Cell cycle related proteins in hyperplasia of usual type in breast specimens of patients with and without breast cancer

Luciene SA Tafuri, Gislene FS Rocha and Helenice Gobbi*

Address: Department of Anatomic Pathology, School of Medicine, Federal University of Minas Gerais, Av. Alfredo Balena, 190, Belo Horizonte, Minas Gerais, 30150-270, Brazil

Email: Luciene SA Tafuri - lutafuri@gmail.com; Gislene FS Rocha - fatimag22@hotmail.com; Helenice Gobbi* - hgobbi@medicina.ufmg.br

* Corresponding author

Abstract

Background: Hyperplasia of usual type (HUT) is a common proliferative lesion associated with a slight elevated risk for subsequent development of breast cancer. Cell cycle-related proteins would be helpful to determine the putative role of these markers in the process of mammary carcinogenesis. The aim of this study was to analyze the expression of cell cycle related proteins in HUT of breast specimens of patients with and without breast cancer, and compare this expression with areas of invasive carcinomas.

Results: Immunohistochemical evaluation was performed using antibodies against cell cycle related proteins ER, PR, p53, p21, p63, and Ki-67 in hyperplasia of usual type (HUT) in specimens of aesthetic reduction mammaplasty (ARM), in specimens of mammaplasty contralateral to breast cancer (MCC), and in specimens of invasive mammary carcinomas (IMC) presenting HUT in the adjacent parenchyma. The results showed that the immunoexpression of ER, PR, p21, p53, p63, and KI-67 was similar in HUT from the three different groups. The p63 expression in myoepithelial cells showed discontinuous pattern in the majority of HUT, different from continuous expression in normal lobules. Nuclear expression of p53 and p21 was frequently higher expressed in IMC and very rare in HUT. We also found cytoplasmic expression of p21 in epithelial cells of hyperplastic foci and in neoplastic cells of IMC.

Conclusion: Our data failed to demonstrate different expression of cell cycle related proteins in HUT from patients with and without breast cancer. However, we found discontinuous expression of p63 in myoepithelial cells around HUT adjacent to carcinomas and cytoplasmic expression of p21 in epithelial cells of hyperplastic foci. Further studies are needed to determine how these subgroups relate to molecular abnormalities and cancer risk.
is not necessarily a direct precursor of invasive breast carcinoma but may identify individuals whose breast tissue has acquired a molecular alteration that can facilitate the eventual development of this disease [5].

The defective function of regulatory cell cycle elements, like estrogen and progesterone receptors, Ki-67, p53, p21 \textsuperscript{WAF1} and p63 leads toward increased proliferation and, in addition, expansion of genome damaged cells. Cell cycle-related markers would be helpful to determine the putative role of these markers in the process of mammary carcinogenesis [6].

Many studies evaluated cell cycle-related proteins in invasive breast carcinomas, but there are few studies evaluating these proteins in HUT [7-9]. Some molecular alterations may already be present in the earliest stages of breast cancer development. Detection of these alterations may be important for understanding the pathogenesis and also for risk assessment of premalignant breast lesions.

Incidental cancers or precursor lesions are rare in specimens of cosmetic mammoplasty compared to reduction mammoplasty specimens performed for symmetry of contralateral breast in women with breast cancer undergoing mastectomy or conservative surgery. Our hypothesis is that the expression of cell cycle related proteins would be different in HUT lesions from women at higher risk of breast cancer or with breast cancer, compared to those HUT from women without breast cancer. The aim of this study is to analyze the expression pattern of cell cycle related proteins ER, PR, p53, p21 \textsuperscript{WAF1}, p63, and Ki-67 in hyperplasia of usual type (HUT) of breast specimens of patients with and without breast cancer, and compare this expression with neoplastic cells of invasive carcinomas.

**Results**

The age of patients submitted to ARM ranged from 30 to 67 years (mean 43.9 years; SD = ± 7.4 years), of patients with IMC ranged from 30 to 86 years (mean 55.7; SD = ± 13.1 years), and age of patients submitted to MCC ranged from 30 to 75 years (mean 51.6; SD = ± 12.4 years). Patients were divided according to the menopausal status into pre-menopausal patients (≤ 50 years) and post-menopausal patients (> 50 years). The mean age of patients with IMC and submitted to MCC was significantly greater (Table 2) than patients submitted to ARM (p < 0.005). There was no statistically significant difference between mean age of patients from IMC and MCC groups.

The histologic review showed that HUT was associated with other benign breast lesions in the majority of the cases. Histologic findings were varied in ARM, MCC, and IMC specimens. Usually, the strongest ER staining was noted at the periphery of the hyperplastic foci (Figure 1A). The majority of the epithelial cells of HUT in all specimens showed positive staining for ER, PR, and Ki-67 (Figure 1; Table 3). The ER immunostaining was localized in the nuclei and showed some variability in intensity even in individual lesions of the same case.

The p63 expression was detected in the majority of the myoepithelial cell nuclei in normal lobules and in HUT. p63-positive cells around HUT foci occurred as a discontinuous layer in 38.1% in ARM, in 73.3% in MCC, and in 64.7% of myoepithelial cells surrounding HUT adjacent to IMC (Figure 2C and 2D). The p63 expression was continuous in myoepithelial cells of normal lobules and ducts. There was no difference in the percentage of positive cells for ER, PR, p21 \textsuperscript{WAF1}, p53, p63, and Ki-67 in HUT of ARM, MCC and IMC (p > 0.05). The mean percentage of ER+, PR+, Ki-67+ in epithelial cells and, p63+ in myoepithelial cells of HUT from all groups was significantly higher than positivity in neoplastic cell of IMC (Table 3).
Usually, neoplastic cells of IMC showed intermediate to high proliferative index of Ki-67 positivity (73.5%) compared to positive cells of HUT from ARM specimens (5.9%), MCC specimens (13.3%) and HUT-IMC (14.7%), (Table 3). No significant difference was observed between Ki-67 expression in cells of HUT-ARM and HUT-IMC. All proliferative cells of HUT of the three different groups were negative for p53.

The p21 expression in IMC was predominantly nuclear (55.9%). Cytoplasmatic staining was seen in neoplastic cells in 23.5% of cases (Figure 2F). Nuclear staining was detected in cells of HUT-MCC in 2 cases (5.9%; Figure 1D) and 6 cases showed cytoplasmatic staining in hyperplastic cells (Figure 2E).

**Discussion**

The aim of this study was to determine alterations in the expression of proteins involved in proliferation and cell cycle in HUT cells of patients with and without invasive breast cancer. Our analysis showed no difference in the cell cycle related proteins immunoexpression in HUT from the three different groups, in spite of age and menopausal status. Similar results were obtained by Gobbi et al. (2005) evaluating ER expression in usual hyperplasia without atypia of patients who developed breast cancer compared with patients who did not.

In our study, we found that ER and PR immunoexpression was significantly higher in HUT cells than in neoplastic cells of IMC specimens. Even in all 16 cases of ER-negative
tumors the epithelial cells of HUT were positive for ER. Our results are in agreement with other investigators who found higher levels of ER expression in benign breast epithelium of patients who developed breast cancer compared to controls [7,9-11]. The presence of positive ER staining in normal lobules increases the breast cancer risk and the likelihood of progression to cancer [7]. It occurs through the increase of the rate of cell proliferation by both recruiting non-cycling cells into the cell cycle and by shortening the overall cell cycle time due to a reduction in the length of G1 phase [11].

Previous comparison between normal and precancerous breast biopsies has shown that ER expression is relatively low in normal epithelium and slightly increased in HUT [12]. Recent studies indicate that HUT is a heterogeneous entity containing subgroups identified according to the criterion of ER-α (+) proliferating cells and this fact could explain the different biologic behavior of HUT [13]. In our study, the ER positive cells were more often found at the periphery of hyperplastic lesions. Similar pattern of ER positivity was previously described by Gobbi et al(2005)[7]. It is possible that the ER+ epithelial cells at the periphery of HUT represent the most proliferative group of cells, in spite of low positivity for Ki-67 in sequential sections of the same lesion. The estrogen exposure may stimulate a clonal proliferation of some ER+ cells or may increase the chance of spontaneous mutations [9].

In normal breast, there is a negative association between expression of ER and Ki-67, indicating either that ER+ cells are non-dividing or that the receptor is down-regulated as cells enter cycle [12,14]. This important correlation breaks down in many ER+ cancers, where the receptor is often detected in proliferating cells [14]. However, cells co-expressing ER-α and Ki-67 have been found in precancerous lesions and correlate positively with the level of risk of developing breast cancer [11]. In our series, we found higher ER and Ki-67 immunoeexpression in HUT areas compared to the immunonegativity for these markers in adjacent normal lobules. Our results are similar to those described by Schmitt (1995) [11] who found the existence of a positive correlation between ER status and proliferation in hyperplastic epithelium and a progressive inversion of this relationship in lesions evolving towards malignancy. The observation of higher rates of proliferation in ER positive benign proliferative breast lesions fits with the concept of an initial hormone-dependent status in breast carcinogenesis [11]. In addition, HUT with higher expression of ER and Ki-67 could represent a subset of hyperplastic lesions with increased risk of subsequent breast cancer development.

Some previous studies suggest that at least some HUTs are clonal [9,15]. Nevertheless, it remains unclear whether the dysregulation of ER has arisen prior to clonal expansion of the HUT, since cells with apparently abnormal regulation of ER during cell division are scattered randomly throughout the HUT in a non-contiguous pattern in some cases. The variable number of ER+ cells might indicate that in HUT the dysregulation of ER expression is incomplete and may be absent in some lesions, or apparent under certain conditions [13].

We detected p53 and p21 positivity in neoplastic cells of IMC, especially in high grade carcinomas. Mutations in tumor suppressor gene TP53, which mediates G1 arrest and apoptosis leads to an increased half-life and accumulation of the p53 protein [16]. A way to investigate the functional status of TP53 is to evaluate some of its downstream effectors such as p21 gene whose product acts by blocking cyclin-dependent kinases [17]. In our study, we found a positive association between expression of p21

| Antibody | HUT-ARM n (%) | HUT-MCC n (%) | HUT-IMC n (%) | IMC n (%) |
|----------|--------------|--------------|--------------|----------|
| RE       | 33 (97.1)    | 13 (86.7)    | 32 (94.1)    | 18 (52.9) |
| RP       | 34 (100)     | 12 (80.0)    | 33 (97.1)    | 18 (52.9) |
| p53      | 0 (0)        | 0 (0)        | 0 (0)        | 19 (55.9) |
| p21      | 0 (0)        | 0 (0)        | N+ 2 (5.9)   | N+ 19 (55.9) |
| p63      | 34 (100)     | 15 (100)     | 34 (100)     | 3 (8.8)   |
| Ki-67 ** | L- 20 (58.8) | L- 4 (26.7)  | L- 22 (64.7) | L- 9 (26.5) |

* Localization of p21 staining: N- nuclear, C- cytoplasmatic
** Ki-67 expressed as: L-low, I-intermediate, and H-high proliferative index
Positive immunostaining for ER (400×) (A) and for Ki-67 (400×) (B) in nuclei of epithelial cells of HUT, note in both the marked staining of the most of the cells at the periphery of the spaces. Positive immunostaining for p63 in nuclei of myoepithelial cells of HUT occurred as a continuous layer (C) (200×); and occurred as a discontinuous layer pattern (200×)(D)in nuclei of myoepithelial cells of HUT. Positive immunostaining for p21 in cytoplasm of epithelial cells of HUT (400×) (E) and in invasive mammary carcinoma (400×) (F).
and p53 in neoplastic cells, which is in agreement with the studies of Bankfalvi et al (2000)[18] and Pelikainen et al (2003)[19].

High expression of p21 would result in decreased cell proliferation subsequent to inhibition of cyclin/CDK activity [20]. However, in our study p21 expression in neoplastic cells was related to higher proliferative index. Our results are in agreement with the theory of p53 independent pathways of p21 regulation in breast cancer [21,22]. High amounts of p21 in high proliferating cells may reflect an unsuccessful effort to halt proliferation. This may result from the presence of other cell cycle regulatory pathways, which bypass the p21 mediated cell cycle block, such as c-Myc or B-myb [17] or due to mutant non-functional forms of p21 which posses prolonged half lives [23]. In addition, p21 expression can indeed be up-regulated by epidermal growth factor receptor and transforming growth factor β1 [17] which are associated with higher tumor grade and disease progression in breast carcinoma [24,25].

In our study, cytoplasmic expression of p21 was found in 17.6% of benign hyperplastic cells and in 23.5% of neoplastic cells of IMC. Previous studies have reported exclusive nuclear localization of p21 in neoplastic cells of breast carcinomas [19,23], and in epithelial cells of HUT [8]. However, other authors reported p21 immunopositivity in the cytoplasm of breast and ovarian tumors and it was considered critical for promoting cell transformation [26,27]. There is no other data in current literature concerning cytoplasmatic p21 expression in HUT similar to our findings. It remains unclear how the elevated cytoplasmic p21 expression might contribute to tumorigenesis. One possibility is that p21 is sequestered away from the nucleus thereby preventing it from binding to nuclear cyclin/CDK complexes, thus allowing sufficient cyclin/CDK activity for cell cycle progression [28]. Alternately, relocalization of p21 to the cytoplasm may target cytoplasmic molecules such as apoptosis signal-regulating kinase 1 (ASK1) thereby promoting cell survival [29].

In our study, p63 was exclusively expressed in myoepithelial cells of normal breast lobules and ducts, partially expressed around the HUT cells, and rarely expressed in invasive breast carcinoma. We observed that p63 staining was discontinuous in 38.1% in HUT-ARM, in 73.3% in HUT-MCC, and in 64.7% in HUT-IMC. The discontinuous p63 expression pattern in HUT was different from continuous expression in normal lobules and could suggest that there is loss of p63 expression in the progression to invasive carcinoma. Our data is similar to the findings of Wang et al (2002)[30] that demonstrate non-continuous expression of p63 in usual ductal hyperplasia. P63 expression has been useful to differentiate DCIS from microinvasive and invasive carcinomas based on lack of myoepithelial cells in invasive tumors without continuous distribution [31-33]. Although p63 is the most specific marker for myoepithelial cells, limitations exist because discontinuous myoepithelial layer seen in benign lesions, such as in our study may potentially cause diagnostic problems in clinical practice [34].

Although our data and genetic studies failed to demonstrate molecular changes in HUT, that are present in columnar cell lesions, ADH cells and in neoplastic cells of DCIS and IMC [36] the argument that HUT may be an early precursor is still supported by consistent data from epidemiological studies [1-4,37,38].

**Conclusion**

Our findings and previously published data [36,37] demonstrate that the immunoprofile of HUT is different from other accepted precursor lesions, since they are composed of a mixed population of cells types with variable proportions of cell-cycle related protein expression, and some alterations could be present in the latest stages of breast cancer development.

**Methods**

We selected slides and formalin-fixed, paraffin-embedded blocks from 83 female mammary specimens examined in the Breast Pathology Laboratory of Hospital das Clínicas of Federal University of Minas Gerais received from 1996 to 2004. The specimens selected were 34 specimens of aesthetic reduction mammaplasty (ARM), 15 specimens of mammaplasty contralateral to breast cancer (MCC), and 34 specimens of invasive mammary carcinomas (IMC) presenting HUT in the adjacent parenchyma. The aesthetic reduction mammaplasty was indicated only for cosmetic reasons or for back pain related to hypertrophic breast. There was no clinic or mammography alteration in the breasts of these patients. The mammaplasties contralateral to breast cancer were indicated in order to obtain an aesthetic balance and equilibrium related to the contralateral lumpectomy or mastectomy indicated because of breast cancer. Clinical and pathological data were obtained from the Breast Pathology Laboratory and hospital files. Clinical features evaluated were age, and menopausal status. Slides were reviewed by two observers and criteria used to classify HUT were those from Page & Anderson (1987)[39] and the terminology adopted by the WHO classification [40]. We performed immunostainings using monoclonal antibodies (summarized in Table 1) and the streptavidin-biotin method (Biogenex, USA) with previous heat-induced epitope retrieval. Immunoreactivity for ER, PR, p53, p21WAF1, p63, and Ki-67 was evaluated in HUT of ARM specimens (HUT-ARM), in HUT of MCC specimens (HUT-MCC), in HUT adjacent to invasive...
mammary carcinomas (HUT-IMC), and in neoplastic cells of IMC specimens.

Only nuclear staining was considered in the evaluation of ER, PR, p53, p63, and Ki-67. For p21WAF1, both nuclear and cytoplasmatic staining were considered positive [35]. We also evaluated ER expression in normal lobules of ARM and MCC specimens, and in adjacent normal lobules of IMC. Two to four lobular units adjacent to HUT areas were evaluated in each case.

Cases were classified as ER, PR, and p53 positive when more than 10% of cells exhibited positive nuclear staining [7,41,42]. The Ki-67 labeling index was obtained by the percentage of neoplastic and HUT cells showing nuclear staining. The tumors and HUT were grouped in three categories: < 10%, low proliferative index; 10–25%, intermediate proliferative index; and > 25%, high proliferative index [43]. We considered p63 positive cases when at least 10% of myoepithelial cells exhibited positive nuclear staining [32]. Cases were classified as p21WAF1 positive when more than 2% of cells exhibited positive nuclear staining [35].

**Abbreviations**

HUT- hyperplasia of usual type
ER- estrogen receptor
PR- progesterone receptor
Ki-67- Ki-67 protein
p53- p53 protein
TP53- p53 gene
p21WAF1- protein p21
p63- protein p63
MCC- mammaplasty contralateral to breast cancer
IMC- invasive mammary carcinomas
ARM- aesthetic reduction mammaplasty
HUT-ARM- hyperplasia of usual type in aesthetic reduction mammaplasty
HUT-MCC in mammaplasty contralateral to breast cancer
HUT-IMC in invasive mammary carcinomas
WHO- world health organization

DCIS- ductal carcinoma in situ
ADH- atypical ductal hyperplasia
G1- cell cycle phase gap 1

**Authors' contributions**

LSAT: obtained the samples, carried out the histopathological and immunohistochemical analysis and wrote the first draft of manuscript. GFSR: carried out the histology and immunohistochemistry. HG: conceived and designed the study, confirmed the histopathological and immunohistochemical analysis and provided expert input for writing and supervised the study.

All authors have read and approved the final manuscript.

**Acknowledgements**

Supported by grants from Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG; CDS 560/01) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq: 520177/00-0 NV)

We thank Sandra J. Olson for revising the English manuscript.

**References**

1. Allred DC, Harvey JM, Berardo M, Clark GM: Prognostic and predictive factors in breast cancer by immunohistochemical analysis. *Mod Pathol* 1998, 11:155-68.
2. Andersen TI, Holm R, Nesland JM, Heimdal KR, Ottestad L, Borresen AL: Prognostic significance of TP53 alterations in breast carcinoma. *Br J Cancer* 1993, 68:540-8.
3. Asada M, Yamada T, Ichijo H, Della D, Miyazono K, Fukumuro K, Mizutani S: Apoptosis inhibitory activity of cytoplasmic p21 (Cip1/WAF1) in monocytic differentiation. *Embo J* 1999, 18:1233-4.
4. Bankfalvi A, Tory K, Kemper M, Breukelmann D, Cubick C, Poremba C, Fuzesi L, Lelle RJ, Bocker W: Clinical relevance of immunohistochemical expression of p53-targeted gene products mdm2, p21 and bcl-2 in breast carcinoma. *Pathol Res Pract* 2000, 196:489-501.
5. Barbaresci M, Caffo O, Dogliani C, Fina P, Marchetti A, Buttitta F, Leek R, Morelli L, Leonardi E, Bevilacqua G, et al.: p21WAF1 immunohistochemical expression in breast carcinoma: correlations with clinicopathological data, oestrogen receptor status, MIB1 expression, p53 gene and protein alterations and relapse-free survival. *Br J Cancer* 1996, 74:208-15.
6. Barbaresci M, Pecciarini L, Cangi MG, Maci E, Rizzo A, Viale G, Dogliani C: p63, a p53 homologue, is a selective nuclear marker of myoepithelial cells of the human breast. *Am J Surg Pathol* 2001, 25:1054-60.
7. Barboule N, Baldin V, S JO, Vidal S, Valette A: Increased level of p21 in human ovarian tumors is associated with increased expression of cdk2, cyclin A and PCNA. *Int J Cancer* 1998, 76:891-6.
8. Caffo O, Dogliani C, Veronese S, Bonzanini M, Marchetti A, Buttitta F, Fina P, Leek R, Morelli L, Palma PD, et al.: Prognostic value of p21(WAF1) and p53 expression in breast carcinoma: an immunohistochemical study in 261 patients with long-term follow-up. *Clin Cancer Res* 1996, 2:1391-9.
9. Clarke RB, Howell A, Potten CS, Anderson E: Dissociation between steroid receptor expression and cell proliferation in the human breast. *Cancer Res* 1997, 57:4987-91.
10. Datto MB, Li Y, Panus JF, Howe DJ, Xiong Y, Wang XF: Transforming growth factor beta induces the cyclin-dependent kinase inhibitor p21 through a p53-independent mechanism. *Proc Natl Acad Sci USA* 1995, 92:5545-9.
11. Di Como CJ, Urist MJ, Babayan M, Drobnjak M, Hedvat CV, Feldstein-Teruya J, Pohar K, Hoos A, Cordon-Cardo C: p63 expression pro-
files in human normal and tumor tissues. Clin Cancer Res 2002, 8:494-501.

12. Dupont WD, Page DL: Risk factors for breast cancer in women with proliferative breast disease. N Engl J Med 1985, 312:146-51.

13. Elston CW, Ellis IO: Pathologic prognostic factors in breast cancer. I. The value of histological grade in breast cancer: experience from a large study with long-term follow-up. C. W. Elston & I. O. Ellis. Histopathology 1991; 19: 403-410. Histopathology 2002, 41:1115.

14. Fitzgibbons PL, Page DL, Weaver D, Thor AD, Allred DC, Clark GM, Dupont WD, Page DL: Molecular and biologic markers of proliferative breast disease and the risk of breast cancer. J Pathol 2001, 193:857-9.

15. Gobbo H, Dupont WD, Parfl FF, Schuyler PA, Plummer WD, Olson SJ, Page DL: Baseline breast cancer risk associated with estrogen receptor expression in epithelial hyperplasia lacking atypia and adjacent lobular units. Int J Cancer 2005, 113:587-92.

16. Grimm SA, Rogers MA, Khurana KK, Meguid MM, Numann PJ: Iqbal M, Davies MP, Shoker BS, Jarvis C, Sibson DR, Sloane JP: Krishnamurthy S, Sneige N: Iqbal M, Davies MP, Shoker BS, Jarvis C, Sibson DR, Sloane JP: Estrogen receptor-positive proliferating cells in the normal and precancerous breast. Am J Pathol 1999, 155:181-8.

17. Gorsch SM, Memoli VA, Stukel TA, Gold LJ, Arrick BA: Immunohistochemical staining for transforming growth factor beta 1 associates with disease progression in human breast cancer. Cancer Res 1992, 52:6949-52.

18. Hartmann LC, Sellers TA, Clark GM, Dupont WD, Page DL: Multistep progression from an oestrogen-dependent growth towards an autonomous growth in breast carcinogenesis. Eur J Cancer 1995, 31A:2049-52.

19. Hentz FC: Multistep progression of an oestrogen-dependent growth towards an autonomous growth in breast carcinogenesis. Curr Opin Genet Dev 1995, 5:611-5.

20. Iqbal M: Expression of estrogen receptor in epithelial hyperplasia of breast defined by proliferation of estrogen receptor-positive cells. Cancer Res 1995, 55:3229-37.

21. Krishnamurthy S, Sneige N: Molecular and biologic markers of premalignant lesions of human breast. Adv Anat Pathol 2002, 9:185-97.

22. Lakhani SR, Collins N, Stratton MR, Sloane JP: Atypical ductal hyperplasia of the breast: clonal proliferation with loss of heterozygosity on chromosomes 16q and 17p. J Pathol 1995, 177:411-6.

23. Lakhani SR, Reis-Filho JS, Fulford L, Penault-Llorca F, van der Vijver M, Parry S, Bishop T, Benitez J, Rivas C, Bigny Y: Prediction of BRCA1 status in patients with breast cancer using estrogen receptor and basal phenotype. Clin Cancer Res 2005, 11:175-80.

24. London SJ, Connolly L, Schnitt SJ, Colditz GA: A prospective study of benign breast disease and the risk of breast cancer. JAMA 1992, 267:941-4.

25. Marshall LM, Hunter DJ, Connolly L, Schnitt SJ, Byrne C, London SJ, Colditz GA: Risk of breast cancer associated with atypical hyperplasia of lobular and ductal types. Cancer Epidemiol Biomarkers Prev 1997, 6:297-301.

26. Mello E, Alves V: Determinação da fração de proliferação celular no câncer de mama pela marcação imunoistoquímica do antígeno nuclear Ki-67: comparação quantitativa e semi-quantitativa. J Bras Patol 1999, 35:200.

27. Mommers EC, van Diest PJ, Leonhart AM, Meijer CJ, Bask JP: Expression of proliferation and apoptosis-related proteins in usual ductal hyperplasia of the breast. Hum Pathol 1998, 29:1539-45.

28. Orend G, Chiquet-Ehrismann R: Adhesion modulation by antiadhesive molecules of the extracellular matrix. Exp Cell Res 2000, 261:104-10.

29. Pellikainen MJ, Pekola TT, Ropponen KM, Kataja VV, Kellokari JK, Eskelinen MJ, Kosma VM: p21WAF1 expression in invasive breast cancer and its association with p53, AP-2, cell proliferation, and prognosis. J Clin Pathol 2003, 56:214-20.

30. Peters MG, Vidal Mdel C, Gimenez L, Mauro L, Armanasco E, Cresta C, Bal de Kier Joffe E, Puricelli L: Prognostic value of cell cycle regulator molecules in surgically resected stage I and II breast cancer. Oncol Rep 2004, 12:1143-50.

31. Reis-Filho JS, Schmitt FC: Taking advantage of basic research: p63 is a reliable myoepithelial and stem cell marker. Adv Anat Pathol 2002, 9:280-9.

32. Rey MJ, Fernandez PL, Jares P, Munoz M, Nadal A, Peiro N, Nayach J, Mallofre C, Muntane J, Campo E, et al.: p21WAF1/CIP1 is associated with cyclin D1CCND1 expression and tubular differentiation but is independent of p53 overexpression in human breast carcinoma. J Pathol 1998, 184:265-71.

33. Ribeiro-Silva A, Ramalho LN, Garcia SB, Brandao DF, Chahud F, Zucoloto S: p63 correlates with both BRCA1 and cytokertatin 5 in invasive breast carcinomas: further evidence for the pathogenesis of the basal phenotype of breast cancer. Histopathology 2005, 47:658-66.

34. Simon P, Reis-Filho JS, Gale T, Lakhani SR: Molecular evolution of breast cancer. J Pathol 2005, 205:248-54.

35. Tavassoli FA, Norris HJ: A comparison of the results of long-term follow-up for atypical intraductal hyperplasia and invasive ductal hyperplasia of the breast. Cancer 1990, 65:18-29.

36. Tavassoli FA, Schnitt S, Hoyofer F, Boecker W, Rossi J: Heywang-Kobrunner H, Monfar RF, Ellis IO, Lakhani SR: Intraductal proliferative lesions. In World Health Organization Classification of Tumors. Pathology and Genetics of Tumors of Breast Female Genital Organs Edited by: Tavassoli FA, Devilee P. Lion: IARC Press; 2003:63-71.

37. Thor AD, Liu S 2nd, Moore DH, Shi Q, Edgerton SM: p21WAF1/CIP1 expression in breast cancers: associations with p53 and outcome. Breast Cancer Res Treat 2000, 61:33-43.

38. Wang X, Mori I, Tang W, Nakamura M, Nakamura Y, Sato M, Sakurai T, Kakudo K: p63 expression in normal, hyperplastic and malignant breast tissues. Breast Cancer Res 2002, 4:R24-9.

39. Winters ZE, Leek RD, Bradburn MJ, Norbury CJ, Harris AL: Cytoplasmic p21WAF1/CIP1 expression is correlated with HER-2/new in breast cancer and is an independent predictor of prognosis. Breast Cancer Res 2003, 5:R24-9.

40. Yang A, Kagiad M, Caput D, McKeon F: The shoulders of giants: p63, p73 and the rise of p53. Trends Genet 2002, 18:90-5.