

## Octamer 4 Expression and Lymph Node Metastasis in Ductal Carcinoma of Breast: Are they Associated?

### Abstract

**Purpose:** Octamer 4 (Oct-4) is a transcription factor which is required for the self-renewal and pluripotency of embryonic stem cells and germ cells. In this study, we tried to examine the association of expression of Oct-4 with lymph node metastasis in ductal carcinoma of the breast.

**Methods:** The study was conducted on a total of 45 cases of invasive ductal carcinoma of breast, no special type. Oct-4 expression was studied on paraffin-embedded sections by immunohistochemistry.

**Results:** Oct-4 expression was seen in 22.2% of cases. No statistically significant association was found between the expression of Oct-4 and histological type, tumor size, histological grade, and lymph node metastasis. Of Oct-4 positive tumor, 80% of cases showed lymph node metastasis, as compared to 62.85% without Oct-4 expression. However, the association was statistically insignificant. **Conclusion:** Oct-4 expression can be a promising biomarker of carcinogenesis, metastatic potential, and prognosis of carcinoma breast. However, the study with larger sample size is needed to establish the clinicopathological potential of this biomarker.

**Keywords:** Breast carcinoma, lymph node metastasis, Octamer 4

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### Introduction

The most common type of breast cancer is ductal carcinoma, and about 80% are of invasive ductal type. Invasive ductal carcinoma affects most commonly elderly women.<sup>[1]</sup> The most important prognostic factor of breast carcinoma is lymph node metastasis.<sup>[2,3]</sup>

Octamer 4 (Oct-4) is a transcription factor containing POU DNA-binding domain.<sup>[4]</sup> Oct-4 expressions are associated with self-renewal of undifferentiated embryonic stem cells.<sup>[5]</sup> Oct-4 has been regarded as a biomarker of adult stem cells, and it is highly expressed in liver, mammary, and gastric stem cells.<sup>[6]</sup> Currently, the expression of Oct-4 has been found in embryonal carcinoma, germ cell tumor, testicular carcinoma *in situ*, seminoma, and dysgerminoma.<sup>[7-9]</sup>

The aim of this study was to examine the Oct-4 expression in invasive ductal carcinoma breast and to correlate Oct-4 expression with tumor size, histological tumor grade, and regional lymph node metastases.

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### Methods

The consecutive study was carried out on the formalin-fixed paraffin-embedded tissue sections of 45 consecutive patients with breast cancer including 30 cases with lymph node metastasis and 15 cases without lymph node metastasis. Patients with infiltrating ductal carcinoma breast, who underwent modified radical mastectomy, were included in the study, while patients with invasive carcinoma of breast other than no special type and male breast ductal carcinoma were excluded from the study.

For all specimens, 4- $\mu$ m thick sections of paraffin-embedded tissue were prepared on poly-L-lysine-coated slides and subjected to Oct-4 immunohistochemical staining. The sections were deparaffinized in xylene and rehydrated through graded alcohol. For microwave, antigen retrieval citrate buffer of pH 6 was used. The slides were incubated overnight with Oct-4 primary antibody (clone: EP143, rabbit monoclonal antibody, BioSB, USA), followed by incubation with the superenhancer for 30 min and then with secondary antibody for 20 min. DAB was added for 10 min. Next, counterstaining in hematoxylin was carried out followed by dehydration, clearing, and mounting. Tissue

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sections from seminoma were included in each run as a positive control for Oct-4.

### Statistical analysis

Associations between categorical variables (side of breast involved, tumor size, tumor grade, lymph node status, estrogen receptor expression, progesterone receptor expression, Her2 expression, and molecular subtypes) were analyzed using the Chi-square test. Two-sided  $P < 0.05$  was considered statistically significant. All statistical analyses were carried out using SPSS 23 (IBM, Armonk, NY, USA).

## Results

### Patient characteristic

A total of 45 patients were enrolled in this study. The mean ( $\pm$ standard deviation) age was 48.15 ( $\pm$ 11.35) years. Out of 45 cases, 66.7% lesions were left sided. The largest diameter of tumor in most (68.9%) cases was 2–5 cm. Invasive ductal carcinoma cases were graded according to the Nottingham modification of Bloom–Richardson grading,<sup>[10]</sup> out of 45 cases, 44.4% of cases were categorized as Grade II.

### Immunohistochemical results

Positive expression of ER was seen in 24.4% of cases, and positive PR expression was present in 24.4% of cases. Her2neu positivity was noted in 44.4% of cases. On molecular subtyping, 44.4% of cases were basal like, 31.1% were Her2+ type, 13.3% were luminal B type, and 11.1% of cases were luminal-A type.

Expression of Oct-4 immunostain was studied in all cases of infiltrating ductal carcinoma, no special type. About 22.2% of cases showed nuclear and/or cytoplasmic positivity in  $\geq 1\%$  tumor cells and were considered positive for Oct-4 [Figures 1 and 2]. About 77.8% of cases were either negative for Oct-4 expression or showed nuclear and/or cytoplasmic staining in  $< 1\%$  tumor cells.

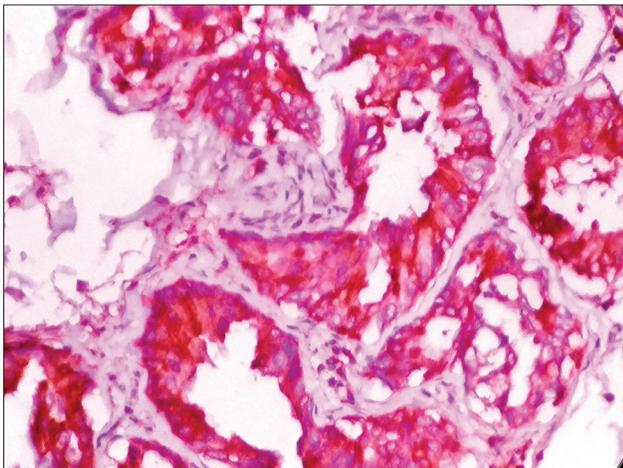


Figure 1: Photomicrograph of invasive breast carcinoma, no special type showing cytoplasmic positivity with Octamer 4 (DAB,  $\times 400$ )

### Correlation between Octamer 4 expression and clinicopathological features

There was no statistically significant association of Oct-4 expression with side involved by tumor ( $P = 0.482$ ), largest tumor diameter ( $P = 0.371$ ), histological grade ( $P = 0.151$ ), ER expression ( $P = 0.194$ ), PR expression ( $P = 0.643$ ), Her2 expression ( $P = 0.108$ ), and molecular subtype ( $P = 0.353$ ). Of Oct-4 positive tumor, 80% of cases showed lymph node metastasis, as compared to 62.85% without Oct-4 expression. However, the difference was not statistically significant ( $P = 0.310$ ). The details are summarized in Table 1.

### Discussion

Expression of Oct-4 immunostain was studied in all cases. Out of 45 cases, 22.2% of cases showed nuclear and/or cytoplasmic positivity in  $\geq 1\%$  tumor cells and were considered positive for Oct-4.

When we compared various previous studies<sup>[11-15]</sup> of expression of Oct-4 in ductal carcinoma of the breast, we found that expression was variable ranging from 15.4% to 62.5%. In these studies,<sup>[11,13,15]</sup> of Oct-4 expressing tumors, about 34.6%–76.92% of cases showed lymph node metastasis.

In this study, Oct-4 expression was not significantly associated with tumor size. This is concordance with previous studies,<sup>[11-14]</sup> while in one study,<sup>[15]</sup> statistically significant association was found.

In the present study, no significant association was found between Oct-4 expression and histological grade. However, other studies reported a statistically significant association.<sup>[11-15]</sup>

In concordance with the study of Cai *et al.*,<sup>[12]</sup> in this study, there was no statistically significant association between Oct-4 expression with hormonal receptor (ER and PR) expression and Her2 expression. However, the study of Gwak *et al.*<sup>[13]</sup> showed a statistically significant association

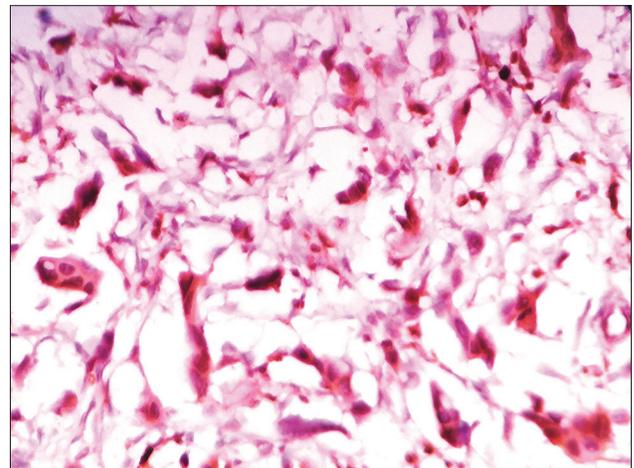


Figure 2: Photomicrograph of invasive breast carcinoma, no special type showing nuclear positivity with Octamer 4 (DAB,  $\times 400$ )

**Table 1: Correlation between Octamer 4 expression and clinicopathological parameters of the studied breast carcinoma (n=45 cases)**

Variables	Total cases	Number of cases (%)		P*
		Oct-4 positive	Oct-4 negative	
Side of breast				
Right	14	5 (35.7)	9 (64.28)	0.482
Left	25	5 (20.0)	25 (80.0)	
Bilateral	1	0 (0.00)	1 (100)	
Tumor size (largest dimension) (cm)				
<2	6	0 (0.00)	6 (100.0)	0.371
2-5	31	8 (25.8)	23 (75.19)	
>5	8	2 (25.0)	6 (75.0)	
Lymph node status				
Positive	30	8 (26.6)	22 (73.3)	0.310
Negative	15	2 (13.3)	13 (86.6)	
Histological grade				
Grade I	7	3 (42.8)	4 (57.1)	0.151
Grade II	20	2 (10.0)	18 (90.0)	
Grade III	18	5 (27.7)	13 (72.2)	
ER status				
Positive	11	4 (36.3)	7 (63.6)	0.194
Negative	34	6 (17.6)	28 (82.3)	
PR status				
Positive	11	3 (27.2)	8 (72.7)	0.643
Negative	34	7 (20.5)	27 (79.4)	
Her2 status				
Positive	20	5 (25.0)	15 (75.0)	0.108
Negative	22	3 (13.6)	19 (86.3)	
Equivocal	3	2 (66.6)	1 (33.3)	
Molecular subtype				
Luminal A	5	1 (20.0)	4 (80.0)	0.353
Luminal B	6	3 (50.0)	3 (50.0)	
Her2+	14	2 (14.28)	12 (85.71)	
Basal like	20	4 (20.0)	16 (80.0)	

\*Chi-square test. ER – Estrogen-receptor; PR – Progesterone receptor; Her2 – Human epidermal growth factor receptor 2; Oct-4 – Octamer 4

between Oct-4 expression with ER expression and Her2 expression, while no statistically significant association was found between Oct-4 expression and PR expression. In a study of Abou Gabal *et al.*,<sup>[15]</sup> a statistically significant association of Oct-4 expression with ER and PR expression was found, while statistically insignificant association was found between Oct-4 expression with Her2 expression.

In the present study, no significant association was found between Oct-4 expression and molecular subtype. However, other studies<sup>[11-15]</sup> demonstrated a statistically significant association.

In this study, we found a statistically insignificant association between lymph node status and Oct-4 expression. This is concordance with the study of Gwak *et al.*,<sup>[13]</sup> however, other studies<sup>[11,12,14,15]</sup> found a statistically significant association.

None of these studies represents the Indian population. In most of the studies observed, they have not defined any study population according to the type of breast carcinoma or with lymph node metastasis. The antibodies used in various studies were different. Furthermore, heterogeneity in the expression of Oct-4 was observed in various studies.<sup>[16,17]</sup>

## Conclusion

In the present study, the expression of Oct-4 was seen in 22.2% of cases. No statistically significant association was found between the expression of Oct-4 and histological type, tumor size, and histological grade. Of Oct-4 positive tumor, 80% of cases showed lymph node metastasis, as compared to 62.85% without Oct-4 expression. In view of limited sample size, in the current study, larger prospective studies should be undertaken for the assessment of association of Oct-4 expression with breast carcinoma.

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## Conflicts of interest

There are no conflicts of interest.

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