INSIGHTS ON AROMATASE IMMUNOHISTOCHEMISTRY: VARIATIONS BETWEEN INTRINSIC MOLECULAR SUBTYPES OF BREAST CANCERS

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Abstract

Context. Aromatase is a key enzyme in local estrogen production by androgen conversion, especially in women post-menopause. There have been controversies concerning aromatase localization in breast carcinomas and its association with current histopathological variables.

Material and Methods. Using polyclonal antibody immunohistochemistry we assessed (by intensity and percentage scores) the immunolocalization of aromatase in 70 tissue samples, and described particularities within the molecular subtypes of breast cancer.

Results. Aromatase was found in all tissue compartments: tumor (95.7%), stroma (58.6%) and adipose tissue (94.3%). Aromatase expression in tumor cells correlated inversely with tumor grading ($p=-0.361$, $p=0.027$), and positively with estrogen receptor status (ER, $p=0.143$, $p<0.001$). Dividing the study group by intrinsic subtypes, a strongly inversely association between tumor aromatase and grading ($p=-0.486$, $p<0.001$), and between stromal aromatase and Ki67-index ($p=-0.448$, $p=0.048$) was observed in luminal A breast cancer. Tumor aromatase and ER percentage scores had stronger correlations in luminal B HER2 negative ($p=0.632$, $p=0.002$), and positive ($p=0.324$, $p=0.026$) tumors. In contrast, in triple negative tumors, a positive association stromal aromatase and Ki67 index ($p=-0.359$, $p=0.007$) was observed.

Conclusion. Local aromatase was linked to better tumor differentiation and proliferation in luminal breast subtypes, and not in triple negative cases, suggesting a potential prognostic role of aromatase in breast carcinomas.

Key words: aromatase, breast cancer, obesity, adipose tissue.

INTRODUCTION

Breast cancer is the most commonly diagnosed cancer and the leading cause of cancer death in females worldwide (1). In 2018, breast cancer accounted for

24.2% (2.1 million) of the total new cancer cases and 15% (627,000) of total cancer-related deaths (1).

Breast cancer is a heterogenous disease and the intrinsic classification of molecular subtypes contributed greatly in establishing prognosis and developing management strategies (2). While this classification constituted a major paradigm shift within both research and clinical arena, the study of new potential biomarkers are always needed, especially to characterize both molecular types and the potential response to specific therapy (3).

Since aromatase is the key enzyme in the estrogen synthesis and is expressed in breast carcinoma cells, surrounding stromal cells as well as endothelial and benign breast cells, many attempts have been made to evaluate the importance of aromatase expression in the breast for local estrogen production and its utility as prognostic and predictive marker in breast cancer, however, with contradictory results (4–9).

Immunohistochemistry is a cost-efficient method for the clinical practice. Currently, the breast cancer cases that may benefit of aromatase inhibitors treatment are selected based on assessments of hormone receptors in the resected tumor tissue, instead of directly assessing aromatase. The development of anti-aromatase antibodies with increased specificity and sensitivity made possible to better describe aromatase immunoreactivity in tissue sections.

OBJECTIVE

The primary aim of our study was to assess the association between aromatase immunoreactivity in all major compartments of the malignant breast (tumor, stroma, adipose tissue) and the common histological variables used for assessing and staging breast tumors. A secondary objective aimed to identify particularities

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in aromatase staining in connection to the histological variable based on the intrinsic subtypes of breast cancer.

PATIENTS AND METHODS

Patients and tumor characteristics

Seventy paraffin-embedded tumor specimens of invasive breast carcinoma from archived tissue at the Department of Pathology, “Sf. Spiridon” Emergency County Hospital Iasi, Romania were included in the present analysis. The tumor tissue had been obtained during curative surgery of the primary tumor in the breast, performed at the Department of Surgery between July 2013 and December 2016.

Clinical and histopathological characteristics were obtained from patients’ records. Patients histological data were grouped in order to better classify the aromatase staining characteristics and included primary tumor size (≤ 20 mm; > 20 mm), lymph node invasion (with or without confirmed lymph node invasion), tumor grading, estrogen (ER) and progesterone (PgR) receptor percentage score, intensity score and status ($<1\%$; $>1\%$ positive in tumor cells) (2), human epidermal growth factor receptor 2 (HER2/neu) status (positive; negative or equivocal) and Ki-67 index ($<15\%$; $\geq 15\%$). The tumor grade had been established according to Elston and Ellis grading system (10) which comprised evaluating tubular differentiation, nuclear pleomorphism, and mitotic counts.

Immunohistochemistry and scoring of immunoreactivity

Aromatase polyclonal antibody (Thermo Fisher Scientific, PA1-21398, Waltham, MA, USA) was used following an EnVision immunohistochemistry protocol (over-night) in preparing the tissue specimens for immunohistochemistry analysis. In brief: 10% formalin-fixed paraffin embedded tissue sections of breast carcinomas were immunostained manually, using epitope retrieval solution pH=6 (Novocastra), dilution 1:100, peroxidase inhibition being done with peroxide (0.3% concentration) for 10 minutes (incubation in a wet-chamber). Placenta was used as a positive control for aromatase.

Slide evaluation was done by using Microscope Nikon Eclipse E200. Aromatase immunoreactivity was detected in the cytoplasm. For assessing aromatase immunoreactivity in breast cancer, percentage and intensity scorings were attributed to each case.

The entire tissue specimens were initially screened at low power fields in the light microscopy assuring that all tissue compartments (tumor, stroma and adipose tissue) were present. Afterwards, the samples were analyzed for identifying maximum aromatase immunolabeling, and eloquent areas were assessed by two pathologists, without using automatic scoring software.

The approximate percentage (proportion score) of stained cells in each tissue compartment was assessed by classifying the proportion score using one of the following scores: 0: $<1\%$, 1: 1–25%, 2: 26–50%, 3: $>50\%$ (adapted after Sasano H *et al.*, 2005, c) (11). Relative intensity of immunopositive tumor cells was determined after selecting the most representative areas of positivity and scoring it as follows: 0: no immunoreactivity, 1: weak, 2: moderate and 3: intense positive.

Differences between results were resolved by final joint probe examination and reconciled values were used in the statistical analyses.

Statistical analysis

Associations between categorical variables were assessed by Chi-square tests. Associations between continuous variables were analyzed using Spearman correlation coefficients. The level of statistical significance was established at $p < 0.05$. All analyses were performed using SPSS version 24.0 software (IBM Corp., Armonk, NY, USA).

Ethics

Ethical approval was obtained from the Ethics Committee of “Grigore T. Popa” University of Medicine and Pharmacy Iasi (no. 1642/25.01.2017). The research protocol was approved by the institutional ethics committees of “Sf. Spiridon” Emergency County Hospital Iasi (no. 78/21.12.2016) and “Grigore T. Popa” University of Medicine and Pharmacy Iasi.

RESULTS

Seventy cases were included in the present analysis. Mean age of patients at surgery was 61.2 ± 12.4 years old (between 36 and 83).

Aromatase was detected immunohistochemically in all tissue compartments of the pathological breast: tumor cells, stromal cells, and adipose tissue. Aromatase immunoreactivity was detected in the cytoplasm, with a slightly increased pattern of intensity localized perinuclear.

Figure 1. Aromatase immunoreactivity in invasive breast carcinoma (x20): intensity score 1 in tumor cells (T, tumor; S, stroma).

Figure 2. Aromatase immunoreactivity in invasive breast carcinoma (x20): intensity score 1 in tumor cells, negative stroma (T, tumor; S, stroma).

Figure 3. Aromatase immunoreactivity in invasive breast carcinoma (x20): intensity score 3 in tumor cells, positive stroma (T, tumor; S, stroma).

Analyzing tumor cells, the majority of samples ($n=67$, 95.7%) were positive for aromatase, with weak immunoreactivity in 17 cases (24.3%, Fig. 1), moderate in 26 (37.1%, Fig. 2) and strong staining in 24 (34.3%, Fig. 3) cases, while only 3 (4.3%) were negative for aromatase. The percentage score attributed to aromatase-positive tumor cells revealed that the majority of cases ($n=67$; 95.7%) had over 50% of tumor cells positive for aromatase (Table 1).

Regarding stromal cells, the aromatase percentage score was evaluated as 3 in 41 (58.6%) cases, 2 in 9 (12.9%) cases, 1 in 11 (15.7%) cases, and 0 in 9 (12.9%) cases. The adipose tissue showed the highest immunoreactive cells as 66 (94.3%) cases had a percentage score of 3, and only 4 cases (5.7%) had a percentage score of 2 (Table 1).

The evaluation of aromatase immunoreactivity in relation to histopathological variables revealed a statistically significant negative correlation between aromatase intensity score in tumor cells and tumor grading ($p=-0.361$, $p=0.027$). Also, a statistically significant positive correlation was identified between the percentage score of ER and aromatase intensity score ($p=0.146$, $p=0.005$), and percentage score ($p=0.143$, $p<0.001$) in tumor cells (Table 2).

Tumor samples were grouped and analyzed by intrinsic subtype: 27 (38.6%) cases were luminal A, 12 (17.1%) cases were luminal B HER2 negative, 14 (20%) cases were luminal B HER2 positive, 5 (7.1%) cases were only HER2 positive, and 10 (14.3%) cases were triple negative forms; in 2 cases the molecular subtype could not be established by available data.

The analysis of aromatase staining based on subtypes of breast carcinoma showed no statistically significant differences between the groups regarding aromatase intensity ($p=0.013$, $p=0.195$) and percentage ($p=-0.028$, $p=0.492$) scores assessed in tumor cells, and also for the scores obtained in stromal cells ($p=0.173$, $p=0.502$,) and adipose tissue ($p=0.100$, $p=0.875$, Table 2).

Due to little variability of aromatase immunoreactivity in adipose tissue and low number of cases included in the HER2 subtype ($n=5$), further analysis focused on the differences in aromatase staining in tumor cells and stroma in the remaining breast cancer subtypes: luminal A, luminal B HER 2 negative and positive, and the triple negative subtypes.

No significant differences were observed regarding age, tumor size or lymph node metastasis (Table 2) within these groups.

In luminal A breast cancer cases, aromatase

Table 1. Aromatase scoring in tumor cells, stroma and adipose tissue in function of intrinsic subtype of breast cancer

Scoring	Luminal A		Luminal B, HER2 negative		Luminal B, HER2 positive		HER2		Triple negative		Total	
	n	(%)	n	(%)	n	(%)	n	(%)	n	(%)	n	(%)
Aromatase - Intensity Score (Tumor cells)	0, negative	1 (3.7%)	1 (8.3%)	0 (0.0%)	1 (20.0%)	0 (0.0%)	1 (20.0%)	0 (0.0%)	3 (4.4%)			
	1, weak positive	6 (22.2%)	1 (8.3%)	3 (21.4%)	1 (20.0%)	5 (50.0%)	16 (23.5%)					
	2, moderate positive	13 (48.1%)	7 (58.3%)	3 (21.4%)	1 (20.0%)	2 (20.0%)	26 (38.2%)					
	3, strong positive	7 (25.9%)	3 (25.0%)	8 (57.1%)	2 (40.0%)	3 (30.0%)	23 (33.8%)					
p = 0.195, p = 0.013												
Aromatase - % Score (Tumor cells)	0, <1%	0 (0.0%)	1 (8.3%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (1.5%)					
	1, 1-25%	1 (3.7%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (10.0%)	2 (2.9%)					
	3, >50%	26 (96.3%)	11 (91.7%)	14 (100.0%)	5 (100.0%)	9 (90.0%)	65 (95.6%)					
p = 0.492, p = -0.028												
Aromatase - % Score (Stroma)	0, <1%	6 (22.2%)	2 (16.7%)	1 (7.1%)	0 (0.0%)	0 (0.0%)	9 (13.2%)					
	1, 1-25%	4 (14.8%)	2 (16.7%)	2 (14.3%)	1 (20.0%)	2 (20.0%)	11 (16.2%)					
	2, 26-50%	2 (7.4%)	4 (33.3%)	1 (7.1%)	1 (20.0%)	1 (10.0%)	9 (13.2%)					
	3, >50%	15 (55.6%)	4 (33.3%)	10 (71.4%)	3 (60.0%)	7 (70.0%)	39 (57.4%)					
p = 0.502, p = 0.173												
Aromatase - % Score (Adipose tissue)	2, 26-50%	2 (7.4%)	1 (8.3%)	1 (7.1%)	0 (0.0%)	0 (0.0%)	4 (5.9%)					
	3, >50%	25 (92.6%)	11 (91.7%)	13 (92.9%)	5 (100.0%)	10 (100.0%)	64 (94.1%)					
p = 0.875, p = 0.100												
Total	27	100.0%	12	100.0%	14	100.0%	5	100.0%	10	100.0%	68	100.0%

n, number of cases.

intensity score in tumor cells was strongly inversely correlated with tumor grading ($p=-0.486$, $p<0.001$), particularly with nuclear polymorphism ($p=-0.356$, $p=0.025$) and mitotic count ($p=-0.169$, $p=0.014$) parameters of this score. Also, a strong negative correlation was found between Ki67 index and aromatase in stroma ($p=-0.448$, $p=0.048$). On the other side, stromal aromatase correlated positively, statistically significant with fibrocystic breast disease ($p=0.487$, $p=0.003$) and PgR ($p=0.380$, $p=0.026$) status. No significant correlations were found between tumor or stromal aromatase and ER scorings in luminal A subtype of breast cancer ($p=-0.240$, $p=0.663$, Table 2).

In luminal B, HER2 negative cases, a similar negative association was observed between aromatase intensity score in tumor cells and tumor grading, including parameters; however, the correlations did not reach the level of significance ($p=-0.597$, $p=0.359$). Most importantly, a strong positive association was observed between aromatase and ER percentage scores in tumor cells ($p=0.632$, $p=0.002$, Table 2). Analysis of aromatase stroma scoring did not show significant results in this subtype.

In luminal B HER2 positive cases, the association between tumor aromatase percentage score and the pathology variables could not be determined as the results were constant in this subgroup. However,

the stromal aromatase was positively associated with ER ($p=0.324$, $p=0.026$) and negatively with PgR percentage scoring ($p=-0.188$, $p=0.031$, Table 2). In this luminal B HER2 positive cases no statistically significant association was found between aromatase and proliferation parameters (Table 2).

In comparison with the rest of the previous groups, the analysis of triple negative cases showed a statistically significant difference regarding stromal aromatase which was a strong positive correlation with Ki67 index ($p=-0.359$, $p=0.007$). In this subgroup, the negative association between tumor aromatase intensity score and tumor grading was also observed, but at a p -value < 0.1 ($p=-0.549$, $p=0.072$). Furthermore, strong associations with fibrocystic breast disease ($p=-0.660$, $p=0.108$) and tumor size ($p=-0.441$, $p=0.274$) were identified; however, correlations did not reach the level of statistical significance. Associations between aromatase and ER could not be determined since the ER scores were constant in this subgroup. Little variation in PgR scoring (with low positivity in tumor) determined a strong correlation between tumor aromatase percentage score and PgR intensity score ($p=-0.745$, $p=0.007$); however, since only ten cases were used for the analysis and very different values were obtained when analyzing various combinations in PgR and aromatase scorings, this finding may be due to a simple overlapping of scores and not be suggestive of a functional connection.

Table 2. Association between aromatase scorings in tumor cells and stroma, and common histological tumor parameters, analyzed in the whole study group and within intrinsic subtypes of breast cancer

Aromatase scoring		Study Group			Luminal A			Luminal B, HER2 negative			Luminal B, HER2 positive			Triple negative		
		Intensity Score	% Score	% Score	Intensity Score	% Score	% Score	Intensity Score	% Score	% Score	Intensity Score	% Score	% Score	Intensity Score	% Score	% Score
		Tumor	Tumor	Stroma	Tumor	Tumor	Stroma	Tumor	Tumor	Stroma	Tumor	Tumor	Stroma	Tumor	Tumor	Stroma
Fibrocystic breast disease	n		70			27		12			14			10		
	p	0.497	0.534	0.258	0.509	0.741	0.003**	0.531	0.583	0.187	0.462	NC	0.524	0.108	0.700	0.202
	p	-0.164	-0.134	0.097	-0.129	-0.116	0.487	-0.138	-0.255	-0.102	-0.154	NC	-0.394	-0.660	-0.218	-0.047
Tumor size	n		70			27		12			14			10		
	p	0.503	0.190	0.822	0.815	0.407	0.084	0.276	0.250	0.446	0.239	NC	0.676	0.274	0.900	0.788
	p	-0.153	0.190	0.053	-0.016	0.237	0.021	0.094	0.522	0.464	-0.435	NC	-0.325	-0.441	-0.111	-0.215
Lymph node status	n		63			27		11			12			7		
	p	0.197	0.197	0.816	0.666	0.556	0.966	0.359	0.455	0.970	0.687	NC	0.091	0.388	0.571	0.233
	p	0.161	0.227	0.097	-0.021	0.175	-0.095	0.302	0.346	0.120	0.245	NC	0.630	0.322	0.354	0.322
Tumor grading	n		70			27		12			14			10		
	p	0.027*	0.687	0.200	<0.001**	0.782	0.473	0.359	0.244	0.016*	0.818	NC	0.790	0.072	0.893	0.139
	p	-0.361	-0.144	-0.094	-0.486	0.013	-0.326	-0.597	-0.457	-0.091	-0.209	NC	-0.273	-0.549	-0.311	-0.281
<i>Tubular differentiation</i>	n		70			27		12			14			10		
	p	0.073	0.719	0.697	0.106	0.572	0.205	0.613	0.761	0.257	0.349	NC	0.857	0.478	0.870	0.899
	p	-0.318	-0.169	-0.158	-0.511	-0.197	-0.484	-0.407	-0.210	0.149	-0.030	NC	0.151	-0.150	-0.166	-0.321
<i>Nuclear polymorphism</i>	n		70			27		12			14			10		
	p	0.125	0.787	1	0.025*	0.737	0.856	0.687	0.466	0.157	0.323	NC	0.713	0.145	0.690	0.022*
	p	-0.318	-0.119	-0.056	-0.356	0.029	0.010	-0.298	-0.340	-0.182	-0.332	NC	-0.430	-0.403	-0.266	-0.069
<i>Mitotic count</i>	n		70			27		12			14			10		
	p	0.535	0.504	0.790	0.014*	0.660	0.401	0.160	0.466	0.390	0.983	NC	0.456	0.221	0.700	0.399
	p	-0.156	-0.086	-0.032	-0.169	0.170	-0.153	-0.289	-0.330	0.004	<0.001	NC	-0.250	-0.371	-0.218	-0.423
ER Status	n		68			27		12			14			10		
	p	0.249	0.548	0.390	0.633	0.741	0.426	0.480	0.500	0.392	0.436	NC	0.444	NC	NC	NC
	p	0.122	0.056	-0.136	-0.240	-0.116	0.228	0.163	0.302	0.251	0.311	NC	0.255	NC	NC	NC
<i>ER – Percentage Score</i>	n		68			27		12			14			10		
	p	0.005**	<0.001**	0.160	0.756	0.889	0.798	0.046	0.002**	0.387	0.100	NC	0.026*	NC	NC	NC
	p	0.146	0.143	-0.067	-0.163	-0.069	-0.025	0.171	0.632	0.125	0.440	NC	0.324	NC	NC	NC
<i>ER – Intensity Score</i>	n		68			27		12			14			10		
	p	0.455	0.007**	0.140	0.633	0.741	0.426	0.342	0.195	0.091	0.397	NC	0.836	NC	NC	NC
	p	0.104	-0.048	-0.036	-0.240	-0.116	0.228	0.443	0.443	0.308	0.311	NC	0.028	NC	NC	NC
PgR Status	n		68			27		12			14			10		
	p	0.637	0.472	0.422	0.905	0.778	0.026*	0.634	0.883	0.187	0.823	NC	0.540	NC	NC	NC
	p	-0.034	-0.149	0.138	-0.129	-0.105	0.380	-0.073	0.135	0.539	0.160	NC	-0.022	NC	NC	NC
<i>PgR – Percentage Score</i>	n		68			27		12			14			10		
	p	0.647	0.207	0.280	0.918	0.627	0.134	0.442	0.351	0.485	0.274	NC	0.031*	0.574	0.900	0.788
	p	0.009	0.030	0.018	-0.007	-0.093	0.264	-0.238	-0.235	0.459	0.008	NC	-0.188	-0.315	0.111	0.215
<i>PgR – Intensity Score</i>	n		68			27		12			14			10		
	p	0.598	0.019	0.263	0.791	0.926	0.056	0.283	0.065	0.690	0.730	NC	0.656	0.645	0.007*	0.316
	p	0.009	0.003	-0.053	-0.196	-0.055	0.422	0.268	<0.001	0.248	-0.211	NC	-0.422	-0.470	-0.745	-0.326
Her2/neu status	n		68			27		12			14			10		
	p	0.179	0.544	0.577	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC
	p	0.163	0.134	0.152	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC
Ki67 index	n		68			27		12			14			10		
	p	0.289	0.568	0.421	0.392	0.481	0.048*	0.854	0.917	0.141	0.494	NC	0.817	0.108	0.900	0.007**
	p	-0.168	-0.129	-0.172	-0.318	-0.204	-0.448	0.049	-0.091	0.273	-0.028	NC	-0.254	-0.126	-0.111	0.359

n, number of specimens; p-value <0.05 in bold; *correlation is significant at the 0.05 level; **correlation is significant at the 0.01 level; Spearman's correlation coefficient $p < -0.1$ and $p > 0.1$ in bold, when p is statistically significant; NC, not computed (analyzed parameter is constant).

Our results described patterns in aromatase immunoreactivity in the major compartments of breast tumors and detailed the differences observed based on the intrinsic subclassification of tumors.

DISCUSSION

In our study, we described aromatase expression in the major tissue compartments of breast malignant tumors by immunohistochemistry method, using anti-aromatase polyclonal antibody PA1-21398 (Thermo Fischer Scientific), which was successfully used in studies detecting aromatase either by immunohistochemistry (12,13) or Western Blot (14) methods.

Previous viewpoints of various investigators researching the localization, association with histopathological variables, and the prognosis prediction value of aromatase varied greatly depending upon the methodology used (immunohistochemistry with monoclonal or polyclonal antibodies, quantitative PCR, etc.) (15). Regarding immunohistochemistry, available data favoured the usage of polyclonal rather than monoclonal antibodies for aromatase detection in archived tumor samples (15–17); however, newer variants of monoclonal antibodies were developed with promising results in retrospective analysis of tissue samples (11,18).

In the early studies, accurate evaluation of aromatase expression and activity in breast cancer was difficult to use clinically due to unsatisfactory specificity and/or restricted availability of anti-aromatase antibodies (15,16,19,20), which is at the base of shifting significance of results reported in latest research. Initial results showed that aromatase was found in both primary and metastatic breast cancer tissue, and its activity was similar for small and large tumors (21), and did not influence either disease-free or overall survival in breast cancer patients (22). Newer immunoassays opened the arena for aromatase studies in relation with various clinical and biological parameters.

Intrinsic molecular classification of breast cancers is currently the most important prognosis and management guiding system. We analyzed aromatase immunoreactivity scorings in the breast cancer subtypes and no statistically significant difference in aromatase staining results for each analyzed tumor compartment in the breast was confirmed. Similar data was previously reported (20).

Further, we analyzed within these subgroups,

different particularities of association between aromatase immunoreactivity scores and common histological parameters of breast tumors.

In our study, tumor aromatase expression was inversely associated with histological tumor grading in the whole study group. When analyzing results based on intrinsic subtypes of cancer, the results showed a stronger correlation between these parameters for the luminal A subtype (tumor grading score: $p=-0.486$, $p<0.001$, and also for tumor grading parameters: tubular differentiation: $p=-0.511$, $p=0.106$; nuclear polymorphism: $p=-0.356$, $p=0.025$; mitotic count: $p=-0.169$, $p=0.014$, Table 2). A similar inverse association was observed for the other subtypes of breast cancer; however, the results did not reach the level of statistical significance.

Regarding ER status, a positive correlation was found between tumor aromatase and ER percentage scores ($p=0.143$, $p<0.001$) in the entire study group. Intrinsic subtypes analysis showed an even stronger association between the tumor aromatase and ER in luminal B HER2 negative tumors ($p=0.632$, $p=0.002$), and moderate level of association in luminal B HER2 positive tumors ($p=0.324$, $p=0.026$), pleading for an autocrine effect of estrogens in tumor cells.

In early studies, tumor aromatase was inversely correlated with ER status (23–25) or had no correlation with ER (20,26). However, in the studies of Brodie *et al.* (27) and Miki *et al.* (28), aromatase mRNA was detected in breast tumoral tissue and the authors observed a trend for ER-positive tumors to express aromatase (27), fact which was also observed in our study, but mainly in luminal B subtype, with higher association in HER2 negative tumors.

Furthermore, stromal aromatase showed a moderate positive association, statistically significant, with fibrocystic breast disease ($p=0.487$, $p=0.003$) in luminal A breast cancer subtype, suggesting a possible paracrine mechanism involved in the disease pathogenesis. Also, in luminal A subtype, the Ki67 proliferation index was negatively associated with stromal aromatase percentage score ($p=0.448$, $p=0.048$), being congruent with the better prognosis of luminal A subtypes of cancer. Aromatase present in stroma within breast tumors, as in surrounding tissues, may be suggestive that estrogen synthesis within the tumor may modulate tumor growth via a paracrine mechanism (20,29,30).

Inversely, in triple negative tumors, a positive correlation was observed between stromal aromatase and the proliferation index Ki67 ($p=0.359$, $p=0.007$),

which is in accordance with a poorer prognosis of these tumors, as they do not express hormone, and particularly, estrogen receptors.

Initial studies, that described the relation between aromatase and tumor proliferation markers, showed no statistically significant correlations (20,30). Other reports attributed a better prognosis of breast cancers expressing aromatase, but it was mainly due to the association with positive ER status (15). However, recent results published by Kanomata *et al.* (17) in 2017, which included a larger database of 221 invasive breast cancer samples, showed that aromatase was significant inversely correlated with tumor and lymph node invasion status (TNM classification), tumor stage, histologic grade, and Ki67 index, results which are similar with our own. However, tumor aromatase expression was independent of ER, PgR and HER2/neu in their results (17), and the analysis did not include variations of aromatase within intrinsic molecular subtypes of tumors.

Even though our study included the analysis of 70 breast cancer tissue samples, an important limitation is represented by the low numbers of cases attributed in each intrinsic subtype based on the molecular expression of tumor cells; however the results are still indicative of different roles aromatase may play in the pathogenesis of each subtype of tumor.

The continuous development of antibodies for aromatase made possible to better detect aromatase immunoreactivity in tissue sections, and the latest studies (11,31,32), including our own, indicate that aromatase may be useful as a prognostic or therapeutic marker in addition to ER and PgR, especially for patients with hormone-dependent breast cancer.

In conclusion, aromatase immunohistochemistry using polyclonal antibodies was proved useful in demonstrating the aromatase expression in breast tumor tissue and local tumor environment, showing important differences between the intrinsic subtypes of breast cancer.

Tumor aromatase was inversely associated with tumor grading and Ki67 index in luminal variants of breast cancer, especially in luminal A, and this effect was independent of ER status. ER status was positively associated with tumor aromatase in luminal B, and not luminal A tumors, with stronger correlations being observed in HER2 negative than in HER2 positive forms of luminal B tumors. In contrast, stromal aromatase in triple negative tumors showed a positive association with Ki-67 index, fact which is congruent to the known less favorable prognosis of these tumors.

Larger studies are needed to better characterize

these relations and evaluate potential connections in response to therapy.

Conflict of interest

The authors declare that they have no conflict of interest.

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