Original Research Article

An analysis of stromal expression of CD10 in invasive ductal carcinoma of breast and its correlation with histological grade

Thiriveni Balajji G. S.[1], Ulaganathan S.[1]*, A. Suresh Venkatachalam[2], K. B. Akila[3], K. B. Lavanya[1], S. Vijayalakshmi[1]

[1]Department of Pathology, Government Coimbatore Medical College and Hospital, Coimbatore, Tamilnadu, India
[2]Department of Surgical Oncology, Regional Cancer centre, Government Coimbatore Medical College and Hospital, Coimbatore, Tamilnadu, India
[3]Department of Medical Oncology, Government Coimbatore Medical College and Hospital, Coimbatore, Tamilnadu, India

*Correspondence:
Dr. Ulaganathan S.,
E-mail: ssdrfpc@gmail.com

ABSTRACT

Background: Breast cancer is one of the most common cancers among women in India. Stroma has an important role in the pathogenesis of carcinoma of breast. Stromal marker can be novel marker for assessing the prognosis of breast cancer.
Methods: With the representative sections of 30 invasive ductal carcinoma of breast NOS type Hematoxylin and eosin staining was done. Immunohistochemistry was done with CD10. CD10 expression in stroma in cases and control slides were studied and statistically analyzed with histopathological grade.
Results: 46% (14 out of 30) of cases showed strong positivity for stromal CD10. Only two cases of strong positivity for CD10 were noted in the adjacent normal breast parenchyma. Stromal expression of CD10 had a statistically significant association with breast carcinoma than in control slides, p value is 0.002. 77% (10 out of 13) of CD10 positive cases were high grade carcinomas. Association of CD10 expression with high grade tumour was statistically significant (p value is 0.04 which is less than 0.05). No association was found with mean age.
Conclusions: As the grade of breast carcinoma increases the stromal expression of CD10 is increased. Stromal CD10 expression is directly correlated with higher tumour grade. CD10 could be used as novel prognostic marker and used to develop newer drugs.

Keywords: Epithelial to mesenchymal transition, Grade of breast carcinoma, Stromal CD10, Stromal marker

INTRODUCTION

Breast cancer is the most common cancer among women in India according to National cancer registry programme 2011 report.[1] Worldwide it is the most common non-skin cancer in females.[2] By the year 2030 global burden of breast cancer will be more than two million every year.[3] At present the mortality rate for breast cancer in India is 11.1per 10,000.[4] Breast tissue is composed of duct (epithelial origin) and stroma (mesenchymal origin).

Epithelial growth of tumour depends partly on chemical mediators between tumour cells and stromal cells.[5] CD10 is a myoepithelial marker.[6] In invasive ductal carcinoma of breast CD10 loss in myoepithelial cell and CD10 expression in stromal cells which is a characteristic feature of epithelial to mesenchymal transition(EMT), is associated with aggressive behaviour.[5] Recent studies suggest that genetic changes in stroma can promote carcinogenesis.[7] There are only few studies regarding stromal expression of CD10 in breast carcinoma

highlighting the role of stroma in tumour growth, progression and prognosis of breast cancer.[8]

CD10 (common acute lymphoblastic leukaemia antigen, CALLA) is a cell surface zinc dependant protease. CD10 act as a stem cell regulator in the breast and prevents uncontrolled proliferation of stem cells.[9] It is expressed in breast myoepithelial cells, lymphoid stem cells, neutrophils, and other epithelial cells.[10] CD10 also expressed in stroma of prostate, lung and colorectal cancers. In gastric carcinoma, CD10 positive stromal cells are correlated with vascular invasion and metastasis.[11] In nasopharyngeal carcinoma, stromal CD10 expression correlates with tumor progression.[12] Routine chemotherapeutic drugs target the epithelial cells while stromal cells are spared which could be responsible for recurrence. It indicates a novel form of therapy and stromal cells could be potential therapeutic targets.

Present study intends to analyse the stromal expression of CD10 in breast carcinoma and correlate with grading of the tumour.

Aim and objectives of the study were to analyse stromal expression of CD10 in invasive ductal breast carcinoma NOS type and to compare it with adjacent normal breast parenchyma and to correlate stromal expression of CD10 with grading of invasive ductal breast carcinoma NOS type.

METHODS

It was a retrospective randomized case control study. For the study cases- specimen with invasive ductal carcinoma of breast received in the department of pathology, Coimbatore Medical College, during the period for one year. Controls-adjacent normal parenchyma in specimen with invasive ductal carcinoma of breast.

For Cases 30 patients with invasive ductal carcinoma of breast diagnosed by clinical and histomorphological method and for Controls 30 representative areas from adjacent breast tissue were considered.

Inclusion criteria

- Age from 18 to 75 years
- Patients with Invasive ductal carcinoma of breast not otherwise specified (NOS) type, stage I, II and III diagnosed by histomorphological studies
- Patients irrespective of whether axillary dissection done for lymph node status or not
- For controls, adjacent breast parenchyma without DCIS component

Exclusion criteria

- Age less than 18 and more than 75 years.
- Breast carcinoma other than invasive ductal breast carcinoma NOS type.

- Patients with Stage I tumour who received neoadjuvant chemotherapy.
- Patients with Stage IV tumour who received chemotherapy and radiotherapy.
- Male patients
- Ill fixed specimen

Data collection

Clinically diagnosed breast carcinoma patient were evaluated with complete blood count, blood urea, blood sugar, serum creatinine, X ray chest, ECG, and Echocardiogram for surgical fitness. The patients underwent modified radical mastectomy procedure after obtaining informed written understandable consent. Specimens were collected in 10% neutral buffered formalin.

Histopathological examination

30 breast carcinoma specimens were fixed in 10% neutral buffered formalin for twenty-four hours. Specimens were grossed and representative bits from carcinomatous areas (cases) and adjacent parenchymal areas (controls) were sampled.
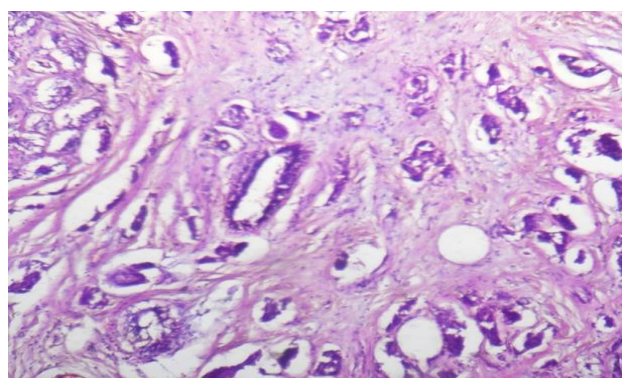

Figure 1: Invasive carcinoma of no special type. Tubules lined by carcinoma cells (10X).
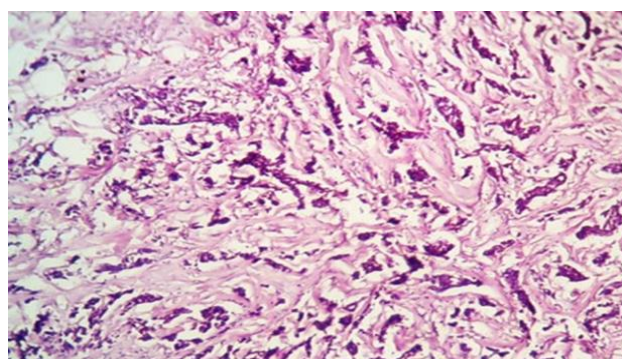

Figure 2: Invasive carcinoma of no special type. Cords of neoplastic cells invading into the stroma (10X).

Hematoxylin and Eosin stained microscopic slides of the primary tumours were reviewed to confirm the diagnosis,

to define tumour subtype and to standardize grading of invasive ductal carcinoma according to the Nottingham Modification of the Bloom and Richardson system (Figure 1-3).
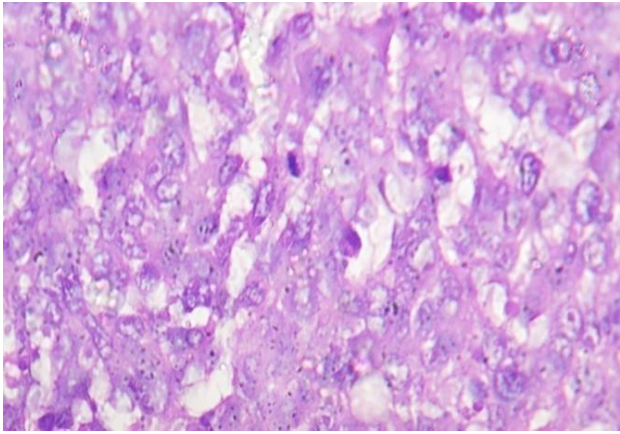

Figure 3: Invasive carcinoma of no special type. Sheets of closely packed pleomorphic cells with mitosis (40X).

Immunohistochemistry (IHC) for CD10

Four micron sections were cut. Sections were deparaffinized in xylene followed by hydration in descending ethanol grades. Antigen retrieval was performed by heating sections at 95°c 4 cycles of 5 min each for CD10 in Tris–EDTA buffer (pH 9.0).

Sections were then incubated with power block for 10 min, followed by incubation with primary antibodies for 1 hour. Mousemonoclonal antibody against human CD10 was used. After two washes with TBS (trisphosphate buffer solution) secondary antibody was added for 30 min.

After two washes with TBS, 3, 3'-diaminobenzidine substrates (DAB tetra hydrochloride) was applied to the sections for 10 min and sections were counterstained with Ehrlich Hematoxylin, dehydrated with ethanol and xylene and mounted permanently with DPX.

Quality control

As part of quality control positive control slide from fibro adenoma (Periductal cells) were used for CD10 (Figure 5). Negative control slides were also used to enhance the accuracy of the results.

Evaluation of staining

CD10 scoring was done as per the following table (Table 1).[13] Pattern of staining for CD10 is cytoplasmic and membranous positivity in stromal cells. Both negative and weak expressions were considered as negative. Only strong CD10 expression was considered as positive for statistical purpose (Figure 4-7).

Table 1: CD10 scoring.

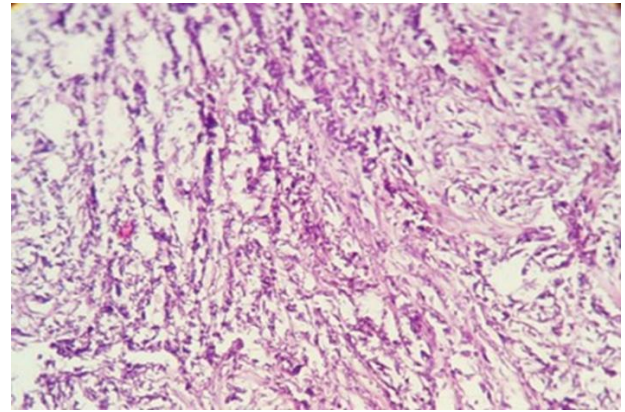| Score	Result	CD10 staining
0	Negative	<10% stromal positive cells (cytoplasmic and membrane positivity)
1	Weak	10%-30% stromal positive cells
2	Strong	>30% stromal positive cells

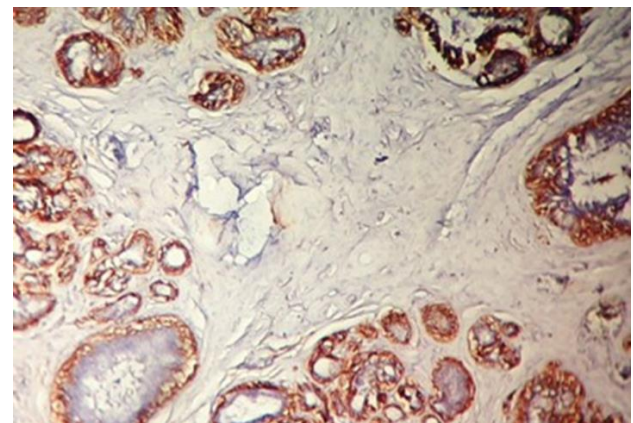**Figure 4: Stromal CD10 negativity in breast carcinoma (10 x).**

Figure 5: Stromal CD10 negativity, but myoepithelial cell positive for CD10 in fibro adenoma (10 x).
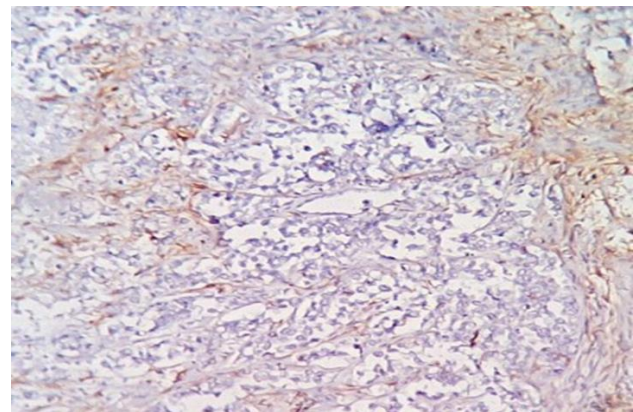

Figure 6: Stromal CD10 positivity in IDC Breast 1+ (10%-30% stromal positive cells) (10x).
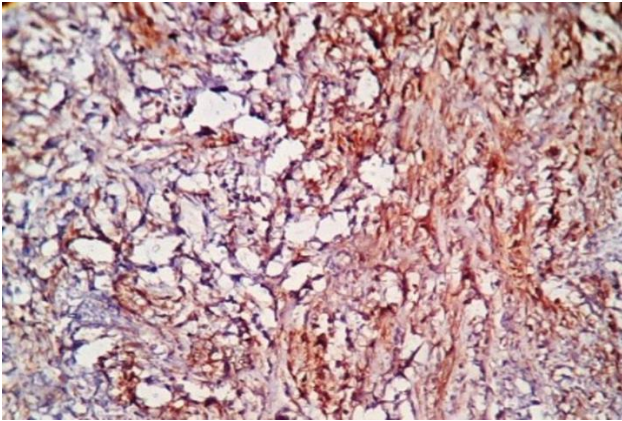
Figure 7: stromal CD10 positivity in IDC Breast 2+ (>30% stromal positive cells) (10 x).

Statistical analysis

The collected data was tabulated and analysed. Continuous data was expressed as mean. Statistical correlation between stromal expression of CD10 and histopathological grade was performed as per Chi square test. P values of less than 0.05 were considered as significant.

Human participant protection

Study was undertaken after obtaining institutional ethical committee clearance. The procedures were carried out with written understandable informed consent from the patients.

RESULTS

Thirty cases and controls were studied and the following results were obtained. The age of the patients ranges from 23 to 72 years with mean age of 48 years (Table 2). Majority of invasive ductal carcinoma of breast cases belong to 41- 50 age group (36.7%).

Table 2: Distribution of IDC of breast according to different age group.

Age	Number	%
21-30	1	3.3%
31-40	6	20%
41-50	11	36.7%
51-60	9	30%
>60	3	10%
Total	30	100%

Table 3: Association of CD10 expression with mean age.

| CD10 | Mean | SD | 95% CI OF Mean | | Lowest age | Highest age | SIG |
			Lower	Upper			
Negative	50.5	11	41.2	59.8	35	65	
Weak	49	12	39	59.1	36	72	
Strong	46.2	9.6	40.7	51.8	23	60	>0.05
Total	48.1	10.5	44.2	52	23	72	

No association is found between CD10 expression and mean age, p value is > 0.05

Table 4: Stromal expression of CD1O in breast carcinoma and normal parenchyma.

Stromal CD10 expression	Negative	Weak positive	Strong positive	Total
Breast carcinoma	8 (27%)	8 (27%)	14 (46%)	30
Normal parenchyma	25 (83%)	3 (10%)	2 (7%)	30

46% (14) cases were strongly positive in breast cancer (cases). Only two cases were strongly positive for CD10 in normal parenchyma (control).

Table 5: Comparison of stromal CD10 expression between cases and controls.

Stromal CD10 expression	Cases	Controls	Total
Positive	14	2	16
Negative	16	28	44
	30	30	60

CD10 positivity significantly associated with breast carcinoma (cases), p value --0.002

73% (22 out of 30) of the cases showed positivity for CD10 in the stroma, of which 46% (14) cases were strongly positive and 27% (8) were weakly positive (Table 4), (Figure 4 to 7). On the other hand, only two cases of strong positivity for stromal CD10 were noted in the adjacent normal breast parenchyma (Table 4), (Figure 8).

Stromal expression of CD10 had a statistically significant association with breast carcinoma than in control slides, p value is less than 0.05 (p value is 0.002) (Table 5).
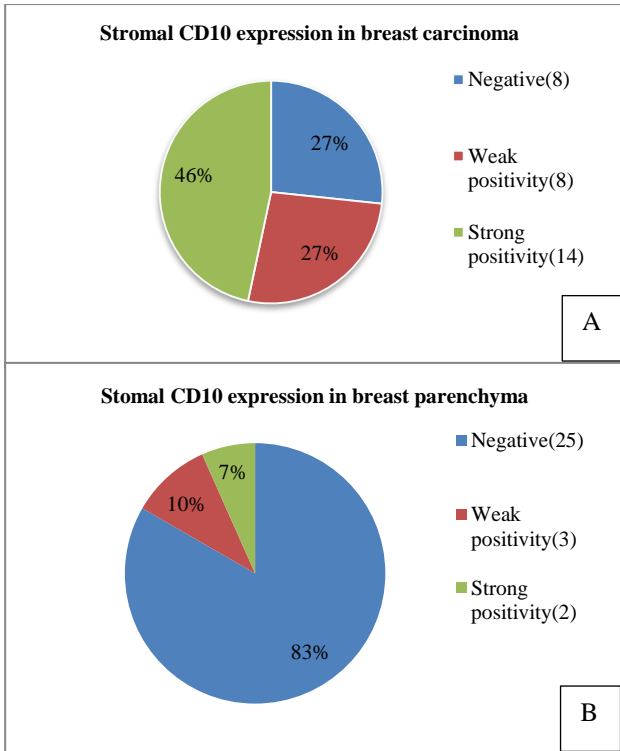
Stromal CD10 expression in breast carcinoma

- Negative(8)
- Weak positivity(8)
- Strong positivity(14)

27% 27% 46%

A

Stomal CD10 expression in breast parenchyma

- Negative(25)
- Weak positivity(3)
- Strong positivity(2)

7% 10% 83%

B

Figure 8: Stromal expression of CD10 in breast carcinoma and normal parenchyma.

77% (10 out of 13) of the grade III Invasive ductal carcinoma of breast showed strong stromal CD10 expression (Table 6).

Table 6: Stromal CD10 expression with histopathological grade.

Grade	CD10			Total
	Negative	Weak positive	Strong positive	
I	2	3	3	8
II	4	4	1	9
III	2	1	10	13
Total	8	8	14	30

Association of CD10 with Grade [N=30][p<0.05]

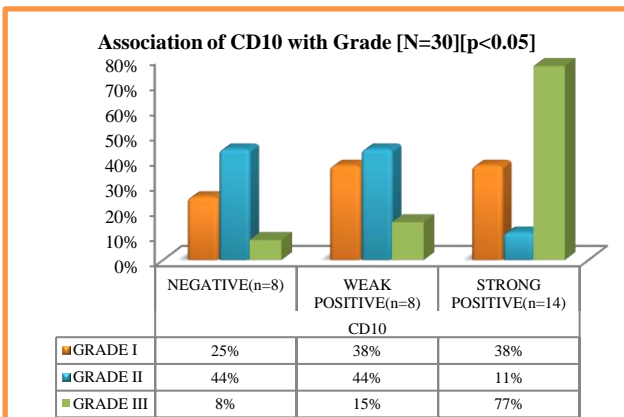|	NEGATIVE(n=8)	WEAK POSITIVE(n=8)	STRONG POSITIVE(n=14)
GRADE I	25%	38%	38%
GRADE II	44%	44%	11%
GRADE III	8%	15%	77%

Figure 9: Association of stromal CD10 with histological grade.

With increasing grade of tumor, the positivity for stromal CD10 expression also increases. The association is statistically significant for high grade carcinoma of breast than low grade carcinoma, p value is less than 0.05 (p value 0.04, Chi -square test) (Figure 9).

DISCUSSION

Stromal cells play an important role in carcinoma breast and metastasis. Tissue microenvironment plays a key role in controlling cell survival, proliferation, migration, polarization, and differentiation.[14,15]

CD10 is a cell surface zinc dependant protease. CD10 act as a stem cell regulator in the breast and prevents uncontrolled proliferation of stem cells.[9] In most of the normal breast tissue, except a small population of stromal cells has been shown to express CD10, there are no CD10 positive stromal cells.[16,17] In gastric carcinoma, CD10 positive stromal cells are correlated with vascular invasion and metastasis.[11] In nasopharyngeal carcinoma, stromal CD10 expression correlates with tumor progression.[12]

The continuous and bilateral molecular interaction between normal epithelial cells and stromal cells is affected by several factors secreted by the tumor cells or by stromal cells under the influence of cancer cells.[14,18,19] The matrix metalloproteinase (MMP) is one such factor. MMP plays an important role in tumor progression, stromal microenvironment in tumor invasion and metastasis.[20] Elevated MMP activities correlate with poor prognosis and promotes tumourigenesis, angiogenesis, invasion and metastasis.[21]

CD10 is a type of MMP which prevents differentiation of progenitor cells into epithelial and myoepithelial cells by cleaving signaling proteins thus it controls unchecked proliferation of stem cells.[22] In breast cancer loss of CD10 in myoepithelial cells leads to proliferation of malignant cells and invasion of in situ cancer. However, in invasive cancer expression of CD10 in stroma which characterize epithelial to mesenchymal transition (EMT), secreted from mesenchymal cells or transformed epithelial cancer stem cells, might prevent differentiation of cancer cells. Thus CD10 helps in maintaining the cancer stem cells.[23] It also explains increased expression of stromal CD10 in high grade undifferentiated breast carcinomas.[9] Recent studies indicate EMT has a role in invasiveness and metastasis of carcinoma.

In the present study majority of invasive ductal carcinoma of breast cases belong to ages between 41 and 50 years. The mean age of invasive ductal carcinoma of breast in this study was 48 years. Puri et al found 48.5 years as mean age of patients in their study.[13]

In the present study, no association was found between stromal expression of CD10 and age. Iwaya et al also

showed similar result in their study.[16] Jana SH et al also observed similar results.[9]

In the present study 73% (22 out of 30) of the cases showed positivity for CD10 in the stroma, of which 46% (14) cases were strongly positive and 27% (8) were weakly positive. Only two cases of strong positivity for CD10 were noted in the adjacent normal breast parenchyma. Stromal expression of CD10 had a statistically significant association with breast carcinoma than in control slides, p value is 0.002.

In a study done by Makretsov et al 79% (205 out of 258) of invasive ductal carcinoma of breast showed stromal CD10 expression.[5] Puri et al also found CD10 expression in 80% (40/50) of invasive ductal carcinoma of breast.[13] Study done by Thomas S et al shows stromal CD10 positivity in 55% (16 out of 29) of cases.[24]

In the present study, Stromal CD10 positivity was found in 77% cases of grade III invasive ductal carcinoma of breast. Association of CD10 expression with grade III invasive ductal carcinoma of breast is statistically significant p value is 0.04 which is less than 0.05. Jana SH et al study shows stromal CD10 positivity in 65% cases of grade III invasive ductal carcinoma of breast and the association is statistically significant.[9] Makretsov et al study shows 59% positivity for stromal CD10 in grade III cases.[5]

CD10 can be a therapeutic target for managing carcinoma breast since it cleaves the chemotherapeutic agent doxorubicin and results in resistance to chemotherapy. CPI0004Na is a CD10 cleavable peptide prodrug of doxorubicin. Experimental studies show CPI0004Na improves antitumor efficacy and reduces the toxicities of chemotherapeutic agents.[14] Thus documenting the stromal CD10 status in carcinoma breast cases before and after chemotherapy is important as a possible prognostic and predictive factor.

To conclude, stromal CD10 expression in invasive ductal carcinoma of breast is directly correlated with higher tumour grade. As the tumour grade increases stromal CD10 expression is increased. It may warrant additional treatment. Thus, analyzing stromal CD10 expression in all invasive ductal carcinoma of breast especially in triple negative patients would help in choosing optimal individualized treatment option. Increased level of CD10 activity from stromal cells leads to inhibition of epithelial cell differentiation. Thus cancer stem cells are maintained and may cause recurrence of carcinoma. Since CD10 cleaves the drug doxorubicin thereby causes chemo resistance. Thus inhibiting the activity of CD10 may have an increased response to chemotherapeutic agents and decreases the recurrence. Experimental studies show CPI0004Na improves antitumor efficacy.

Further studies are needed involving larger number of patients to identify the source of stromal CD10 expression, its role in epithelial to mesenchymal transition, its role in carcinogenesis of breast cancer, effect of chemotherapy on CD10, to develop new therapy targeting CD10 and to correlate with chemotherapeutic response and overall prognosis.

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