Available online at http://www.sjomr.org

SCIENTIFIC JOURNAL OF MEDICAL RESEARCH

Vol. 2, Issue 5, pp 1-6, Winter 2018

ISSN: 2520-5234

ORIGINAL ARTICLE

Prognostic significance of FOXP3+tumor-infiltrating lymphocytes in Basrah women of breast cancer

Hadeel S. Touma[1] , **Wafaa S. Shani**[1] and **Saad Abdullah**[2]

[1] Department of Biology, College of Science, University of Basrah, Basrah, Iraq.
[2] College of Medicine, University of Basrah, Basrah, Iraq.

ARTICLE INFORMATIONS

Article History:
Submitted: 6 December 2017
Revised version received:
25 December 2017
Accepted: 2 January 2018
Published online: 1 March 2018

Key words:
FOXP3
Breast
Cancer
Lymphocyte

Corresponding author:
Hadeel S. Touma
Email: fatimaalzahraa134@yahoo.com
Department of Biology
College of Science
University of Basrah
Iraq.

ABSTRACT

Objective: FOXP3 is a forkhead box transcription factor, a defining hall mark of Treg cells, and its functions as the master regulator in the development and function of regulatory T cell. Treg cells are sub set of T lymphocytes that the main functions regulate the immune response through suppress the proliferation and cytokines production of effector T lymphocyte. The infiltration of FOXP3+ regulatory T cells into invasive tumors has been reported to be associated with survival in a variety of cancers. The prognostic significance of FOXP3+ tumor infiltrating lymphocytes (TILs) in breast cancer, however, remains controversial. We investigated whether there were significant numbers of FOXP3-positive Tregs in breast cancer using immunohistochemistry, and whether the presence of FOXP3-positive Tregs was associated with other prognostic factors, such as stage or histologic grade, receptor status, lymph node involve and metastasis.

Methods: A tissue microarray (TMA) including 24 ductal and 11 lobular breast cancers was stained with antibodies recognizing FOXP3 by immunohistochemical techniques. Foxp3+Lymphocyte counts were correlated with clinicopathological parameters.

Results: The Foxp3-positive was more prevalent whereas Foxp3-negative was rare. Lymph node displayed significantly ($p < 0.01$) higher FOXP3+ lymphocyte infiltration and no metastasis was also significant ($P<0.02$) associated with higher FOXP3+ TIL counts. In contrast, FOXP3+ lymphocyte infiltration was not linked with other clinic pathological parameters.

Conclusion: Our results showed a significant increase in the proportion of these cells in the tissue of breast cancer patients. The prognostic value of FOXP3+ TILs in breast cancer differs depending on lymph node and metastasis.

Citation: Touma H.S., Shani W.S. and Abdullah S. "Prognostic significance of FOXP3+tumor-infiltrating lymphocytes in Basrah women of breast cancer". Sci. J. Med. Res. 2018; 2 (5): 1-6.

INTRODUCTION

Breast carcinoma is one of the leading causes of cancer worldwide, In Iraq; Breast cancer is the most common cancer in women and the second cause of cancer related deaths[1]. FoxP3is a forkhead box transcription factor, also called scurfin, belongs to the forkhead/winged-helix family of transcriptional factors. This transcription factor plays an important role in the development and function of immune regulatory T cells (Tregs), and can be used as a specific biomarker for the identification of Tregs within an inflammatory infiltrate[2] .

Treg cells are sub set of T lymphocytes that the main functions regulate the immune response through suppress the proliferation and cytokines production of effector T lymphocyte. Regulatory T cells represent about 5% of circulating CD4+ T lymphocytes in the human peripheral blood. An increased number of Tregs has been observed in the blood, in the tumor mass and in the draining lymph nodes of patients with different solid tumors[3, 4]. The increased frequency of tumor-infiltrating Tregs have been associated with poor survival in breast[5], gastric[6], ovarian[7], lung[8], hepatocellular[9], renal[10], and pancreatic[11] cancers.

In breast cancer, they are increase in the percentage of Treg cells increases in parallel with the disease stage, from normal to ductal carcinoma in situ (DCIS) and from DCIS to invasive carcinoma. A high frequency of tumor-infiltrating FOXP3+ cells correlates with worse disease-free survival and decreased overall survival in patients with invasive breast carcinoma, suggesting that the presence of Treg cells promotes tumour progression by creating an immunosuppressive environment[5]. Current work was designed to throw a light on the clinical significant of Foxp3 in breast cancer diagnosis that mean as a prognostic factor and investigation of their role in immune suppression in breast cancer patients In breast cancer specifically, only a few studies reported on the role of the marker, in which expression of HLA Class I was related to a better prognosis, Abdul-Jabbar[12] ABO blood group polymorphism, cytogenetic analysis of cultured blood lymphocytes, serum level of estradiol, immunohistochemical evaluation of estrogen, progesterone, HER-1 and HER-2 receptors, tumor-infiltrating immune cells defined by CD4, CD8 and CD68 markers, tumor expression of T-bet, GATA3, IL-17A and FOXP3, and mutational change in exon 3 and exon 7 of estrogen-β gene) of breast tumor (benign and malignant) in a sample of Iraqi female patients and associated with a better prognosis; Alghaliby[13] also focus on mutations of breast cancer susceptibility genes 1and 2 (BRCA1 and BRCA2), Rashed[14] described that the level of interleukin-6 (IL-6) and heat shock protein (HSP70) in the patients serum and responsible role in the evasion from immune surveillance.

MATERIALS AND METHODS

Patients

Between March 2016 and 2017, 35consecutive breast cancer patients who underwent mastectomy at Basrah general Hospital were registered. All patients were women with ages ranging from 21 to 62 years. None of the patients received neo adjuvant chemotherapy. Paraffin-embedded material of 35 breast cancer patients, including 24 IDC and 11DCIS, was used for histological analysis, diagnosed at the Institute for Pathology at Basrah general Hospital (Table 1). Tumour typing and staging were performed according to the classification of the International Union against Cancer[15], TNM classification, tumor diameter and hormone receptor (estrogen receptor (ER), progesterone receptor (PR) HER2 and human epidermal growth factor receptor-2) were obtained from pathology reports.

Detection and evaluation of Foxp3 expression

Cancerous Foxp3 positive infiltrating cells were detected by immunohistochemistry. Paraffin embedded sections of 4 µm thickness were generated. Deparaffinized and soaked in phosphate buffered saline (PBS) for immunohistochemical analysis. Antigen retrieval treatment was performed at 120°C for 5 min in a 10 mM sodium citrate buffer (pH 6.0) as reported previously. Prepared specimens were soaked with 3% H2O2 for 30 min to block endogenous peroxidase activity, and blocked in bovine serum albumin for 30 min to reduce nonspecific binding. Sections were incubated Anti-FOXP3 antibody [236A/E7] ab20034 undiluted according to the manufacturer's instruction. overnight at 4°C, rinsed in PBS and visualized by standard techniques for labeled avidin-biotin immuno-peroxidase staining. Foxp3 positivity was visualized using a DAB Substrate Kit. Finally, sections were counterstained with haematoxylin and mounted with glycerol gelatin. The amounts of tumour infiltrating FoxP3+ cells were evaluated semi quantitatively by analyzing the complete surface of the tissue section A Human tonsil tissue, was obtained and used as the internal positive control Foxp3. For Foxp3, intensity of tumor staining (absent (undetectable or faint in <20% of the cells), weak (faint to weak in 20% but ≤70% of the cells), moderate (weak to moderate in >70% of the cells) or strong intensity (intense in 20-70% of the cells[16].

Statistical analysis

Statistical analysis of the clinical features was performed by the using SPSS version 22 and comparisons of the degree of infiltration between the two groups were performed by Chi square test. A *p* value of less than 0.05 was considered to be statistically significant.

RESULTS

Histological distribution of Foxp3 positive cells in breast cancer

The Foxp3 expression was detected in the nucleus and cytoplasm of breast cancer cells (Figure 1: A, B), interestingly, most analyzed breast cancers were strongly infiltrated by FoxP3+ cells. There was a significant higher Intratumoral foxp3 expression as checked in tumor bed than normal tonsil tissue (as positive control); overall, 85.71%(30/35) of breast cancer lesions were classified as Foxp3 positive (Table 2) . High numbers of FoxP3+ cells were observed with poor prognosis.

FoxP3+ cells were observed in nearly all analyzed breast tissues, and both the tumour epithelium and the surrounding stroma were infiltrated by these cells, but the number and topographic distribution within the tumor varied from case to case. The distribution of the positive cells could be categorized into 3 major immunohistochemical patterns: weak, moderately and strong (Table 2, Figure 1: C, D, E and F).

Table1: **Breast cancer distribution according to stage, grade and receptor.**

Variable		Foxp3	
		No.	**%**
ER	Positive	31	88.57
	Negative	4	11.43
PR	Positive	31	88.57
	Negative	4	11.43
HER	Positive	8	22.86
	Negative	27	77.14
Grade	GI	3	8.57
	GII	20	57.14
	GIII	12	34.29
Stage	T1	8	22.86
	T2	18	51.43
	T3	8	22.86
	T4	1	2.86
Lymph node	Positive	27	77.14
	Negative	8	22.86
Metastasis	Positive	11	31.43
	Negative	24	68.57

Chi square test was used to assess the statistical analysis
ER: estrogen receptor; HER2: human epidermal growth factor receptor-2, PR: progesterone receptor

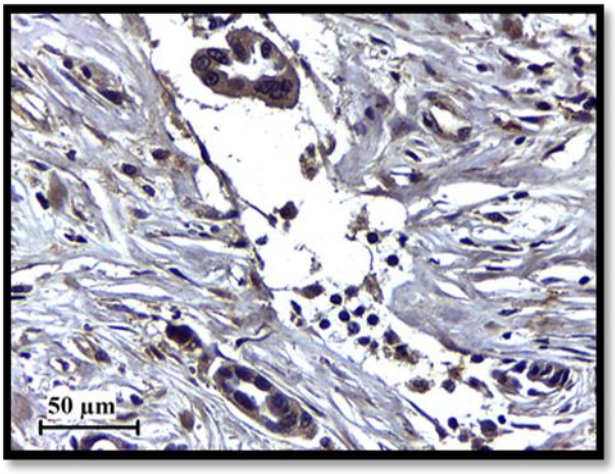
Clinicopathological features of Foxp3 positive breast cancer patients

The tumor size was inversely associated with Foxp3 expression. Foxp3 positive lymphocytic infiltration was not associated with clinicopathological factors except for lymph node and metastasis (Table 3).

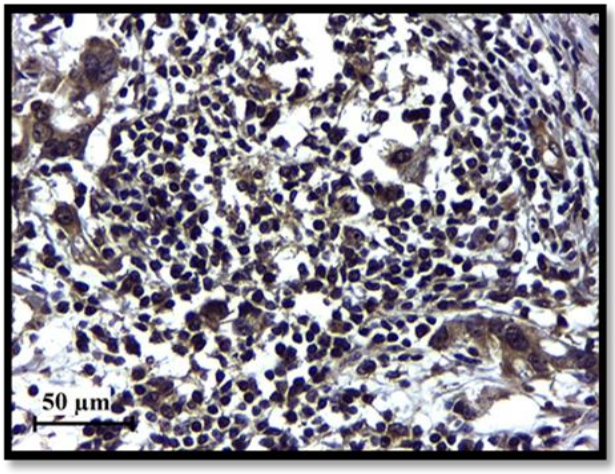
Table 2: **Distribution of Breast cancer patients according to Foxp3 (T regulatory marker) expression.**

Studied marker	Tumor reaction	Total		Reaction score					
				Weak		Intermediate		Strong	
		No	%	No	%	No	%	No	%
Foxp3	Positive	30	85.71	4	13.33	19	63.33	7	23.33
	Negative	5	14.29						
P value					0.012				

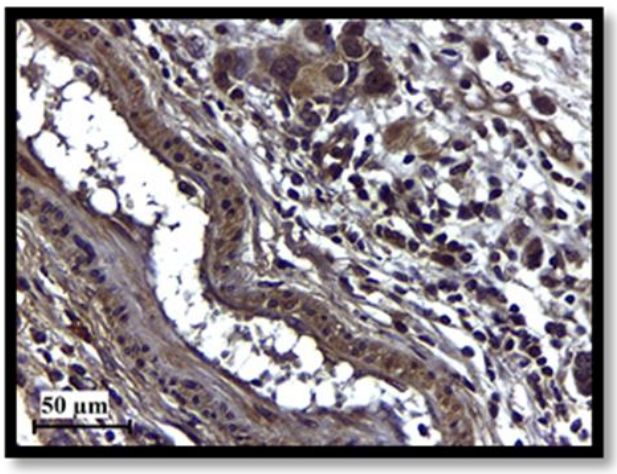
Chi square test was used to assess the statistical analysis



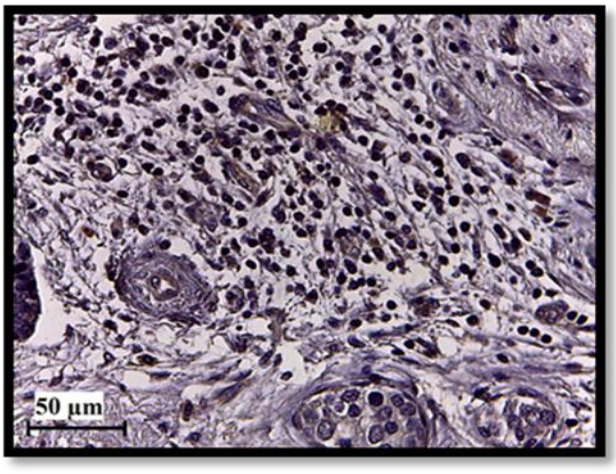
A



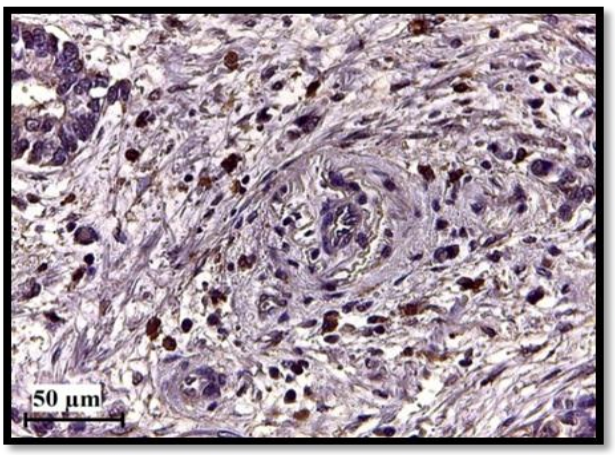
C



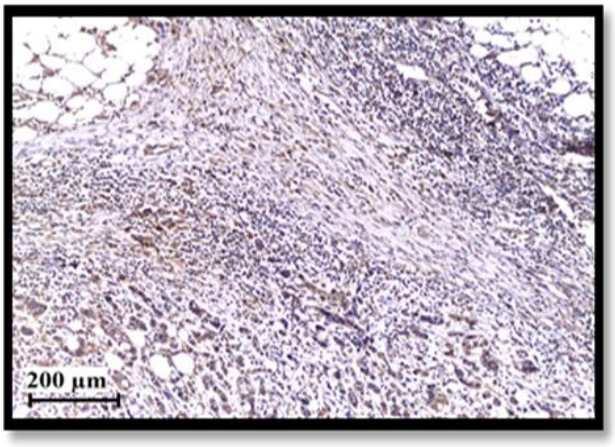
B



D



E



F

Figure1: **Breast cancer section showed Invasive breast carcinoma section showed immunohistochemical stain for Foxp3 within cytoplasm and nucleus (A, B). Immunohistochemical stain for Foxp3 with strong positive staining (C); moderate positive staining (D), weak positive staining (E), (F) Invasive breast carcinoma section showed immunohistochemical stain for Foxp3 (10X).**

Table 3: **Association between clinical factors and foxp3 expression in breast cancer patients.**

Variable		Foxp3 expression		P value
		Positive (n=30)	Negative (n=5)	
Stage	T1	4	2	0.073
	T2	11	0	
	T3	8	0	
	T4	7	3	
Grade	G1	3	1	0.807
	G2	14	2	
	G3	10	2	
Lymph node	Positive	19	0	0.01
	Negative	11	5	
Metastasis	Positive	0	2	0.02
	Negative	30	3	
ER	Positive	19	4	0.467
	Negative	11	1	
PR	Positive	18	2	0.403
	Negative	12	3	
HER-2	Positive	3	0	0.460
	Negative	27	5	

Chi square test was used to assess the statistical analysis

DISCUSSION

In this study, Foxp3 positivity was 85.71%, this finding demonstrates that malignant epithelial tumors are enriched with FOXP3 expressing Tregs whereas weak or no Foxp3 expression was detected in the respective normal counterpart, these might contribute to tumor induced shutdown of an effective antitumor immune response. This is in agreement with previous reports that have described tumour-infiltrating Foxp3 in cancer as follow: Gobert *et al*[16]. reported the Foxp3 Treg presence within lymphoid infiltrates surrounding the tumor was associated with a higher risk of relapse and death. Liu *et al.*[17] found that foxp3 Treg are poor prognostic indicator in ER+ breast cancer, but favorable prognostic factor in HER+/ER- subtype, also, they detected that the prognostic value of FOXP3+ TILs in breast cancer differs depending on ER and HER2 expression status and CD8+ T-cell filtration. According to Banerjee *et al.*[18] Foxp3+Treg were increased in the triple negative tumors, Cause and effect cannot be determined from this type of association study, but for the HER2+/ER– group at least one interpretation consistent with the data is that FOXP3+ TILs are induced secondarily by a robust antitumor CD8+ TIL response.

Foxp3+Treg cells are potentially able to DC and T cell functions which are mediated by IL-10 and transforming growth factor-β respectively. In this series, positive Foxp-3 expression was only associated with lymph node and metastasis but not stage and grade, which disagrees with the significant association with T factor. Nodal involvement and lymphovascular invasion in esophageal, lung and gastric cancer. This agrees with numerous studies of samples from human cancer patients that showed a positive correlation between FOXP3 expression and poor prognosis, especially with metastasis[19]. The association of the prognostic and predictive markers estrogen and progesterone receptors (ER and PR), and her2/neu expression with infiltration by Treg cell (Foxp3 expression) was then explored, no association between Treg infiltration and these receptors was detectable, Furthermore, in triple negative, foxp3 infiltration was also found.

In a more recent investigation, FOXP3 expression in inflammatory breast cancer (an aggressive subtype of breast cancer with the worst survival outcome amongst all breast cancers) was evaluated, and the presented data suggested that FOXP3 may be an effective tumor target in the disease[20].

Notably, Foxp3 has been demonstrated an important role of modulating the expression of various genes implicated in cancer development, including tumor suppressors and oncogenes, For instance, FOXP3 represses the expression of HER2 and SKP2 in breast cancer cells[21, 22] and an inverse correlation between FOXP3 and HER2 mRNA was observed in this type of tumor. Foxp3 also controls the expression of several genes that are all related to the function of Treg cells, such as CD25, GITR, and CTLA-4[23].

Conclusions

In conclusion, the results of our study indicated that quantification of FOXP3-positive Treg in breast tumors is valuable for assessing disease prognosis and progression, and that Treg are an important therapeutic target for breast cancer. T-regulatory cells and their etiopathological role in breast cancer require intensive investigation in terms of expression and mutational changes in the gene that codes for FOXP3.

REFERENCES

1. Saaed A.M., Sheikha A.K., Mohammed S.S., Ameen H.A.M., Sheet M. and Khasraw S.Y. "A survey of suspected familial breast cancer in Iraqi Kurdish women". Journal of Clinical Oncology. 2011, 29(15): 1602. doi: 10.1200/jco.2011.29.15_suppl.1602.
2. Sakaguchi S. "Naturally arising Foxp3-expressing CD25 + CD4+ regulatory T cells in immunological tolerance to self and non-self". Nature Immunology. 2005, 6:345–352. doi:10.1038/ni1178.
3. Mougiakakos D., Choudhury A., Lladser A., Kiessling R. and Johansson C.C. "Regulatory T cells in cancer". Advances in Cancer Research. 2010, 107: 57-117. doi.org/10.1016/S0065-230X(10)07003-X .
4. Whiteside T.L. "Regulatory T cell subsets in human cancer: are they regulating for or against tumor progression?" Cancer Immunol Immunother. 2014, 63(1):67-72. DOI: 10.1007/s00262-013-1490-y
5. Bates G.J., Fox S.B., Han C., Leek R.D., Garcia J.F., Harris A.L. and Banham A.H. "Quantification of regulatory T cells enables the identification of high-risk breast cancer patients and those at risk of late relapse". J Clin Oncol. 2006, 24(34):5373-5380. DOI: 10.1200/JCO.2006.05.9584 .
6. Sasada T., Kimura M., Yoshida Y., Kanai M. and Takabayashi A. "CD4+CD25+ regulatory T cells in patients with gastrointestinal malignancies: possible involvement of regulatory T cells in disease progression". Cancer. 2003, 98(5):1089-1099. DOI:10.1002/cncr.11618 .
7. Sato E., Olson S.H., Ahn J., Bundy B., Nishikawa H., Qian F., Jungbluth A.A., Frosina D., Gnjatic S., Ambrosone C., Kepner J., Odunsi T., Ritter G., Lele S., Chen Y.T., Ohtani H., Old L.J. and Odunsi K. "Intraepithelial CD8+ tumor-infiltrating lymphocytes and a high CD8+/regulatory T cell ratio are associated with favorable prognosis in ovarian cancer". Proc Natl Acad Sci U S A. 2005, 102(51): 18538-18543. DOI: 10.1073/pnas.0509182102 .
8. Petersen R.P., Campa M.J., Sperlazza J., Conlon D., Joshi M.B., Harpole D.H., Jr and Patz E.F. "Tumor infiltrating Foxp3+ regulatory T-cells are associated with recurrence in pathologic stage I NSCLC patients". Cancer. 2006, 107(12):2866-2872. DOI:10.1002/cncr.22282 .
9. Gao Q., Qiu S.J., Fan J., Zhou J., Wang X.Y., Xiao Y.S., Xu Y., Li Y.W. and Tang Z.Y. "Intratumoral balance of regulatory and cytotoxic T cells is associated with prognosis of hepatocellular carcinoma after resection". J. Clin. Oncol. 2007, 25(18):2586-2593. DOI: 10.1200/JCO.2006.09.4565 .
10. Li J.F., Chu Y.W., Wang G.M., Zhu T.Y., Rong R.M., Hou J. and Xu M. "The prognostic value of peritumoral regulatory T cells and its correlation with intratumoral cyclooxygenase-2 expression in clear cell renal cell carcinoma". BJU Int. 2009, 103 (3): 399-405. DOI: 10.1111/j.1464-410X.2008.08151.x .
11. Hiraoka, N., Onozato, K., Kosuge, T. and Hirohashi, S. "Prevalence of FOXP3+ regulatory T cells increases during the progression of pancreatic ductal adenocarcinoma and its premalignant lesions". Clin. Cancer Res. 2006, 12(18):5423-5434. DOI: 10.1158/1078-0432.CCR-06-0369 .
12. Abdul-Jabbar R.A. "Immunohistochemical and Genetic Study in Iraqi Women with Breast Cancer". Thesis, College of Science, University of Baghdad, Iraq. 2014.
13. Alghaliby H.L.A. "Molecular and Immunohistochemical Study in Women with Breast Cancer in Thi-Qar Province". Thesis, College of Science, University of Thi-Qar, Iraq. 2014.
14. Rashed A.R. "Immunohistochemical study and evaluation of hormone receptor and estimation of IL-6 and HSP70 in invasive breast carcinoma patients of Basrah and Thi-qar province". Thesis. Iraq. 2017.
15. Benson J.R., Weaver D.L., Mittra I. and Hayashi M. "The TNM staging system and breast cancer". Lancet Oncol. 2003, 4(1): 56-60.
16. Reimers M.S., Engels C.C., Putter H., Morreau H., Liefers G.J., Cornelis JH van de Velde, C.J.V. and Kuppen P.J. (2014). "Prognostic value of HLA class I, HLA-E, HLA-G and Tregs in rectal cancer: a retrospective cohort study". BMC Cancer. 2014, 14:486. DOI:10.1186/1471-2407-14-486 .
17. Gobert M., Treilleux I., Bendriss-Vermare N., Bachelot T., Goddard-Leon S., Arfi V., Biota C., Doffin A.C., Durand I., Olive D., Perez S., Pasqual N., Faure C., Ray-Coquard I., Puisieux A., Caux C., Blay J.Y. and Ménétrier-Caux C. "Regulatory T cells recruited through CCL22/CCR4 are selectively activated in lymphoid infiltrates surrounding primary breast tumors and lead to an adverse clinical outcome". Cancer Res. 2009, 69(5): 2000-2009. DOI:10.1158/0008-5472.CAN-08-2360 .
18. Liu P., Wang Z., Brown S., Kannappan V., Tawari P. E., Jiang J., Irache J.M., Tang J.Z., Armesilla A.L., Darling J.L., Tang X. and Wang W. "Liposome encapsulated Disulfiram inhibits NFκB pathway and targets breast cancer stem cells *in vitro* and *in vivo*". Oncotarget. 2014, 5(17): 7471–7485. DOI: 10.18632/oncotarget.2166 .
19. Banerjee S., Reis-Filho J.S. and Ashley S. "Basal-like breast carcinomas: clinical outcome and response to chemotherapy". J. Clin. Pathol. 2006, 59(7):729–35. DOI:10.1136/jcp.2005.033043 .
20. Uva V., Sfondrini L., Triulzi T., Casalini P., Tagliabue E. and Balsari A. "FOXP3 expression in tumor cells and its role in cancer progression". *Atlas* Genet Cytogenet Oncol Haematol. 2015, 19 (3): 234-239. DOI: 10.4267/2042/56419 .
21. Nair S., Aldrich A.J., McDonnell E., Cheng Q., Aggarwal A., Patel P., Williams M.M., Boczkowski D., Lyerly H.K., Morse M.A. and Devi G.R. "Immunologic targeting of FOXP3 in inflammatory breast cancer cells". PLoS One. 2013, 8(1):e53150. doi.org/10.1371/journal.pone.0053150 .
22. Zuo T., Liu R., Zhang H., Chang X., Liu Y., Wang L., Zheng P. and Liu Y. "FOXP3 is a novel transcriptional repressor for the breast cancer oncogene SKP2". J. Clin. Invest. 2007a, 117(12):3765-3773.
23. Zuo T., Wang L., Morrison C., Chang X., Zhang, H., Li, W., Liu, Y., Wang Y., Liu X., Chan M.W., Liu J.Q., Love R., Liu C.G., Godfrey V., Shen R., Huang T.H., Yang T., Park B.K., Wang

C.Y., Zheng P. and Liu Y. "FOXP3 is an X-linked breast cancer suppressor gene and an important repressor of the HER-2/ErbB2 oncogene". Cell. 2007b, 129(7):1275-86.

24. Benevides L., Cardoso C.R.B., Tiezzi D.G., Marana H.R.C., Andrade J.M. and Silva J.S. "Enrichment of regulatory T cells in invasive breast tumor correlates with the upregulation of IL-17A expression and invasiveness of the tumor". Eur. J. Immunol. 2013, 43(6): 1518-1528. DOI:10.1002/eji.201242951 .