Breast carcinogenesis

Transition from hyperplasia to invasive lesions

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ABSTRACT

Objectives: To examine the balance loss between proliferation and apoptosis that play a role in breast cancer development, and to explore the places of various genes and molecules within this process in this supposed multistep process.

Methods: We obtained the specimens from 40 patients between 2002 and 2004 at the Department of Pathology, Medical Faculty, Adnan Menderes University, Aydin, Turkey. We categorized the lesions ductal hyperplasia (DH), atypical ductal hyperplasia (ADH), in situ ductal carcinoma (DCIS), and invasive ductal carcinoma (IDC). We determined the tumor size, histological grade and lymph node status of invasive cases and we used nottingham prognostic index (NPI). We applied ER, PR, c-erbB2, p53, Ki-67, bcl-2, dUTP nick end labeling (TUNEL), breast cancer gene-1, matrix metalloproteinases-1 and tissue inhibitor matrix metalloproteinases-1 stains to each lesion using the immunohistochemical method.

Results: We observed that ER and PR decreased in ADH when compared with DH (p=0.0001 and p=0.019). However, we determined that in DCIS as c-erbB2 (p=0,005) and Ki-67 (p=0,004) increase, TUNEL (p= 0,04) and bcl-2 (p=0,005) decrease, when compared with ADH. When compared with DCIS lesions, we observed the existence of a higher c-erbB2 (p=0,003) and a lower TUNEL (p=0,012) in invasive tumors. Furthermore, we found that there is a higher MMP-1 (p=0,04) in invasive lesions, when compared with non-invasive lesions. We detected higher PR (p=0,049), lower TUNEL and c-erbB2 (p=0,017) in low grade group of NPI, when compared with high grade group of NPI.

Conclusion: As a result, it has been shown that together with increase in proliferation, decrease in apoptosis, too, contributes to the proliferation/apoptosis imbalance that occurs in breast carcinogenesis. Increase in proliferation and decrease in apoptosis are parallel with the progression of lesions. We also showed that the changes, beginning with loss of ER and PR in ADH step, can cause malign transformation, which is especially notable both in DCIS step due to Ki-67 and c-erbB2 increase, and also with bcl-2 and TUNEL decrease.

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Breast cancer is appearing to represent an enormous public health problem. The etiology of breast cancer involves a complex interplay of genetic, hormonal and environmental factors that influence the physiological status of the host.[1] Previous studies discussed about the development of this tumor and many risk factors have been detected. It is reported that while women with breast cancer gene-1 (BRCA-1) have more risk of cancer development, the role of the BRCA-1 in the carcinogenesis is not entirely known.[2,3] There are 2 approaches, one of which supports that breast cancer is a multistep process, which develops in forms of "hyperplasia, atypical hyperplasia, in situ carcinoma, and invasive carcinoma", whereas the other defends de novo cancer development.[1]

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Supporting the first approach, reports state that not only in the in situ and invasive breast carcinoma relative to the normal breast tissue but also in the benign proliferative lesions, the mitotic activity increases and the apoptotic ratio decreases.[4] Matrix metalloproteinases (MMP) plays an important role in tumor cell growth and tumor invasion. Tissue inhibitor matrix metalloproteinases (TIMP) is an endogen substance that regulates the MMPs. It is known that the invasion takes place along with the increase of MMPs in carcinogenesis and the decrease of TIMP, which functions as an inhibitor. Behrens et al[5] and Ishigaki et al[6] discussed the effects of these endogen substances on prognosis, as well as the improvement of new treatment methods. This study aims to analyze the balance between apoptosis and proliferation in breast carcinogenesis, the invasion growth mechanisms and the assumption that multistep breast cancer might be incurred. Therefore, the role of BRCA-1, MMP and TIMP in breast cancer development and the significance of proliferative and antiapoptotic mechanisms in the transition assumed to occur between benign and malign lesions have been examined and have eventually been compared with the well-known prognostic factors.

Methods.

Cases and tissue samples. Formalin-fixed, paraffin-embedded tissue samples from 40 patients were used. Specimens were selected from the files of the Department of Pathology, Medical Faculty, Adnan Menderes University, Aydin, Turkey. All of the hematoxylin and eosin (HE) stained slides were reviewed, independently by 2 pathologists. The corresponding tissue blocks were then used for immunostaining. The criteria of Page and Rogers[7] were used to identify hyperplasia of usual type and atypical hyperplasia. Invasive carcinomas were classified according to the criteria of World Health Organization (WHO) tumor classification,[1] whereas histological grade was determined based on the classification of Bloom and Richardson and in situ ductal carcinomas according to the criteria of Holland et al.[8] Nottingham prognostic index (NPI), was calculated as follows: NPI = (0.2 x tumor size) + grade + axillary metastatic lymph nodes.[9]

Immunohistochemistry. Four µm-thick sections from formalin-fixed, paraffin-embedded tissue obtained were placed on coated slides. Immunostaining was performed using the avidin-biotin complex method. After deparaffinization and dehydration, sections were treated twice for 5 minutes in citrate buffer (0.01 mol/l, pH 6.0) in a microwave oven at 700 W. The slides were then cooled to room temperature for one hour. Endogenous peroxidase activity was blocked by immersing the sections in 3% hydrogen peroxide in methanol for 30 minutes. Sections were then incubated with primary antibody for one hour at room temperature. Biotinylated goat anti-rabbit secondary antibody was applied for 60 minutes at room temperature. Bound antibody was visualized with avidin–biotin–peroxidase complex (Histostain-plus Kits, Zymed, San Francisco, USA, code no: 85-9843) for one hour at room temperature. The color was developed by 3,3'-diaminobenzidine tetrahydrochloride. Between steps, the slides were rinsed 3 times for 10 minutes in tris-buffered saline (pH 7.6). The slides were counterstained lightly in Harris' hematoxylin, and then were dehydrated and mounted. The antibodies used were as follows: ER, (Novocastra, Newcastle, United Kingdom, code no: NCL-L-ER-6F11), 1/80 dilution, PR, (Novocastra, Newcastle, United Kingdom, code no: RTU-PGR-312), c-erbB2, (Neomarkers, CA, USA, code no: MS-730-R7), p53,(Novocastra, Newcastle, United Kingdom, code no: RTU-p53-DO7), Ki-67, (Novocastra, Newcastle, United Kingdom, code no: RTU-Ki67-MM1), MMP-1, (Neomarkers, CA, USA, code no: MS-1687-R7), TIMP-1, (Neomarkers, CA, USA, code no: MS-1567-R7), bcl-2, (Neomarkers, CA, USA, code no: MS-1844-R7), and BRCA-1 (Zymed, San Francisco, USA, code no: 33-7500). In the immunohistochemical staining, internal positive control, as well as various tissue samples, were used. As negative control the primary antibody phase was skipped and the staining process continued. For dUTP nick end labeling (TUNEL) staining, the recommended protocol for In Situ Cell Death Detection Kit, Pod (Roche, Germany, code no:1 684 817) was applied. As for the positive control, HE stained sections were examined under light microscope. Out of the necrotic areas, the cells showing the characteristic morphologic of apoptosis were chosen. All samples HE stained and exposed to immunohistochemical staining were examined through a light microscope. In addition to microscopic examination, in one of the stages in TUNEL stain, fluorescent attachment was used. By searching the existence of cytoplasmic being stained in samples stained with MMP-1 and TIMP-1, in epithelial cells, and around it, in stromal cells which are in lesions, in 50 high magnification areas, the number of positive stained cells was registered. In other preparations, exposed to immunohistochemical staining, rates of positive stained cells were calculated using the Histoscore (HSCORE) method, which is applied for identification of the ER positiveness and regarded a more reliable method.[1] With this aim, by evaluating the cells showing nuclear staining for ER, PR, p53, Ki-67, TUNEL, BRCA-1 and cytoplasmic membrane staining for c-erbB2, at least 3000 cells, of 1000 in each area showing most intensive staining, medium staining, most weak staining, were counted and stained cell percents were identified. The cell percent in the area showing most intensive staining, was multiplied with 3, in the area showing medium staining was multiplied with 2, in the area showing

most weak staining was multiplied with one and finally the results were added.

Statistical analysis. With the aim of statistical study, dimension and histological grade of invasive tumors, number of lymph node metastasis, and values of NPI were divided into groups and compared. In addition, the differences of HSCOREs of immunohistochemical markers that were applied to all lesions, among lesions were inspected. In order to make statistical analysis in invasive and non-invasive lesions, the results of TIMP-1 and MMP-1 staining were divided into 2 groups as present and absent. It was tested whether a relationship between all markers existed or not. For the statistical evaluation of the results, SPSS Data analysis program (SPSS) for Windows has been used. Differences among the tissue groups were assessed by Kruskall–Wallis analysis of variance. Mann–Whitney test was applied for comparison of the breast lesions. Correlations between the various indices were identified by Spearman's rank test. All values were expressed as medians (range), and $p<0.05$ was regarded as statistically significant.

Table 1 - Features of cases with invasive tumors

Features of cases	Number of case (%)	
Grade		
I	4	(14.3)
II	13	(46.4)
III	11	(39.3)
Tumor size		
<2 cm	15	(53.6)
2-5 cm	6	(21.4)
>5 cm	7	(25)
Nottingham prognostic index		
<3.4	6	(21.6)
3.4-5.4	18	(64.2)
>5.4	4	(14.2)
Lymph node metastasis		
0	10	(35.7)
1-3	5	(17.9)
>4	13	(46.4)
Stage, (Tumor node matastasis)		
I	2	(7.1)
II A	6	(21.6)
II B	11	(39.2)
III A	9	(32.1)

Results. In this study, 73 lesions, belonging to 40 cases, were examined. The patient's ages were ranged from 36-71 years (median age=51 years). In 25 of the cases (62.5%), more than one lesion was evaluated. Twenty-eight of the lesions were classified as IDC, 18 lesions were classified as DCIS, 12 lesions were classified as ADH and 15 were classified as DH (low-intermediate grade). The features of cases with invasive tumors were summarized in **Table 1**. Of 18 DCIS in total, 9 were evaluated as high grade (comedo type) and 9 were evaluated as low-intermediate grade (non-comedo type). In 14 lesions of 18 lesions, which were diagnosed as DCIS, invasive tumor focus existed in the next tissue. Six of 12 ADH lesions (50%), and 12 of 15 DH lesions (80%) were again observed in breast tissue, which was next to in situ or invasive tumor. The highest staining HSCORE of ER and PR was detected in DH lesions. In ADH lesions, HSCORE of both receptors was decreasing when compared with DH and this decrease was statistically significant ($p=0.0001$ and $p=0.0019$). Decrease in DCIS's and in IDC's was continuing indeed (**Figure 1a & 1b**). The change in lesions of ER and PR HSCORE has been shown in **Figure 2**.

The highest staining HSCORE of c-erbB2 was found in IDCs. In DCIS, when compared with invasive lesions, lower staining HSCORE was observed. In one of the c-erbB2 and 15 DH's, and in 3 of 12 ADH's, being stained with low HSCORE was determined (**Figure 1c & 1d**). In being stained with c-erbB2 HSCOREs, the differences between DCIS and ADH, and, between DCIS and IDC were found statistically significant ($p=0.005$ and $p=0.03$). In one of the p53, and 15 DH, and in 2 of 12 ADH, being stained was observed. Highest being stained was observed in IDC. A significant difference between the lesions, in being stained HSCOREs, was not to be discovered. dUTP nick end labeling apoptotic cell HSCORE, verified by light microscope was observed highest in ADH (**Figure 1e & 1f**). Being stained with TUNEL, HSCORE, showed statistically a significant difference between ADH and DCIS and between DCIS and IDC ($p=0.04$ and $p=0.012$) (**Figure 3**). The highest HSCORE with Ki-67 was found in DCIS. Low HSCORE was detected in DH and ADH. In invasive lesions, higher HSCORE than these 2 lesions and lower HSCORE than DCIS were observed (**Figure 1g & 1h**). It was found that the difference between ADH and DCIS was statistically significant ($p=0.004$) (**Figure 3**). Breast cancer gene-2 HSCOREs were observed in DH in highest rates. While in ADH and in DCIS, bcl-2 HSCORE decreased, in IDC, when compared with DCIS, a small increase was observed (**Figure 1i & 1j**). Due to these changes among lesions, it was found that difference between ADH and DCIS was statistically significant ($p=0.005$) (**Figure 3**). In order to study the difference with MMP-1 statistically, lesions were divided into 2 groups as non-invasive (DH, ADH, DCIS) and invasive and results of being stained were evaluated as present or absent. While in 2 of non-invasive lesions (4%) being stained was

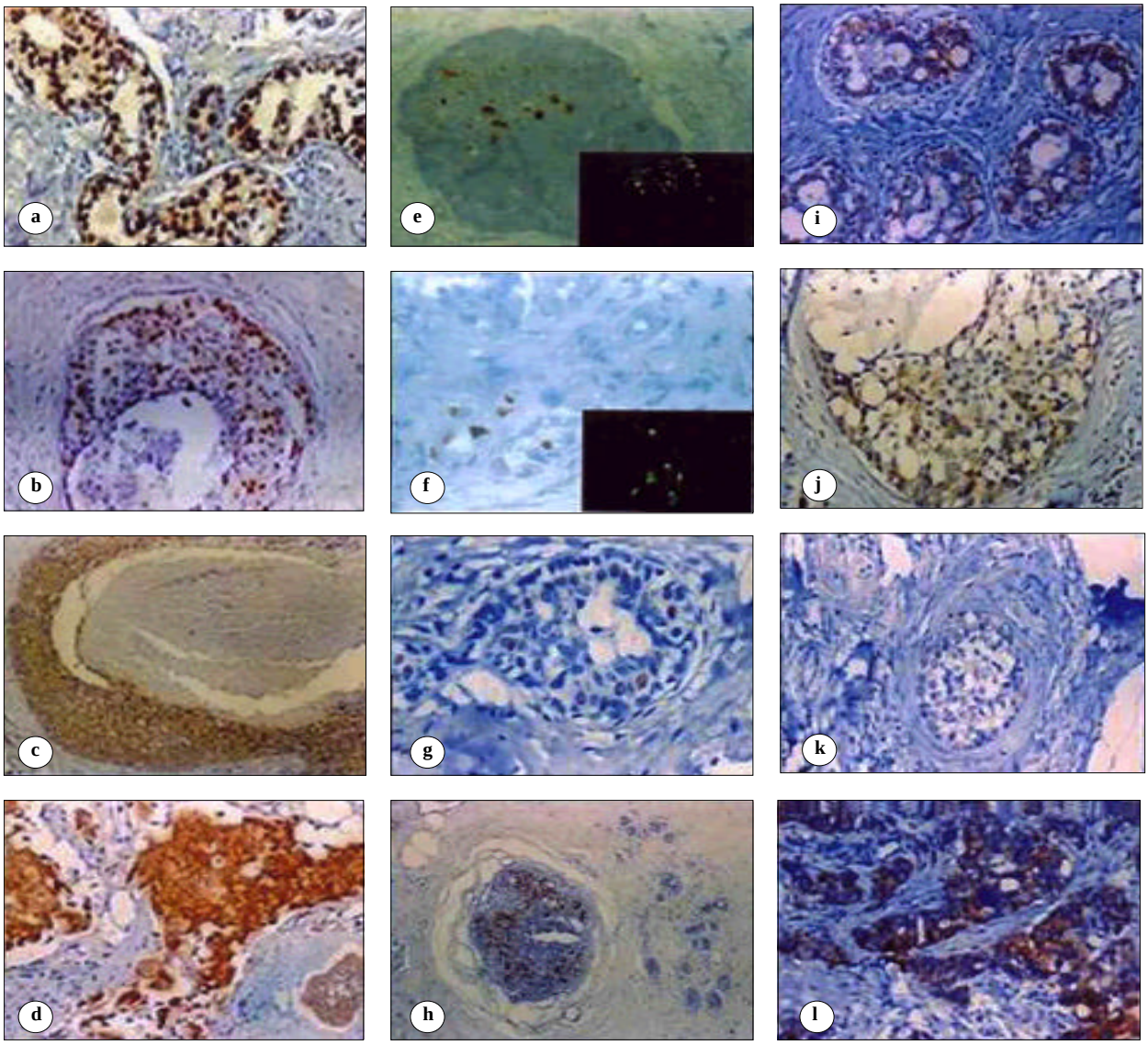

Figure 1 - Immunoreactivity of ER, c-erbB2, dUTP nick end labeling (TUNEL), Ki-67, bcl-2, Matrix metalloproteinases-1 (MMP-1) in different lesions: **a)** Ductal hyperplasia (anti-ER, x 200), **b)** Ductal carcinoma in situ (anti-ER x 100), **c)**-Ductal carcinoma in situ (anti-c-erbB2, x 200), **d)** Invasive ductal carcinoma (anti-c-erbB2, x 200), **e)** Ductal carcinoma in situ (TUNEL, x 200, insert immunofluorescent-fluorescein isothiocyanate (FITC) visualization x 200), **f)** Invasive ductal carcinoma (TUNEL, x 400, insert immunofluorescent-FITC visualization x 400), **g)** Atypical ductal hyperplasia (anti-Ki-67, x 400), **(h)** Ductal carcinoma in situ (anti-Ki-67, x 100),**i)** Atypical ductal hyperplasia (anti-bcl-2, x 200), **j)** Ductal carcinoma in situ (anti-bcl-2, x 200), **k)** Ductal carcinoma in situ (anti-MMP-1, x 100), **l)** Invasive ductal carcinoma (anti-MMP-1, x 200).

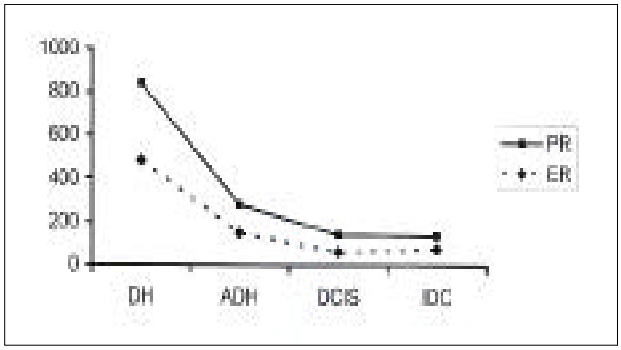

Figure 2 - The changes in lesions of ER and PR Histoscore.

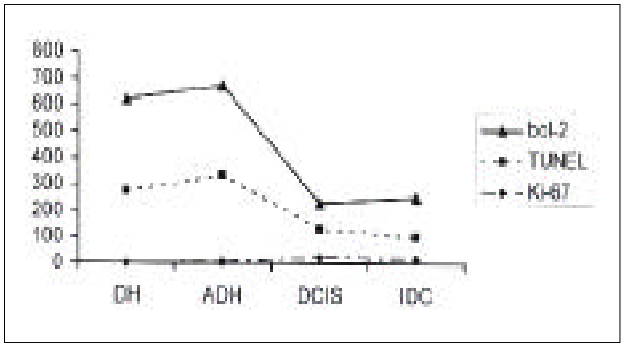

Figure 3 - The changes in lesions of bcl-2, Ki-67 and dUTP nick end labeling (TUNEL) Histoscore.

observed, in 8 of invasive lesions (28%) being stained existed (**Figure 1k & 1l**). It was found that difference of staining between these 2 groups was statistically significant (p=0.04). Being stained with TIMP-1 was evaluated using the method applied to MMP-1. In 26 of non-invasive lesions (57%) and 17 of invasive lesions (60%) being stained was detected. It was observed that being stained difference between 2 groups was not statistically significant (p=0.082). Being stained with BRCA-1 HSCOREs was found in DH in highest rates. While in ADH, DCIS and IDC's being stained HSCOREs decreased, it was observed that being stained difference among lesions was not statistically significant. Nottingham prognostic index, while in IDC cases which had good prognostic index (lower than 3.4), there were higher ER, PR, BRCA-1, bcl-2 and Ki-67 being stained HSCOREs; lower c-erbB2, p53 and TUNEL HSCOREs were detected, when compared with the cases which had bad prognostic index (higher than 5.4). It was found that being stained HSCORE difference between identifiers PR (p=0.049), c-erbB2 (p=0.017) and TUNEL (p=0.009) was statistically significant.

Discussion. The hypothetical multistep model for carcinogenesis within the breast indicates that invasive carcinoma arises as a result of a series of intermediate hyperplastic (with and without atypia) and neoplastic (in situ carcinoma) stages. Within the colon, there is a well-defined preinvasive lesion in the form of an adenoma and this has facilitated the delineation of genetic alterations in this putative precursor lesion. The morphological heterogeneity of the preinvasive lesions complicates breast studies. Tavassoli[1] showed that in the development of breast cancer, many risk factors play a role; but, clarification regarding at which steps these factors are effective. Allred et al[10] claimed that this tumor develops in a multistep process, which starts with DH and results in leading to ADH, DCIS and invasive carcinoma. When the cases, which include hyperplastic lesions but in which development of cancer was not found, on the contrary of which lesions regress, were taken into consideration, there is no consensus regarding the view that breast cancer is a multistep process. In addition, in order to determine prognosis and treatment and to create new treatment chances, studies carry on. Page[11] showed through many studies that exogen and endogen estrogen contribute to the development of breast cancer. It is known that normal breast epithelial cells carry receptors for estrogen and progesterone and therefore they respond to hormonal effects. Due to this, Cauley et al[12] believed that ER and PR are effective in early periods of breast cancer development. It has been claimed that ER and PR do not play a role in all these tumors, some tumors develop independently of these.[1,12] In our study, especially in ADH lesions, a remarkable ER and PR decrease has been observed. In in situ and invasive carcinomas, this decrease continues, too. This result leads us to think that in multistep process, which is supposed to be in breast carcinogenesis, in early stages, estrogen and progesterone play a role. A number of different studies supports that exogen and endogen steroids, particularly estrogen play a worsening role in the development of breast cancer.[1] It is thought that steroid receptors, which play a role in the development of ductal epithelium, are effective in the development of cancer through various ways. Among these, the effects on apoptosis via bcl-2, such as c-erbB2 on protoonkogenes, which regulates cell cycle and on direct cell DNA, which has been brought up recently might be mentioned.[11,12] The results of our study are consistent with the idea that atypical ductal cells, which show ER and PR loss, release from regulatory effects of steroid hormones, thus damages in growth mechanisms appear and help the carcinogenesis together with other factors, which will be added in future steps. It is accepted that today presence of ER and PR is a very important indication in breast cancer prognosis.[12] The good unity of ER and PR positive tumors with prognostic indications has been proved by researches.[13] Breast cancer cells carry ER and PR and this has a critical importance regarding the respond to hormonotherapy. In our study, the negative correlation of ER and PR with poor prognostic factors, such as Ki-67, c-erbB2, and p53, has been shown too. In our study, a positive unity between bcl-2, which is accepted as a good prognostic indication when found in invasive tumors in high rates, and ER, PR has also been found. All these results suggest that loss of ER and PR can play a role in the early stages of breast carcinogenesis by causing damage in cell balance and it is an important indication in prognosis. Today parallel with the basically known mechanisms of the development of cancer, it is accepted that the development of breast cancer occurs as a result of uncontrolled cell increase, that originates from abnormal cell proliferation and apoptosis being hindered, too.[3] In our study, it is observed that Ki-67, which is an indication of proliferation, exists in invasive carcinomas in higher rates when compared with non-invasive lesion (DH, ADH, DCIS). Moreover, we observed the number of apoptotic cells, determined with TUNEL in our study, in lower rate in IDC when compared with non-invasive lesions. Beside Ki-67 rate which increased from DH towards invasive lesions, existence of decreased TUNEL positiveness support the generally accepted, increased proliferation and decreased apoptosis view. This result is consistent with the view that the abnormal cells, originated from the uncontrolled increase in the number of cell, lead to the

development of cancer. In our study, it is observed that Ki-67 starts to increase, especially, DCIS step and this increase persists in invasive lesions. The apoptosis rates shown by TUNEL decrease from DH lesions towards invasive tumors. It is notable that this decrease is evident especially in DCIS step and invasive tumors. The increase in cell proliferation and decrease in apoptosis are meaningful especially in DCIS lesions and this shows that the balance between proliferation/ apoptosis has considerably been deteriorated in this step. The role of bcl-2, known as a gene, which hinders the apoptosis in the development of breast cancer is not clear.[14-20] It is known that this gene, reported to be a good prognostic indication in breast carcinomas, exists in normal breast epithelium.[21] In studies conducted before, it is found that bcl-2 decreases in breast epithelium during the malign transformation.[22] It is found in our study that bcl-2, which has an effect of preventing apoptosis, decreases from DH towards IDC. It is observed that this decrease is especially evident in DCIS lesions. This result shows that bcl-2 plays an important role in preventing apoptosis in DCIS. In this study, besides the existence of bcl-2, which prevents apoptosis, in invasive tumors in lower rate, low apoptotic cell rate shown by TUNEL has suggested that apoptosis can occur with different mechanisms and is interpreted as an indication of structural differences of tumoral cells. There are studies in relation to the topic that MMP, which is responsible especially in the formation of extracellular matrix, and TIMP which is their inhibitor, are effective on apoptosis as well as on the development of invasion.[23-26] It is claimed that such as high MMP secretion in invasive tumors, low TIMP secretion, too, is effective on the development of invasion.[25,26] In our study, MMP-1 is found in a higher rate in invasive lesions. This result supports the view, which has been claimed in former studies that MMPs play an important role in the development of invasion. Matrix metalloproteinases is observed in invasive lesions in a higher rate; but it is detected in a small number of invasive tumor, which shows that, as in the subject of apoptosis in tumoral cells, in the progress of invasion, too, many different mechanisms can function. In our study, positiveness is determined that in TIMP-1 and in more than 50% of all lesions in epithelial cells. This result supports the view that TIMP-1 is not only responsible for MMP-1 inhibition but also has a task to apoptosis as stated recently.[25] Clarifying the different functions of molecules, such as MMP and TIMP that is thought to have a task in tumor invasion and progression, fully, will help in planning the new treatment chances that are thought to be realized in future. One of the important oncogenes, which play a role in the development of breast cancer, is c-erbB2.[27] This gene has been reported in many studies as a poor prognostic indication for years and the unity of this gene with other bad prognostic indications is known, too.[28] In our study, weak c-erbB2 positiveness has been found in few (14%) of the DH and ADH lesions. It has been shown in our study that this gene does not play a role in the hypothesis of multistep breast cancer development process, which begins with DH in the early steps. Earlier studies have stated that the c-erbB2 positiveness found in DH and ADH cause these lesions to carry a higher risk in cancer development later.[1] In our study, 67% of DH and ADH lesions are placed in the neighborhood of in situ and invasive carcinoma, nevertheless positiveness is found in very few of them; and this shows that these lesions can be affected by mechanisms independent of c-erbB2 during the alleged malign transformation. In a study conducted before, it has been shown that c-erbB2 does not play an important role in progression from DCIS to invasive tumor.[29] In our study c-erbB2 positiveness, which starts to increase in DCIS lesions, has been found notably high in invasive tumors and this suggests that this oncogenes plays a more important role tumor progression which occurs after invasion. It is claimed that p53, which has been stated in many studies as a poor prognostic indication, causes damaged DNAs as a result of stopping the cell cyclus and through this it has a task in carcinogenesis.[30] In our study, the existence of p53 cell is only observed in 3 of 27 DH and ADH lesions and it has been observed that p53 positiveness between in situ and invasive carcinoma lesions, which display a higher being stained rate, does not change notably. Results show that p53, too, has started to become effective in DCIS lesions. It has been shown in the studies made before that in invasive tumors, which have a high apoptotic index, p53 decreases[31] and causes an increase in the number of cell by damaging the cell proliferation control of mutant p53.[32] Findings of our study support these views also. According to this, existence of mutant p53 in high rate in DCIS and invasive tumors suggests that this gene can play a role in proliferation and apoptosis imbalance, which is shown by Ki-67 and TUNEL, in these lesions. There are studies regarding BRCA-1 that prevents the apoptosis by playing a role in p53 activation.[33,34] In some studies, it has been claimed that in sporadic breast cancer, BRCA-1 directs apoptosis independently of p53.[35] It is reported that in invasive tumors the secretion of BRCA-1 decreases; but it shows a strong secretion through immunohistochemical methods in normal breast epithelial cells.[35] In our study, it has been observed that in DH and ADH lesions, there is BRCA-1 positiveness in more cells, when compared with DCIS and invasive tumors; but a significant difference could not be found. This result is consistent with decreased BRCA-1 positiveness in invasive tumors, which has

been reported before; however, it is not enough to state the effect of this gene on apoptosis and so its role in carcinogenesis. Nottingham prognostic index is a simple index which is determined with some prognostic indications such as dimension of tumor in invasive breast carcinomas, histological grade and lymph node status. In studies, it has been reported that tumor with low index live longer.[1,4] In our study, the meaningful unity of high PR positiveness in low NPI values, and high c-erbB2 and TUNEL positiveness in high NPI values has been brought up. Higher c-erbB-2 is found in group which has a high index and this supports our view that this gene is especially effective on progression of invasive tumors. In a study conducted before, it has been reported that in invasive tumors cell turn-over increases as a result of increased proliferation and apoptosis.[3] In our study, in high index group which has loss of differentiation, increased tumor dimension and positiveness of lymph node, the rates of high Ki-67 and high TUNEL score have been determined. Then it is observed that the cell turn-over increase occurs together with increase in the progression of invasive tumors. These findings show that high NPI degree invasive tumors can have different behaviors when compared with low degree ones. As a result, with this study, it has been shown that together with increase in proliferation, decrease in apoptosis, too, contributes to the proliferation/ apoptosis imbalance that occurs in breast carcinogenesis. Increase in proliferation and decrease in apoptosis are parallel with the progression of lesions. In our study, it has been observed that loss of receptors, which provide the effect of estrogen and progesterone in cell regulation, starts at ADH step. In our study, the increased positiveness of c-erbB2 and p53 especially in DCIS, and again in this step high proliferation shown with Ki-67 and decreased apoptosis shown with TUNEL has been observed. This shows that these lesions are at a critical step where malign transformation occurs. Gene known for preventing apoptosis such as bcl-2, is decreased especially at the early stages of DCIS step and this is consistent with our critical transformation view that we suggested in this step. Our results show that MMP-1 which exists in increasing rates in invasive tumors contributes to the invasion progression. Moreover, in our study it has been shown that c-erbB2 can contribute to the progression of tumor especially after tumor proves to be in invasion situation. It has been seen that together with c-erbB2, increased cell turn-over, too, contribute to the different behaviors in the further stages of invasive tumors. In this study the effects of molecules, such as BRCA-1 and TIMP-1, could not be completely clarified in the development of breast cancer.

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