Metabolic coupling in phyllodes tumor of the breast and its association with tumor progression

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Abstract. There are markers of metabolic coupling in breast cancer. Loss of caveolin-1 (Cav-1) and upregulation of monocarboxylate transporters (MCTs), especially MCT1 and MCT4, serve an important role in metabolic coupling necessary for release and uptake of metabolites. However, the occurrence of these phenomena in phyllodes tumors (PTs) of the breast is unclear. A total of 101 PTs (60 benign, 26 borderline and 15 malignant) and nine breast tissue samples with no pathological lesions were analyzed. Immunohistochemical staining for Cav-1, MCT1 and MCT4 was performed using tissue microarray and their expression in both stromal and epithelial components was assessed. Cav-1 expression in PTs demonstrated a significant decrease in the stromal component compared with that in the normal breast tissues (P<0.001). MCT1 expression in both epithelial and stromal components was significantly increased in PTs, compared with that in normal breast tissues (both P<0.001). Stromal MCT1 and MCT4 expression were different depending on tumor grade of PTs, and stromal MCT1 expression significantly increased with increasing tumor grade (P<0.001). Although not statistically significant, stromal Cav-1 expression notably decreased with increases in PT grade. High stromal MCT1 expression was significantly associated with lower disease-free survival rate in comparison with low stromal MCT1 expression (P<0.05). These results suggested that changes in protein expression of Cav-1, MCT1 and MCT4 may be associated with tumorigenesis and progression of PTs of the breast.

Introduction

Phyllodes tumors (PTs) are rare biphasic neoplasms that account for <1% of all breast tumors (1,2). PTs are histologically classified as benign, borderline or malignant (1). The primary standard treatment for PTs is adequate surgical resection with negative margins (2). Local relapse occurs in 10-65% of all PTs and distant metastasis develops in 5-40% (3,4). Recurrent and metastatic PTs pose therapeutic challenges as effective treatment options are yet to be elucidated (3-6). Therefore, the development of novel treatment options or therapeutic targets for PTs is needed.

Metabolic reprogramming is an emerging hallmark of cancer that enables tumor growth and progression (7). Aerobic glycolysis is one of the characteristics of cancer cell metabolic reprogramming (8). However, metabolic reprogramming of cancer cells is complicated because it involves multiple metabolic compartments connected by transfer of metabolites (9-12). Rapidly proliferating cancer cells induce oxidative stress in surrounding stromal cells, causing aerobic glycolysis and formation of metabolites such as lactate and pyruvate, which are taken up by anabolic cancer cells. This metabolic coupling is also present within tumor cells, between tumor cells adjacent to blood vessels and tumor cells distant from blood vessels.

Profiling markers associated with metabolic coupling is an active area of research to identify drivers of tumor progression and elucidate the prognostic and predictive biomarkers as well as novel targets for cancer treatment (9-12). Loss of caveolin-1 (Cav-1) in stromal cells and upregulation of monocarboxylate transporters (MCTs), particularly MCT1 and MCT4, in both stromal and cancer cells serve a key role in metabolic coupling required for the release and uptake of metabolites (9,10,12). Several studies have evaluated the relationship between protein expression of Cav-1, MCT1 and MCT4 in cancer and stromal cells and outcome in patients with breast cancer (13-18). Most studies report that these markers are associated with tumor progression and clinical outcomes (13-18). Nonetheless, information on the protein expression of Cav-1, MCT1 and MCT4 in PTs of the breast is limited (19,20).

The aim of the present study was to evaluate the protein expression of Cav-1, MCT1 and MCT4 in PTs and to assess associations between the protein expression data and the clinicopathological factors of PTs. Representative areas from 101 PTs (60 benign, 26 borderline and 15 malignant) and nine breast tissue samples with no pathological lesions were selected to construct tissue microarrays (TMAs) that were immunohistochemically stained for Cav-1, MCT1 and MCT4. Cav-1, MCT1 and MCT4 protein expression was evaluated in both stromal and epithelial components.

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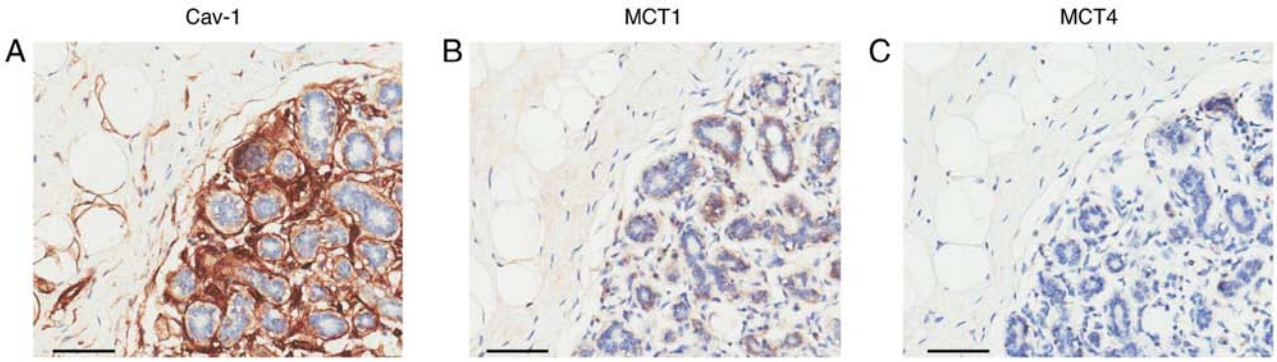

Figure 1. Protein expression and localization of Cav-1, MCT1 and MCT4 was determined via immunohistochemistry in the epithelial and stromal components of normal breast tissue samples. (A) Cav-1 expression was not demonstrated in the luminal epithelial cells, but was observed in the stromal component including fibroblasts, endothelial cells and adipocytes. (B) MCT1 expression was almost exclusively confined to the epithelial component. (C) MCT4 expression was not demonstrated in epithelial or stromal components. Magnification, x200; scale bar, 100 μm. Cav-1, caveolin-1; MCT, monocarboxylate transporter.

Materials and methods

Tumor samples. PT samples with a follow-up period of 10-20 years were used to compare patient clinical results (recurrence and progression). Formalin-fixed, paraffin-embedded (FFPE) PT specimens collected from January 1999 to December 2012 were obtained. As the incidence of phyllodes tumors is low (<1%) (1,2), samples were obtained from the following four hospitals: Chonnam National University Hospital (Gwangju, South Korea), Chonnam National University Hwasun Hospital (Hwasun, South Korea), Cell In All Private Clinics (Gwangju, South Korea) and Foryou Private Clinics (Gwangju, South Korea). A total of nine breast tissue samples collected from January 2007 to December 2009 with no pathological lesions were obtained from Chonnam National University Hwasun Hospital.

Archived hematoxylin and eosin-stained slides of PTs were reviewed by two pathologists. PTs were classified as benign, borderline or malignant according to the 2019 World Health Organization criteria (1). A total of 101 PTs (60 benign, 26 borderline and 15 malignant) were selected. A total of 20, 47, 21 and 13 specimens were obtained from Chonnam National University Hospital, Chonnam National University Hwasun Hospital, Cell In All Private Clinics and Foryou Private Clinics, respectively. Among the 47 specimens from Chonnam National University Hwasun Hospital, 19 specimens were provided by the Biobank of Chonnam National University Hwasun Hospital Biobank of Korea.

TMA construction. Histologically representative sites of each PT and normal breast tissue were selected for inclusion in TMA blocks. For each FFPE block, two cores (diameter, 2 mm) were punched to form a TMA block.

Immunohistochemistry and evaluation of immunohistochemical staining. Immunohistochemical staining for Cav-1, MCT1 and MCT4 was performed on TMA sections (4 μm) using an automated BOND-MAX immunostainer (Leica Microsystems, Inc.) as previously described (21). Mouse monoclonal antibodies for Cav-1 (cat. no. 610407, 1:50; clone 2297/Caveolin 1; BD Transduction Laboratories; BD Biosciences), MCT1 (cat. no. MA5-18288; 1:200; clone P14612; Thermo Fisher Scientific, Inc.), and MCT4 (cat. no. sc-376140; 1:50; clone D-1; Santa Cruz Biotechnology, Inc.) were used. BOND Primary Antibody Diluent (cat. no. AR9352; Leica Microsystems, Inc.) was used to dilute the primary antibodies. Primary antibodies binding to tissue sections were visualized using the BOND Polymer Refine Detection system (cat. no. DS9800, Leica Microsystems, Inc.).

The immunostained TMA slides were digitized using a scanning microscope Leica Aperio AT2 (Leica Microsystems, Inc.), annotations were made using Aperio ImageScope 12.3 (Leica Microsystems, Inc.). Immunoreactivity of Cav-1, MCT1 and MCT4 was assessed in both stromal and epithelial components, with slight modifications of previously reported methods (14). Immunoreactivity was scored based on the intensity (0, no reaction; 1, weak staining; 2, moderate staining; 3, strong staining) and proportion of positive cells (0, 0; 1, <5; 2, 5-50; 3, >50%). The intensity and extent scores were added to obtain a final staining score. In addition to previously reported methods (14), the immunoexpression of Cav-1, MCT1, and MCT4 were classified into two groups. Final staining scores ≤3 were considered low expression, whilst those >3 were considered high expression.

Statistical analysis. The number of replicates is one and all categorized variables are presented as the count (percentage). Differences in Cav-1, MCT1 and MCT4 protein expression between groups were analyzed using Pearson's χ^2 or Fisher's exact test. Linear-by-linear association test was used to analyze the trends of Cav-1, MCT1 and MCT4 expression according to grades of PTs. χ^2 test with Bonferroni post hoc test was used for each possible paired comparison. To measure a linear correlation, a Pearson correlation coefficient test was performed. A total of 101 patients were classified as low and high expression based on the expression of Cav-1, MCT1 and MCT4. Disease-free survival was estimated using the Kaplan-Meier estimate and survival curve comparisons were performed using log-rank tests. When the log-rank test could not be applied to survival plots where late-stage crossover between the groups was observed, the weighted Renyi test was used instead. SPSS Statistics version 27 (IBM Corp.) was used for statistical analyses. $P<0.05$ was considered to indicate a statistically significant difference.

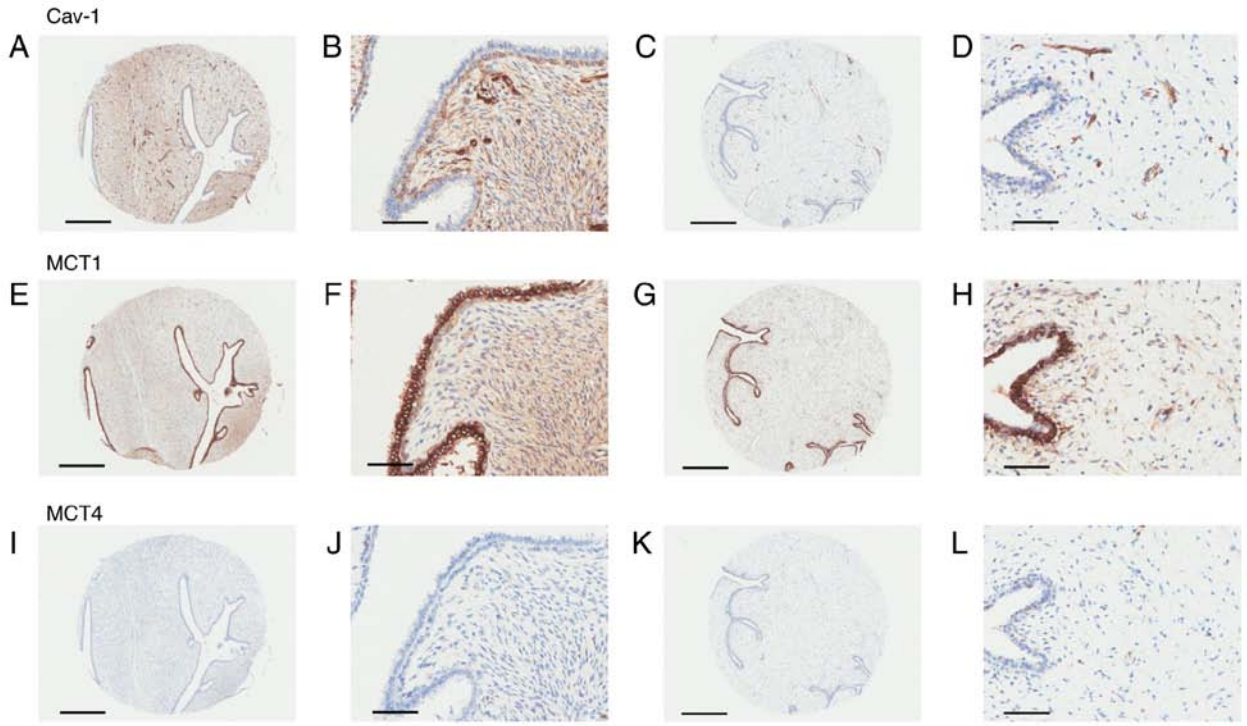

Figure 2. Protein expression and localization of (A-D) Cav-1, (E-H) MCT1 and (I-L) MCT4 were determined via immunohistochemistry in the epithelial and stromal components of two benign phyllodes tumors. (A and B) High expression of Cav-1 in the stromal component. (C and D) Cav-1 expression was not demonstrated in epithelial or stromal components, except in endothelial cells. (E and F) Positive immunoreactivity of MCT1 in both epithelial and stromal components. (H and K) High MCT1 expression in the epithelial component and low expression in the stromal component. (I-L) Negative immunoreactivity of MCT4. Magnification, x4; Scale bar, 500 μm for A, C, E, G, I and K. Magnification, x200; Scale bar, 100 μm for B, D, F, H, J and L. Cav-1, caveolin-1; MCT, monocarboxylate transporter.

Results

Clinicopathological data. All patients were female. Age of patients with no pathological breast lesions was 24-50 years (median, 40 years; mean, 39 years). PT tissue samples were obtained from diagnostic and therapeutic vacuum-assisted breast biopsy or surgical excision. Wide excision was performed immediately for lesions diagnosed as borderline or malignant. Postoperative follow-up was performed without adjuvant therapy. This treatment was in accordance with the medical insurance program controlled by the Ministry of Health and Welfare of Korea. Age of patients with PTs was 16-77 years (median, 43 years; mean, 41 years). Tumor size of PTs was 1.9-21.0 cm in diameter (median, 4.0 cm; mean, 4.9 cm). Mean age of patients and tumor size were as follows: 39.6 years and 4.2 cm for benign; 43.7 years and 5.9 cm for borderline and 46.5 years and 6.1 cm for malignant PTs. Tumor size significantly increased with increase in grade of PTs ($P<0.05$). Mean follow-up period was 61 months, with a range of 5-212 months. Local recurrence occurred in 15 patients, including three (20.0%) malignant, four (15.4%) borderline and eight (13.3%) benign PT cases. Although not statistically significant, the local recurrence rate was notably higher in patients with positive surgical margins than in those with negative surgical margins (20.0 and 12.8%, respectively). Distant metastasis was observed in one case of malignant PTs. Deaths related to PTs were not recorded.

Protein expression of Cav-1, MCT1 and MCT4 in normal breast tissue and PTs. Cav-1, MCT1 and MCT4 expression levels were assessed in both epithelial and stromal components in nine normal breast tissue samples. For epithelial component of the 101 PT cases, a total of 99, 97 and 100 cases were assessed for Cav-1, MCT1 and MCT4, respectively. For stromal elements, all cases of PTs were assessed.

In normal breast tissue samples, Cav-1 expression was not demonstrated in the epithelial component, whereas its expression was observed in the stromal component including stromal fibroblast, endothelial cells, vascular smooth muscles and adipocytes (Fig. 1A). Cav-1 expression was only demonstrated in the cytoplasm. MCT1 expression in normal breast tissue was variable in the epithelial components, whereas its expression was absent in stromal components (Fig. 1B). MCT1 expression was demonstrated in both the plasma membrane and cytoplasm. MCT4 expression was absent in normal breast tissue (Fig. 1C). With a final staining score of >3 being defined as high expression, high expression of Cav-1 (9/9; 100%) was demonstrated in the stromal component of normal breast tissue and MCT1 (4/9; 55.6%) in the epithelial component (Table I).

In PTs, the expression and localization of Cav-1 and MCT1 was similar to that in normal breast tissue (Figs. 2 and 3). MCT4 expression in PTs was not notable and the expression was localized to the cell membrane (Fig. 3). High expression of Cav-1, MCT1 and MCT4 expression were observed in 0 (0/99), 99 (96/97) and 2% (2/100) of the epithelial component and 23.8 (24/101), 72.3 (73/101) and 3.0% (3/101) of the stromal component, respectively (Table I). Compared with normal breast tissue, Cav-1 expression in PTs was significantly decreased in the stromal component ($P<0.001$). MCT1 expression demonstrated a significant increase in both epithelial and

Table I. Expression of Cav-1, MCT1 and MCT4 in normal breast tissue and phyllodes tumors.

	Cav-1		MCT1		MCT4	
Sample	Epithelial	Stromal[a,b]	Epithelial[a,b]	Stromal[a,b]	Epithelial	Stromal
Normal	0/9 (0.0)	9/9 (100.0)	4/9 (55.6)	0/9 (0.0)	0/9 (0.0)	0/9 (0.0)
Phyllodes tumor	0/99 (0.0)	24/101 (23.8)	96/97 (99.0)	73/101 (72.3)	2/100 (2.0)	3/101 (3.0)

Data are presented as count (%). [a]Data analyzed by Fisher's exact test. [b]P<0.001. Cav-1, caveolin-1; MCT, monocarboxylate transporter.

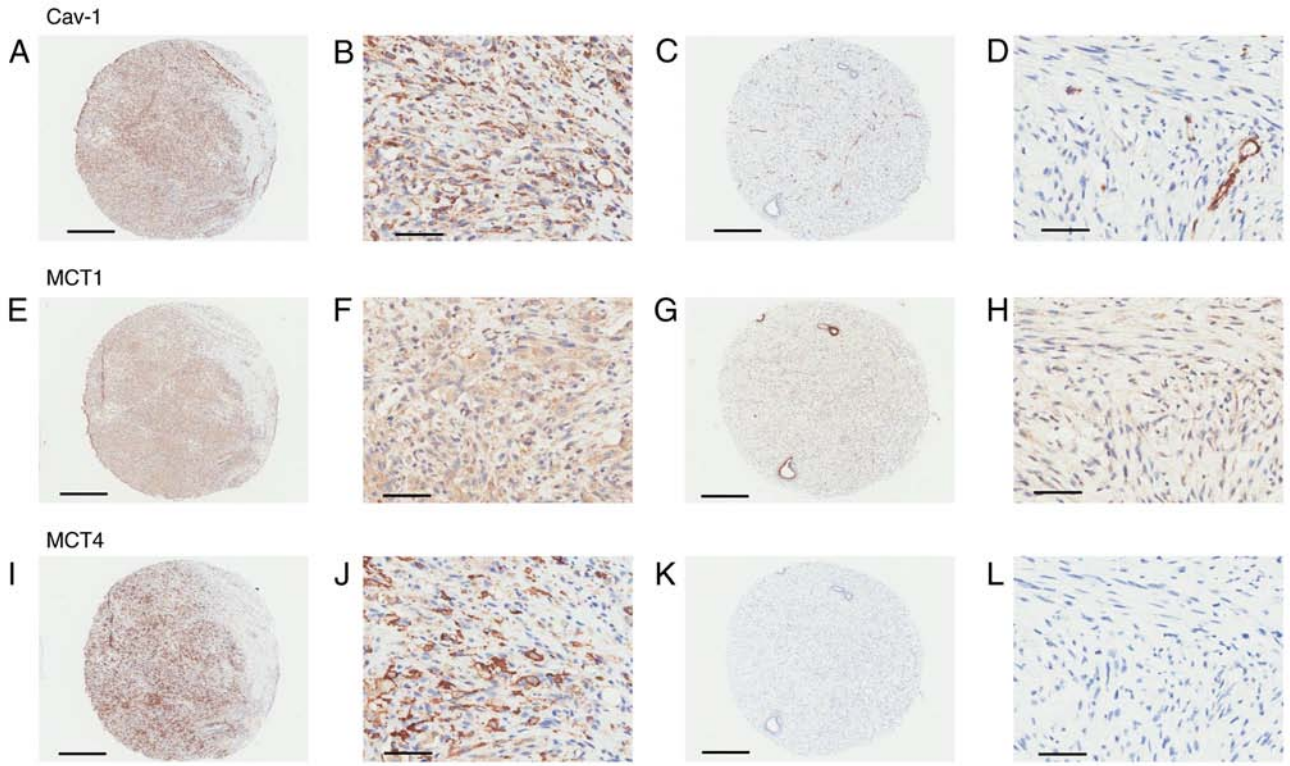

Figure 3. Protein expression and localization of Cav-1 (A-D) MCT1 (E-H) and MCT4 (I-L) was determined via immunohistochemistry in the epithelial and stromal components of two malignant phyllodes tumors. (A and B) Immunoreactivity of Cav-1 in stromal components. (C and D) Epithelial and stromal components, excluding endothelial cells, demonstrated negative immunoreactivity of Cav-1. Strong immunoreactivity was demonstrated in endothelial cells. (E and F) Immunoreactivity of MCT1 in stromal components. (G and H) MCT1 expression is high in epithelial components, but low in stromal components. (I and J) Immunoreactivity of MCT4 in stromal components and cell membranes. (K and L) Negative immunoreactivity of MCT4. Magnification, x4; Scale bar, 500 μm for A, C, E, G, I and K. Magnification, x200; Scale bar, 100 μm for B, D, F, H, J and L. Cav-1, caveolin-1; MCT, monocarboxylate transporter.

stromal components of PTs compared with that in normal breast tissue (both P<0.001). There was no significant difference in MCT4 expression between normal breast tissue and PTs (Table I).

Associations between Cav-1 expression and MCT1 and MCT4 expression in PTs were evaluated. High Cav-1 expression was not observed in epithelial components, therefore no additional statistical tests were performed for epithelial Cav-1 expression. No associations were demonstrated between stromal Cav-1 and MCT 1 or MCT4 expression (Table II).

The expression of Cav-1, MCT1 and MCT4 in benign, borderline and malignant PTs is summarized in Table III. Stromal MCT1 expression varied according to the tumor grade of PTs (P<0.001) and significantly increased with increasing tumor grade (r=0.343, P<0.001) (data not shown). Stromal MCT4 expression was also significantly different according to the tumor grade of PTs (P<0.01). Conversely, there was a notable decreasing trend of stromal Cav-1 expression with increasing tumor grade of PTs, however, the difference was not statistically significant (r=-0.136; P=0.174). As the linear-by-linear association test showed statistically significant differences in the stromal expression of MCT1 and MCT4 depending on PT grade, an additional subgroup analysis was performed (Table III). Stromal MCT1 expression in borderline and malignant PTs was significantly higher than that in benign PTs (both P<0.01). However, there was no significant difference in the stromal MCT1 expression between borderline and malignant PTs. Stromal MCT4 expression in malignant PTs was significantly higher than that in benign and borderline PTs (P<0.01 and P<0.05, respectively). However, no significant difference in stromal

Table II. Association between stromal Cav-1 and MCT1 and MCT4 expression in phyllodes tumors.

	MCT1						MCT4					
	Epithelial			Stromal			Epithelial			Stromal		
Expression	Low	High	P-value[b]	Low	High	P-value[a]	Low	High	P-value[b]	Low	High	P-value[b]
Stromal Cav-1			1.000			0.166			1.000			0.140
Low	1/76 (1.3)	75/76 (98.7)		24/77 (31.2)	53/77 (68.8)		75/77 (97.4)	2/77 (2.6)		76/77 (98.7)	1/77 (1.3)	
High	0/21 (0.0)	21/21 (100.0)		4/24 (16.7)	20/24 (83.3)		23/23 (100.0)	0/23 (0.0)		22/24 (91.7)	2/24 (8.3)	

Data for high and low expression are presented as count (%) and analyzed by [a]χ^2 or [b]Fisher's exact test. Cav-1, caveolin-1; MCT, monocarboxylate transporter.

Figure 4. Prognostic analysis of stromal expression of Cav-1, MCT1 and MCT4 expression in phyllodes tumor. (A) Stromal Cav-1 expression had no prognostic value. (B) Patients with phyllodes tumor with high stromal MCT1 expression had a significantly lower disease-free survival rate. (C) Stromal MCT4 expression had no prognostic value. Cav-1, caveolin-1; MCT, monocarboxylate transporter.

MCT4 expression was observed between benign and borderline PTs.

Fig. 4 illustrates the Kaplan-Meier curves of disease-free survival on the basis of stromal Cav-1, MCT1 and MCT4 expression. High stromal MCT1 expression demonstrated a significantly lower disease-free survival rate than low stromal MCT1 expression (Fig. 4B). Stromal Cav-1 and MCT4 expression had no notable prognostic value (Fig. 4A and C). As the disease-free survival curves for stromal MCT4 expression crossed-over (Fig. 4C), an additional Renyi test was performed, but the results did not affect the original interpretation results, with a non-significant value of P=0.918.

Discussion

A substantial fraction of patients with PTs experience recurrence or metastases (3,4). Markers of metabolic coupling are

Table III. Expression of Cav-1, MCT1 and MCT4 in benign, borderline and malignant phyllodes tumors.

	Cav-1 expression		MCT1 expression		MCT4 expression	
Tumor	Epithelial	Stromal	Epithelial	Stromal[a]	Epithelial	Stromal[b]
Benign	0/60 (0.0)	17/60 (28.3)	58/59 (98.3)	34/60 (56.7)[c,d]	1/60 (1.7)	0/60 (0.0)[e]
Borderline	0/26 (0.0)	5/26 (19.2)	26/26 (100.0)	24/26 (92.3)	1/26 (3.8)	0/26 (0.0)[f]
Malignant	0/13 (0.0)	2/15 (13.3)	12/12 (100.0)	15/15 (100.0)	0/14 (0.0)	3/15 (20.0)

Data are presented as count (%) and analyzed using linear-by-linear association test. [a]P<0.001, [b]P<0.01. Subgroup analysis using χ^2 test with a Bonferroni post hoc test. [c]P<0.01 benign vs. borderline, [d]P<0.01 benign vs. malignant, [e]P<0.01 benign vs. malignant, [f]P<0.05 borderline vs. malignant. Cav-1, caveolin-1; MCT, monocarboxylate transporter.

associated with clinical outcomes in breast cancer (13-18). To the best of our knowledge, however, metabolic coupling remains to be evaluated in PTs. Therefore, the present retrospective study was performed to assess expression of Cav-1, MCT1 and MC4 in benign, borderline and malignant PTs. It was demonstrated that stromal MCT1 and MCT4 expression was different according to the tumor grade of PT and the stromal MCT1 expression increased with increasing tumor grade. Moreover, high stromal MCT1 expression was associated with lower disease-free survival rate.

The mechanisms of metabolic coupling in cancer are an active area of research that may elucidate prognostic and predictive cancer biomarkers as well as novel therapeutic targets (9-12). MCTs are a family of proton-bound membrane transporters responsible for migration of MCs, such as lactate and pyruvate (22). MCT1 and MCT4 serve an important role in metabolic coupling between cancer and cancer-associated stromal cells. Furthermore, caveolae are small invaginations of plasma membrane that are involved in a variety of signaling processes specifically related to stress signaling (23). Caveolins are the primary protein component of caveolae and consist of three members: Cav-1, Cav-2 and Cav-3. Cav-1 is found on mesenchymal cells such as fibroblasts, endothelial cells, adipocytes and muscle cells (24). Decreased Cav-1 expression in cancer-associated fibroblasts decreases mitochondrial metabolism and induces aerobic glycolysis (25).

Changes in protein expression levels of Cav-1, MCT1 and MCT4 in different compartments within tumors serve a key role in metabolic coupling and have been reported in numerous types of cancer, including breast cancer (9,10,12). Cav-1 is not expressed in the epithelium of normal breast tissue, however its expression has been reported in the stromal component (14). MCT1 and MCT4 expression in normal breast tissue is low or absent (13,15,18). Loss of stromal Cav-1 expression has been reported in breast cancer tissue compared with normal breast tissue (14,15,17). MCT1 expression is increased in cancer cells (13,16) and MCT4 expression is increased in cancer cells (18) and cancer-associated stromal cells (14,15).

Despite numerous studies on expression of Cav-1, MCT1 and MCT4 in breast cancer (13-18), their role in PTs is not well-explored. In the current study, expression of Cav-1, MCT1 and MCT4 was determined using immunohistochemistry in 101 PT and nine breast tissue samples with no pathological lesions. Cav-1, MCT1 and MCT4 expression in normal breast tissue was similar to that reported previously (13-15,18). Compared with the expression levels in normal breast tissue, decreased stromal Cav-1 and increased MCT1 expression in both epithelial and stromal components were demonstrated in PTs. However, there was no difference in MCT4 expression between normal breast tissue and PTs. These results suggested that Cav-1 and MCT1 may serve an important role in the development of PTs and their roles vary depending on the epithelial component and stromal component.

Agelopoulos *et al* (19) reported Cav-1 expression in the cytoplasm of 9/53 (17%) PTs and Cav-1 staining in both stromal and epithelial components. This contradicts the findings of Martins *et al* (15), which reported stromal cells as a unique source of Cav-1 expression in breast cancer. In the present study, Cav-1 expression was only demonstrated in the stromal components of PTs.

The role of MCT1 expression in PTs has not yet been elucidated. One of the characteristic features of malignant PTs is overgrowth of sarcomatous stromal component (1). Pinheiro *et al* (26) evaluated MCT1 expression in 86 soft tissue sarcomas and reported MCT1 expression in 52 cases (60.5%). The present study demonstrated stromal MCT1 expression in 73 (72.3%) of 101 PTs.

Kwon *et al* (20) immunohistochemically evaluated MCT4 expression in 207 cases of PTs. MCT4 expression was observed in 16 (8.1%) of 198 epithelial components and 30 (14.5%) of 207 stromal components. The present study demonstrated MCT4 expression in 2.0% (2/100) of epithelial and 3.0% (3/101) of stromal components of PTs. Although the reason for these discrepancies is unclear, differences in criteria for evaluating positive staining may explain them. Kwon *et al* (20) considered positive staining to be >10% of cells stained. The present study used a final staining score that combined intensity and extent, and final staining scores >3 were considered to demonstrate high expression.

An evaluation of the association between Cav-1, MCT1 and MCT4 expression in breast cancer demonstrated that levels of stromal Cav-1 and MCT4 are inversely related, and high levels of stromal MCT4 directly correlate with a loss of stromal Cav-1 immunostaining (14,15). In the present study, stromal Cav-1 expression was not associated with MCT 1 or MCT4 expression in PTs. Jensen *et al* (27) evaluated Cav-1, MCT1 and MCT4 expression in oral squamous cell carcinoma and did not report any association between decreased Cav-1 expression and MCT4. Collectively these results suggest that expression of MCT1 and MCT4 in PTs may be regulated by

mechanisms other than those demonstrated in breast cancer. Further studies are warranted to elucidate the regulatory mechanisms involved in the expression of MCT1 and MCT4 in PTs.

In the present study, stromal MCT1 expression varied according to tumor grade of PTs and tended to increase with increasing tumor grade. Stromal MCT1 expression was significantly different between borderline or malignant and benign PTs, but not between borderline and malignant PTs. Stromal MCT4 expression of malignant PTs was significantly higher than that of borderline PTs. Kwon *et al* (20) reported that MCT4 expression in the stromal component increases with increasing tumor grade of PTs. These data suggest that stromal MCT1 and MCT4 expression have different roles in the progression of PTs; MCT1 is involved in the progression of benign to borderline PTs and MCT4 in the progression to malignant PTs.

Downregulation of Cav-1 expression has been reported during tumor progression (15,28). Martins *et al* (15) evaluated Cav-1 expression in breast cancer samples, including matched *in situ* and invasive components, and reported a significant decrease in stromal Cav-1 expression in progression of ductal carcinoma *in situ* (13%) to invasive cancer (76%). Wiechen *et al* (28) evaluated Cav-1 expression in normal mesenchymal tissue, benign mesenchymal tumors and sarcoma and reported that Cav-1 expression is increased in normal mesenchymal tissue and benign mesenchymal tumors but decreased in the majority of sarcomas of certain histological types, such as fibrosarcoma, leiomyosarcoma, angiosarcoma, malignant fibrous histiocytoma and synovial sarcoma. In the present study, although differences were not significant, stromal Cav-1 expression notably decreased with increasing PT grade. Due to the small number of borderline and malignant cases in the present study, these results need to be evaluated in additional case series.

In the present study, high stromal MCT1 expression was associated with lower disease-free survival rate compared with low stromal MCT1 expression. This suggested that stromal MCT1 expression may be associated with a more aggressive phenotype in PTs and may serve as marker of a poor prognosis in patients with PTs. Kwon *et al* (20) observed that stromal MCT4 expression is associated with shorter disease-free and overall survival in patients with PTs. In breast cancer, low Cav-1 expression in cancer-associated stroma, high MCT1 expression in cancer cells and high MCT4 expression in cancer-associated stroma or cancer cells are associated with poor prognostic clinicopathological factors and patient outcomes (13,14,16-18). In soft tissue sarcoma, expression of MCT1 and MCT4 is associated with poor prognostic parameters such as high tumor grade, disease progression and shortened overall survival (26). However, in the present study, expression of Cav-1 and MCT4 was not associated with recurrence. Given the potential prognostic value of Cav-1 or MCT4 in patients with PT, further studies in large cohorts of patients with PT with longer follow-up period are needed.

The present study highlighted that MCT1 was upregulated in a subset of patients with PT recurrence, however these patients do not have effective treatment options (3-6). The development of therapies targeting MCT1 may be a promising strategy for treating relapsed PTs. Further studies, including *in vitro* approaches, are needed to assess this hypothesis.

There were a few limitations in the present study. One limitation was the small number of borderline and malignant PT cases. In addition, the number of recurrences was low during a relatively limited follow-up time, which restricted the correlation with outcome. Additionally, the assessment of protein expression was somewhat limited using TMA technology, which uses only a small part of tumor samples. However, the significance of this limitation was reduced by including two 2 mm-sized representative cores per case to account for tumor heterogeneity and possible sampling issues.

In conclusion, the current study demonstrated that MCT 1 and MCT4 were involved in progression of PTs and stromal MCT1 expression was associated with recurrence of PTs. Efforts are needed to develop therapeutic approaches targeting metabolic coupling, specifically MCT1, for treatment of PTs.

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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

NK and JL conceived the experiments. MP prepared the samples. NK, MP and JL performed the experiments and analyzed the data. SSK performed statistical analysis and edited the manuscript. NK and JL wrote the manuscript. NI and JL confirm the authenticity of all the raw data. All authors read and approved the final manuscript.

Ethics approval and consent to participate

This retrospective study utilized archived normal and PTs tissues and did not impact patient care; approval was granted by the Institutional Review Board of Chonnam National University Hwasun Hospital (Jeollanam, South Korea) and the requirement for patient consent was waived at the time of tissue collection. (approval no. CNUHH-2018-068).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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