



## Integrin $\alpha\beta6$ as a Prognostic Indicator in Breast Cancer: a comparative study of China and India.

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### ABSTRACT

**Background and Objective:** Integrin  $\alpha\beta6$  serves a vital role in the progressive development of cancer. The distribution of  $\alpha\beta6$  integrin are seen in proliferating epithelia especially around the lungs and mammary cells. In this study our aim was to determine the expression of Integrin  $\alpha\beta6$  and compare between China and India.

**Methodology:** The level of expression for integrin  $\alpha\beta6$  was evaluated in breast cancer tissues and paraneoplastic tissues of 80 cases from China and 80 cases from India via immunohistochemical stain.

**Results:** The level of expression for integrin  $\alpha\beta6$  was notably related with histological grade (China group  $P=0.0471$ , India group  $P=0.0051$ ) and axillary lymph node involvement (China group  $P=0.0471$ , India group  $P=0.0003$ ). The survival analysis by Kaplan-Meier indicated the median follow-up time of two groups was lower with positive integrin  $\alpha\beta6$  (China group  $P=0.016$ , India group  $P=0.004$ , the log-rank test).

**Conclusion:** Integrin  $\alpha\beta6$  is the prognostic indicator for invasiveness and metastatic development of breast cancer in both China and India and could be an important factor for treatment in the near future.

### KEYWORDS

Integrin  $\alpha\beta6$ , breast cancer, immunohistochemistry, prognostic indicator

### Introduction

Breast cancer is one of the most commonly diagnosed malignancies in women worldwide, with an increasing annual incidence. Despite the advances in multidisciplinary treatment for breast cancer, the clinical outcome of patients is different widely. Our previous studies have shown that integrin  $\alpha\beta6$  plays an important role in the development and progression of colon cancer. As members of the cell adhesion molecular family, integrin  $\alpha\beta6$  is a subtype of integrin's, expressed strictly in epithelia, up-regulated in parallel with embryo formation, oncogenesis and epithelial repair, and rarely expressed in normal tissue. Previously studies showed that integrin  $\alpha\beta6$  played an important role in invasion, proliferation, apoptosis, tumor immunity and EMT of malignant tumors. It is worth noting that integrin  $\alpha\beta6$  expression was closely related with clinicopathological features of many carcinomas, as well as patient prognosis. The functional association of  $\alpha\beta6$  in breast cancer is confirmed but the clinical outcome is yet to be explored. Morbidity and mortality rates are very high in breast cancer, especially in developing countries like China and India where it is detected at the last stage. Lack of awareness among women adds to the already existing shortage of medical facilities.

In this research we studied about the expression of Integrin  $\alpha\beta6$  for a series of breast cancer from China and India. Moreover we made a comparison between China and India by investigating the relationship between clinicopathological features and Integrin  $\alpha\beta6$  expression of patients with breast carcinoma.

### Methodology

#### 1. Clinical samples

A total of 160 cases (80 cases from China and 80 cases from India) were collected from breast cancer patients whose surgical resection was performed between February 2010 to July 2012 for IHC, from Qilu Hospital of Shandong University China and Swadesh Basu Hospital of Kolkata, India respectively. None of

patients in this study had received radiation therapy or chemotherapy before surgical resection. In China group, the average age of patient was 54.5 (range 27-76), with a median follow up time of 48 months. 28 patients had negative lymph node, while 52 patients had positive lymph node. 18 cases were grade I, 47 cases were grade II and 15 cases were grade III. In India group, the average age of patients was 64.5 (range 38-72) along with a median follow up time of 49 months. 19 patients had negative lymph node, while 61 patients had positive lymph node. 14 cases were grade I, 36 cases were grade II and 30 cases were grade III. The study complied with the requirements of The Ethics Committee of Qilu Hospital, Shandong University and Swadesh Basu Hospital of Kolkata, India. All the details of the case characteristics are figured in Table 1 and Table 2.

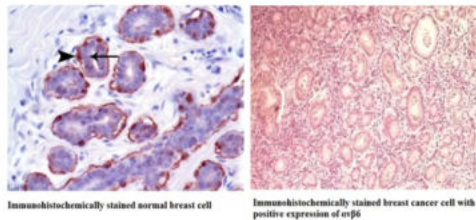
#### 2. IHC and Antibodies

Firstly sections were dried for 50 minutes at a temperature of 65°C, then used xylene and ethanol serially to deparaffinize and rehydrate. Following with 250 ml citrate buffer (pH 6.4) for microwave antigen retrieval, at high temperature for 5 minutes and finally 40°C for 15 minutes. Then the sections were allowed to cool down to room temperature, washed in PBS and endogenous peroxidase was blocked by incubating 30 minutes in 0.3% H<sub>2</sub>O<sub>2</sub>. Slides were incubated overnight at 4°C, with the anti-Integrin  $\beta6$  polyclonal rabbit antibody (1:300; Proteintech Group, Inc., Cat. No. 19695-1-AP, Chicago, USA). The second day, sections were incubated with secondary antibody for 30 minutes. Then stained and terminated tissues using DAB and Hematoxylin (Beijing ZSJQB CO., Ltd., Beijing, China). Finally the samples were observed under light microscope (Olympus Corp, Tokyo, Japan).

#### 3. Immunohistochemical analysis and scoring

The tumor cells were considered positive in which cytoplasm were observed stained dark brown under light microscope. Integrin  $\alpha\beta6$  was expressed both in cytoplasm and on cellular membrane. For the quantification of  $\alpha\beta6$  expression; both staining intensity

and the percentage of stained cells were evaluated. Cells without staining were scored as 0 points, weak staining intensity (light yellow), 1 point, moderate staining intensity (yellow brown), 2 points, and strong staining intensity (brown), 3 points. And the percentage of stained cells was scored as 0 points( 0% positive cells), 1 point(less than 25%), 2 points(25%-50%) 3 points(50%-75%),4 points(more than 75%).The final score for  $\alpha v\beta 6$  expression was presented as the summation of the above two kinds of scores. A score ranging within 0 to 3 points were considered as negative group and a score more than 3 points was considered as positive group.



**4. Statistical analysis**

Statistical analyses were performed using IBM SPSS Statistics, version 23.0 software. The association between integrin  $\alpha v\beta 6$  and clinicopathological factors was examined using the chi-square test. Survival analyses were conducted by using Kaplan-Meier method and comparison between the survival curves were estimated using log-rank tests. A Cox proportional hazards model was constructed to identify factors that were associated with cancer deaths independently. P values<0.05 was considered to be statistically significant.

**Results**

**1. The expression of integrin  $\alpha v\beta 6$  in breast cancer of China and India**

In China group, 45 cases were positive expression, and 35 cases were negative (Figure), positive rate was 56.25%. In India group, 44 cases were positive expression, and 36 cases were negative (Figure), positive rate was 55%. And the normal para-neoplastic tissues for the two groups were all negative staining.

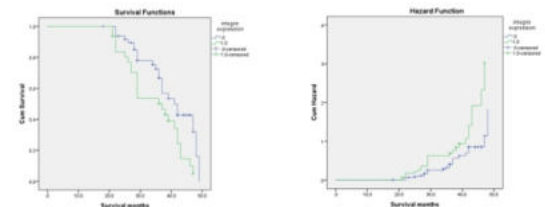
**2. Association between clinicopathological characteristics and integrin  $\alpha v\beta 6$  expression**

Correlations between clinicopathological characteristics and integrin  $\alpha v\beta 6$  expression of two groups are shown in Table 1 and 2, respectively. In China group, there was significant association between integrin  $\alpha v\beta 6$  expression and axillary lymph node status (P=0.0407) and histological grade (P=0.0471). And the positive rate of grade II was much more than that of grade I and grade III. In contrast, based on the standard of P<0.05, there was no significant association between integrin  $\alpha v\beta 6$  expression and other clinicopathological features such as tumor size, expression of PR and ER. In India group, integrin  $\alpha v\beta 6$  expression was significantly different between patients with and without axillary lymph node metastasis (P=0.0003). Analyzing the results from India group showed that integrin  $\alpha v\beta 6$  expression was higher in grade III than that in grade I and grade II. The chi-square analysis of the other factors showed expression level of integrin  $\alpha v\beta 6$  was significantly correlated with histological grade (P=0.0051). But tumor size, expression of PR and ER tended to have no association with integrin  $\alpha v\beta 6$  expression.

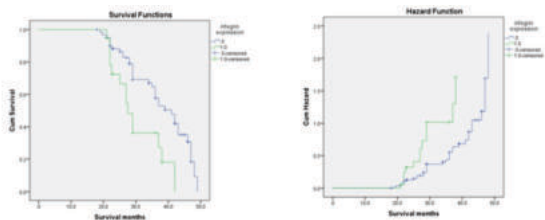
Table 1				Table 2			
Clinicopathological Factors	#	Integrin $\alpha v\beta 6$ expression	P value	Clinicopathological Factors	#	Integrin $\alpha v\beta 6$ expression	P value
		Negative				Negative	
		Positive				Positive	
Tumor size				Tumor size			
≤ 2	32	13	19	≤ 2	55	19	36
> 2	48	23	25	> 2	25	10	15
Axillary lymph node status			0.0407	Axillary lymph node status			0.0003
Without metastasis	28	12	16	Without metastasis	19	14	5
Metastasis	52	11	41	Metastasis	61	17	44
Histological grade			0.0471	Histological grade			0.0051
I	18	10	8	I	14	10	4
II	47	21	26	II	36	19	17
III	15	3	12	III	30	7	23
ER				ER			
Negative	27	12	14	Negative	33	14	19
Positive	51	22	31	Positive	47	21	26
PR			0.9734	PR			0.8412
Negative	22	8	14	Negative	38	13	25
Positive	38	20	18	Positive	42	17	25

**Correlation of integrin  $\alpha v\beta 6$  expression and overall survival in patients with breast cancer**

To evaluate the prognostic power of integrin  $\alpha v\beta 6$  for breast cancer, we generated survival curves through the Kaplan-Meier survival analysis. In China group, patients with positive expression had a lower overall survival rate than those with negative expression (P=0.016). In India group, the survival analysis revealed patients with positive expression showed a worse prognosis for overall survival compared to those with negative expression (P=0.004). Patient survival over time on integrin  $\alpha v\beta 6$  is illustrated in Figure 1 and 2.



**Figure 1.** Overall survival according to integrin  $\alpha v\beta 6$  in China group and Cox proportional hazards model. P= 0.016



**Figure 2.** Overall survival according to integrin  $\alpha v\beta 6$  in India group and Cox proportional hazards model. P= 0.004

**Discussion**

The mechanism in development and progress of breast cancer remains still unclear. In recent years, several studies have proved that tumor invasion and metastasis was associated with integrin expression. Integrins are a family of heterodimeric cell-adhesion molecules, comprising of two non-covalently bound transmembrane subunits. As a member of integrin family,  $\alpha v\beta 6$  occurs in many epithelial tumors and has been considered to play a prominent role in tumor invasion and metastasis. Bates et al reported that in colon cancer, the median survival time of patients with high expression of integrin  $\alpha v\beta 6$ , compared with patients with no or low expression of integrin  $\alpha v\beta 6$ , was significantly shorter. Kawashima et al found that the expression of integrin  $\alpha v\beta 6$  in stomach carcinoma was significantly higher than that of paraneoplastic tissues, and integrin  $\alpha v\beta 6$  expression was related to the lymph node metastasis. Additionally, a report published by Elayadi et al analyzed 311 cases lung cancer tissue and revealed the positive rate was 54%, integrin  $\alpha v\beta 6$  expression was higher in nonsmall-cell lung cancer(NSCLC) than that of in SCLC.

In our study, our results showed that integrin  $\alpha v\beta 6$  expression was significantly associated with clinical grade and lymph node metastasis both in China and India group, similar to the report of Jemal. But from the clinical data, we can find some differences between China and India. Breast cancer patients in India are diagnosed at later stages than those in China, which might contribute to variable clinical outcomes and lower 5-year survival rate. In India, breast cancer is the leading incident cancer amongst women. In India, the overall incidence of breast cancer is less as compared to the US. But if you see the actual number of cases, India is not far behind. In the year 2012, there were about 2,32,000 breast cancer cases reported in the US, whereas in India, 1,45,000 new cases were diagnosed. This implies that, though, because of India's population, the percentage of total women affected seems less, the breast cancer burden in India has almost reached about 2/3rds of that of the US and is steadily rising. In contrast to what is seen in China, in India, people are lack of knowledge of the importance of screening to detect early stage disease. Therefore, early breast cancer detection and disease

down-staging remains the cornerstone of breast cancer control to improve outcome and survival in low income countries. Although the diagnosis and treatment for patients with breast cancer have a great progress over the past few years, but 5-year survival rates for patients in China remained 76%-82%. These consequences indicated that patients with different genetic backgrounds may have same prognoses. A further study needs to be conducted for more details about patients in China and India.

### Conclusion

Our finding provides evidence that integrin  $\alpha\beta6$  expression is associated with clinical factors and outcomes of patients with breast cancer. The high level of expression predicts a poor prognosis. Additionally, integrin  $\alpha\beta6$  may be an important biomarker for predicting unfavorable biological behavior. As we know, the study is the first report to compare between China and India. Although the findings need to be conducted further to confirm the results, integrin  $\alpha\beta6$  may provide important indicator for prognosis of breast cancer and even a target for therapy.

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