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Adipose Triglyceride Lipase (ATGL) Expression Is Associated with Adiposity and Tumor Stromal Proliferation in Patients with Pancreatic Ductal Adenocarcinoma

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Abstract. *Background: Obesity is an established risk factor for the development of pancreatic ductal adenocarcinoma (PDAC). However, the pathophysiology of how increased adiposity increases the risk for PDAC has not been fully elucidated. Adipose triglyceride lipase (ATGL) is a lipase that catabolizes triglyceride hydrolysis and has been implicated in the development of breast cancer. We hypothesized that overweight patients with PDAC would demonstrate higher tumor ATGL expression compared to non-overweight patients with PDAC. Materials and Methods: Immunohistochemical analysis for ATGL expression was performed on PDAC tissues from 44 patients after Whipple procedure or distal pancreatectomy. Correlation of ATGL expression with clinicopathological features was evaluated. Results: A total of 23/44 (52.2%) PDACs showed low level ATGL immunoreactivity, while 21/44 (47.8%) showed a high level, with moderate to strong positive ATGL immunoreactivity in more than 50% of the tumor cells. Chi-squared testing revealed a statistically significant association between high ATGL expression and both BMI >25 kg/m^2 (χ^2=5.74, p=0.017) and increased tumor stroma (χ^2=19.14, p<0.001). Chi-squared testing failed to reveal a statistically significant association when comparing ATGL expression by lymph node metastasis, histological grade, tumor size, patient age, patient sex and presence of fat invasion. Conclusion: Our results suggest that increased ATGL expression is associated with increased adiposity and stromal proliferation in patients with PDAC, making it a possible key protein in how obesity increases the risk of PDAC.*

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Pancreatic ductal adenocarcinoma (PDAC) carries an extremely poor prognosis, with a 5-year survival rate of less than 6% in the United States (1). The low survival rate is due to the late stage at which patients are usually diagnosed, with only 20% of patients being eligible for tumor resection at the time of diagnosis (1). Several risk factors for PDAC are firmly established, such as chronic pancreatitis (2), cigarette smoking (3), family history of pancreatic cancer (4), and diabetes mellitus (5).

Both epidemiological and experimental studies have also linked obesity to the development of PDAC. In a large case–control study, Silverman *et al.* (6) showed obesity portends a 50-60% increased risk of PDAC in both men and women. Similarly, a pooled case–control analysis using data from the Pancreatic Cancer Cohort Consortium (PanScan) comparing 2170 PDAC cases to 2209 controls showed a positive association between increasing body mass index (BMI) and risk of PDAC [adjusted odds ratio (aOR) for the highest *vs.* lowest BMI quartile=1.33, 95% confidence interval (CI)= 1.12-1.58, $p<0.001$ (7).

The mechanism of how obesity increases PDAC risk is poorly understood. Previous studies have shown that increased peritumoral fat density promotes metastasis and increases mortality in patients with PDAC (8). Adipose triglyceride lipase (ATGL) is a lipase in adipose tissue that catabolizes the first step in triglyceride hydrolysis and is responsible for the release of free fatty acids. Another lipase called monoacylglycerol lipase has been shown to be overexpressed in human cancer cell lines and to promote *in vivo* tumor growth *via* 'feeding' cancer cells with free fatty acids (9). Previous studies of breast cancer demonstrated increased peritumoral ATGL expression in fat cells of obese

patients compared to non-obese patients, suggesting that increased adiposity contributes to increased tumor growth (10). However, currently no studies have investigated the relationship between ATGL expression and obesity in patients with PDAC. We hypothesized that overweight patients with PDAC would demonstrate higher tumor ATGL expression compared with non-overweight patients with PDAC.

Materials and Methods

Patients and clinicopathological data. This study was approved by the Institutional Review Board at Saint Louis University (St. Louis, MO) (approval number 25281). Clinicopathological information was collected for each patient from 2010 to 2014 *via* retrospective review of the pathology database. Patient demographics included age, sex and BMI). A BMI over 25 kg/m^2 was considered overweight and BMI ≤25 kg/m^2 was considered non-overweight. We chose BMI of 25 kg/m^2 as a cutoff because we had very few patients with BMI >30 kg/m^2 (n=4, 9%). Tumor characteristics included size, histological grade, tumor (T) stage, and nodal status. Tissue arrays were constructed using 33.1 and 20 mm^2 of PDAC tumor tissue from 44 patients and used for immunohistochemistry of ATGL.

Immunohistochemistry (IHC). Analysis by IHC was performed on 4-μm thick sections using antibodies for ATGL (Cell Signaling Technology, MA, USA) as previously described (Figure 1) (11). IHC stains were graded on a scale from 0 to 3: score 0 represented absence of staining; score 1 represented <30% of tumor cells with positive staining; score 2 represented between 30-50% of tumor cells with positive staining; and score 3 represented >50% tumor cells with positive staining. Each specimen with a score ≥1 was also given a density score. High ATGL expression was characterized as moderate-to-strong staining density present in more than 50% of the tumor cells. Additionally, each specimen was analyzed for the presence or absence of fat invasion by tumor. The amount of stroma within each tumor specimen was also scored on a scale from 0 to 3, depending on the ratio of stroma to malignant glands: score 0 represented absence of stroma; score 1 represented a stroma/tumor cell ratio of <30%; score 2 represented a stroma/tumor cell ratio between 30-50%; and score 3 represented a stroma/tumor cell ratio of >50%. Scoring of the immunostain was blindly performed by two board-certified gastrointestinal pathologists (JL and YC).

Statistical analysis. Statistical analyses were performed using SPSS software, version 23.0 (IBM Corp., Armonk, NY, USA). Correlation between ATGL expression and clinicopathological characteristics were evaluated by the chi-square test. *p*-Values less than 0.05 were considered statistically significant.

Results

Patient and tumor characteristics. Patient demographic and tumor characteristics are presented in Table I. ATGL levels were analyzed in a total of 44 patients with PDAC. The mean age of our study population was 66.5 years (range=43-84 years). The mean BMI was 24.2 kg/m^2 (median=24.6 kg/m^2; range=15.8-39.5 kg/m^2) and the mean tumor size was 3.5 cm (range=1.2-6.4 cm). The majority of the tumors were moderately differentiated (G2) (26/44, 59%); a minority were well-differentiated (G1) (11/44, 25%) or poorly differentiated (G3) (7/44, 16%).

Table I. *Clinicopathological correlation of adipose triglyceride lipase (ATGL) expression in 44 patients with pancreatic ductal adenocarcinoma.*

Variable	Low ATGL, n	High ATGL, n	χ^2	*p*-Value
Age at diagnosis (years)			0.002	0.989
≥65	12	11		
<65	11	10		
Gender			0.091	0.763
Male	12	10		
Female	11	11		
Body mass index (kg/m^2)			5.74	0.017
>25	6	13		
≤25	17	8		
Tumor size (cm)			0.878	0.349
≥3.5	12	8		
<3.5	11	13		
Nodal metastasis			0.781	0.377
Yes	4	6		
No	19	15		
Tumor fat invasion			0.71	0.400
Yes	18	14		
No	20	10		
Tumor score			2.17	0.338
1	7	4		
2	14	12		
3	2	5		
Stroma score			19.14	<0.001
1	5	1		
2	17	6		
3	1	14		

n, Number of patients.

PDACs of overweight patients had higher ATGL expression compared to those from non-overweight patients. IHC analysis was performed to compare tumor ATGL expression in overweight patients (BMI >25 kg/m^2) compared to non-overweight patients (BMI ≤25 kg/m^2) (Table I). Six cases (6/44, 13.6%) showed negative-to-weakly positive ATGL staining in fewer than 30% of the tumor cells. Seventeen cases (17/44, 38.6%) showed weak-to-moderate ATGL expression in 30-50% of the tumor cells. Twenty-one cases (21/44, 47.8%) showed moderate-to-strong ATGL expression in more than 50% of the tumor cells. Twenty-three cases (23/44, 52.2%) with a score 1-2 were considered to have low expression and 21 (21/44, 47.8%) were considered to have high expression. Chi-squared testing revealed a statistically significant association between high ATGL expression and BMI over 25 kg/m^2 (p=0.017; Table I).

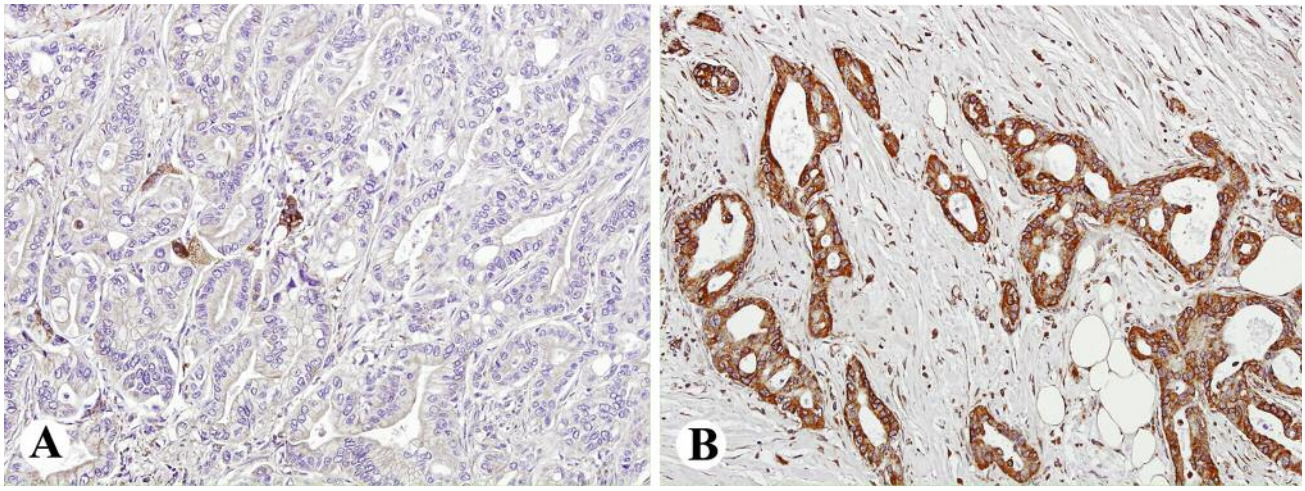

Figure 1. *A: A case of moderately differentiated pancreatic ductal adenocarcinoma with minimal desmoplasia, showing essentially negative reactivity for antibody to adipose triglyceride lipase (ATGL). B: Another case of moderately differentiated PDAC with marked desmoplasia, showing a high level of ATGL immunoreactivity. Original magnification, ×200.*

PDACs with higher desmoplasia showed higher ATGL expression compared to PDACs with low desmoplasia. ATGL expression was compared between the PDACs with low desmoplasia (Figure 1A) and high desmoplasia (Figure 1B). Chi-squared testing revealed a statistically significant association between high ATGL expression and increased tumor stroma ($p<0.001$; Table I).

IHC analysis was also performed to compare ATGL expression in tumors without fat invasion compared to those with. When tumors demonstrated fat invasion, ATGL grading was performed on the tumor cells directly contiguous to the fat cells rather than the whole tumor. Chi-squared testing failed to identify a statistically significant association between high ATGL expression and the presence of fat invasion ($p=0.400$).

No statistically significant association was found when comparing ATGL expression by lymph node metastasis or histological grade ($p>0.05$). Additionally, no significant association was found when comparing ATGL expression in tumors <3.5 cm to tumors ≥3.5 cm, patients older than 65 compared to those younger, or by patient sex ($p<0.05$; Table I).

Discussion

Adipose tissue is a complex endocrine organ that regulates fatty acid metabolism and secretes cytokines and hormones that affect distant targets. Strong evidence suggests that obesity is implicated in the etiology of many different cancer types, including PDAC (12, 13). Increased adiposity has also been shown to lead to poorer outcomes in a variety of cancer types. However, the pathophysiology behind how obesity increases risk and leads to poorer outcomes for pancreatic cancer has not been fully elucidated. ATGL is a lipase in adipose tissue that catabolizes the first step in triglyceride hydrolysis and is responsible for the release of free fatty acids. In a study of patients with breast cancer, Gnerlich *et al*. found increased ATGL expression in the peritumoral and distant fat of obese patients compared to non-obese patients (10). As far as we are aware, the current study is the first to examine the relationship between tumor ATGL expression and PDAC. We found increased ATGL expression in the PDACs of overweight patients compared to non-overweight patients, suggesting that ATGL could be a key enzyme in explaining how obesity increases PDAC risk. Additionally, we found an association between tumor ATGL expression and the amount of stromal proliferation (desmoplasia), suggesting ATGL might mediate a desmoplastic response.

Cancer cells reprogram their metabolic pathways to meet increased demand for proliferation (14). One source of energy is lipids and altered lipid metabolism in oncogenesis has been studied extensively over the past decade. Cancer cells mostly depend on endogenous *de novo* synthesis for fatty acids, as evidenced by increased activity of intracellular enzymes involved in lipogenesis such as ATP citrate lyase, acetyl-CoA carboxylase and fatty acid synthase (14-16). Lipid metabolism in PDAC has not been studied in the human population. However, lipids are likely to be an important source of energy for PDAC tumor cells. Human PDAC cell lines incubated in the presence oleic and linoleic acid demonstrated increased proliferative rates (17), which could be oncogenic *via* an increase in reactive oxygen species (18). Philip *et al*. showed pancreatic tissue from mice

that were fed a high-fat diet demonstrated increased oncogenic KRAS activity and accelerated pancreatic intraepithelial neoplasia development compared to mice on a low-fat diet, suggesting that dietary lipids may contribute to pancreatic cancer (19). Gnerlich *et al*. found increased ATGL expression in both peritumoral and tumor-free distant fat in obese patients with breast cancer, suggesting that ATGL might be implicated in how obesity increases the risk for breast cancer (10). Our study is the first to demonstrate increased ATGL expression within tumor cells of PDAC in overweight patients (BMI >25 kg/m^2).

Additionally, this is also the first study to demonstrate an association between ATGL expression in tumor cells of PDAC and intratumoral stromal content in a human population. Histopathologically, PDAC consists of malignant glands infiltrating into fibrotic stroma composed mostly of extracellular matrix, which consists of type 1 collagen, fibronectin, hyaluronan and proteoglycans (20). Pancreatic stellate cells (PSCs) are resident cells of the pancreas, comprise approximately 5% of the pancreatic parenchyma (21) and have the important function of maintaining the balance of extracellular matrix synthesis and degradation (22). PSCs undergo a phenotypic transformation from a quiescent state to a myofibroblast-like state during times of pancreatic injury in response to stressors such as ethanol, oxidants and various cytokines (21-24). The results of our study suggest that ATGL might mediate an increase in tumor stroma, possibly by increasing free fatty acid content in the tumor microenvironment, thereby contributing to the phenotypic change of PSCs into an extracellular matrix-secreting state.

In summary, obesity is a well-established risk factor for the development PDAC. However, the pathophysiology of how increased adiposity increases the risk of PDAC is currently not well understood. Our results suggest that increased ATGL expression is associated with increased adiposity and stromal proliferation in patients with PDAC, making it a possible key protein in how obesity increases the risk of PDAC. Further studies to elucidate the role of ATGL in PDAC development and progression are warranted.

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