$P2Y_2R$ has a significant correlation with Notch-4 in patients with breast cancer

DONG CHUL KIM[1*], HANA JIN[2*], JONG SIL LEE[1], EUNA SON[3], GYEONG WON LEE[4] and HYE JUNG KIM[2]

Departments of [1]Pathology and [2]Pharmacology, College of Medicine, Institute of Health Sciences, Gyeongsang National University, Jinju, South Gyeongsang 52727; [3]Division of Life Science, College of Natural Sciences, Gyeongsang National University, Jinju, South Gyeongsang 52828; [4]Division of Hematology-Oncology, Department of Internal Medicine, College of Medicine, Gyeongsang National University Hospital, Gyeongsang National University, Jinju, South Gyeongsang 52727, Republic of Korea

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Abstract. Our previous study found that highly metastatic breast cancer cells, such as MDA-MB-231 cells, release higher levels of ATP and exhibit greater $P2Y_2$ receptor ($P2Y_2R$) activity than lowly metastatic breast cancer cells, and that $P2Y_2R$ activation mediated by ATP plays a significant role in tumor progression and metastasis. In addition, we reported that radiotherapy-resistant (RT-R) breast cancer cells promote invasion and tumor growth through the activation of $P2Y_2R$ by ATP released from RT-R-breast cancer cells than breast cancer cells. Moreover, increased numbers of cancer stem cells (CSCs) were observed among the RT-R-breast cancer cell population. Therefore, in this study, we investigated the expression level of five CSC markers (CD24, CD44, Oct3/4, Notch-4 and ALDH1A1) as well as $P2Y_2R$ in the tumor tissues of patients with breast cancer and determined which CSC marker correlates with $P2Y_2R$ in breast cancer. According to the immunohistochemical analysis, CD44, Oct3/4 and Notch-4 but not ALDH1A1 were significantly expressed in the tumor tissues (n=180) compared with the normal epithelial tissues (n=20) of patients with breast cancer. It was demonstrated that $P2Y_2R$ expression was increased in tumor tissues of patients with breast cancer compared with normal epithelial tissue. Notably, it was identified that $P2Y_2R$ expression has a significant correlation with only the CSC marker Notch-4 in patients with breast cancer. The results of this study suggested for the first time to the best of our knowledge that Notch-4 has a notable correlation with $P2Y_2R$, which has important roles in tumor progression and metastasis.

Correspondence to: Professor Hye Jung Kim, Department of Pharmacology, College of Medicine, Institute of Health Sciences, Gyeongsang National University, 816 Bungil 15, Jinjudaero, Jinju, South Gyeongsang 52727, Republic of Korea
E-mail: hyejungkim@gnu.ac.kr

Professor Gyeong Won Lee, Division of Hematology-Oncology, Department of Internal Medicine, College of Medicine, Gyeongsang National University Hospital, Gyeongsang National University, 816 Bungil 15, Jinjudaero, Jinju, South Gyeongsang 52727, Republic of Korea
E-mail: brightree@naver.com

*Contributed equally

Abbreviations: ALDH, aldehyde dehydrogenase; AM, adhesion molecule; CSC, cancer stem cell; EC, endothelial cell; EMT, epithelial mesenchymal transition; HIF, hypoxia-inducible factor; LOX, lysyl oxidase; MMP, matrix metalloproteinase; TNBC, triple-negative breast cancer

Key words: breast cancer, CSC markers, CD44, Notch-4, Oct3/4, $P2Y_2$ receptor

Introduction

Breast cancer is one of the leading causes of cancer-related death in women (1). Although most breast cancer patients respond to traditional treatments, such as tumor removal surgery, chemotherapy and radiation, the disease becomes difficult to treat and causes death with recurrence and metastasis to distant organs. Metastasis is the process by which cancer cells migrate from a site of origin and develop in neighboring locations, and rather than the primary tumor itself, metastasis is responsible for the majority of cancer-related deaths (2-4). Recently, it has been proposed that cancer stem cells (CSCs) exist in tumors and contribute to tumorigenesis, aggressiveness, metastasis to distant organs, resistance to different types of anticancer therapeutic strategies, including radiation therapy and chemotherapy, and disease recurrence. CSCs are known to be able to self-renew and regenerate heterogeneous populations that consist of tumor cells after treatment (5). Some or all of these cells, which are present in a tumor in specific microenvironments or niches, render tumor cells more resistant to radiation therapy and chemotherapy, ultimately leading to tumor regrowth and distant metastasis (6-10).

The $P2Y_2$ receptor ($P2Y_2R$) belongs to the family of G protein-coupled P2Y receptors and is most consistently expressed (or overexpressed) by tumor cells; $P2Y_2R$ mediates various responses, including proliferation, in many

tumors (11-14) upon activation by ATP, which is released in the tumor microenvironment (15). In our previous study, we reported that MDA-MB-231 breast cancer cells, which show highly metastatic properties, release higher levels of ATP and show greater $P2Y_2R$ activity than lowly metastatic breast cancer cells, such as MCF-7 cells, and that $P2Y_2R$ activation by ATP plays an important role in tumor progression and metastasis by regulating various responses in tumor cells and by modulating crosstalk between cancer cells and endothelial cells (ECs) (16). In addition, ATP-mediated activation of $P2Y_2R$ in monocytes induces their recruitment toward a tumor and promotes inflammatory conditions around primary tumors by secreting matrix metalloproteinase. In breast cancer cells, $P2Y_2R$ activation by ATP induces hypoxia-inducible factor (HIF)-1α expression, lysyl oxidase (LOX) secretion, and collagen crosslinking, which results in premetastatic niche formation (17). Moreover, we found that radiotherapy-resistant (RT-R) breast cancer cells, particularly RT-R-MDA-MB-231 cells derived from the highly metastatic breast cancer cell line MDA-MB-231, release high levels of ATP, promoting invasion and tumor growth through activation of $P2Y_2R$ (18). Furthermore, more CSCs developed among RT-R-MDA-MB-231 cells, contributing to the acquisition of tolerance to other anticancer therapies in addition to radiation therapy (19). Thus, we hypothesized that $P2Y_2R$ may have a relationship with CSCs in breast cancer, and we aimed to evaluate CSC marker expression in human breast cancer and to determine their relationship with $P2Y_2R$ in human breast cancer. In this study, we investigated the expression level of 5 CSC markers (CD24, CD44, Oct3/4, Notch-4 and ALDH1A1) and $P2Y_2R$ in human breast cancer patients and examined the correlation of CSC markers with $P2Y_2R$.

Materials and methods

Case selection. This retrospective study was approved by the Institutional Review Board of Gyeongsang National University Hospital with a waiver of informed consent (GNUH-2018-05-010). Specimens from 180 breast cancer patients who underwent surgery with wide excision or mastectomy between January 2010 and December 2012 at Gyeongsang National University Hospital, Jinju, Korea, were selected. For each sample, formalin-fixed, paraffin-embedded and hematoxylin and eosin-stained sections were prepared on glass slides and assessed by two pathologists. Data from electronic medical records, including sex, age, menstrual status, tumor size, lymph node status, distant metastasis, and tumor stage, were reviewed. Cancer stages were determined according to the eighth edition of the American Joint Committee on Cancer (AJCC). Histological type and grade were determined per the fifth edition of the World Health Organization (WHO) classification. As shown in Table I, all patients except one were female (179), with a mean age of 51.5 years old (range, 25~81). Patients <50 years old accounted for 50.6; 56.1% of the patients were premenopausal; 91.7% of patients had a tumor diameter classified as T1/T2, whereas 8.3% had a tumor diameter classified as T3/T4; 85.0% had an N0/N1 lymph node grade, whereas 15.0% had an N2/N3 lymph node grade; 80.6% were at stage I/II, whereas 19.4% were at stage III/IV. Cases were divided into three groups according to ER, PR and HER-2 expression: i) triple-negative breast cancer (TNBC), ER, PR and HER-2 negative (n=20; 11.1%); ii) HER-2-breast cancer, ER and PR negative (n=7; 3.9%; iii) luminal breast cancer, ER and/or PR positive, HER-2 negative or positive (n=153; 85.0%). The criteria for ER- and PR-positive staining was a score >3 by the Allred scoring system. HER-2 was considered positive if >10% of the cancer cells presented with strong and complete brown cell membrane staining (Table II).

Tissue microarray and immunohistochemistry. Representative hematoxylin and eosin-stained sections on glass slides containing prominent intratumoral regions from 180 breast cancer patient specimens were chosen. Two 2-mm tissue cores were obtained from each representative paraffin block and transferred to recipient tissue microarray (TMA) blocks, and immunohistochemial staining was performed on the TMA blocks using anti-$P2Y_2R$ polyclonal (1:200 dilution, #PA1-46150; Thermo Fisher Scientific, Inc.), anti-CD24 monoclonal (1:100 dilution, #ab31622; Abcam), anti-CD44 monoclonal (1:100 dilution, #ab51037; Abcam), anti-OCT3/4 polyclonal (1:100 dilution, #ab18976; Abcam), anti-Notch-4 polyclonal (1:50 dilution, #ab199295; Abcam), and anti-ALDH1A1 monoclonal (1:100 dilution, #ab52492; Abcam) primary antibodies.

Evaluation of immunohistochemistry. CD24 and CD44 were interpreted as positive when staining in the cell membranes was observed in at least 10% of the cells. ALDH1A1 was interpreted as positive when staining in the cytoplasm was observed in at least 10% of the cells. Cytoplasmic expression of Oct3/4 was detected and classified into two groups: Low expression (0~50% cells) or high expression (51~100% cells). Membrane and nuclear expression of Notch-4 was recorded. A semi-quantitative scoring system was used, evaluating both the staining intensity (0, no stain; 1+, weak stain; 2+, moderate stain; 3+, strong stain) and percentage of stained cells (0, <5%; 1, 5-25%; 2, 26-50%; 3, 51-75%; and 4, >75%). Scores for staining intensity and the percentage of positive cells were then multiplied to generate the immunoreactivity score (IS) for each case. All cases were sorted into two groups according to IS. High expression of Notch-4 was defined as detectable immunoreactivity in the cytoplasm and nucleus with IS ≥4. Only nuclear expression of $P2Y_2R$ was recorded, given a score from 0 to 12 according to the intensity (0, 1+, 2+, 3+) and percentage of positive tumor cells (0=all negative, 1+=0~10%, 2+=11~50%; 3+=51~80% and 4+=more than 81% of cells).

Statistical analysis. All statistics were analyzed using GraphPad Prism7 software (GraphPad Software, Inc.). One-way ANOVA followed by Newman-Keuls post hoc test was carried out to compare different groups. Data are presented as the mean ± SEM. Coefficients of correlation (r) were determined by the Pearson correlation method. $P<0.05$ was considered to indicate a statistically significant difference. Correlation analyses were performed using the chi-square test and Fisher's exact test. SPSS version 25.0 (IBM Corp.) was used for the analysis.

Results

CD44, Oct3/4 and Notch-4 CSC markers are significantly expressed in the tumor tissue of breast cancer patients. First,

Table I. Association between cancer stem cell markers and clinicopathological characteristics in patients with breast cancer.

Clinicopathological characteristics	Total patients: 180 (%)	CD24 (%)			CD44 (%)			Oct3/4 (%)			Notch-4 (%)			ALDH1A1 (%)		
		Negative	Positive	P-value	Negative	Positive	P-value	Low	High	P-value	Low	High	P-value	Negative	Positive	P-value
Age, years				0.6504			0.2627			0.3424			0.4559			0.6187
<50	91 (50.6)	55 (60.4)	36 (39.6)		25 (27.5)	66 (72.5)		20 (22.0)	71 (78.0)		42 (46.1)	49 (53.9)		90 (99.0)	1 (1.0)	
≥50	89 (49.4)	50 (56.2)	39 (43.8)		32 (36.0)	57 (64.0)		14 (15.7)	75 (84.3)		36 (40.4)	53 (59.6)		87 (97.7)	2 (2.3)	
Menstrual status				0.1268			0.075			0.4451			0.8802			0.2582
Premenopausal	101 (56.1)	54 (53.5)	47 (46.5)		38 (37.6)	63 (62.4)		17 (16.8)	84 (83.2)		44 (43.6)	57 (56.4)		98 (97.0)	3 (3.0)	
Postmenopausal	78 (43.3)	51 (65.4)	27 (34.6)		19 (24.4)	59 (75.6)		17 (21.8)	61 (78.2)		33 (42.3)	45 (57.7)		78 (100.0)	0 (0.0)	
None (male)	1 (0.6)	1 (100.0)	0 (0.0)		0 (0.0)	1 (100.0)		0 (0.0)	1 (100.0)		1 (100.0)	0 (0.0)		1 (100.0)	0 (0.0)	
ER status				0.0812			0.5931			0.8332			<0.0001			0.1871
Negative	50 (27.8)	24 (48.0)	26 (52.0)		14 (28.0)	36 (72.0)		10 (20.0)	40 (80.0)		37 (74.0)	13 (26.0)		48 (96.0)	2 (4.0)	
Positive	130 (72.2)	81 (62.3)	49 (37.7)		43 (33.1)	87 (66.9)		24 (18.5)	106 (81.5)		41 (31.5)	89 (68.5)		129 (99.2)	1 (0.8)	
PR status				0.5021			0.8587			0.141			0.0185			
Negative	50 (27.8)	27 (54.0)	23 (46.0)		15 (30.0)	35 (70.0)		13 (26.0)	37 (74.0)		29 (58.0)	21 (42.0)		49 (98.0)	1 (2.0)	
Positive	130 (72.2)	78 (60.0)	52 (40.0)		42 (32.3)	88 (67.7)		21 (16.2)	109 (83.8)		49 (37.7)	81 (62.3)		128 (98.5)	2 (1.5)	
HER-2 status																
Negative	151 (83.9)	91 (60.3)	60 (39.7)	0.3039	45 (29.8)	106 (70.2)	0.2757	29 (19.2)	122 (80.8)	1	61 (40.4)	90 (59.6)	0.1006	148 (98.0)	3 (2.0)	
Positive	29 (16.1)	14 (48.3)	15 (51.7)		12 (41.4)	17 (58.6)		5 (17.2)	24 (82.8)		17 (58.6)	12 (41.4)		29 (100.0)	0 (0.0)	
Tumor size																
T1/T2	165 (91.7)	98 (59.4)	67 (40.6)	0.4152	55 (33.3)	110 (66.7)	0.15	30 (18.2)	135 (81.8)	0.054	69 (41.8)	96 (58.2)	0.1861	162 (98.2)	3 (1.8)	
T3/T4	15 (8.3)	7 (46.7)	8 (53.3)		2 (13.3)	13 (86.7)		4 (26.7)	11 (73.3)		9 (60.0)	6 (40.0)		15 (100.0)	0 (0.0)	
Lymph node involvement																
N0/N1	153 (85.0)	88 (57.5)	65 (42.5)	0.6751	45 (29.4)	108 (70.6)	0.1768	29 (18.9)	124 (81.1)	1	63 (41.2)	90 (58.8)	0.207	150 (98.0)	3 (2.0)	
N2/N3	27 (15.0)	17 (63.0)	10 (37.0)		12 (44.4)	15 (55.6)		5 (18.5)	22 ((81.5)		15 (55.6)	12 (44.4)		27 (100.0)	0 (0.0)	
Distant metastasis																
No	175 (97.2)	102 (58.3)	73 (41.7)	0.65	55 (31.4)	120 (68.6)	0.6524	33 (18.9)	142 (81.1)	1	75 (42.9)	100 (57.1)	0.6537	172 (98.3)	3 (1.7)	
Yes	5 (2.8)	4 (80.0)	1 (20.0)		2 (40.0)	3 (60.0)		1 (20.0)	4 (80.0)		3 (60.0)	2 (40.0)		5 (100.0)	0 (0.0)	
Tumor stage																
I/II	145 (80.6)	85 (58.6)	60 (41.4)	1	44 (30.3)	101 (69.7)	0.4273	27 (18.6)	118 (81.4)	0.8136	58 (40.0)	87 (60.0)	0.0868	142 (97.9)	3 (2.1)	
III/IV	35 (19.4)	21 (60.0)	14 (40.0)		13 (37.1)	22 (62.9)		7 (20.0)	28 (80.0)		20 (57.1)	15 (42.9)		35 (100.0)	0 (0.0)	

Table II. Expression of cancer stem cell markers in different subtypes of breast cancer.

	CD24 (%)			CD44 (%)			Oct3/4 (%)			Notch-4 (%)			ALDH1A1 (%)		
Breast cancer type	Negative	Positive	P-value	Negative	Positive	P-value	Low	High	P-value	Low	High	P-value	Low	High	P-value
TNBC	11 (55.0)	9 (45.0)	0.3456	3 (15.0)	17 (85.0)	0.2964	3 (15.0)	17 (85.0)	0.9795	15 (75.0)	5 (25.0)	0.0013	19 (95.0)	1 (5.0)	0.4504
$HER2^+$ (ER^-/PR^-)	6 (85.7)	1 (14.3)		3 (42.9)	4 (57.1)		1 (14.3)	6 (85.7)		5 (71.4)	2 (28.6)		7 (100.0)	0 (0.0)	
Luminal	92 (60.1)	61 (39.9)		43 (28.1)	110 (71.9)		25 (16.3)	128 (83.7)		56 (36.6)	97 (63.4)		151 (98.7)	2 (1.3)	

we examined the expression of the CSC markers CD24, CD44, Oct3/4, Notch-4 and ALDH1A1 in tumor tissues (n=180) and normal epithelial tissues (n=20) obtained from breast cancer patients. As shown in Fig. 1, expression of CD44, Oct3/4 and Notch-4, but not CD24 and ALDH1A1, was significantly induced in the tumor tissues compared to the normal epithelial tissues of breast cancer patients.

$P2Y_2R$ expression is also significantly increased in tumor tissue and has a significant correlation only with Notch-4 of the cancer stem cell markers evaluated. In our previous study, we suggested that $P2Y_2R$ has an important role in tumor progression and metastasis in highly metastatic breast cancer cells, such as MDA-MB-231 and RT-R breast cancer cells (16-19). In addition, Ko *et al* (19). showed that induced expression of CSC markers in RT-R-MDA-MB-231 cells. Therefore, in the present study, we examined whether expression of $P2Y_2R$ and CSC markers is induced in breast cancer patients and, if so, whether there is a relationship between $P2Y_2R$ and CSC markers in these patients. As depicted in Fig. 2A, we confirmed that $P2Y_2R$ expression was increased in the tumor tissues of breast cancer patients compared to normal epithelial tissues. We also found that $P2Y_2R$ expression had a significant correlation only with Notch-4 in breast cancer patients (Fig. 2B). Immunohistochemical staining results showed that Notch-4 and $P2Y_2R$ were highly expressed in tumor tissues compared to normal tissues (Fig. 2C).

Discussion

Breast CSCs are characterized by high expression of CD44 and low expression of CD24 ($CD44^+/CD24^{-/low}$), and Notch-4, Oct3/4 and ALDH1 have also been suggested as CSC markers (20-24). The present study shows that of CSC markers, expression of CD44, Oct3/4 and Notch-4, but not CD24 and ALDH1A1, was significantly induced in tumor tissues compared to normal epithelial tissues obtained from breast cancer patients. Recent studies have suggested that ALDH1, a detoxifying enzyme responsible for the oxidation of retinol to retinoic acid, may be a potent marker of breast CSCs (24-27). However, there is controversy regarding the use of ALDH1 as a breast CSC marker. Resetkova *et al* (28). reported that ALDH1-positive cells did not significantly increase following neoadjuvant chemotherapy in surgical specimens, whereas other researchers (25-27), including Tanei *et al* (24), reported that ALDH1 was a more significant predictive marker than $CD44^+/CD24^-$ for the identification of breast CSCs with respect to resistance to chemotherapy. In our previous *in vitro* study (19), ALDH1 levels were significantly increased in RT-R-MDA-MB-231 cells, indicating an increased number of CSCs, compared to MDA-MB-231 cells; furthermore, ALDH1 was not expressed in MCF-7 and T47D cells, even in RT-R-MCF-7 and RT-R-T47D cells, suggesting that ALDH1 may be a potent marker for breast CSCs and that ALDH1-positive breast CSCs may play an important role in radioresistance. ALDH1 has three main isotypes, ALDH1A1, ALDH1A2, and ALDH1A3 (28). Recent reports suggest that ALDH1, and its isotype ALDH1A1 in particular, are useful CSC markers that may be used to enrich tumor-initiating subpopulations from various cell lines and primary tumors

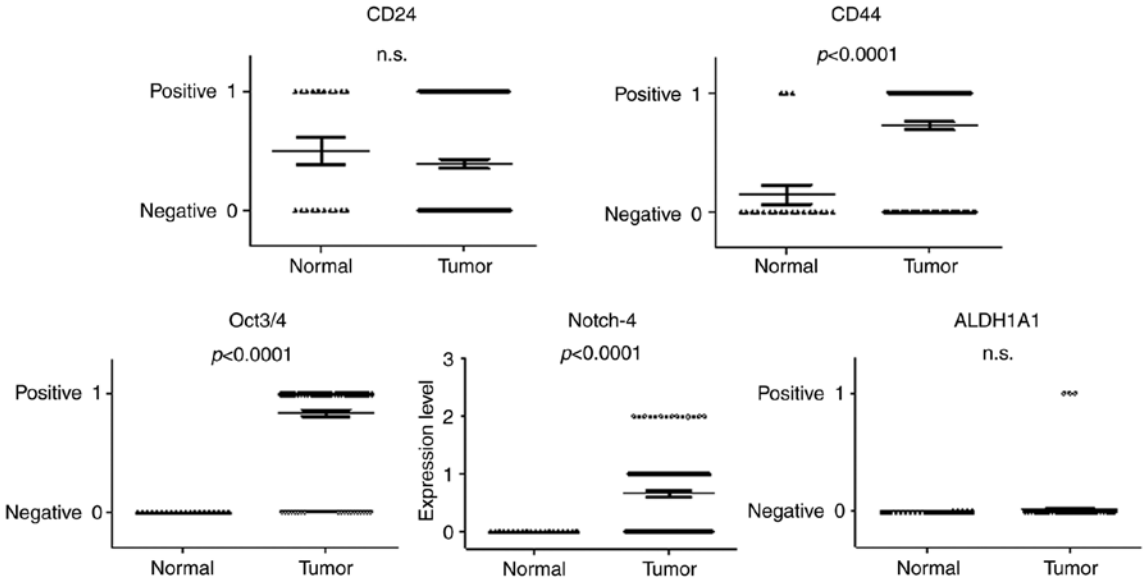

Figure 1. CD44, Oct3/4 and Notch-4 cancer stem cell markers are significantly expressed in the tumor tissues compared with normal epithelial tissues of patients with breast cancer. The expression levels of cancer stem cell markers (CD24, CD44, Oct3/4, Notch-4 and ALDH1A1) were compared in normal epithelial tissues (n=20) and tumor tissues (n=180) of patients with breast cancer (n=180). ALDH, aldehyde dehydrogenase.

and that they are associated with cancer progression (29-31). Therefore, among the isotypes of ALDH1, we investigated expression of ALDH1A1 in tumor tissues of human breast cancer patients. However, among our 180 breast cancer patients, expression of ALDH1A1 was not induced in tumor tissues compared to normal epithelial tissues. Thus, more work is needed to clarify this issue.

As mentioned above, $P2Y_2R$ has, according to an *in vitro* study, an important role in tumor progression and metastasis. We also wondered whether $P2Y_2R$ is highly expressed in the tumor tissues of breast cancer patients. As expected, $P2Y_2R$ expression was significantly induced in tumor tissues compared to normal epithelial tissues. More interestingly, we found that $P2Y_2R$ expression has a significant correlation with Notch-4 in breast cancer patients. Many studies have confirmed increased expression of the Notch receptor and its ligands in breast cancer tissue compared with normal breast tissue (32-34). The Notch receptor family comprises four transmembrane proteins. Rizzo *et al* (34) reported that Notch-1 and Notch-4 expression was low in normal breast tissue but that invasive ductal carcinoma and invasive lobular carcinoma exhibited 81 and 93% Notch-4 positivity, respectively. In particular, the role of Notch-4 in epithelial tumors was established by insertional mutagenesis in mice infected with mouse mammary tumor virus (35,36). Notch-4 is expressed in stem cells of the mammary gland terminal duct and is involved in the formation of branching structures that precede poorly differentiated adenocarcinoma and the incorporation of TAC-2 cells into duct branches. Notch-4 has also been implicated in growth factor β function, aggressive tumor phenotypes, and the transformation from normal mouse mammary epithelial cells to heterotypic cells (35-38). These results establish that the Notch-4 signaling pathway has an important role in regulating the growth and development of the mammary gland. Abnormal expression of Notch-4 may inhibit mammary stem cell differentiation, and Notch-4 gene mutations may enhance mammary epithelial cell proliferation, thus leading to the occurrence of breast cancer. According to Wang *et al* (39), Notch-4 expression was significantly higher in patients with TNBC and HER-2-overexpressing breast cancer than in those with luminal breast cancer. They also suggested that Notch-4 expression is associated with aggressive clinicopathological and biological phenotypes and may predict poor prognosis in luminal breast cancer patients. Nonetheless, in the present study, Notch-4 was not significantly associated with tumor size, lymph node involvement, or clinical TNM stage (Table I). Moreover, as shown in Table II, Notch-4 levels were increased in non-TNBC and luminal breast cancer types compared with TNBC (Table II). In survival analysis, Notch-4 expression did not show any significance in the breast cancer patients enrolled in this study (data not shown). Interestingly, Notch-4 expression was significantly associated with $P2Y_2R$ expression in breast cancer cells, even though expression of $P2Y_2R$ was also not associated with tumor size, lymph node involvement, clinical TNM stage, TNBC (Table SI) or the overall survival rate (P=0.245; data not shown). As this study was performed with specimens from breast cancer patients who underwent surgery with wide excision or mastectomy, the patients enrolled were in the early phase rather than in the late phase. This fact is a possible reason why no significant relationship between several CSC markers and survival rate or recurrence was found. Although Notch-4 does not appear to be associated with the presence of hormone receptors, tumor size, and clinical TNM stage in human breast cancer patients, it is very meaningful that Notch-4 showed a notable correlation with $P2Y_2R$, which has important roles in tumor progression and metastasis, as noted in a previous study. As we mentioned in the Introduction, in *in vitro* study and *in vivo* mice model, $P2Y_2R$ was closely related with the tumor progression, metastasis and acquisition of tolerance to other anticancer therapies (16~19). Accordingly, we surmise that we might obtain more impressive results regarding the relationship between Notch-4

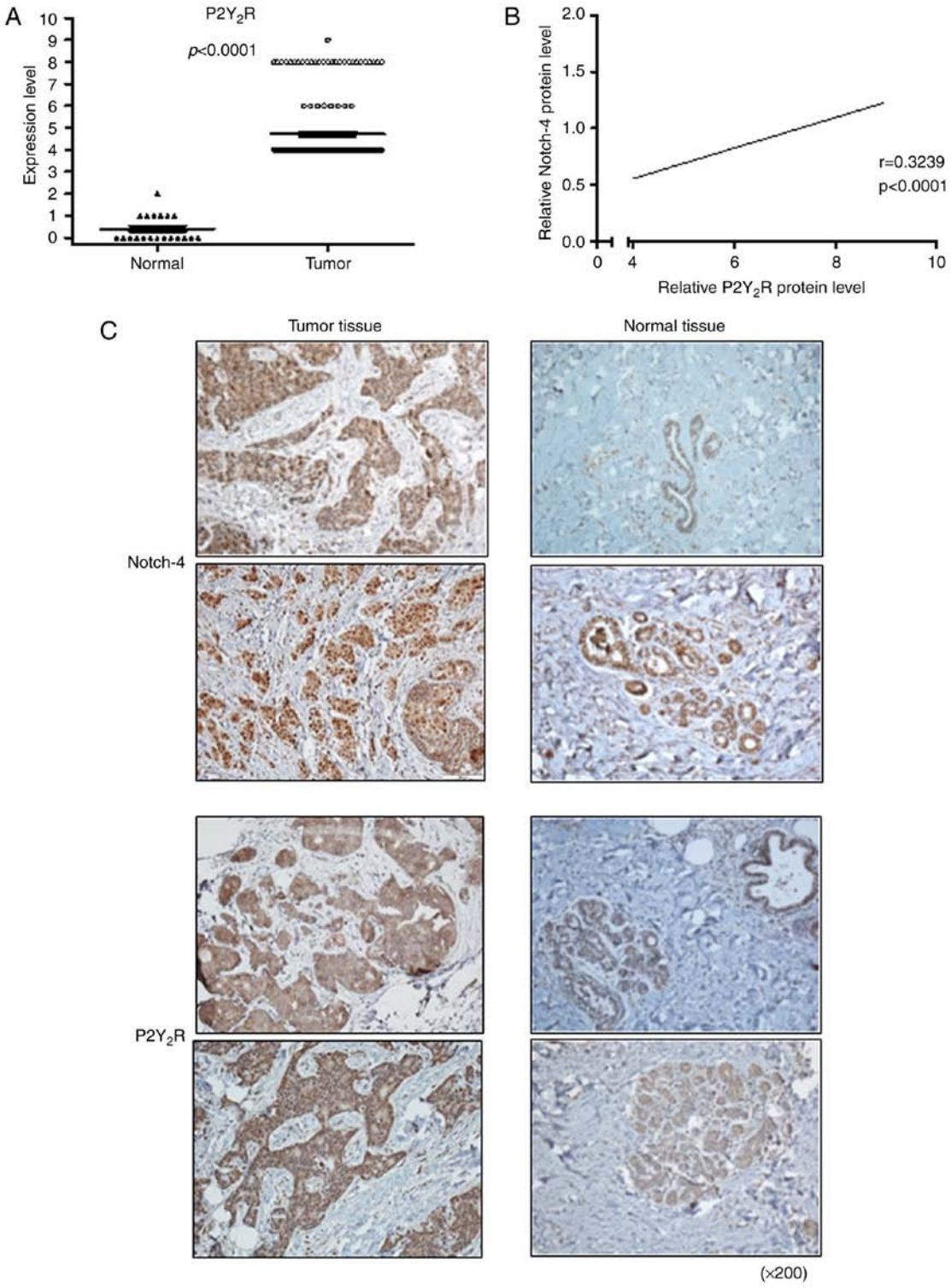

Figure 2. $P2Y_2R$, which is also significantly expressed in the tumor tissues of breast cancer patients, correlates only with Notch-4, of the cancer stem cell markers. (A) $P2Y_2R$ expression is significantly increased in the tumor tissue (n=180) compared with normal epithelial tissue (n=20) of patients with breast cancer (n=180). (B) $P2Y_2R$ expression has a significant correlation with Notch-4 cancer stem cell marker in patients with breast cancer. (C) Immunohistochemical staining of $P2Y_2R$ and Notch-4 in tumor tissues and normal epithelial tissues of patients with breast cancer.

and $P2Y_2R$ in the prognosis of breast cancer patients, including the survival rate, by studying the tumor tissues of breast cancer patients in a later phase.

The above limitation might also be applied to the fact that expression of other CSCs, namely, CD44 and Oct3/4, in tumor tissues displayed no significant correlation with $P2Y_2R$ expression in breast cancer patients. As we stated above, RT-R-MDA-MB-231 cells derived from the highly metastatic breast cancer cell line MDA-MB-231 exhibited increased expression of CSC markers, including CD44 and Oct3/4, with promotion of invasion and tumor growth through activation of $P2Y_2R$. Thus, it is also expected that expression of CD44 and Oct3/4 is related to $P2Y_2R$ expression as well as Notch-4. Regardless, we found no significant relationship between CD44

or Oct3/4 and $P2Y_2R$. CD44 is a transmembrane receptor that is associated with cancer-initiating cell development and tumor metastasis (40-42). CD44 binds to hyaluronic acid (HA), resulting in effective activation of STAT-3 pathways, which play important roles in the regulation of the growth and maintenance of CSCs (43). In addition, STAT-3 activation has been associated with the resistance of tumor cells to chemotherapeutic agents and γ radiation (44,45). Oct3/4, also known as Oct3, Oct4 or Pit-Oct0Unc class 5 homeobox 1 (POU5F1), is a transcription factor (46), and overexpression of Oct3/4 has been shown to enhance the stemness of CSCs and to induce the pathogenesis of several human cancers (47-49). Based on these reports, it is possible to expect that data based on tumor tissues of breast cancer patients in a later phase may reveal a significant correlation between CD44 or Oct3/4 and $P2Y_2R$.

Taken together, there is no report to date on the correlation between Notch-4 and $P2Y_2R$ in tumor progression or tumorigenesis. Thus, it might be valuable to investigate how $P2Y_2R$ correlates with Notch-4, and further study may uncover a novel strategy for developing targeted therapy.

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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

DCK performed the experiments, analyzed the data and drafted the initial manuscript. HJ performed the experiments, analyzed and interpreted the data. JSL collected patients' samples, performed experiments and analyzed the data. ES performed experiments and analyzed the data. GWL and HJK designed the study, interpreted the data and revised the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The study was conducted in accordance with the Good Clinical Practice guidelines and the Declaration of Helsinki. This retrospective study was approved by the Institutional Review Board of Gyeongsang National University Hospital with a waiver of informed consent (GNUH-2018-05-010).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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