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CASE REPORT

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Demonstration of polyclonal origin of giant fibroadenoma of the breast

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Abstract We have shown that fibroadenoma of the breast is polyclonal and that phyllodes tumour is monoclonal in origin. It is not known whether a giant fibroadenoma which is histologically identical to the more usual type of fibroadenoma but grows to be a huge mass, like a phyllodes tumour, is polyclonal or monoclona. Clonal analysis was conducted on the DNA samples extracted from the paraffin sections of a giant fibroadenoma resected from a 21-year-old woman. The method used was based on trinucleotide repeat polymorphism of the X-chromosome-linked androgen receptor gene and on random inactivation of the gene by methylation. Clonal analysis showed that the giant fibroadenoma and the adjacent normal breast tissue are polyclonal in origin. Although the term giant fibroadenoma has often been used interchangeably with the term benign phyllodes tumour, because of their similarity in clinical appearance, our present results demonstrate that a giant fibroadenoma is a polyclonal fibroadenoma that has attained an immense size and is different from the monoclonal phyllodes tumour.

Key words Giant fibroadenoma · Phyllodes tumor · Clonality

Introduction

Both fibroadenoma and phyllodes tumour present as well-demarcated masses in the breast, and histologically the presence of both epithelial and stromal components is common to both. In spite of histological similarity, the clinical course of these two tumours is quite different. Fibroadenoma is benign and often ceases to grow after reaching 2–3 cm in diameter, a considerable number of fibroadenomas are reported to regress spontaneously [4]. Phyllodes tumour grows to be a huge mass measuring more than 10 cm in diameter, and some have malignant potential with metastasis to the lungs [5].

Clonal analysis provides valuable information on the histogenesis of a lesion and is extremely useful for differentiating a neoplastic (monoclonal) from a hyperplastic (polyclonal) lesion [2]. To elucidate the distinct difference in clinical course between fibroadenoma and phyllodes tumour, we conducted clonal analysis of these two tumours, using the method based on restriction fragment length polymorphism of the X-chromosome-linked phosphoglycerokinase (PGK) gene and on its random inactivation [7]. We found that fibroadenoma was composed of polyclonal epithelial and stromal components, indicating that fibroadenoma is not a neoplasm but an example of hyperplasia of a lobule. Phyllodes tumour, however, has been found to be composed of a polyclonal epithelial component and a monoclonal stromal component, indicating that it is a neoplasm of stromal cells. These observations appear to be consistent with the clinical behaviour of these tumours.

Occasionally, fibroadenomas grow into huge tumours, which are then called giant fibroadenomas. Although the clinical appearance of a giant fibroadenoma is similar to that of a phyllodes tumour, this disease is histologically identical to the usual type of fibroadenoma and, unlike phyllodes tumour, it behaves as a benign lesion and never metastasizes [4, 5]. It seems to be of interest to study clonality of this rare disease, to elucidate whether it is a neoplasm or an example of hyperplasia. In this paper, results of clonal analysis of giant fibroadenoma are presented.

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Case report

A 21-year-old woman developed a well-demarcated tumour measuring 12 cm×12 cm in the left breast. This was diagnosed clinically as phyllodes tumour or giant fibroadenoma (Fig. 1). Following surgery, the specimen was fixed in 3.7% buffered formalin and

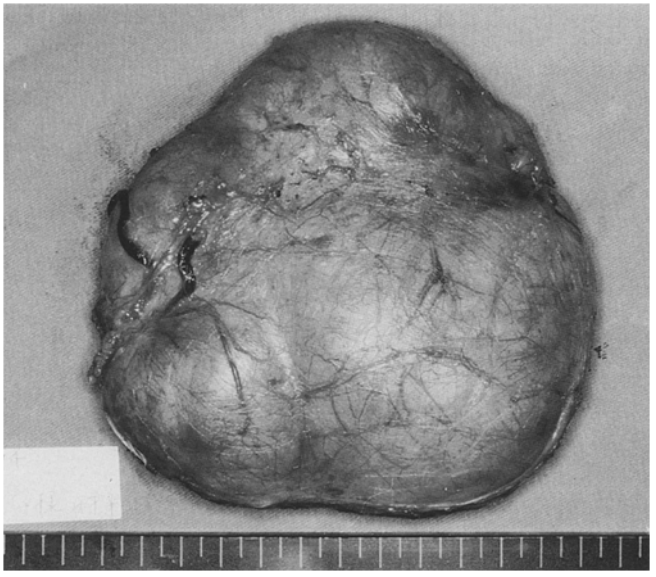

Fig. 1 The gross appearance of giant fibroadenoma (12 cm ×12 cm)

embedded in paraffin. Histological examination revealed that the tumour was a giant fibroadenoma with no characteristics of phyllodes tumour such as leaf-like projection of the stroma and hypercellular stroma. The patient is well and has no evidence of the disease 20 months after surgery.

Materials and methods

DNA extgraction

In addition to giant fibroadenoma mentioned above, a typical fibroadenoma of an ordinary size (2×2 cm) obtained from a 24-year-old woman and a benign phyllodes tumour (6×4 cm) from a 42-year-old woman were also analysed in the present study.

Since we have previously shown that the difference in clonality between fibroadenoma and phyllodes tumour lies in the stromal component, DNA samples were extracted from the stromal component of each of these tumours after microdissection from ten 10-μm paraffin sections under a dissection microscope, with a reference haematoxylin and eosin section [7]. Special attention was paid to selective collection of the stromal component without contamination by normal breast tissue, inflammatory cells and blood. DNA samples were also extracted from the adjacent normal breast tissue. The samples were each finally resuspended in 20 μl of water and divided into halves. One half of each was digested with Hpa II in a 20 μl reaction mixture at 37° C for 24 h, and the other half was left undigested.

Polymerase chain reaction

Since the clonal analysis method using the PGK gene is not applicable to the fragmented DNA samples extracted from the formalin-fixed and paraffin-embedded sections, another method using the androgen receptor (AR) gene was used in the present study [6]. Primers for the polymerase chain reaction (PCR) were designed to amplify the region of the AR gene containing trinucleotide repeats and Hpa II restriction sites [1] as shown in (Fig. 2A). In females heterozygous for the trinucleotide repeat polymorphism of the AR gene, a somatic cell contains a longer allele (Xa in Fig. 2A) and a shorter allele (Xb in Fig. 2A) of the AR gene because of the difference in the number of trinucleotide repeats. The length of the PCR products was around 220 bp.

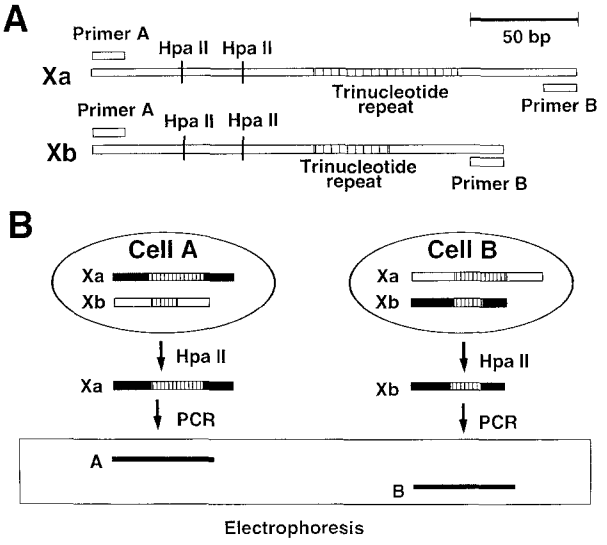

Fig. 2 **A** Restriction map of the androgen receptor gene and primers for PCR. Primers for PCR were designed in order to amplify the region containing HpaII restriction sites and trinucleotide repeats. A female somatic cell contains a longer allele (Xa) and a shorter allele (Xb) of the AR gene due to trinucleotide repeat polymorphism (*hatched bars*). Primer sequences were 5'-TCCAGA ATCTGTTTCCAGAGC for primer A and 5'-TGGGGAGAACCA TCCTCACC for primer B (*bp* base pair). **B** Strategy for clonal analysis. One of two alleles of the AR gene is randomly inactivated by methylation (*solid bars*, inactive (methylated) alleles; *open bars*, active (unmethylated alleles). Thus, female somatic cells are composed of two types of cells, i.e., cell A (inactive Xa and active Xb) and cell B (active Xa and inactive Xb). The extracted DNA samples from these cells were digested with HpaII, and then they were amplified by polymerase chain reaction (PCR) in the presence of [γ-^{32}P]ATP-labelled primers. The amplicons were analysed by means of polyacrylamide gel

Primers were labelled with [γ-^{32}P]ATP (3000 Ci/mmol) using T4 polynucleotide kinase. Two microlitres of each DNA reaction mixture (Hpa II digested or undigested) was added to an 18-μl PCR reaction mixture containing 10 pmol of each labelled primer, dATP, dGTP, dTTP, dCTP (each 200 μM), and 0.5 U Taq polymerase in standard PCR reaction buffer (Perkin Elmer Cetus, Norwalk, Conn.). Thirty-five cycles of amplification were carried out using cycling parameters of 94° C for 1 min, 64° C for 1 min, and 72° C for 1 min. Of each reaction mixture, 5 μl was added to an equal volume of formamide containing 0.1% bromophenol blue and 0.1% xylene cyanol, loaded onto 7.2 M urea-6.0% polyacrylamide gel, and electrophoresed at 60 W for 3.5 h. Gels were dried and autoradiographed for 12–48 h at room temperature using Kodak XAR film (Rochester, N.Y.).

Strategy for clonocal analysis

Clonal analysis was conducted according to the method described by Mashal et al. [6]. The strategy is shown in Fig. 2B. One of two X-chromosomes in a female somatic cell is randomly inactivated by methylation at some time early in embryogenesis. While the initial choice of which X-chromosome will be inactive in a given cell is made at random, once it is made it is stable to cell division and is therefore fixed not only for that cell, but for all its descendants. Since the AR gene is on the X-chromosome, one of the two alleles of the AR gene is randomly inactivated by methylation. Thus, somatic cells are composed of two types of cells, viz those containing inactive (methylated) Xa and active (unmethylated) Xb (cell A in Fig. 2B) and those containing active (unmethylated) Xa and inactive (methylated) Xb (cell B in Fig. 2B). In the method of

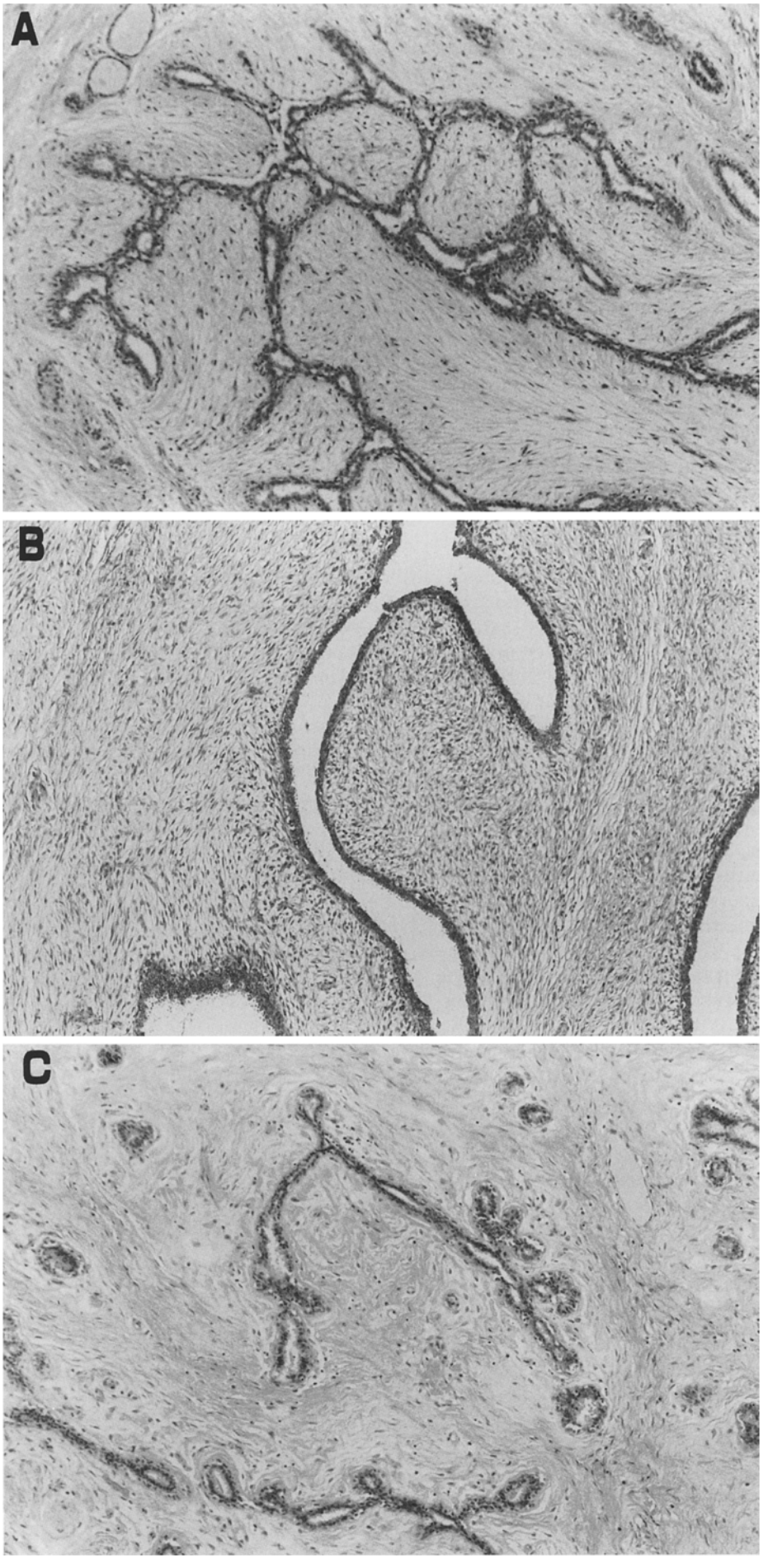

Fig. 3A–C Haematoxylin and eosin sections of **A** fibroadenoma, **B** phyllodes tumour and **C** giant fibroadenoma ×100

clonal analysis by PCR, DNA samples were first digested with a methylation-sensitive restriction enzyme, HpaII, to cleave the unmethylated, active alleles of the AR gene (open bars in Fig. 2B). The methylated, inactive alleles of the AR gene (solid bars in Fig. 2B) were preserved after HpaII digestion. If tumours are polyclonal, tumour cells should be composed of two types of cells, cell A (inactive Xa and active Xb in Fig. 2B) and cell B (active Xa and inactive Xb in Fig. 2B). Thus, both Xa and Xb were preserved after HpaII digestion. Amplification by PCR of the AR gene resulted in an amplification of Xa and Xb, giving rise to two bands in the electrophoresis (bands A and B in Fig. 2B). If tumours are monoclonal, the cells should be composed of one type of cell, either A or B. Thus, either Xa or Xb was preserved after HpaII digestion. Amplification by PCR of the AR gene resulted in an amplification of Xa or Xb, giving rise to one band in the electrophoresis (band A or B in Fig. 2B).

When DNA samples were amplified without HpaII digestion, both alleles of the AR gene were preserved. Thus, electrophoretic analysis of the amplified samples by PCR gave rise to two bands (bands A and B in Fig. 2B) regardless of the clonality of tumours.

Results

Haematoxylin and eosin sections of fibroadenoma (ordinary size), phyllodes tumour, and giant fibroadenoma are shown in Fig. 3. Leaf-like projections of hypercellular stroma were observed in phyllodes tumour (Fig. 3B). The histological appearance of giant fibroadenoma (Fig. 3C) was similar to that of fibroadenoma (Fig. 3A), and neither of these tumours had the histological characteristics observed in phyllodes tumour. The results of clonal analyses are shown in Fig. 4. Each allele of the AR gene generated a set of tightly clustered multiple bands in the autoradiogram produced from the denaturing polyacrylamide gel used to resolve the products of amplification. This phenomenon is frequently observed with PCR performed on DNA containing oligonucleotide repeats, for the reasons discussed by Mashal et al. [6]. Clonal analysis of normal breast tissue obtained from the patient with a fibroadenoma resulted in the appearance of bands corresponding to the two alleles of the AR gene in the absence of an HpaII precut, since both alleles of the AR gene were preserved (Fig. 4). Even in the presence of an HpaII precut two allelic bands appeared, indicating that normal breast tissue was polyclonal in origin. Clonal analysis of a fibroadenoma also showed a polyclonal pattern evidenced by the appearance of two allelic bands, indicating that a fibroadenoma is polyclonal in origin. However clonal analysis of the phyllodes tumour resulted in the appearance of two allelic bands in the absence of an HpaII precut but only one allelic band in the presence of this precut, indicating a monoclonal origin. These results were consistent with our previous results obtained with the method based on the PGK gene [7].

Clonal analysis of the normal breast tissue, which was obtained from the patient with a giant fibroadenoma, showed a polyclonal pattern characterized by the appearance of both alleles of the AR gene regardless the presence or absence of a HpaII precut. Clonal analysis of the giant fibroadenoma showed a polyclonal pattern (Fig. 4), indicating that this tumour is not a neoplasm but represents hyperplasia. DNA samples extracted from the other sites of this tumour were also analysed, and all of them were found to be polyclonal in origin.

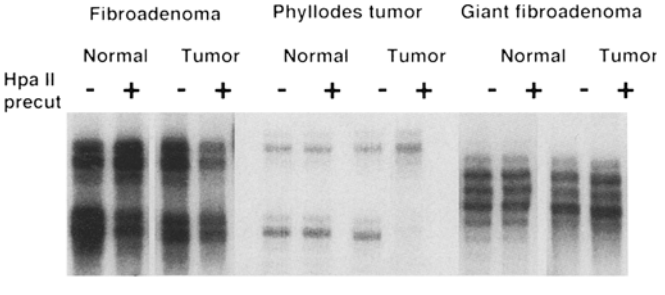

Fig. 4 Results of clonal analysis of fibroadenoma, phyllodes tumour, and giant fibroadenoma and of adjacent normal breast tissues. Clonal analysis was conducted according to the method described in "Materials and methods"

Discussion

We have shown that a giant firboadenoma is polyclonal in origin, indicating that this lesion is merely a fibroadenoma that has attained an immense size and is different from a phyllodes tumour, which is a neoplasm consisting of monoclonal stromal cells. Although the term giant fibroadenoma has often been used interchangeably with the term benign phyllodes tumour, our results have provided strong evidence that these two lesions should be considered to have different histogeneses; hyperplasia (giant fibroadenoma) vs neoplasia (phyllodes tumour).

Fletcher et al. conducted cytogenetic analysis of nine fibroadenomas and found that four of them had clonal chromosome aberrations [3]. Since these aberrations were confined to the stromal component, the authors concluded that fibroadenomas were made up of a neoplastic (monoclonal) stromal and a reactive (polyclonal) epithelial component. Their results are inconsistent with our findings that both stromal and epithelial components of fibroadenomas are polyclonal in origin [7]. Interestingly, their results are identical to our clonal analysis results for phyllodes tumours. Fibroadenoma and phyllodes tumour are often difficult to differentiate with certainty. Some of these tumours are diagnosed as fibroadenomas and others as phyllodes tumors, according to subjective criteria that are very difficult to standardize. The discrepancy between the clonal analysis results obtained by Flechter et al. and by us may be attributable, least in part, to difficulty in differential diagnosis.

The distinction between fibroadenoma and phyllodes tumour is often difficult, and we propose new criteria for the differential diagnosis. These criteria are based on the difference in clonality of the stromal component; a fibroepithelial tumour consisting of a polyclonal stromal component is fibroadenoma and one with a mono-

clonal stromal component is phyllodes tumour. This new criterion would be superior to histological criteria, as it is based on the biological features of the tumours and not just on morphological appearance and will thus be more closely related to the natural course of the disease than the histological criteria. Even in complicated cases, differential diagnosis between fibroadenoma and phyllodes tumour can be made objectively, and since the PCR-based method for clonal analysis is applicable even to a small DNA sample such as is obtained from fine needle aspirates of a tumour, preoperative differential diagnosis between fibroadenoma and phyllodes tumour will be possible. The validity of this newly proposed criterion should be assessed by a future study of a large number of patients with fibroadenomas and phyllodes tumours.

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