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Bcl-2 protein in normal, hyperplastic and neoplastic breast tissues. A metabolite of the putative stem-cell subpopulation of the mammary gland

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Summary. This investigation, though initially designed to examine the possible influence of the Bcl-2 protein on the node-metastasizing capacity of breast carcinomas, was amplified to study the expression of this anti-apoptotic protein in normal breast lobules and hyperplastic lesions. We examined paraffin sections of 508 breast carcinomas, stained for Bcl-2, estrogen (ER) and progesterone receptors (PgR) and epithelial membrane antigen, and occasionally for other antigens as well. Only a few cells showing a strong Bcl-2 positivity spotted the tubulo-lobular units of normal resting glands, whereas such cells were relatively numerous in atrophic lobules, and very scarce in the terminally differentiated lactating breast. Columnar and usual types of hyperplasia were exclusively, or almost exclusively, composed of Bcl-2(+), ER(+) and PgR(+) cells. The foci of carcinoma in situ and those of invasive carcinomas were respectively 83% and 66% positive for Bcl-2 in at least 25% of their cells. Even among the invasive carcinomas, Bcl-2(+) cases included 83% and 87% of the ER(+) and PgR(+) cases, respectively (p=0.0001). Though there was a statistically significant inverse relation between Bcl-2 and tumor grade (p=0.0001), no significant association was found between Bcl-2 and lymph node stage. In conclusion, we suggest that normal, hyperplastic and neoplastic breast epithelial cells expressing the anti-apoptotic protein Bcl-2 are immature cells that ought to form part of the stem-cell subpopulation, which is committed to the development and to the maintenance of the normal gland and which gives rise to hyperplastic and neoplastic disorders when its proliferation is deregulated. In ductal proliferative changes Bcl-2 assays may be useful for diagnostic but not for prognostic purposes.

Key words: Bcl-2, Hyperplasia, Carcinoma, Stem, Apoptosis

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Introduction

The bcl-2 gene (B cell leukemia/lymphoma-2) is cancer-related and the protein it expresses (Bcl-2) can be localized inside the mitochondria, the endoplasmic reticulum and the nuclear membrane of the cell (Wang et al., 2001). Bcl-2 protein is present in long-lived cells because it is involved in the regulation of cell death by blocking cell apoptosis (Larsen, 1994; Yang et al., 1999). Even though bcl-2 is linked to the t (14;18) translocation in some hematological neoplasms, it is not so in the breast (Diaz-Cano et al., 1997). Bcl-2 protein has been linked to blastic and stem cells in bone marrow (Naumovski and Cleary, 1994), soft tissues (Suster et al., 1998), and squamous and glandular epithelia (Lu et al., 1993, 1996). Bcl-2 protein is already present in the fetal mammary plaque and it later influences the specific morphogenetic behavior of mammary cells (Nathan et al., 1994; Lu et al., 1995). Bcl-2 protein expression has been reported to be present in virtually all epithelial cells of the normal breast and its hyperplastic lesions (Siziopikou et al., 1996; Mommers et al., 1998; Fraser et al., 2000). Bcl-2 protein expression in carcinomas reaches a high rate, is directly related to hormone receptors and, like these, disappears in anaplastic cases (Doglioni et al., 1994; Friedrich et al., 1995; Visscher et al., 1996; Berardo et al., 1998; Holmqvist et al., 1999; Silver and Tavassoli, 2000). While some authors (Zhang et al., 1997; Berardo et al., 1998; Buckholm et al., 2002) have found a positive influence of Bcl-2 expression on regional disease and/or survival, others have failed to find any statistically significant relationship with tumor stage (Doglioni et al., 1994), any prognostic effect (Alsabeh et al., 1996; Castiglione et al., 1999; Gonzalez-Campora et al., 2000; Villar et al., 2001) or any predictive value in breast cancer therapy (Hamilton and Piccart, 2000). With the idea of studying the probable influence of Bcl-2 protein expression on the node-metastasizing capacity of breast cancers we studied five hundred consecutive cases of breast cancer using antibodies to Bcl-2, hormone receptors and epithelial membrane antigen (EMA), among others. However,

during the course of the study the Bcl-2 protein expression in the normal and abnormal peritumoral breast tissues also awoke our interest, and after examining both the tumoral and the peritumoral breast tissue of the five hundred cases we realized that Bcl-2(+) cells might be related to the yet unveiled mammary stem-cell.

Materials and methods

We studied the results of immunohistochemical staining for Bcl-2 in 508 consecutive breast carcinomas treated by extirpation with axillary dissection between 1998 and 1999 and which were collected in the surgical pathology files of the Carlos Haya Regional University Hospital, Malaga, Spain. The mean and median ages of the patients were 54.82 and 55.00 years, respectively, and the general characteristics of the carcinomas are shown in Table 1. For immunohistochemical studies, representative paraffin blocks from formalin-fixed tissues were selected from all cases and their tissue sections were incubated with prediluted ready-to-use antibodies to Bcl-2 (clone 124), estrogen receptors (clone 1D5), progesterone receptors (clone 1A6), and EMA (clone E29, 1:10). Forty cases were incubated with antibodies to cytokeratin 18 (CK18, clone DC10), high molecular weight keratin (HMWK) for cytokeratins 1, 5, 10 and 14 (clone CK34,E12), S100 (H 0066), vimentin (clone V9), and actin (clone HHF35). All antibodies were supplied by Dako A/S, Glostrup, Denmark. Sections were mounted on Dako ChemMate slides, dried by heating at 55 ºC for 12 hours, dewaxed, rehydrated and boiled in a pressure cooker in Dako ChemMate Buffer for Retrieval for 2 minutes, followed by cooling to room temperature for 20 minutes. The staining protocol was made in a Dako TechMate 500 immunostainer using the Dako ChemMate HRP/DAB System kit. The criteria used for the histological grading of these tumors, and for evaluating the hormone receptors, EMA pattern, and lymph node staging were those reported previously (Luna-Moré et al., 2001) and each case was evaluated by two of the authors (SL-M and BW). The results of Bcl-2 immunostaining were semiquantitated according to the following scale: 1: negative or <25%; 2: 25-50%; and 3: >50%, and the required intensity of staining was that of lymphocytes. The univariate relationships of Bcl-2 with other carcinoma features were studied using the chi square test and logistic regression analysis performed with the SPSS/PC+ version 9.0 computer program.

Table 1. General features of the 508 invasive breast carcinomas studied.

	No. OF CASES (%)
Tumor size	
pT1	249 (49.0)
pT2	220 (43.3)
pT3	39 (7.7)
Tumor type	
NOS	439 (86.4)
Tubular	6 (1.2)
Papillary	6 (1.2)
Micropapillary	15 (3.0)
Mucinous	7 (1.4)
Medullary	7 (1.4)
Lobular	18 (3.5)
Others	10 (2.0)
Tumor grade	
1	103 (20.3)
2	190 (37.4)
3	215 (42.3)
ER	
Positive	335 (65.9)
Negative	173 (34.1)
PgR	
Positive	218 (42.9)
Negative	290 (57.1)
Bcl-2	
Positive	336 (66.1)
Negative	172 (33.9)
EMA	
Lineal	77 (15.2)
Cytoplasmic	390 (76.8)
Negative	41 (8.1)
Nodal stage	
A	266 (52.4)
B	147 (28.9)
C	95 (18.7)

ER: Estrogen receptors; PgR: Progesterone receptors; EMA: Epithelial membrane antigen.

Results

Normal breast tissue and hyperplastic conditions

The peritumoral breast tissue present in the majority of the slides was normal in 247 (48.6%), presented areas of columnar cell hyperplasia in 100 (19.7%), areas of hyperplasia of the usual type in 22 (4.3%), and foci of both types of hyperplasia in 100 (19.7%). Normal breast tissue showed Bcl-2(+) pyramidal and cuboid cells irregularly dispersed throughout ducts and lobules (Fig. 1). After a subjective evaluation it was seen that these cells were relatively more numerous in atrophic lobules (including microcysts) but very scarce in lactational lobules (2 cases) as well as in lobules with pseudo-lactational and clear cell changes (10 cases). In the very common apocrine metaplastic changes, cells were Bcl-2(–), ER(–) and PgR(–). Initial manifestations of hyperplasia of Bcl-2(+) cells developed in two different ways: a) by adopting a columnar cell morphology crowned with apical snouts; and b) by maintaining a polyhedric morphology whilst forming plates between mature epithelial and myoepithelial cells (Fig. 2). Once the hyperplastic process was definitely established, the columnar cell type appeared exclusively formed by Bcl-2(+) cells with apical snouts (Fig. 3). All cases of the

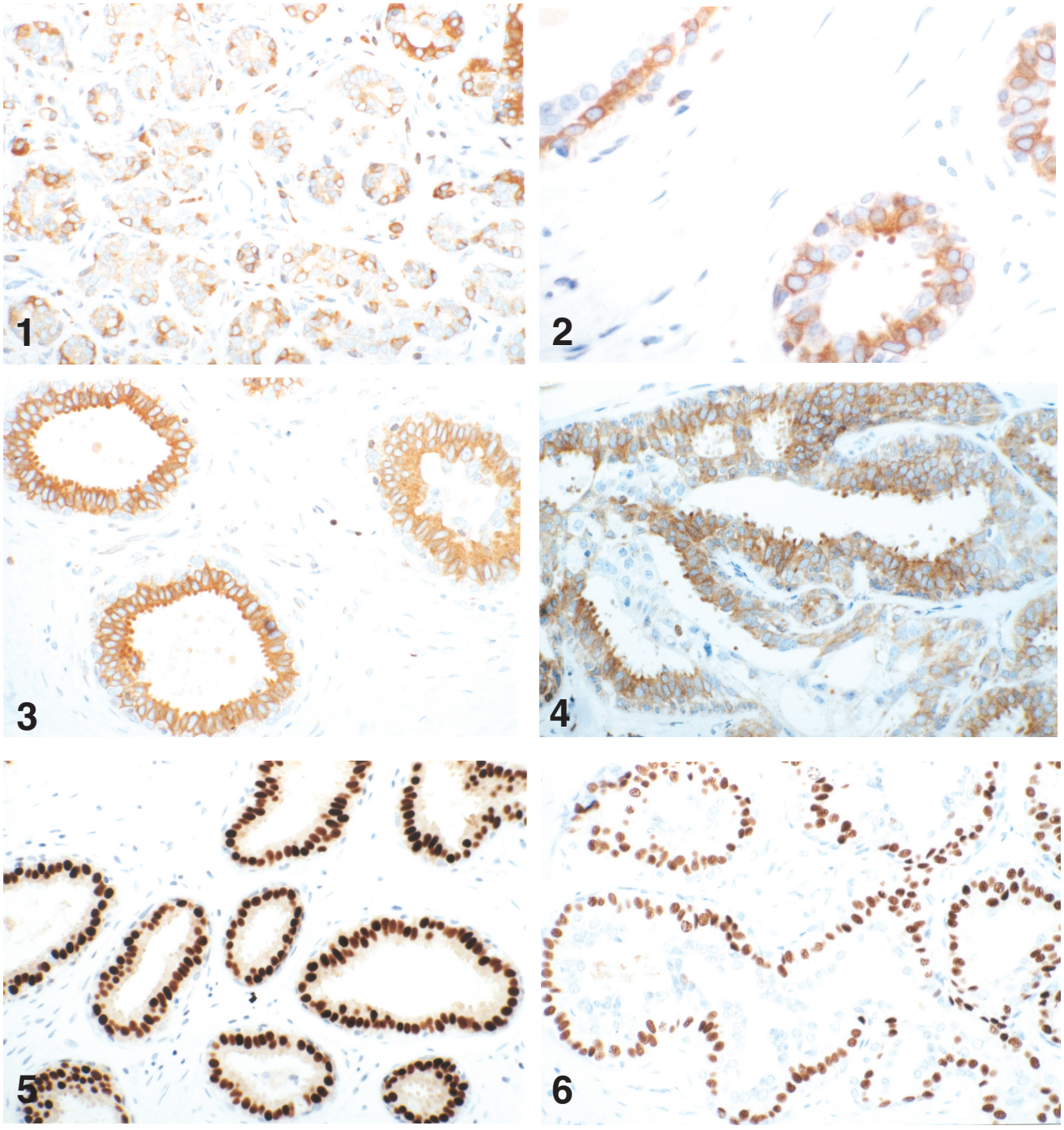

Fig. 1. Normal breast lobule rich in terminal ductules composed of two kinds of epithelial cells. Those Bcl-2(+) are pyramidal or cuboid and dispersed among the other Bcl-2(-) epithelial and myoepithelial cells. x 189

Fig. 2. Initial Bcl-2(+) cell hyperplasia showing columnar morphology with apical snouts in two terminal lobular ducts and forming a continuous layer of polygonal cells between layers of Bcl-2(-) epithelial and myoepithelial cells in the wall of a primary lobular duct. x 378

Fig. 3. Blunt duct adenosis formed almost exclusively by hyperplastic Bcl-2(+) columnar cells with apical snouts resting upon the Bcl-2(–) myoepithelial layer. x 189

Fig. 4. Ductal epithelial hyperplasia of the usual type showing columnar Bcl-2(+) cells and centrally placed polygonal Bcl-2(-)cells. x 189

Fig. 5. Columnar cell hyperplasia with general nuclear progesterone-receptor-positive staining in the columnar cells and negativity of the myoepithelial cells. x 189

Fig. 6. Ductal epithelial hyperplasia of the usual type showing nuclear estrogen-receptor positivity in the columnar cells and negativity in the centrally placed polygonal ductal epithelial cells and the peripheral myoepithelial cells. x 189

usual type of hyperplasia, whether in isolated ducts or in ducts of tumoral adenosis (3 cases), fibroadenomas (4 cases) or papillomas (4 cases), were composed of plates of Bcl-2(+) cells covered by mosaics of polygonal secretory or metaplastic squamous and apocrine cells, which were Bcl-2(–), ER(–) and PgR(–) (Fig. 4) but CK34ßE12(+) or S100(+). Be it in normal or atrophic ductules or in hyperplastic lesions, we could see the habitual coincidence in Bcl-2(+) cells of ER and PgR positivity (Figs. 5, 6), and a strong staining for CK18 in the same cells in all cases where this antibody was used. Actin faithfully stained the continuous peripheral layer of myoepithelial cells in ducts and lobules, and this layer was also easily stained with S100 and vimentin. S100 also stained the secretory cells in lactational, pseudolactational and clear changes.

Neoplastic conditions

Intraductal growth was seen in 316 out of the 508 slides stained for Bcl-2, with Bcl-2 expression being positive in 262 (82.9%) and negative in 54 (17.1%). Most of the comedo carcinomas (85.71%) were negative for Bcl-2 and also for ER and PgR. Bcl-2 protein expression in at least 25% of the neoplastic cells was present in 336 cases (66.1%) of the invasive carcinomas. Cellular Bcl-2 expression differed according to the histological tumor type (Papillary and Colloid, 100%; Tubular and Lobular 83.3%, NOS 64.9%, Medullary 28.6%), although the differences were not statistically significant. Bcl-2 protein expression was inversely related to WHO grades and each one of their parameters (Table 2). Bcl-2 also presented a statistically significant direct relation to ER and PgR and EMA pattern (p=0.0001) and Bcl-2(+) invasive breast carcinomas included 83% and 87% of the ER(+) and PgR(+) tumors, respectively (Table 2). Although there were more Bcl-2(+) carcinomas without node metastasis and relatively more Bcl-2(–) carcinomas in node stage C, there was no statistical relation at all between Bcl-2 expression and the presence or absence of axillary node metastasis (Table 2). Logistic regression analysis showed tumor size (p=0.0001) and EMA pattern (p=0.0001) to be the only factors with independent and direct influence on tumoral node stage.

Table 2. Bcl-2 protein expression in relation to other histological characteristics with reference to the statistical P value.

	Bcl-2(+) Nº (%)	Bcl-2(–) Nº (%)	P
Grade 1	90 (87.4)	13 (12.6)	
Grade 2	144 (75.8)	46 (24.2)	
Grade 3	102 (47.4)	113 (52.6)	0.0001
G1 Tub formation	45 (86.5)	7 (13.5)	
G2 Tub formation	126 (74.6)	43 (25.4)	
G3 Tub formation	164 (57.3)	122 (42.7)	0.0001
G1 Nuclear atypia	61 (87.1)	9 (12.9)	
G2 Nuclear atypia	184 (78.3)	51 (21.7)	
G3 Nuclear atypia	90 (44.6)	112 (55.4)	0.0001
G1 Mitosis	123 (81.5)	28 (18.5)	
G2 Mitosis	111 (70.3)	47 (29.7)	
G3 Mitosis	101 (51.0)	97 (49.0)	0.0001
ER positive	279 (83.3)	56 (16.7)	
ER negative	57 (32.9)	116 (67.1)	0.0001
PgR positive	190 (87.2)	28 (12.8)	
PgR negative	146 (50.3)	144 (49.7)	0.0001
EMA lineal	60 (77.9)	17 (22.1)	
EMA cytoplasmic	252 (64.6)	138 (35.4)	
EMA negative	24 (58.5)	17 (41.5)	0.044
Node stage A (0 nodes)	181 (68.0)	85 (32.0)	
Node stage B (1-3 nodes)	101 (68.7)	46 (31.3)	
Node stage C (≥ 4 nodes)	56 (58.9)	39 (41.1)	0.3422

ER: Estrogen receptors; PgR: Progesterone receptors; Tub formation: Tubular formation; EMA: Epithelial membrane antigen.

Discussion

It is common knowledge that tissue homeostasis requires a balance between proliferation, differentiation and apoptosis, and the committed cell for this function is the so-called stem, precursor, or progenitor cell. Although since the nineteenth century there has been no doubt about the necessity of the existence of such cells, there is so far no agreement among pathologists about markers for their recognition. In recent years some connection has been reported between Bcl-2 protein and stem cells in diverse organs and tissues (Neumovski and Cleary, 1994; Lu et al., 1996; Suster et al., 1998) and it seems logical that these elusive progenitor cells are endowed with apoptotic and proliferative controls. Although Bcl-2 positivity has been reported in virtually all mammary epithelial cells (Siziopikou et al., 1996; Mommers et al., 1998) others recognize cyclic variations in the number of Bcl-2(+) cells (Sabourin et al., 1994; Gompel et al., 2000). We realized that in normal resting lobules there were only a few epithelial Bcl-2(+) cells scattered among other Bcl-2(–) epithelial and myoepithelial cells and, with the limits afforded by a subjective evaluation, we suspect that the number of ductal epithelial Bcl-2(+) cells is more or less stable, because they are numerous in atrophic lobules and very scarce in lactating or pseudolactating lobules. Thus, the size variations of the lobules would depend mainly on the number of mature Bcl-2(–) cells. Accepting that Bcl-2(+) cells may form part of the stem cell subpopulation, their number in resting lobules might seem excessive unless it is recalled that breast parenchyma is terminally differentiated only during lactation. We could also confirm that Bcl-2 expression is highly increased in hyperplastic lesions (Visscher et al., 1996; Strange et al., 2001), an increase which is not restricted to the breast and has been reported in hyperplastic lesions of the skin (Nakagawa et al., 1994), stomach (Bronner et al., 1995), rectum (Garnieri et al., 2000) and endometrium (Gompel et al., 1994). According to our findings, columnar cell hyperplasia and usual ductal hyperplasia were,

exclusively in the former and almost exclusively in the latter, formed by epithelial Bcl-2(+), ER(+) and PgR(+), and strongly CK18(+) cells. It is thanks both to the occasional intermediate location of Bcl-2(+) cells and to their easily recognizable apical snouts that we did not see any need to use double staining techniques to confirm the actual coincidence of Bcl-2, ER, PgR and CK18 in the same cells. The coincidence of the columnar morphology with Bcl-2 and ER positivity had already been noted (Siziopikou et al., 1996; Mommers et al., 1998) and even these immature cells were distinguished from the mature apocrine cells because the former are Bcl-2(+) and ER(+) while the latter are Bcl-2(–) and ER(–) (Fraser et al., 2000). Independently of this, Bcl-2 protein negativity of the apocrine cells is a well known feature (Siziopikou et al., 1996; Visscher et al., 1996; Mommers et al., 1998; Fraser et al., 2000; Feuerhake et al., 2001) and this protein loss is indicative of some form of cellular maturation. Bcl-2 protein expression was seen in the majority (83%) of the intraductal foci of our carcinomas; its incidence varying according to tumor grade, as also seen by others (Siziopikou et al., 1996; Moreno et al., 2001). Bcl-2 protein expression in 25% or more of the neoplastic cells was recognized in 336 out of the 508 invasive breast carcinomas (66.1%) in this report, and we found a very close and statistically significant direct relationship between Bcl-2 positivity, low cancer grade and ER and PgR positivity (p=0.0001). Bcl-2(+) invasive tumors included 83% of the ER(+) and 87% of the PgR(+) cases but, paradoxically, no significant positive effect of the Bcl-2 expression was found on lymph node stage (Table 2). Only tumor size and EMA pattern were found to be independent predictors of the node stage, confirming our own previous findings with a lower number of cases (Luna-Moré et al., 2001).

Although this is the first time Bcl-2 has been proposed as a stem cell marker of the breast, several other markers for the stem cell of the breast have been suggested in the past. Rudland et al. (1993) believed that those cultured epithelial cells of still indeterminate type which stained for CK18 formed the stem cell population of the breast. Trask (1990) also saw that immortalized preneoplastic and cultured tumor-derived mammary epithelial cells showed decreased expression of CK5 and increased amounts of CK18. We are in total agreement with Rudland and Trask, since by using formalin-fixed and paraffin-embedded breast tissue sections we realized that tubulo-lobular Bcl2(+) cells showed a much stronger positivity for CK18 than any normal, hyperplastic or neoplastic epithelial cell. On the other hand, Bocker et al. (1992a) adopted those luminal superficial cells that stained for HMWK in ductal hyperplasia as candidates for stem cells, but we do not share this assumption, since HMWK(+) cells were Bcl-2(–), ER(–) and PgR(–) and were not anchored basally, as stem cells should be. Furthermore, since HMWK marks not only basal cells but also stratified epithelia (Schaafsma and Ramaekers, 1994), we assume that the centrally placed intraductal HMWK(+) cells are mature metaplastic rather than stem cells. This interpretation would also explain why the presence of HMWK(+) cells favors hyperplasia instead of neoplasia in difficult diagnoses of intraductal cellular proliferations (Raju et al., 1990; Bocker et al., 1992a,b; Moinfar et al., 1999; Otterbach et al., 2000). Another candidate proposed as the stem cell of the breast parenchyma was the S100(+) cell (Bassler and Katzer, 1992), but we confirmed that besides staining the myoepithelial cells, S100 also stained the secretory cells of the lactating breast and of the pseudolactational and clear cell changes in the resting breast (Viña and Wells, 1989) and consequently, it could be said that S100(+) cells are all terminally differentiated epithelial or myoepithelial cells but not stem cells. It has also been speculated (Barbareschi et al., 2001) that the breast progenitor cells are those stained for p63, but again this would be unlikely, because p63 is a selective nuclear marker of terminally differentiated myopithelial cells which do not participate in ductal hyperplasias or carcinomas. Stem-related cells must be long-lived cells (Bcl-2 effect) open to proliferative stimuli (ER and PgR effect) and therefore it is hard to accept that ER(–) cells are the breast stem cells, as was proposed by Anderson et al. (1998). ER negativity seen by them in proliferating cells could be the result of a simple masking effect after the activating binding of ER to DNA. Finally, we have no basis for evaluating whether those cultured MUC(–) and ESA(+) cells function as precursor cells (Gudjonsson et al., 2002).

In conclusion, we suggest that Bcl-2(+) cells, which are usually ER(+) PgR(+) and CK18(+), and are dispersed in normal lobules but massive in breast hyperplasias and carcinomas might represent the precursor or stem cell subpopulation already present in the fetal mammary plaque, committed to the development and thereafter to the maintenance of the gland and the first target for the development of hyperplastic and neoplastic lesions. We also think that Bcl-2 staining might be used for differential diagnostic purposes in combination with stains for hormone receptors, keratins, and S100 to distinguish the heterogeneous cellularity of hyperplasias from the clonal cellularity of intraductal carcinomas. Finally, as no relation was found between Bcl-2 positivity and lymph node stage in invasive carcinomas, we were unable to derive a prognostic value from their Bcl-2 protein expression.

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References

Alsabeh R., Wilson C.S., Ahn C.W., Vasef M.A. and Battifora H. (1996). Expression of bcl-2 by breast cancer: a possible diagnostic application. Mod. Pathol. 9, 439-444.

Anderson E., Clarke R.B. and Howell A. (1998). Estrogen responsiveness and control of normal human breast proliferation. J. Mammary Gland Biol. Neoplasia 3, 23-35.

Barbareschi M., Pecciarini L., Cangi M.G., Macri E., Viale G. and Doglioni C. (2001). P63, a p53 homologue, is a selective nuclear marker of myoepithelial cells of the human breast. Am. J. Surg. Pathol. 25, 1054-1060.

Bassler R. and Katzer B. (1992). Histopathology of myoepithelial (basocellular) hyperplasias in adenosis and epitheliosis of the breast demonstrated by the reactivity of cytokeratins and S100 protein. An analysis of heterogenic cell proliferations in 90 cases of benign and malignant breast diseases. Virchows Arch. (A) 421, 435-442.

Berardo M.D., Elledge R.M., de Moor C., Clark G.M., Osborne C.K. and Allred D.C. (1998). Bcl-2 and apoptosis in lymph node positive breast carcinoma. Cancer 82, 1296-1302.

Bocker W., Bier B., Freytag G., Brommelkamp B., Jarasch E-D., Edel G., Dockhorn-Dworniczak B. and Schmid K.W. (1992a). An immunohistochemical study of the breast using antibodies to basal and luminal keratins, alpha-smooth muscle actin, vimentin, collagen IV and laminin. Part I: Normal breast and benign proliferative lesions. Virchows Arch. (A) 421, 315-322.

Bocker W., Bier B., Freytag G., Brommelkamp B., Jarasch E-D., Edel G., Dockhorn-Dworniczak B. and Schmid K.W. (1992). An immunohistochemical study of the breast using antibodies to basal and luminal keratins, alpha-smooth muscle actin, vimentin, collagen IV and laminin. Part II: Epitheliosis and ductal carcinoma in situ. Virchows Arch. (A). 421, 323-330.

Bronner M.P., Culin C., Reed J.C. and Furth F.E. (1995). The bcl-2 proto-oncogene and the gastrointestinal epithelial tumor progression model. Am. J. Pathol. 146, 20-26

Bukholm I.R.K., Bukholm G. and Nesland J.M. (2002). Reduced expression of both Bax and Bcl-2 is independently associated with lymph node metastasis in human breast carcinomas. APMIS 110, 214-220.

Castiglione F., Sarotto I., Fontana V., Destefanis M., Venturino A., Ferro S., Cardaropoli S., Orengo M.A. and Porcile G. (1999). Bcl2, p53 and clinical outcome in a series of 138 operable breast cancer patients. Anticancer Res. 19, 4555-4563.

Diaz Cano S.J., Garcia Moliner M., Carney W. and Wolfe H.J. (1997). Bcl-2 expression and DNA fragmentation in breast carcinoma, pathologic and steroid hormone receptors correlates. Diagn. Mol. Pathol. 6, 199-208.

Doglioni C., Dei Tos A.P., Laurino L., Chiarelli C., Barbareschi M. and Viale G. (1994). The prevalence of Bcl-2 immunoreactivity in breast carcinomas and its clinicopathological correlates, with particular reference to oestrogen receptor status. Virchows Arch. 424, 47-51.

Feuerhake F., Unterberger P. and Hofter E.A. (2001). Cell turnover in apocrine metaplasia of the human mammary gland epithelium: apoptosis, proliferation, and immunohistochemical detection of Bcl-2, Bax, EGFR, and c-erbB2 gene products. Acta Histochem. 103, 53-65.

Fraser J.L., Pliss N., Connolly J.L. and Schnitt S.J. (2000). Immunophenotype of columnar alteration with prominent apical snouts and secretions (CAPSS) Abstract Annual Meeting, United States and Canadian Academy of Pathology March 25-31, New Orleans, Louisiana

Friedrich K., Dimmer V., Haroske G., Lossnitzer A., Kasper M., Theissig F. and Kunze K.D. (1995). Expression of p53 and bcl-2 in correlation to clinicopathological parameters, hormone receptor status and DNA ploidy in breast cancers. Pathol. Res. Pract. 191, 1114-1121.

Garnieri E., Nagar C., Valli C., Brunetti E., Palmieri M.B. and Vecchione A. (2000). Bcl2 and BAX expression in hyperplastic and dysplastic rectal polyps. Hepatogastroenterology 47, 159-162.

Gompel A., Sabourin J.C., Martin A., Yaneva H., Audouin J., Decroix Y. and Poitout P. (1994). Bcl-2 expression in normal endometrium during the menstrual cycle. Am. J. Pathol. 144, 1195-1202.

Gompel A., Somai S., Chaouat M., Kazem A., Kloosterboer H.J., Beusman I., Forgez P., Mimoun M. and Rostene W. (2000). Hormonal regulation of apoptosis in breast cells and tissues. Steroids 65, 593-598.

Gonzalez-Campora R., Galera-Ruiz M.R., Vazquez-Ramirez F., Rios-Martin J.J., Fernandez-Santos J.M., Ramos-Martos M.M. and Gomez-Pascual A. (2000). Apoptosis in breast cancer. Pathol. Res. Pract. 196, 167-174.

Gudjonsson T., Villadsen R., Nielsen H.L., Ronnov J.L., Bissell M.J. and Petersen O.W. (2002). Isolation, immortalization, and characterization of a human breast epithelial cell line with stem cell properties. Genes Dev. 16, 693-706.

Hamilton A. and Piccart M. (2000). The contribution of molecular markers to the prediction of response in the treatment of breast cancer: a review of the literature on HER-2, p53, and BCL-2. Ann. Oncol. 11, 647-663.

Holmqvist P., Lundstrom M. and Stal O. (1999). Apoptosis and Bcl-2 expression in relation to age, tumor characteristics and prognosis in breast cancer. South-East Sweden Breast Cancer Group. Int. J. Biol. Markers 14, 84-91.

Larsen C.J. (1994). The Bcl2 gene, prototype of a gene family that controls programed cell death (apoptosis). Ann. Genet. 37, 121-134.

Lu P.J., Lu Q.L., Rughetti A. and Taylor-Papadimitriou J. (1995). Bcl-2 overexpression inhibits cell death and promotes the morphogenesis, but not tumorigenesis of human mammary epithelial cells. J. Cell Biol. 129, 1368-1378.

Lu Q.L., Abel P., Foster C.S. and Lalani E.N. (1996). bcl-2: Role in epithelial differentiation and oncogenesis. Hum. Pathol. 27, 102-110.

Luna-Moré S., Rius F., Weil B., Jimenez A., Bautista M.D. and Perez-Mellado A. (2001). EMA: A differentiation antigen related to node metastatic capacity of breast carcinomas. Pathol. Res. Pract. 197, 419-425.

Moinfar F., Man Y.G., Lininger R.A., Bodian C. and Tavassoli FA. (1999). Use of keratin 34,E12 as an adjunct in the diagnosis of mammary intraepithelial neoplasia-ductal type –benign and malignant intraductal proliferations. Am. J. Surg. Pathol. 23, 1048-1058.

Mommers E.C.M., van Diest P.J., Leonhart A.M., Meijer C.J.L.M. and Baak J.P.A. (1998). Expression of proliferation and apoptosis-related proteins in usual ductal hyperplasia of the breast. Hum. Pathol. 29, 1539-1545.

Moreno A., Figueras A., Lloveras B., Escobeo A., Griera E., Sierra A. and Fabra A. (2001). Apoptosis in ductal carcinoma in situ of the breast. Breast J. 7, 245-248.

Nakagawa K., Yamamura K., Maeda S. and Ichihashi M. (1994). bcl-2 expression in epidermal keratinocytic diseases. Cancer 74, 1720-

1724.

Nathan B., Anbazhagan R., Clarkson P., Bartkova J. and Gusterson B. (1994). Expression of BCL-2 in the developing human fetal and infant breast. Histopathology 24, 73-76.

Naumovski L. and Cleary M.L. (1994). Bcl2 inhibits apoptosis associated with terminal differentiation of HL-60 myeloid leukemia cells. Blood 83, 2261-2267.

Otterbach F., Bankfalvi A., Bergner S., Decker T., Krech R. and Boecker W. (2000). Cytokeratin 5/6 immunohistochemistry assists the differential diagnosis of atypical proliferations of the breast. Histopathology 37, 232-240.

Raju U., Crissman J.D., Zarbo R.J. and Gottlieb C. (1990). Epitheliosis of the breast. Am. J. Surg. Pathol. 14, 939-947.

Rudland P.S. (1993). Epithelial stem cells and their possible role in the development of the normal and diseased human breast. Histol. Histopathol. 8, 385-404.

Sabourin J.C., Martin A., Baruch J., Truc J.B., Gompel A. and Poitout P. (1994). bcl-2 expression in normal breast tissue during the menstrual cycle. Int. J. Cancer 59, 1-6.

Schaafsma H.E. and Ramaekers F.C.S. (1994). Cytokeratin subtyping in normal and neoplastic epithelium: Basic principles and diagnostic approaches. In: Pathology Annual 1994 Part 1, pp 21-62. Rosen P.P. and Fechner R.E. (eds). Appleton and Lange, Norwalk, Connecticut.

Silver S.A. and Tavassoli FA. (2000). Pleomorphic carcinoma of the breast: clinicopathological analysis of 26 cases of an unusual high-grade phenotype of ductal carcinoma. Histopathology 36, 505-514.

Siziopikou K.P., Prioleau J.E., Harris J.R. and Schnitt S.J. (1996). Bcl-2 expression in the spectrum of preinvasive breast lesions. Cancer 77, 499-506.

Strange R., Metcalfe T., Thackray L. and Dang M. (2001). Apoptosis in normal and neoplastic mammary gland development. Microsc. Res. Tech. 52, 171-181.

Suster S., Fisher C. and Moran C.A. (1998). Expression of bcl-2 oncoprotein in benign and malignant spindle cell tumors of soft tissue, skin, serosal surfaces, and gastrointestinal tract. Am. J. Surg. Pathol. 22, 863-872.

Trask D.K., Band V., Zajchowski D.A. Yaswen P., Suh T. and Sager R. (1990). Keratins as markers that distinguish normal and tumor-derived mammary epithelial cells. Proc. Natl. Acad. Sci. 87, 2319-2323.

Villar E., Redondo M., Rodrigo I., Garcia J., Avila E. and Matilla A. (2001). Bcl-2 expression and apoptosis in primary and metastatic breast carcinomas. Tumour Biol. 22, 137-145.

Viña M. and Wells C.A. (1989). Clear cell metaplasia of the breast: a lesion showing eccrine differentiation. Histopathology 15, 85-92.

Visscher D.W., Sarkar F., Tabaczka P. and Crissman J. (1996). Clinicopathologic analysis of bcl-2 immunostaining in breast carcinoma. Mod. Pathol. 9, 642-646.

Wang N.S., Unkila M.T., Reineks E.Z. and Distelhorst C.W. (2001). Transient expression of wild-type or mitochondrially targeted Bcl-2 induces apoptosis, whereas transient expression of endoplasmic reticulum-targeted Bcl-2 is protective against Bax-induced cell death. J. Biol. Chem. 276, 44117-44128.

Yang Q., Sakurai T., Jing X., Utsunomiya H., Shan L., Nakamura Y., Nakamura M., Oura S., Suzuma T., Yoshimura G., Umemura T., Kokawa Y. and Kakudo K. (1999). Expression of Bcl-2, but not Bax, correlates with estrogen receptor status and tumor proliferation in invasive breast carcinoma. Pathol. Int. 49, 775-780.

Zhang G.J., Tsuda H., Adachi I., Fukutomi T., Yamamoto H. and Hirohashi S. (1997). Prognostic indicators for breast cancer patients with one to three regional lymph node metastases, with special reference to alterations in expression levels of bcl-2, p53 and c-erbB-2 proteins. Jpn. J. Clin. Oncol. 27, 371-377.

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