Association between fatty acid synthase and adipophilin expression in triple-negative breast cancer

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Abstract. It is well known that cancer cells produce energy via anaerobic glycolysis. Lipid metabolism is often upregulated in numerous types of cancer. Our previous study demonstrated that adipophilin (ADP), a lipid-associated protein, was a poor prognostic indicator in patients with triple-negative breast cancer (TNBC). However, the mechanism of ADP expression in TNBC remains unclear. Fatty acid synthase (FASN) is a crucial enzyme in *de novo* fatty acid synthesis, and its upregulation has been reported in several types of carcinomas; however, to the best of our knowledge, the association of FASN and ADP in TNBC remains unclear. The present study analysed the association between FASN and ADP expression and the prognostic significance of FASN in TNBC. Using immunohistochemical methods and tissue microarrays, the present study examined FASN expression in 61 patients with TNBC. Overall and relapse-free survival and their risk factors were analysed for FASN expression and compared with ADP expression. A total of 40 (65.6%) patients were classified as FASN-high (score ≥120), and this was significantly associated with a lower Ki-67 labelling index (P=0.011). FASN expression was not associated with relapse-free survival and overall survival. FASN-high was negatively associated with ADP expression (P=0.041). The results of the present study revealed that FASN-high was associated with a lack of ADP expression and a lower Ki-67 labelling index. These results indicated that *de novo* fatty acid synthesis by FASN is not the main pathway of lipogenesis and the source of energy in cancer cells of ADP-positive highly proliferative TNBC.

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Abbreviations: ADP, adipophilin; FASN, fatty acid synthase; HER2, human epidermal growth factor receptor 2; LI, labelling index; OS, overall survival; RFS, relapse-free survival; TNBC, triple-negative breast cancer

Key words: triple-negative breast cancer, adipophilin, fatty acid synthase, lipid metabolism

Introduction

Triple-negative breast cancer (TNBC), characterised by the lack of oestrogen and progesterone receptors and human epidermal growth factor receptor 2 (HER2) expression, occurs in approximately 12-17% of breast cancer patients (1,2). It shows an aggressive clinical behaviour and a high rate of local and distant relapse after treatment compared to other subtypes of breast cancer (2-4). Therefore, there is an urgent need to develop new treatments and biomarkers for TNBC. While normal cells mainly produce energy by aerobic phosphorylation through the tricarboxylic acid cycle, cancer cells produce energy via anaerobic glycolysis and other metabolic pathways. Oncogenic metabolic pathways differ depending on the tumour type; therefore, developing therapies against tumour metabolism is not straightforward (5). Lipid metabolism is a crucial pathway in tumour progression, and cancer cells typically accumulate lipids (6,7).

Adipophilin (ADP) is a lipid-associated protein that coats the surface of intracytoplasmic lipid droplets (8,9) ADP expression in tumour cells is correlated with a poor prognosis in some types of carcinomas, including lung adenocarcinoma (10) and pancreatic ductal adenocarcinoma (11). Recently, we demonstrated via multivariate analysis that ADP expression is an independent indicator of a poor prognosis for patients with TNBC, while widely used prognostic factors, such as the Ki-67 labeling index (LI) and the Nottingham Prognostic Index, and tumor size were not independent (12). Fatty acid synthase (FASN) is a critical lipogenic enzyme overexpressed in various human cancers, including salivary gland tumours (13,14). FASN expression has been reported to be associated with a poor prognosis in several types of tumours (15,16); thus, ADP expression in carcinoma cells might be related and occur via overexpression of FASN. In addition, FASN expression has been reported to correlate with the frequency of lymph node metastasis but is uncorrelated with prognosis in TNBC (17). Although an inverse correlation between FASN and ADP expression has been reported in salivary duct carcinomas (18), the relationship between FASN and ADP in TNBC remains unclear. The present study aimed

to evaluate the prognostic role of FASN expression and assess the correlation between FASN and ADP expression in TNBC patients.

Materials and methods

Patient selection. We selected 165 consecutive patients with TNBC who underwent surgical resection at the Department of Surgery of the Kansai Medical University Hospital between January 2006 and December 2018. Patients who were diagnosed with invasive breast carcinoma of no special type according to the recent World Health Organization Classification of Breast Tumors (19) were selected. The exclusion criteria of the present study were as follows: patients who were administered neoadjuvant chemotherapy and who had a particular type of invasive carcinoma, such as apocrine carcinoma. The study cohort comprised 61 TNBC patients.

The patient cohort in the present study overlaps with that of our previous studies (12,20,21). Our previous study analysed the prognostic significance of ADP expression in tissue microarrays using operative specimens from patients with TNBC (12). The present study included information regarding the ADP expression status of operative specimens from the previous study (12). Moreover, we previously examined the relationship between clinicopathological features and PD-L1-positive cancer-associated fibroblasts (20) or CD155, an immune-checkpoint protein (21), in patients with TNBC using tissue microarrays from operative specimens. The contents of the present study do not overlap with those of these two studies (20,21).

This retrospective single-institution study was conducted following the principles of the Declaration of Helsinki, and the study protocol was approved by the Institutional Review Board of the Kansai Medical University Hospital (Approval #2019234). All the data were anonymised. The institutional review board waived the requirement for informed consent because of the retrospective design of the study using medical records and archival samples, with no risk to the participants. Moreover, the present study does not include minors. Information regarding this study, such as the inclusion criteria and opportunity to opt out, was provided through the institutional website (https://www.kmu.ac.jp/hirakata/hospital/2671t800000136cd-att/a1582783269511.pdf).

Histopathological analysis. Surgically resected specimens were fixed with formalin, sectioned, and stained with hematoxylin and eosin. More than two experienced pathologists independently evaluated histopathological features. We used the TNM Classification of Malignant Tumours, Eighth edition. The histopathological grading was based on the Nottingham histological grade (22). The Ki-67 labelling index (LI) was considered high when ≥40% of neoplastic cells were labelled (23).

Tissue microarray. Hematoxylin and eosin-stained slides were used to select the most morphologically representative carcinoma regions; three tissue cores of 2 mm in diameter were punched out from the paraffin-embedded blocks for each patient. Tissue cores were arrayed in the recipient paraffin blocks. These specimens were also used in our previous study (12,20,21).

Immunohistochemistry. Immunohistochemical analyses were performed using an autostainer (Discovery Ultra System; Roche Diagnostics, Basel, Switzerland) according to the manufacturer's instructions [OptiView DAB Universal Kit (cat. no. 518-111427; Roche)]. Primary mouse monoclonal antibody against FASN (clone 23: BD Biosciences; diluted 1:200) was used. Secondary antibody was pre-diluted [OptiView DAB Universal Kit (cat. no. 518-111427; Roche)]. At least two researchers independently evaluated immunohistochemical staining.

FASN was analysed using a combined scoring system based on the proportion of positive tumour cells (0-100%) and the predominant staining intensity in the tumour (18,24). The FASN staining intensity was scored as follows: 0, negative; 1, weak; 2, moderate; 3, strong (Fig. 1). The FASN score (0-300) was calculated by multiplying the percentage by the staining intensity. FASN was classified into two groups based on the FASN score: low (<120) and high (≥120), according to a previous report (18).

Statistical analysis. All analyses were performed using SPSS Statistics 27.0 (IBM, Inc.). Correlations between two groups were determined using the chi-squared test or Fisher's exact test for categorical variables and the Mann-Whitney U test for continuous variables. The rates of relapse-free survival (RFS) and overall survival (OS) were evaluated using Kaplan-Meier analysis. Log-rank tests were used to compare the groups. The statistical significance was set at $P<0.05$.

Results

Patient characteristics. Table I summarises the clinicopathological features of the present cohort. The cohort of this study is fundamentally identical to that previously reported regarding ADP expression in TNBC (12). This study included 61 women with TNBC. The median age at the time of initial diagnosis was 58 years (range, 31-93 years). All patients were diagnosed with TNBC based on biopsy results. All samples were invasive carcinomas of no special type. No discrepancy was found in the pathological diagnosis and molecular subtype between the preoperative biopsy and operative specimens. The median observation period was 61 months (range: 11-173 months). Eleven (18.0%) patients experienced relapse (all had distant metastasis, and none experienced local recurrence), and nine (14.3%) patients died of the disease.

Correlation between clinicopathological factors and FASN expression. Table II shows the correlation between FASN expression and the clinicopathological factors in the study cohort. Forty patients (65.6%) were FASN-positive and 21 (34.4%) were FASN-negative. Typically, FASN expression was observed in the cytoplasm of neoplastic cells (Fig. 1).

FASN expression did not correlate with any clinical factors, including age, menopausal status, body mass index, or adjuvant chemotherapy. A lower Ki-67 LI was significantly correlated with FASN expression ($P=0.011$), but not with other factors, such as tumour diameter, pathological stage, histological grade, lymphatic and venous invasion, or lymph node status.

Table I. Clinical characteristics of patients with triple-negative breast cancer.

Factors	Value
Total, n	61
Median age, years (range)	68 (31-93)
Menopausal status, n (%)	
Premenopausal	9 (14.8)
Postmenopausal	51 (83.6)
Unknown	1 (1.6)
Median BMI (range)	23.3 (16.2-32.2)
Median tumor size, mm (range)	20 (2-55)
Pathological stage, n (%)	
I	25 (41.0)
IIA	23 (37.7)
IIB	5 (8.2)
IIIA	4 (6.6)
IIIB	3 (4.9)
IIIC	1 (1.6)
Lymph node status, n (%)	
Positive	14 (23.0)
Negative	33 (54.1)
Not tested	14 (23.0)
Lymphatic invasion, n (%)	
Positive	53 (86.9)
Negative	8 (13.1)
Venous invasion, n (%)	
Positive	37 (60.7)
Negative	24 (39.3)
Nottingham histological grade, n (%)	
1	2 (3.3)
2	27 (44.3)
3	32 (52.5)
Ki-67 labeling index, n (%)	
High	37 (60.7)
Low	21 (34.4)
Not tested	3 (4.9)
Adjuvant chemotherapy, n (%)	
Performed	35 (57.4)
Not performed	23 (37.7)
Undetermined	3 (4.9)

Correlation between FASN expression and prognosis. The median RFS of FASN-high and -low patients was 53 and 64 months, respectively, and the median OS of FASN-high and -low patients was 59 and 64 months, respectively. FASN expression was not correlated with RFS or OS (Fig. 2, P=0.611 and P=0.727, respectively).

Correlation between ADP and FASN expression. As previously reported, ADP expression was positive in 14 patients (23%) and negative in 47 patients (77%) (12). The correlations between ADP and FASN expression are shown in Table III. A significant negative correlation was observed between ADP and FASN expression (P=0.041).

Discussion

The present study demonstrated that FASN-high was significantly negatively correlated with ADP expression and a lower Ki-67 LI. FASN expression was not correlated with RFS and OS in patients with TNBC.

Fatty acids are essential components of all cells as they constitute the lipid membrane and are important substrates for energy metabolism. FASN synthesises long-chain fatty acids using acetyl-CoA as a primer, malonyl-CoA as a two-carbon donor, and the predominant product of this enzyme is a 16-carbon fatty acid, palmitate (13). Under normal conditions, FASN converts excess carbohydrates into fatty acids, leading to esterification to store triacylglycerols. In non-neoplastic tissues, FASN expression is observed in the high lipid metabolic tissues, including adipocytes, hepatocytes, sebaceous glands, and hormone-sensitive tissues, such as the endometrium, prostate, and adrenal cortex, and its expression is low in other non-neoplastic cells (24). It is well known that lactic acid synthesis via anaerobic glycolysis is highly upregulated in cancer cells (Warburg effect), and excess pyruvate is synthesised for *de novo* fatty acid synthesis via acetyl-CoA to maintain cell membrane production in proliferative cancer cells (13). Therefore, upregulation of FASN has been reported in some types of carcinomas (25,26), including non-small cell lung cancer (27), oral squamous cell carcinoma (28), colon cancer (15), bladder cancer (16), salivary gland tumour (18) and malignant melanoma (29).

In breast cancer, FASN expression has been addressed in some studies. FASN expression was significantly higher in the HER2 subtype and lower in the luminal subtype and TNBC (30). In one report, high FASN was significantly correlated with lymph node metastasis but not with pathological stage, tumour cell proliferative activity (Ki-67), and disease-free and OS in patients with TNBC (17). In another report, FASN expression was significantly correlated with pathological stage and lymph node metastasis in patients with TNBC (31). In the present cohort, FASN-high was significantly correlated with a lower Ki-67 LI and was not correlated with patient prognosis.

Interestingly, ADP expression was significantly negatively correlated with FASN expression. A previous study demonstrated that ADP expression was significantly correlated with a higher Ki-67 LI (12); therefore, lower FASN expression was significantly correlated with ADP expression and a higher Ki-67 LI. The correlation between ADP and FASN has only been evaluated in salivary duct carcinoma, a highly aggressive type of salivary gland carcinoma (18), and the present study is the first to address this correlation in TNBC. In salivary duct carcinoma, ADP expression was also a significantly poor prognostic marker of progression-free and OS by multivariate analysis and was negatively correlated with FASN expression, which is consistent with the results of our present and previous studies in patients with TNBC (12). These results suggest that *de novo* fatty acid synthesis by FASN is not the main pathway of lipogenesis and a source of energy for cancer cells in ADP-positive highly proliferative TNBC and salivary duct carcinoma.

Table II. Association between clinicopathological factors and FASN expression.

Factors	FASN-high (n=40)	FASN-low (n=21)	P-value
Age, years (median ± SD)	64±15	66±15	0.773
Body mass index, kg/m^2 (median ± SD)	23.5±3.5	23.3±4.1	0.820
Menopausal status, n			
Premenopausal	6	3	>0.999
Postmenopausal	34	17	
Unknown	0	1	
Tumor size, n			
≤20 mm	16	13	0.104
>20 mm	24	8	
Pathological stage, n			
I+II	34	19	0.703
III	6	2	
Lymph node status, n			
Positive	11	3	0.321
Negative	20	13	
Not tested	9	5	
Lymphatic invasion, n			
Positive	33	20	0.243
Negative	7	1	
Venous invasion, n			
Positive	25	12	0.684
Negative	15	9	
Nottingham histological grade, n			
1+2	19	10	0.993
3	21	11	
Ki-67 labeling index, n			
High	19	18	0.011
Low	18	3	
Not tested	3	0	
Adjuvant chemotherapy, n			
Performed	24	11	0.546
Not performed	14	9	
Undetermined	2	1	

FASN, fatty acid synthase.

ADP expression reflects the intracellular lipid accumulation in cancer cells (10-12,18). ADP expression was significantly associated with higher proliferative activity in cancer cells in breast cancer, including TNBC (12,32) and salivary duct carcinoma (18). Thus, ADP expression might be associated with higher proliferative activity, leading to a poor prognosis. Although the detailed mechanism of ADP expression in cancer cells remains unclear, ADP expression in cancer cells might reflect upregulation of lipid metabolism correlating with a higher proliferative capacity and production of cell membranes of cancer cells in a hypoxic tumour microenvironment (12). As described earlier, FASN is well known to be a central enzyme complex in *de novo* fatty acid synthesis, and both ADP and FASN have been known to be activated under hypoxic conditions (13,33,34). ADP expression was significantly negatively correlated with FASN expression in TNBC and salivary duct carcinoma (18). Therefore, lipid accumulation in TNBC and salivary duct carcinoma was not correlated with upregulation of *de novo* fatty acid synthesis. Lipid acumination can be derived from lipid uptake and neutral lipid synthesis (35). Thus, the mechanism of ADP expression in TNBC other than the FASN pathway must be clarified to address the new therapeutic strategy in ADP-positive TNBC patients with a poorer prognosis.

Although the prognostic significance of FASN expression in TNBC remains controversial, FASN is considered a potential therapeutic target (36). It has been shown that blocking FASN has anticancer effect via the apoptotic pathway *in vitro* and

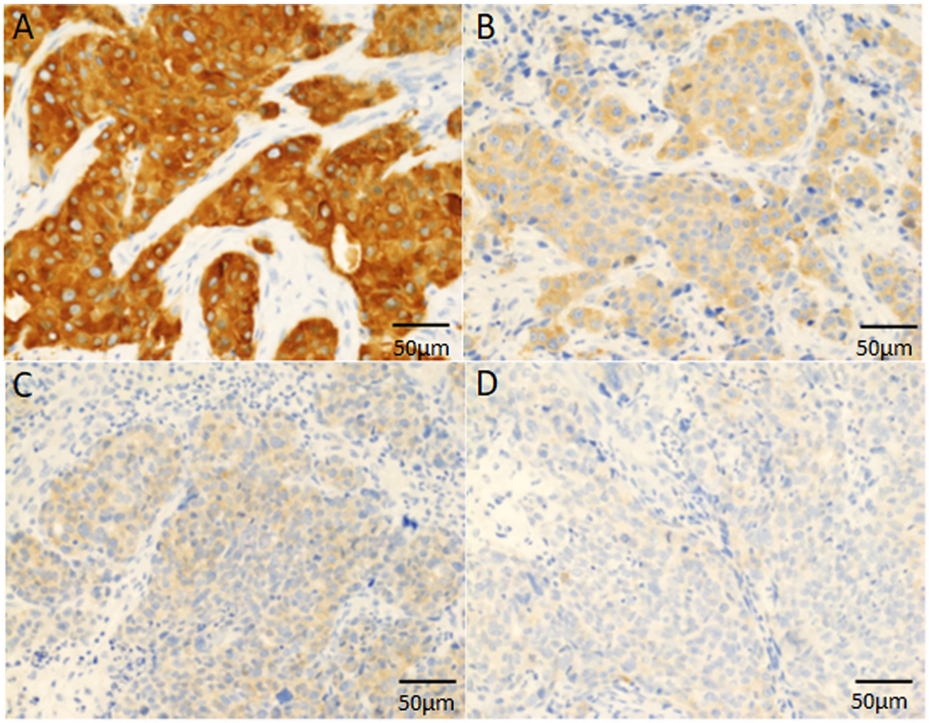

Figure 1. Typical immunohistochemical features of fatty acid synthase. (A) Strong, (B) moderate, (C) weak and (D) negative expressions. Magnification, x400. Scale bar, 50 μm.

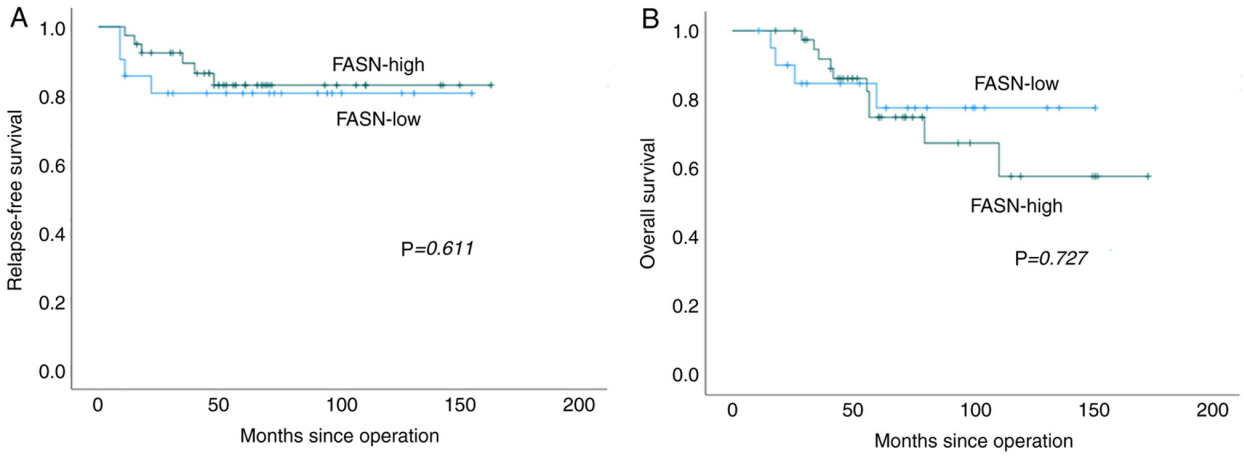

Figure 2. Kaplan-Meier curves of (A) relapse-free and (B) overall survival in patients in the FASN-high (green) and FASN-low (blue) groups. FASN, fatty acid synthase.

Table III. Association between adipophilin and fatty acid synthase expression.

	Fatty acid synthase		
Adipophilin	High, n	Low, n	P-value
Positive	6	8	
Negative	34	13	0.041

in vivo (37-39). Moreover, the effectiveness of simultaneous blocking of FASN and epidermal growth factor receptors has also been reported in preclinical models of chemoresistant TNBC (40). The usefulness of orlistat, an anti-obesity drug, in epidermal growth factor receptor mutated non-small cell lung cancer has also been reported (28). Accordingly, FASN can be a potential therapeutic target for patients with TNBC, and the detailed mechanism of FASN expression and correlation of ADP expression in TNBC using both experimental animal model and human cultured cells must be clarified.

There are some limitations to the present study. First, this was a retrospective single-institution study with a small sample size, which could have led to selection bias. Second, tissue microarray cores of 2 mm diameter were used to determine FASN and ADP expression. Hence, there could have been a heterogeneous expression in the cancer tissues, despite our

selection of regions that were morphologically most representative of cancer. Third, since chemotherapy may affect FASN expression, this study excluded patients who had undergone neoadjuvant chemotherapy. Therefore, additional studies with larger patient populations are needed to clarify these issues.

In conclusion, FASN expression was significantly negatively correlated with ADP expression in TNBC. ADP expression reflects lipid acumination in cancer cells; however, its mechanism other than *de novo* lipogenesis synthesised by FASN via acetyl-CoA might be present. Thus, additional studies are needed to analyse the mechanism of ADP expression, a significantly poor prognostic marker, leading to a new therapeutic strategy for patients with ADP-positive TNBC.

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Availability of data and materials

All data generated or analysed during this study are included in this published article.

Authors' contributions

KY and MI conceived and designed the study. KY and MI performed immunohistochemical analyses. KY, MI, HY, KT, MS and TS acquired and analyzed data. KY and MI confirm the authenticity of all the raw data. KY and MI drafted the manuscript and prepared tables and figures. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

The present study was conducted in accordance with the Declaration of Helsinki, and the study protocol was approved by the Institutional Review Board of the Kansai Medical University Hospital (protocol no. 2019234; Hirakata, Osaka, Japan). The institutional review board waived the requirement for informed consent.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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