Comparing the clinical, histopathological and myoepithelial features of estrogen receptor positive and negative mammary carcinomas

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ABSTRACT

Objectives: The purpose of this study is to examine the relationship between hormone-receptor status and histological parameters, considering that some estrogen receptor (ER)-negative breast carcinoma are suggested to be of myoepithelial origin or differentiation; and to examine the presence of significant difference by myoepithelial markers and define their morphologies.

Methods: For this research, 30 estrogen receptor-negative and 31 estrogen receptor-positive breast carcinomas diagnosed at the Pathology Department, Istanbul Training and Education Hospital, Istanbul, Turkey, between February 2003 and October 2004 were considered and compared clinically, microscopically and immunohistochemically considering myoepithelial markers using SMA, S100, keratin14.

Results: We found a higher amount of grade 3 frequency pushing margins, solid islets, and presence of central necrosis in the estrogen receptor-negative group than in the positive group ($p<0.001$ and $p<0.05$). Six estrogen-negative and 2 estrogen-positive cases were found positive for myoepithelial markers; a difference which is non-significant ($p=0.147$). The presence of solid islets, fusiform, and clear cells was detected higher in myoepithelial positive tumors than in negative group ($p<0.05$).

Conclusion: For daily pathologic applications, some morphological properties of a breast carcinoma can give clues about ER and myoepithelial features. In estrogen receptor-negative tumors, there is a remarkable myoepithelial marker positivity. Studies involving broader series and different myoepithelial markers could give more reliable results.

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Presence of estrogen receptor (ER) is vital in prediction and in prognosis of breast carcinoma.[1-4] A progressive breast carcinoma leads to loss of estrogen receptors and an increase in nuclear grade.[5,6] Specific types of tumors such as apocrine, medullar, metaplastic, and myoepithelial carcinoma do not express estrogen receptors in any stage of development regardless of progression. Myoepithelial carcinoma is known to be the tumor of myoepithelial cells between luminal epithelium and basal membrane. Myoepithelial cells were thought to be inert cells that were not involved in carcinoma; later they were defined in increasing numbers in different lesions and neoplasia. Myoepithelioma, adenomyoepithelioma, low grade adenosquamous carcinoma, syringomatous carcinoma and adenoid cystic carcinoma are some well-known pathologies. In the last few years, pure myoepithelial carcinoma, poorly differentiated myoepithelial-rich carcinoma and matrix producing myoepithelial carcinoma have been included in this group.[1-3,5,7-9] Lately, invasive ductal carcinoma was defined as not otherwise specified with a high grade,

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wide central acellular zone; and estrogen receptor-negative are suggested to be of myoepithelial origin or differentiation.[5,6] Myoepithelial cells' main function is transporting excretion due to its contractile property. Also, it was located peripherally they store extracellular matrix and excrete proteinase inhibitors preventing invasion.[6,10] Myoepithelial cells can be in different forms such as fusiform, epithelioid, plasmacytoid and clear cytoplasmic. In applied pathology, immunohistochemical stains were widely used. Aside from markers, we could use smooth muscle actin, smooth muscle myosin heavy chain, S100 protein, GFAP, also Cd10, CK14, p63, calponin, caldesmon, 14-3-d antibodies.[3,5,8-12] In this study, we aim to examine the relationship between hormone receptor status and histological parameters, and considering that some estrogen receptor-negative breast carcinoma could be considered myoepithelial; and to examine the presence of significant difference by myoepithelial markers and define their morphologies.

Methods. Among the cases studied and diagnosed by the Pathology Department, Istanbul Training and Research Hospital, Istanbul, Turkey, from February 2003 to October 2004 we considered 31 estrogen receptor-positive and 30 negative. In 4 of these samples, only excision material was present and lymph nodes were not evaluated. Metastatic carcinomas of breast and specimens other than mastectomies or excisional biopsies were excluded from this study. For ER results, pathology reports were taken. In estrogen receptor-negative cases, the negativity was verified by reapplying receptor dyes. Staining extension more than 10% was considered positive. In all hematoxylin and eosin stained sections of the cases, tumor type, histological and nuclear degree, pushing or infiltrating margins, central hyalinization and necrosis, development in solid islets, comedonecrosis presence, lymphoplasmacytic infiltration, clear and fusiform cell appearance, in situ component, angioinvasion, perineural invasion, and SMA (Novo Castra 1:200 dilution), S100 (Neo Markers, 1:100 dilution) and CK14 (Novo Castra, ready to use) immunohistochemically as myoepithelial marker were applied to detect dying properties. Standard staining protocol was applied. Two groups, estrogen positive (31 cases) and negative (30 cases), have been compared under patient's age, tumor diameter and metastatic lymph node number using Student's t test and Mann Whitney U test. These groups were compared under the above parameters using x^2 and Fisher exact tests. The myoepithelial markers of the 2 groups were compared histologically using Fisher exact test.

Results. In the positive group, the age range was 28-87 years and in the negative group the age range was 31-80 years. The range of the tumor diameter in the positive group was1.5-7.0 cm (average 3.48 cm) and in negative group was 1.2-10 cm (average 3.66 cm). Number of metastatic lymph nodules in the estrogen positive group was 0-22 (average 8.54) and in the negative group was 0-16 (average 4.37). There were no difference among the 2 groups considering the age, tumor diameter and metastatic lymph nodules (**Table 1**). Tumor types in ER-positive and negative groups are shown in **Table 2**. When we compared the histopathological features of the negative group, we found the following features:pushing margin, central necrosis, development in solid islets and histological and nuclear grade 3 presence ($p<0.05$, $p<0.01$, $p<0.001$) (**Figures 1 & 2, Table 3**). Infiltrative development pattern in the estrogen receptor-positive group was found reasonably high ($p<0.05$). Six estrogen negative and 2 positive cases were positive for myoepithelial markers (**Table 3**). Although there were significant differences between the 2 groups; we found it statistically non-significant ($p=0.147$). Myoepithelial marker staining characteristics was summarized on **Table 4**. Accordingly, 3 estrogen receptor-negative cases were stained with smooth muscle actin, 3 cases with S100 and 3 cases with CK14 (**Figures 3 & 4**) and no case was defined to express all of these: 2 cases with SMA and S100, one with SMA and CK14, one with only S100 and one expresses positivity with only CK14. Two cases of receptor positive group expressed limited staining with CK14 merely over 10% (+1). It was observed that myoepithelial positive and negative groups show no difference by means of age, diameter and lymph node metastasis (**Table 5**). When we compared the 6 myoepithelial marker positive cases with the remaining 24 cases in estrogen-negative group histologically (**Table 6**), we detected that the presence of solid islets ($p=0.026$), fusiform ($p=0.041$) and clear cells ($p=0.016$) were statistically significant ($p<0.05$).

Discussion. One of the models suggested for development of breast carcinoma in insitu carcinoma while proceeding to metastatic carcinoma goes through changes in phenotype and turns aggressive due to accumulation of molecular anomaly. According to clonal hypothesis, some successive genetic changes occur in the carcinoma tissue, increasing aggressivity, proliferation, adhesion, proteolysis, motility, angiogenesis abilities are achieved by clonal populations derived from the modified cells, finally tumor progresses. It is suggested that differences

among tumors is caused by this clonal diversity. Therefore, cells in the in-situ carcinoma zones in the breast and cells in invasive and metastatic zones must have morphological, immunophenotypical, genetic and molecular differences. On the other hand, it was observed that molecular and genetic profile of the carcinoma cell remains mostly static during progression.[6,13,14] Actual difference is detected in different tumors by means of grade and estrogen hormone receptor status. Thus, different neogenetic pathways are present for different types of genetic changes and grade and ER status is defined right from the beginning of its formation. Factors leading to the spread of the tumor are direct and paracrine interactions between the tumor itself and the surrounding tissue.[6] In situ and invasive carcinoma parts are closely parallel in grade, molecular markers, DNA content and keratin expressions. It is observed that ER 96%, PR 82%, p53 76%, ERB-B2 84% and Ki67 85% are concordant to one another. It is also found that primary tumors and tumors in metastatic lymph nodes are 80% correlated for ER. Tumor phenotype is also closely parallel to this.[6] Estrogen receptor- positive tumors are low grade and have well differentiated luminal in phenotype but ER-negative tumors have generally high proliferative/apoptotic index and basal-like poorly differentiated features.[6] In all primary breast carcinoma, ER positivity is 55-80%. This percentage decreased to 2% in grade 3 carcinoma.[6,7] Eighty-five percent of ER-negative carcinoma are invasive ductal carcinoma not involved in a specific group, 8% of them are atypical medullary carcinoma.[4] Comparing ER-positive and ER-negative tumors, it was observed that in the receptor negative group, genes which code proteins such as Cyclin E1, p-cadherin, p-16, cathepsins, EGFR, metallothionein, interleukin-8, S100A4 and vimentin are expressed in higher levels.[6] Resources state an increase in p53 expression, epidermal growth factor secretion c.erb. B2 over-expression along with high expression of Ki67 and topoisomerase 2α in ER-negative tumors. On the other hand, higher levels of cyclin dependent kinase inhibitors p21 and p27 are observed in ER-positive tumors.[6] Estrogen and progesterone receptor, bcl-2, p27 and cyclin-D positivity in low grade tumors and ERB-B2, p-53, cyclin-E, CK5/6 like basal type keratin and Mib1(Ki67) positivity in high grade tumors are key indicators. There is no specific change in the number of chromosomes during progression. Through the use of comparative genomic hybridization (CGH), chromosome 3q and 7q gains in grade 3 / ER (-) tumors and 16q and 20q gain in low grade / ER (+) tumors are significant events. Again, in low degree 16q LOH, in high degree 11p and 17p LOH have been detected.[6] All of these, show that ER-positive and ER-negative breast carcinomas are actually different neoplasms. Therefore, it would be expected to have different clinical and

Table 1 - Clinical features and estrogen receptor status.

Parameters	Estrogen receptor (-) (n=30) Mean ±SD	Estrogen receptor (+) (n=31) Mean±SD	*P* value
Age (years)	50.17 ± 13.78	52 ± 11.79	0.578
Size (cm)	3.66 ± 1.73	3.48 ± 1.90	0.707
No. of metastatic lymph nodes	4.37 ± 5.23	6.33 ± 8.54	0.294

Table 2 - Distribution of tumor types and estrogen receptor status

Estrogen receptor	n
Positive receptor	
Invasive ductal Ca not otherwise specified	19
Invasive ductal with extensive DCIS	2
Cribriform carcinoma	2
Tubulolobular carcinoma	1
Invasive ductal carcinoma with mucinous component	1
Invasive micropapillary carcinoma	2
Invasive papillary carcinoma	1
Classic type invasive lobular carcinoma	1
Pleomorphic lobular carcinoma	1
Mixture invasive ductal-lobular carcinoma	1
Negative receptor	
Invasive ductal carcinoma not otherwise specified	20
Adenosquamous carcinoma	3
Apocrine carcinoma	1
Invasive micropapillary carcinoma	1
Invasive papillary carcinoma	1
Invasive ductal with extensive DCIS	1
Invasive ductal carcinoma - mucinous component	1
Invasive lobular carcinoma - mucinous component	1
Signet ring cell carcinoma	1

DCIS - ductal carcinoma insitu

Table 3 - Histopathological features in estrogen receptor positive and negative cases.

Histopathological features	No. of estrogen receptor (%)						SD	*P* value
	Negative		Positive		Total			
Myoepithelial markers								
Negative	24	(80)	29	(93.5)	**53**	**(86.9)**		
Positive	6	(20)	2	(6.5)	**8**	**(13.1)**		0.147
Nuclear grade								
Grade 1-2	11	(36.7)	21	(67.7)	**32**	**(52.5)**		
Grade 3	19	(63.3)	10	(32.3)	**29**	**(47.5)**	5.90	0.015
Histological grade								
Grade 1-2	9	(45)	22	(95.6)	**49**	**(80.3)**		
Grade 3	11	(55)	1	(4.4)	**12**	**(19.7)**	19.64	0.001
Infiltrative margins								
Negative	13	(43.3)	4	(12.9)	**17**	**(27.9)**		
Positive	17	(56.7)	27	(87.1)	**44**	**(72.1)**	7.02	0.008
Pushing margins								
Negative	18	(60)	27	(87.1)	**45**	**(73.8)**		
Positive	12	(40)	4	(12.9)	**16**	**(26.2)**	5.78	0.016
Central hyalinisation								
Negative	19	(63.3)	13	(41.9)	**32**	**(52.5)**		
Positive	11	(36.7)	18	(58.1)	**29**	**(47.5)**	2.79	0.094
Central necrosis								
Negative	17	(56.7)	28	(90.3)	**45**	**(73.8)**		
Positive	13	(43.3)	3	(9.7)	**16**	**(26.2)**	8.92	0.003
Solid nests								
Negative	18	(60)	29	(93.5)	**47**	**(77)**		
Positive	12	(40)	2	(6.5)	**14**	**(23)**	9.70	0.002
Comedonecrosis								
Negative	24	(80)	29	(93.5)	**53**	**(86.9)**		
Positive	6	(20)	2	(6.5)	**8**	**(13.1)**		0.147
Desmoplasia								
Negative	24	(80)	18	(58.1)	**42**	**(68.9)**		
Positive	6	(20)	13	(41.9)	**19**	**(31.1)**	3.42	0.064
Lymphoplasmacytic infiltration								
Negative	20	(66.7)	25	(80.6)	**45**	**(73.8)**		
Positive	10	(33.3)	6	(19.4)	**16**	**(26.2)**	1.54	0.215
Spindle cell features								
Negative	25	(83.3)	29	(93.5)	**54**	**(88.5)**		
Positive	5	(16.7)	2	(6.5)	**7**	**(11.5)**		0.255
Clear cell features								
Negative	23	(76.7)	25	(80.6)	**48**	**(78.7)**		
Positive	7	(23.3)	6	(19.4)	**13**	**(21.3)**	0.14	0.704
In situ component								
Negative	10	(33.3)	11	(35.5)	**21**	**(34.4)**		
Positive	20	(66.7)	20	(64.5)	**40**	**(65.6)**	0.03	0.860
Angiolymphatic invasion								
Negative	26	(86.7)	22	(81.5)	**48**	**(84.2)**		
Positive	4	(13.3)	5	(18.5)	**9**	**(15.8)**		0.722
Perineural invasion								
Negative	26	(86.7)	20	(71.4)	**46**	**(79.3)**		
Positive	4	(13.3)	8	(28.6)	**12**	**(20.7)**	2.04	0.152

Table 4 - Presence of myoepithelial markers and spread of staining in estrogen receptor (ER) positive and negative groups.

Myoepithelial markers	ER-positive group n (spread)	ER-negative group n (spread)
SMA	-	-
S100	-	1 (++)
CK14	2 (+)	2 (+++), (+)
SMA-S100	-	2 (++/+)
SMA-CK14	-	1 (+/+)
S100-CK14	-	-
SMA-S100-CK14	-	-

Table 5 - Clinical features in myoepithelial marker positive and negative tumors.

Clinical features	Myoepithelial marker (negative) (n=24) Mean ± SD	Myoepithelial marker positive (n=6) Mean±SD	*P* value
Age (years)	50.75 ± 14.18	47.83 ± 13.00	0.836
Size (cm)	3.85 ± 1.85	3.67 ± 1.25	0.854
No. of lymph node metastases	4.83 ± 5.50	2.50 ± 3.78	0.383

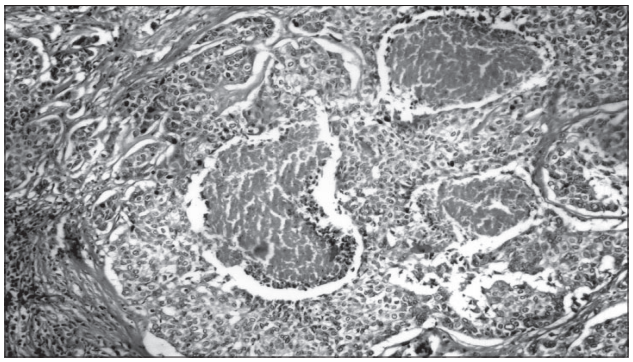

Figure 1 - Solid nests and comedonecrosis in estrogen receptor-negative case.

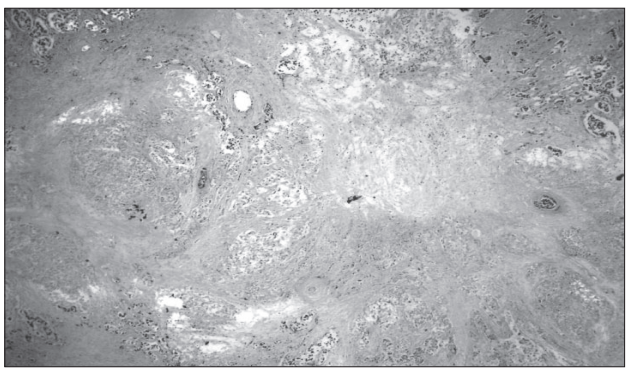

Figure 2 - Central necrosis in estrogen receptor-negative case.

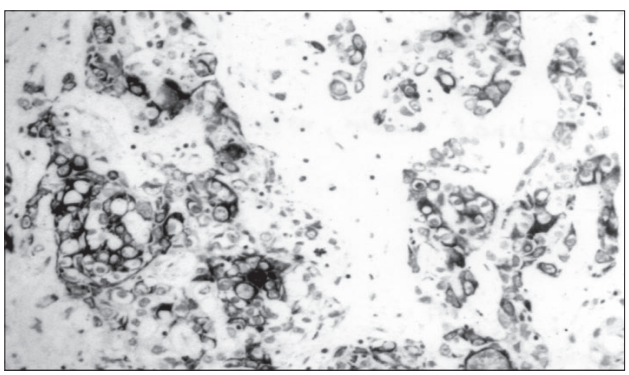

Figure 3 - S100-positive staining in estrogen receptor-negative case.

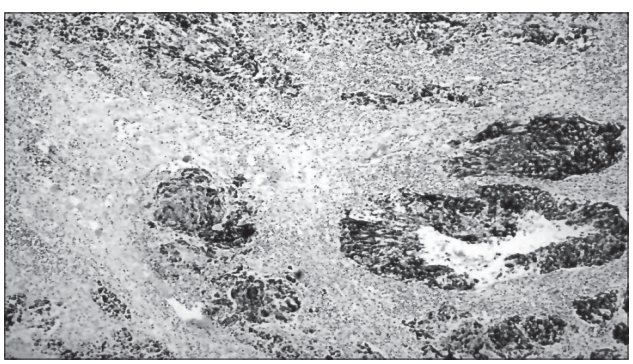

Figure 4 - Extensive CK14-positive staining in ER-negative case.

Table 6 - Comparison of histopathological features between myoepithelial marker positive and negative groups in oestrogen receptor negative tumors.

Features	No. of myoepithelial markers (%)		*P*-value
	Negative markers (n=24)	Positive markers (n=6)	
Nuclear grade			
Grade 1-2	10 (41.7)	1 (16.7)	
Grade 3	14 (58.3)	5 (83.3)	0.372
Histological grade			
Grade 1-2	15 (62.5)	4 (66.7)	
Grade 3	9 (37.5)	2 (33.3)	-
Infiltrative margins			
Negative	8 (33.3)	5 (83.3)	
Positive	16 (66.7)	1 (16.7)	0.061
Pushing margins			
Negative	16 (66.7)	2 (33.3)	
Positive	8 (33.3)	4 (66.7)	0.184
Central hyalinization			
Negative	14 (58.3)	5 (83.3)	
Positive	10 (41.7)	1 (16.7)	0.372
Central necrosis			
Negative	14 (58.3)	3 (50)	
Positive	10 (41.7)	3 (50)	-
Solid nests			
Negative	17 (70.8)	1 (16.7)	
Positive	7 (29.2)	5 (83.3)	0.026*
Comedonecrosis			
Negative	20 (83.3)	4 (66.7)	
Positive	4 (16.7)	2 (33.3)	0.571
Desmoplasia			
Negative	19 (79.2)	5 (83.3)	
Positive	5 (20.8)	1 (16.7)	-
Lymphoplasmacytic infiltration			
Negative	18 (75.0)	2 (33.3)	
Positive	6 (25.0)	4 (66.7)	0.141
Spindle cell features			
Negative	22 (91.7)	3 (50)	
Positive	2 (8.3)	3 (50)	0.041*
Clear cell features			
Negative	21 (87.5)	2 (33.3)	
Positive	3 (12.5)	4 (66.7)	0.016*
In situ component			
Negative	7 (29.2)	3 (50)	
Positive	17 (70.8)	3 (50)	0.372
Angio lymph invasion			
Negative	22 (91.7)	4 (66.7)	
Positive	2 (8.3)	2 (33.3)	0.169
Perineural invasion			
Negative	20 (83.3)	6 (100)	0.557
Positive	4 (16.7)		

*Significant due to Fisher exact test

histopathological features. Ninety-five percent of the ER-negative breast carcinoma are caused by grade 3 tumors. Common morphological features for these tumors are comedonecrosis, lymphoid stroma, central necrosis and fibrosis and pushing type margins existence.[4] In this study, no difference has been found by means of age, tumor diameter and number of metastasis. Frequency of grade 3, pushing margins, solid islets and central necrosis were found in higher amounts in ER-negative tumors. Infiltrative margins were found significant in ER-positive tumors. In ER-negative tumors, histological grade 3 frequency was found to be 55% and nuclear grade 3 frequency to be 63%. Experimental, immunohistochemical, ultrastructural, cell cultural, genetic and molecular researches show that multipotent stem cells capable of both regenerating and generating epithelial and myoepithelial cells are present in the breast.[15-21] During organogenesis in breast, stem cell first differentiates into ductal and lobular epithelial cell types specific to the tissue. Later, these differentiate into luminal epithelial and myoepithelial cells.[15,20,21] Stem cell may carry out mutations during organogenesis and this mutation can be conserved in the following replications. Therefore, stem cell carries a major potential risk for carcinogenesis. Genetic molecular researches show that the changes of myoepithelial and epithelial cells have in common; this are the 17p. It is suggested that this change in 17p goes with the stem cell model.[10] Myoepithelial carcinoma are rare in breast. Genetically, basal/myoepithelial tumors are not always pure myoepithelial carcinoma. Some of them are high grade ER-negative carcinoma showing basal/myoepithelial differentiation.[6] Kesse-Adu et al have detected in ER-negative breast carcinoma (29%) or taking S100 singly into consideration (47%) myoepithelial marker positivity. This shows that some of the ER-negative breast carcinoma are of myoepithelial cell origin or tumors differentiated in that direction.[3] In the presented study, 6 of the 30 ER-negative cases have been stained with at least one of the myoepithelial markers. From the 31 ER-positive cases, 2 were colored with focal dyes and traced with CK14. Statistically, the result was found insignificant but it seems one positive or negative case could affect the result. Therefore, one cannot say that the result of our study denies the literature. Using wider series and different markers in the research would be appropriate.

In conclusion, ER-negative and ER-positive breast carcinomas bear no difference by means of age, tumor diameter and lymph node metastasis. While ER-negative group has grade 3 frequency, includes pushing type progression, solid islets and central necrosis, ER-positive group bears infiltrating margins. Although in ER-negative tumors, presence of myoepithelial markers had no statistically meaningful results, it has shown significant difference in the number of cases and staining diversity. Fusiform and clear cells along with solid islets have been found in common in these cases.

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