Effect of curcumin on the cell surface markers CD44 and CD24 in breast cancer

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Abstract. Human breast cell lines are often characterized based on the expression of the cell surface markers CD44 and CD24. CD44 is a type I transmembrane glycoprotein that regulates cell adhesion and cell-cell, as well as cell-extracellular matrix interactions. CD24 is expressed in benign and malignant solid tumors and is also involved in cell adhesion and metastasis. The aim of the present study was to investigate the effects of curcumin on the surface expression of CD44 and CD24 in breast epithelial cell lines. An established breast cancer model derived from the MCF-10F cell line was used. The results revealed that curcumin decreased CD44 and CD24 gene and protein expression levels in MCF-10F (normal), Alpha5 (premalignant) and Tumor2 (malignant) cell lines compared with the levels in their counterpart control cells. Flow cytometry revealed that the $CD44^+/CD24^+$ cell subpopulation was greater than the $CD44^+/CD24^-$ subpopulation in these three cell lines. Curcumin increased $CD44^+/CD24^+$ to a greater extent and decreased $CD44^+/CD24^-$ subpopulations in the normal MCF-10F and the pre-tumorigenic Alpha5 cells, but had no significant effect on Tumor2 cells compared with the corresponding control cells. Conversely, curcumin increased CD44 and decreased CD24 gene expression in MCF-7 breast cancer cells, and decreased CD44 gene expression in MDA-MB-231 cell line, while CD24 was not present in these cells. Curcumin did not alter the $CD44^+/CD24^+$ or $CD44^+/CD24^-$ subpopulations in the MCF-7 cell line. However, it increased $CD44^+/CD24^+$ and decreased $CD44^+/CD24^-$ subpopulations in MDA-MB-231 cells. In breast cancer specimens from patients, normal tissues were negative for CD44 and CD24 expression, while benign lesions were positive for both markers, and malignant tissues were found to be negative for CD44 and positive for CD24 in most cases. In conclusion, these results indicated that curcumin may be used to improve the proportion of $CD44^+/CD24^+$ cells and decrease the proportion of $CD44^+/CD24^-$ cells. Therefore, it may be suggested that curcumin decreased cancerous types of breast cells.

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Introduction

Human breast epithelial cells are frequently characterized based on the expression of cell surface markers, including CD44 and CD24 (1-8). CD44 is expressed in basal-like and CD24 in luminal-like cell lines. CD44 is a transmembrane glycoprotein that normally regulates cell-cell adhesion and cell-matrix interactions, as well as cell migration. This glycoprotein binds mainly to hyaluronic acid, collagen, fibronectin, laminin and chondroitin-sulfate, which are all important epithelial and mesenchymal components (6-11). Either independently or in collaboration with other cell surface receptors, CD44 can promote uncontrolled growth, evasion of apoptosis, angiogenesis, cell motility and invasion, which are hallmarks of cancer progression (10,12,13). The association of CD44 with breast cancer progression has been evaluated *in vivo* using mouse models. One of the earliest indications of the role of CD44 in metastasis derived from pancreatic cancer. The transfection of CD44 variants into a non-metastatic rat pancreatic carcinoma cell line conferred metastatic potential in these cells when injected into syngeneic rats (14).

CD24 is a small, heavily-glycosylated protein core that consists of 27 amino acids attached to cell membranes. It has been shown to be expressed at lower levels in progenitor cells compared with differentiated cells (15). CD24 is a cell surface protein that, depending on the cell or tissue type, presents highly variable glycosylation (4). CD24 is overexpressed during cancer progression, supporting its usefulness as a marker for diagnosis and prognosis in breast, ovarian, prostate and pancreatic cancers (15-19). Previous studies have indicated that CD24 may promote cell proliferation (20) and invasion of cancer cells (21). In breast cancer cells, CD24 increased cell proliferation, motility and invasiveness (22).

Curcumin (diferuloylmethane) is a natural yellow pigment derived from the rhizome of the herb *Curcuma longa*. It has been demonstrated that curcumin has a variety of pharmacological effects, including potent antiproliferative, anti-inflammatory, antioxidant and anti-carcinogenic

activities, conferring chemopreventive potential (9-11,23). Recent evidence indicated that curcumin had a diverse range of molecular targets, supporting the concept that it acted upon numerous biochemical and molecular cascades. Curcumin can induce apoptosis-like changes in some cells, whereas it can inhibit proliferation in others (23,24). Since research has demonstrated the importance of developing novel preventive strategies that can target cancer cells, the aim of the present study was to investigate the effect of curcumin on breast epithelial cell lines with regard to the expression of the cell surface markers CD44 and CD24, to assess its potential capacity for breast cancer prevention and to determine whether curcumin can increase the ratio of $CD44^+/CD24^+$ cells and decrease that of $CD44^+/CD24^-$ cells.

Materials and methods

Breast cancer cell lines. An *in vitro* experimental breast cancer model, termed the Alpha model, was used in these studies; it was derived from MCF-10F (ATCC®; CRL-10318™), an immortalized human epithelial cell type (25), which was exposed to low doses of high linear energy transfer (LET) α-particle radiation (150 keV/μm) and subsequently, to 17β-estradiol (25). The cell lines used in this model were as follows: i) MCF-10F, a normal cell line used as a control; ii) Alpha5, a pre-malignant and tumorigenic cell line; and ii) Tumor2, derived from Alpha5 after the injection of that cell line into nude mice. These cell lines were cultured in Dulbecco's modified Eagle's medium (DMEM)/F-12 (1:1) supplemented with antibiotics [100 U/ml penicillin, 100 μg/ml streptomycin, 2.5 μg/ml amphotericin B (all from Life Technologies; Thermo Fisher Scientific, Inc., Waltham, MA, USA)], 10 μg/ml 5% equine serum (Biofluids, Rockville, MD, USA), 0.5 μg/ml hydrocortisone (Sigma-Aldrich; Merck KGaA, Darmstadt, Germany) and 0.02 μg/ml epidermal growth factor (Collaborative Research, Bedford, MA, USA). Two other breast carcinoma cell lines, MDA-MB-231 (ATCC®; HTB26™) and MCF-7 (ATCC®; HTB22™), were cultured in RPMI-1640 and minimum essential medium (MEM) (Life Technologies; Thermo Fisher Scientific, Inc.), respectively. All the cell lines were maintained at 37˚C in a humidified atmosphere containing 5% CO_2. Curcumin at 30 μM was applied for 48 h in all the experimental designs.

Reverse transcription-quantitative polymerase chain reaction (RT-qPCR). Total RNA was extracted with TRIzol (Invitrogen, Carlsbad, CA, USA) from untreated and treated breast cell lines, following the manufacturer's instructions. A UV spectrophotometer was used to determine RNA purity and concentration (Thermo Fisher Scientific, Inc., Rochester, NY, USA). A High-Capacity cDNA Reverse Transcription kit (Applied Biosystems, Carlsbad, CA, USA) and 10 units of RNase inhibitor (Applied Biosystems) were used to reverse-transcribe the RNA into cDNA, according to the manufacturer's protocol. For qPCR, we used 2 μl cDNA with SYBR-Green PCR Master Mix (Agilent Technologies, Inc., La Jolla, CA, USA) and primers for target gene (CD44 and CD24; Table I) at a concentration of 5 μM. A CFX96 Real-Time PCR Detection system (Bio-Rad Laboratories, Hercules, CA, USA) was used to perform the reactions under the following conditions: 95˚C for 10 min; and 40 cycles of a 2-step program consisting of 95˚C for 10 sec and 61˚C for 45 sec, when fluorescence-reading occurred. PCR products were monitored through dissociation curve analysis (measurement of fluorescence during stepped-increases in temperature of 2˚C/min from 61 to 95˚C). Bio-Rad CFX Manager 2.1 software was used to obtain the threshold cycle (Ct) and a reference housekeeping gene (β-actin) was used to normalize the average gene expression.

Table I. Primers for genes selected to develop cDNA probes.

Gene name	Product length (bp)	Primer sequence
CD44	116	F: CGGACACCATGGACAAGTTT
		R: CACGTGGAATACACCTGCAA
CD24	16	F: AACTAATGCCACCACCAAGG
		R: GACGTTTCTTGGCCTGAGTC

Determination of the protein expression of cell surface markers CD44 and CD24 in cells by immunoperoxidase staining. Cells were placed on a glass chamber slide (Nunc Inc., Naperville, IL, USA) at a density of $1x10^4$ cells/ml of medium and allowed to grow until 70% confluent. Subsequently, they were fixed with buffered paraformaldehyde, incubated with 1% H_2O_2 in methanol for 20 min to block endogenous peroxidase and washed with buffer solution, and then covered with normal horse serum for 30 min. The cells were then incubated with mouse monoclonal antibodies anti-CD44 (DF1485) (cat. no. sc-7297) and anti-CD24 (BA-1) (cat. no. sc-65257; both were obtained from Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA) at 1:500 dilution overnight at 4˚C. Subsequently they were incubated for 45 min with diluted 1:500 biotinylated secondary antibody solution (cat. no. PK-6102 mouse IgG; Vector Laboratories, Burlingame, CA, USA) and Vectastain Elite ABC reagent (Vector Laboratories). The localization of bound antibodies was visualized with DAB (Peroxidase substrate kit; Vector Laboratories) for 5 min and counter-staining with Mayer's hematoxylin solution (MHS128-4L; Sigma-Aldrich; Merck KGaA) for 30 sec was performed.

Flow cytometric analysis of cell surface markers CD24 and CD44 in different cell lines. Breast cells were washed with Dulbecco's phosphate-buffered saline (DPBS/modified; HyClone, Logan, UT, USA) and then harvested with Trypsin, 2.2 mM of ethylenediaminetetraacetic acid (EDTA; Corning, Manassas, VA, USA). Detached cells were re-suspended in phosphate-buffered saline supplemented with 0.5% fetal bovine serum (1x106 cells/50 μl). Fluorochrome-conjugated antibodies (1:1) against human CD44 (FITC-conjugated; cat. no. 555478) and CD24 (PE-conjugated; cat. no. 555428) were obtained from BD Biosciences (San Diego, CA, USA). Antibodies were added to the cell suspension, as recommended by the manufacturer, and incubated at 4˚C in the dark for 30-40 min. The labeled cells were analyzed in a Cytomics FC500 flow cytometer (Beckman Coulter, Inc., Fullerton,

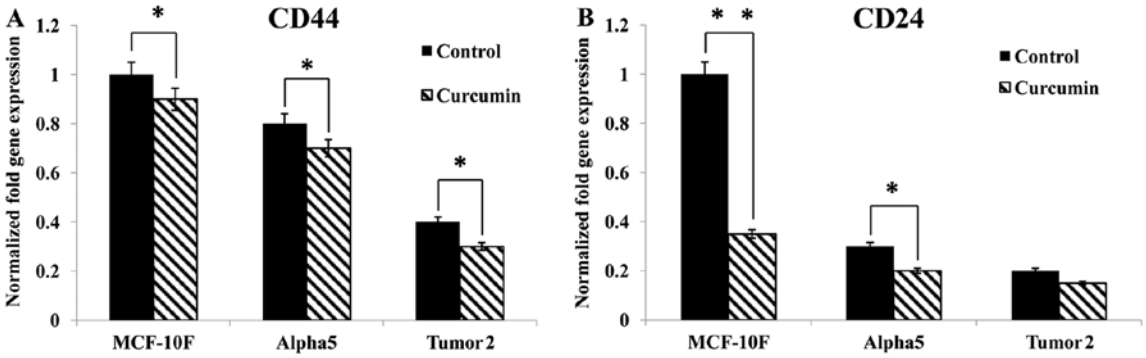

Figure 1. Effect of curcumin (30 μM for 48 h) on (A) *CD44* and (B) *CD24* gene expression in MCF-10F, Alpha5 and Tumor2 cell lines analyzed by RT-qPCR. Graphs represent the relative fold-change of *CD44* and *CD24* gene expression compared with control cells (vehicle-treated). *P<0.05; **P<0.01. All of the experiments were conducted in triplicate.

CA, USA), and the data were analyzed using CXP software (Beckmann Coulter, Inc.).

Determination of protein expression of cell surface markers CD24 and CD44 in breast tissue samples by immunoperoxidase staining. Breast tissue samples were obtained from the archives of the School of Medicine, Saint-Luc Hospital, IMAG Unit (IREC), of University of Louvain (Brussels, Belgium), entrusted by Professor P. Maldague and Professor A. Trouet. The study was approved by the Ethics Committee of the same Institution and conducted in accordance with Institutional Guidelines. Tissue samples from primary surgery were used in the present study, and none of the patients received neo-adjuvant therapy prior to the surgery. Hematoxylin/eosin-stained sections from specimens were evaluated to confirm the diagnosis and to select the most representative area for each sample. Lesions were diagnosed and classified according the World Health Organization guidelines (26). Samples were obtained from 33 patients (all patients had provided written informed consent prior to obtaining the samples), including 3 normal tissues, 8 dysplastic tissues, 9 ductal carcinomas and 13 reduction mammoplasty tissues.

Immunocytochemical studies were performed using a streptavidin-biotin immunoperoxidase method. Tissue sections (5-μm-thick), obtained after formalin fixation and paraffin-embedding, were deparaffinized in xylene for 1 h and rehydrated with TBS. Each section was treated with citrate buffer for 10 min (pH 6.0) for antigen retrieval and with hydrogen peroxide for quenching of the endogenous peroxidase activity, then washed with blocking reagent for 20 min. The aforementioned monoclonal antibodies were applied to all samples, and the tissues were then placed in a moist chamber overnight at 4˚C. A biotinylated secondary antibody was applied, then the tissues were washed and streptavidin-peroxidase was added. The localization of bound antibodies was visualized with DAB for 7 min, and counterstaining with Mayer's hematoxylin solution for 2 min was performed. Table III describes the analysis of CD44 and CD24 surface markers in breast biopsy specimens.

Statistical analysis. Numerical data are expressed as the mean ± standard error of the mean (SEM). Comparisons between treated groups and controls were carried out by ANOVA and Dunnett's test. P<0.05, P<0.01, P<0.001 were considered to indicate statistically significant differences. All the experiments were performed at least three times.

Results

The potential effect of curcumin on the gene and protein expression of CD24 and CD44 was examined in an established breast cancer model as explained above (25). MCF-10F, the normal breast cell line, did not demonstrate any of the features that characterize malignant cells, such as anchorage-independent growth in soft agar, invasion or tumor growth in nude mice, whereas the Alpha5 cell line formed colonies in soft agar, had invasive capability and formed mammary gland tumors in immunosuppressed mice after injection. Tumors derived from such animals gave rise to the Tumor2 cells (25).

The results revealed that curcumin significantly (P<0.05) inhibited CD44 and CD24 gene expression in MCF-10F, Alpha5 and Tumor2 cell lines (Fig. 1), compared with the corresponding control cells. Notably, CD44 and CD24 surface markers were lower in MCF-10F cells than in the premalignant and malignant cell lines. As displayed in Fig. 2A and B MCF-10F cells had lower CD44 and CD24 protein expression than Alpha5 and Tumor2 cell lines. Curcumin decreased such expression in the malignant cell lines. Protein expression was consistent with gene expression with regard to the effect of curcumin on Alpha5 and Tumor2 cells. Representative images of the effect of curcumin on CD44 and CD24 protein expression in these three cell lines are displayed in Fig. 2C and D.

The effect of curcumin on CD44 and CD24 was also analyzed by flow cytometry in MCF-10F, Alpha5 and Tumor2 cell lines. The percentages of $CD44^{+}/CD24^{+}$ and $CD44^{+}/CD24^{-}$ subpopulations of cells determined by dot-plots are illustrated in Fig. 3A. The histogram of the expression of CD44 and CD24 is displayed in Fig. 3B and the graphs in Fig. 3C depict the percentages of cells with the $CD44^{+}/CD24^{+}$ and $CD44^{+}/CD24^{-}$ phenotypes. The $CD44^{+}/CD24^{+}$ subpopulation was significantly larger than the $CD44^{+}/CD24^{-}$ subpopulation in MCF-10F, Alpha5 and Tumor2 cells. Notably, curcumin decreased $CD44^{+}/CD24^{-}$ and increased $CD44^{+}/CD24^{+}$ subpopulations in normal MCF-10F and pre-tumorigenic Alpha5 cells, whereas there was no significant effect in Tumor2 cells compared with the control cells of the same type.

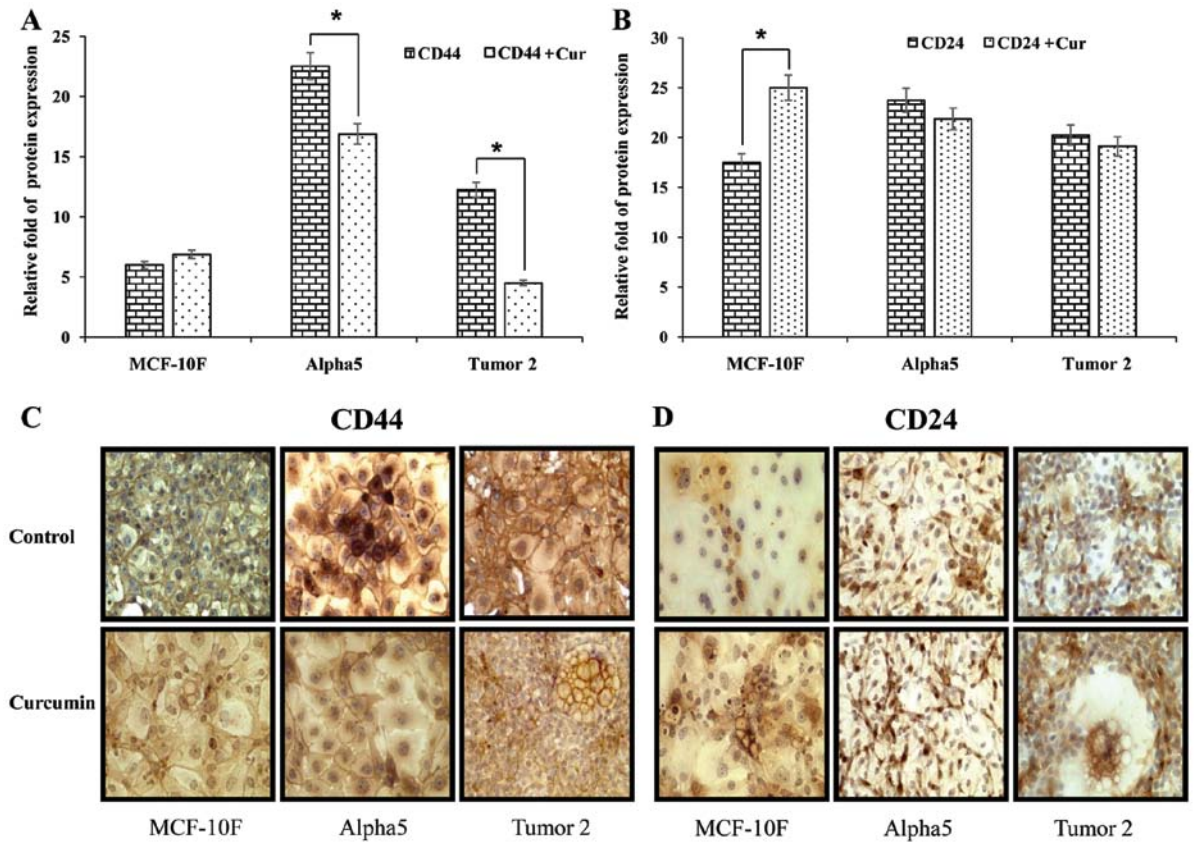

Figure 2. Relative grade of intensity of (A) CD44 and (B) CD24 protein expression after treatment of the cells with curcumin (30 μM for 48 h). Representative images of (C) CD44 and (D) CD24 protein expression in MCF-10F, Alpha5 and Tumor2 cell lines analyzed by immunocytochemistry. *P<0.05. All of the experiments were conducted in triplicate.

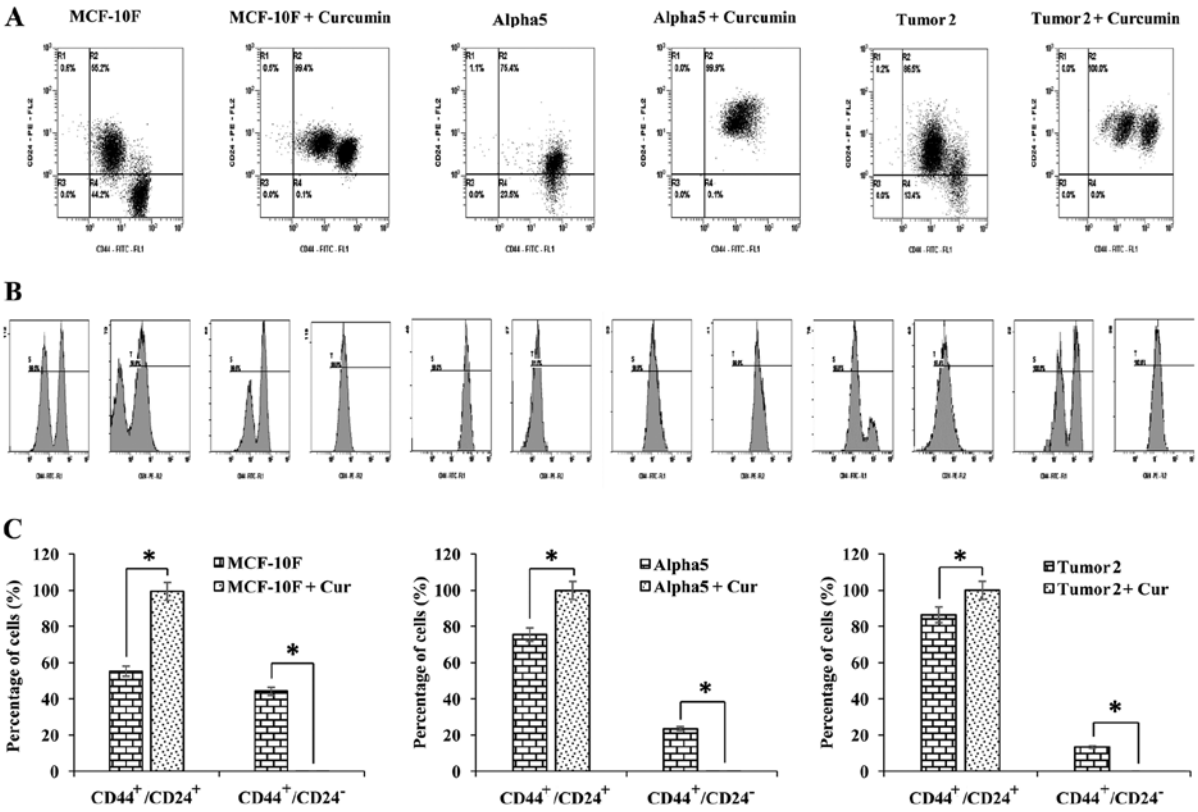

Figure 3. Effect of curcumin (30 μM for 48 h) on CD44 and CD24 markers. (A) Dot-plot, (B) histograms and (C) percentages of $CD44^+/CD24^+$ and $CD44^+/CD24^-$ subpopulations in MCF-10F, Alpha5 and Tumor2 cell lines, as determined by flow cytometry. *P<0.05. All of the experiments were conducted in triplicate.

Table II. Percentage of CD44/CD24 cell surface markers in breast cell lines untreated and treated with curcumin.

Breast cell lines	$CD44^+/CD24^+$ (%)	$CD44^+/CD24^-$ (%)	$CD44^-/CD24^+$ (%)	$CD44^-/CD24^-$ (%)
MCF-10F	55.2	44.2	0.6	0.0
MCF-10F + Cur	99.4	0.1	0.5	0.0
Alpha5	75.4	23.5	1.1	0.0
Alpha5 + Cur	99.9	0.1	0	0.0
Tumor2	86.5	13.4	0.2	0.0
Tumor2 + Cur	100.0	0.0	0.0	0.0
MCF7	90.7	0.0	9.2	0.0
MCF7 + Cur	97.3	0.9	1.8	0.0
MDA-MB-231	0.6	99.2	0.0	0.2
MDA-MB-231 + Cur	99.2	0.0	0.8	0.0

Cur, curcumin.

Table III. Analysis of CD44 and CD24 surface markers in breast biopsy specimens.

No.	Breast biopsies	CD44	CD24
1	Normal tissue	-	-
2	Normal tissue	-	-
3	Normal tissue	-	-
4	Dysplasia	+	+
5	Dysplasia	+	+
6	Dysplasia	+	+
7	Dysplasia	+	+
9	Dysplasia	+	+
10	Dysplasia	+	+
11	Dysplasia	+	+
12	Dysplasia	+	+
13	Ductal carcinoma	+	-
14	Ductal carcinoma	+	+
15	Ductal carcinoma	+	-
16	Ductal carcinoma	-	+
17	Ductal carcinoma	+	+
18	Ductal carcinoma	-	+
19	Ductal carcinoma	-	+
20	Ductal carcinoma	-	+
21	Ductal carcinoma	-	+

Table II summarizes the effects of curcumin on the percentage of CD44 and CD24 in the MCF-10F, Alpha5, Tumor2, MCF-7 and MDA-MB-231 breast cell lines, as determined by flow cytometry.

As displayed in Fig. 4, curcumin increased CD44 and decreased CD24 gene expression in MCF-7 cells. Conversely, curcumin decreased CD44 gene expression in the MDA-MB-231 cell line. Notably, CD24 was not present in MDA-MB-231 cells. The effect of curcumin on CD44 and CD24 was analyzed via flow cytometry in MCF-7 and MDA-MB-231 cells (Fig. 5A-C). Curcumin did not affect the $CD44^+/CD24^+$ or the $CD44^+/CD24^-$ subpopulations in the MCF-7 cell line. However, the $CD44^+/CD24^+$ subpopulation was increased and the $CD44^+/CD24^-$ subpopulation was decreased in MDA-MB-231 cells following treatment with curcumin. The percentages of $CD44^+/CD24^+$ and $CD44^+/CD24^-$ cells are indicated in Fig. 5C.

Biopsy specimens obtained from 33 patients diagnosed with benign and breast cancer lesions were studied. Among them were three normal tissues, which were negative for CD44 and CD24 (Fig. 6A-a and B-a), 8 dysplastic tissues positive for CD44 and CD24 (Fig. 6A-b and B-b), 9 positive ductal carcinomas, of which some were positive for CD44 (Fig. 6A-c1-5) and all of which were positive for CD24 (Fig. 6B-c). Benign specimens contained atypia, subnormal ductules and atypical ductal hyperplasia. The 13 samples from reduction mammoplasties were all negative for both markers.

Discussion

The present study was conducted to analyze the effect of curcumin on the cell surface markers and adhesion molecules CD44 and CD24 in breast cancer cell lines. The results demonstrated that curcumin decreased CD44 and CD24 gene expression levels in the Alpha model (MCF-10F, Alpha5 and Tumor2 cell lines). Peroxidase staining results corroborated that CD44 and CD24 protein expression were also decreased in Alpha5 and Tumor2 cells. However, in MCF-10F cells, there were increases in both markers. Furthermore, curcumin increased CD44 and decreased CD24 gene expression in MCF-7 cells, but decreased CD44 expression in MDA-MB-231 cells, while having no significant effect on CD24.

Certain previous studies (27-29) have indicated that CD44 has several roles, including its activity as an adhesion molecule and as a signal regulator. It has been reported that CD44-positive cells exhibit a more mesenchymal-like profile, are usually enriched for genes involved in cell motility, proliferation and angiogenesis and form tumors in mice (27).

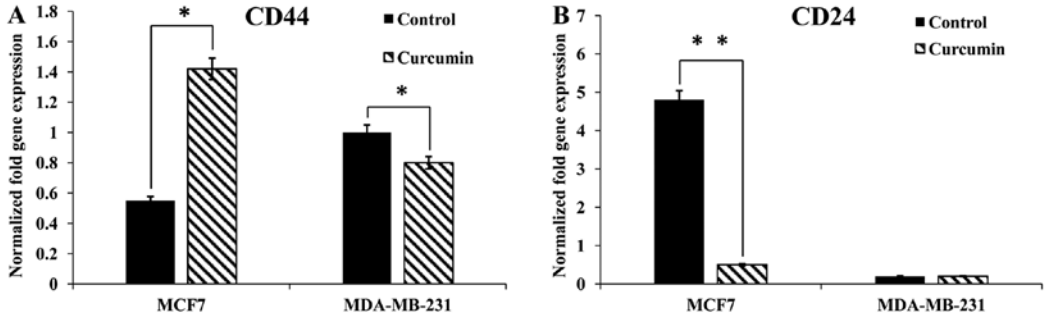

Figure 4. Effect of curcumin (30 μM for 48 h) on (A) *CD44* and (B) *CD24* gene expression in MCF-7 and MDA-MB-231 cells analyzed by RT-qPCR. Graphs represent the fold-change of CD44 and CD24 gene expression relative to vehicle-treated cells. *P<0.05; **P<0.01. All of the experiments were conducted in triplicate.

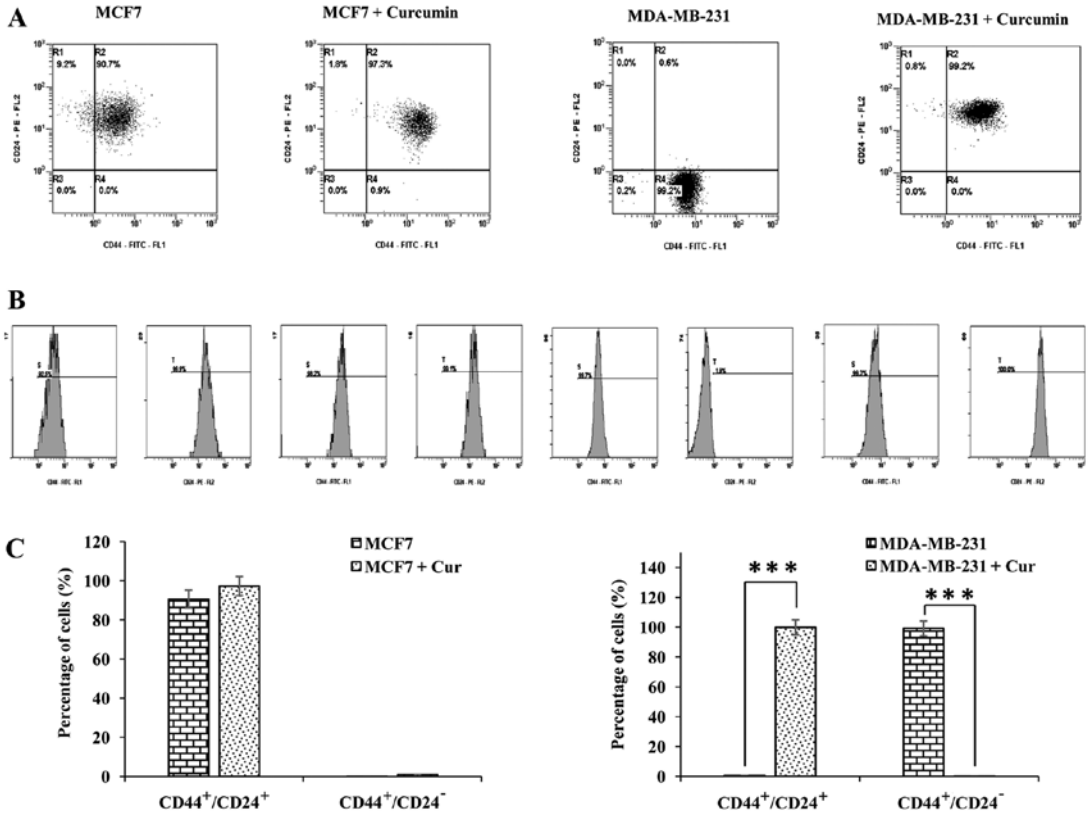

Figure 5. Effect of curcumin (30 μM for 48 h) on CD44 and CD24 markers. (A) Dot-plot, (B) histograms and (C) percentages of $CD44^{+}/CD24^{+}$ and $CD44^{+}/CD24^{-}$ subpopulations in MCF-7 and MDA-MB-231 cell lines, as determined by flow cytometry. ***P<0.001. All of the experiments were conducted in triplicate.

CD24-positive cells usually express genes implicated in carbohydrate metabolism and RNA splicing (27). CD24 is a mucin-like adhesion molecule that increases the metastatic potential of malignant cells and is associated with poor clinical outcomes in breast carcinomas (27).

In the present study, the effects of curcumin were examined via flow cytometry to determine the percentages of two subpopulations of cells, $CD44^{+}/CD24^{+}$ and $CD44^{+}/CD24^{-}$, among MCF-10F, Alpha5 and Tumor2 cell lines. It was found that the $CD44^{+}/CD24^{+}$ and $CD44^{+}/CD24^{-}$ subpopulations were present in all three cell lines. When curcumin treatment was applied, the $CD44^{+}/CD24^{+}$ subpopulation increased and the $CD44^{+}/CD24^{-}$ subpopulation decreased in the normal MCF-10F and the pre-tumorigenic Alpha5 cells. Such an effect was not observed in Tumor2 cells in comparison to the corresponding control cells of the same type.

When subpopulations were analyzed by flow cytometry it was observed that curcumin increased the $CD44^{+}/CD24^{+}$ and decreased the $CD44^{+}/CD24^{-}$ subpopulations of MDA-MB-231 cells. However, curcumin did not affect the $CD44^{+}/CD24^{+}$ or the $CD44^{+}/CD24^{-}$ subpopulations of MCF-7 cells. The prognostic value of these markers in breast cancer remains controversial and requires further research (30,31).

A pioneering study (32) in human breast cancer cells, such as MCF-7 and MDA-MB-231, demonstrated that $CD44^{+}/CD24^{-}$ cells can be recognized as prospective cancer stem cells. Later, these findings were supported by those of Sheridan *et al* (33). Evidence indicates that such phenotypes are not present in all breast cancers, only in breast cancer stem cells (30,34,35).

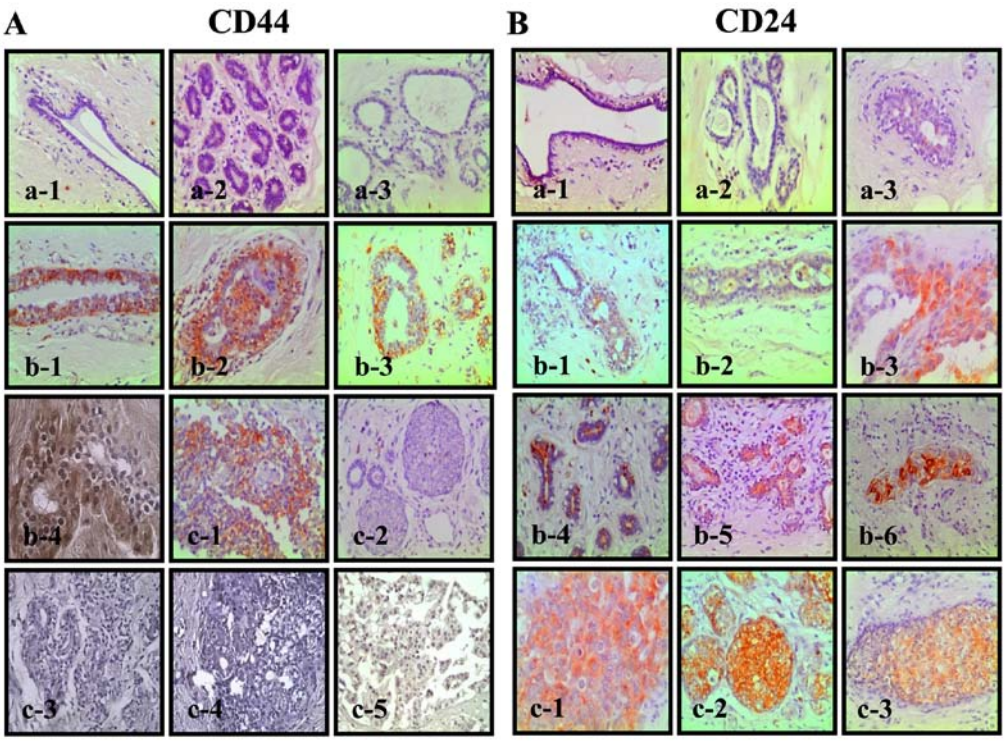

Figure 6. Representative images of (A) CD44 protein expression in cross sections of subnormal mammary glands where normal ducts, lobules and cysts can be seen (a-1-3), and in benign (b-1-4) and ductal carcinoma (c-1-5) breast lesions. (B) CD24 protein expression in cross sections of subnormal mammary glands (a-1-3), and in benign (b-1-6) and ductal carcinoma (c-1-3) breast lesions stained using the immunoperoxidase method. CD44 (sc-7297) and CD24 (sc-65257) monoclonal antibodies (Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA), each at a 1:500 dilution, were used. All of the experiments were conducted in triplicate.

It is important to point out that the $CD44^+/CD24^+$ phenotype Is highly expressed in differentiated epithelial cell types (30) and $CD44^+/CD24^-$ cells exhibit undifferentiated basal/mesenchymal cell properties (33). It can be suggested that curcumin can be used to improve the ratio of $CD44^+/CD24^+$ cells and decrease the $CD44^+/CD24^-$ subpopulation, as well as the cancerous types of breast cells. Such results have some kind of significance to be taken into consideration concerning the use of curcumin for breast cancer treatment. Evidence of migration, colony formation and invasion in $CD44^+$ MDA-MB-468 cells supports the concept of regulation of phenotypes (36). In similar studies (37), evidence indicated that there can be inter-conversion between the phenotypes and that epithelial-like $CD44^+/CD24^+$ cells can readily give rise to $CD44^+/CD24^-$ cells during tumor initiation (38).

In summary, the present study investigated the expression of CD44 and CD24 in epithelial lesions derived from biopsy specimens obtained from the archived tissues of patients with benign and breast cancer lesions. The results indicated that normal tissues were negative for CD44 and CD24 expression. However, benign lesions were positive for both markers. Malignant tissues were negative for CD44 and positive for CD24 in most of the cases. In conclusion, these results indicated that a natural substance such as curcumin may be used to improve the ratio of $CD44^+/CD24^+$ cells and to decrease $CD44^+/CD24^-$ subpopulations, which have the characteristics of undifferentiated basal/mesenchymal cells. Our next study will be conducted with the aim to validate this conclusion and further explore it in animal models where we will study the effects of curcumin on the expression of CD44 and CD24 *in vivo* by delivering it orally in nude mouse xenografts of Alpha5 and Tumor2 cells. Furthermore, we will consider the effects of curcumin on malignant behavior, such as the proliferation or motility of breast cancer cells used in the present study in association with studies on the expression of CD44 and CD24.

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Availability of data and materials

The datasets used during the present study are available from the corresponding author upon reasonable request.

Authors' contributions

GMC and JAQ conceived and designed the study. GMC, RPC and JAQ performed the experiments. GMC and RPC wrote the the manuscript. GMC, RPC and JAQ reviewed and edited the manuscript. All authors read and approved the manuscript and agree to be accountable for all aspects of the research in ensuring that the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Ethics approval and consent to participate

The present study was approved by the Ethics Committee School of Medicine, Saint-Luc Hospital, IMAG Unit (IREC) of University of Louvain (Brussels, Belgium). All patients had provided written informed consent prior to obtaining the samples.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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