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Original Research

PRMT5 and FOXP1 expression profile in invasive breast cancer patients undergoing neoadjuvant chemotherapy

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Abstract: Neoadjuvant chemotherapy is the standard treatment for patients with advanced localized breast cancer, but today it has found a good place in the early stages to achieve a negative surgical margin and increase the possibility of breast preservation. Numerous studies have shown that patient survival increases with a complete pathological response in the relationship of some immunological molecules known as immunohistochemistry markers. The aim of this study was to investigate the complete pathological response in the relationship between PRMT5 and FOXP1 expression. In a cross-sectional study of breast cancer patients in stages I to III, who were treated with Neoadjuvant chemotherapy at the Breast Cancer Research Institute during the years 2018 to 2019, were examined. A complete pathological response was obtained in cases where no tumors remained in the breast and axillary tissue after surgery. Immunohistochemical analyzes for FOXP1, PRMT5, and PR and ER biomarkers of the tumor were conducted. Data were analyzed using descriptive and analytical statistics by SPSS v. 21 software. 157 patients with a mean age of 47.5 ±16.2 years were included in the study. Our results revealed that there was no significant difference between the foxp1 Positive and Negative patients, in terms of cancer stage, metastasis, being PR or ER-positive (P>0.05). While being PRMT5+/- had a significant relationship with FOXP1 expression (p=0.001). In the case of the response to treatment, there was a significant association between a complete response and being foxp1 + (p=0.01). While in other immunohistochemistry markers, no significant association was found (P>0.05). Our study revealed no association of foxp1 and PRMT5 with other biomarkers of breast cancer and clinical progress of the disease. Our study revealed no association of foxp1 and PRMT5 with other biomarkers of breast cancer and clinical progress of the disease.

Key words: Breast cancer; PRMT; FOXP1; Chemotherapy.

Introduction

Breast cancer is not only the second leading cause of cancer-related death in American women but also this disease is a common cause of death in other women globally (1). Malignant cells in this disease are getting uncontrollable development Many genetic factors, as well as immunohistochemical markers, are used to determine the disease in addition to the conventional diagnostic criteria such as size and cancer stage (2). Receptors of progesterone (PR) and estrogen (ER), HER2 and Ki67 are several molecular markers for cancer patients' diagnosis and treatment (3). Based on these factors, breast cancer is classified into the following categories: Luminal A, Luminal B, HER2, Basal, and Regular (4). For example, the HER2, PR, and ER markers are all negative in the basal-like group. One way to diagnose cancer is by using data from the expression of patient genes (5). Advances in bioinformatics technology, in particular microarray technology, have led to the simultaneous extraction of gene expression data from thousands of genes linked to a single cancer sample (6). Some breast cancers (e.g. basal-like subtypes) are more susceptible to preoperative chemotherapy bearing doxorubicin or paclitaxel than that of the luminal and normal-like cancers (7). Post-translational methylation of arginine is responsible for controlling the many biological processes. Protein arginine methyltransferase 5 (PRMT5, Capsuleen, Jbp1, Skb1, Dart5) is mainly responsible for mono- and symmetric dimethylation of arginine (8). A growing literature shows its essential biological work across a wide range of cellular processes (9)

Protein methylation, including histone by PRMT5 coordinates primordial germ cells (PGCs), cell cycle, genome organization, spliceosome assembly, transcription, stem cells as well as proliferation (10). Furthermore, recently collected evidence shows that concentrations of PRMT5 in cancer tumorigenesis label them as potential oncogenesis as indicators of poor clinical outcomes (11). PRMT5 is vital for the regulation of stem cell survival of breast cancer through Forkhead box P1 (FOXP1) epigenetic regulation. This molecule is also associated with different types of tumors (12). Since several works indicate that this molecule might have a remarkable role in breast cancer cell proliferation via

regulation of estrogen signaling and also this molecule may be correlated with clinical breast cancer depend on estrogen (13). Furthermore, this molecule is affected by repeated translocations of chromosomes and overexpression of this molecule gives a poor prognosis in a number of lymphoma forms and in this way, it might act as an oncogene. FOXP1, on the other hand, locates a tumor suppressor locus at 3p14.1, and expression loss in this molecule in case breast cancer is linked with worse results, indicating that this molecule may act as a tumor suppressor in other tissue types (14). Therefore, in this study, it was to test both PRMT5 and FOXP1 in subjects with breast cancer besides other molecular factors.

Materials and Methods

In this descriptive-analytical study, records of all women with breast cancer referred to Pathology Centers and Diagnosis of Breast Cancer between the years 2018 to 2019 were evaluated. At first, the histopathological slides of patients were examined based on the type of tumor and its degree using Bloom Richardson's system. Throughout this work (unless stated), formalin-fixed paraffin-embedded specimens were obtained through biopsy or surgery. These studies were confirmed by the ethics committee, and all patients filled informed consent. Patients underwent breast surgery after chemotherapy, and breast and axial tissue samples were sent to pathology. Patients who had no invasive tumor in the tissue sample were placed in the complete pathological response group, and patients who had remaining invasive tumor cells were considered as the relative response group. Demographic characteristics, the status of the immunohistochemistry profile of cancer including PR and ER biomarkers of the tumor, and the patient's condition in the follow-up in terms of disease recurrence and death of the patient were extracted from records. Inclusion criteria were the availability of information in the records and the biopsy sample.

Immunohistochemically method

Immunohistochemical analyzes for FOXP1 were carried out by using an EnVision+visualization kit (Dako, Carpinteria, CA). 6 μm tissue parts were deparaffinized, dehydrated by ethanol, and rinsed with 0.05 percent Tween 20 in Tris-buffered saline. Sections were heated in a 121 ° C autoclave for 15 min in a 10 mM sodium citrate buffer (pH 6.0) to extract antigens. 0.3 percent hydrogen peroxide was used to block endogenous peroxidase activity, and the parts were incubated for 30 min in 10 percent bovine fetal serum. FOXP1 antibody (polyclonal; 1:1,000 dilution) was applied and samples were left for incubation overnight at 4 °C. They were rinsed in TBST and then incubated at room temperature for 1 h with EnVision+HRP-labeled polymer (antirabbit). Using the 3,3′-diaminobenzidine substrate kit for peroxidase (Vector Laboratories, Burlingame, CA), the antigen-antibody complex was visualized. Rabbit IgG was applied as a negative control in place of the primary antibody. All slides were assessed for the proportion of positively stained cells. Based on the scoring method provided by Allred et al. (16), two experts separately analyzed the score of the tissues. FOXP1 Immunostainig scores of 0 to 2 were considered negative and 3 to 8 as positive (14).

Again, tumor tissues trapped in formalin-fixed paraffin were used for the PRMT gene analysis assay. Representative areas of the invasive carcinomas in the breast were identified by an expert pathologist. Tissue parts were boiled for 40 min after deparaffinization and rehydration in a 10 mM citrate buffer (pH 8.0 at 95 ° C). The slides were then incubated in sterile water with 5 percent hydrogen peroxide to inhibit the development of endogenous peroxidases, then with the anti-LKB1 or anti-PRMT5 antibody at 37 ° C for 1 hour. Subsequently, all immunohistochemical slides were incubated with a secondary biotinylated antibody attached to a streptavidin peroxidase conjugate (Envision Flex kit Ref: K800021–2, Dako). By incorporating the substrate 3,3-diaminobenzidine, bound antibodies were visualized. Blinded to clinical evidence, the detection of PRMT5 was reported as same as previous genes by two pathologists, who separately measured the percentage and strength of nuclear and cytoplasmic staining. PRMT5-amount of each sample got a score based on the number of stained cells and intensity of staining according to the previously published study (17).

Statistical analysis

Statistical analysis was performed by using SPSS v.21. Dischominous variables were compared for verities in the distribution of data by Chi-square. Continues variables were compared between the Dischominous grouping of patients by T-test and ANOVA in case of parametric data or man-witney and Kruskal walis test in case of non-parametric data. *p*-values less than 0.05 were considered statistically significant.

Results

In the present study, 157 patients were examined. The mean age of participants was 47.5 ±16.2 years. According to the results of Table 1, the most prevalent stage of disease was stage III (37.0% of patients), and there was metastasis in 54.5% of cases. PR+ happened in 51.9 % of patients; while ER+ happened in 52.2%. Foxp1 was positive in 44.8% of cases and PRMT5 in 44.2%.

The association of foxp1 and PRMT5 with the Patient's characteristics and immunohistochemistry profile was evaluated. There was no remarkable difference

Table 1. Patient's characteristics and immunohistochemistry profile.

		Frequency	Percent
Stage	I	56	36.4
	II	41	26.6
	III	57	37.0
Metastasis	Positive	70	45.5
	Negative	84	54.5
Er	Positive	82	52.2
	Negative	72	45.8
Pr	Positive	80	51.9
	Negative	74	48.1
Foxp1	Positive	69	44.80
	Negative	85	55.2
Prmt5	Positive	68	44.2
	Negative	86	55.8

Table 2. Association of foxp1 and PRMT5 with immunohistochemistry profile.

		Positive Foxp1	Negative Foxp1	*p*	Positive Prmt5	Negative Prmt5	*p*
Stage	I	22	34	0.959	31	25	0.916
	II	16	25		24	17	
	III	21	36		31	26	
Metastasis	Yes	43	27	0.952	36	34	0.314
	No	52	32		50	34	
PR	Positive	53	27	0.226	49	31	0.160
	Negative	42	32		31	25	
ER	Positive	61	21	0.067	48	34	0.894
	Negative	48	24		39	33	

Table 3. The correlation of studied variables and age.

	r	*p*
foxp1 vs. Age	0.101	0.351
PRMT5 vs. Age	0.067	0.407

Table 4. Association of immunohistochemistry profile and response to neoadjuvant treatment.

	foxp1 +		PRMT5 +		ER +		PR +	
	n	%	n	%	n	%	n	%
Relative response	26	16.88	31	26.62	41	24.03	35	19.48
Complete response	43	27.92	33	29.22	41	24.03	39	21.43
p-value	0.001		0.623		1		0.519	

between the foxp1 Positive as well as Negative patients, in terms of cancer stage, metastasis, being PR or ER-positive (p>0.05). While being PRMT5+/- had a significant relationship with FOXP1 expression (p=0.001) (Table 2).

The Pearson correlation test was performed to see the relationship between the foxp1 gene and two variables, PRMT5, and age. No significant correlation was observed between the foxp1 gene with age (p=0.351, r=0.101, n= 154). Also, no significant correlation was found between PRMT5 gene with age (p=0.351, r=0.067, n= 0.407) (Table 3).

In the case of the response to treatment, no significant association was found between a complete response and being foxp1 + (p=0.01)., please check this sentence, meaning? (I think significant association was found between a complete response and being foxp1), but no significant association was seen (p>0.05) in the other immunohistochemistry markers (Table 4).

Discussion

Our analysis showed that foxp1 and PRMT5 are not associated with other markers of breast cancer immunohistochemistry and not the stage of disease or metastasis. Although there was a major correlation between maximum response and foxp1 + being.

FOXP1 plays a remarkable role in breast cancer cell proliferation by modulating estrogen signaling. This molecule is also correlated with clinical breast cancer dependency on estrogen that could help to predict favorable prognosis in patients treated with tamoxifen (14). This current study has not found any connection between progesterone or estrogen and FOXP1. The estrogen receptor positivity in this study was measured as raw, regardless of the degree or extent of its positivity, which may be one of the explanations why the findings of this study were specific and unrelated to the pathological response or the FOXP1 response. Many studies have found an inverse association between the status of hormonal receptors and a complete pathologic response. In the ECTO experiment, the estrogen receptor status was the only predictor that affected the full pathological response (18). PRMT5 is a vital regulator for stem cell survival of breast cancer through FOXP1 epigenetic regulation. As a result, inhibitors of PRMT5 could potentially kill stem cells of cancer and thus prevent tumor recurrence (19).

Yang and his coworkers have been previously reported that PRMT5 was overexpressed in the breast cancer cells and tissues. In their work, they proposed that PRMT5 could play a significant role by activating the signaling pathway to the NF-κB (20). Huang et al. have also revealed that PRMT5 is a prognostic factor for survival of patients with this disease. In breast cancer cases, high expression of PRMT5 favors a better prognosis. In the previous research, reported that there was no substantial difference in the stage of breast cancer (21). Koon and his coworkers proposed that FOXP1 could be useful in predicting prognosis and could also be used to establish therapeutic strategies oriented to FOXP1 (22).

The overall current study indicated that there was no association of FOXP1 and PRMT5 with other biomarkers of breast cancer and clinical progress of the disease even though FOXP1 was associated with better response to neoadjuvant chemotherapy. This study also had some limitations. First of all, the sample size is small. Second, we had a lack of information related to the detail of the patient's treatment regimen. Studies with larger sample sizes and follow-ups of patients in terms of overall survival and disease-free survival might be useful to bet-

ter understand the underline mechanism of FOXP1 and PRMT5 in breast cancer patients.

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