Oct4 Is Not a Crucial Factor in Breast Invasive Ductal Carcinoma in Contrast to Recent Beliefs

Ata Abbasi[1], Farahnaz Noroozinia[1], Sonia Hosseinzadeh[1], Mohammad Amin Abbasi[2], Samira Anvar[1], Seyed Arman Seyed Mokhtari[3]

[1] *Department of Pathology, School of Medicine, Urmia University of Medical Sciences, Urmia, Iran*
[2] *Firoozabadi Hospital Clinical Research Development Unit (FHCRDU), Iran University of Medical Sciences, Tehran, Iran*
[3] *Student Research Committee, Urmia University of Medical Sciences, Urmia, Iran*

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Abstract- We aimed to determine the frequency of Octamer binding transcription factor 4 (Oct4) expression in human invasive ductal carcinoma. 72 paraffin-embedded samples of breast cancer were enrolled. All blocks were stained for estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor 2(HER 2/neu), ki67, and Oct4 by immunohistochemistry (IHC) method. Of 72 enrolled cases, the mean age was 49.6±1.42 years. 18 (25%) of cases were luminal A, 14 (19.4%) were Her2 positive, 31 (43%) were luminal B, and 9 (12.5%) were triple-negative. IHC staining for Oct4 revealed no Oct4 expression in breast cancer samples. The staining was repeated twice, and seminoma was used as a positive control in each run. The results of both repeats were the same, and none of the examined samples showed Oct4 expression. We found no Oct4 expression in breast cancer samples examined in our study. We also did not find Oct4 expression in normal breast tissue. Our study is one of the few studies which has evaluated Oct4 expression in human breast cancer on tissue samples and is one of the least that has reported no expression of Oct4 in breast cancer.

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Introduction

Octamer binding transcription factor 4 (Oct4) plays a crucial role in self-renewal, pluripotency, and lineage commitment in embryonal stem cell (1-4) and is considered a master regulator of pluripotency during embryogenesis. It also plays a pivotal role in mammalian development during embryogenesis (5,6). The role of Oct4 in initiating malignant tumors is also well documented in germ cell tumors, and it is now a useful marker in the diagnosis of Seminoma and embryonal carcinoma (6-8). Recent studies have evaluated the role of Oct4 in other malignancies, including lung and bladder, which have reached to promising results but studies on breast cancer are very rare (9,10). As there are only a few studies evaluating the role of Oct4 in human breast cancer and no studies from our region (the Middle East and Iran) on this topic exist, we performed this study to determine the frequency of Oct4 expression in human invasive ductal carcinoma. We also evaluated the selected samples for the estrogen receptor (ER), progesterone receptor (PR), ki67, and Her2/neu expression to evaluate the probable correlation between them and Oct4 expression.

Materials and Methods

In this study, 72 paraffin-embedded samples of breast cancer collected by the pathology department of Urmia University of medical sciences, Urmia, Iran, were enrolled. The inclusion criteria were: (a) undergoing a curative operation with axillary dissection, (b) resected tumor specimens were pathologically examined, and (c) a complete medical record was available. All embedded paraffin blocks were sectioned at 4micrometer, and sections were stained by hematoxylin and eosin (H & E) method for routine histologic examination and tumor grading. Tumor grading was performed according to the Nottingham modification of the Bloom-Richardson system. The cases were categorized according to their

Corresponding Author: A. Abbasi
Department of Pathology, School of Medicine, Urmia University of Medical Sciences, Urmia, Iran
Tel: +98 9124397395, Fax: +98 4433433854, E-mail addresses: aabbasi@razi.tums.ac.ir, ata.abasi@gmail.com

IHC staining profile of ER, PR, ki67, and Her2/neu into four groups as follows: Luminal A: ER-positive and Her2/neu negative; Luminal B: ER and HER2/neu positive with ki67 index less than 14%; Her2/neu positive group: ER-negative, Her2/neu positive and ki67 index more than 14%; Basal-like: triple (ER, PR, Her2/neu) negative.

IHC staining

For immunohistochemistry (IHC), five consecutive sections were obtained and stained for estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor 2(HER 2/neu), ki67, and octamer binding transcription factor 4 (OCT 4) according to manufacturer's guidelines.

Briefly, slides were deparaffinized in Xylen and hydrated in a washing solution. Prior to antibody staining, the slides were pretreated with microwave irradiation to unmask binding epitopes (Heat-induced epitope retrieval). Endogenous peroxide activity was blocked with a 3% solution of hydrogen peroxide in methanol. Then the slides were incubated with oct4, ER, PR, and Her2/neu antibodies. Then Envision was added as a secondary antibody, and after incubation, staining was visualized by adding diaminobenzidine (DAB) for 5 min at room temperature.

All antibodies and associated reagents were obtained from DAKO Corporation, Glostrup, Denmark, and the clone used for Oct4 staining were N1NK. We also used Seminoma (germ cell tumor) samples as a positive control for Oct4 and stained in each staining run.

Finally, all the slides were examined by a single pathologist using a light microscope.

ER and PR IHC reporting

The immunohistochemistry results for ER, PR, and Her2/neu were interpreted according to the College of American Pathologists (CAP) protocols.

Briefly, for ER and PR staining, nuclear positivity was scored 0 to 5 as following: 0 (0%), 1 (<1%), 2 (1-10%), 3 (11-33%), 4 (34-66%), and 5 (>67%) (11).

The intensity of staining (IS) for the nuclear positivity of the cells was graded as 0, 1, 2, and 3 when there was none, mild, moderate, or strong staining, respectively.

Finally, the Allred score was calculated (according to CAP protocol) by summing up the values for proportion and intensity of staining. The Allred score range was between 0-8.

Her2/neu IHC reporting

Her2/neu staining results were reported as following: 0 (negative) if there was no immunoreactivity, 1+ (negative) faint or weak incomplete membranous immunoreactivity in >10% of tumor cells, 2+(equivocal) weak to moderate complete membrane immunoreactivity in > 10% of tumor cells or circumferential (complete) intense membranous staining <10% of cells and 3+ (positive) more than 10% of the tumoral cells showed circumferential (complete) intense and uniform membranous staining with the homogenous chicken-wire pattern.

Ki67 IHC reporting

Ki67 shows mitotic activity and stains tumoral cell nucleus. Percent of positive cells (nuclear staining) in hot spot areas were reported as ki67 index. The cases were divided into two groups: index ≤14% and more than 14%

Oct4 IHC reporting

Oct 4 is a nuclear transcription factor, and only nuclear staining pattern was considered a positive result (even weak or focal staining patterns). The staining was repeated twice, and seminoma was used as a positive control in each run (Figure 1).

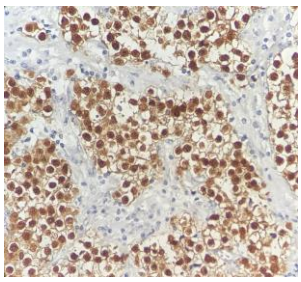

Figure 1. Showing strong nuclear staining of testis Seminoma (as positive control) for Oct4 marker by immunohistochemistry (IHC) method (IHC, 20x)

Statistical analysis

Quantitative and descriptive data were expressed as mean±SD and frequencies (percentages), respectively. Statistical analysis was performed using SPSS version 16.0 (SPSS Inc., Chicago, IL, USA). The normality of data was evaluated with the Kolmogorov Smirnov test. The statistical differences between proportions were determined by $\chi 2$ analysis following the exact Fischer test. Numerical data were evaluated using *student t-test*, and ordinal data were evaluated by the Kruskal-Wallis test. $P<0.05$ was considered as significant.

Results

Seventy-two cases were enrolled in this study. The patients' mean age was 49.6±1.42 years. Demographic data are shown in table 1.

Table 1. Showing histologic parameters of the examined tumors

		Frequency	Percent
Histologic grade	**Grade I**	3	4.2 %
	Grade II	36	50 %
	Grade III	33	45.8 %
Tumor side	**Right**	30	41.7 %
	Left	42	58.3 %
Tumor size	**< 2cm**	3	4.2 %
	2cm – 5 cm	53	73.6 %
	> 5cm	16	22.2%
Lymph-vascular invasion	**present**	51	71 %
	Not identified	14	19 %
	indeterminate	7	10 %
Perineural invasion	**present**	22	30.6 %
	Not identified	50	69.4 %
Nipple involvement	**Present**	13	18.1 %
	Not identified	59	81.9 %
Skin involvement	**Present**	15	20.8 %
	Not identified	57	79.2 %
Axillary lymph node Involvement	**Present**	59	81.9 %
	Not identified	13	18.1 %
Hormone profile	**Luminal A**	18	25%
	Luminal B	31	43%
	Her2/neu	14	19.4%
	Basal like (triple negative)	9	12.5%

Of 72 cases, 18 (25%) were luminal A, 14 (19.4%) were Her2 positive, 31 (43%) were luminal B, and 9 (12.5%) Were triple-negative (Figures 2-4).

The hormone profile and its relation with tumor grade are summarized in Table 2. IHC staining for Oct4 revealed no Oct4 expression in breast cancer samples. The results of both repeats were the same, and no Oct4 expression was observed in any of the examined samples (Figure 5). Normal breast tissue was also negative for Oct4 staining.

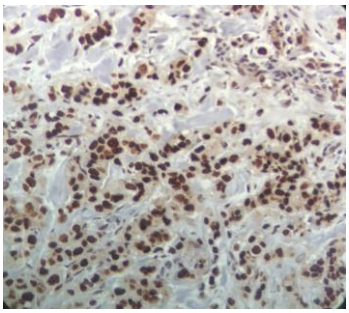

Figure 2. Showing strong nuclear staining of invasive breast carcinoma for ER marker by immunohistochemistry (IHC) method (IHC, 20x)

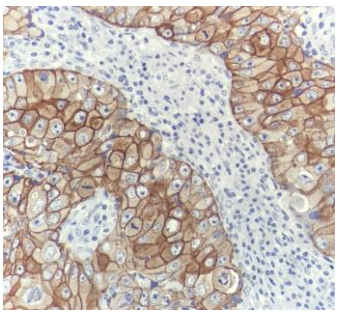

Figure 3. Showing strong nuclear staining of invasive breast carcinoma for PR marker by immunohistochemistry (IHC) method (IHC, 20x)

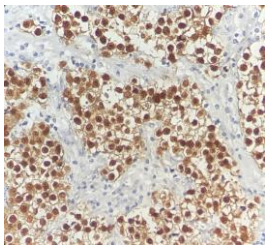

Figure 4. Showing a strong and complete membranous staining of invasive breast carcinoma for Her2/neu marker by immunohistochemistry (IHC) method (IHC, 20x)

Table2. Showing hormone profile and its relation with tumor grade

Molecular/ Subtype/ Grade	Luminal A	Luminal B	HER2/neu	Triple negative (basal-like)	Ki67 ≤ 14 % (N=26)	Ki 67 > 14 % (N=46)
Grade I	1 (5.5%)	2 (6.5%)	0	0	2 (7.5%)	1 (2.3%)
Grade II	11* (61.5%)	17* (54.5%)	2 (14%)	3 (33%)	15 (58%)	21 (45.7%)
Grade III	6 (33%)	12 (39%)	12* (86%)	6* (67%)	9 (34.5%)	24 (52%)
	18 (100%)	31 (100%)	14 (100%)	9 (100%)	26 (26%)	46 (100%)

*: *P*=0.001

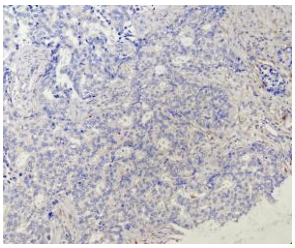

Figure 5. Showing invasive breast carcinoma with negative results for Oct4 marker by immunohistochemistry (IHC) method (IHC, 20x)

Discussion

In this study, we evaluated the expression of Oct4 in invasive breast carcinoma and also explored its relationship with tumor histologic parameters, including grade, size, lymphatic involvement, lymph node metastasis, and also hormone receptors expression profile. In our study, we found no Oct4 expression in either normal breast tissue or breast carcinoma in any grades. We also evaluated ER-positive, ER-negative, Her2 positive, and high or low-grade tumors but found no Oct4 expression in any of the mentioned conditions, and none of the examined tumors, neither low grades nor high-grade ones, expressed Oct4.

Oct4 is a transcriptional factor that plays an important role in breast development during embryogenesis (6). It has been shown that even up or down-regulation of Oct4 during embryogenesis could initiate different differentiation pathways (12). Additionally, recent studies have shown that re-expression of Oct4 in adult mature somatic cells can induce pluripotency and dedifferentiation (13,14). Oct4 is not expressed in normal breast tissue (13), but its reactivation has been observed in breast cancer cell lines (15). In this study, we did not observe Oct4 expression in normal breast tissue, which is consistent with previous findings and the knowledge about oct4 expression in normal breast epithelium. There are very few studies evaluating the role of Oct4 in human breast cancer, most of which are in vitro evaluation or stem cell studies, and only a few are on human cancer samples (16-19). In contrast to most of the mentioned studies, which support the potential role of Oct4 in breast tumorigenesis, our data showed no expression of Oct4 in human breast cancer. In order to omit any staining mistakes and reduce any probable errors during IHC staining, we repeated IHC staining for Oct4 on all of the enrolled samples and also used a positive control in every staining run but found no Oct4 expression in our enrolled samples. But as mentioned above, there are few studies on human breast cancer specimens that have observed Oct4 expression. Qian and Zhao have shown that Oct4 transcription and also expression was significantly increased in human breast cancer comparing to normal breast tissue (13). Wang *et al.*, have also found the same results and have mentioned Oct4 as a potential prognostic marker in breast cancer (20), and Cho *et al.*, have reported that Oct4 has some roles in cancer stem cells stability and targeting Oct4 post-regulation process can be one of the targeted therapy approaches in the treatment of breast cancer (21). Actually, we were not able to fully explain why our results were in contrast to others and why we have not observed any Oct4 expression in our study, but we concluded that all of these studies which have shown Oct4 expression in breast cancer support the hypothesis that stem cells are target cells in tumorigenesis (13) and our results showed that at least a proportion of breast cancers are not developed through the mentioned stem cell pathway.

Additionally, there are various isoforms and different splicing products for Oct factors, and the antibodies which were used in different studies for Oct4 identification may be different and cause discordant results. Also, according to genetic diversity and ethnical factors, cancer samples from different regions and populations may have different molecular and genetic profiles, and our study is the first of its kind in our region, and all of the studies with contrary results have evaluated different populations. We assumed that genetic diversity among populations would be one of the

main reasons for our different results .

It should be noted that we have evaluated only infiltrative ductal carcinoma of the breast in our study. Since there are other subtypes of breast cancer, Oct4 could be expressed in those subtypes which were not evaluated in our study. We also recommend evaluating the criteria and the staining quality of the previous studies, which have reported positive results for Oct4 in their breast tumors.

We recommend further studies from different regions and populations to better understand the role of Oct4 in breast cancer. We also suggest that the Oct4 results in breast cancer and its probable diagnostic and prognostic value be interpreted with caution until sufficient results from different regions are present.

In summary, we found no Oct4 expression in human breast cancer samples in our study. Our study is one of the few studies which has evaluated Oct4 expression in human breast cancer on tissue samples and is one of the least that has reported no expression of Oct4 in breast cancer. As the data on this topic is very new, we recommend further studies, especially on breast tissue samples, to explore Oct4 expression and its role in breast cancer.

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