Brazilian Journal of Medical and Biological Research (2001) 34: 1003-1006
ISSN 0100-879X Short Communication

Cytogenetic description of breast fibroadenomas: alterations related solely to proliferation?

R.R. Burbano[1,3], E.M. Lima[3], A.S. Khayat[3], J. Barbieri Neto[2], I.R. Cabral[4], L. Bastos Jr.[6], M.O. Bahia[5] and C. Casartelli[1]

Departamentos de [1]Genética and [2]Patologia, Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto, SP, Brasil
Departamentos de [3]Biologia, [4]Genética, and [5]Patologia, Centro de Ciências Biológicas, Universidade Federal do Pará, Belém, PA, Brasil
[6]Instituto da Saúde da Mulher, Belém, PA, Brasil

Abstract

Twelve breast fibroadenomas were analyzed cytogenetically and only four were found to have clonal alterations. The presence of chromosomal alterations in fibroadenomas must be the consequence of the proliferating process and must not be related to the etiology of this type of lesion. In contrast, the few fibroadenomas that exhibit chromosomal alterations are likely to be those presenting a risk of neoplastic transformation. Clonal numerical alterations involved chromosomes 8, 18, 19, and 21. Of the chromosomal alterations found in the present study, only monosomy of chromosomes 19 and 21 has been reported in breast fibroadenomas. The loss of chromosome 21 was the most frequent alteration found in our sample. The study of benign proliferations and their comparison with chromosome alterations in their malignant counterparts ought to result in a better understanding of the genes acting on cell proliferation alone, and of the genes that cause these cells to exhibit varied behaviors such as recurrences, spontaneous regression and fast growth.

Correspondence
R.R. Burbano
Departamento de Biologia
Centro de Ciências Biológicas, UFPA
Campus Universitário do Guamá
Av. Augusto Correia, 1
66075-900 Belém, PA
Brasil
Fax: +55-91-211-1601
E-mail: rommel@ufpa.br

Research partially supported by FAPESP, CAPES, FAEPA, USP, UFPa and CNPq.

Received February 7, 2001
Accepted June 12, 2001

Key words
- Fibroadenomas
- Chromosome alterations
- Breast cancer

Fibroadenomas are the most common benign solid tumors of the female breast. Women can present with these lesions at any age, but the tumors are most commonly diagnosed when the patients are in their 20s, an age when breast cancer is extremely rare (1). Fibroadenomas are responsible for about 10% of consultations in Brazilian breast clinics (2).

Histologically, fibroadenomas are primarily stromal proliferations with compression, distortion, or atrophy of the epithelial component. Fibroadenoma stromas have the loose mucopolysaccharide-rich appearance of normal intralobular breast stromas (3). Proliferation is both epithelial and mesenchymal, but the initial neoplastic component arises from the stroma (mesenchyme) (4). Malignant changes of the epithelial component of fibroadenomas have been described in 0.02-0.3% of cases (5).

Although these lesions are common, especially among young women, little is known about the cytogenetic alterations of fibroadenomas. Few cytogenetic studies of fibroadenomas are available (4-10) and the data

remain inconclusive. With the exception of Petersson et al. (7) and Tibiletti et al. (10), the number of samples analyzed in each of these studies did not exceed 10 cases.

In the present study, 12 untreated patients were submitted to biopsy and the material was sent for tissue culture and cytogenetic analysis. The histopathological diagnosis was breast fibroadenomas in all cases. Patient age ranged from 18 to 35 years (mean = 26.5 years). All patients exhibited a single tumor and all the tumors were primary. Furthermore, there was no family history of breast cancer. Clinical follow-up of these patients showed no recurrence of the tumor to date. According to Cant et al. (11), the resolution of fibroadenomas was significantly more frequent in women aged 20 years or less than in those who were older. The mean diameter of our samples was ±2 cm, involving a favorable prognosis associated with tumor resolution (11) since fibroadenomas have a propensity to evolve to phyllode tumors. Clinical data are summarized in Table 1.

Cytogenetic study of the samples analyzed was approved by the Ethics Committee from the "Instituto da Saúde da Mulher", Belém, PA, Brazil. Fragments of surgical specimens received under sterile conditions were cut into very small fragments, treated with 0.8% collagenase IV (Sigma, St. Louis, MO, USA) and plated into sterile bottles containing HAM-F10 medium (Sigma) supplemented with 20% fetal calf serum and antibiotics. Cells were grown at 37°C for 6 to 12 days. For cytogenetic analysis, cells from primary cultures in the exponential growth phase were submitted to cell synchronization (12), collected by trypsin treatment (0.05%), treated with hypotonic 0.075 M KCl for about 20 min at 37°C, and fixed with methanol:acetic acid (3:1). Metaphases were submitted to standard Giemsa staining and banded with trypsin-Giemsa (G-banding). Chromosome abnormalities were described according to the Cancer Cytogenetics Supplement recommendations (13). Only clonal abnormalities were considered in the description of the tumor karyotype.

Table 1. Clinical data and abnormal clonal karyotypes.

Case	Age (years)	Fibroadenoma size (cm)	Obstetric history	Breast	Karyotype	Cells analyzed
1	18	1.7	No	Right	45,XX; -18[3]/45,XX; -19[3]	31
2	35	1.8	P2/B2	Right	45,XX; -21[3]	30
3	35	2.2	P4/B3	Left	45,XX; -21[7]	37
4	34	2.3	P1/B1	Right	45,XX; -8 [4]	30
5	22	1.9	P1/B1	Left	-	31
6	34	1.4	P2/B1	Left	-	28
7	22	2.0	No	Left	-	31
8	18	2.2	No	Left	-	24
9	18	2.4	P1/B1	Right	-	28
10	35	1.9	P3/B2	Left	-	23
11	18	2.1	No	Right	-	25
12	20	2.0	No	Right	-	36

The number of cells that constitute a clone is given in brackets. P, pregnancy; B, birth.

The modal chromosome number was 46 in all cases. Under GTG banding, 66 to 88% of the cells in all the fibroadenomas analyzed in the present study presented normal karyotypes (46,XX). Only four (cases 1-4) of the 12 fibroadenomas in our sample exhibited clonal chromosomal alterations (Table 1), which is consistent with the 18 to 30% frequency of cases presenting chromosomal abnormalities in previous studies of this kind (7-11). Clonal numerical alterations involved chromosomes 8, 18, 19, and 21. All these abnormalities have been previously detected in fibroadenomas.

According to Sandberg (14), the information acquired with the cytogenetics of benign tumors will be of crucial importance to the understanding of cellular events involved in neoplasia. The loss of chromosome 21 (Figure 1) was the most frequent alteration found in our sample (cases 2 and 3). This monosomy has also been described in mammary epithelial hyperplasias (15), but is not frequent in breast cancer, even though it is found in some types of leukemias (16).

No fibroadenoma-specific aberrations have emerged from the reported studies. The most frequent alterations reported in the lit-

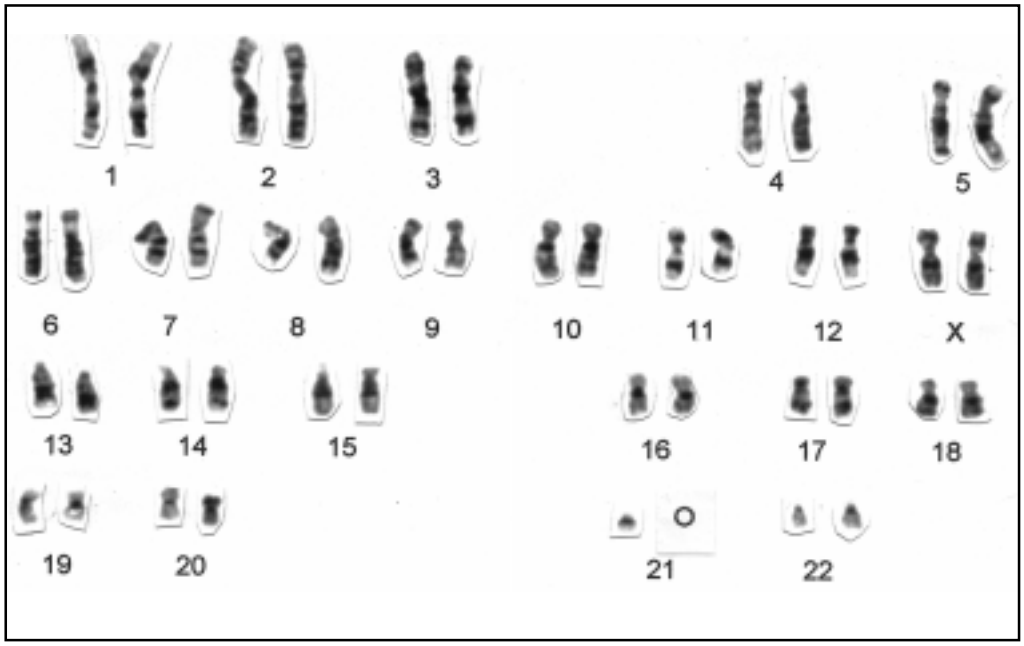

Figure 1. G-banded karyotype: 45,XX; -21 (case 2).

erature involve rearrangements of chromosomes 1, 6 and 3 (11). These abnormalities were not found in the present study, probably because fibroadenomas have not been extensively studied and the literature agrees that no consistent chromosomal alteration has been found thus far which characterizes these tumors. The presence of chromosomal alterations in fibroadenomas must be the consequence of the proliferating process and must not be related to the etiology of this type of lesion.

Other forms of genetic alterations occur in fibroadenomas, such as loss of heterozygosity of chromosome 11 and microsatellite instability, but the incidence is low (17).

All the chromosomal alterations found in the present study have been described in breast cancer, even though they are not frequent (16). This suggests that the monosomies found should not be related to malignant transformation in breast cancer. Our hypothesis is based on the fact that many fast-growing tumor cells may present random chromosomal alterations (18), and if these alterations give the cell an adaptive advantage, they may be fixed in the tissue population, further increasing the rate of proliferation. The increased rate of cell division would provide a greater opportunity for the development of variant cells, with different random numerical chromosome alterations (19). According to Wolman (20), the presence of aneuploidies in proliferating tissue may provide a basis for continuing nondisjunction.

The relationship between the presence of chromosomal aberrations and a predisposition to cancer has been well established in the so-called chromosomal instability syndromes (ataxia telangiectasia, Fanconi's anemia, and Bloom's syndrome). The alterations found in the fibroadenomas analyzed here must be related to the development of benign breast tumors, given that malignant changes of the epithelial component of the fibroadenomas are rare. On the other hand, the few fibroadenomas that exhibit chromosomal alterations are likely to be those presenting a risk of neoplastic transformation. Further research will be necessary to evaluate the usefulness of these markers as tools for the evaluation of the malignant potential of benign breast tissue.

References

1. Dupont WD, Page DL, Parl FF, Vnencak-Jones CL, Plummer Jr WD, Rados MS & Schuyler PA (1994). Long-term risk of breast cancer in women with fibroadenoma. *New England Journal of Medicine*, 331: 10-15.
2. Barros ACSD (1994). Alterações fibrocísticas mamárias. In: Halbe HW (Editor), *Tratado de Ginecologia*. 1st edn. Roca, São Paulo.
3. Belda F, Lester SC, Pinkus JL, Pinkus GS & Fletcher JA (1993). Lineage-restricted chromosome translocation in a benign fibrous tumor of the breast. *Human Pathology*, 24: 923-927.
4. Fletcher JA, Pinkus GS, Weidner N & Morton CC (1991). Lineage-restricted clonality in biphasic solid tumors. *American Journal of Pathology*, 138: 1199-1207.
5. Ozisik YY, Meloni AM, Stephenson AP, Moore GE & Sandberg AA (1994). Chromosome abnormalities in breast fibroadenomas. *Cancer Genetics and Cytogenetics*, 77: 125-128.
6. Calabrese G, Di Virgilio C, Cianchetti E, Franchi PG, Stuppia L, Parruti G, Bianchi PG & Palka G (1991). Chromosome abnormalities in breast fibroadenomas. *Genes, Chromosomes and Cancer*, 3: 202-204.
7. Petersson C, Pandis N, Rizou H, Mertens F, Dietrich CU, Adeyinka A, Idvll I, Bondeson L, Georgiu G, Ingvar C, Heim S & Mitelman F (1997). Karyotypic abnormalities in fibroadenomas of the breast. *International Journal of Cancer*, 70: 282-286.
8. Lundin C & Mertens F (1998). Cytogenetics of benign breast lesions. *Breast Cancer Research and Treatment*, 51: 1-15.
9. Stephenson CF, Davis RI, Moore GE & Sandberg AA (1992). Cytogenetics and fluorescence *in situ* hybridization analysis of breast fibroadenomas. *Cancer Genetics and Cytogenetics*, 63: 32-36.
10. Tibiletti MG, Sessa F, Bernasconi B, Cerutti R, Broggi B, Furlan D, Acquati F, Bianchi M, Russo A, Capella C & Taramelli R (2000). A large 6q deletion is a common cytogenetic alteration in fibroadenomas, pre-malignant lesions, and carcinomas of the breast. *Clinical Cancer Research*, 6: 1422-1431.
11. Cant PJ, Madden MV, Coleman MG & Dent DM (1995). Non-operative management of breast masses diagnosed as fibroadenoma. *British Journal of Surgery*, 82: 792-794.
12. Yunnis JJ (1981). New chromosome techniques in the study of human neoplasia. *Human Pathology*, 12: 540-549.
13. International System for Human Cytogenetic Nomenclature (ISCN) (1995). *Guidelines for Cancer Cytogenetics Supplement*. S. Karger, Basel, Switzerland.
14. Sandberg AA (1991). Chromosome abnormalities in human cancer and leukemia. *Mutation Research*, 247: 231-240.
15. Burbano RR, Medeiros A, Bastos Jr L, Lima EM, Mello A, Barbieri Neto J & Casartelli C (2000). Cytogenetic description of epithelial hyperplasias of the human breast. *Cancer Genetics and Cytogenetics*, 119: 62-66.
16. Mitelman F (1998). *Catalog of Chromosome Aberrations in Cancer*. 3rd edn. Wiley Liss, New York.
17. McCulloch RK, Sellner LN, Papadimitrou JM & Turbett GR (1998). The incidence of microsatellite instability and loss of heterozygosity in fibroadenoma of the breast. *Breast Cancer Research and Treatment*, 49: 165-169.
18. Seizinger BR, Klinger HP, Junien C, Nakamura Y, Le Beau M, Cavenee W, Emanuel B, Ponder B, Naylor S, Mitelman F, Louis D, Menon A, Newshan I, Decker J, Kaelbling M, Henry I & Deimling AV (1991). Report of the Committee on Chromosome and Gene Loss in Human Neoplasia. *Cytogenetics and Cell Genetics*, 58: 1080-1096.
19. Burbano RR, Barbieri Neto J, Philbert PMP, Lemos JA & Casartelli C (2000). Chromosome 4 trisomy in a case of gynaecomastia. *Cancer Genetics and Cytogenetics*, 117: 143-145.
20. Wolman SR (1986). Cytogenetic heterogeneity: its role in tumor evolution. *Cancer Genetics and Cytogenetics,* 19: 129-140.