Int J Clin Exp Pathol 2016;9(5):5148-5157
www.ijcep.com /ISSN:1936-2625/IJCEP0022936

*Original Article*

# Expression of β-catenin and axin in metaplastic breast carcinomas

Le Wang[1*], Jingxuan Wang[1*], Xingjian Niu[1], Huatian Liu[2], Wenjing Tian[1], Tieying Dong[1], Liqian Su[1], Hang Liu[1], Qingyuan Zhang[1]

[1]*Department of Medical Oncology, The Third Affiliated Hospital of Harbin Medical University, Harbin, China;*
[2]*Department of Medical Anesthesiology, The First Affiliated Hospital of Harbin Medical University, Harbin, China.*
[*]*Equal contributors and co-first authors.*

Received December 30, 2015; Accepted March 17, 2016; Epub May 1, 2016; Published May 15, 2016

**Abstract:** Background: Metaplastic breast carcinoma (MBC) is a rare subtype of invasive breast cancer that tends to have a suboptimal response to standard chemotherapy regimens as well as an aggressive clinical manifestation. It is urgent to find prognostic markers and therapeutic targets. The β-catenin, Tcf-4 and Axin protein are key molecules in wnt signaling pathway. The aim of the present study is to explore wnt signaling pathway protein expression in MBC and their association with clinicopathological factors and prognosis. Methods: The expression of β-catenin, Tcf-4 and Axin proteins were examined in 40 MBC tissues and 43 invasive ductal carcinoma (IDC) tissues with immunohistochemistry. Results: Compared with IDCs, the expression of β-catenin and TCF-4 were significantly higher, whereas Axin protein expression was significantly lower in the 40 MBC specimens. Interestingly, β-catenin expression is inverse correlated with Axin expression. Concomitantly, lower Axin or higher β-catenin expression is associated with high clinical stages and larger tumors. Furthermore, MBC patients had visibly reduced PFS compared to IDC patients and the β-catenin-positive/Axin-negative was distinctly associated with short PFS when the two factors were combined together. Conclusions: β-catenin up-regulation and Axin down-regulation are potentially useful prognostic markers for MBC and also are significantly unfavorable prognostic factors for patients with MBC.

**Keywords:** β-catenin, Tcf-4, Axin, metaplastic breast carcinoma, prognosis

## Introduction

Metaplastic breast carcinoma (MBC) is a rare and heterogeneous subtype of breast carcinoma with a generally poor outcome. MBC was defined as a distinct histologic entity by World Health Organization (WHO) in 2000, and it was classified into several subtypes: squamous subtype, low-grade adenosquamous subtype, fibromatosis-like subtype, mesenchymal and myoepithelial subtype [1]. MBC usually presents with larger tumor size, less nodal involvement, higher histological grade, and lack hormone receptors and HER2 expression. Since MBC is almost negative for hormone receptors and HER-2 expression, it can be classified as triple-negative breast cancer (TNBC) [2]. However, the invasiveness of MBC is higher than the TNBC [3]. Up to date, the molecular mechanism leading to MBC remains unknown. Thus, once metastasis occur, there are currently no effective treatments for patients with MBC [4].

Using immunohistochemistry and mutational analysis, Hayes and colleagues have demonstrated that MBC is a type of breast cancer with frequent wnt pathway activation [4]. Monteiro et al [5] have stated that constitutive activation of Wnt signaling affected self-renewal and differentiation capacity of stem/progenitor cells in the mouse mammary gland which lead the establishment of MaCSCs and form TNBC-like tumors at the primary site. These findings indicated that MBC had a closer relationship with wnt signaling pathway activation than other types of breast cancer.

The *Wnt* signaling pathway is known to be critical in the mammary gland development and oncogenesis [4]. *Wnt*-driven gene expression regulates cell fate, differentiation, survival,

**Table 1.** Clinical characteristics of 40 metaplastic carcinomas and 43 invasive ductal carcinomas

| Clinical characteristics | Metaplastic carcinomas | % | Invasive ductal carcinomas | % | *P*-value |
|---|---|---|---|---|---|
| Age | | | | | 0.18 |
| ≤50 | 16 | 40.0 | 17 | 39.5 | |
| >50 | 24 | 60.0 | 26 | 60.5 | |
| Tumor size (cm) | | | | | 0.18 |
| ≤2 | 14 | 35.0 | 16 | 37.2 | |
| >2 | 26 | 65.0 | 27 | 62.8 | |
| Lymph node | | | | | 0.16 |
| 0 | 22 | 55.0 | 22 | 51.2 | |
| 1-3 | 8 | 20.0 | 10 | 23.3 | |
| >3 | 10 | 25.0 | 11 | 25.6 | |
| Clinical stage | | | | | 0.14 |
| I II | 30 | 75.0 | 29 | 67.4 | |
| III IV | 10 | 25.0 | 14 | 32.6 | |
| Menstrual status | | | | | 0.17 |
| Pre-menopause | 13 | 32.5 | 16 | 37.2 | |
| Post-menopause | 27 | 67.5 | 27 | 62.8 | |
| p53 | | | | | 0.18 |
| Negative | 25 | 62.5 | 26 | 60.5 | |
| Positive | 15 | 37.5 | 17 | 39.5 | |
| Ki67 | | | | | 0.22 |
| ≤14% | 7 | 17.5 | 8 | 18.6 | |
| >14% | 33 | 82.5 | 35 | 81.4 | |
| Relapse | 23 | 57.5 | 18 | 41.9 | 0.06 |
| Bone/Soft tissue | 6 | 15.0 | 5 | 11.6 | |
| Visceral | 13 | 32.5 | 11 | 25.6 | |
| Both | 4 | 10.0 | 2 | 4.7 | |

stem cell self-renewal as well as epithelial to mesenchymal transition (EMT) which is essential for MBC formation [6-8]. In the *Wnt* signaling pathway, β-catenin is a multifunctional protein that plays an important role in cell-cell adhesion and is a critical regulatory gene responsible for transduction of the signal to the nucleus. However, Axin is a negative regulator, which coordinates β-catenin degradation through the ubiquitin-proteasome way. When the *Wnt* signaling pathway is activated, the membranous β-catenin will be reduced and then accumulate in cytoplasm and nuclear where it triggers transcriptional activators (TCF/LEF family) to regulate the Wnt-specific downstream genes, such as cyclin D1, c-myc and etc [4, 6, 9, 10].

So far, only a few studies performed on the MBC about the expression of *Wnt* signaling pathway proteins. The purpose of the present study was to investigate the expression of β-catenin, Tcf-4 and Axin proteins in MBC to determine their prognostic importance, as well as their relation to clinicopathological features. It's essential to explore the mechanism to understand thoroughly about the tumorigenesis of MBC and offer an effective therapeutic target for women of this subtype and poor prognoses.

## Patients and methods

### *Patients*

From July 2006 to December 2012, 40 MBC patients were enrolled from the Department of Pathology at the Third Affiliated Hospital of Harbin Medical University, China. Since MBC was the main components of TNBC, we collected 43 invasive ductal carcinoma of TNBC tissues during the same period as the control. Clinical characteristics of the carcinoma cases were summarized in **Table 1**. All sections of the tumor blocks were initially stained by hematoxylin and eosin (H&E) staining to verify the primary pathological diagnosis and to select a cross-sectional border. All protocols were approved by the Ethical Committee of Harbin Medical University. Written consent was taken from all the participating patients in this research.

### *Determination of β-catenin, Tcf-4 and Axin*

Immunohistochemical staining was performed by two steps plus Poly-HRP method. Briefly, the sections were de-paraffinized and rehydrated with xylene and alcohol respectively. After antigen retrieval in pressure heating environment, the sections were soaked in 3% $H_2O_2$ solution for 15 mins, then incubated with monoclonal antibodies (β-catenin, 1:200; Tcf-4, 1:50; Axin, 1:500, MILLIPORE Technologies, USA) at 4°C overnight. After washing through the phosphate-buffered saline (PBS), the slides were incubated with secondary antibodies (ZSGB-Bio, Beijing, China) at room temperature for 30 minutes, then visualized by DAB. Eventually, the immunostained sections were counterstained,

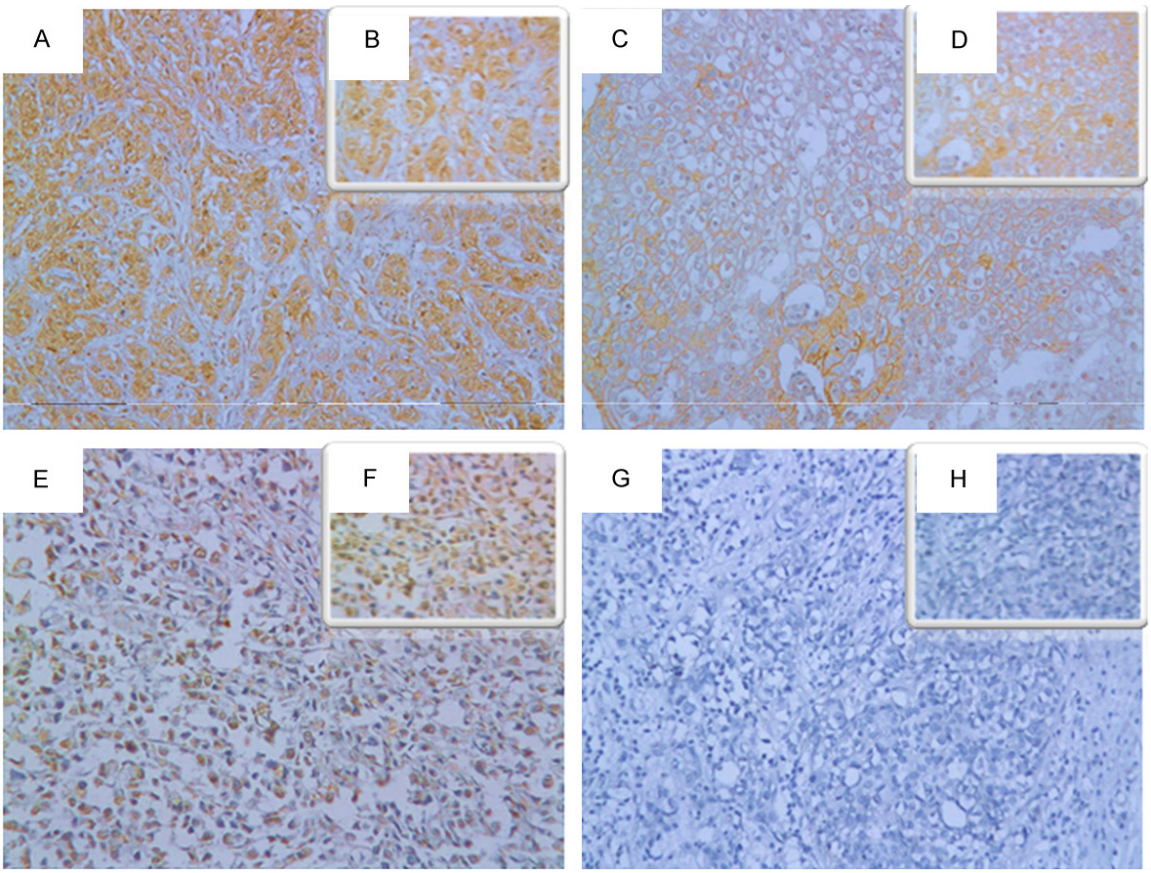


**Figure 1.** Immunohistochemical pattern of β-catenin expression in MBC and IDC tissue. A. β-catenin-positive specimen in MBC (×200). B. β-catenin-positive specimen in MBC (×400). C. β-catenin-negative specimen in MBC (×200). D. β-catenin-negative specimen in MBC (×400). E. β-catenin-positive specimen in IDC (×200). F. β-catenin-positive specimen in IDC (×400). G. β-catenin-negative specimen in IDC (×200). H. β-catenin-negative specimen in IDC (×400).

dehydrated and detected. Every staining section was treated with PBS instead of primary antibody for negative control.

*Evaluation of immunohistochemical staining*

Sections were blindly evaluated by two experienced investigators using light microscopy. Immunostaining was assessed following previously published methods [4] as normal when β-catenin was observed in membrane staining, or abnormal when greater than 5% of tumor cells had nuclear or cytoplasmic accumulation, or reduced or absent membrane staining. The abnormal expression was taken as positive. The evaluation of Axin and TCF4 staining was based on staining intensity and staining cell area. For staining intensity: Grade 0, no staining; Grade 1, mild staining; Grade 2, moderate staining; Grade 3, strong staining. The positively stained area was graded by the percentage of the whole area and scored as follows: 0 (0-10% positive cells); 1 (11-25% positive cells); 2 (26-50% positive cells); 3 (51-100% positive cells). The combined score (intensity plus extension) was evaluated as follows: <2 and =2, negative staining (-); >2, positive staining (+) [11].

*Statistical analysis*

Statistical analysis was carried out using SPSS 22.0 software. We used Chi-square test and Spearman correlation coefficient to analyze the association between the proteins staining and the clinical parameters as well as themselves. The Kaplan-Meier method was performed to estimate PFS. $P<0.05$ was considered statistically significant.

## Results

*Clinical results*

A total of 83 participants (40 MBC patients and 43 IDC patients) were included in this study. All

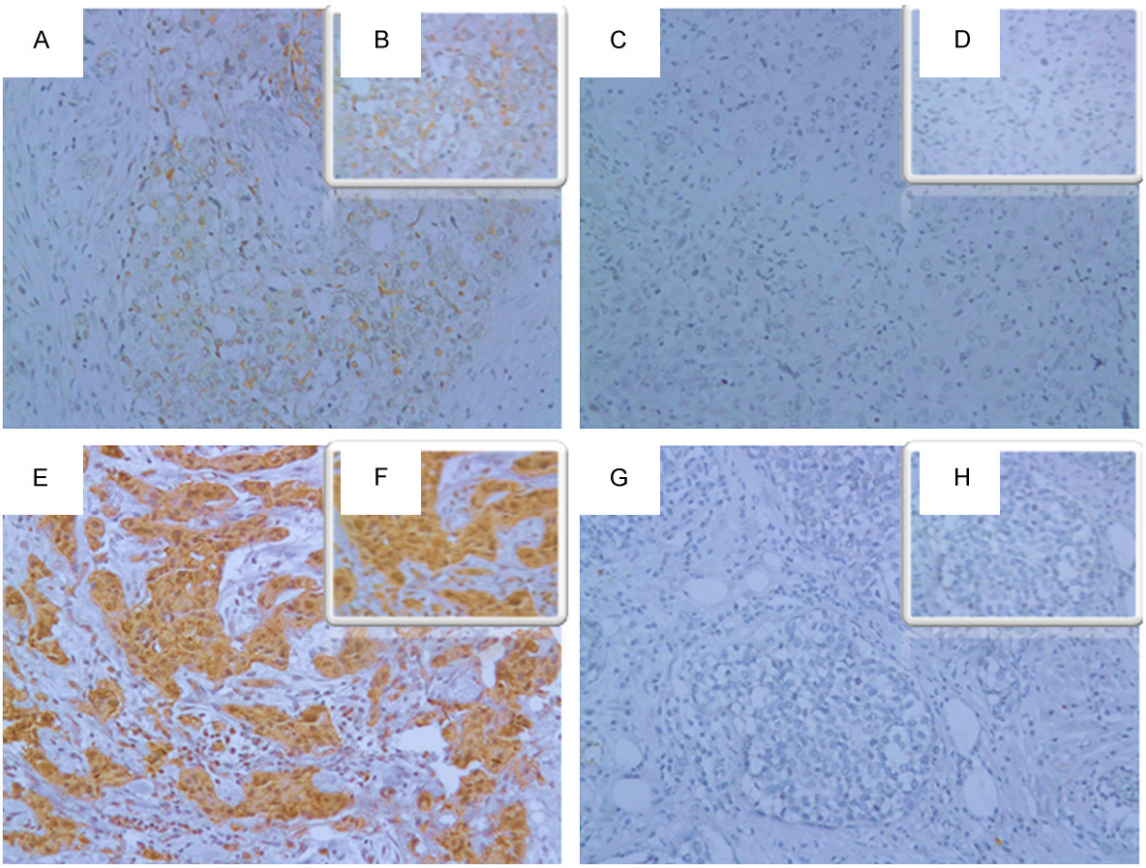


**Figure 2.** Immunohistochemical pattern of Axin expression in MBC and IDC tissue. A. Axin-positive specimen in MBC (×200). B. Axin-positive specimen in MBC (×400). C. Axin-negative specimen in MBC (×200). D. Axin-negative specimen in MBC (×400). E. Axin-positive specimen in IDC (×200). F. Axin-positive specimen in IDC (×400). G. Axin-negative specimen in IDC (×200). H. Axin-negative specimen in IDC (×400).

carcinomas were triple-negative breast cancer. For MBC cases, the mean age was 52.5 (range 25-79; median age 52). When comparing with controls, the mean age was 49.5 (range 24-77; median age 50) and the *P* value was not considered statistically significant. The clinical stage and lymph node metastases didn't show statistical differences in groups of MBC and IDC. The recurrence rate of MBC patients was higher than the IDC patients (23/40, 57.5% vs. 18/43, 41.9%; P=0.06) (**Table 1**).

*Immunohistochemical pattern of β-catenin, Tcf-4 and Axin expression in metaplastic carcinoma and invasive ductal carcinoma tissues*

In the present study, β-catenin was detected predominantly in nuclei and/or in cytoplasm of the tumor tissues in 45% and 23.3% of the MBC and IDC cases, respectively (**Figure 1A**, **1B**, **1E**, **1F**). Positive expression of Axin was mainly located in the cytoplasm, while the Tcf-4 staining was observed in the cell nucleus mostly. Our results demonstrated the positive rates of Axin protein and Tcf-4 were 47.5% (19/40) and 67.5% (27/40), respectively in MBC (**Figures 2A**, **2B**, **3A**, **3B**). In the control group, the overexpression of Axin protein and Tcf-4 was observed in 66.7% (38/43) and 37.2% (16/43) cases, respectively (**Figures 2E**, **2F**, **3E**, **3F**).

The β-catenin was higher expressed in MBC than IDC in tumor tissues (45.0% vs. 23.3%; P=0.041). Compared with IDC, Tcf-4 expression rate was higher (67.5% vs. 37.2%; P=0.008) while Axin protein expression was lower (47.5% vs. 66.7%; P<0.001) in MBC (**Table 2**).

*Correlation between the expression of Tcf-4, Axin and β-catenin*

To explore the relationship between the expression of Tcf-4, Axin and β-catenin, we found a significant negative connection between the expression of β-catenin and Axin (P=0.031). Among β-catenin positive group (n=18), 13

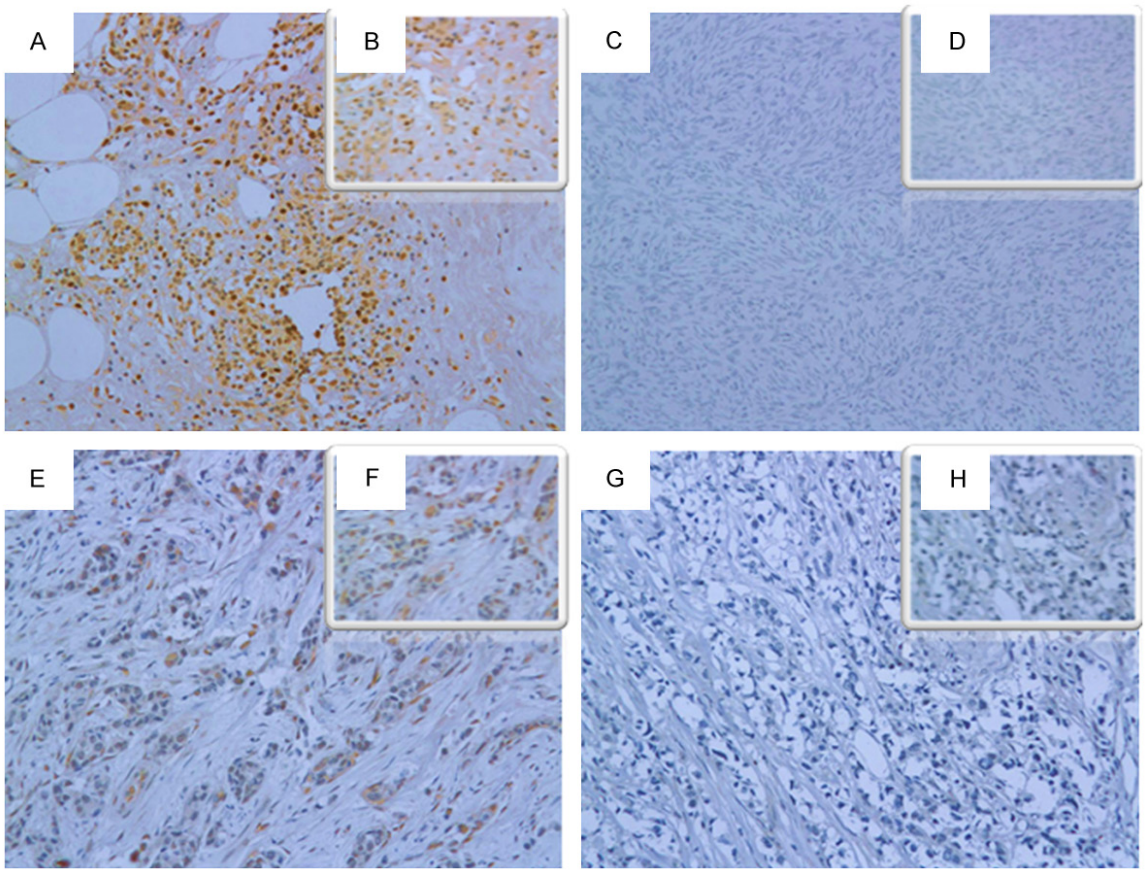


**Figure 3.** Immunohistochemical pattern of TCF-4 expression in MBC and IDC tissue. A. TCF-4-positive specimen in MBC (×200). B. TCF-4-positive specimen in MBC (×400). C. TCF-4-negative specimen in MBC (×200). D. TCF-4-negative specimen in MBC (×400). E. TCF-4-positive specimen in IDC (×200). F. TCF-4-positive specimen in IDC (×400). G. TCF-4-negative specimen in IDC (×200). H. β-catenin-negative specimen in IDC (×400).

**Table 2.** Comparison between the expression of β-catenin, axin and Tcf-4 in metaplastic carcinomas and invasive ductal carcinomas

| Histological type | n | β-catenin | | axin | | Tcf-4 | |
|---|---|---|---|---|---|---|---|
| | | Positive (%) | *P*-value | Positive (%) | *P*-value | Positive (%) | *P*-value |
| Metaplastic carcinoma | 40 | 45.0 (18/40) | | 47.5 (19/40) | | 67.5 (27/40) | |
| Invasive ductal carcinoma | 43 | 23.3 (10/43) | 0.041 | 66.7 (38/43) | <0.001 | 37.2 (16/43) | 0.008 |

patients were Axin negative (72.2%), 5 patients were Axin positive (27.8%). By contrast, among β-catenin negative group (n=22), only 8 patients were Axin negative (36.4%), 14 patients were Axin positive (63.6%). In contrast, no correlation was obtained when analyzing the relationship between the other factors (**Table 3**).

*Correlations among the expression of β-catenin, Tcf-4, Axin and various clinicopathological features*

Correlations among the expression of β-catenin, Tcf-4, Axin and parameters in MBC were summarized in **Table 4**. β-catenin immunoreactivity in cancer tissues showed positive association with the classic clinicopathological markers such as tumor size and clinical stage (P=0.046 and P=0.025, respectively), but not for age, lymph node and menstrual status. However, none of the classic clinicopathological features was related to TCF4 expression in tumor tissues. An inverse relation was found between Axin expression and tumor size as well as clinical stage (P=0.007 and P=0.009, respectively), while there was no relation to the other clinicopathological indices.

**Table 3.** Correlation between the expression of Tcf-4, axin and β-catenin

| | Cases | Tcf-4 | | P | R | axin | | P | R |
|---|---|---|---|---|---|---|---|---|---|
| β-catenin | 40 | - | + | 0.737 | 0.091 | - | + | 0.031 | -0.357 |
| - | 22 | 8 | 14 | | | 8 | 14 | | |
| + | 18 | 5 | 13 | | | 13 | 5 | | |

**Table 4.** Correlations among expression of β-catenin, Tcf-4, axin and clinical characteristics

| Clinical characteristics | n | β-catenin | | | Tcf-4 | | | axin | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 40 | - | + | *P*-value | - | + | *P*-value | - | + | *P*-value |
| Age (years) | | | | 1.000 | | | 0.503 | | | 0.349 |
| ≤50 | 16 | 9 | 7 | | 4 | 12 | | 10 | 6 | |
| >50 | 24 | 13 | 11 | | 9 | 15 | | 11 | 13 | |
| Tumor size (cm) | | | | 0.046 | | | 0.316 | | | 0.007 |
| ≤2 | 14 | 11 | 3 | | 3 | 11 | | 3 | 11 | |
| >2 | 26 | 11 | 15 | | 10 | 16 | | 18 | 8 | |
| Lymph node | | | | 0.059 | | | 0.905 | | | 0.758 |
| 0 | 22 | 12 | 10 | | 8 | 14 | | 12 | 10 | |
| 1-3 | 8 | 7 | 1 | | 2 | 6 | | 3 | 5 | |
| >3 | 10 | 3 | 7 | | 3 | 7 | | 6 | 4 | |
| Clinical stage | | | | 0.025 | | | 0.246 | | | 0.009 |
| I II | 30 | 20 | 10 | | 8 | 22 | | 12 | 18 | |
| III IV | 10 | 2 | 8 | | 5 | 5 | | 9 | 1 | |
| Menstrual status | | | | 1.000 | | | 0.722 | | | 1.000 |
| Premenopause | 13 | 7 | 6 | | 5 | 8 | | 7 | 6 | |
| Post-menopause | 27 | 15 | 12 | | 8 | 19 | | 14 | 13 | |
| p53 | | | | 0.747 | | | 0.498 | | | 0.745 |
| Negative | 25 | 13 | 12 | | 7 | 18 | | 14 | 11 | |
| Positive | 15 | 9 | 6 | | 6 | 9 | | 7 | 8 | |

*Kaplan-Meier survival analysis*

Kaplan-Meier survival curves were shown in **Figures 4**, **5**. Among the 83 study patients, MBC patients experienced significantly poorer outcomes in terms of PFS (P=0.049) in comparison with patients of IDC. Among the 40 MBC patients, the prognosis of Axin-negative patients was distinctly poorer than that of Axin-positive patients with regard to PFS (P=0.018; **Figure 5B**). Similarly, β-catenin-positive patients had a worse survival rate than β-catenin-negative patients (P=0.002; **Figure 5A**). However, the levels of TCF4 had no prognostic significance for breast cancer (P=0.269; **Figure 5C**).

To assess whether β-catenin-positive and Axin-negative are prognostic indicators for MBC patients, we found that the expression level of β-catenin-positive and Axin-negative were correlated with shorter PFS than the other types. (P<0.001; **Figure 5D**).

*Univariate and multivariate analyses of wnt signaling pathway protein expression and clinicopathological variables*

Univariate analysis of PFS using Cox regression analysis identified β-catenin-positive expression (P= 0.004), Axin-positive expression (P=0.025) and clinical stage (P=0.022) as significant prognostic predictors. Age, Tumor size, Lymph node, menstrual status, P53 and TCF4-positive expression had no prognostic value. Multivariate analysis was performed on the same set of patients. The result indicated that β-catenin status (HR: 2.968; P=0.018) were independent unfavorable prognostic factor (**Table 5**).

## Discussion

Metaplastic breast cancer (MBC) is a rare subtype of invasive breast cancer that accounts for less than 1% of all breast cancers [12, 13]. It is characterized by a larger tumor size at presentation, lower rates of axillary nodal involvement as well as higher rates of ER, PR and Her2 negativity when compared to other invasive breast cancers. MBC patients have a higher rate of relapse and a worse prognosis than other triple negative breast cancers [14, 15] with fewer therapeutic options. Therefore, it needs useful prognostic markers for the better diagnosis and treatment. The activation of Wnt signaling pathway is common in MBC [4].

β-catenin plays essential roles in the Wnt signal pathway and in cell-cell adhesion along with E-cadherin [16]. It has been reported that β-catenin was significantly associated with the

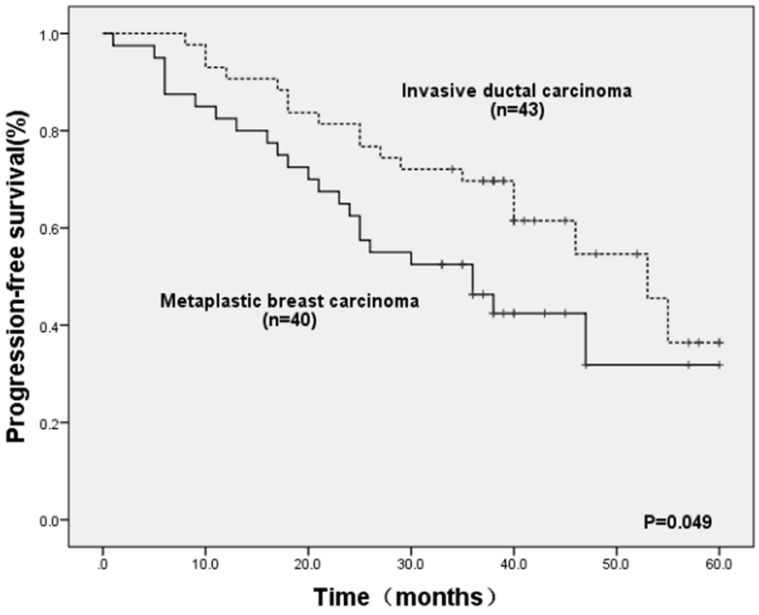


**Figure 4.** Kaplan-Meier analysis for PFS based on MBCs and IDCs (P=0.049).

invasion and metastasis of carcinomas including the esophagus, stomach, colon, liver and melanomas [17-20]. Deregulated β-catenin signaling occurs frequently in human breast cancer [9]. Usually, the β-catenin level is controlled by Wnt signaling for signal transduction through phosphorylation and ubiquitin-mediated degradation. In the absence of Wnt ligands, cytoplasmic β-catenin is recruited into a destruction complex that consists of the core proteins Axin, adenomatous polyposis coli (APC), glycogen synthase kinase-3β (GSK3β) and casein kinase 1 (CK1). This complex formation induces the subsequent degradation of β-catenin [21, 22]. Axin is a multidomain protein that interacts with multiple proteins and acts as a negative regulator of Wnt signaling by down regulating β-catenin levels. Clearly, it serves as a central platform for the APC-GSK-3β-β-catenin degradation complex. In fact, Axin participates in different signaling pathways besides the Wnt pathway, such as JNK pathway and TGF-β pathway. Axin regulates multiple signaling pathways by serving as a scaffold protein, controlling diverse cellular functions in proliferation, fate determination, and suppression of tumorigenesis [23]. Several studies have identified Axin expression in various human cancers, and have demonstrated an inverse association between axin expression and tumor invasion and metastasis [24].

In the present study, we collected 40 cases of primary MBCs and analyzed the expression of Wnt signaling components in order to find out a potential target to treat this invasive breast cancer. We found that β-catenin expression was overexpressed while Axin expression was reduced in MBC patients comparing with controls. The upregulation of β-catenin and downregulation of Axin were associated with higher TNM stage, larger tumor size and short PFS. From all prognostic factors analyzed in this study, β-catenin aberrant expression was the only independent prognostic factor for recurrence. Furthermore, we also found that β-catenin (+)/Axin (-) patients have significantly shorter PFS. Our results are comparable to those reported in previous studies. Elena et al has undertaken an immunohistochemical study measuring the levels of β-catenin in 292 invasive ductal breast cancers. An association was identified between high cytoplasmic β-catenin expression, and high tumor grade, positive Ki67, negative estrogen receptor (ER). Furthermore, cytoplasmic accumulation of β-catenin was associated with poor prognosis in invasive ductal carcinoma [25]. Another study using Immunofluorescence staining has reported that high levels of β-catenin protein expression predicted worse prognosis in breast cancer. They also found that β-catenin was an independent predictor of breast cancer by multivariate analysis [26]. He Y et al detected the positive expression of Axin in breast carcinomas was lower compared with intraductal proliferative lesions [6]. However, knowledge regarding the patients with tumors coexpressing of abnormal β-catenin and Axin in human breast cancer is not well studied. Therefore, further investigation is required in order to elucidate the exact role of these two molecules in breast tumorigenesis.

However, when Wnt ligands are secreted, cytoplasmic β-catenin degradation is inhibited by an inactive destruction complex. Binding of the Wnt ligand to the receptors Frizzled (FZD) and

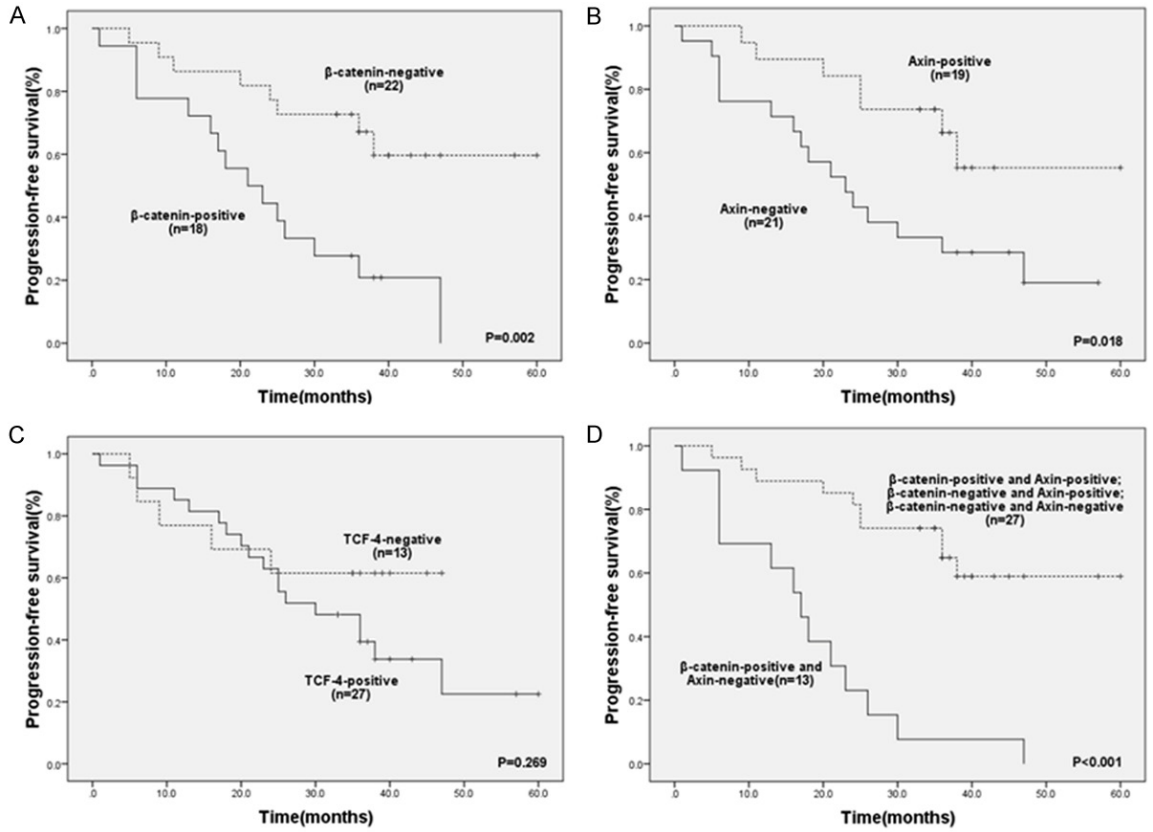


**Figure 5.** Kaplan-Meier analysis for PFS based on the expression of Wnt signaling protein in MBC. A. Kaplan-Meier analysis for PFS based on β-catenin expression in patients with MBC (P=0.002). B. Kaplan-Meier analysis for PFS based on Axin expression in patients with MBC (P=0.018). C. Kaplan-Meier analysis for PFS based on TCF-4 expression in patients with MBC (P=0.269). D. MBC patients with low Axin and high β-catenin had worse PFS (P<0.001).

**Table 5.** Prognostic factors in the Cox proportional hazards model

| Varible | Univariate | | | Multivariate | | |
|---|---|---|---|---|---|---|
| | *P*-value | Hazard Ratio | 95% CI | *P*-value | Hazard Ratio | 95% CI |
| Age (>50 vs ≤50) | 0.738 | 1.161 | 0.485-2.780 | | | |
| Tumor size (>2 vs ≤2) | 0.327 | 1.600 | 0.625-4.096 | | | |
| Lymph node (Positive vs Negative) | 0.097 | 2.024 | 0.880-4.659 | | | |
| Clinical stage (III/IV vs I/II) | 0.022 | 2.819 | 1.158-6.866 | 0.355 | 1.572 | 0.602-4.106 |
| Menstrual status (Post-menopause/Premenopause) | 0.198 | 1.919 | 0.711-5.185 | | | |
| p53 (Positive vs Negative) | 0.267 | 0.603 | 0.246-1.473 | | | |
| β-catenin (Positive vs Negative) | 0.004 | 3.522 | 1.478-8.396 | 0.018 | 2.968 | 1.203-7.321 |
| Axin (Positive vs Negative) | 0.025 | 0.358 | 0.146-0.878 | 0.112 | 0.463 | 0.179-1.195 |
| TCF4 (Positive vs Negative) | 0.279 | 1.730 | 0.641-4.669 | | | |

low-density lipoprotein receptor-related protein 5/6 (LRP5/6) activates Wnt signaling pathway. Concomitantly, the scaffolding protein Dishevelled (Dsh) is recruited to the cell membrane, and the destruction complex is out of action. As a consequence, β-catenin is no longer ubiquitinated or degraded, leading to complex saturation and inhibition. The cytoplasmic β-catenin stabilization allows its translocation to the nucleus where it binds to members of the T-cell factor/lymphoid enhancing factor (TCF/LEF) family of transcription factors, such as TCF1,

TCF4, and finally induces the expression of Wnt target genes [21, 22]. In this study, we found that TCF4 was overexpressed in MBC patients comparing with controls. Somehow unexpectedly, we did not observe any association between TCF4 expression with the other factors. We analyzed the reason that lack of association was in line with little sample size of patients taken in this research. Further detailed analyses of Wnt signaling components are needed to determine the mechanisms that lead to MBC.

In summary, we have shown that MBC patients expressed high levels of β-catenin, TCF4 and low levels of Axin. The upregulation of β-catenin and downregulation of Axin are associated with higher TNM stage and short PFS, respectively. Concomitantly, β-catenin expression negatively correlated with Axin expression, and the β-catenin (+)/Axin (-) group have the shortest PFS. These results suggested that the loss of Axin and the gain of β-catenin increase the risk of MBC patients recurrence, thus β-catenin and Axin combination may be a better prognostic marker for MBC. In addition, they are possibly good targets in prevention and treatment of MBC.

## Acknowledgements

This experiment was finished in Oncology Key Lab of Heilongjiang Province Institution of Higher Education. We thank all staff in medical record room for their support and participation.

## Disclosure of conflict of interest

None.

**Address correspondence to:** Dr. Qingyuan Zhang, Department of Medical Oncology, The Third Affiliated Hospital of Harbin Medical University, Harbin 150040, China. Tel: +86-451-86298681; Fax: +86 451-86298681; E-mail: zqysci@163.com

## References

[1] Frank GA, Danilova NV, Andreeva I, Nefedova NA. WHO classification of tumors of the breast, 2012. Arkh Patol 2013; 75: 53-63.

[2] Gaspar C, Franken P, Molenaar L, Breukel C, van der Valk M, Smits R, Fodde R. A targeted constitutive mutation in the APC tumor suppressor gene underlies mammary but not intestinal tumorigenesis. PLoS Genet 2009; 5: e1000547.

[3] Jung SY, Kim HY, Nam BH, Min SY, Lee SJ, Park C, Kwon Y, Kim EA, Ko KL, Shin KH, Lee KS, Park IH, Lee S, Kim SW, Kang HS, Ro J. Worse prognosis of metaplastic breast cancer patients than other patients with triple-negative breast cancer. Breast Cancer Res Treat 2010; 120: 627-37.

[4] Hayes MJ, Thomas D, Emmons A, Giordano TJ, Kleer CG. Genetic changes of Wnt pathway genes are common events in metaplastic carcinomas of the breast. Clin Cancer Res 2008; 14: 4038-44.

[5] Monteiro J, Gaspar C, Richer W, Franken PF, Sacchetti A, Joosten R, Idali A, Brandao J, Decraene C, Fodde R. Cancer stemness in Wnt-driven mammary tumorigenesis. Carcinogenesis 2014; 35: 2-13.

[6] He Y, Liu Z, Qiao C, Xu M, Yu J, Li G. Expression and significance of Wnt signaling components and their target genes in breast carcinoma. Mol Med Rep 2014; 9: 137-43.

[7] Zeng YA, Nusse R. Wnt proteins are self-renewal factors for mammary stem cells and promote their long-term expansion in culture. Cell Stem Cell 2010; 6: 568-77.

[8] Geyer FC, Lacroix-Triki M, Savage K, Arnedos M, Lambros MB, MacKay A, Natrajan R, Reis-Filho JS. beta-Catenin pathway activation in breast cancer is associated with triple-negative phenotype but not with CTNNB1 mutation. Mod Pathol 2011; 24: 209-31.

[9] Incassati A, Chandramouli A, Eelkema R, Cowin P. Key signaling nodes in mammary gland development and cancer: beta-catenin. Breast Cancer Res 2010; 12: 213.

[10] Khramtsov AI, Khramtsova GF, Tretiakova M, Huo D, Olopade OI, Goss KH. Wnt/beta-catenin pathway activation is enriched in basal-like breast cancers and predicts poor outcome. Am J Pathol 2010; 176: 2911-20.

[11] Nakajima M, Fukuchi M, Miyazaki T, Masuda N, Kato H, Kuwano H. Reduced expression of Axin correlates with tumour progression of oesophageal squamous cell carcinoma. Br J Cancer 2003; 88: 1734-9.

[12] Luini A, Aguilar M, Gatti G, Fasani R, Botteri E, Brito JA, Maisonneuve P, Vento AR, Viale G. Metaplastic carcinoma of the breast, an unusual disease with worse prognosis: the experience of the European Institute of Oncology and review of the literature. Breast Cancer Res Treat 2007; 101: 349-53.

[13] Tavassoli FA. Classification of metaplastic carcinomas of the breast. Pathol Annu 1992; 27: 89-119.

[14] Okada N, Hasebe T, Iwasaki M, Tamura N, Akashi-Tanaka S, Hojo T, Shibata T, Sasajima Y, Kanai Y, Kinoshita T. Metaplastic carcinoma of the breast. Hum Pathol 2010; 41: 960-70.

[15] Bae SY, Lee SK, Koo MY, Hur SM, Choi MY, Cho DH, Lee JE, Kim JH, Kim JS, Nam SJ, Yang JH. The prognoses of metaplastic breast cancer patients compared to those of triple-negative breast cancer patients. Breast Cancer Res Treat 2011; 126: 471-8.

[16] Umbreit C, Flanjak J, Weiss C, Erben P, Aderhold C, Faber A, Stern-Straeter J, Hoermann K, Schultz JD. Incomplete epithelial-mesenchymal transition in p16-positive squamous cell carcinoma cells correlates with beta-catenin expression. Anticancer Res 2014; 34: 7061-9.

[17] Rubinfeld B, Robbins P, El-Gamil M, Albert I, Porfiri E, Polakis P. Stabilization of beta-catenin by genetic defects in melanoma cell lines. Science 1997; 275: 1790-2.

[18] Osterheld MC, Bian YS, Bosman FT, Benhattar J, Fontolliet C. Beta-catenin expression and its association with prognostic factors in adenocarcinoma developed in Barrett esophagus. Am J Clin Pathol 2002; 117: 451-6.

[19] Kim HS, Hong EK, Park SY, Kim WH, Lee HS. Expression of beta-catenin and E-cadherin in the adenoma-carcinoma sequence of the stomach. Anticancer Res 2003; 23: 2863-8.

[20] Mao TL, Chu JS, Jeng YM, Lai PL, Hsu HC. Expression of mutant nuclear beta-catenin correlates with non-invasive hepatocellular carcinoma, absence of portal vein spread, and good prognosis. J Pathol 2001; 193: 95-101.

[21] Bilir B, Kucuk O, Moreno CS. Wnt signaling blockage inhibits cell proliferation and migration, and induces apoptosis in triple-negative breast cancer cells. J Transl Med 2013; 11: 280.

[22] MacDonald BT, Tamai K, He X. Wnt/beta-catenin signaling: components, mechanisms, and diseases. Dev Cell 2009; 17: 9-26.

[23] Luo W, Lin SC. Axin: a master scaffold for multiple signaling pathways. Neurosignals 2004; 13: 99-113.

[24] Xu HT, Wang L, Lin D, Liu Y, Liu N, Yuan XM, Wang EH. Abnormal beta-catenin and reduced axin expression are associated with poor differentiation and progression in non-small cell lung cancer. Am J Clin Pathol 2006; 125: 534-41.

[25] Lopez-Knowles E, Zardawi SJ, McNeil CM, Millar EK, Crea P, Musgrove EA, Sutherland RL, O'Toole SA. Cytoplasmic localization of beta-catenin is a marker of poor outcome in breast cancer patients. Cancer Epidemiol Biomarkers Prev 2010; 19: 301-9.

[26] Dolled-Filhart M, McCabe A, Giltnane J, Cregger M, Camp RL, Rimm DL. Quantitative in situ analysis of beta-catenin expression in breast cancer shows decreased expression is associated with poor outcome. Cancer Res 2006; 66: 5487-94.