Expression of LXR-β, ABCA1 and ABCG1 in human triple-negative breast cancer tissues

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Abstract. Previous studies have reported that liver X receptor (LXR), ATP-binding cassette sub-family G number 1 (ABCG1) and ATP-binding cassette transporter number 1 (ABCA1), which are associated with cholesterol metabolism, may be associated with the development and progression of breast cancer. The expression levels of LXR-β, ABCA1 and ABCG1 in triple-negative breast cancer (TNBC) tissues and in non-cancerous mammary tissues were observed by immunohistochemistry, quantum dot-based immunohistochemistry, western blot analysis and reverse transcription-quantitative polymerase chain reaction. The present study identified that the expression of ABCA1 in TNBC tissues was higher than that in non-cancerous mammary tissues. A high expression of ABCA1 in the TNBC tissues was significantly associated with the histological grade. However, no significant differences were identified between the expression levels of LXR-β and ABCG1 in the TNBC tissues compared with the non-cancerous mammary tissues. Therefore, the findings of this study suggest that ABCA1 is a specific marker for TNBC.

Introduction

Breast cancer has the highest cancer incidence rate among women in the USA and it is also the second leading cause of cancer-associated mortality in women following lung cancer (1). The American Cancer Society (ACS) conducted a statistical analysis of the incidence and mortality rates of breast cancer in the USA, which revealed that although the incidence rate is increasing, the mortality rate is declining (2). The study identified that the reduction in breast cancer-associated mortality may be attributed to both improvements in treatment, including the development of adjuvant chemotherapy and hormonal therapy in the 1980s, and targeted therapies in the 1990s, as well as the popularization of breast cancer screening (2). The ACS statistics have also demonstrated that in the USA, 74% of breast cancer cases are hormone receptor $(HR)^+$/human epidermal receptor $(HER2)^-$ (luminal A) and 12% are $HR^-/HER2^-$ [triple-negative breast cancer (TNBC)], which indicates that TNBC accounts for a relatively high proportion of cases among the different molecular classifications of breast cancer (2). In China, breast cancer has the highest incidence rate among all cancer types among females; however, the mortality rate is second following lung cancer (3).

According to different expression levels of estrogen receptor (ER), progesterone receptor (PR), HER2 and Ki-67, breast cancer can be divided into four different types: Luminal A, luminal B, HER2-positive and TNBC (4). Luminal A and B type breast cancers are positive for HRs; therefore, adjuvant endocrine therapy can be used for treatment (4,5). HER2-overexpressing breast cancers can be treated with targeted therapy, including trastuzumab (4,5). However, for TNBC, as there is no targeted therapy index, adjuvant chemotherapy is commonly used for treatment (4,5). Therefore, the study of the targeted therapy index of TNBC is of utmost clinical significance.

Obesity and being overweight are risk factors for breast cancer (6). Metabolic syndrome has been confirmed as a risk factor for breast cancer among post-menopausal women and is defined as having at least three of the following: Abdominal obesity, hyperlipidemia, high levels of low-density lipoprotein, high blood glucose and high blood pressure (7). According to the National Enhanced Cancer Surveillance System in Canada, a high-cholesterol diet can increase breast cancer incidence by 48% in post-menopausal women (8). As for pre-menopausal women, low levels of high-density lipoprotein (HDL) can also increase the incidence of breast cancer (9,10). In animal experiments, a high level of cholesterol has been

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shown to promote the growth and metastasis of mammary tumors (11). Nelson *et al* (12) demonstrated that a metabolite termed 27-hydroxycholesterol (27HC) was produced following cholesterol metabolism. Their study also identified that sterol 27-hydroxylase, which converts cholesterol into 27HC, was highly expressed in breast cancer; In addition, 27HC can activate two receptors [ER and liver X receptor (LXR)] in ER^+ breast cancer cells to promote their growth and dissemination. Through animal experiments, Nelson *et al* (12) also revealed that the growth of ER^+ breast cancer relies on ER activation and the activation of LXR promotes cancer metastasis. This previous study demonstrated that the metabolism of cholesterol plays an important role in HR-positive breast cancer. However, to the best of our knowledge, the effects of cholesterol metabolism in HR-negative TNBC remain to be investigated.

Cholesterol is a major component of the cell membrane. As important carriers of cholesterol metabolism, ATP-binding cassette transporter number 1 (ABCA1) and ATP-binding cassette sub-family G number 1 (ABCG1) can increase the levels of HDL and decrease intermediate-density lipoprotein to promote cholesterol metabolism and transport from cells (13). ABCA1 and ABCG1 are regulated by the lipid metabolism homeostasis regulator, LXR. An LXR agonist can increase the expression levels of ABCA1 and ABCG1. By contrast, an LXR inhibitor can downregulate the expression levels of ABCA1 and ABCG1 (14,15). LXR mediates cholesterol out-flow through the ABCA1/apolipoprotein A1/HDL or ABCG1/HDL pathway (16,17).

LXR has two subtypes: LXR-α and LXR-β. LXR-α is highly expressed in adipose tissue, the liver, adrenal glands, lungs and the gastrointestinal tract, while LXR-β is widely expressed (18). ABCA1 is highly expressed in normal mammary epithelial cells and neoplastic breast tissues. ABCA1 is also associated with lymph node metastasis in breast cancer (19). The present study detected the expression levels of LXR-α, LXR-β, ABCA1 and ABCG1 in TNBC tissues and in non-cancerous mammary tissues in preliminary experiments (data not shown). LXR-α did not localize to the cell nucleus; therefore, the current study investigated the expression of LXR-β, ABCA1 and ABCG1, which were associated with cholesterol metabolism in the TNBC tissues and in the non-cancerous mammary tissues.

Materials and methods

Clinical samples. A total of 96 TNBC tissue chips embedded in paraffin were obtained from Guilin Fanpu Biotech, Inc. In addition, the present study obtained 20 paraffin-embedded non-cancerous mammary tissue samples, 10 clinical samples of TNBC tissue and 5 clinical samples of non-cancerous mammary tissue, which were stored at -80˚C. Both types of tissues were provided by the Department of Pathology, Traditional Chinese Medicine Hospital of Wenling. All patients were female, aged 29 to 79 years and of Han ethnicity. The specimens were collected from January, 2005 to December, 2015. Patients with recurrent tumors were excluded. The Ethics Board of the Traditional Chinese Medicine Hospital of Wenling reviewed and approved the experimental protocol for assaying the surgical materials and written informed consent was obtained from the patients.

Immunohistochemistry (IHC). Paraffin-embedded tissue samples were sectioned (serial 4-μm-thick sections), baked, dewaxed and hydrated. Antigen retrieval was performed with Tris-EDTA (pH 9.0) at a high pressure for 6 min. The sections with nuclear expression were treated for 30 min using 0.3% Triton solution at 37˚C to permeabilize the membranes. Primary antibodies were diluted in PBS and incubated overnight at 4˚C with the tissue sections. The following antibodies were used: Anti-LXR-β (1:100; rabbit polyclone; cat. no. SC-1001; Santa Cruz Biotechnology, Inc.), anti-ABCA1 (1:250; mouse monoclonal; cat. no. ab18180; Abcam) and anti-ABCG1 (1:250; mouse monoclonal; cat. no. ab52617; Abcam). PBS was used instead of the primary antibody as a negative control. The sections were then incubated with horseradish peroxidase (HRP)-conjugated second antibody (Dako REAL EnVision Detection System, cat. no. K4063, Agilent) for 30 min at room temperature. The sections were stained with 3,3'-diaminobenzidine for 2-10 sec at room temperature and then completely rinsed with water. In addition, hematoxylin staining for 3 min at room temperature was performed prior to washing with water. Differentiation for 2 sec was performed using 1% hydrochloric acid in alcohol. Lithium carbonate was added as a blue stain for 30 sec and then dehydrated. The sections were treated with clearing agents to ensure transparency before neutral resins were used for sealing.

Quantum dot-based IHC (QD-IHC). Until the secondary antibody was added, the protocol for QD-IHC was the same as for that described above for IHC. The biotinylated goat anti-rabbit or anti-murine IgG (1:200; cat. no. 111-065-006; Jackson ImmunoResearch) was diluted in 2% bovine serum albumin (BSA; Gibco; Thermo Fisher Scientific, Inc.) and incubated for 30 min at room temperature prior to blocking for 20 min in 2% BSA. Quantum dot-conjugated streptavidin probes (QD-SA) (1:400; QS605; Wuhan Jianyuan Quantum Dots Co., Ltd.) diluted in 2% BSA were dripped onto the sections and incubated for 1 h in a 37˚C incubator, as previously described (20,21). The sections were sealed with 90% glycerol buffer.

The same ranking criteria were used for IHC and QD-IHC, and a double-blind experiment was performed for both. Two senior pathological experts observed the sections under x200 magnification and the positive area (PA) was calculated. The PA ranking was as follows: 0 (PA, ≤20%), 1 (PA, 21-40%), 2 (PA, 41-60%) and 3 (PA >60%). The intensity staining (IS) was evaluated under high magnification and ranked as follows: 0 (negative), 1 (weak) and 2 (strong). The final results were determined by intensity distribution (ID), where ID=PA x IS. An ID ≤2 represented a negative or weak expression, while an ID >2 represented a strong expression (21). The criteria were suitable for LXR-β, ABCA1 and ABCG1 indices. A high expression was a strong expression, a low expression was a weak expression and a negative expression was described as negative.

Extraction of tissue proteins. Breast tissues obtained from surgery that were stored at -80˚C were cut into sections (1-2 mm thickness). Subsequently, 500 μl pre-cooled RIPA lysis buffer (Wuhan Kerui Biotech Co., Ltd.) was added to

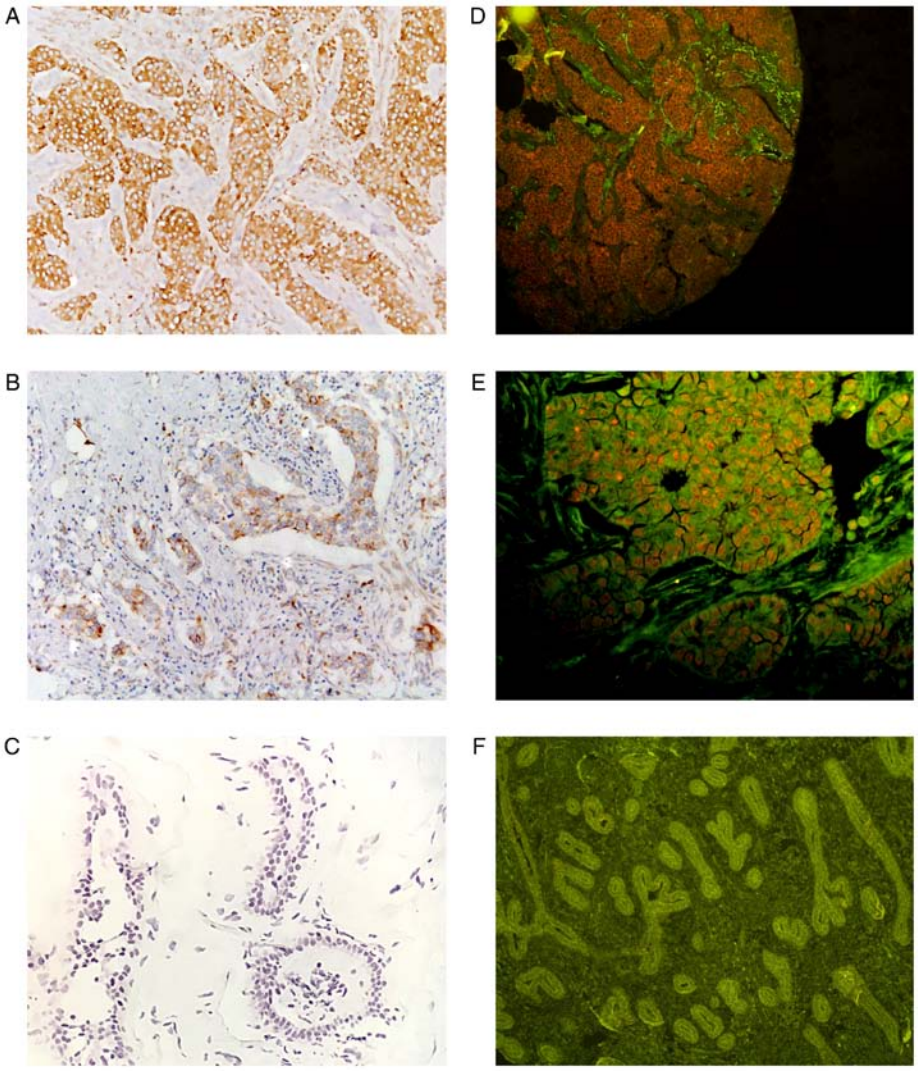

Figure 1. Staining intensity following IHC and QD-IHC. (A, B and C) The difference in staining intensity with x20 magnification following IHC. The brown areas represent positive expression, while the blue areas represent hematoxylin staining. (D, E and F) The difference in staining intensity with (D and F) x10 magnification and (E) x20 magnification following QD-IHC. The red areas represent the positive expression of the cells, while the green areas represent a negative expression. (A and D) Strong staining (score, 2); (B and E) weak staining (score, 1); (C and F) negative staining (score, 0). (A, B, D and E) are TNBC tissues, while (C and F) are non-cancerous mammary tissues. IHC, immunohistochemistry; QD-IHC, quantum dot-based immunohistochemistry; TNBC, triple-negative breast cancer.

100 mg tissue and homogenized with a homogenizer [Tiangen Biotech (Beijing) Co., Ltd.]. The homogenates were lysed on ice for 30 min prior to centrifugation with 12,000 x g for 15 min at 4˚C for later use. The protein concentration was detected using the BCA method (Wuhan Kerui Biotech Co., Ltd.), according to the manufacturer's protocol.

Western blot analysis. SDS-PAGE (8% resolving gel and 5% stacking gel) was used to resolve proteins prior to transfer onto polyvinylidene difluoride membranes (EMD Millipore). The membranes were blocked for 1 h in 5% skim milk at room temperature prior to incubation with primary antibody overnight at 4˚C. The reference antibody used was β-actin (Sigma-Aldrich; Merck-KGaA). The primary antibodies used were the same as for IHC. The antibody dilutions were as follows: Anti-ABCA1 (1:800), anti-ABCG1 (1:800) and anti-LXR-β (1:300). The membranes were incubated with the secondary antibody (1:800; cat. no. 111-065-006; Jackson ImmunoResearch) for 1 h at room temperature. An enhanced chemiluminescent detection system (Pierce; Thermo Fisher Scientific, Inc.) was used to examine the signals, according to the manufacturer's instructions. Quantity One software (version 4.52; Bio-Rad, Inc.) was used to examine the densitometry, according to the manufacturer's instructions. This experiment was repeated 3 times.

Extraction of total RNA from tissue samples and reverse transcription (RT). Frozen breast tissue samples obtained from surgery were sectioned and TRIzol solution (Invitrogen; Thermo Fisher Scientific, Inc.) was added at 1 ml/100 mg tissue prior to homogenization. Total RNA was extracted using phenol-chloroform. RNA was reverse transcribed to cDNA using the RevertAid First Strand cDNA Synthesis kit (Thermo Fisher Scientific, Inc.), according to the manufacturer's instructions.

Quantitative polymerase chain reaction (qPCR). qPCR was performed on a 96-well plate with SYBR-Green Real-time

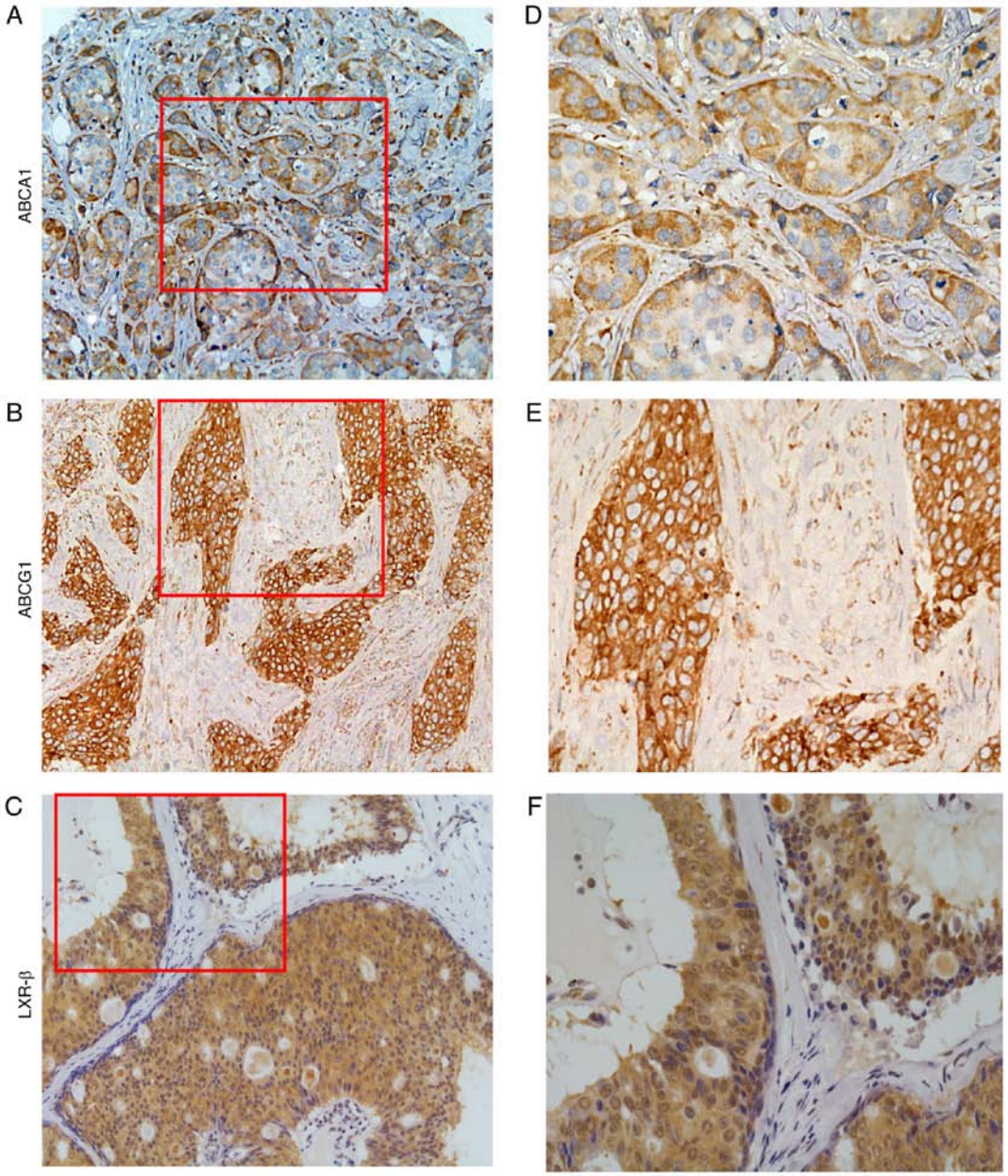

Figure 2. Positive expression of ABCA1, ABCG1 and LXR-β in TNBC tissues detected by IHC. The brown areas represent a positive expression, while the blue areas represent hematoxylin staining. (A, B and C) Positive expression with x20 magnification. (D, E and F) Positive expression with x40 magnification. IHC, immunohistochemistry; ABCA1, ATP-binding cassette transporter number 1; LXR-β, liver X receptor-β; ABCG1, ATP-binding cassette sub-family G number 1; TNBC, triple-negative breast cancer.

PCR Master mix (Toyobo Life Science), according to the manufacturer's instructions. Bio-Rad CFX Manger 3.1 software (Bio-Rad Laboratories, Inc.) was used for data collection. Each experiment was conducted with at least 3 independent replicates. The qPCR conditions were as follows: Two initial cycles at 95˚C for 180 sec and 95˚C for 6 sec and 39 cycles of 60˚C for 9 sec and 72˚C for 12 sec with the primers (LXR-β forward, 5'-CAGCGAGTCTTCCAGAAGGG-3' and reverse, 5'-TGGGCTTGAGGTGTAAGCTG-3'; ABCA1 forward, 5'-GGGAGAGCACAGGCTTTGAC-3' and reverse, 5'-CACTCACTCTCGCTCGCAA-3'; ABCG1 forward, 5'-CCTGTCTGATGGCCGCTTT-3' and reverse, 5'-CACCTCATCCACCGAGACAC-3'; and β-actin forward, 5'-TCACCATGGATGATGATATCGC-3' and reverse, 5'-GAATCCTTCTGACCCATGCC-3). RT-qPCR quantification was performed using the $2^{-\Delta\Delta Cq}$ method (22).

Statistical analysis. SPSS 21.0 software (IBM Corp.) and GraphPad Prism 5.0 software (GraphPad Software, Inc.) were used for the analysis. The χ^2 test was used to analyze the differences in the protein expression levels of LXR-β, ABCA1 and ABCG1 detected by IHC and QD-IHC between the TNBC and non-cancerous mammary tissues, as well as to determine the association between ABCA1 immunostaining and the clinicopathological parameters of the patients with TNBC. All values for LXR-β, ABCA1 and ABCG1 expression detected by western blot analysis and RT-qPCR are expressed as the means ± standard error of the mean.

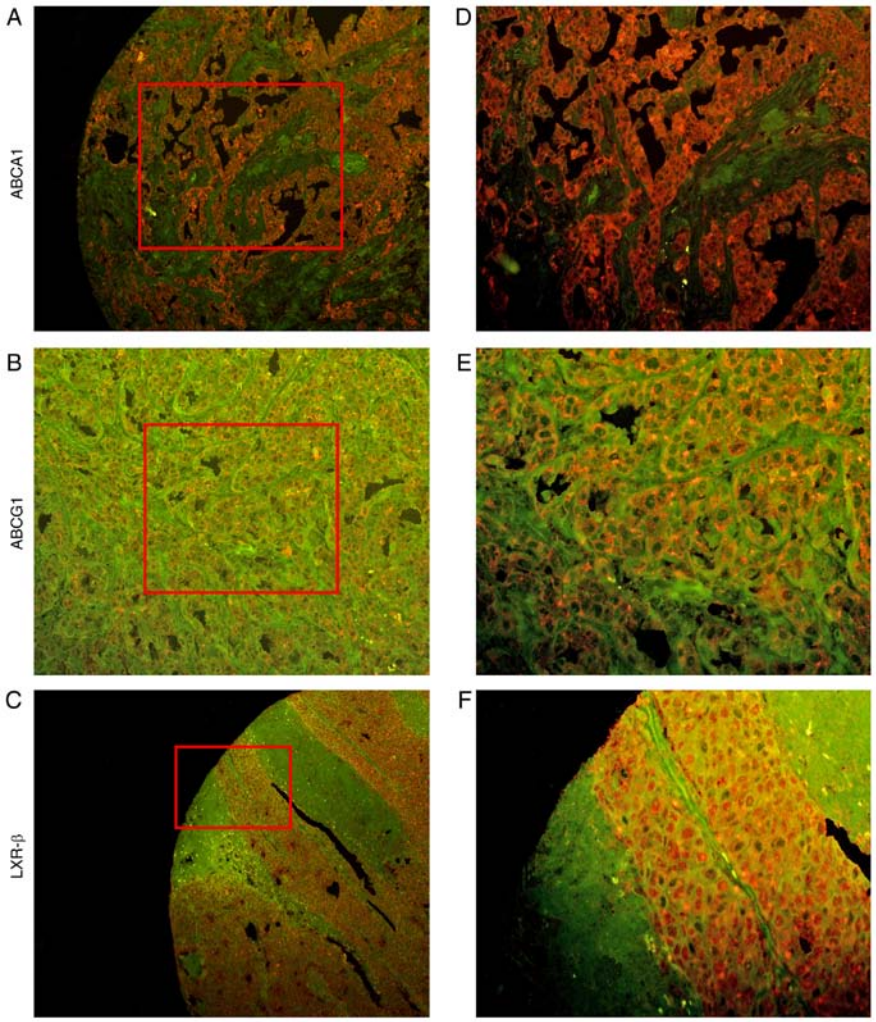

Figure 3. Positive expression of ABCA1, ABCG1 and LXR-β in TNBC tissues detected by QD-IHC. The red areas represent positive expression of the cells, while the green areas represent negative expression. (A and B) Positive expression with x20 magnification. (C) Positive expression with x10 magnification. (D, E and F) Positive expression with x40 magnification. QDs-IHC, quantum dots-immunohistochemistry; ABCA1, ATP-binding cassette transporter number 1; LXR-β, liver X receptor-β; ABCG1, ATP-binding cassette sub-family G number 1; TNBC, triple-negative breast cancer.

Statistical analysis was performed using a Student's t-test for 2 groups. $P<0.05$ was considered to indicate a statistically significant difference.

Results

A high expression of ABCA1 is associated with the breast cancer histological grade. IHC and QD-IHC were used to observe the expression levels of LXR-β, ABCA1 and ABCG1 in the 96 TNBC tissue samples and the 20 non-cancerous mammary tissue samples (Figs. 1-3). The results, which are presented in Tables I and II, demonstrated that the expression of ABCA1 was significantly higher in the TNBC tissues, compared with the non-cancerous mammary tissues ($P<0.05$), while the expression levels of LXR-β and ABCG1 in the TNBC tissues and non-cancerous mammary tissues did not exhibit any significant difference ($P>0.05$). Evidently, ABCA1 is highly expressed in the TNBC tissues, which indicates that ABCA1 is a specific marker for TNBC.

Statistical analysis was performed according to the pathological characteristics of the 96 patients. It was identified that the expression of ABCA1 was associated with the breast cancer histological grade (Table III; IHC, $P<0.001$; QD-IHC, $P<0.001$). However, age and tumor-node-metastasis (TNM) stage were not significantly associated with the expression of ABCA1 ($P>0.05$).

TNBC tissues exhibit higher ABCA1 protein and mRNA expression levels compared with non-cancerous mammary tissues. Western blot analysis and RT-qPCR were used to examine the protein and mRNA expression levels of LXR-β, ABCA1 and ABCG1 in tissues from 10 patients with TNBC and 5 non-cancerous mammary tissues that were stored at -80˚C following surgery (Fig. 4). It was identified that the protein and mRNA expression levels of ABCA1 were significantly higher in the TNBC tissues, compared with the non-cancerous mammary tissues ($P<0.05$). LXR-β mRNA was highly expressed in the TNBC tissues and expressed at low levels in the non-cancerous mammary tissues, although the difference was not statistically significant ($P>0.05$). The mRNA and protein expression levels of LXR-β and ABCG1 did not exhibit any significant difference between the TNBC tissues and non-cancerous mammary tissues ($P>0.05$). Notably, the mRNA and protein expression levels of LXR-β were high

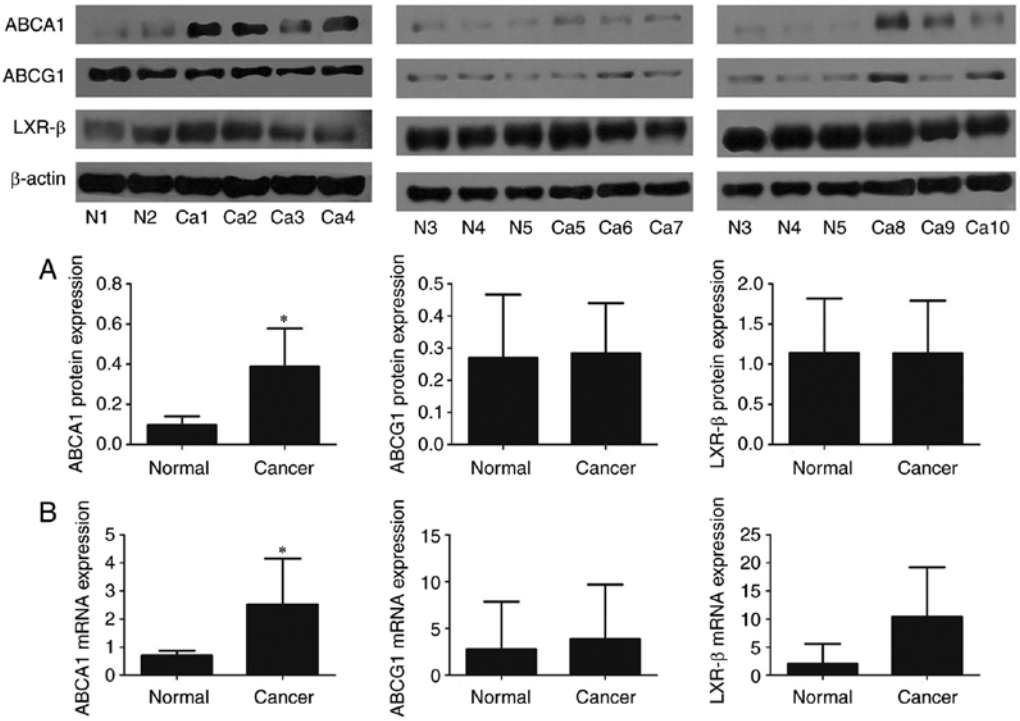

Figure 4. mRNA and protein expression levels of ABCA1, ABCG1 and LXR-β in TNBC tissues and non-cancerous mammary tissues. (A) Protein expression levels. (B) mRNA expression levels. β-actin served as an internal control. Data are representations of 3 repeated experiments and are presented as the average ± standard deviation. Western blot analysis (ABCA1, $^{*}P<0.05$; LXR-β and ABCG1, $P>0.05$). RT-qPCR (ABCA1, $^{*}P<0.05$; LXR-β and ABCG1, $P>0.05$). N, non-cancerous mammary tissue; Ca, TNBC tissues; ABCA1, ATP-binding cassette transporter number 1; LXR-β, liver X receptor-β; ABCG1, ATP-binding cassette sub-family G number 1; TNBC, triple-negative breast cancer.

Table I. Positivity rate in the expression of LXR-β, ABCA1 and ABCG1 in TNBC tissues and non-cancerous mammary tissues examined by IHC.

	TNBC tissues			Non-cancerous tissues			
	n	High, n (%)	Low, n (%)	n	High, n (%)	Low, n (%)	P-value
LXR-β	96	60 (62.5)	36 (37.5)	20	8 (40)	12 (60)	0.063
ABCA1	96	62 (64.6)	34 (35.4)	20	6 (30)	14 (70)	0.004
ABCG1	96	53 (55.2)	43 (44.8)	20	11 (55)	9 (45)	0.986

IHC, immunohistochemistry; ABCA1, ATP-binding cassette transporter number 1; LXR-β, liver X receptor-β; ABCG1, ATP-binding cassette sub-family G number 1; TNBC, triple-negative breast cancer; non-cancerous tissues, non-cancerous mammary tissues. High, strong expression; Low, weak expression and negative expression; N, number. Strong, weak and negative expression were obtained from intensity distribution (ID) statistics. ID ≤2 represented a negative or weak expression, while an ID >2 represented a strong expression.

in the breast cancer tissues; however, no significant difference was observed compared with the non-cancerous mammary tissues. Thus, these results demonstrate that ABCA1 is highly expressed in TNBC tissues, and suggest that ABCA1 may be closely associated with the occurrence and development of TNBC.

Discussion

To the best of our knowledge, to date, there is no similar study available on cholesterol metabolism and ABCA1 expression in TNBC examined using IHC and QD-IHC. From the histological experiments, the present study identified that ABCA1 was a specific marker for differentiating TNBC tissues from non-cancerous tissues. Further statistical analysis revealed that the expression of ABCA1 was associated with the histological grade of TNBC; however, no significant association with age or TNM stage was identified. From the above-mentioned results, it is suggested that ABCA1 may be an oncogene in TNBC; however, further *in vitro* studies are required to confirm our findings.

Due to a lack of studies regarding ABCA1 as an oncogene, the mechanisms of action of ABCA1 as regards the development and progression of cancer remain unknown. Studies have demonstrated that ABCA1 is highly expressed in M14 melanoma cell lines (23), and in LDL1 colon cancer

Table II. Positivity rate in the expression of LXR-β, ABCA1, and ABCG1 in TNBC tissues and non-cancerous mammary tissues examined by QD-IHC.

	Breast cancer tissues			Non-cancerous tissues			
	n	High, n (%)	Low, n (%)	n	High, n (%)	Low, n (%)	P-value
LXR-β	96	63 (65.6)	33 (34.4)	20	9 (45)	11 (55)	0.084
ABCA1	96	64 (66.7)	32 (33.3)	20	6 (30)	14 (70)	0.002
ABCG1	96	55 (57.3)	41 (42.7)	20	12 (60)	8 (40)	0.823

QD-IHC, quantum dot-based immunohistochemistry; ABCA1, ATP-binding cassette transporter number 1; LXR-β, liver X receptor-β; ABCG1, ATP-binding cassette sub-family G number 1; TNBC, triple-negative breast cancer; non-cancerous tissues, non-cancerous mammary tissues. High, strong expression; Low, weak expression and negative expression; N, number. Strong, low and negative expression were obtained from intensity distribution (ID) statistics. ID ≤2 represented a negative or weak expression, while an ID >2 represented a strong expression.

Table III. The association between ABCA1 protein expression and the clinicopathological parameters of the 96 patients with TNBC.

		ABCA1 by IHC			ABCA1 by QD-IHC		
Parameters	n	High (%)	Low (%)	P-value	High (%)	Low (%)	P-value
Age (years)				0.151			0.749
<50	68	47 (49.0)	21 (21.9)		48 (50.0)	20 (20.8)	
≥50	28	15 (15.6)	13 (13.5)		19 (19.8)	9 (9.4)	
T stage				0.460			0.898
T2	72	48 (50.0)	24 (25.0)		50 (52.1)	22 (22.9)	
T3 + 4	24	14 (14.6)	10 (10.4)		17 (17.7)	7 (7.3)	
N stage				0.606			0.581
N0	57	38 (39.6)	19 (19.8)		41 (42.7)	16 (16.7)	
N1 + 2	39	24 (25.0)	15 (15.6)		26 (27.1)	13 (13.5)	
Grade				<0.001			<0.001
I + II	51	42 (43.8)	9 (9.4)		44 (45.8)	7 (7.3)	
III	45	20 (20.8)	25 (26.0)		23 (24.0)	22 (22.9)	

T, tumor size; N, regional lymph node metastasis; No distant metastasis was found in 96 TNBC patients; Grade, histological grade; N, number; IHC, immunohistochemistry; QD-IHC, quantum dot-based immunohistochemistry; ABCA1, ATP-binding cassette transporter number 1; TNBC, triple-negative breast cancer; High, strong expression; Low, weak expression and negative expression; Strong, low and negative expression were obtained from intensity distribution (ID) statistics. ID ≤2 represented a negative or weak expression, while an ID >2 represented a strong expression.

cell lines and colon cancer tissues (24). The silencing of ABCA1 has been shown to promote the apoptosis of cancer cells (23). Schimanski *et al* (19) identified that ABCA1 was highly expressed in the cell membrane and cytoplasm of breast cancer cells, and that it was associated with lymph node metastasis. Another study reported that the over-expression of the ABC superfamily members, including ABCA1, is an important barrier against chemotherapy, and sensitivity can be increased by downregulating or silencing their expression (25).

However, numerous studies have reported that ABCA1 is a tumor suppressor gene that promotes cholesterol metabolism to inhibit cancer development (26-28). Studies have demonstrated that ABCA1 exerts antitumor effects and exhibits a low-to-medium expression in colon cancer cells, as insufficient levels of ABCA1 can cause an accumulation of cholesterol in cancer cells, inhibit the release of tumor necrosis factor in the mitochondria and promote cancer progression (26). Another study demonstrated that LXR agonists can upregulate ABCA1 expression to inhibit cancer cell proliferation (27,28). Cells in the S phase require twice the amount of cholesterol compared with those in the G1 phase for the completion of DNA synthesis and preparation for the G2 phase and subsequent mitosis. An LXR agonist can upregulate ABCA1 expression to inhibit the proliferation of ER-positive breast cancer cells, promote the efflux of cholesterol from cells and cause intracellular cholesterol levels in cancer cells to be lower than that required for the S phase. This interferes with DNA synthesis in cancer cells, causing them to be unable to complete mitosis, thereby inhibiting the proliferation of cancer cells (29).

However, the aforementioned mechanism is for ER-positive breast cancer and other types of cancer cells. To the best of our knowledge, there is no such study currently available for TNBC cells. The overexpression of ER can promote reverse cholesterol transport (29). Unlike ER-positive breast cancer, TNBC lacks the intermediary effects of ER. Therefore, further experimental research is warranted to confirm whether ABCA1 serves as an oncogene in TNBC.

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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

HP conducted most of the experiments and wrote the manuscript; YZ analyzed the experimental data, and was a major contributor in writing the manuscript; QP collected the clinical specimens; HC and FC were involved in the conception and design of the study; JW and DD performed partial of the immunohistochemistry and quantum dot-immunohistochemistry. All authors have read and approved the final manuscript and agree to be accountable for all aspects of the research.

Ethics approval and consent to participate

For the use of clinical materials, prior approval was obtained from Traditional Chinese Medical Hospital of Wenling and written informed consent forms were signed by all the patients.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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