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Metaphase and Interphase Cytogenetics in Fibroadenomas of the Breast

HELEN RIZOU[1], GEORGIA BARDI[1], MARIA ARNAOURTI[2], NIKIFOROS APOSTOLIKAS[2], KOSTAS SFIKAS[3], ANTONIOS CHARLAFTIS[4], ATHANASSIOS POLICHRONIS[4], NIKI J. AGNANTIS[5] and NIKOS PANDIS[1]

Departments of [1]Genetics and [2]Pathology, Saint Savas Anticancer Hospital, 171 Alexandras Ave, Athens; [3]Department of Pathology, Helena Venizelou Hospital, Athens; [4]Breast Unit, 6th IKA, Athens; [5]Department of Pathology, Medical School, University of Ioannina, Ioannina, Greece

Abstract. *Short-term cultures of fifty-two samples of fibroadenomas were cytogenetically analyzed. Thirty-three of the successfully karyotyped fibroadenomas were further investigated for the presence of amplifications in the CCND1, c-MYC and HER/2-neu genes by means of FISH analysis. Compared to carcinomas, fibroadenomas seem to have less complex cytogenetic rearrangements and limited alterations on HER-2/neu, CCND1 and c-MYC loci. A cytogenetic subgroup of fibroadenomas with hyperdiploid karyotypes and only numerical changes was observed. Amplification of CCND1 seems to play a more substantial role in benign tumor progression. These findings confirm that fibroadenomas do have genetic alterations and support the hypothesis that a fibroadenoma subset displays changes also found in carcinomas, thus indicating that patients belonging to this group might have an increased risk for subsequent breast cancer.*

It is well-documented that cancer is characterized by the accumulation of genetic changes. Breast carcinomas, in particular, have been linked to a number of genetic alterations, including chromosomal rearrangements (1) and amplification of certain proto-oncogenes. Some of the most common amplified genes in breast cancer are *HER-2/neu* (2), *CCND1* and *c-MYC* (2-4). Amplification of these three oncogenes occurs in about one-third of breast cancers.

Benign Breast Lesions is a heterogeneous group of lesions that mainly comprises benign breast tumors and fibrocystic disease (proliferative and non-proliferative). Some types of proliferative breast disease (*e.g.* atypical hyperplasia) have been epidemiologically associated with high risk for subsequent breast cancer development. Additionally, non-random cytogenetic aberrations and alterations in genes involved in carcinogenesis have currently been detected in these benign breast lesions (5).

Benign breast tumors include fibroadenomas, papillomas, adenomas and benign phyllodes. Among them, fibroadenomas are the most frequent and usually affect women between 20-50 years old. Fibroadenoma is a sharply circumscribed mass of fibrous and epithelial elements. Ten to 20% of fibroadenomas are multiple when they are first detected and approximately 3% of the patients have at least one fibroadenoma in each breast (6).

Traditionally, the risk for subsequent development of breast cancer in patients with typical fibroadenomas remains uncertain. Nonetheless, some data suggest there may be a 2-3 times increased risk in women with fibroadenomas (7).

Compared to malignant breast tumors and to fibrocystic disease, the cytogenetic findings in fibroadenomas have been meager, though it seems that some of the characteristics of invasive carcinomas may also be found in fibroadenomas (8). However, it remains obscure whether alterations in those proto-oncogenes (such as *HER2, MYC* and *CCND1*), that play a central role in pathogenesis of breast cancer, can also be found in fibroadenomas.

Fluorescence *In Situ* Hybridization (FISH) is one of the most popular and accurate molecular cytogenetic methods of testing alterations in proto-oncogenes. Its main advantages are that each specimen can be assessed on a cell-specific basis and that very little tissue, only a single section, is required for the hybridization procedures (9). On the other hand, classical Cytogenetics is a screening method where every chromosome of an individual cell is examined, so an overview of both balanced and unbalanced karyotypic rearrangements can be obtained (10).

In the present investigation, chromosome banding and FISH analysis were performed in fifty-two and thirty-three samples of fibroadenomas, respectively. As the transition

Correspondence to: Dr Helen Rizou, Department of Genetics, Saint Savas Hospital, 171 Alexandras Str., G-15224 Athens, Greece. Tel: +302106409168, Fax: +302106420146, e-mail: helen_rizou@yahoo.com

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Table I. Histopathological and metaphase cytogenetic data on 53 examined breast fibroadenomas.

Histopathological data	Abnormal karyotypes	Normal karyotypes
Fibroadenomas with hyperplastic epithelial phase	3(60%)	2(40%)
Fibroadenomas situated next to foci of typical hyperplasia	0(0%)	3(100%)
Fibroadenomas situated next to atypical hyperplasia, Ca *in situ*	1(100%)	0(0%)
Fibroadenoma with phyllodes progression	1(100%)	0(0%)
Fibroadenomas with no further characteristic	16(38%)	26(62%)
Total	21(40%)	31 (60%)

from normal epithelium to cancer cell still remains unknown, the investigation of the genetic relationship between fibroadenoma and breast carcinoma seems indispensable.

The objectives of the present study were not only to detect genetic changes (chromosome aberrations and alterations in certain proto-oncogenes) in the same samples of fibroadenomas, but also to compare the findings to those already obtained from breast carcinomas, in order to identify potential genetic markers indicative of the early steps of the tumorigenic proccess. More specific aims were to correlate the findings to clinicopathological data and investigate genetic alterations in multiple fibroadenomas.

Materials and Methods

Patients and tumor samples. Fifty-two fresh tumor samples of benign breast tumors were obtained by the surgeon or the pathologist and brought directly to the laboratory. All specimens were diagnosed as fibroadenomas. Some of the samples were multiple and situated either in the same breast or bilaterally.

Cytogenetic analysis. All specimens were processed for cytogenetic examination according to the technique described by Pandis *et al.* (11) and modified by Dietrich *et al.* (12). Briefly, the samples were mechanically minced by scissors and enzymatically disaggregated by collagenase and hyaluronidase for 6-12 hours. The cell suspension was then rinsed and transferred to plastic culture flasks. The cultures were inspected daily and the medium was changed every second day. After 3-6 days, the cells were harvested and the chromosomes were banded with the use of Wright's stain. In the subsequent cytogenetic analysis, all the available metaphases were analysed. The clonality criteria and the description of karyotypes followed the recommendation of ISCN (1995) (13).

FISH analysis. Thirty-three of the samples, that had been successfully analyzed by G-banding, were further investigated for the presence of gene alterations on *HER-2/neu*, *CCND1* and *c-MYC* loci by means of FISH analysis. Archival formalin-fixed, paraffin-embedded biopsy blocks from each specimen were selected by a pathologist. Sections of 4 μm were cut from the selected blocks and applied to silinized slides. Different slides were prepared for each locus. Additional serial sections from the representative blocks were stained by hematoxylin-eosin in order to confirm the presence of tumor cells and to choose the appropriate area for the hybridization procedures. Three sections from normal breast tissue and two sections from breast carcinomas, that had previously been found amplified on the *HER-2/neu* locus, were used as negative and positive controls, respectively.

All slides were dried at 37˚C overnight, baked at 60˚C for one hour, deparaffinized in three changes of fresh xylene for 10 minutes, dehydrated in 100% ethanol solutions (twice for 5 minutes each) and allowed to air-dry before application of the pretreatment kit (Vysis, France), according to the manufacturer's instructions.

For hybridization procedures, the following FISH probes were used: 1) LSI HER-2 Spectrum orange/CEP 17 spectrum green (commercially known as Path Vysion) ready for use in hybridization buffer; 2) LSI Cyclin D1 spectrum orange/CEP 11 Spectrum green (prepared by mixture of 1 μl probe+2 μl distilled water+7 μl hybridization buffer); 3) LSI *c-MYC* spectrum orange (prepared by mixture of 1 μl probe+2 μl distilled water+7 μl hybridization buffer). All probes were obtained from Vysis (France). Ten μl of the hybridization mixtures were applied onto the areas of interest on the slides. Target areas were, afterwards, covered with glass coverslips and sealed with rubber cement. The sections and the probes were simultaneously denaturated at 85˚C for 5 minutes. Hybridization was carried out overnight at 37˚C in a moist chamber. Two post-hybridization washes were performed in 2x SSC/0.3%NP40 as follows: the first at room temperature for 2 minutes and the second at 72˚C for 4 minutes (*HER-2, CCND1*) and for 2 minutes (*c-MYC*). Slides were air-dried and counterstained using 10 μl DAPI. The prepared slides were stored in the dark at 4˚C until analysis occurred 24-48 hours later.

Hybridization signals were counted by the use of a Zeiss Axioplan fluorescence microscope equipped with the appropriate filter combination and the ISIS digital imaging system and software (Metasystems, Germany). FISH was considered successful if evaluation of the tissue section met the criteria proposed by Pauletti (9). Hybridization signals from at least 60 nuclei were counted at magnification x 1,000. Nuclei partially or totally overlapping were not scored. A sample was determined to carry an amplification on the *HER-2/neu* or *CCND1* loci if the total number of gene probe signals divided by the total number of centromere signals in all the examined nuclei was >2. A case was considered amplified on the *c-MYC* locus if signal gain (*i.e.*, >4) was observed in more than 10% of its examined nuclei.

Results

The histopathological data of all the examined fibroadenomas are listed in Table I, whereas the fibroadenomas that displayed abnormalities in mitotic or interphase cells are summarized in Table II.

Cytogenetic findings. Three out of 5 fibroadenomas, that displayed a hyperplastic epithelial phase, exhibited

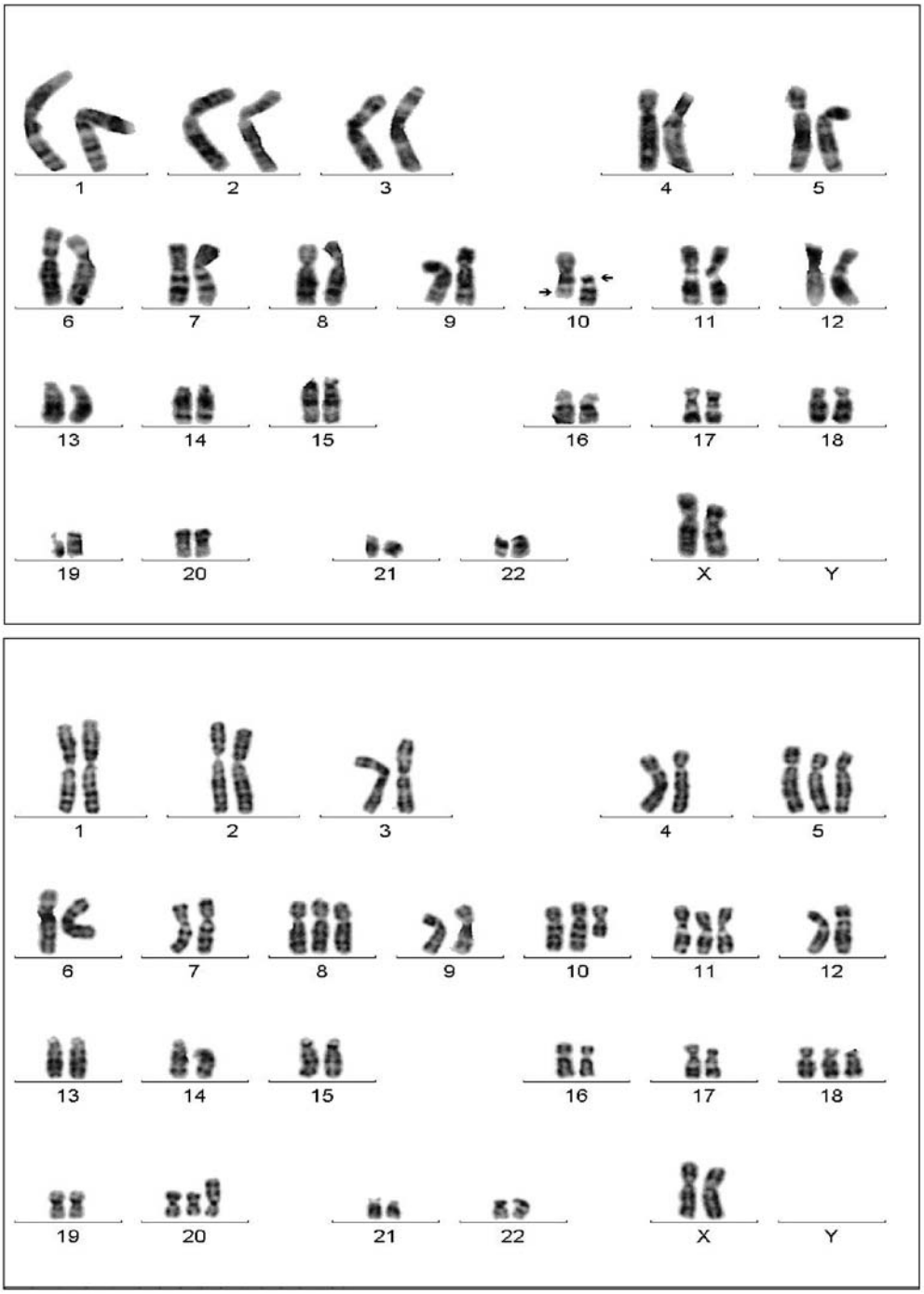

Figure 1. *Representative karyograms of cases: a) 252/98 with unbalanced rearrangements on both chromosomes 10 (up) and b) 95/97 with hyperdiploidy of certain chromosomes and a balanced rearrangement between the chromosome arms 10q and 20p (down).*

abnormal karyotypes. Three more samples, that were situated adjacently to typical hyperplasias, were found cytogenetically normal, whereas the only sample that was found cytogenetically abnormal was situated next to an atypical hyperplasia and to a carcinoma *in situ*. One more abnormal case displayed a progression to phyllodes tumor and the remaining 16 out of 42 were fibroadenomas with no further characteristics. Totally, clonal chromosome changes were found in 21 (40%) of the examined fibroadenomas. The remaining 31 samples displayed normal karyotypes since only non-clonal chromosome aberrations were observed (Table I).

Table II. *Metaphase and interphase cytogenetic results of all the abnormal cases.*

CASE No	KARYOTYPE	*ERBB2*	*CCND1*	*c-MYC*
A. Fibroaedenomas with no further characteristic				
1. 082/96	**46,XX,del(3)(p12p21)inv(3)(p25p21)[24]/46,XX[4]**	not done	not done	not done
2. 067/97	46,XX [60]	normal	**extra signals**	normal
3. 069/97	**42-45,XX,-3,-16 [cp 5]/46,XX[50]**	not done	not done	not done
4. 094/97	**46,XX,t(4;12)(p16;q15)[23]/46,XX [112]**	normal	**extra signals**	normal
5. 095/97	**52,XX,+5,+8,+10,+11,+18,+20[33]/ 52,XX,idemt(10;20)(q23;p12)[19]/46,XX[12]**	normal	normal	normal
6. 141/97	**46,XX,t(1;4)(q23;p15.1)[26]/46,XX[165]**	**extra signals**	normal	normal
7. 156/97	**46,XX,del(12)(q22) [2]/46,XX[60]**	not informative	not informative	not informative
8. 141/98	**47,XX,+20[26]/48,XX,idem+5[10]/46,XX[25]**	normal	normal	not informative
9. 229/98	46,XX[97]	**extra signals**	normal	normal
10.249/98	**46,XX,tas(15;22)(p11.2;p11.2)[15] /46,XX[32]**	normal	normal	normal
11.252/98	**46,XX,del(10)(q22q24),der(10)del(p11)add(1)(q11)[3]/46,XX[18]**	normal	normal	normal
12.258/98	**49,XX,+5,+11,+20 [22]/46,XX [46]**	normal	normal	normal
13.267/98	**46,XX,inv(3)(q13.2q21)[3]/46,XX[162]**	normal	**extra signals**	normal
14.279/98	**46,XX,der(7)t(7;13)(p10;q10)[3]/46,XX[132]**	normal	normal	normal
15.281/98	**46,XX,del(17)(p13)[4]/46,XX[78]**	normal	normal	normal
16.298/98	**47,XX,+11[2]/46,XX[15]**	normal	normal	normal
17.021/99	46,XX[6]	normal	**extra signals**	normal
18.073/99	**52,XX,+5,+7,+11,+18,+19,+20[13]/46,XX[30]**	normal	**extra signals**	normal
19.074/99	**41-45,XX,-8[cp4]/46,XX[38]**	normal	**extra signals**	normal
20.075/99	**43-44,XX,-8[cp4]/46,XX[52]**	normal	**amplified**	normal
21.115/99	**35-44,XX,-17[cp4]/46,XX[86]**	not done	not done	not done
B. Fibroadenomas with hyperplastic epithelial phase				
22.161/98	**46,XX,+add(1)(p22),-7[4]/46,XX[25]**	not done	not done	not done
23.244/98	**46,XX,der(?)t(?;17)(?;p11)[3]/46,XX[72]**	normal	normal	normal
C. Fibroadenoma situated next to proliferative disease with atypia and to a carcinoma in-situ				
24.127/97	**46,XX,del(1)(q31q42)[2]/46,XX,[138]**	normal	**amplified**	**amplified**
D. Fibroadenoma situated next to fibrocystic disease				
25.017/99	46,XX[69]	normal	**extra signals**	normal

All abnormal cases demonstrated simple chromosomal changes and were diploid or near diploid, except from 5 specimens that had hyperdiploid karyotypes with only numerical changes (cases 95/97, 141/98, 258/98, 298/98, 73/99) (Figure 1).

Two tumors, cases 94/97 and 141/98, had a balanced translocation as sole anomaly between chromosome arms 4p;12q and 1q;4p, respectively, whereas 2 more tumors (244/98, 279/98) displayed unbalanced translocations.

Chromosome 3 was rearranged in more than one case. In particular, in case 82/96, a derivative chromosome 3, that combined a deletion and an inversion at 3p arm, was found. Additionally a 3q inversion was shown in case 267/98.

Deletions of chromosome arms 1q, 12q, 17p were found in cases 127/97, 156/97 and 281/98, respectively, whereas case 252/98 exhibited deletions in both chromosomes 10.

Clonal loss of chromosomes 3, 7, 8, 16, 17 was shown in 5 cases (69/97, 161/98, 74/99, 75/99, 115/99). Monosomy of chromosome 8 was found in 2 samples from the same patient as a part of a composite karyotype.

Two samples (95/97, 141/98) exhibited two related clones each, as a result of clonal evolution.

Interphase cytogenetic findings. The abnormal findings of FISH analysis are shown in detail in Table II. Amplification of *CCND1* was detected in 2 (127/97, 75/99) out of 31 (6.5%) informative cases (Figure 2). Only one case (127/97) out of 26 (3.8%) was amplified on the *c-MYC* locus as well as on the *CCND1* (Figure 2), whereas amplification of *HER/2-neu* was not observed. However, extra signals (ratio for counted signals oncogene/centromere > 1.30) for *CCND1* and *HER/2-neu* were found on 7 (cases 67/97, 94/97, 267/98, 17/99, 21/9973/99, 74/99) and 2 fibroadenomas (cases 141/97, 229/98) (Figure 2), respectively, in a significant portion (>12%) of the examined nuclei.

Discussion

Excluding our results, about fifty more fibroadenomas have been reported to display clonal chromosome aberrations (14). Our cytogenetically abnormal cases demonstrated simple chromosomal rearrangements and, the majority of them, a near diploid karyotype. Compared to breast carcinomas, whose treatment followed the same technical procedure, fibroadenomas exhibited a simpler pattern of chomosome aberrations as well as a smaller number of abnormal metaphases per case. The interpretation of these results is that either we failed to receive all the abnormal metaphases due to a number of technical reasons, or that the tumor cells are proliferating at a lower rate than the normal ones.

Some of the cytogenetic aberrations that were found in the present study have also been reported by other investigators in fibroadenomas (8,15,16), as well as in breast carcinomas (17). For example, rearrangements in chromosome arms 1q, 6q, 8q, 20q that were found in the present study, and the involvement of 3p both in breast carcinomas and in fibroadenomas, have already been discussed (18,19). Band 12q15, which was involved in a translocation, harbors the *HMGIC* gene and has been repeatedly found rearranged in mesenchymal tumors including fibroadenomas (20,21).

A balanced translocation between bands 10q23; 20p12 (case 95/97) was revealed. Band 10q23, which was also deleted in case 252/98 (Figure 1), has not been found rearranged in benign breast tumors before, although rearrangements of 10q have been referred to breast carcinomas, but not as a primary abnormality (14).

Monosomy of chromosome 8 was found in two fibroadenomas of the same patient (cases 74/99,75/99). Loss of a whole copy of chromosome 8 has not been mentioned in benign breast tumors, except from a CGH study, where loss of 8q24.1-pter was reported in fibroadenomas (15). In carcinomas, monosomy of chromosome 8 appears to be more frequently detected in ductal than in lobular ones, though it is not a common finding in malignant tumors of the breast either (22). Although monosomy 8 was part of a composite karyotype, the fact that it was found twice in the same patient creates a question about its pathogenetic importance.

Five fibroadenomas (cases 95/97,141/98,258/98,298/98, 73/99) of the present study displayed numerical changes only, with chromosome number ranging from 47 to 52 chromosomes (Figure 1). The most frequently found trisomies were these of chomosomes 5,11,20,18,7,8 and 10, in decreasing order of frequency. Other investigators have reported cases of fibroadenomas with such multiple numerical extra chromosome copies (19,21,23,24). Multiple trisomies without any structural aberrations have also been reported in a subset of breast carcinomas (25). The time sequence in which the chromosome copies were obtained is difficult to define. However, case 141/98, where two related clones were detected, indicates that the acquisition of the extra chromosome 20 preceded the trisomy of chromosome 5. Extra copy of chromosome 20 is considered as an early event in breast cancer development (26,27). In total six fibroadenomas, either of the present study or referred to in the bibliography, share the following common characteristics: the age of all patients except one (case 258/98) was below 35 years old and their karyotypes displayed numerical changes only, in a substantial percentage of the examined metaphases. Other types of tumors with numerical changes only have been correlated to low malignancy grade (24). It seems that the above-mentioned fibroadenomas comprise a dinstinct cytogenetic subgroup, whose biological significance remains to be clarified due to the limited number of cases and the lack of sufficient clinicopathological data.

All our G-banded abnormal samples can be categorized into three groups according to the chromosome abnormalities exhibited a) those that had balanced rearrangements, *e.g.* translocations between two chromosomes (the involved bands in these rearrangements have been discussed above); b) those that displayed unbalanced rearrangements with loss of chromosome material or their karyotypes had less than 46 chromosomes; c) those whose karyotypes had a supernumerary number of chromosomes. Exclusively, unbalanced rearrangements were found in nine out of the sixteen abnormal fibroadenomas with no further characteristics, and in all the cytogenetically abnormal cases that had either a hyperplastic epithelium, a phyllodes progression or were situated next to a proliferative lesion with foci of atypical hyperplasia. (Table I). Whether and in what way the presence of hyperplasia is correlated to the presence of unbalanced rearrangements in benign tumors remains to be confirmed.

Interphase cytogenetic findings. As reported above, fibroadenomas and breast carcinomas share common cytogenetic features. Our FISH results indicate that there might be similarities between the two different entities at

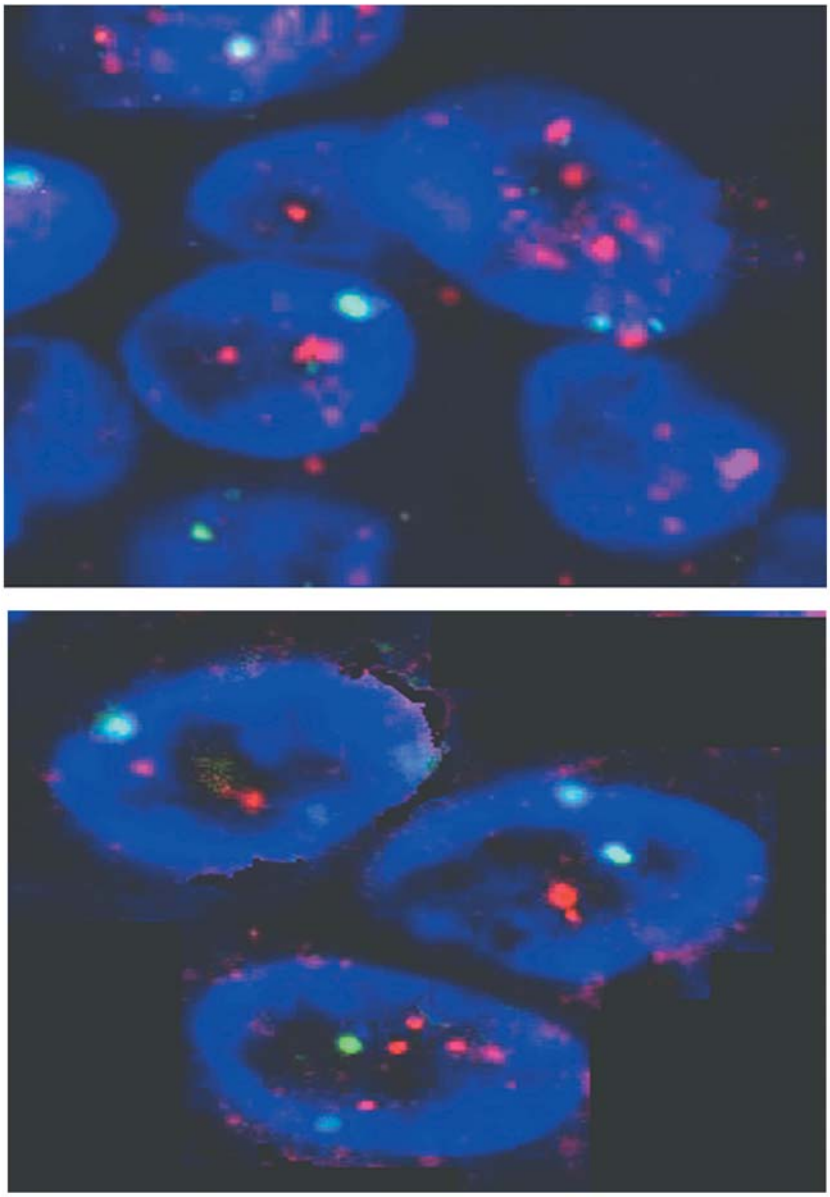

Figure 2. *Amplification on CCND1 gene of case 127/97 (up) and extra signals on the HER-2/neu locus of case 229/98 (down). CCND1, HER-2/neu probes: red spots, centromeres: green spots*

the molecular level, too. The number of molecular cytogenetic studies in fibroadenomas is still meager. They have mainly revealed numerical abnormalities by means of FISH analysis (28). With CGH analysis, the findings are controversial. Two recent reports suggest genetic imbalances (15,29), whilst a previous one does not (30). To the best of our knowledge, this is the first study that combines metaphase and interphase cytogenetic data on the same samples of fibroadenomas. Among the plethora of genes that have been studied in breast cancer, we chose to detect alterations in *HER-2/neu, CCND1* and *c-MYC*, not only because they have been frequently found altered in infiltrating and *in situ* carcinomas, but because of their role as cycle regulators. Therefore, any observed alterations in these three proto-oncogenes could be considered as early steps in the tumorigenic process.

A total of eleven out of thirty-three cases examined by FISH were found to be aberrant on at least one of the three examined loci. Amplification on the *HER-2/neu* locus was not detected in any of the informative cases, which is in accordance with results by other investigators in benign tumors (31,32). However, in two of our samples (one

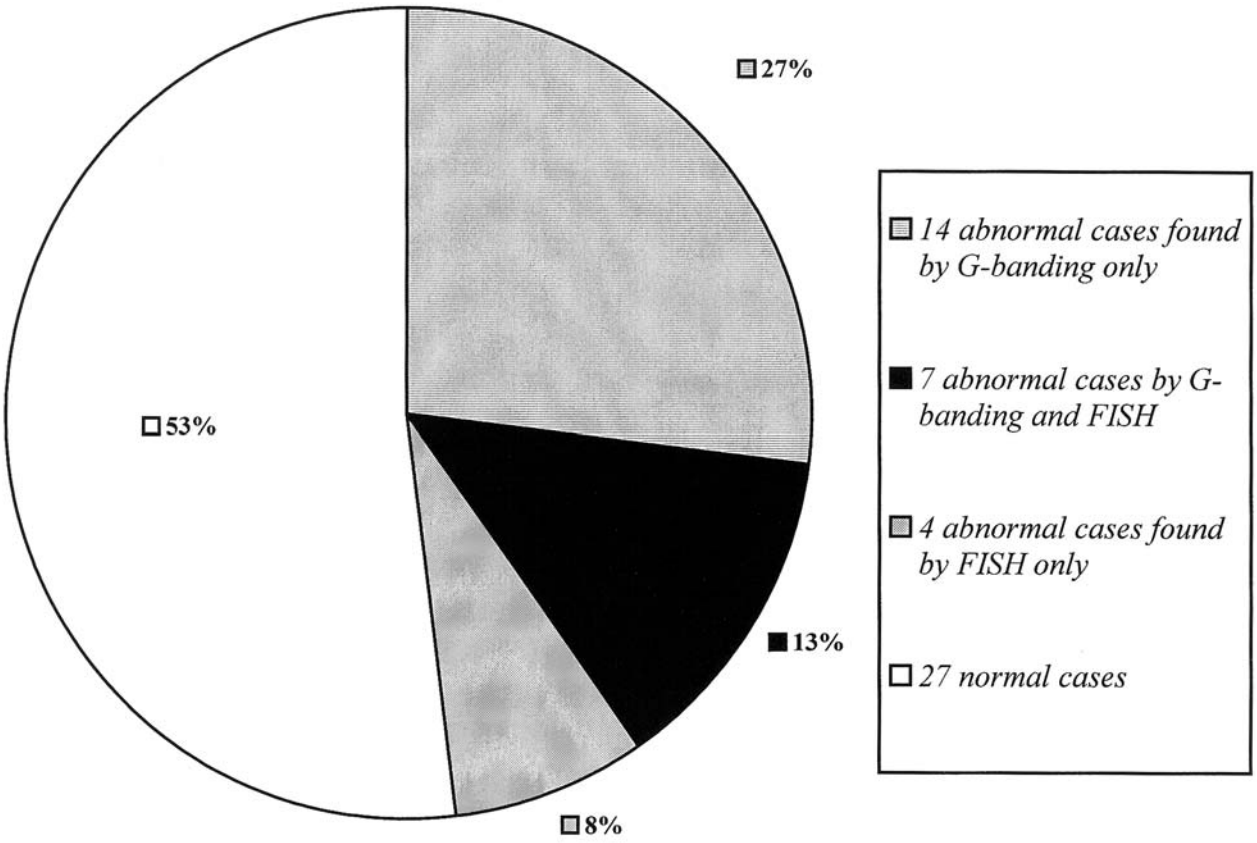

Figure 3. *Diagram of all the abnormal and normal cases. Forty-seven % of the samples displayed either metaphase, or interphase cytogenetic abnormalities, or both.*

cytogenetically normal and one abnormal) extra signals on the *HER-2/neu* locus were detected. Stark *et al.* (33) have reported *HER-2/neu* amplification in one fibroadenoma. The latter report and our data allow us to assume that low-grade amplification of *HER/2-neu* can be detected in a few fibroadenomas, at least in some of their cells.

Gene amplification on the *c-MYC* locus was detected in one cytogenetically abnormal case. Rao *et al.* (34) have reported amplification on the *c-MYC* locus in benign breast disease, whilst other studies have failed to detect any genetic alterations on this locus in benign breast tumors (35). These controversial findings can be explained by the different technical approaches that were used by different investigators.

In our study, the most frequently genetically-altered locus was *CCND1*. It was found to be amplified in two cases (one co-amplified with *c-MYC*) and to exhibit extra signals in another seven cases. Six out of the nine above-mentioned samples were cytogenetically abnormal, too. Amari *et al.* have reported genetic alterations on the *CCND1* locus in benign breast disease as well as in normal breast tissue (36). In breast carcinomas, it has also been supported that amplification of the *CCND1* gene is an earlier event than those of *ERBB2* and *MYC* (37). Therefore, the higher incidence of alterations on the *CCND1* locus compared to the other two loci may indicate that the gene of cyclin D1 has a more substantial role in benign breast tumor progression.

Combination of metaphase and interphase cytogenetic findings. Forty % of the examined cases displayed metaphase cytogenetic aberrations, whereas 33% of the FISH-treated samples demonstrated, more or less, a degree of alterations on the studied loci.

Two different and complementary reasons can explain the smaller percentage of interphase cytogenetically abnormal cases when compared to metaphase ones. Either because of technical difficulties (*e.g.* less available tissue in fibroadenomas than in carcinomas) the procedure was not applied successfully in all cases and/or genetic alterations were not detected since there were not any in these specific loci, since only three genes, among the vast majority that are involved in breast carcinogenesis, were investigated.

Fourteen fibroadenomas displayed cytogenetic aberrations only; seven samples exhibited cytogenetic abnormalities along with extra signals in interphase cells, whereas aberrations at the molecular level were detected in four more samples (Figure 3). Therefore, it seems that the

combination of different technical approaches increases the number of abnormal cases (from 40% to 47%) and permits an in-depth study of the *in vivo* situation within a tumor, not only in fibroadenomas but in carcinomas as well.

Four out of eleven cases that carried alterations at the molecular level were found normal at the chromosome level. The fact that the cytogenetic and molecular cytogenetic data are not in full concordance can be explained if we assume that molecular changes either precede the chromosomal ones or at least occur simultaneously. This remark strengthens the hypothesis that alterations at the chromosome and gene level seem to occur independently, as is also the case in breast carcinomas, thus implying the existence of different genetic mechanisms than those observed in hematological diseases (38), although we can not exclude the possibility that the results are biased due to the limited number of cases examined (39).

One fibroadenoma (case 127/97), which was situated next to proliferative disease with atypia and to an *in situ* carcinoma, was found to be co-amplified on the *CCND1* and *c-MYC* loci and also to display a cytogenetic clone with a deletion of 1q, a recurrent finding in breast carcinomas (14). In this particular case, the histological and genetic profiles seem to be in full agreement and establish the point of view that breast carcinogenesis is a process that comes from the accumulation of several genetic changes.

Multiple fibroadenomas. Some samples of the present study were multiple fibroadenomas situated in groups of two or three, either in the same breast or bilaterally. In these cases, no cytogenetically specific characteristic features were observed that can differentiate multiple fibroadenomas from single ones.

Two groups of fibroadenomas, belonging to two differrent patients, exhibited controversial results. Samples 94/97 and 95/97, that were situated in the same breast of a woman, revealed different cytogenetic abnormalities. Additionally, case 94/97 showed extra signals on the *CCND1* locus; whilst in sample 95/97, only normal signals on the three examined loci were observed. These different genetic patterns are in concordance with the hypothesis established mainly by histological observations that fibroadenomas are different breast entities. The existence of genetic heterogeneity, not only across the parenchyma-stroma borderline but also within the same tumor (different subpopulations of neoplastic cells), has already been proven in a substantial portion of breast carcinomas (40).

Unlike the above-mentioned results, both fibroadenomas of cases 74/99 and 75/99 exhibited monosomy of chromosome 8 in their karyotypes as well as extra signals in the *CCND1* locus. This genetic "homogeneity" may have two possible explanations. First, the observed similarities may be coincidental, even though the likelihood of the absence of the same chromosome, even as a part of a composite karyotype, is very low. The other possible explanation is probably related to a genetic "predisposition" of this patient's breast cells to develop a certain kind of abnormality. As has already been shown in the case of breast carcinomas, some of their secondary changes are related to the presence of certain primary abnormalities (41). Correspondingly, in the cells of fibroadenomas, the existence of the above-mentioned predisposition may have caused identical abnormalities to both tumors. Some of these abnormalities were finally revealed at the time when the cells were harvested.

The evaluation of our results suggests that benign and malignant tumors of the breast share common genetic features, although at a lower rate and in simpler patterns at the DNA and chromosome level respectively, in fibroadenomas compared to carcinomas. These findings support the hypothesis that there might be a transition from benign to malignant breast tumors, since genetic aberrations characteristic of the latter were also detected in fibroadenomas. A closer relationship between fibroadenomas and carcinomas is implied, which may, in turn, indicate that some of these benign breast lesions could be precursors of invasive carcinomas.

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