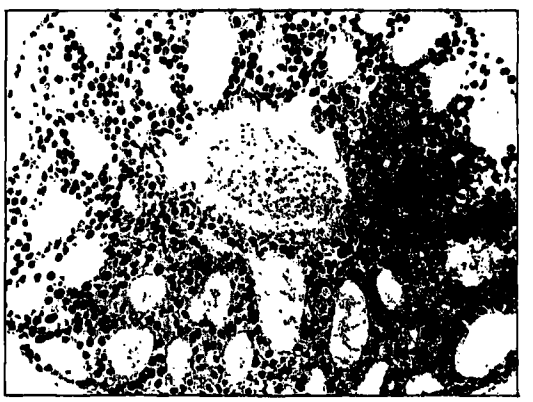

Fig. 3. Duct carcinoma *in situ* stained with immunohistochemical methods (x 200)

medullary carcinomas were not given a grade. The invasive duct carcinomas were graded as I, II, III taking into consideration the tubular formation, nuclear pleomorphism and the number of mitoses (8). The 4 mucoid carcinomas had the highest score when compared with others. The positive staining of medullary carcinomas can be attributed to its poorly differentiated histological appearance. The duct carcinoma *in situ* had only moderate staining compared with invasive duct carcinoma grade II. This is probably due to the carcinoma acquiring more steroid receptors when the tumour starts invading. The grade II invasive duct carcinomas had significantly higher ($P < 0.001$) levels of progesterone receptors than grade III invasive duct carcinomas.

It is concluded that the immunohistological method described here is reliable for the demonstration of progesterone receptors in routinely processed paraffin wax sections of breast tissue. Further the better differentiated invasive carcinoma have higher percentage of progesterone receptors than the poorly differentiated (grade III) carcinomas.

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