Expression of yes-associated protein, β-catenin and smoothened, and their clinical significance in invasive breast cancer

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Abstract. The expression profile and role of yes-associated protein (YAP) in occurrence and development of breast cancer is ambiguous. The present study aimed to explore the relationship among the YAP, β-catenin and smoothened (SMO) signaling pathways to provide a theoretical basis for the clinical diagnosis and treatment of invasive breast cancer. Immunohistochemistry was used to determine the protein expression levels of YAP, β-catenin and SMO in tumor, tumor-adjacent and normal breast tissue. The possible association between the expression levels of these three proteins and the clinicopathological features of patients with breast cancer was then analyzed by the χ^2 test. The protein expression of YAP was found to be downregulated, whilst β-catenin and SMO expression were found to be upregulated in tumor tissues as compared with that in normal breast tissues. In addition, the expression of YAP in breast cancer tissues was found to be associated with that of human epidermal growth factor receptor 2 (HER2), progesterone and estrogen receptors. By contrast, the protein expression of β-catenin and SMO in breast cancer tissues was only associated with HER2. There was a negative correlation between the expression of YAP and SMO protein in breast cancer tissues. Compared with that in the changes in each of YAP, β-catenin and SMO protein expression levels individually, their combined changes in expression were demonstrated to associate significantly with the tumor histological grade. To conclude, data from the present study suggest that the combined protein expression of YAP, β-catenin and SMO can be used as a prognostic indicator for the treatment of invasive breast cancer.

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Introduction

Breast cancer is one of the most prevalent cancer worldwide, accounting for 11.7% of all cancer incidence and 6.9% of total mortality in 2020 (1). It often develops drug resistance and has high recurrence rates (2). In addition, the high rates of metastasis during clinical treatment greatly worsens the prognosis of patients (3). Therefore, identifying novel molecular markers that can be applied to effectively predict the progression and prognosis of breast cancer would be of great imortance for prevention and treatment.

Yes-associated protein (YAP) is a transcriptional co-factor of the hippo signaling pathway, the expression of which has been frequently reported to be upregulated in numerous types of cancers, such as esophageal, lung, liver and colon cancer (4-7). YAP is encoded by proto-oncogenes, where its expression can be enhanced by binding to a combination of transcriptional enhancers and residue domain transcription factors, which in turn promotes cell proliferation (8-10). Aberrant alterations in the hippo signaling pathway have been shown to promote nuclear localization of YAP to induce gene expression, leading to the progression of different types of tumors, including breast cancer (11,12). By contrast, one previous study has found that the expression levels of YAP in Breast tumor are lower compared with those in normal breast tissues (13). As a transcriptional regulator, it lacks a binding domain and cannot directly bind DNA; however, it can bind other transcription factors to regulate the transcription and expression of downstream target genes (14). In recent years, YAP has been reported to be a potential target for the treatment of breast cancer (12). Owing to its role as a transcription co-factor, YAP may need to combine with other regulators to mediate its role as a potential therapeutic target.

β-catenin and smoothened (SMO) are core proteins of the Wnt and hedgehog signaling pathways, respectively (15,16). The activities of these two pathways serve a key role in tumor physiology (17), since the self-renewal and differentiation capabilities of breast cancer stem cells are regulated by these two signaling pathways (18). Furthermore, previous studies have found the possibility of cross-talk between hippo and Wnt signaling pathways (19,20). In basal-like breast cancer, YAP and β-catenin were reported to synergistically regulate tumor stem cells to drive breast cancer pathology (21). In addition, overexpression of YAP has been found to suppress

the hedgehog signaling pathway, whereas knocking down YAP expression enhanced its activity (22). Although YAP, β-catenin and SMO all apparently serve important roles in the occurrence and development of breast cancer, the relationship among them remains unclear. Therefore, the present study aimed to elucidate the association between the expression of these three proteins and breast cancer, in addition to exploring the significance of this association.

Materials and methods

Tissue samples. A total of 60 patients with breast cancer who underwent surgical treatment from January 2020 to January 2021 were selected. All patients had complete medical records with related clinicopathological data and were diagnosed with invasive breast cancer by the pathology department of The Second Affiliated Hospital of Fujian Medical University (Quanzhou, China). All patients were female, who did not receive neoadjuvant therapy before surgery (mean age was 49.97±10.25 years). We excluded patients with advanced breast cancer or breast cancer with other malignancy. The tumor histological grades and stages in the present study were determined based on the 2020 diagnostic criteria of the National Comprehensive Cancer Network (23). Clinicopathological data, such as the expression status of HER2, estrogen receptor (ER), progesterone receptor (PR) and Ki-67, were obtained from postoperative patient reports and determined by referring to the 2021 Chinese Society of Clinical Oncology guidelines for the diagnosis and treatment of breast cancer (24). All patients underwent modified radical mastectomy for breast cancer. Tumor, tumor-adjacent (breast tissues taken 2 cm from the edge of the cancerous tissue) and normal tissues (breast tissue taken >5 cm away from the edge of the cancerous tissue without cancer cell infiltration as confirmed by pathology) were taken 30 min after the removal of breast tissue. All tissue specimens were fixed with 4% paraformaldehyde (room temperature, 20-25°C) and embedded in paraffin blocks. The present study complied with the Declaration of Helsinki. The present study was approved by the Ethics committee of The Second Affiliated Hospital of Fujian Medical University. Written informed consent was obtained from all patients. Clinicopathological data of the 60 patients with breast cancer are presented in Table I.

Main reagents and materials. Citric acid (pH 6.0) antigen retrieval solution (cat. no. G1202), 4% paraformaldehyde (cat. no. G1102), bovine serum albumin (cat. no. G5001), hematoxylin stain solution (cat. no. G1004), 3, 3'-diaminobenzidine (DAB) chromogenic reagent (cat. no. G1211), normal rabbit serum (cat. no. G1209) were all purchased from Wuhan Servicebio Technology Co., Ltd. Primary antibodies against YAP (cat. no. ab52771), β-catenin (cat. no. ab231305), SMO (cat. no. ab235183), universal HRP-conjugated secondary antibody against rabbit (cat. no. ab205718) were all purchased from Abcam.

Immunohistochemical staining. Paraffin-embedded tissue sections were deparaffinized in xylene, rehydrated in a series of ethanol solutions (100 to 50%) at room temperature. For antigen retrieval, tissue sections were placed in a box filled with the citric acid (pH 6.0) antigen retrieval solution in a microwave oven, heated on medium power for 8 min until boiling. 0.3% hydrogen peroxide solution for 20 min to block the endogenous peroxidase activity. Samples were blocked for 30 min at room temperature by using bovine serum albumin. Next, the sections were incubated overnight with primary antibodies against YAP, β-catenin and SMO (1:200 dilution) at 4˚C. Subsequently, the sections were incubated with secondary antibodies against rabbit (1:400 dilution) for 50 min at room temperature. The sections were then stained with DAB and counterstained using hematoxylin stain solution for 3 min at room temperature. Placed the section in a series of ethanol solutions (100 to 50%) to dehydrate, and than mounting was performed. Finally, the stained tissue sections were observed under a microscope and images were captured for analysis.

Scoring criteria. Each region of the tissue sections was initially examined with a low magnification (x100), before higher magnification (x200) was used to observe the local area and to select a representative area for image capture and analysis. A comprehensive staining score was created by counting the total number of cells as well as the number of YAP-, β-catenin- and SMO-positive cells in the measurement area. Cells were scored according to the staining intensity and the percentage of stained cells [(number of stained cells/total number of cells) x100]. Staining scoring criteria were as described previously (25): i) 0, no color; ii) 1, light yellow; iii) 2, brownish-yellow; and iv) 3, brown. Scoring based on the extent of stained cells was as follows: i) 0, 0-5%; ii) 1, 6-25%; iii) 2, 26-50%; iv) 3, 51-75%; and v) 4, >75%. Multiplying the staining intensity score with the percentage of stained cells yielded the comprehensive staining score, wherein 0-3 was considered to be low expression, and a score ≥4 was considered high expression.

Statistical analysis. SPSS 26.0 (IBM Corp.) and GraphPad Prism 8.0 (GraphPad Software, Inc.) were used for the statistical analyses. Friedman's test and Nemenyi's test were used to compare the staining scores among each group. The counted data were analyzed using the χ^2 test. Spearman's correlation analysis was used for the correlation of the IHC data. All data are presented in this study by mean ± SD, $P<0.05$ was considered to indicate a statistically significant difference.

Results

Differential expression of YAP, β-catenin and SMO in tumor, tumor-adjacent and normal breast tissues. The strongly positive expression of YAP is mainly localized to the cytoplasm and nucleus (Fig. 1C). The expression levels of YAP in the three different types of breast tissue samples were found to be significantly different ($P<0.001$; Figs. 2A and 1A-C). Pairwise comparisons revealed significantly decreased YAP expression levels in the tumor tissues compared with those in the normal breast tissues ($P<0.01$). In addition, the expression levels of YAP in the tumor-adjacent breast tissues had no significant differences compared with those in the normal tissues.

The strongly positive expression of β-catenin is mainly localized to the cytoplasm and cell membrane (Fig. 1C). The expression levels of β-catenin in the three breast tissue samples were also

Table I. Clinicopathological features of the 60 patients with invasive breast cancer.

Clinicopathological feature	N	Percentage
Age, years		
≥50	28	46.7
<50	32	53.3
Histological grade		
I	1	1.7
II	27	45
III	32	53.3
Tumor size, cm		
<2	21	35
2-5	36	60
>5	3	5
Lymph node metastasis		
0	34	56.7
1-3	15	25
4-9	7	11.6
≥10	4	6.7
Human epidermal growth factor receptor 2		
Positive	22	36.7
Negative	38	63.3
Estrogen receptor		
Positive	41	68.3
Negative	19	31.7
Progesterone receptor		
Positive	40	66.7
Negative	20	33.3
Ki-67		
Positive	42	70
Negative	18	30
Tumor stage		
I	16	26.7
II	33	55
III	11	18.3

found to be significantly different (P=0.016; Figs. 2B and 1D-F). Pairwise comparisons showed significantly increased expression levels of β-catenin in the tumor tissues compared with those in the normal breast tissues (P<0.05). The expression levels of β-catenin in the tumor-adjacent tissue showed no significant difference compared with those in the normal breast tissues.

The strong positive expression of β-catenin is mainly localized to the cytoplasm (Fig. 1G). The expression levels of SMO in the three breast tissue samples were significantly different (P=0.005; Fig. 2C and 1G-I). Pairwise comparisons showed significantly increased expression levels of SMO in the tumor tissues compared with those in the normal breast tissues (P<0.05). The expression levels of SMO in the tumor-adjacent tissues had no significant difference compared with those in the normal breast tissues.

Relationship between the expression of YAP, β-catenin and SMO and the clinicopathological characteristics of patients with breast cancer. The expression of YAP in the tumor tissues of the patients with breast cancer was not found to be associated with age, tumor histological grade and size, lymph node metastasis, Ki-67 expression index or tumor stage (Table II). However, it was significantly associated with the expression of HER2 (χ^2=8.735; P=0.003), PR (χ^2=5.735; P=0.017) and ER (χ^2=4.45; P=0.035).

The expression of β-catenin in the tumor tissue of the patients with breast cancer was not associated with age, tumor histological grade and size, lymph node metastasis, Ki-67 expression index, ER, PR or tumor stage (Table II). However, it was significantly associated with the expression of HER2 (χ^2=11.579; P<0.001).

The expression levels of SMO in the tumor tissues of the patients with breast cancer were not associated with age, tumor histological grade, size, lymph node metastasis, Ki-67 expression index, ER, PR or tumor staging (Table II). However, a significant association was observed between SMO and HER2 expression (χ^2=5.833; P=0.016).

Correlation of YAP with β-catenin and SMO expression in the tumor tissues. Spearman's analysis revealed a negative correlation between expression levels of YAP and SMO in the tumor tissue (ρ=-0.31; P=0.015; Fig. 3A). No correlation was identified between the expression levels of YAP and β-catenin (ρ=0.13; P=0.32; Fig. 3B) or between the β-catenin and SMO (ρ=-0.1; P=0.45; Fig. 3C).

Relationship between the combined expression changes of YAP, β-catenin and SMO in tumor tissue and the clinicopathological characteristics. Compared with individual changes in each of YAP (Decreased expression: IHC staining Score<4), β-catenin and SMO expression (Increased expression: IHC staining Score≥4), their combined changes were found to be significantly associated with the tumor histological grade (P=0.013; Table III). In particular, a significant association was found with grade III (low differentiation) compared with grades I-II (medium and high differentiation). There was no significant association with age, tumor size, lymph node metastasis, HER2, ER, PR, Ki-67 or tumor stage. This observation suggested that the combined expression changes of these three proteins may be associated with the histological grade of breast cancer.

Discussion

A number of studies have previously reported that YAP expression is upregulated in malignant tumors, such as esophageal, lung, liver and colon cancers (4-7). Therefore, YAP is considered to be an oncogenic protein. However, data regarding YAP from previous studies on breast cancer remain controversial, since the expression profile of YAP in this type of cancer is ambiguous (13,26). Results from the present study revealed that the expression levels of YAP in normal breast tissues is higher compared with those in tumor tissues, suggesting that YAP may be a tumor suppressor in invasive breast cancer. Yuan *et al* (27) previously reported that YAP may serve as a tumor suppressor in breast cancer. They found that YAP

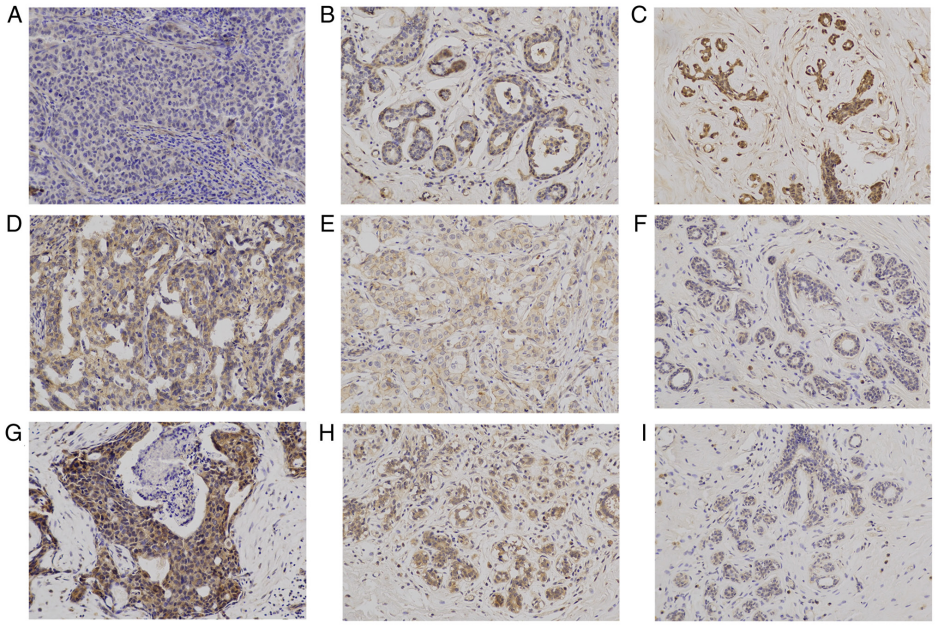

Figure 1. Immunohistochemical analysis of YAP, β-catenin and SMO expression in tumor, tumor-adjacent and normal breast tissues. (A) YAP expression in tumor tissue with a negative staining score. (B) YAP expression in tumor-adjacent tissue with a weakly positive or moderately positive staining score. (C) YAP expression in normal breast tissue with a strongly positive staining score, which is mainly localized to the cytoplasm and nucleus. (D) β-catenin expression in tumor tissue with a strongly positive staining score, which is mainly localized in the cytoplasm and cell membrane. (E) β-catenin expression in tumor-adjacent tissue with a moderately positive staining score. (F) β-catenin expression in normal breast tissue with a negative staining score. (G) SMO expression in tumor tissue with a strongly positive staining score, which is mainly localized in the cytoplasm. (H) SMO expression in tumor-adjacent tissue with a moderately positive staining score. (I) SMO expression in normal breast tissue with a negative staining score. Magnification, x200. SMO, smoothened; YAP, yes-associated protein.

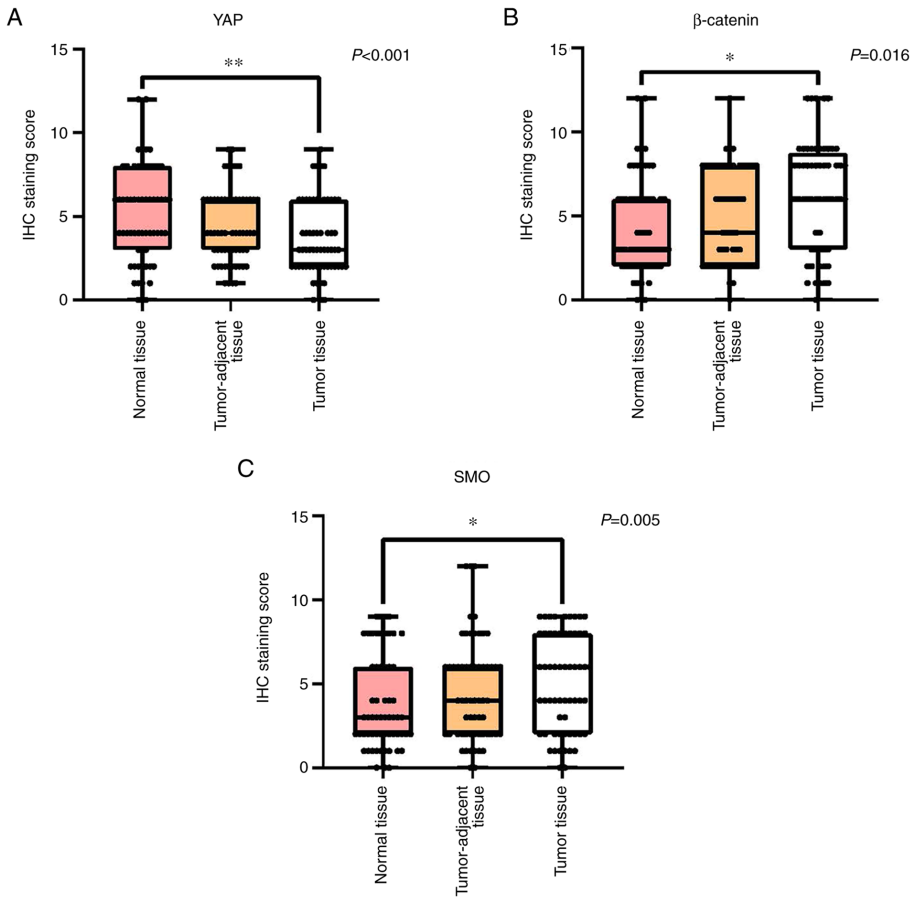

Figure 2. Expression levels of YAP, β-catenin and SMO in normal, tumor-adjacent and tumor breast tissues. The protein expression scores of (A) YAP, (B) β-catenin and (C) SMO in normal breast, tumor-adjacent and cancer tissues were determined from immunohistochemical staining. *P<0.05 and **P<0.01. SMO, smoothened; YAP, yes-associated protein.

Table II. Relationship between the expression levels of YAP, β-catenin and SMO and the clinicopathological features of patients with breast cancer.

Group	N	High YAP expression, n (%)	P-value	High β-catenin expression, n (%)	P-value	High SMO expression, n (%)	P-value
Age, years							
≥50	28	14 (50)	0.33	22 (78.6)	0.55	18 (64.3)	0.91
<50	32	12 (37.5)		23 (71.9)		21 (65.6)	
Histological grade							
I-II	28	14 (50)	0.33	19 (67.9)	0.37	15 (53.6)	0.083
III	32	12 (37.5)		25 (78.1)		24 (75)	
Tumor size, cm							
≤2	21	8 (38.1)	0.55	15 (71.4)	0.81	16 (76.2)	0.18
>2	39	18 (46.2)		29 (74.4)		23 (59)	
Lymph node metastasis							
No	34	18 (52.9)	0.09	19 (55.9)	0.98	20 (58.8)	0.25
Yes	26	8 (30.8)		26 (100)		19 (73.1)	
Human epidermal growth factor receptor 2							
Positive	22	15 (68.2)	0.003	22 (100)	<0.001	10 (45.5)	0.016
Negative	38	11 (28.9)		23 (60.5)		29 (76.3)	
Estrogen receptor							
Positive	41	14 (34.1)	0.035	30 (73.2)	0.63	27 (65.9)	0.84
Negative	19	12 (63.2)		15 (78.9)		12 (63.2)	
Progesterone receptor							
Positive	40	13 (32.5)	0.017	28 (70)	0.21	27 (67.5)	0.57
Negative	20	13 (65)		17 (85)		12 (60)	
Ki-67							
Positive	42	20 (47.6)	0.65	32 (76.2)	0.9	27 (64.3)	0.32
Negative	18	6 (33.3)		13 (72.2)		12 (66.7)	
Tumor stage							
I	16	6 (37.5)	0.09	11 (68.8)	0.55	12 (75)	0.16
II	33	15 (45.5)		26 (78.8)		18 (54.5)	
III	11	9 (81.8)		8 (72.7)		9 (81.8)	

SMO, smoothened; YAP, yes-associated protein.

expression was decreased in breast cancer, such that the metastatic and invasive capabilities of breast cancer cells without YAP expression were increased.

In the present study, association analysis between YAP expression and the clinicopathological characteristics of patients with breast cancer revealed that it was associated with HER2, ER and PR expression. This suggested that YAP may mediate an important role during the endocrine or targeted therapy of invasive breast cancer. A study by Zhu *et al* (28) previously revealed that YAP serves as co-regulators of ERα for estrogen-regulated enhancer activation, which suggests YAP to be a potential therapeutic target for ER-positive breast cancer. In addition, a recent study found that after targeting HER2 with trastuzumab, a monoclonal antibody, the expression of YAP was significantly higher in trastuzumab-sensitive BT474 cell lines compared with that in their trastuzumab-resistant counterparts, suggesting its involvement in preventing trastuzumab resistance in HER2-positive breast cancer cells (29). Therefore, YAP can be used as a prognostic marker for trastuzumab neoadjuvant therapy in patients with HER2-positive breast cancer (29). The present study demonstrated that YAP may serve a key role in the treatment of invasive breast cancer. Previous studies have shown that the expression levels of YAP in different breast cancer subtypes also differs (13,30), meaning that the biological role of YAP in breast cancer cells is likely to be dependent on their pathological subtypes or microenvironments. Therefore, the viability of YAP as a potential target for the treatment of breast cancer remains unclear and further research is needed to identify the specific factors and pathways that can regulate YAP expression in different subtypes of breast cancer.

Table III. Relationship between the combined changes in tumor expression of YAP, β-catenin SMO and the clinicopathological characteristics.

Group	N	YAP^-/β-$catenin^+/SMO^+$	P-value
Age, years			
≥50	28	8 (28.6)	0.82
<50	32	10 (31.3)	
Histological grade			
I-II	28	4 (14.3)	0.013
III	32	14 (43.8)	
Tumor size, cm			
≤2	21	5 (23.8)	0.44
>2	36	13 (36.1)	
Lymph node metastasis			
No	34	7 (20.6)	0.07
Yes	26	11 (42.3)	
Human epidermal growth factor receptor 2			
Positive	22	4 (18.2)	0.13
Negative	38	14 (36.8)	
Estrogen receptor			
Positive	41	15 (36.6)	0.1
Negative	19	3 (15.8)	
Progesterone receptor			
Positive	40	14 (35)	0.23
Negative	20	4 (20)	
Ki-67			
Positive	42	12 (28.6)	0.71
Negative	18	6 (33.3)	
Tumor stage			
I	16	3 (18.8)	0.51
II	33	11 (33.3)	
III	11	4 (36.4)	

SMO, smoothened; YAP, yes-associated protein.

In the present study, immunohistochemistry results revealed that β-catenin was expressed at a higher levels in the tumor tissues compared with those in the normal breast tissues, which was mainly localized to the cell membrane and cytoplasm. After the Wnt signaling pathway is activated, β-catenin enters the cytoplasm from the cell membrane and undergoes a series of reactions, resulting in its accumulation in the cytoplasm. Eventually, nuclear translocation occurs, activating the transcription of target genes associated with tumor development and metastasis (15). These results are consistent with those reported by previous studies. For example, Jang *et al* (31) found that Wnt/β-catenin signaling activity was enhanced in the breast tumors compared with that in the normal breast tissues. In addition, mouse models of breast cancer were used in this study to demonstrate that inhibition of the Wnt/β-catenin signaling pathway suppressed breast cancer stem cell activity, thereby reducing the metastatic potential of breast cancer cells (31). Upregulation of the Wnt/β-catenin signaling pathway was also previously found to increase the metastatic ability of primary breast tumors (32). Therefore, β-catenin can be considered to be an oncogene involved in the occurrence and metastasis of breast cancer.

β-catenin expression was found to associate with the expression levels of HER2 in the present study, which suggested the potential of targeting the Wnt/β-catenin signaling pathway for breast cancer therapy. A previous study reported that HER2 activated Wnt/β-catenin signaling pathway through its downstream regulators, AKT and MAPK; this can inhibit glycogen synthase kinase-3 expression, resulting in the translocation of β-catenin to the nucleus, thereby promoting the transcription of target genes (33). Another study found that Wnt3 ligand-mediated activation of the Wnt/β-catenin signaling pathway induced epithelial-mesenchymal transition and trastuzumab resistance in HER2-positive breast cancer cells (34). At present, no Wnt inhibitors have been approved for breast cancer treatment. Therefore, further research on the Wnt/β-catenin signaling pathway can potentially provide solutions for the clinical treatment of breast cancer.

SMO is one of the core components of the hedgehog signaling pathway (16). The hedgehog signaling pathway is involved in the regulation of mammary gland development during embryogenesis, the development of duct structures and the differentiation of the breast during lactation (35). During the early stages of embryogenesis, the hedgehog pathway is inhibited to allow breast parenchyma formation. Subsequently, ductal morphology typically develops during puberty, when the hedgehog signaling pathway must be activated to promote elongation of the terminal buds. Shortly after puberty, its activity decreases again in the mammary glands (36). Aberrant activation of the hedgehog signaling pathway can lead to the development and metastasis of breast cancer (37). The present results revealed that the expression levels of SMO in tumor breast tissues is higher compared with those in normal tissues. Therefore, findings from the present study suggested that SMO may be an oncogenic gene product that can mediate the abnormal activation of the hedgehog pathway to promote the progression of breast cancer.

Association analysis between SMO and the clinicopathological characteristics of patients with breast cancer showed that SMO was associated with HER2, which suggested that SMO is important for the targeted therapy of invasive breast cancer. Several studies have previously shown that SMO inhibitors can be combined with other inhibitors or chemotherapeutic agents to effectively inhibit breast cancer progression (38-40). In particular, a clinical trial targeted SMO in the hedgehog signaling pathway in female patients with breast cancer (41). The results of these trials suggested that targeting this pathway can be an effective treatment option for patients with cancer (41).

The present study demonstrated that the expressions of YAP and SMO in breast cancer tissues were negatively associated, suggesting that there may have been an interaction between the hippo and hedgehog signaling pathways. Tariki *et al* (22)

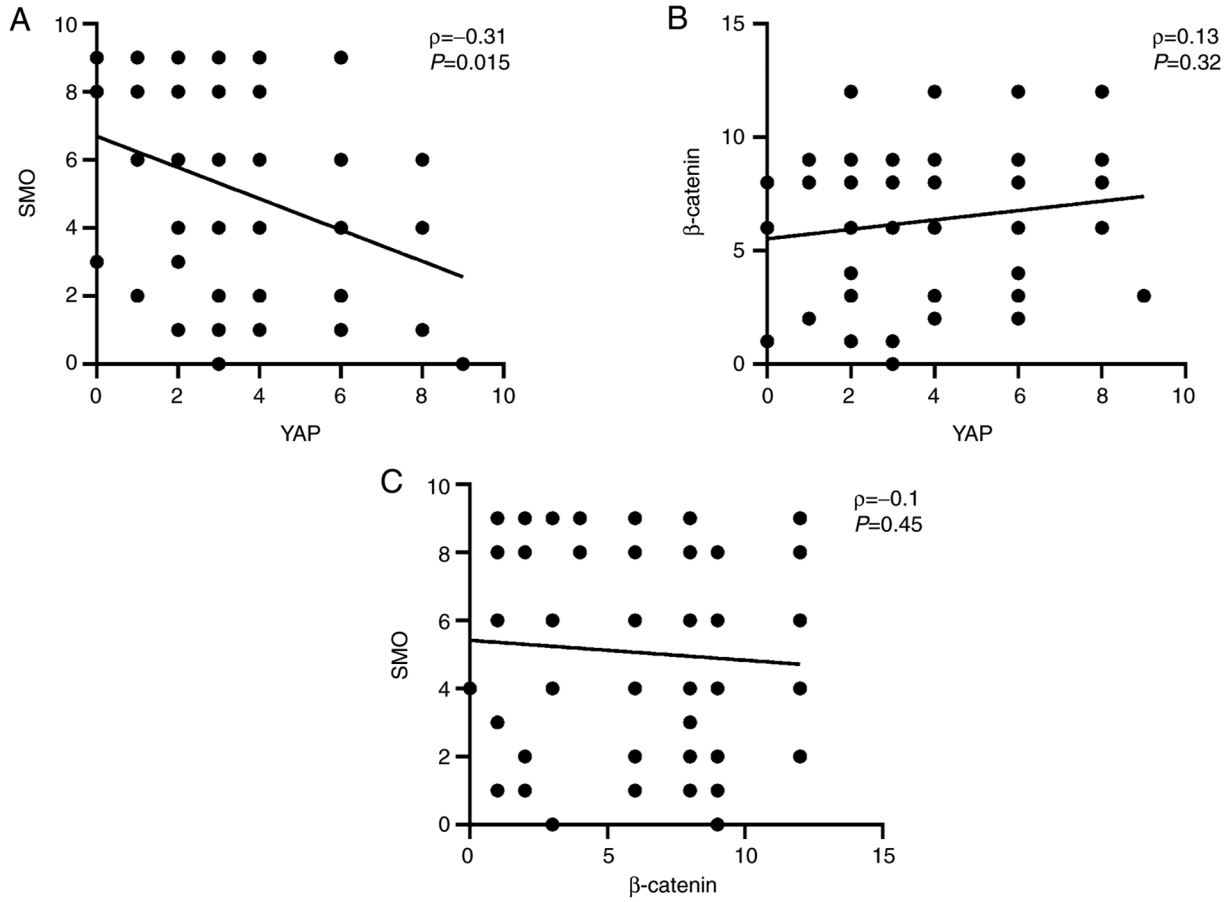

Figure 3. Spearman's correlation analysis of YAP, β-catenin and SMO immunohistochemistry staining scores in breast cancer tissues. (A) Correlation of YAP and β-catenin expression in the tumor tissues. (B) Correlation of YAP and SMO expression in the tumor tissues. (C) Correlation of β-catenin and SMO expression in the tumor tissues. SMO, smoothened; YAP, yes-associated protein.

previously revealed that the overexpression of YAP blocked the hedgehog signaling pathway, whereas knocking down YAP expression using siRNA enhanced its activity, demonstrating a negative regulatory relationship between these two pathways; however, hedgehog signaling pathway can enhance YAP activity through a post-transcriptional mechanism, which in turn forms a negative feedback loop to turn off hedgehog signaling.

Zheng *et al* (42) revealed an oncogenic function of YAP in reprogramming glucose metabolism, The lncRNA breast cancer anti-estrogen resistance 4 (BCAR4) is required for YAP-dependent glycolysis. Mechanistically, The overexpression of YAP leads to therapeutic efficacy of BCAR4-targeted locked nucleic acids (LNA) by inducing the transcription of BCAR4 in order to promote the expression of BCAR4, which coordinated with the hedgehog signaling pathway to enhance the expression of glycolysis activators hexokinase-2 and 6-phosphofructo-2-kinase/fructose-2, 6-bisphosphatase 3. This resulted in the therapeutic delivery of LNA, which attenuated YAP-dependent glycolysis and breast tumor growth, suggesting that YAP, the core protein of the hippo signaling pathway in breast cancer, has both inhibitory and potentiation effects on the hedgehog pathway. When the hedgehog signaling pathway is aberrantly activated, the activity of YAP may be enhanced, thereby decreasing the activity of the hedgehog pathway. Therefore, it was speculated that YAP may regulate the growth and development of normal breast cells by stabilizing protein expression in the hedgehog signaling pathway. The absence of YAP in cells may lead to the abnormal activation of the hedgehog signaling pathway to promote the occurrence and development of breast cancer.

By analyzing the relationship between the combined changes in the expression of YAP (decreased), β-catenin (increased) and SMO (increased) in breast tumors and the clinicopathological characteristics of the patients, the present study found that these changes in expression were significantly associated with the tumor histological grade. Specifically, grade III (low differentiation) showed higher association compared with grades I-II (medium and high differentiation). These findings suggested that the downregulation of YAP and the upregulation of β-catenin and SMO in breast cancer can promote disease progression. In addition, the present data suggested that the possibility of recurrence or malignant transformation in tissues adjacent to tumors can be determined by detecting the combined expression levels of these three proteins in the tumor-adjacent tissues. This can provide a basis for determining the surgical margin and scope of preserving healthy breast tissues when planning radical mastectomy surgeries. However, further research is required to verify this hypothesis.

In summary, YAP, β-catenin and SMO were found to serve key roles in the occurrence, development, diagnosis and

treatment of breast cancer in the present study. The association between YAP and SMO provides a new direction for exploring the mechanisms in the physiology of invasive breast cancer. Because the expression of YAP is downregulated, whereas β-catenin and SMO are upregulated in breast cancer, this may promote the progression of this disease. Therefore, analyzing the expression profile of all three of these may provide important information on the physiology of breast cancer. However, the follow-up time of patients in the present study was short, meaning that it was not possible to evaluate the relationship between the expression levels of these proteins and the prognosis of the patients. In future studies, the prognostic information of patients are also required, which should be combined to assess the importance of these three proteins in invasive breast cancer.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

PL and JZ conceived and designed the project. PL and GY provided materials and patient samples and collected the data. PL and GY performed IHC staining. PL analyzed and interpretated the data. PL and JZ confirm the authenticity of all the raw data. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

The present study was approved by the Ethics Committee of The Second Affiliated Hospital of Fujian Medical University [Quanzhou, China; approval no. (2019)(196)]. Written informed consent was obtained from all patients.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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