Cancer Stem Cell-Related Gene Periostin: A Novel Prognostic Marker for Breast Cancer

Dongyang Xu1, Hong Xu2, Ying Ren3, Caigang Liu4*, Xuemei Wang4*, Hao Zhang1, Ping Lu1

1 Ultrasound Department, First Hospital of China Medical University, Shenyang, China, 2 Department of Breast Surgery, Tumor Hospital of Liaoning Province, Shenyang, Liaoning Province, People’s Republic of China, 3 Radiology Department, Shengjing Hospital of China Medical University, Shenyang, Liaoning Province, People’s Republic of China, 4 Department of Breast Surgery, General Surgery, First Hospital of China Medical University, Shenyang, Liaoning Province, People’s Republic of China

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* E-mail: angel-s205@163.com (CL); xuemeiwangcmu@163.com (XW)

Abstract

We investigated the expression status of periostin in breast cancer stem cells and its clinical implications in order to lay a foundation for managing breast cancer. CD44+/CD24−/low tumor cells (CSC) from clinical specimens were sorted using flow cytometry. Periostin expression status was detected in CSC cells and 1,086 breast cancer specimens by Western blot and immunohistochemistry staining, with the CSC ratio determined by immunofluorescence double staining. The relationship between the periostin protein and clinico-pathological parameters and prognosis was subsequently determined. As a result, CSC cells are more likely to generate new tumors in mice and cell microspheres that are deficient in NOD/SCID compared to the control group. Periostin protein was expressed higher in CSC cells compared to the control cells and was found to be related to CSC chemotheraphy resistance. Moreover, periostin expression was found to be related to the CSC ratio in 1,086 breast cancer specimens (P = 0.001). In total, 334 (30.76%) of the 1,086 breast cases showed high periostin expression. After universal and Spearman regression correlation analysis, periostin was observed to be related to histological grade, CSC ratio, lymph node metastasis, tumor size, and triple-negative breast cancer (all P < 0.05). Furthermore, periostin was shown to attain a significantly more distant bone metastasis and worse disease-specific survival than those with none or low-expressed periostin protein (P = 0.001). In the Cox regression test, periostin protein was detected as an independent prognostic factor (P = 0.001). In conclusion, periostin was found to be related to the CSC and an independent prognostic factor for breast cancer. It is also perhaps a potential target to breast cancer.

Introduction

Breast cancer is the most common cause of death in female malignant tumor disease [1]. In 2008, 1,380,000 new occurrences of breast cancer were diagnosed worldwide, with 438,400 persons dying from breast cancer that same year [2,3]. Currently, surgical treatment is directed mainly at primary treatment, chemotherapy, radiotherapy, and endocrine therapy, whereas targeted treatment aims to eliminate the residual tumor cells and thus reduce the risk of recurrence and metastasis. Some patients, however, still relapse or metastasize after chemotherapy, radiotherapy, endocrine therapy, and targeted therapy. What causes the poor effects of chemotherapy? Why does targeted treatment have no effect on some patients? These questions remain unanswered.

Stem cells, which represent only a very small percentage of the total tumor mass, have been found to be the source of some, and possibly most, cancers [4]. Breast cancer stem cells are a small group of tumor cells with the capacity to self-renew, a strong ability to form solid breast tumors, and the ability to differentiate into a relatively quiescent primitive group of cancer cells that are considered the underlying factor of tumor recurrence on the main reason breast cancers resist therapies [5].

Following a better understanding of cancer stem cell theory, stem cell-related genes in malignant tumors have gained more academic attention. Recently, Malanchi et al. found that periostin allows cancer stem cells to maintain, and blocking periostin’s function prevents metastasis [6]. Notably, blocking the periostin protein rarely caused side effects in mice [6]. In another study, Kyutoku et al. [7] reported that periostin plays a pivotal role in how breast cancer progresses and metastasizes. Administering the periostin antibody prolonged cell survival by inhibiting the progression and metastasis of 4T1 cells; therefore, developing the periostin antibody further, including generating a humanized antibody, may provide a new therapeutic agent against breast cancer.

Periostin is a kind of bone adhesion molecule that regulates osteoblast adhesion and differentiation and is classified among the extracellular matrix (ECM) proteins [8]. It presents as part of the extracellular matrix in natural conditions and plays an important role in fetal development. In adults, only some specific organs such as the breast, bone, skin, and intestines have periostin activity [9]. Periostin is reported to be involved in tumor EMT, extracellular matrix degradation, tumor invasion, and distant metastasis, but the mechanism by which it operates is still unclear [10,11]. Currently, the periostin expression status in breast cancer stem cells (CSC) and clinical implications for breast cancer are also unclear. In the present study, we try to sort and identify breast cancer stem cells investigate the expression status of periostin in those cells, and evaluate the clinical implications of periostin in breast cancer. Gaining this knowledge will lay a foundation for managing breast cancer.
Materials and Methods

Patients and Tissue Specimens

A total of 1,086 patients who had histologically confirmed breast cancer and who underwent radical operations in the Tumor Hospital of Liaoning province and China Medical University between January 2001 and January 2006 were enrolled for immunohistochemical and immunofluorescence double staining and prognostic analysis. The mean age was 50.73±10.28 years (range from 27 to 80 years). The criteria to include a patient in this

Figure 1. CD44+/CD24- tumor cells got a strong ability to form solid breast tumors and mammospheres. A representative FACS plots to demonstrate the CD44+/CD24- cancer stem cells (A) and a representative tumor in the NOD/SCID mouse showed at the CSC-cell injection site (B1), but not at the non-CSC cell injection site (B2). CSC cells can form mammospheres in the non-adherent culture condition (1-week culture) (C1), whereas no mammosphere was produced in non-CSC cells (C2).

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study were as follows: (1) curative operations were performed; (2) resected specimens were pathologically examined; (3) more than 10 lymph nodes were pathologically examined after the operation; and, (4) a complete medical record including the ER, PR, Her2, p53, and Ki67 status was available. The study protocol was approved by the Ethics Committee of China Medical University and Liaoning Tumor Hospital and a written informed consent was obtained from all participants involved in the study. The animal experiments were also approved by the Ethics Committee of China Medical University.

**Identifying the Ability of CSC Cells to Form Tumors**

The clinical specimens were digested into single tumor cells using collagenase III. The tumor cells were suspended in 100 µl/10⁶ cells of HBSS with 2% HICS. The samples were then washed twice with HBSS/2% HICS and suspended. Antibodies, including anti-CD2, -CD3 -CD10, -CD16, -CD18, -CD31, and anti-CD326 were added and incubated for 20 min on ice and then washed twice with HBSS/2% HICS. Lineage+ cells were first eliminated using anti-CD2, -CD3 -CD10, -CD16, -CD18, -CD31, and anti-CD326 during flow cytometry. Dead cells were eliminated using the viability dye 7AAD. Next, CD44+ tumor cells were sorted by CD44 and CD24 in flow cytometry. The selected cells to be injected were then suspended in 1640/Matrigel mix (1:1 volume) and injected into the appropriate area of the mammary fat pad. The tumorigenicity experiments were repeated three times.

**Mammosphere Generation Test**

For this step, Complete MammoCult™ Medium (Human) was prepared by adding 50 mL of thawed MammoCult™ Prolifera-

**siRNA Transfection and the Effect Confirmation**

Periostin siRNA and control siRNA were designed and synthesized by Santa Cruz Biotechnology, Inc. Transfection was performed in 50 to 60% confluent cells using Lipofectamine 2000 Reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacture’s protocol. Briefly, siRNA and Lipofectamin 2000 in Opti-MEM I Reduced Serum Medium (Invitrogen, Carlsbad, CA, USA) were diluted separately and mixed gently. After incubation for 15 min at room temperature, they were combined and incubated for another 15 min at room temperature. Next, the complexes were added to the culture plates or wells with cells and the medium without serum, and then the final concentration of siRNA to 25 nM was made. After incubating the cells at 37°C in a humidified CO₂ incubator for 6 h, the medium was replaced with complete medium. Transfecting only with Lipofectamine 2000 acted as a mock transfection. The downregulation of periostin by siRNA was confirmed by RT-PCR and Western blot at the indicated time points.

**Treatments with Chemotherapeutic Agents and Measuring Cell Viability**

When the above cells cultured as monolayers were healthy and were 80 to 90% confluent, they were washed with warm Hank's Balanced Salt Solution (HBSS). The cells were scraped gently from the dish using a sterile cell scrape. The scraped cells were then suspended in Complete MammoCulttm medium and counted. The sensitivity of the cells to three chemotherapeutic drugs were examined using the Cell Counting Kit-8 (CCK-8) technique. Cells were plated at a density of 5 × 10⁴/mL cells per well into Ultra-Low Adherent 96-well plates containing 100 µl Complete MammoCult medium and treated with for concentration of DDP (2.5 µg/ml/PPC (plasma peak concentration)), EPI (0.78 µg/ml/PPC), and DTX (3.7 µg/ml/PPC) as follows: 0.2, 1.0, 5.0, 10.0 PPC. CCK-8 reagent was added to each well and incubated for 2 h before reading at wavelength of 450 nm. The cells were counted at 24, 48, 72 and 96 h with CCK-8.

![Figure 3.](https://example.com/figure3.png)

**Figure 3.** MTT assay shows that the cells exposed to periostin siRNA showed a significant decrease in IC₅₀ among the DDP, EPI, and DTX compared with the control siRNA group (control group) (P<0.01).

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Western Blot Analysis

For Western blot analysis, cells were lysed with the buffer [0.1% SDS, 50 mmol/L Tris-HCl (pH 7.6), 1% NP-40, 150 mmol/L NaCl, 2 mg/ml aprotnin, 2 mg/ml leupeptin and 7 mg/ml PMSF]. The protein concentrations were determined using the BCA Protein Assay kit (Pierce Biotechnology, Inc., Rockford, IL, USA). Thirty micrograms of protein were separated on 10% SDS-

Figure 4. Periostin was located in the cytoplasm and membrane of the breast cancers. It was observed that Periostin was either not expressed or expressed low in paracancerous tissues (A, x400) and atypical hyperplasia tissues (B, x400); expressed low in cases without lymph node metastasis (C, x400) and expressed high in cases with lymph node metastasis (D, x400); expressed low in the non-triple-negative breast cancers (E, x400) and expressed high in triple-negative breast cancers (F, x400).
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Thin slices of tumor tissue for all cases received in our histopathology unit were fixed in 4% formaldehyde solution (pH 7.0) for periods not exceeding 24 h. The tissues were processed routinely for paraffin embedding, and 4 µm-thick sections were cut and placed on glass slides coated with 3-aminopropyl triethoxysilane for immunohistochemistry. Tissue samples were stained with hematoxylin and eosin, and the pathologist Chen B and Wang MX determined the histological type and tumor grade.

Briefly, immunohistochemical staining was performed using the standard streptavidin-peroxidase (SP) method with the UltraSensitive TM S-P Kit (Maxin-Bio, China) according to the manufacturer’s instructions, and signals were visualized using the DAB substrate, which stains the target protein yellow. Briefly, one paraffin-embedded block of the tissue was cut at 4 µm and placed on poly-L-lysine coated slides. The slides were deparaffinized in xylene, rehydrated in a gradient of ethanol solutions, and then immersed in 10 mM sodium citrate buffer (pH 6.0), pretreated in a microwave oven for 10 min, followed by a 10-minute rinse with phosphate-buffered saline (PBS). The sections were incubated with 3% hydrogen peroxide for 10 min to block endogenous peroxidase activity at room temperature. Nonspecific reactions were blocked by incubating the sections in a solution containing normal serum. Then the slides were incubated in a humid chamber at 4°C overnight with primary antibody. Following washings with PBS, sections were incubated for 30 min in the secondary biotinylated antibody (Multilink Swine anti-goat/mouse/rabbit immunoglobulin; Dako, Inc.). Following washings, Avidin Biotin Complex (ABC) overnight. After washing, the membrane was incubated with a secondary antibody at a dilution of 1:2,000 at room temperature for 1 h. Proteins were detected with the ECL Kit (Varsal Instruments, Beijing, China), and anti-β-actin antibody (Sigma-Aldrich, St. Louis, MO, USA) at 4°C the membrane was incubated with an ABC complex at 37°C. Sections were then counterstained in Gill’s Hematoxylin and dehydrated in ascending grades of methanol before clearing in xylene, then mounting under a coverslip.

To score periostin as immuno-positive staining, the positive cells appeared as a yellow to brown color in the nucleus and/or cytoplasm. Periostin expression was classified semi-quantitatively according to the following criteria: 0 if <1% of neoplastic cells discretely expressed periostin; 1+ if ≥1% and <10% of morphologically unequivocal neoplastic cells discretely expressed periostin; and, 2+ if ≥10% of morphologically unequivocal neoplastic cells discretely expressed periostin. Samples scored as 1+ or 2+ were considered positive. Nuclear staining for ER, PR, and P53 was graded 1+ if <10% of the cells were stained, 2+ if 10–50% of the cells were stained, and 3+ if >50% of the cells were stained. Grades 2+ and 3+ were considered positive, whereas absence of staining and 1+ staining were considered negative. Similar standards were used for staining intensity in HER-2 / neu; only grade 3+ (high intensity) was considered positive.

**Table 1. Correlations between Periostin expression and clinico-pathological features (n = 1086).**

| Variables            | n    | Periostin | Periostin+ | X² value | p value |
|----------------------|------|-----------|------------|-----------|---------|
| Age                  |      |           |            |           |         |
| <40 Y                | 174  | 110       | 64         | 3.534     | 0.073   |
| ≥40 Y                | 912  | 642       | 270        |           |         |
| Tumor size           |      |           |            |           |         |
| T1                   | 173  | 104       | 69         | 20.665    | 0.001   |
| T2                   | 836  | 580       | 256        |           |         |
| T3                   | 69   | 62        | 7          |           |         |
| T4                   | 8    | 6         | 2          |           |         |
| Histological grade   |      |           |            | 11.576    | 0.003   |
| I                    | 84   | 63        | 21         |           |         |
| II                   | 732  | 524       | 208        |           |         |
| III                  | 270  | 165       | 105        |           |         |
| Histological type    |      |           |            | 2.477     | 0.290   |
| Ductal               | 831  | 582       | 249        |           |         |
| Lobular              | 96   | 66        | 30         |           |         |
| Others               | 159  | 104       | 55         |           |         |
| HER2 status          |      |           |            | 0.391     | 0.218   |
| negative             | 814  | 558       | 256        |           |         |
| positive             | 272  | 194       | 78         |           |         |
| Metastatic nodes     |      |           |            | 34.133    | 0.001   |
| negative             | 467  | 437       | 130        |           |         |
| positive             | 519  | 315       | 204        |           |         |
| Distant metastasis   |      |           |            | 185.466   | 0.001   |
| negative             | 798  | 644       | 154        |           |         |
| positive             | 288  | 108       | 180        |           |         |
| Triple-negative breast cancer | 39.052 | 0.001 |
| Yes                  | 295  | 162       | 133        |           |         |
| No                   | 781  | 590       | 201        |           |         |
| CSC ratio            |      |           |            | 536.135   | 0.001   |
| <5%                  | 621  | 603       | 18         |           |         |
| 5–10%                | 364  | 128       | 236        |           |         |
| >10%                 | 101  | 21        | 80         |           |         |

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**Immunohistochemistry Experimental Procedures**

Thin slices of tumor tissue for all cases received in our histopathology unit were fixed in 4% formaldehyde solution (pH 7.0) for periods not exceeding 24 h. The tissues were processed routinely for paraffin embedding, and 4 µm-thick sections were cut and placed on glass slides coated with 3-
Statistical Analysis

All data were analyzed with SPSS Statistics software (Version 13.0, Chicago, IL, USA). Relationships between periostin and other parameters were studied using the chi-square test, Fisher’s exact test, or independent t tests. Disease-specific survival was analyzed using the Kaplan-Meier method. The log-rank test was used to analyze survival differences. Multivariate analysis was performed using the Cox proportional hazards model selected in forward stepwise. A P value of less than 0.05 was considered statistically significant.

Table 2. Spearman correlation analysis between clinic-pathological features and Periostin.

| Clinic-pathological features | Periostin expression (p; Spearman correlation) |
|-----------------------------|-----------------------------------------------|
| age                         | 0.060(0.057)                                  |
| Histological grade          | 0.001(0.100)                                  |
| Histological type           | 0.460(0.001)                                  |
| HER2 status                 | 0.075(0.036)                                  |
| CSC ratio                   | 0.001(0.170)                                  |
| Lymph node metastasis       | 0.001(0.177)                                  |
| Tumor size                  | 0.001(0.118)                                  |
| Triple-negative breast cancer| 0.001(0.190)                                  |

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Table 3. Multivariate analysis of the factors related to post-operative distant metastasis.

| Characteristic               | Exp(B) | 95% CI for Exp(B) | P value |
|------------------------------|--------|-------------------|---------|
| age                          | 0.335  | 0.211–0.532       | 0.001   |
| Histological grade           | 3.574  | 2.540–5.028       | 0.001   |
| Histological type            | 1.208  | 0.755–1.824       | 0.125   |
| CSC ratio                    | 1.193  | 0.845–1.685       | 0.316   |
| Lymph node metastasis        | 4.836  | 3.239–7.219       | 0.001   |
| Tumor size                   | 1.475  | 1.029–2.114       | 0.035   |
| Triple-negative breast cancer| 8.434  | 5.701–12.476      | 0.001   |
| Periostin                    | 5.829  | 3.582–9.485       | 0.001   |
| Constant                     | 0.005  |                   |         |

CI = confidence interval.
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Figure 5. The CSC ratios in 1,086 cases were observed. Images show <5% (A), 5 to 10% (B), and >10% (C) CSC ratios in the specimens. Staining was performed with: mouse anti-human CD44 antibody and PE conjugated secondary (1); rabbit anti-human CD24 antibody with FITC conjugated secondary (2); and DAPI to detect nuclei (3). An overlay of panels (1) and (2) is presented in (4).
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samples in Fig. 1A). A quantity of 10^3, 10^4, 10^5, and 10^6 CSC cells total tumor cells was from 3.75% to 33.11% (representative of the same number of non-CSC cells was injected into the left mammary fat pad as a control. Eight weeks post-injection, 10^3 CSC cells successfully formed a tumor (2/4), while non-CD44+/CD24- tumor cells failed to form tumors until attaining 10^6 cells (1/4) (P<0.05, Fisher’s extract test, Fig. 1B1 and B2).

After 7 days of culture, single-cell suspensions of CSC cells that were separated from the solid tumors produced viable mammospheres (20–100 μm), which could be passaged further. No mammosphere was produced by the non-CSC cells in the same culture condition (see Fig. 1C1 and C2). CSC cells generated new tumors in mice and cell microspheres that were deficient in NOD/SCID compared to the control group. Furthermore, periostin protein was expressed higher in CSC cells compared to the control cells (Figure 2). Moreover, periostin expression was found to be related to the CSC ratio in 1,086 breast cancer specimens (P = 0.001).

Expression of Periostin was Blocked Efficiently by RNAi

By replacing the serum culture medium at 6 h after siRNA transfection, we observed that the successfully transfected CSC cells had green fluorescent when examined using fluorescence microscopy. The positive rate of transfected cells was 66.12±8.46% in the CSC cells. The expression of periostin was examined by RT-PCR and Western blot at 48 h after siRNA transfection. The efficiency reached more than 82% at the protein level in the CSC cells.

### Table 4. Correlations between periostin expression and distant metastasis(n(%)).

| Organs metastasis | n   | Periostin- | Periostin+ |
|-------------------|-----|------------|------------|
| Bone              | 134 | 42(38.89)  | 92(51.11)  |
| Lung              | 59  | 22(20.37)  | 37(20.56)  |
| Liver             | 46  | 20(18.52)  | 26(14.44)  |
| Ovarian           | 22  | 8(7.41)    | 14(7.78)   |
| Others            | 27  | 16(14.81)  | 11(6.11)   |

### Results

**Identifying the Stemness of CSC Cells**

We found that the mean percentage of CD44+CD24- cells in total tumor cells was from 3.75% to 33.11% (representative samples in Fig. 1A). A quantity of 10^3, 10^4, 10^5, and 10^6 CSC cells that were separated from the solid tumors were separately injected into the right mammary fat pad of four SCID mouse. Meanwhile, the same number of non-CSC cells was injected into the left mammary fat pad as a control. Eight weeks post-injection, 10^3 CSC cells successfully formed a tumor (2/4), while non-CD44+/CD24- tumor cells failed to form tumors until attaining 10^6 cells (1/4) (P<0.05, Fisher’s extract test, Fig. 1B1 and B2).

### Table 5. Correlations between periostin expression and chemotherapeutic resistance in breast cancers (n = 135; n(%)).

| Chemosensitivity | n   | Periostin- | Periostin+ | X² value | P value |
|------------------|-----|------------|------------|----------|---------|
| CR               | 15  | 11         | 42(26.67)  | 8.017    | 0.046   |
| PR               | 60  | 43         | 17(28.33)  | 14(43.75)|         |
| SD               | 32  | 18         | 16(57.14)  |          |         |
| PD               | 28  | 12         |            |          |         |

CR: complete response; PR: partial response; SD: stable disease; PD: progressive disease.

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**Periostin Downregulation Sensitizes to Chemotherapy Drugs in CSC Cells**

To investigate whether downregulation of periostin expression has the potential to sensitize CSC cells to chemotherapy, a combination treatment of periostin-specific siRNA with chemotherapeutic drugs was performed. Twenty-four hours after transfection with siRNA, cells were treated with DDP, EPI, and DTX at 0, 0.2 PPC, 1 PPC, 5 PPC and 10 PPC scaled concentrations for 72 h. The IC50 was determined by MTT assay. Figure 3 shows that the cells exposed to periostin siRNA showed a significant decrease in IC50 among the three drugs when compared with the control siRNA or no treatment (P<0.01).

**Periostin Expression in Breast Cancer and its Relationship with Clinicopathological Characteristics**

Immunohistochemical examination showed that periostin was located in the cytoplasm and membrane of the breast cancers (Figure 4). It was also observed that periostin protein was expressed significantly higher in breast cancer tissues compared to paracancerous tissue and atypical hyperplasia tissues (30.76% vs 7.92% vs 5.99%, respectively) (Fig. 4A and 4B). The cases with high periostin expression intended to develop into lymph node and postoperative distant metastasis (P=0.001 and 0.001, respectively) (Fig. 4C and 4D). Moreover, 133 (43.08%) of the 295 triple-negative breast cancers showed periostin expression compared to 201 (25.74%) of the 781 cases of non-triple-negative breast cancers (P=0.001) (Fig. 4E and F).

We also determined the CSC ratio in the 1,086 patients. CSC ratios in 621 (57.18%) cases were <5%; 364 (33.52%) were 5 to 10%; and 101 (9.3%) were >10% (Table 1). In total, 334 (30.76%) of the 1,086 breast cancer cases showed high periostin expression. After universal analysis, periostin was observed to be related to tumor size, histological grade, lymph node metastasis, postoperative distant metastasis, triple-negative breast cancer, and CSC ratio (P=0.01, 0.003, 0.001, 0.001, respectively) (Table 1).

Spearman correlation regression analysis showed that periostin expression has a linear correlation to histological grade, CSC ratio, lymph node metastasis, tumor size and triple-negative breast cancer (P=0.001, 0.001, 0.001, 0.001, respectively) (Table 2). After multivariate analysis, age, histological grade, lymph node metastasis, postoperative distant metastasis, triple-negative breast cancer, and periostin expression were related to post-operative distant metastasis (P=0.01, 0.001, 0.001, 0.001, 0.001, respectively) (Table 3).

**Postoperative Recurrence Pattern and Chemotherapeutic Resistance**

In the present study, the patients with positive periostin expression attain a significantly more distant metastasis rate. Of the 334 cases with high periostin expression, 180 (53.89%) developed 5-year postoperative distant metastasis, whereas only 12.68% of patients without periostin expression developed 5-year postoperative distant metastasis (P=0.001). After subgroup analysis, a significant association between periostin expression and postoperative bone metastasis (Table 4) was observed.

We further studied the relationship among age, tumor size, histological grade, histological type, molecular type, CSC ratio, periostin expression and chemotherapeutic sensitivity in 135 neoadjuvant chemotherapy breast cancers. Finally, molecular type, CSC ratio and periostin expression were observed significantly related to the chemosensitivity (P=0.01, 0.012 and 0.046 respectively). Periostin expression was expressed in 26.67%,
Prognostic Analysis

In the prognostic analysis, patients with breast cancer expressing periostin, along with age, histological grade, lymph node metastasis, and triple-negative breast cancer, were shown to attain a poorer disease-specific survival than those with no or low expressed periostin protein (\(P = 0.001, 0.002, 0.001, 0.004,\) and 0.001, respectively) (Figure 6). In the Cox regression test, periostin protein was detected as an independent prognostic factor (\(P = 0.001\)) (Table 6).

Table 6. Cox model regression analysis of the breast cancer prognostic factors.

| varies | OR | 95% CI for OR | \(P\) value |
|--------|----|--------------|-------------|
| age    | 1.410 | 1.133–1.755 | 0.002       |
| Histological grade | 1.545 | 1.355–1.762 | 0.001       |
| Lymph node metastasis | 1.245 | 1.074–1.443 | 0.004       |
| Triple-negative breast cancer | 2.597 | 2.121–3.179 | 0.001       |

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Discussion

Periostin is a 93.3-kD secreted cell adhesion protein, which has been shown to be a critical regulator of bone metabolism, hypertensive nephropathy, and wound repair [12,13]. Recent studies have revealed that periostin plays an important role in tumor epithelial-mesenchymal transition, tumor angiogenesis, and tumor development. It is upregulated in a wide variety of cancers including colon, pancreatic, ovarian, head, and gastric cancer [14,15].

Recently, Malanchi et al demonstrated that stromal periostin is crucial for metastatic colonization by regulating the interactions between cancer stem cells and their metastatic niche [6]. Periostin mediates the crosstalk between cancer stem cells and their niche. It is considered required to maintain cancer stem cells, and blocking its function prevents metastasis. Moreover, blocking the periostin protein rarely caused side effects in mice. Hence, periostin may be a potential breast cancer treatment target. Currently, the expression status of periostin protein in breast cancer and its relationship to the biological behavior of the disease are still unclear [16]. Furthermore, studies that have addressed the relationship between periostin and chemotherapy sensitivity and prognosis of breast cancer are still sparse [17].

Tumor stem cells have been found to be the source of most cancers and the culprit of tumor recurrence and metastasis and drug resistance [18]. In recent studies, periostin was reported as a bridge between cancer stem cell and metastasis [6,9]. No studies to date, however, have examined the relationship among periostin expression status and breast cancer CSC ratio, chemotherapeutic sensitivity, and the clinical implications of breast cancer. In the current study, we sorted and identified the breast cancer CSC from clinical specimens, observing that periostin was expressed high in breast cancer CSC compared to the control group. Moreover, drug sensitivity tests showed that a combination treatment of periostin-specific siRNA with chemotherapy drugs could significantly increase the apoptosis of breast cancer CSC. The outcome demonstrated that periostin plays an important role in breast cancer’s resistance to chemotherapy.

We also investigated the relationship between periostin expression and the biological behavior of breast cancer stem cells and the clinicopathological characteristics of breast cancer. Periostin protein was observed to be expressed significantly higher in cancerous tissues than adjacent-tumor tissues. Moreover, periostin protein was found to be related to tumor size, histological grade, lymph node metastasis, postoperative distant metastasis, triple-negative breast cancer, and CSC ratio in the 1,086 breast cancers studied. We also studied the relationship between periostin expression and chemotherapeutic sensitivity. We found that periostin expression was significantly related to a poor chemotherapy response in breast neoadjuvant chemotherapy.

After survival analysis, periostin was shown to attain a significantly more distant postoperative bone metastasis and attained significantly poorer postoperative disease-specific survival. Indeed, the Cox regression test showed periostin protein was detected as an independent prognostic factor. These outcomes suggest that periostin is associated with breast cancer CSC, suggesting that its expression may be implicated in self-renewal and tumorigenesis by activating its downstream target genes. Periostin may also play a role in breast cancer oncogenesis and may be a potential biomarker for metastasis and chemotherapy resistance of breast cancer.

Conclusion

The present study found that periostin was highly expressed in CSC cells and could be a potential biomarker for the bone metastasis and chemotherapy resistance of breast cancer tumors. The underlying genetic mechanism of periostin regulating the breast cancer CSC is still unclear, however, and needs further investigation.

Author Contributions

Conceived and designed the experiments: CL DX XW YR. Performed the experiments: CL HX HZ PL DX XW YR. Analyzed the data: CL HX DX XW YR. Contributed reagents/materials/analysis tools: HX HZ PL XW. Wrote the paper: CL DX XW YR.

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