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Original Article

CD44 positive/CD24 negative (stem cell like property) breast carcinoma cells as marker of tumor aggression

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ABSTRACT

Background: Cells with stem cell like properties in solid organ malignancies like breast and pancreas have been studied over the last decade and have been found to be associated with poor prognosis. Presence of CD44 positive and CD24 negative tumor cells in breast carcinoma (cells with 'stem cell' like property) as marker of aggressiveness and poor prognosis was checked for association with various markers of disease aggression like age at presentation, size of tumor, histological grade of tumor, triple negative status, level of micro-vessel density, and nodal status.

Methods: Single and double staining immunohistochemistry protocol was used for CD24 and CD44 staining. The staining protocol was repeated with more contemporary techniques using fluorescent chromogen also.

Results: 52 cases, all females who underwent modified radical mastectomy at a tertiary care hospital over a period of 3 years, were evaluated. No association was found between presence of stem cells and size of lesion, histological grade, triple negative status or micro-vessel density. However, significant association was found with respect to younger age of presentation (p value = 0.044). 20 out of 25 cases with nodal metastasis were positive for presence of stem cells (p value is 0.0003). Further, 18 of these 20 cases also had stem cells in the metastatic nodule. Fluorescent chromogens (FITC and Cyanine Red) revealed similar results.

Conclusion: Cases positive for stem cells showed earlier onset of disease and proneness to nodal metastasis.

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Introduction

The CD44 antigen is a trans-membrane cell-surface glycoprotein involved in cell–cell interactions, cell adhesion, and migration. In humans, the CD44 antigen is encoded by the CD44 gene on chromosome 11.¹ CD44 (HCAM) molecule plays an important role in the cascade of metastasis and progression of human malignant tumors. CD44 antigen is present on T lymphocytes, granulocytes, red blood cells, brain, and epithelial cells. The standard isoforms, CD44s, are also expressed in a wide range of normal tissues such as tonsil, skin, bladder, and cervical squamous epithelium.²

The CD24 antigen is a glycoprotein expressed at the surface of most B lymphocytes and differentiating neuroblasts.³

The cancer stem cell hypothesis was proposed to explore breast cancer heterogeneity and the risk of breast cancer recurrence, and these cell subpopulations may contribute to drug resistance that drives tumor recurrence or metastasis.⁴ Several studies implicated a subset of human breast cancer cells as having enhanced ability to form tumors in immunocompromised mice. These cells were distinguished from their non-tumorigenic counterparts by a specific cell surface marker profile: the CD44+/CD24– lineage.^{5,6}

On review of literature, limited number of studies could be found and none from India. The tumors with presence of these lineage and phenotypic expression of cells have been found to be more aggressive in distant metastasis.^{7,8} The breast carcinomas with presence of this population (CD44+/CD24–) of cells have been shown more likely to be 'triple negative'.⁹ The breast carcinomas with presence of these stem cell lineage tumor cells have also been found to be more commonly relapsing postsurgical resection.¹⁰

The present study evaluated presence of CD44 positive and CD24 negative tumor cells in Breast carcinoma (cells with 'stem cell' like property) as marker of aggressiveness and poor prognosis.

Materials and methods

Cases of carcinoma breast, who had undergone modified radical mastectomy over 3 years from 2010 to 2012 at a tertiary care, were included in the study.

The age and sex were noted in each case from clinical data sheet. The size of tumor and number of nodes positive among the total number of nodes dissected out of axillary tail were noted from the gross notes and as per microscopic evaluation respectively. The T1, 2, 3 for TNM was allotted as per the size of the tumor in each case.

Routine Haematoxylin and Eosin stained sections from the tumor in each case were reviewed for the histological type of tumor and routine Nottingham's Modification of Scarf Bloom Richardson grading score. The scoring was performed in each case and the cases were graded into I, II, and III.

In each case, the representative tumor block was chosen for immuno-histochemical evaluation. Estrogen receptor (ER) (Biogenix mouse clone-ER88), progesterone receptor (PR) (Biogenix mouse clone-IA 6), human epidermal growth factor receptor 2 (HER2/neu) (Biogenix rabbit clone-EP6) and platelet

endothelial cell adhesion molecule-PECAM-1 (CD31) receptor (Biocare mouse clone BC2) staining were done. All antibodies were ready to use and incubation time was 30 min. Routine technique for immunohistochemistry was applied in all cases. ER, PR status using Allred scoring¹¹ and HER2/neu status using ASCO guidelines¹² was noted in each case. Cases were segregated into two groups of triple negative status positive/negative. Microvessel density (MVD) was calculated in the area showing maximum CD31 staining. 5 high power fields (HPF) were evaluated in this area and the number of CD31 positive vessels and even discrete cell aggregates were counted as number of micro-vessels per 5 hpf. The cases were grouped into three categories: <10/5 hpf, 10–20/5 hpf, and >20/5 hpf.¹³

Since single antibody-chromogen staining is a standardized technique of immunohistochemistry and it is a more sensitive and accurate technique for assessing both CD44 and CD24 individually for their level of positivity, the protocol was adopted before selection of cases for sequential double staining. Moreover, for sequential double staining, both antibodies available in the market are anti-mouse; hence, an elution step was needed to be adopted to get satisfactory results with both traditional chromogen-based detection technique and fluorescent-based technique. Section from the tumor was stained with CD44 monoclonal antibody (Biocare mouse clone-BC8, Isotype IgG1, in dilution of 1:100 in Biocare Da Vinci Green Antibody Diluent with incubation of 30 min) and CD24 monoclonal antibody (Biocare mouse monoclonal-SN3b in dilution of 1:100 in Biocare Van Gogh Yellow Antibody Diluent with incubation of 45 min). In both cases, DAB was used as a chromogen. The positivity of tumor cells was noted for CD44 and CD24. The cases were grouped under CD44+/– and CD24+/–.

Cases were labeled as CD44 positive only if more than 10% of tumor cells are positive for CD44.⁹ CD44 positive cases, totally negative for CD24 staining by single staining protocol were taken as breast carcinomas having stem cells (CD44+ and CD24–). CD44 positive cases, which also showed CD24 positive tumor cells in single staining protocol mentioned above, were taken up for sequential double staining. Protocol included first staining for CD24 with DAB as chromogen. HRP conjugate was used for this step. Elution step was applied using Biocare denaturing solution – incubation 5 min (working solution A 1 part + solution B 2 parts) before sequential staining for CD44 with alkaline phosphatase as a conjugate. FAS red was used as a chromogen. The co-localization of both the antigens on the same tumor cells was checked, CD44 being localized in the membrane and CD24 localized to the membrane and granular positivity in the cytoplasm. Thus, CD44+ and CD24– tumor cells (stem cells) were identified. Now, all cases in this group were finally accepted as having stem cells in case they had more than 10% of tumor cell as CD44+ and CD24– (stem cells).⁹

As the study was of contemporary interest, the CD44 and CD24 staining were also conducted with fluorescent chromogen tagging as the last step instead of the traditional DAB and wrap red to visualize these antigens under fluorescence and evaluate whether there is superiority over the traditional chromogens. Single staining of CD44 with fluorescent detection tagging was first done. Postincubation with CD44 antibody Biocare fluorescent enhancer was used with incubation for 30 min in dark. Fluorescent chromogen used was Biocare Goat

Table 1 – Distribution of cases as per age group.

n = 52			Percentage
Sl No.	Age group in decade	N	
1.	20–30	04	7.7%
2.	31–40	12	23.1%
3.	41–50	09	17.3%
4.	51–60	13	25%
5.	61–70	10	19.2%
6.	71–80	03	5.8%
7.	>80	01	1.9%

anti-mouse Dylight-FDM 488 (spectral characteristics of green fluorescein isothiocyanate (FITC) diluted in fluorescence antibody diluents, supplied along with in 1:100) for 30 min in dark. The slides were air dried in dark and mounted with normal cover slip with ready to use BiocareFluoro Care Anti-Fade Mountant medium. Similar technique was used for CD24 fluorescent staining. Here, Biocare Goat anti-mouse Dylight (FDM 549) (spectral characteristics of red fluorescent cyanine dye (Cy3)) was used. Double sequential staining of CD44 and CD24 with fluorescent detection tagging protocol was done. An elution step was used in the sequential staining protocol using Biocare denaturing solution – incubation 5 min (working solution A 1 part + solution B 2 parts) in dark. The fluorescent protocol was undertaken for all cases and results compared with traditional immunohistochemistry.

The stem cell positive and negative cases were checked for association with age at presentation, gross size of tumor, histological grade of tumor, level of micro-vessel density and histological nodal status.

The statistical analysis used to evaluate the results was 'Fischer Exact' probability test as the number of cases was less. Significant statistical association was looked for.

Results

A total of 52 cases of carcinoma breast who had undergone modified radical mastectomy were studied during the period of study from 01 January 10 to 31 December 12. The ages of the patient varied from 24 to 89 years. The age distribution of cases is appended in Table 1. All cases were females.

The results of CD44 and CD24 staining status of the cases are appended in Table 2. The 22 cases in column (a) in the table were only CD44 positive (at least 10% of tumor cells) but were negative for CD24 by single staining protocol; hence, they were

tumors with stem cells. The six cases in column (b) in the table were positive for both CD44 and CD24 by single staining protocol. Thus, they were subjected to double sequential staining protocol for individual cell CD44/CD24 localization and all these six cases were found to have at least 10% of tumor cells⁹ which were CD44 positive but CD24 negative though it showed cells which were CD44 and CD24 positive and only CD24 positive also. Thus, they were also included in the category of cases positive for stem cells. 24 cases in categories (c) and (d) were excluded from further evaluation as these cases were totally negative or had less than 10% of tumor cells staining for CD44 antigens and thus were considered negative for presence of stem cells.

Figs. 1 and 2 illustrate the results of single staining protocol used with DAB as chromogen and sequential double staining protocol using DAB (CD24) and FAS red (CD44) as chromogen respectively for identifying cases qualifying for presence of stem cells.

For the various associations that follow with different parameters like age of onset, histological grade of lesion, triple negative status, vascular density, and nodal metastasis, 28 cases of columns (a) and (b) of Table 2 were taken.

The age span of 20–80 years was divided into two halves: first group of 20–50 years and second group 51–80 years. Only one case was above 80 years which is included in the second group. Distribution of cases is appended in Table 3. The one-tailed Fischer's exact probability test *p* value was 0.044 (<0.05). Hence, a significant association of presence of tumor stem cells with early age of onset was found. The median age of 28 cases positive for presence of stem cells was 41.5 years whereas those negative for presence of stem cells was 56 years. The median age for cases, which were positive for presence stem cells, was lower by 14.5 years when compared with those who were negative for stem cells.

As appended in Table 3, the association of presence or absence of stem cells with tumor size (*p* = 0.3), histological grade (*p* = 0.3), triple negative status (0.055), and micro-vessel density (*p* = 0.18) was not found to be significant whereas significant association was found of presence of tumor stem cells with presence of nodal metastasis (*p* = 0.0003).

Double sequential staining following protocol was performed on all these 28 cases to check upon presence of stem cells and their percentage in the metastatic nodules. 20 out of 28 stem cell positive cases were positive for nodal metastasis. 18 out of these 20 cases also had stem cells in the metastatic nodule. The percentage of stem cells in all these 18 cases was more than 70% of metastatic tumor cells (refer Fig. 2d).

Table 2 – Summary of CD44 and CD24 status of all cases.

n = 52			
CD44 positive (n = 28)		CD44 negative (n = 24)	
(Positive for stem cells)		(Negative for stem cells)	
(a)	(b)	(c)	(d)
CD44+ and CD24– 22	CD44+ and CD24+ 06 ^a	CD44– and CD24+ 16	CD44– and CD24– 08

^aAll six cases had at least 10% of tumor cells which were CD44+ but CD24– by in double staining protocol.

Fig. 1 – (a) Case 1 – membranous CD44+ tumor cells (20×), (b) Case 10 – CD44+ (black arrow) and CD44– (white arrow) tumor cells (20×), (c) Case 2 – granular cytoplasmic CD24+ tumor cells (20×) and (d) Case 34 – granular cytoplasmic CD24+ tumor cells (100×).

Fig. 2 – Sequential double staining (a) Case 5: CD44+/CD24– (black arrow), (b) Case 9: CD44+/CD24+ (black arrow) (10×), (c) Case 30: tumor cells with membranous CD44– (10×), (d) Case 1: CD44+/CD24– tumor cells (white arrow) with CD44+ T lymphocytes all around in the lymphnode (black arrow) (10×).

Immunohistochemical staining using fluorescent tagging showed similar results to the traditional IHC qualitatively. However, it was difficult to do semiquantitative assessment of percentage cells positive for the two antibodies in respect to all the tumor cells as background is not available.

So traditional IHC is a better technique where besides qualitative, semiquantitative assessment is also required. Non-availability of broadband filters did not allow simultaneous capture of both fluorescence in the same shot (refer Fig. 3).

Table 3 – Summary of association of stem positive and negative cases for age of onset with tumor size, histological grade, triple negative status, microvessel density, and nodal status.

Stem cell	Age of onset ($p = 0.044$)		Tumor size (T) ($p = 0.3$)			Grade ($p = 0.3$)			Triple negative ($p = 0.055$)		Microvessel count/5 hpf ($p = 0.18$)			Nodal metastasis ($p = 0.0003$)	
	≤50 years ($n = 25$)	>50 years ($n = 27$)	T1	T2	T3	I	II	III	+	–	<10	10–15	>15	+	–
+	17	11	11	14	3	11	16	1	8	20	14	7	7	20	8
–	8	16	13	7	4	11	5	8	13	11	8	12	4	6	18

Fig. 3 – (a) Membranous CD44 positive cells in Case 30 (FITC) (40×), (b) granular cytoplasmic with membranous accentuation – CD24+ (Cyanine dye) tumor cells – Case 34 (40×), (c) and (d) membranous CD44 and membranous with granular cytoplasmic CD24 positivity in the same tumor cells in double staining protocol in Case 9 (40×).

Discussion

52 cases of carcinoma breast were evaluated in the present study. These cases had undergone modified radical mastectomy at this tertiary care center over a period of 3 years from 01 January 2010 till 31 December 2012. Various case parameters were evaluated and the results correlated with the presence or absence of stem cell like cells in these cases. The stem cell defining criterion was CD44 positivity with CD24 negativity in an individual cell.

The age of the patient at mastectomy was evaluated. In our study, the number of cases below or equal to 50 years was 25 out of a total of 52 (48%). In India, around 50% of the breast carcinomas in females occur below the age of 50 years (premenopausal).¹⁴ Nearly half (48%) of female breast cancers in UK on the other hand occur a decade later in 50–69 age group.¹⁵

In our study as appended in Table 2, a significant ($p = 0.044$) association was found for early onset of breast carcinoma in cases which were positive for tumor stem cells as compared to cases which were negative. The median age of cases positive for presence of stem cells was 41.5 years whereas those negative for presence of stem cells was 56 years. The median age for cases, which were positive for presence stem cells, was lower by 14.5 years when compared with those who were negative for stem cells. Thus, this indicates towards an earlier onset of disease in cases, which have presence of stem cells. In a study⁹ of 139 cases of carcinoma breast, the cases with CD44 (+)/CD24(–) phenotype had a 10-year lower median age at presentation.

The size of lesion (T1, T2, and T3-TNM) (Table 3) at diagnosis in tumors with or without stem cells did not show any significant association in our study. On literature search, no study correlating presence of stem cells with size of tumor at mastectomy was found.

The three histological grades as reflected in Table 3 did not show any trend towards presence or absence of stem cells. A study¹³ of 94 cases of ductal carcinoma breast showed significant association of higher histological grade with presence of stem cells. Here CD133 a separate marker for stem cells beside CD44 & CD24 content was been evaluated. Studies with 117 special histological type and 466 NOS have shown very high content of stem cells in special histological variants of breast carcinomas like medullary, tubular, and papillary. Content of stem cells in these carcinomas was 80%, 100%, and 100% respectively as compared to NOS/NST where 45.3% of tumor cells were stem cells.¹⁶ 117 special types of breast carcinomas were compared with 466 NOS/NST in this study for stem cell content.

The presence of triple negative status was correlated in our study with the presence of stem cells. The results are appended in Table 3. No significant association was found in our study. Triple negative tumors have been found to be associated with CD44+/CD24– phenotype in a study of 139 breast carcinomas.⁹

Association of micro-vessel density was evaluated with presence or absence of stem cells. The results are appended in Table 3. The statistical evaluation did not reveal any association of micro-vessel density with presence or absence of stem cells in our study. Association of a separate stem cell marker aldehyde dehydrogenase-1 (ALDH-1) has been found to be significantly associated with increased micro-vessel density, p value <0.002 in a study of 94 cases¹³ of invasive ductal carcinoma. The ALDH-1 positive cells were found to colonize areas of more increased micro-vessel density in this study. No association for presence or absence of CD44+/CD24– cells was found with micro-vessel density in this study.

The presence of nodal metastasis was seen in 25 out of 52 cases and 20 of these 25 cases were positive for presence of stem cells whereas 5 cases of nodal metastasis were negative for stem cells. The data are appended in Table 3. The one-tailed p -value = 0.0003. Thus, the association of nodal metastasis with presence of stem cells was found to be significant.

Further 18 out of the 20 cases which were positive for stem cells in the primary tumor showed large number of CD44+/CD24– phenotype cells among the metastatic tumor cells. Both the findings support the hypothesis that cases positive for stem cells in the primary tumor are more likely to metastasize to regional axillary lymphnodes and predominantly the metastasized cells are also stem cells with CD44+/CD24– phenotype. The findings are comparative to the findings in the study by Wei⁸ which proved statistically significant association of the stem cells having metastasized to the axillary lymph nodes. They studied 32 lymphnodes which had been mechanically enzyme digested for recognition of the CD44+/CD24– stem cells among the metastasized tumor cells.

Studies have shown significant association of the presence of stem cells in the primary tumor to reduced disease-free survival, overall survival, and presence of nodal metastasis.¹⁰ Few studies have however found CD24 positivity as poor prognostic marker in hormonal receptor positive tumors and CD44 positivity as good prognostic feature in hormonal receptor negative tumors¹⁷ as far overall survival and disease-free survival.

Targeted therapy against CD44 may be the future towards management of carcinoma breast. Thus, it is felt that evaluation of the primary tumor for presence of stem cells must be undertaken in all cases of carcinoma breast for prognostication and chances of lymph node metastasis.

Our studies showed qualitatively similar results in fluorescent tagged immunohistochemical staining and traditional IHC with chromogens like DAB and FAS red. Difficulty was felt in semi-quantitatively evaluating fluorescent chromogens by being ignorant of the background and due to the fact that absence of broadband filters for 488 and 549 nm both fluorescent chromogens could not be captured in the same exposure. Laser capture micro-dissection method with triple staining has been successfully conducted¹⁸ for semi-quantitative assessment of stem cells in a fluorescent setting.

Conclusion

Significant association was found in the present study, of 52 cases of MRM for two features of aggression i.e. early onset of disease and nodal metastasis with presence of tumor stem cells. The median age for cases positive for presence of tumor stem cells was lower by 14.5 years in the present study than the cases where tumor stem cells were absent. Even stem cells were detected in the nodal metastatic deposits.

No association was however found for presence of tumor cells towards other parameters of aggression like size of primary tumor at mastectomy, histological grade of lesion, triple negative status, and micro-vessel density in the present study.

It is felt though the study is limited by the number of cases which could be evaluated during the period of the study (most international studies being of more than 100 cases), definitely the routine inclusion of CD44 and CD24 for phenotypic evaluation for presence of stem cells in cases of carcinoma breast needs to be done along with ER, PR, and HER/2neu. It is felt that it has prognostic significance.

Conflicts of interest

The authors have none to declare.

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REFERENCES

1. Spring FA, Dalchau R, Daniels GL, et al. The Ina and Inb blood group antigens are located on a glycoprotein of

- 80,000 MW (the CDw44 glycoprotein) whose expression is influenced by the In(Lu) gene. *Immunology*. 1998;64(1):37–43.
2. Goodison S, Urquidi V, Tarin D. CD44 cell adhesion molecules. *Mol Pathol*. 1999;52(4):189–196.
 3. Hough MR, Rosten PM, Sexton TL, Kay R, Humphries RK. Mapping of CD24 and homologous sequences to multiple chromosomal loci. *Genomics*. 1994;22(1):154–161.
 4. Reya T, Morrison SJ, Clarke MF, Weissman IL. Stem cells, cancer, and cancer stem cells. *Nature*. 2001;414:105–111.
 5. Ponti D, Costa A, Zaffaroni N, et al. Isolation and in vitro propagation of tumorigenic breast cancer cells with stem/progenitor cell properties. *Cancer Res*. 2005;65:5506–5511.
 6. Mani SA, Guo W, Liao MJ, et al. The epithelial–mesenchymal transition generates cells with properties of stem cells. *Cell*. 2008;133:704–715.
 7. Sheridan C, Kishimoto H, Fuchs RK, et al. CD44⁺/CD24[−] breast cancer cells exhibit enhanced invasive properties: an early step necessary for metastasis. *Breast Cancer Res*. 2006;8(5):R59.
 8. Wei W, Hu H, Tan H, Chow LWC, Yip AYS, Loo WTY. Relationship of CD44⁺CD24^{−/low} breast cancer stem cells and axillary lymph node metastasis. *J Transl Med*. 2012;10(suppl 1):S1–S6.
 9. Giatromanolaki A, Sivridis E, Fiska A, Koukourakis MI. The CD44⁺/CD24[−] phenotype relates to 'triple-negative' state and unfavorable prognosis in breast cancer patients. *Med Oncol*. 2011;28(3):745–752.
 10. Lin Y, Zhong Y, Guan H, Zhang X, Sun Q. CD44⁺/CD24[−] phenotype contributes to malignant relapse following surgical resection and chemotherapy in patients with invasive ductal carcinoma. *J Exp Clin Cancer Res*. 2012;31:59.
 11. Collins LC, Botero ML, Schnitt SJ. Bimodal frequency distribution of estrogen. Receptor immunohistochemical staining results in breast cancer. *Am J Clin Pathol*. 2005;123:16–20.
 12. Wolff AC, Hammond ME, Schwartz JN, et al. American Society of Clinical Oncology/College of American Pathologists guideline recommendations for human epidermal growth factor receptor 2 testing in breast cancer. *J Clin Oncol*. 2007;25:118–145.
 13. Currie MJ, Beardsley BE, Harris GC, et al. Immunohistochemical analysis of cancer stem cell markers in invasive breast carcinoma and associated ductal carcinoma in situ: relationships with markers of tumor hypoxia and microvasculature. *Hum Pathol*. 2013;44(3):402–411.
 14. Agarwal G, Pradeep PV, Aggarwal V, Yip CH, Cheung PS. Spectrum of breast cancer in Asian women. *World J Surg*. 2007;31:1031–1040.
 15. Data-Office for National Statistics on Request. <http://www.ons.gov.uk/ons/search/index.html> [accessed June 2012].
 16. de Beçaetal FF. Cancer stem cells markers CD44, CD24 and ALDH1 in breast cancer special histological types. *J Clin Pathol*. 2013;66:187–191.
 17. Kim HJ, Kim MJ, Ahn SH, et al. Different prognostic significance of CD24 and CD44 expression in breast cancer according to hormone receptor status. *Breast*. 2011;20(February (1)):78–85.
 18. Kai M, Onishi H, Souzaki M, et al. Semi-quantitative evaluation of CD44⁺/CD24[−] tumor cell distribution in breast cancer tissue using a newly developed fluorescence immunohistochemical staining method. *Cancer Sci*. 2011;102:2132–2138.

IMPORTANT

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