EJGO European Journal of Gynaecological Oncology

Expression analysis of the Beclin-1 in premalignant and malignant tissues of the uterine cervix

Z. Protrka[1], A. Dimitrijevic[1], N. Jovic[1], T. Kastratovic[1], J. Djuric[1], J. Nedovic[2], S. Zivanovic[3], A. Vujic[3], S.M. Drakulic[3], N. Djonovic[3], P. Arsenijevic[1]

[1]Department of Obstetrics and Gynecology, Faculty of Medicine, University of Kragujevac, Kragujevac
[2]Department of Oncology, Clinical Center Kragujevac, Kragujevac; [3]Faculty of Medicine, University of Kragujevac, Kragujevac (Serbia)

Summary

Background: Beclin-1 is the key regulator gene for autophagy and plays a significant role in regulating tumor cell growth and death. *Materials and Methods:* To assess the expression and clinical significance of autophagic gene Beclin-1 in carcinogenesis of the uterine cervix, the authors performed immunohistochemistry in 138 tissue samples of: low grade cervical squamous intraepithelial lesions (SIL) (n=32), high grade SIL (n=22), portio vaginalis uteri (PVU) carcinoma *in situ* (n=22), and PVU invasive carcinoma, Stage IA-IIA (n=26) (study group) and 36 samples of normal uterine cervix (control group). Fisher's exact test ($p < 0.05$) was used to assess statistical significance. The level of reliability of specificity and sensitivity was determined as a possible screening method for detection of changes in the uterine cervix. *Results:* There is no important difference in the frequency of overexpression of Beclin-1 between the patients with normal cervix and with low grade SIL, in this study. On the other hand there was a large difference in the frequency of overexpression in patients with high grade SIL (18/22, $p = 0.008$), PVU carcinoma *in situ* (20/22, $p = 0.008$), and PVU invasive carcinoma (18/26, $p = 0.033$), in relation to the control group. High sensitivity values show diagnostic significance in noticing these types of changes in the uterine cervix. Regarding high predictive values, there is a conclusion that patients with overexpression of Beclin-1 gene probably have premalignant or malignant changes in the uterine cervix. *Conclusions:* The current achievements show that the evaluation of Beclin-1 expression might provide additional and independent prognostic information to predict the clinical course of uterine cervical cancer. It is confirmed that overexpression of these genes proposes with great certainty that there are premalignant or malignant changes in the uterine cervix.

Key words: Carcinogenesis; Uterine cervix carcinoma; Beclin-1; Immunohistochemistry.

Introduction

Invasive carcinoma of the uterine cervix is the most lethal gynecological malignancy and a growing problem worldwide. Molecular-genetic research, in the past decade, plays a significant role in prevention, early diagnosis, and determination of effective treatment for premalignant lesions and malignant tumors of the uterine cervix.

As a physiological process in cell metabolism, autophagy is involved in facilitating the cellular clearance of aggregation-prone proteins, and by that expending a cytoprotective function [1]. At the same time, as a genetically determined process, autophagy enables the recycling of damaged organelles or long-lived proteins, thus promoting the cell survival by purging the cell of toxic metabolites and intracellular pathogens, or by generating the essential proteins required to maintain vital functions during nutrient-limiting conditions [2]. Various conditions such as hypoxia, inflammatory stimulation, and lack of nutrients, may act as a potential trigger for the autophagy [3,4]. Depending on the environment, autophagy can have two potential effects, it can either facilitate adaptive cell survival, or promote programmed cell death [5-7].

The ability of the cancer cells to survive under hypoxic and hypo-nutrient microenvironments, is due to their enhancement of autophagic ability and utilization of the recyclable materials [8]. Despite autophagy being a critical focus in cancer research, it is still unclear whether autophagy acts fundamentally as a cell survival or cell death pathway, or both. [9-12].

Beclin-1 as one of 30 specific genes that regulate the process of autophagy and plays a key role in mammalian autophagy [13, 14]. Beclin-1 gene is found in human chromosome 17q21 [15]. It is expressed in wild type of mice, and heterozygous mutations of this protein increase cell proliferation and cancer development [16, 17]. The expression of Beclin-1 also correlates with decrease in the level of monoallelic deletions which is found in 40-75% of sporadic breast, prostate, and ovarian cancers [18]. Overexpression of Beclin-1 protein were frequently found in advanced stages of various cancers and were shown to be associated with tumor progression [19-25], but several other studies have detected a decrease of Beclin-1 expression in cancer compared to normal tissues [26-28]. However, there are no studies regarding specifically the roles of

Revised manuscript accepted for publication January 17, 2017

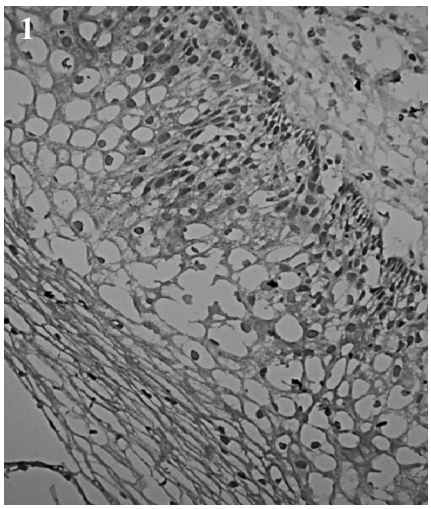
Eur. J. Gynaecol. Oncol. - ISSN: 0392-2936
XXXIX, n. 6, 2018
doi: 10.12892/ejgo4028.2018

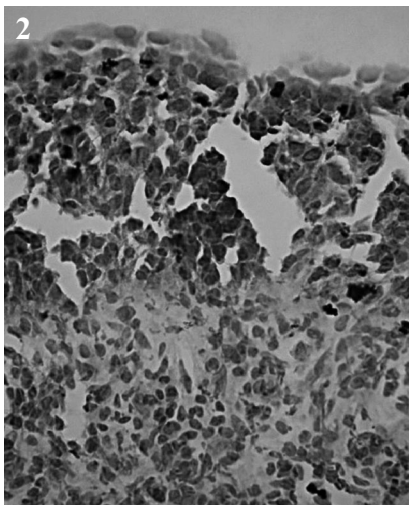
7847050 Canada Inc.
www.irog.net

Table 1. — *Expression of Beclin-1 in the control and study group.*

Beclin-1 expression	Control group, n (%)	Low grade SIL, n (%)	High grade SIL, n (%)	PVU in situ carcinoma, n (%)	PVU invasive carcinoma, n (%)
Negative(-)	8/36 (22.2)	2/32 (6.2)	0/22 (0)	0/22 (0)	2/26 (7.6)
Negative (1+)	18/36 (50.0)	12/32 (37.5)	4/22 (18.2)	2/22 (9.1)	6/26 (23.1)
Positive (2+)	2/36 (5.6)	8/32 (25.0)	4/22 (18.2)	14/22 (63.6)	6/26 (23.1)
Positive (3+)	8/36 (22.2)	10/32 (31.3)	14/22 (63.6)	6/22 (27.3)	12/26 (46.2)

Squamous intraepithelial lesions (SIL), portio vaginalis uteri (PVU).





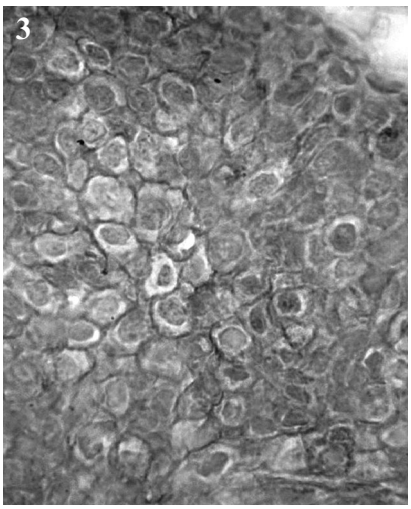


Figure 1. — Beclin-1 overexpression (3+) in high grade SIL (×40).
Figure 2. — Beclin-1 overexpression (3+) in Ca PVU *in situ* (×40).
Figure 3. — Beclin-1 overexpression (3+) in Ca invasive PVU (×40).

autophagy in carcinoma of the uterine cervix.

The aims of the present study were to assess of Beclin-1 expression and clinical significance in the uterine cervix carcinogenesis.

Materials and Methods

This prospective experimental study was performed between January 2013 and December 2015, at the Department of Obstetrics and Gynecology and at the Laboratory for Experimental and Clinical Immunology, Faculty of Medicine, University of Kragujevac, Serbia.

In patients who had undergone surgeries for hysterectomy, punch biopsy or conization, because of premalignant and/or malignant changes of the uterine cervix, the tissue specimens were collected from the operative material. Cryostat sections were sent for histopathological diagnosis.

Sections of tissue deriving from the operative material or biopsy material were taken after obtaining informed consent of patients in accordance with the Declaration of Helsinki and recommendations of the World Health Organization (WHO) for experiments on human material and after getting approval of the Ethics Committee.

To evaluate Beclin-1 expressions in control group, the authors performed immunohistochemistry in 36 cases of normal cervix, from patients in whom Papanicolaou indicated through ambulatory biopsy of the uterine cervix, did not show malignant changes or SIL (cervicitis chronica of mild to moderate degree).

To evaluate Beclin-1 expressions in study group, we performed immunohistochemistry on 32 cases with histopathological diagnosis of low grade squamous intraepithelial lesions (SIL), 22 with high grade SIL, 22 with portio vaginalis uteri (PVU) carcinoma *in situ*, and 26 with PVU invasive carcinoma, Stage IA-IIA.

Extra sections of cervical lesions were snap-frozen in liquid nitrogen and stored at -70°C, until they were used for immunohistochemistry analysis. The primary antibody used for investigation was polyclonal rabbit Beclin-1 antibody diluted with phosphate buffered saline (PBS, pH-7.2, 1:500). The frozen sections, four-micrometer in diameter, were fixed in 100% acetone for five minutes and the endogenous peroxidase activity was suppressed by ten-minute incubation in 0.5% hydrogen peroxide. After fixation, the slides were washed with PBS. Non-specific antibody binding was blocked with PBS containing 10% rabbit anti-human polyclonal primary antibodies against Beclin-1 serum, for ten minutes at room temperature in the humidity chamber. The slides were blotted and primary antibodies applied. For Beclin-1 antibody the incubation period lasted 60 minutes at room temperature (concentration of 5 μg/ml). The slides were washed three times in PBS. Secondary antibody, diluted with PBS, was applied for 60 minutes in a humidity chamber at room temperature. Streptavidin/horse-

Table 2. — *Table of contingency (low grade SIL).*

Test	Disease present (low grade SIL)	Disease absent (control group)	Total
Beclin-1 overexpression	18 ($p = 0.163$)	10	28
Beclin-1 negative	14	26	40
Total	32	36	68

Squamous intraepithelial lesions (SIL).

Table 3. — *Table of contingency (high grade SIL).*

Test	Disease present (high grade SIL)	Disease absent (control group)	Total
Beclin-1 overexpression	18 ($p = 0.008$)	10	28
Beclin-1 negative	4	26	30
Total	22	36	58

Squamous intraepithelial lesions (SIL).

Table 4. — *Table of contingency (PVU carcinoma in situ).*

Test	Disease present (PVU carcinoma in situ)	Disease absent (control group)	Total
Beclin-1 overexpression	20 ($p = 0.008$)	10	30
Beclin-1 negative	2	26	28
Total	22	36	58

Portio vaginalis uteri (PVU).

Table 5. — *Table of contingency (PVU invasive carcinoma).*

Test	Disease present (PVU invasive carcinoma)	Disease absent (control group)	Total
Beclin-1 overexpression	18 ($p = 0.033$)	10	28
Beclin-1 negative	8	26	34
Total	26	36	62

Portio vaginalis uteri (PVU).

radish-peroxidase complex technique was used for visualization with diaminobenzidine as chromogen. Sections were counterstained with hematoxylin for 30 seconds and washed in cold tap water. At the end, the slides were dehydrated in alcohol, cleared in xylene, and enveloped in Canada balsam.

For the positive controls the authors used hepatocellular carcinoma with cytoplasmic Beclin-1 and NET-1. The samples were taken through the procedure with exclusion of the primary antibody, for negative controls.

Slides were evaluated by two of the authors, unaware of immunohistochemical or clinical data using a semiquantitative method on a light microscope. The percentage of immunopositive cells in presentational areas of the sections was determined. The intensity of immunostaining was split into four categories, namely: negative -(0-5%), 1+(5-25%), and positive 2+(25-50%), and 3+ (50-100%).

Patients were split into 4 subgroups, based on the frequency of the lesions found and 2×2 contingency tables were formed from where specificity and sensitivity were calculated. Also, based on the receiver operating characteristic (ROC) curve, the discrimination power of the test was determined.

For the assessment of statistical significance the authors used Fisher's exact test. The established levels of the sensitivity and specificity was used as a possible screening method for the early detection of the uterine cervical changes.

Results

The expression levels of Beclin-1 gene in the study and control group is shown in Table 1. Beclin-1 was overexpressed in 27.8% of patients with normal cervix and in 56.3% of patients with low grade SIL (Fisher's exact test, $p = 0.163$).

Sensitivity and specificity, as a method for low grade SIL detection, were calculated in a 2×2 table of contingency, on the basis of the frequency of Beclin-1 overexpression. Sensitivity was 56.25% and specificity was 72.2% (Table 2).

On the basis of the positive predictive value of 64.32%, may be expected in patients with Beclin-1 overexpression that have low grade SIL in this percentage. On the basis of the negative predictive value of 65%, this percentage of patients with Beclin-1 negative expression are not expected to have low grade SIL.

Overexpression of Beclin-1 was present in 27.8% of patients with normal cervix and in 81.8% with high grade SIL (Fisher's exact test, $p = 0.008$) (Figure 1).

As a method for the detection of this type of the uterine cervical changes, the sensitivity of determining Beclin-1 was 81.82% and specificity was 72.2% (Table 3).

With the aim of determining high grade SIL, the discrimination power of overexpression Beclin-1 was greater than the discrimination power of negative expression of this gene, in patients with normal findings in the cervix. In high grade SIL changes, the positive predictive value was 64.18% and the negative predictive value was 86.65%. Table 1 shows that Beclin-1 was overexpressed in 27.8% of patients with normal cervix and in 90.9% of patients with PVU carcinoma *in situ* (Fisher's exact test, $p = 0.008$), (Figure 2). Overexpression of Beclin-1 oncogene was significantly different in normal cervix than in PVU carcinoma *in situ* (Fisher's exact test, $p = 0.008$). The sensitivity for diagnosing PVU carcinoma *in situ* was 90.9% and specificity was 72.2% (Table 4). The positive predictive value was 64.18% and the negative predictive value was 86.65%.

Beclin-1 was overexpressed in 27.8% of patients with normal cervix and in 69.3% PVU *invasive* carcinoma (Fisher's exact test, p = 0.033) (Table 1, Figure 3). The sensitivities was 69.3%, specificity 72.2%, the positive predictive values 64.13%, and the negative predictive values 76.46%, respectively (Table 5).

Discussion

Beclin-1 and Beclin-2 belong to the Beclin family. Human cancer studies have assessed the role of Beclin 1, and the outcomes showed that cytoplasmic Beclin-1 expression may be specific to the cancer type. There is low expression of

Beclin-1 in breast cancer [29, 30], ovarian cancer [31], lung cancer [32], hepatocellular carcinoma [33], cholangiocarcinoma [34], pancreatic cancer [35], hypopharyngeal cancer [36], laryngeal cancer [37], esophageal cancer [38], duodenal cancer [39], gastric cancer [40], lymphomas [41–43], and so on, and this is associated with poor prognosis. On the other hand there is high expression of Beclin-1 which is connected to tumor aggressiveness in nasopharyngeal carcinoma [44], endometrial cancer [45], and colorectal cancer [46]. After studying Beclin-1 in oral cancer tissues for a while, it was concluded that high expression was related to poor prognostic factors and/or aggressive clinical outcomes [47, 48].

There is no important difference in the frequency of overexpression of Beclin-1 between the patients with normal cervix and with low grade SIL, in the present study. On the other hand there is a large difference in the frequency of overexpression in patients with high grade SIL, PVU carcinoma *in situ*, and PVU invasive carcinoma in relation to the control group. High sensitivity values show diagnostic significance for noticing these kinds of changes in the uterine cervix. Regarding high predictive values, there is a conclusion that patients with overexpression of Beclin-1 gene probably have premalignant or malignant changes in the uterine cervix.

Dysfunction in autophagy and metabolism is suggested by high or low Beclin-1 expression in cancer tissues. Autophagy has been involved in tumor suppression and tumor progression. Autophagy prevents malignant transformation of normal cells by providing cellular homeostasis, genomic stability, and normal metabolism [49]. Tumor cell growth is frequent promoted by autophagy which maintains metabolism in stressful conditions on established tumors [50, 51]. The present impact of autophagy on tumorigenesis might be context-dependent. Cell growth might be regulated by autophagy in a different manner according to tissue types and specific genetic settings [52, 53]. Autophagy and Beclin proteins have special associations which have been well documented. Beclin proteins interact with components in class III phosphoinositide 3-kinase complex, which is important for initiation of autophagy [49, 54]. Beclin-1 is included in the creating of phagophore in the early stage of autophagy, and is colocalized with LC3 punctae [55].

Basically Beclin-1 has a tumor suppressor function. High expression of Beclin-1 was accompanied with tumor progression in some cancers, as described earlier [44–48]. Allelic loss of the Beclin 1 prevented the creating of tumor in certain genotypes of mice [56,5 7]. Beclin-1 interacts with positive and negative regulators of autophagy and also participates in several non-autophagic processes [58]. The influence of Beclin-1 on tumor progression might be independent from autophagy. However, the present study established the connection of Beclin-1 and tumor progression in the uterine cervix. The connection of Beclin-1 and tumorigenesis can be more complex. The tumor progression which is caused by Beclin-1 overexpression and depletion can be due to different mechanisms. The tumor growth may be evoked by autophagy and/or autophagy-independent mechanisms. Beclin-1 has several interacting partners of Beclin-2, and additionally required for degradation of GASP1-regulated G protein-coupled receptors (GPCRs). Many GPCRs are overexpressed in cancers and contribute to cancer cell proliferation [59]. Accumulation of some GPCRs may be caused by loss of Beclin-2 and lead to tumor progression. High expression of Beclin-1 or Beclin-2, which have been found in previous steps of autophagy, could be issued from either autophagic activation or late step impairment. It can be supposed by all of these factors why patients with either high or low Beclin-1 expression had poorer prognosis than those with moderate expression. There should be more studies to address the biological mechanisms and related signaling pathways of Beclin proteins in tumorigenesis. Most of Beclin-1 is found in the intracytoplasmic organelles, which include endoplasmic reticulum, mitochondria, and perinuclear membrane. Its nuclear expression was seldom noted.

Conclusion

The current achievements show that the evaluation of Beclin-1 expression might provide additional and independent prognostic information to predict the clinical course of uterine cervix cancer. It is confirmed that overexpression of these genes proposes with great certainty, that there are premalignant or malignant changes in the uterine cervix.

Realising Beclin-1 overexpression, modulation, and interaction with other factors which were involved in the autophagic pathways might contribute to the development of new approaches to the prevention of uterine cervical cancer.

References

[1] Klionsky D.J., Emr S.D.: "Autophagy as a regulated pathway of cellular degradation". *Science*, 2000, *290*, 1717.

[2] Kundu M., Thompson C.B.: "Autophagy: basic principles and relevance to disease". *Annu. Rev. Pathol*., 2008, *3*, 427.

[3] Sun P.H., Zhu L.M., Qiao M.M., Zhang Y.P., Jiang S.H., Wu Y.L., Tu S.P.: "The XAF1 tumor suppressor induces autophagic cell death via upregulation of Beclin-1 and inhibition of Akt pathway". *Cancer Lett*., 2011, *310*, 170.

[4] Liu J., Zhang Y., Qu J., Xu L., Hou K., Zhang J., Qu X., Liu Y.: "β s human gastric cancer cells from undergoing apoptosis". *BMC Cancer*, 2011, *11*, 183.

[5] Kim H.S., Lee S.H., Do S.I., Lim S.J., Park Y.K., Kim Y.W.: "Clinicopathologic correlation of beclin-1 expression in pancreatic ductal adenocarcinoma". *Pathol. Res. Pract*., 2011, *207*, 247.

[6] Rasul A., Yu B., Zhong L., Khan M., Yang H., Ma T.: "Cytotoxic effect of evodiamine in SGC-7901 human gastric adenocarcinoma cells via simultaneous induction of apoptosis and autophagy". *Oncol. Rep.,* 2012, *27*, 1481.

[7] Pan W.R., Chen P.W., Chen Y.L., Hsu H.C., Lin C.C., Chen W.J.: "Bovine lactoferricin B induces apoptosis of human gastric cancer cell line AGS by inhibition of autophagy at a late stage". *J. Dairy Sci*., 2013, *96*, 7511.

[8] Park J.M., Huang S., Wu T.T., Sinicrope F.A.: "Association of autophagy regulator beclin 1 with response to neoadjuvant chemoradiation in rectal carcinoma". *Gastroenterology*, 2012, *142*, 111.
[9] Karantza-Wadsworth V., Patel S., Kravchuk O., Chen G., Mathew R., Jin S., White E.: "Autophagy mitigates metabolic stress and genome damage in mammary tumorigenesis:. *Genes Dev*., 2007, *21*, 1621.
[10] Corcelle E.A., Puustinen P., Jäättelä M.: "Apoptosis and autophagy: Targeting autophagy signalling in cancer cells -'trick or treats'?" *FEBS J.*, 2009, *276*, 6084.
[11] Livesey K.M., Tang D., Zeh H.J., Lotze M.T.: "Autophagy inhibition in combination cancer treatment". *Curr. Opin. Investig. Drugs*, 2009, *10*, 1269.
[12] Turcotte S., Giaccia A.J.: 'Targeting cancer cells through autophagy for anticancer therapy". *Curr. Opin. Cell. Biol*., 2010, *22*, 246.
[13] Cheong H., Klionsky D.J.: "Biochemical methods to monitor autophagy-related processes in yeast". *Methods Enzymol*., 2008, *451*, 1.
[14] Eskelinen E.L., Saftig P.: "Autophagy: a lysosomal degradation pathway with a central role in health and disease". *Biochim. Biophys. Acta,* 2009, *1793*, 664.
[15] Kihara A., Kabeya Y., Ohsumi Y., Yoshimori T.: "Beclin-phosphatidylinositol 3-kinase complex functions at the trans-Golgi network". *EMBO Rep.*, 2001, *2*, 330.
[16] Sun Q., Fan W., Zhong Q.: "Regulation of Beclin 1 in autophagy". *Autophagy*, 2009, *5*, 713.
[17] Yue Z., Jin S., Yang C., Levine A.J., Heintz N.: "Beclin 1, an autophagy gene essential for early embryonic development, is a haplosufficient tumor suppressor". *Proc. Natl. Acad. Sci. USA,* 2003, *100*, 15077.
[18] Qu X., Yu J., Bhagat G., Furuya N., Hibshoosh H., Troxel A., et al.: "Promotion of tumorigenesis by heterozygous disruption of the beclin 1 autophagy gene". *J. Clin. Invest.*, 2003, *112*, 1809.
[19] Lebovitz C., Bortnik S.B., Gorski S.M.: "Here, there be dragons: charting autophagy-related alterations in human tumors". *Clin. Cancer Res.*, 2012, *18*, 1214.
[20] Shen Y., Li D.D., Wang L.L., Deng R., Zhu X.F.: "Decreased expression of autophagy-related proteins in malignant epithelial ovarian cancer". *Autophagy*, 2008, *4*, 1067.
[21] Ding Z., Shi Y.H., Zhou J., Qiu S.J., Xu Y., Dai Z., et al.: "Association of autophagy defect with a malignant phenotype and poor prognosis of hepatocellular carcinoma". *Cancer Res*., 2008, *68*, 9167.
[22] Shi Y., Ding Z.B., Zhou J., Qiu S.J., Fan J.: "Prognostic significance of Beclin 1-dependent apoptotic activity in hepatocellular carcinoma". *Autophagy*, 2009, *5*, 380.
[23] Jiang Z., Shao L.J., Wang W.M., Yan X.B., Liu R.Y.: "Decreased expression of Beclin-1 and LC3 in human lung cancer". *Mol. Biol. Rep.*, 2012, *39*, 259.
[24] Won K., Kim G.Y., Kim Y.W., Lim S.J., Kim Y.W.: "Decreased Beclin-1 expression is correlated with the growth of the primary tumor in patients with squamous cell carcinoma and adenocarcinoma of the lung". *Hum. Pathol.*, 2012, *43*, 62.
[25] Chen Y., Lu Y., Lu C., Zhang L.: "Beclin-1 expression is a predictor of clinical outcome in patients with esophageal squamous cell carcinoma and correlated to hypoxia-inducible factor (HIF)-1a". *Pathol. Oncol. Res.*, 2009, *15*, 487.
[26] Dong L., Hou Hou Y.J., Tan Y.X., Tang L., Pan Y.F., Wang M., et al.: "Prognostic significance of Beclin 1 in intrahepatic cholangiocellular carcinoma". *Autophagy*, 2011, *7*, 1222.
[27] Li B., Li C.Y., Peng R.Q., Wu X.J., Wang H.Y., Wan D.S., *et al.*: "The expression of beclin 1 is associated with favorable prognosis in stage IIIB colon cancers". *Autophagy,* 2009, *5*, 303.
[28] Ahn C., Jeong E.G., Lee J.W., Kim M.S., Kim S.H., Kim S.S., *et al.*: "Expression of beclin-1, an autophagy-related protein, in gastric and colorectal cancers". *APMIS*, 2007, *115*, 1344.
[29] Dong M., Wan X.B., Yuan Z.Y., Wei L., Fan X.J., Wang T.T., *et al.*: Low expression of Beclin 1 and elevated expression of HIF-1alpha refine distant metastasis risk and predict poor prognosis of ER-positive, HER2-negative breast cancer". *Med. Oncol.,* 2013, *30*, 355.
[30] Yao Q, Chen J, Lv Y, Wang T, Zhang J, Fan J, et al. The significance of expression of autophagyrelated gene Beclin, Bcl-2, and Bax in breast cancer tissues. *Tumour Biol.*, 2011, *32*, 116.
[31] Lin H.X., Qiu H.J., Zeng F., Rao H.L., Yang G.F., Kung H.F., et al.: "Decreased Expression of Beclin 1 correlates closely with Bcl-xL expression and poor prognosis of ovarian carcinoma". *Plos One*, 2013, *8*, 60516.
[32] Won K.Y., Kim G.Y., Lim S.J., Kim Y.W.: "Decreased Beclin-1 expression is correlated with the growth of the primary tumor in patients with squamous cell carcinoma and adenocarcinoma of the lung". *Hum. Pathol.*, 2012, *43*, 62.
[33] Ding Z.B., Shi Y.H., Zhou J., Qiu S.J., Xu Y., Dai Z., *et al.*: "Association of autophagy defect with a malignant phenotype and poor prognosis of hepatocellular carcinoma". *Cancer Res.*, 2008, *68*, 9167.
[34] Dong L.W., Hou Y.J., Tan Y.X., Tang L., Pan Y.F., Wang M., *et al.*: "Prognostic significance of Beclin 1 in intrahepatic cholangiocellular carcinoma". *Autophagy*, 2011, *7*, 1222.
[35] Kim H.S., Lee S.H., Do S.I., Lim S.J., Park Y.K., Kim Y.W.: "Clinicopathologic correlation of beclin-1 expression in pancreatic ductal adenocarcinoma". *Pathol. Res. Pract.*, 2011, *207*, 247.
[36] Wang J., Pan X.L., Ding L.J., Liu D.Y., Da-Peng L., Jin T.: "Aberrant expression of Beclin-1 and LC3 correlates with poor prognosis of human hypopharyngeal squamous cell carcinoma". *Plos One.* 2013, *8*, 69038.
[37] Huang L., Wang S., Li S.S., Yang X.M.: "Prognostic significance of Beclin-1 expression in laryngeal squamous cell carcinoma". *Pathol. Oncol. Res.*, 2013, *19*, 771.
[38] Chen Y., Lu Y., Lu C., Zhang L.: "Beclin-1 expression is a predictor of clinical outcome in patients with esophageal squamous cell carcinoma and correlated to hypoxia-inducible factor (HIF)-1alpha expression". *Pathol. Oncol. Res.,* 2009, *15*, 487.
[39] Wu X.Y., Chen J., Cao Q.H., Dong M., Lin Q., Fan X.J., *et al.*: "Beclin 1 activation enhances chemosensitivity and predicts a favorable outcome for primary duodenal adenocarcinoma". *Tumour Biol.*, 2013, *34*, 713.
[40] Yu M., Gou W.F., Zhao S., Xiao L.J., Mao X.Y., Xing Y.N., *et al.*: "Beclin 1 expression is an independent prognostic factor for gastric carcinomas". *Tumour Biol.*, 2013, *34*, 1071.
[41] Huang J.J., Zhu Y.J., Lin T.Y., Jiang W.Q., Huang H.Q., Li Z.M.: "Beclin 1 expression predicts favorable clinical outcome in patients with diffuse large B-cell lymphoma treated with R-CHOP". *Hum. Pathol.*, 2011, *42*, 1459
[42] Nicotra G., Mercalli F., Peracchio C., Castino R., Follo C., Valente G., *et al.*: "Autophagy-active beclin-1 correlates with favourable clinical outcome in non-Hodgkin lymphomas". *Modern Pathol.*, 2010, *23*, 937.
[43] Huang J.J., Li H.R., Huang Y., Jiang W.Q., Xu R.H., Huang H.Q., *et al.*: "Beclin 1 expression: a predictor of prognosis in patients with extranodal natural killer T-cell lymphoma, nasal type". *Autophagy*, 2010, *6*, 777.
[44] Wan X.B., Fan X.J., Chen M.Y., Xiang J., Huang P.Y., Guo L., *et al.*: "Elevated Beclin 1 expression is correlated with HIF-1alpha in predicting poor prognosis of nasopharyngeal carcinoma". *Autophagy*, 2010, *6*, 395.
[45] Giatromanolaki A., Koukourakis M.I., Koutsopoulos A., Chloropoulou P., Liberis V., Sivridis E.: "High Beclin 1 expression defines a poor prognosis in endometrial adenocarcinomas". *Gynecol. Oncol.*, 2011, *123*, 147.
[46] Han Y., Xue X.F., Shen H.G., Guo X.B., Wang X., Yuan B., *et al.*: "Prognostic significance of Beclin-1 expression in colorectal cancer: a meta-analysis". *Asian Pac. J. Cancer Prevent.*, 2014, *15*, 4583.
[47] Sakakura K., Takahashi H., Kaira K., Toyoda M., Oyama T., Chikamatsu K.: "Immunological significance of the accumulation of autophagy components in oral squamous cell carcinoma". *Cancer Sci.*, 2015, *106*, 1.
[48] Tang J.Y., Fang Y.Y., Hsi E., Huang Y.C., Hsu N.C.H., Yang W.C., *et*

al.: "Immunopositivity of Beclin-1 and ATG5 as indicators of survival and disease recurrence in oral squamous cell carcinoma". *Anticancer Res.*, 2013, *3*, 5611.

[49] Galluzzi L., Pietrocola F., Bravo-San Pedro J.M., Amaravadi R.K., Baehrecke E.H., Cecconi F., *et al.*: "Autophagy in malignant transformation and cancer progression". *EMBO J.*, 2015, *34*, 856.

[50] Guo J.Y., Xia B., White E.: "Autophagy-mediated tumor promotion". *Cell*, 2013, *155*, 1216.

[51] Kenific C.M., Debnath J.: "Cellular and metabolic functions for autophagy in cancer cells". *Trends Cell Biol.*, 2015, *25*, 37.

[52] Perez E., Das G., Bergmann A., Baehrecke E.H.: "Autophagy regulates tissue overgrowth in a contextdependent manner". *Oncogene*, 2015, *34*, 3369.

[53] Rosenfeldt M.T., O'Prey J., Morton J.P., Nixon C., MacKay G., Mrowinska A., *et al.*: "p53 status determines the role of autophagy in pancreatic tumour development". *Nature*, 2013, *504*, 296.

[54] Kihara A., Kabeya Y., Ohsumi Y., Yoshimori T.: "Beclin–phosphatidylinositol 3-kinase complex functions at the trans-Golgi network". *EMBO Rep.*, 2001, *2*, 330.

[55] Buraschi S., Neill T., Goyal A., Poluzzi C., Smythies J., Owens R.T., *et al.*: "Decorin causes autophagy in endothelial cells via Peg3". *Proc. Nat. Ac. Sci.*, 2013, *110*, 2582.

[56] Valentin-Vega Y.A., MacLean K.H., Tait-Mulder J., Milasta S., Steeves M., Dorsey F.C., *et al.*: "Mitochondrial dysfunction in ataxia-telangiectasia". *Blood*, 2012, *119*, 1490.

[57] Parkhitko A., Myachina F., Morrison T.A., Hindi K.M., Auricchio N., Karbowniczek M., *et al.*: "Tumorigenesis in tuberous sclerosis complex is autophagy and p62/sequestosome 1 (SQSTM1)-dependent". *Proc. Nat. Ac. Sci.*, 2011, *108*, 12455.

[58] Dorsam R.T., Gutkind J.S.: "G-protein-coupled receptors and cancer". *Nat. Rev. Canc.*, 2007, *7*, 79.

[59] He C., Wei Y., Sun K., Li B., Dong X., Zou Z., *et al.*: "Beclin 2 functions in autophagy, degradation of g protein-coupled receptors, and metabolism". *Cell*, 2013, *154*, 1085.

Corresponding Author:
Z. PROTRKA, M.D.
Department of Obstetrics and Gynecology
Kragujevac Stevana Visokog 28
34000 Kragujevac (Serbia)
e-mail: protrka.zoran@gmail.com