



Expression of tetraspanins NET-6 and CD151 in breast cancer as a potential tumor biomarker

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Abstract

Tetraspanins have been implicated in multiple biological functions including protein networking and cell signaling. NET-6 (TSPAN 13) has been demonstrated to be a tumor suppressor gene in breast cancer, while CD151 is more likely to act as an oncogene. However, the biological function of both proteins is still inconclusive. Immunohistochemistry was used to analyze the expression of NET-6 and CD151 proteins in breast tumors and benign epithelial cells. The cellular expression of both markers was correlated with HER2, ER, and PR status as well as tumor grade, Ki-67 scores, invasion, and metastasis. Expression of NET-6 and CD151 was variable both in tumors and in benign epithelial cells. Expression of NET-6 and CD151 was stronger in tumors than in benign epithelial cells. The expression of NET-6 was also stronger in HER2-negative, low-grade, lymphovascular invasion-negative, and non-metastatic breast tumors. There was no correlation between NET-6 expression and ER, or PR, or triple-negative status. There was no correlation between CD151 expression and HER2, ER, PR, or triple-negative status, tumor grade, or Ki-67 scores, invasion, and metastasis. The expression of tetraspanins NET-6 and CD151 may indicate an alteration of their biological function during neoplastic transformation. NET-6 expression in tumors might be a potential marker indicating the outcome of breast cancer.

Keywords Tetraspanin · NET-6 (TSPAN 13) · CD151 · Breast cancer · Metastasis

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Introduction

Tetraspanins are a large group of membrane proteins possessing four transmembrane domains. The large extracellular loop contains four conserved cysteine residues which promote the formation of disulfide bridges, and thus, the tetraspanins function as membrane protein organizers [1]. There are 34 tetraspanin family members that have been identified so far, and 33 of them exist in humans [2]. Tetraspanins interact laterally among themselves and with partner proteins to control the spatial organization of membrane proteins in large networks called the tetraspanin web and play an important role in cellular signaling and tumorigenesis [3–10].

NET-6 (also known as TSPAN 13) is a member of the tetraspanin family and its chromosomal location is 7p21.1. The *NET-6* gene encodes a 204-amino acid protein which we demonstrated to be a tumor suppressor gene in breast cancer [11]. The mRNA level of NET-6 was very low in breast cancer cells with aggressive characteristics [12]. However, the expression of NET-6 protein in breast tumors and breast cancer cell lines has not been reported thus far.

CD151 is another member of the tetraspanin family which possesses the characteristics of other tetraspanins. The chromosomal localization of the *CD151* gene is 11p15.5, and it encodes a protein of 253 amino acids. It has been implicated in the development and progression of several cancers including breast cancer [7, 13–18]. A handful of reports mentioned that CD151 played a role in breast cancer invasion and metastasis associated with HER2 expression and it could be a potential prognostic biomarker in the clinic [13–18]. However, there is no report on the observation of the concomitant expression of NET-6 and CD151 in breast tumors thus far.

Since NET-6 has not been studied extensively, its expression in breast tumors and benign epithelial cells is scarcely described, and its correlation with clinical pathological features is not known. In addition, the expression of CD151 in breast cancer is also inconclusive so far. In this study, we demonstrate the expression of tetraspanins NET-6 and CD151 in breast tumors and benign epithelial cells by immunohistochemistry. The expression of the two proteins was correlated with HER2, ER, and PR status, tumor grade, lymphovascular invasion, Ki-67 scores, and metastasis.

Materials and methods

Patients and samples

Ninety-six (for NET-6) and 65 (for CD151) formalin-fixed paraffin-embedded (FFPE) breast cancers tissue blocks and 73 or 37 adjacent non-cancerous tissues (benign epithelium) from the tissue bank of Guangzhou Huayin Medical Laboratory of South China Medical University and the Department of Pathology of the People's Hospital of Guangxi Zhuang Autonomous Region were used in this study. The tissue blocks were collected between 2011 and 2017. The study was approved by the Institute Review Boards of Guangzhou Huayin Medical Laboratory and the People's Hospital of Guangxi Zhuang Autonomous Region for the use of human materials. The HER2, ER, PR, Ki-67, and tumor grade data were obtained from the clinical pathology records.

Immunohistochemistry

The NET-6 and CD151 immunohistochemistry analysis was carried out by using a goat anti-NET-6 polyclonal antibody (SC-160582) from Santa Cruz Biotechnology, Inc. (Dallas, TX, USA) and mouse anti-CD151 monoclonal antibody (ab33315) from Abcam (Cambridge, MA, USA). Briefly, the FFPE tissues or cell blocks were cut into 5- μ m-thick sections, followed by de-waxing according to routine histological protocols. The de-waxed slides were pretreated with 1 mM of pH 9.0 EDTA buffer in a microwave oven at 600 W

for 5 min, followed by blocking with 1% H₂O₂ in methanol to block the endogenous peroxidase activity for 30 min at room temperature. The sections were stained with goat anti-NET-6 (1:200) or mouse anti-CD151 (1:100) antibodies and incubated at 37 °C for 1 h. Slides were rinsed with PBS for three times, and then, the horseradish peroxidase-labeled secondary antibody was applied and incubated for 1 h at room temperature following three times of PBS washing. The reaction was visualized by DAB and counterstained with hematoxylin, followed by mounting in glycerol jelly.

Interpretation of immunostains

The scoring criteria for the immunostains were according to Nishimura et al. [19]. For NET-6 and CD151, the staining results were scored as 0, 1+, 2+, or 3+ after reviewing all the samples combining the criteria of Nishimura et al. Results were scored by two pathologists separately. Specifically, if there was no staining, the result was scored as 0 (negative); weak staining was scored as 1+, moderate staining was scored as 2+, and strong staining was scored as 3+. A score of 1+ and above was considered positive.

Statistical analysis

The IHC results of NET-6 and CD151 were correlated with the HER2, ER, PR status, and Ki-67 scores from routine IHC analysis as well as with tumor grade, extent of invasion, and metastasis. The statistical analysis was performed using the SPSS version 18.0, of which the Mann–Whitney *U* test was performed. A *p* value of less than 0.05 was considered significant.

Results

Expression of NET-6 and CD151 in breast tumors and benign breast tissue

Tables 1 and 2 show the basic characteristics of patient collectives in this study. The expression of both NET-6 and CD151 in tumors and benign epithelial cells was variable (Figs. 1, 2); however, the expression of both tetraspanins was significantly stronger in tumors than in benign epithelial cells (Table 3, *p* = 0.001 and *p* = 0.003, respectively).

Correlation of NET-6 expression with hormone receptor status, tumor grade, Ki-67 scores, lymphovascular invasion (LVI), and metastasis in breast tumors

HER2 overexpression is associated with rapid progression and breast cancer metastasis, while ER and PR expression

Table 1 Basic characteristic of patient collectives for NET-6

Characteristic	<i>n</i>	Median (range)
Age		50 (32–78)
Histological type		
Minimally invasive carcinoma	5	
Invasive carcinoma		
Invasive carcinoma of no special type (NST)	87	
Special subtypes of invasive carcinoma	4	
Tumor grade		
I	4	
II	43	
III	49	
Molecular subtype		
Luminal A	37	
Luminal B	34	
HER-2+	11	
Basal-like	14	

Table 2 Basic characteristic of patient collectives for CD151

Characteristic	<i>n</i>	Median (range)
Age		50 (32–78)
Histological type		
Minimally invasive carcinoma	8	
Invasive carcinoma		
Invasive carcinoma of no special type (NST)	48	
Special subtypes of invasive carcinoma	9	
Tumor grade		
I	9	
II	30	
III	26	
Molecular subtype		
Luminal A	21	
Luminal B	15	
HER-2+	11	
Basal-like	18	

are favorable biomarkers of hormonal therapy response. Tumor grade and Ki-67 score are markers of breast cancer aggressiveness. Results showed that the expression of NET-6 was stronger in HER2-negative breast tumors than in HER2-positive cases ($p = 0.037$, Table 4), and it was higher in grade I, II tumors than in grade III tumors ($p = 0.024$). There was no correlation between NET-6 expression and ER, or PR, or triple-negative receptor status (Table 4). There was no correlation between NET-6 expression and Ki-67 scores in tumors ($p = 0.346$, Table 4). In addition, NET-6 expression was stronger in tumors with no LVI than in LVI-positive tumors ($p = 0.012$, Table 4), in

non-lymph node involvement (metastasis) than with lymph node involvement ($p = 0.028$, Table 4).

Correlation of CD151 expression with hormone receptor status, tumor grade, Ki-67 scores, lymphovascular invasion, and metastasis in breast tumors

There was no statistically significant correlation between CD151 expression and HER2, ER, PR status, tumor grade, Ki-67 scores, LVI, and metastasis in tumors (Table 5).

Discussion

NET-6 and CD151 belong to the tetraspanin family and play an important role in the networking of membrane proteins and cellular signaling [17, 20, 21]. Deletion of the *NET-6* gene on chromosome 7p15-21 was found to be associated with Wilms tumor [22]. Our previous study found that NET-6 inhibited breast cancer growth both in vitro and in vivo [11, 12]. Nevertheless, the expression of NET-6 and CD151 in cancers and their role in cancer biology are still not fully understood [7, 13–15, 18, 23].

In this study, the expression of NET-6 and CD151 in breast tumors and benign epithelial cells was studied in archival tissue blocks by immunohistochemistry. Results showed that the expression of NET-6 and CD151 was stronger in tumors than in benign epithelial cells (Table 3). The expression of both tetraspanins in tumors and benign epithelial cells was variable as shown in Figs. 1 and 2. One study mentioned that in prostate cancer, 80% of cases had NET-6 overexpression by immunohistochemistry, and NET-6 expression was inversely correlated with Gleason score and prostate-specific antigen (PSA) preoperative levels and directly correlated with the presence of prostatic intraepithelial neoplasia in tumor tissue [21]. NET-6 was expressed in low-grade prostatic intraepithelial neoplasia. NET-6 expression correlated with factors of favorable outcome [21]. Our results are similar to those findings. This indicates that NET-6 expression in tumors may be a favorable prognostic factor.

CD151 is generally considered to be an oncoprotein. A previous study found that tetraspanins CD151 and Tspan 8 were found in the exosome of cancer cells to interact with their surroundings [24]. Another report indicated that CD151 was highly expressed in endothelial cells of hepatic sinusoids and neovessels developing in fibrotic septa and tumor margins and it co-localized with the endothelial adhesion molecule/immunoglobulin superfamily member, vascular cell adhesion molecule-1 (VCAM-1) [17]. This suggests that the expression and cellular localization of CD151 may play a role in cellular signaling pathways.

Fig. 1 Representative examples of expression of NET-6 in breast tumors and in benign epithelial cells. **a–d** Tumor. **a** Strong expression; **b** moderate expression; **c** weak expression; **d** no expression. **e–h** Benign epithelial cells. **e** Strong expression; **f** moderate expression; **g** weak expression; **h** no expression $\times 200$

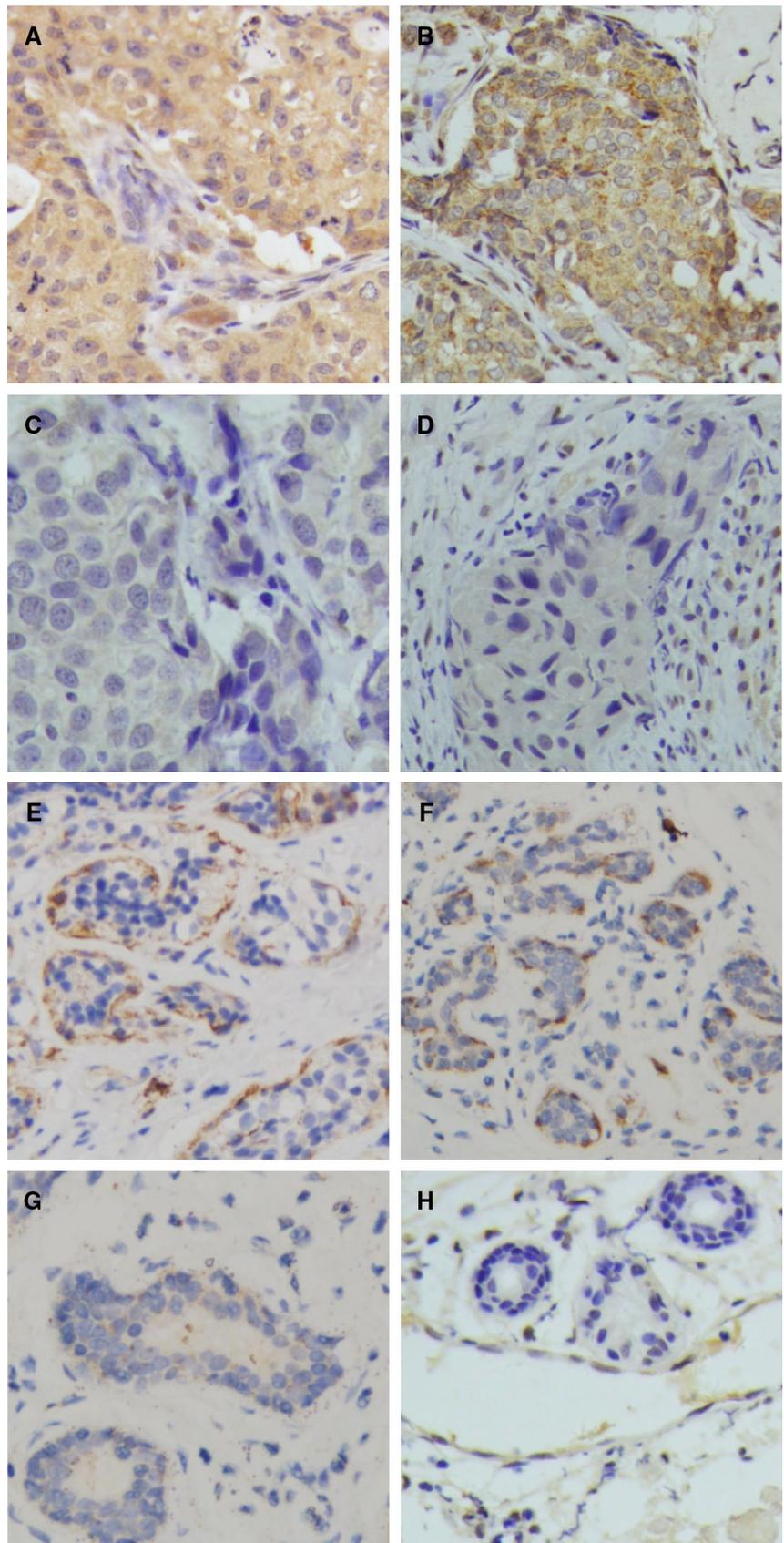


Fig. 2 Representative examples of expression of CD151 in breast tumors and in benign epithelial cells. **a–d** Tumor. **a** Strong expression; **b** moderate expression; **c** weak expression; **d** no expression. **e–h** Benign epithelial cells. **e** Strong expression; **f** moderate expression; **g** weak expression; **h** no expression $\times 200$

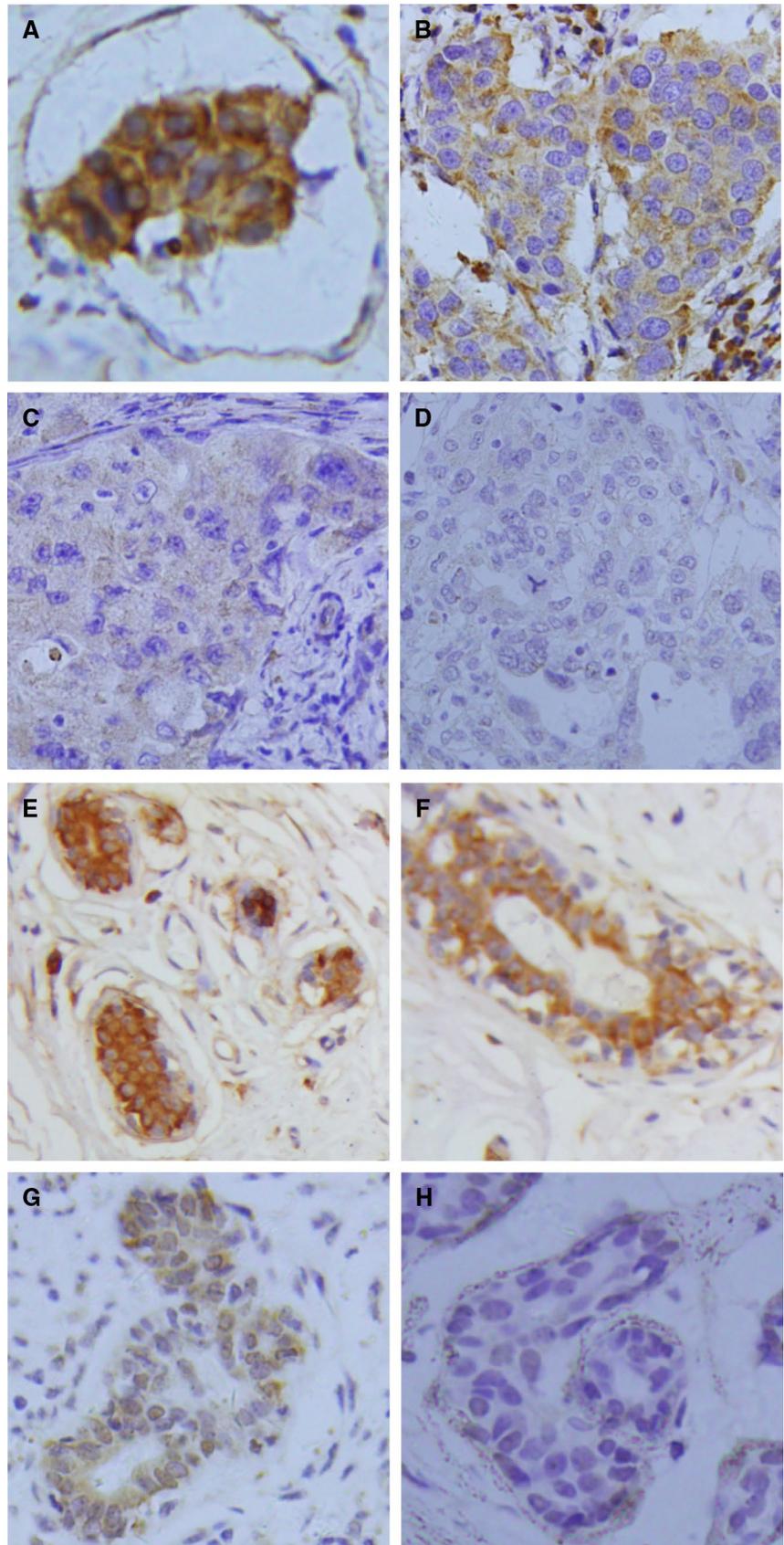


Table 3 Expression of NET-6 and CD151 in breast tumors and benign epithelial cells (Mann–Whitney *U* test)

	NET-6 IHC scores (mean rank)	CD151 IHC scores (mean rank)
Tumors	94.79	57.54
Benign tissues	72.13	40.89
<i>p</i>	0.001	0.003

IHC scores of NET-6 and CD151 (Mean rank 94.79 and 57.54) were stronger in tumors than in benign epithelial cells (Mean rank 72.13, 40.89) ($p=0.001$ and $p=0.003$)

Table 4 Correlation of NET-6 expression with hormone receptor status, Ki-67 scores and clinical features in breast tumors (Mann–Whitney *U* test)

	<i>n</i>	NET-6 IHC scores Mean Rank	<i>p</i>
HER2 status			0.037
Negative	51	53.60	
Positive	45	42.72	
ER status			0.712
Negative	26	46.92	
Positive	70	49.09	
PR status			0.386
Negative	41	45.89	
Positive	55	50.45	
HER2 + ER + PR status			0.617
Triple-negative	14	51.64	
Non-triple-negative	82	47.96	
Tumor grade			0.024
Grade III	45	42.26	
Grade I, II	51	54.01	
Ki-67			0.346
< 15%	13	35.65	
≥ 15%	67	41.44	
Lymphovascular invasion			0.012
No lymphovascular invasion	55	54.14	
Lymphovascular invasion	41	40.94	
Lymph node involvement			0.028
No lymph node involvement	72	51.78	
Lymph node involvement	24	38.67	

Expression of NET-6 was stronger in HER2-negative breast tumors, in tumors with low grades (grade I-II), in LVI-negative and in lymph node-negative groups than in HER2-positive tumors ($p=0.037$), in tumors with high grade (grade III, $p=0.024$), in tumors with lymphovascular invasion ($p=0.012$), and in lymph node positive groups ($p=0.028$)

HER2 overexpression is an indicator of fast progression and metastatic potential of breast cancer, while ER and PR expressions are favorable biomarkers of hormonal therapy response. Tumor grade, Ki-67 scores, and lymphovascular

Table 5 Correlation of CD151 expression with hormone receptor status, tumor grade, Ki-67 scores, and clinical features in breast tumors (Mann–Whitney *U* test)

	<i>n</i>	CD151 IHC scores Mean Rank	<i>p</i>
HER2 status			0.589
Negative	39	32.04	
Positive	26	34.44	
ER status			0.060
Negative	30	28.57	
Positive	35	36.80	
PR status			0.377
Negative	40	31.48	
Positive	25	35.44	
HER2 + ER + PR status			0.932
Triple-negative	19	32.88	
Non-triple-negative	46	33.29	
Tumor grade			0.148
Grade III	31	36.31	
Grade I, II	34	29.99	
Ki-67			0.538
< 15%	6	16.67	
≥ 15%	27	17.52	
Lymphovascular invasion			0.569
No lymphovascular invasion	28	31.57	
Lymphovascular invasion	37	34.08	
Lymph node involvement			0.279
No lymph node involvement	48	34.41	
Lymph node involvement	17	29.03	

Expression of CD151 did not have significant correlation with hormone status, tumor grade, Ki-67 scores, extent of invasion, and lymph node involvement

invasion are indicators of cancer aggressiveness, while lymph node involvement indicates distant metastatic potential. Our previous study found that NET-6 mRNA levels were lower in HER2-negative breast carcinoma cells. In addition, NET-6 was markedly down-regulated in estrogen receptor-negative breast cancers, and its expression was lowest in “basal-like” tumors [12]. In this study, results indicate that the protein expression of NET-6 was stronger in HER2-negative breast tumors than in HER2-positive cases ($p=0.037$, Table 4). Whether HER2 directs the expression of NET-6 remains to be determined. The role of tetraspanins in cellular signaling was described in other reports [1]. We did not confirm the correlation of NET-6 expression with ER, PR, and triple-negative status. In addition, the expression of NET-6 was stronger in tumors with low grade, no lymphovascular invasion, and no lymph node involvement (Table 4). These results further suggest that NET-6 protein expression is a favorable prognostic factor in human breast cancer.

For CD151, results showed that there was no correlation among CD151 and HER2, ER, PR status, tumor grade, Ki-67 scores, extent of invasion, and metastasis in tumors (Table 5).

The tetraspanins NET-6 and CD151 showed variable expression and no correlation with hormonal receptor status. The expression of the two proteins might indicate an important role in tumorigenesis and progression. In addition, only NET-6 expression was correlated with HER2 expression, tumor grade, extent of invasion, and metastasis which may suggest utility as a potential prognostic factor.

Conclusion

The expression of tetraspanins NET-6 and CD151 in breast tumors and benign epithelial cells has been observed and was correlated with hormonal receptors status, tumor grade, Ki-67 scores, extent of invasion, and metastasis features. The findings in this study suggest that the tetraspanin NET-6 and possibly CD151 may play a role in breast cancer tumorigenesis and progression; thus, especially, NET-6 may possess clinical utility as a prognostic marker.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval The study was approved by the Institute Review Boards of Guangzhou Huayin Medical Laboratory and the People's Hospital of Guangxi Zhuang Autonomous Region for the use of human materials.

Informed consent This study was waived for the patients' informed consent from the above-mentioned institutions because the study was a retrospective with pathology archived tissues.

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