RESEARCH ARTICLE

Expression of autophagy-related proteins Beclin-1 and LC3A and proliferation marker Ki-67 in calculous and acalculous human gallbladder epithelium

Oikonomou P[1], Giatromanolaki A[2], Tsaroucha AK[1], Balaska K[2], Tsalikidis CH[1], Nikolaou CH[1], Pitiakoudis M[1], Simopoulos C[1]

[1]2nd Department of Surgery and Laboratory of Experimental Surgery & Surgical Research
[2]Department of Pathology
Medical School, Democritus University of Thrace, Alexandroupolis, Greece

Abstract

Background: Autophagy is an inducible intracellular process that has been studied mostly in cancer and less in inflammatory diseases. To establish the relation between cholecystitis (calculous and acalculous) and autophagy, we studied the expressions of immunohistochemical markers Beclin-1, LC3A, and Ki-67 in gallbladder epithelium and their significance in the induction of autophagy.

Methods: Adult human gallbladder tissues were obtained from 100 patients (45 male, 55 female) who underwent cholecystectomy. According to the findings, the patients were divided into two groups: group A (calculous gallbladder: 24 male, 46 female; mean age 52.6 ± 16.0 years) and group B (acalculous gallbladder: 21 male, nine female; mean age 65.3 ± 12.4 years). The expressions of immunohistochemical markers Beclin-1, LC3A, and Ki-67 in gallbladder epithelium were studied using immunohistochemistry techniques.

Results: Beclin-1 expression was correlated with LC3A expression in group A with increased Beclin-1 expression promoting LC3A expression (p =0.0001). In group B, the LC3A expression did not follow Beclin-1 expression (p =0.09). The mean percentage of Beclin-1 expression in group A patients was 23.8 % compared to group B patients, where the corresponding percentage was only 17.3 %. Corresponding mean percent expressions of LC3A in groups A and B were 38.9 % and 50.7 %, respectively. The expression of Ki-67 was higher in group A patients compared to group B patients. The mean percentage of Ki-67 expression in group A patients was 3.75 %, whereas, in group B patients, it was only 0.5 % (statistically significantly different; p =0.0003).

Conclusion: In the epithelium of calculous cholecystitis, overexpression of LC3A is related to Beclin-1 overexpression, which reinforces the view that Beclin-1 promotes autophagy in stone cholecystitis. HIPPOKRATIA 2019, 23(2): 64-69.

Keywords: Autophagy, gallbladder epithelium, Beclin-1, cholecystitis, Ki-67, LC3A, lithogenesis

Corresponding author: Panagoula Oikonomou, MD, 2nd Department of Surgery, Medical School, Democritus University of Thrace, 68100, Dragana, Alexandroupolis, Greece, tel: +306985577973, e-mail: pen.ek@hotmail.com

Introduction

Cholelithiasis is a common disease in the western population, causing cholecystitis, occurring mostly in aged and female patients. Significant risk factors for cholelithiasis include gallbladder hypomotility, hyperlipidemia, obesity, and total parenteral nutrition. The gallstone disease is accompanied by inflammation of the gallbladder epithelium, mainly due to irritation and chemical injury[1]. More frequent gallstones in the western population are cholesterol stones. The physicochemical basis of cholesterol gallstone formation in humans is well studied. Lithogenesis is a multifactor procedure, which was initially researched by Admirand and Small in 1968[2]. Inflammation, infection, and hypoxia constitute situations that promote autophagy. Contrariwise, the first breakthrough in autophagy research was made in the early 1990s[3].

Autophagy is an inducible intracellular process, which is involved in cellular homeostasis, recycling, and removal of damaged organelles and intracellular pathogens, promoting cellular viability. In order to be maintained, tissue integrity and function need appropriate cellular stress response[1]. Autophagy is regulated by specific proteins, which are coded as autophagy-related genes[4-6].

The autophagy expression markers LC3A, Beclin-1 are related to chronic inflammation conditions, such as asthma[7], which enhances the evidence of correlation of autophagy with inflammation. Currently, there are no previous studies that relate inflammation with the three immunohistochemistry markers studied here. The study of autophagy-related proteins LC3A and Beclin-1 and proliferation marker Ki-67 expression in calculous and acalculous human gallbladder epithelium will enhance this hypothesis furthermore.

Microtubule-associated protein 1 light chain 3 (LC3) is an essential element of the autophagy procedure. LC3A is one isoform of LC3. LC3A arises from the activation of the autophagy pathway and is related to poor patient prognosis in several cancer types. According to other studies, high LC3 expression appears in colorectal, gastrointestinal, and pancreatic cancer and is related to poor patient prognosis[8].

Beclin-1 is the first mammalian autophagy identified modulator and maybe the main target that is activated or deactivated in order to coordinate autophagy proteins with the needs of the cell to enhance or suppress autophagy in a context-dependent manner[9,10]. The role of Beclin-1 expression in cancer is still questioned. Recent studies maintain that autophagy suppresses tumor formation, while other researchers suggest that autophagy enhances tumorigenesis. Beclin-1 is highly expressed and constitutes a non-dependent biomarker associated with a better prognosis in gallbladder cancer[11].

The Ki-67 antigen is a nuclear protein and is expressed in every active phase of the cell cycle. Ki-67 is absent in non-proliferating cells. During interphase, the antigen can be detected in the nucleus, whereas in mitosis, most of the protein is transported to the surface of the chromosomes. The antigen is degraded as the cell enters the non-proliferated state, and there is no expression of Ki-67 during DNA repair processes. Therefore, it constitutes a possible autophagy index.

We investigated the expression of Beclin-1, LC3A, and Ki- 67 in specimens of calculous and acalculous gallbladder epithelia in order to compare the expression of these immunohistochemical biomarkers and find their correlation with calculous cholecystitis and autophagy.

Materials and methods

Our prospective study was conducted at Democritus University of Thrace (DUTH) Medical School, in the University Hospital of Alexandroupolis, Greece, during the period from 2016 till 2019. Ethical approval for the research protocol was obtained from the Ethical and Scientific Committee of the Institution (decision No 306, date: 12/04/2016).

Human gallbladder full-thickness specimens were obtained from 100 patients (45 male, 55 female) who underwent cholecystectomy. The patients were not consecutive and were selected according to the degree of inflammation. The gallbladder tissues were examined in the Pathology Laboratory of DUTH. The patients were divided into two groups: group A (calculous gallbladder group: 24 male, 46 female; mean age 52.6 ± 16.0 years); and group B (acalculous gallbladder group: 21 male, nine female; mean age 65.3 ± 12.4 years). Tissue specimens were fixed in formalin solution 10 % and embedded in paraffin (FFPE).

Immunohistochemistry

Tissue blocks containing material from the body of the gallbladder were retrieved from the archive of the Department of Pathology of our University Hospital. Three μm sections were cut from all FFPE blocks and mounted on positively charged glass slides. Immunohistochemistry for Beclin-1, LC3A, and Ki-67 was performed on serial sections. The whole immunohistochemical procedure was performed with the Envision Flex Kit (Dako Cytomation, Glostrup, Denmark). The slides were deparaffinized at 80 °C for 30 min, and then, the epitope retrieval procedure was carried out. Analytically, the sections were placed in citrate buffer (DAKO Envision Flex Target Retrieval Solution, 1:10 dilution, pH 6.0) and heated in a microwave oven at 97 °C for 3 x 5 min. Then, the slides were left to cool for 20 min. The sections were placed in tris-buffered saline (TBS) buffer for 5 min. Subsequently, the sections were incubated with the primary antibodies LC3A (1:20 dilution; overnight incubation; Abgent, San Diego), Beclin-1 (1:50 dilution; overnight incubation; Santa Cruz Biotechnology, Europe), and Ki-67 (ready to use; 60 min incubation; Dako Cytomation, Glostrup, Denmark). The slides were washed with tris-buffered saline (TBS) buffer for six min twice, and Endogenous peroxidase blocking was carried out using the Envision Flex Peroxidase-Blocking Solution (DAKO) for ten min. The slides were rewashed with tris-buffered saline (TBS) buffer for six min twice and incubated with Envision Flex, Mouse (Linker) as stabilizing protein. The slides were rewashed with TBS six min twice, and Envision Flex/HRP was applied for 30 min. Washing with TBS was repeated twice, and then, Envision Flex DAB+ Chromogen was applied for five min. Following that step, double washing was performed, and counter-staining with hematoxylin Q5 (vector) was performed for two min. Finally, the sections were washed once more with TBS, dehydrated through descending alcohol solutions in xylene and mounted in synthetic resin. All slices were evaluated without knowledge of the clinical outcome. Beclin-1, LC3A, and Ki-67 expression in the 100 cases were evaluated under an Olympus BX40 microscope (Olympus, Center Valley, PA). Staining was scored by two different observers.

Evaluation of immunohistochemical staining

Evaluation of the stained tissue slides was performed at x200 magnification, and all available tissue areas were inspected. The samples were selected based on the degree of inflammation, which was moderate to severe. Areas of metaplastic epithelium were seldom noted and were excluded from the assessment. Strong cytoplasmic expression of LC3A and Beclin-1 was taken into account to score each case. The Ki-67 proliferation marker was assessed as the percentage of epithelial cells with nuclear expression. The percentage of epithelial cells with strong cytoplasmic (for LC3A and Beclin-1) or nuclear (for Ki-67) staining was recorded, and cases were grouped as 0 =negative, 1 =low expression, 2 =moderate expression, and 3 =high expression.

Each immunostain was evaluated independently by two independent observers. Areas of regenerative epithe-

lium expressed similar expression patterns with the rest of the gallbladder epithelium and were included together in the analysis.

As a positive control, we used tissue slides from colon carcinomas with known intense expression of LC3A, Beclin-1, or Ki-67. As LC3A and Beclin-1 proteins are poorly expressed in healthy tissues, there was no internal positive control to consider.

Statistical analysis

The R software (version 3.4.1) was used for statistical analyses. The results are presented as mean ± standard deviation (SD). Positive biomarker expression was determined when its value was higher than the median value, which was used as dichotomous. Chi-square test/Fisher's exact test was used for the comparison of categorical/dichotomous variables. A p-value of ≤0.05 was considered significant.

Results

The expressions of the three biomarkers in the tissues of the gallbladders were studied in the two groups. Ki-67 and Beclin-1 expression were higher in patients with calculous than acalculous gallbladder (Figure 1, Figure 2, Figure 3).

Beclin-1 expression was higher in the gallbladder epithelium of group A patients compared to group B patients (Figure 4C). This is also shown from the comparison in Figure 2. The expression of LC3A was higher in the gallbladder epithelium of group B patients than in group A patients (Figure 4A). The immunohistochemical expression of LC3A in groups A and B is shown in Figure 3.

Regarding the correlation of Beclin-1 expression with LC3A expression in group A, it was observed that increased Beclin-1 expression promoted LC3A expression (p =0.0001; Table 1). Table 1 and Figure 5 present the simultaneous expression of the biomarker in each group. Positive simultaneous biomarker expression was determined when

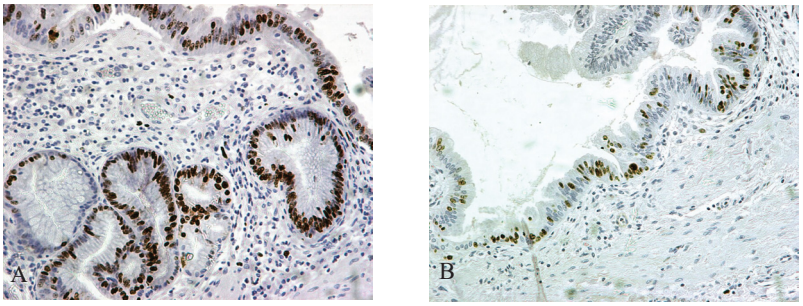

Figure 1: Expression of biomarker Ki-67 in the gallbladder epithelium (magnification x20): A) in the calculous gallbladder, and B) in acalculous gallbladder tissue.

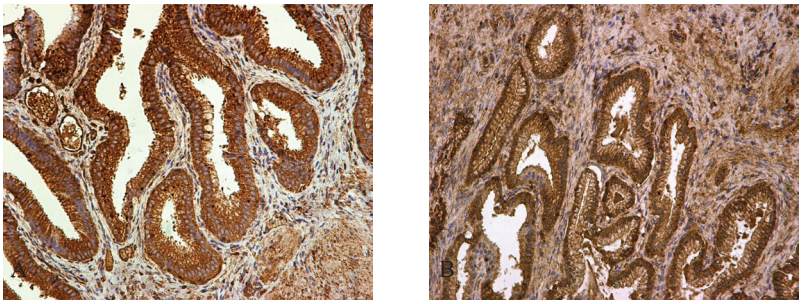

Figure 2: Expression of biomarker Beclin-1 in the gallbladder epithelium (magnification x20): A) in the calculous gallbladder, and B) in acalculous gallbladder tissue.

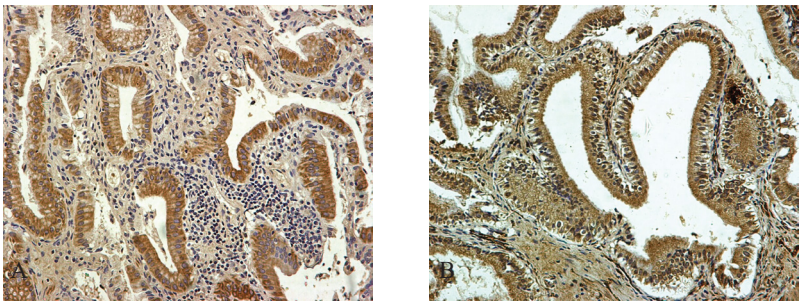

Figure 3: Expression of biomarker LC3A in the gallbladder epithelium (magnification x20): A) in the calculous gallbladder, and B) in acalculous gallbladder tissue.
LC3A: isoform of microtubule-associated protein 1 light chain 3.

its value was higher than the median value, which was used as dichotomous. In group B patients, the LC3A expression did not follow Beclin-1 expression (p =0.09; Table 1). The mean percentage of Beclin-1 expression in group A patients was 23.8 % compared to group B patients, where the corresponding percentage was only 17.3 % (Table 2). In Table 2, variants Min, Max, Mean, and SD refer to the percentage of patients with the minimum, maximum, and mean expression of biomarkers, respectively, while SD is the standard deviation of the biomarker expression. Corresponding mean percent expressions of LC3A in groups A and B were 38.9 % and 50.7 %, respectively (Table 2).

The expression of the third immunohistochemical markers Ki-67 was higher in group A patients compared to group B patients (Figure 4B; Table 3). In Table 3, positive biomarker expression was determined when its value was higher than the median value. Figure 1A shows that in group A, staining intensity with the Ki-67 antibody is more intense compared to Figure 1B (group B). The mean percentage of Ki-67 expression in group A patients was 3.75 %, whereas, in group B patients, it was only 0.5 % (statistically significantly different; p =0.0003). In group A, expression of Ki-67 >0.5 % was observed in 43 patients, and in group B in six patients (Table 3, Table 4).

The median expression of LC3A in all patients was 40 % (Table 4: LC3A >40 %, 53 patients; LC3A <40 %, 47 patients). In Table 4, the classification of biomarkers was done using the median value. Biomarker expression was positive when the expression was higher than the median value. Chi-square test/Fisher's exact test was used to compare categorical/dichotomous variables. The median expression of Ki-67 in all groups was 0.5 % (Table 4: Ki-67 >0.5 %, 49 patients; Ki-67 <0.5 %, 51 patients). The median expression of Beclin-1 in all groups was 10 % (Table 4: Beclin >10 %, 40 patients; Beclin-1 <10 %, 60 patients).

Finally, we correlated the expression of Beclin1 and LC3A expression (Figure 5) using the chi-squared test/ Fisher's exact test in the two groups, as shown in Table 1.

The expression of Beclin-1, LC3A, and Ki-67 in relation to demographic characteristics of groups A and B (gender and age of patients) was also studied. Our results have shown that in calculous gallbladder epithelium, the patient age influences (but not statistically significantly; p =0.06) the immunohistochemistry marker expression. However, in the acalculous gallbladder epithelium, the patient's age is not related to the immunohistochemistry marker expression (p =0.63). The patient's gender does not seem to affect the expression of the immunohistochemistry markers in both groups.

Table 1: LC3A and Beclin-1 expression in the calculous and acalculous gallbladder epithelium.

		LC3A <40 % (low)	LC3A >40 % (high)
Calculous Gallbladder, p =0.0001	Beclin-1 <10 % (low)	30 (81 %)	7 (19 %)
	Beclin-1 >10 % (high)	7 (21.2 %)	26 (78.8 %)
Acalculous Gallbladder, p =0.09	Beclin-1 <10 % (low)	10 (43.5 %)	13 (56.5 %)
	Beclin-1 >10 % (high)	0 (0 %)	7 (100 %)

LC3A: isoform of microtubule-associated protein 1 light chain 3.

Table 2: Expression of biomarkers LC3A, Ki-67, and Beclin-1, and the mean age of patients in groups A (calculous gallbladder tissue derived from 24 male and 46 female patients) and group B (acalculous gallbladder tissue derived from 21 male and 9 female patients).

	Min	Max	Mean	SD
Group A				
LC3A (%)	0	100	38.9	33.9
Ki-67 (%)	0	50	3.7	7.6
Beclin-1 (%)	0	90	23.8	25.3
Age (years)	11	88	52.6	16.0
Group B				
LC3A (%)	0	100	50.7	29.4
Ki-67 (%)	0	10	0.5	1.8
Beclin-1 (%)	0	100	17.3	28.4
Age (years)	34	82	65.3	12.4

Min, Max, Mean, and SD refer to the percentage of patients with the minimum, maximum, and mean expression of biomarkers, respectively, while SD is the standard deviation of the biomarker expression.

Table 3: Expression of biomarker Ki-67 in the calculous and acalculous gallbladder epithelium.

	Ki-67 <0.5 % (low)	Ki-67 >0.5 % (high)
Calculous gallbladder	27 (38.6 %)	43 (61.4 %)
Acalculous gallbladder	24 (80 %)	6 (20 %)

Table 4: Presence of bile stones, sex, and biomarkers expression frequencies.

Variables	Classification	Frequency (%)
Calculous gallbladder	Yes	70.0
Acalculous gallbladder	No	30.0
Sex	Female	55.0
Sex	Male	45.0
LC3A	>40 %	53.0
LC3A	<40 %	47.0
Ki-67	>0.5 %	49.0
Ki-67	<0.5 %	51.0
Beclin-1	>10 %	40.0
Beclin-1	<10 %	60.0

LC3A: isoform of microtubule-associated protein 1 light chain 3.

Discussion

The role of gallbladder epithelium in calculous and acalculous cholecystitis continues to present research interest. To our knowledge, this is the first study of the autophagy process in the gallbladder epithelium. The pres-

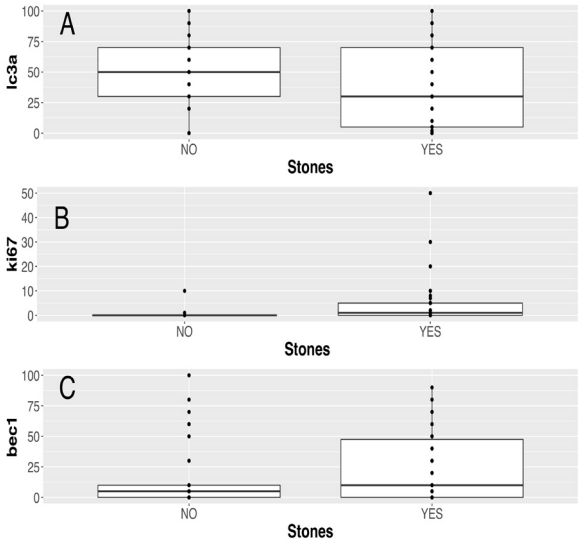

Figure 4: A) LC3A, B) Ki-67, and C) Beclin-1 expression in (YES): Group A, calculous gallbladder, and (NO): Group B, acalculous gallbladder.
LC3A: isoform of microtubule-associated protein 1 light chain 3.

ence of autophagy markers Beclin-1, LC3A, and Ki-67 were examined using immunohistochemistry techniques in tissue samples from patients who underwent cholecystectomy with diagnosis cholecystitis.

Beclin-1, LC3A, and Ki-67 were originally identified as excellent markers for autophagic structures[12]. Nowadays, Ki-67 is used as a prognostic index in non-muscle invasive bladder cancer[13], superficial non-invasive papillary urothelial neoplasms of bladder[14], chordoma[15], prostate cancer[16], breast phyllodes tumors[17], sarcoma and neuroendocrine tumors of tubular gastrointestinal tract and pancreas[18,19]. Furthermore, Beclin-1 is associated with tumorigenesis and appears in various neoplastic situations such as ovarian[20] and gastric cancer[21]. Additionally, LC3A is expressed in keratoacanthomas[22], glioblastomas[23], follicular, and B-cell lymphomas[24].

Sivridis et al have shown that LC3A reactivity was recognized in cutaneous squamous cell carcinomas with diffuse cytoplasmic, "stone-like" structures (SLS), and cytoplasmic/perinuclear structures, that are rounded, large, amorphous densely stained material, enclosed within cytoplasmic vacuoles[22,25]. Previous studies have proved that LC3A follows the same pattern under stressed conditions[25]. This fact is confirmed by Giatromanolaki et al, who studied the LC3A and Beclin-1 expression in gastric cancer[21]. In our patients, both Beclin-1 and LC3A have shown cytoplasmic expression.

In the current study, the samples were selected based on the degree of inflammation, which was moderate to severe, and there was no staging of inflammation in another way, which is a limitation of our study. It was found that the epithelium of calculous gallbladder presents significantly higher Beclin-1 and Ki-67 expressions compared to the acalculous gallbladder epithelium, and LC3A presents a high expression pattern in both groups, which was characterized as a diffuse cytoplasmic pattern. To date, there is no other study of the three markers in the gallbladder epithelium in cholecystitis.

Autophagy expression has been related to chronic inflammation conditions, such as asthma in a mouse experimental model[7]. There is limited literature on the relation of chronic inflammation conditions and autophagy. This study presents for the first time in human gallbladder epithelium three autophagy immunohistochemistry markers comparing their expression with calculous and acalculous cholecystitis.

The fact that the mechanism of autophagic regulation plays an essential role in carcinogenesis and cancer thera-

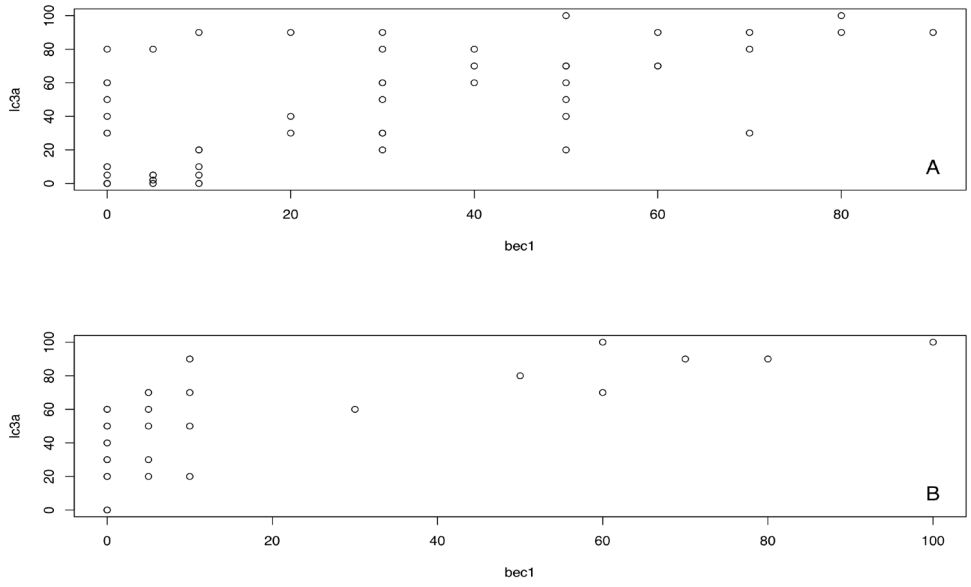

Figure 5: Distribution of LC3A expression relative to the Beclin-1 expression in A) Group A (calculous gallbladder tissue), and B) Group B (acalculous gallbladder tissue).
LC3A: isoform of microtubule-associated protein 1 light chain 3.

peutics constitutes a mandatory necessity for the investigation of autophagy procedure in chronic inflammation conditions, which could potentially be neoplastic[26]. It has been studied that chronic inflammation is responsible for genetic and epigenetic diversions, which can be correlated with a higher possibility of tumor development in the calculous epithelium of the gallbladder[27]. Although autophagy is related to the inflammatory process, there is no clear evidence for its participation in this situation[28,29].

In our study, the expression of autophagy-related proteins LC3A and Beclin-1, and proliferation marker Ki-67 were examined in calculous and acalculous human gallbladder epithelium. For the first time, these findings provide evidence of a high correlation between LC3A and Beclin-1 expression in calculous gallbladder epithelium. In the calculous gallbladder epithelium, an overexpression of biomarkers Beclin-1 and LC3A was found. Therefore, stone presence promotes inflammation and autophagy in gallbladder epithelium, and the mechanism of autophagic regulation plays a significant role in chronic inflammation as well. This constitutes a necessity for further investigation of the correlation of autophagy in gallbladder epithelium in the presence of bile stone as a precancerous stage.

Conflict of interest

The authors declare no financial interests and conflicts of interest regarding any of the products or methods used in this study. This research did not receive any specific grant or funding from agencies in the public, commercial, or not-for-profit sectors.

References

1. Tsaroucha AK, Chatzaki E, Lambropoulou M, Despoudi K, Laftsidis P, Charsou C, et al. Megalin and cubilin in the human gallbladder epithelium. Clin Exp Med. 2008; 8: 165-170.
2. Admirand WH, Small DM. The physicochemical basis of cholesterol gallstone formation in man. J Clin Invest. 1968; 47: 1043-1052.
3. Ohsumi Y. Historical landmarks of autophagy research. Cell Res. 2014; 24: 9-23.
4. White E, Lowe SW. Eating to exit: autophagy-enabled senescence revealed. Genes Dev. 2009; 23: 784-787.
5. Levine B, Kroemer G. Autophagy in the pathogenesis of disease. Cell. 2008; 132: 27-42.
6. Fink MY, Weichseldorfer MR, White TL, Snyder ML. Lipopolysaccharide-Enhanced Bactericidal Activity is Mediated by Induction of the Autophagosomal Process. Hayat M (ed). Autophagy: Cancer, Other Pathologies, Inflammation, Immunity, Infection, and Aging. Academic Press, New York, 2017, 435-449.
7. Ran Q, Zhang L, Qiu YH, Wing X, Li GP. [Effects of 3-methyladenine on airway inflammation, airway hyperresponsiveness and mucus secretion in asthmatic mice]. Zhonghua Jie He He Hu Xi Za Zhi. 2019; 42: 185-192.
8. Sivridis E, Koukourakis MI, Zois CE, Ledaki I, Ferguson DJ, Harris AL, et al. LC3A-positive light microscopy detected patterns of autophagy and prognosis in operable breast carcinomas. Am J Pathol. 2010; 176: 2477-2489.
9. Liang XH, Kleeman LK, Jiang HH, Gordon G, Goldman JE, Berry G, et al. Protection against fatal Sindbis virus encephalitis by beclin, a novel Bcl-2-interacting protein. J Virol. 1998; 72: 8586-8596.
10. Liang XH, Jackson S, Seaman M, Brown K, Kempkes B, Hibshoosh H, et al. Induction of autophagy and inhibition of tumorigenesis by beclin 1. Nature. 1999; 402: 672-676.
11. Park JY, Kim HS, Cho H, Kim NC, Chae KH, Park WS, et al. Clinicopathologic correlation of autophagy-related Beclin-1 expression in gallbladder cancer. Hepatogastroenterology. 2014; 61: 1494-1500.
12. Klionsky DJ, Abdelmohsen K, Abe A, Abedin MJ, Abeliovich H, Acevedo Arozena A, et al. Guidelines for the use and interpretation of assays for monitoring autophagy (3rd edition). Autophagy. 2016; 12: 1-222.
13. Ko K, Jeong CW, Kwak C, Kim HH, Ku JH. Significance of Ki-67 in non-muscle invasive bladder cancer patients: a systemic review and meta-analysis. Oncotarget. 2017; 8: 100614-100630.
14. Ogata DC, Marcondes CA, Tuon FF, BusatoWF Jr, Cavali G, Czeczko LE. Superficial papillary urothelial neoplasms of the bladder (PTA E PT1): correlation of expression of P53, KI-67 and CK20 with histologic grade, recurrence and tumor progression. Rev Col Bras Cir. 2012; 39: 394-400.
15. von Witzleben A, Goerttler LT, Lennerz J, Weissinger S, Kornmann M, Mayer-Steinacker R, et al. In chordoma, metastasis, recurrences, Ki-67 index, and a matrix- poor phenotype are associated with patients' shorter overall survival. Eur Spine J. 2016; 25: 4016-4024.
16. Fisher G, Yang ZH, Kudahetti S, Møller H, Scardino P, Cuzick J, et al. Prognostic value of Ki-67 for prostate cancer death in a conservatively managed cohort. Br J Cancer. 2013; 108: 271-277.
17. Kieer CG, Giordano TJ, Braun T, Oberman HA. Pathologic, immunohistochemical, and molecular features of benign and malignant phyllodes tumors of the breast. Mod Pathol. 2001; 14: 185-190.
18. Dhall D, Mertens R, Bresee C, Parakh R, Wang HL, Li M, et al. Ki-67 proliferative index predicts progression-free survival of patients with well-differentiated ileal neuroendocrine tumors. Hum Pathol. 2012; 43: 489-495.
19. Lloyd RV, Osamura RY, Klöppel G, Rosai J. Neoplasms of the neuroendocrine pancreas. Lloyd RV, Osamura RY, Klöppel G, Rosai J (eds). WHO Classification of Tumours of Endocrine Organs, 4th Edition, World Health Organization, Geneva, 2017, 209-239.
20. Kulshrestha A, Katara GK, Ibrahim SA, Riehl V, Sahoo M, Dolan J, et al. Targeting V-ATPase Isoform Restores Cisplatin Activity in Resistant Ovarian Cancer: Inhibition of Autophagy, Endosome Function, and ERK/MEK Pathway. J Oncol. 2019; 2019: 2343876.
21. Giatromanolaki A, Koukourakis MI, Georgiou I, Kouroupi M, Sivridis E. LC3A, LC3B and Beclin-1 Expression in Gastric Cancer. Anticancer Res. 2018; 38: 6827-6833.
22. Sivridis E, Koukourakis IM, Arelaki S, Balaska K, Karpouzis A, Giatromanolaki A. Patterns of LC3A Autophagy Protein Expression in Keratoacanthomas. Head Neck Pathol. 2019.
23. Mitrakas AG, Kalamida D, Giatromanolaki A, Pouliliou S, Tsolou A, Kyranas R, et al. Autophagic flux response and glioblastoma sensitivity to radiation. Cancer Biol Med. 2018; 15: 260-274.
24. Giatromanolaki A, Koukourakis MI, Pouliliou S, Gatter KC, Pezzella F, Harris AL, et al. Overexpression of LC3A autophagy protein in follicular and diffuse large B-cell lymphomas. Hematol Oncol Stem Cell Ther. 2013; 6: 20-25.
25. Zois CE, Giatromanolaki A, Sivridis E, Papaiakovou M, Kainulainen H, Koukourakis MI. "Autophagic flux" in normal mouse tissues: focus on endogenous LC3A processing. Autophagy. 2011; 7: 1371-1378.
26. Panda PK, Mukhopadhyay S, Das DN, Sinha N, Naik PP, Bhutia SK. Mechanism of autophagic regulation in carcinogenesis and cancer therapeutics. Semin Cell Dev Biol. 2015; 39: 43-55.
27. Murata M. Inflammation and cancer. Environ Health Prev Med. 2018; 23: 50.
28. Sasaki M, Miyakoshi M, Sato Y, Nakanuma Y. Autophagy mediates the process of cellular senescence characterizing bile duct damages in primary biliary cirrhosis. Lab Invest. 2010; 90: 835-843.
29. Haq S, Grondin J, Banskota S, Khan WI. Autophagy: roles in intestinal mucosal homeostasis and inflammation. J Biomed Sci. 2019; 26: 19.