

AUTOPHAGY AND RENAL CELL CARCINOMA: WHAT DO WE  
KNOW SO FAR?AUTOFAGIJA I KARCINOM BUBREŽNIH ĆELIJA: ŠTA ZNAMO DO  
SADA?Milan Radovanović<sup>1</sup>, Zoran Džamić<sup>1</sup><sup>1</sup> Klinički centar Srbije, Klinika za urologiju, Beograd, Srbija

Correspondence: milan\_950@hotmail.com

**Abstract**

Renal cell carcinoma (RCC) is the most common type of kidney tumor in adults, accounting for approximately 90% of kidney malignancies, occurring usually between the ages of 60 and 70. The 5-year overall survival rate for all RCC types is 49%. Since RCCs are resistant to numerous different radio and chemotherapeutics that act via apoptosis induction, the development of new approaches to RCC treatment is still in the focus of modern urology. In particular, in recent years, autophagy in RCC has been widely studied as a mechanism of cell extinction through which tumor cells can overcome resistance to apoptosis activation therapy. Autophagy is often referred to as a double-edged sword because it can be a process that allows cells of cancer to survive and, on the other hand and under other conditions, it can be a cell dying mechanism, independent or closely related to other cell death modalities, like apoptosis and necrosis. The central role in the tempering of the process of autophagy, in general, belongs to the mTOR complex (mammalian target of rapamycin), which integrates numerous signals that affect autophagy, such as growth factors, nutrients, various stressors and the energy status of the cell. In RCC, the most important is PI3K/AKT/mTOR signaling pathway, since activation of this signaling leads to survival of tumor cells through mTOR activation and thus, autophagy inhibition. Up to now, it was found that autophagy markers such as Beclin-1 and LC3-II can be considered as prognostic markers for RCC since the high level of Beclin-1 was detected in tissues and cells of RCC (A498 and ACHN cell lines) and that tumor cell mobility is promoted by the up-regulated expression of LC3.

Therefore, a progress in RCC therapy can be expected from the development and synthesis of specific compounds targeting autophagy, as well as the therapy based on their combination.

**Keywords:**renal cell carcinoma,  
autophagy,  
mTOR complex

## Sažetak

Karcinom bubrežnih ćelija je najčešća forma karcinoma bubrega kod odraslih, čineći otprilike 90% svih bubrežnih maligniteta i javlja se najčešće između 60. i 70. godine. Ukupno petogodišnje preživljavanje za sve tipove karcinoma bubrega iznosi 49%. S obzirom na to da je karcinom bubrežnih ćelija rezistentan na mnoge vidove radio i hemioterapije koji se baziraju na indukciji apoptoze, razvoj novih pristupa lečenju ovog karcinoma i dalje je u fokusu moderne urologije. Shodno tome, prethodnih godina autofagija kod karcinoma bubrežnih ćelija široko je ispitivana kao alternativni mehanizam ćelijske smrti kojim bi tumorske ćelije mogle da prevaziđu otpor terapiji koja aktivira apoptozu. Autofagija se često može predstaviti dvojako, imajući u vidu da to može biti mehanizam koji, s jedne strane, dozvoljava tumorskim ćelijama da prežive, a, s druge strane, u drugačijim uslovima može biti mehanizam umiranja ćelija, u zavisnosti ili u bliskoj vezi sa drugim modalitetima ćelijske smrti, kao što su apoptoza i nekroza. Centralnu ulogu u regulaciji autofagije zauzima *mTOR* kompleks, koji integriše brojne signale koji utiču na autofagiju kao što su faktori rasta, nutrienti, različiti faktori stresa i energetska status ćelije. Za karcinom bubrežnih ćelija najveći značaj ima *PI3K/AKT/mTOR* signalni put čija aktivacija vodi u preživljavanje tumorskih ćelija preko *mTOR* aktivacije i, samim tim, u inhibiciju autofagije. Do sada je utvrđeno da bi markeri autofagije *Beclin-1* i *LC3-II* mogli da budu razmatrani kao prognostički markeri kod karcinoma bubrežnih ćelija usled registrovane visoke ekspresije *Beclin-1* u tkivima i ćelijama karcinoma bubrežnih ćelija (*A498* i *ACHN* ćelijske linije) kao i usled povećane mobilnosti tumorskih ćelija, podstaknute povišenom ekspresijom *LC3*.

### Ključne reči:

karcinom  
bubrežnih ćelija,  
autofagija,  
*mTOR* kompleks

Dakle, napredak u terapiji karcinoma bubrežnih ćelija može se očekivati kroz razvoj i sintezu specifičnih jedinjenja koja targetiraju autofagiju i terapiju koja se bazira na njihovoj kombinaciji.

## Introduction

This paper aims to concisely present the modern research aimed at understanding and explaining the role of autophagy in both, the development and the progression of kidney tumors, primarily, renal cell carcinoma (RCC) as the most commonly occurring renal malignancy.

Kidney cancer represents around 3% of all cancers and it is the 14th most common malignancy in world population, with global age standardized incidence rate of 4 per 100 000 (1, 2). The incidence of this cancer varies widely in terms of geographical distribution and the incidence is particularly high in the most developed parts of the world, namely in Europe, North America and Australia (3). More specifically, during the last twenty years, until recently, there has been an annual growth of about 2% in incidence both worldwide and in Europe, which lead to approximately 99 200 new RCC cases and 39 100 kidney cancer-related deaths within the European Union in 2018 (1).

Renal cell carcinoma (RCC) is the most commonly occurring type of kidney tumor in adults, representing nearly 90% of kidney malignance and it occurs usually between the ages of 60 and 70 (4, 5). It often displays no symptoms until the late stage of the disease. The classic symptoms are often represented by a triad that consists of flank pain, visible haematuria and palpable abdominal mass, seen in only 6-10% of cases. As the stage and histopathological grade become higher, the prognosis for all RCC types worsens. Hence, the 5-year overall survival rate for all RCC types is 49% (2). Renal cell carcinoma shows great

heterogeneity at histological, morphological and molecular levels and, according to its histological presentation, it is classified in different types: the most frequent ones are the clear cell carcinoma (ccRCC, 80-90%), the papillary carcinoma (6-15%) and the chromophobe carcinoma (2-5%) (6, 7). Thanks to the diagnostics made by abdominal ultrasound (US), computered tomography (CT) imaging and magnetic resonance (MR), the detection of RCC becomes faster. However, despite the earlier detection of RCC and the improvement of tumor treatment, the rate of RCC related mortality is still high.

Therefore, the great efforts are placed in investigation of molecular biomarkers in RCC, such as carbonic anhydrase IX (CaIX), vascular endothelial growth factor (VEGF), hypoxia-inducible factor (HIF), Ki67 (proliferation), p53, p21 (8), PTEN (phosphatase and tensin homolog), E-cadherin, osteopontin (9) CD44 (cell adhesion) (10, 11), CXCR4 (12), and other cell cycle and proliferative markers that are being investigated (13, 14) as biomarkers, but also the targets for specific antitumor therapy (15). Unfortunately, these markers failed to repair the predictive shortcomings of the prognostic systems currently in use and, so far, they have not received external validation.

For that reason, the development of new therapeutic approaches to RCC is still in the focus of modern urology. This is mainly because RCCs are resistant to different radio and chemotherapeutics that induce apoptosis. The molecular mechanism of apoptosis is complex and malignant transformation or resistance to anticancer drugs usually occurs as the result of apoptotic signaling pathway disruption. Therefore, it would be useful to focus to other

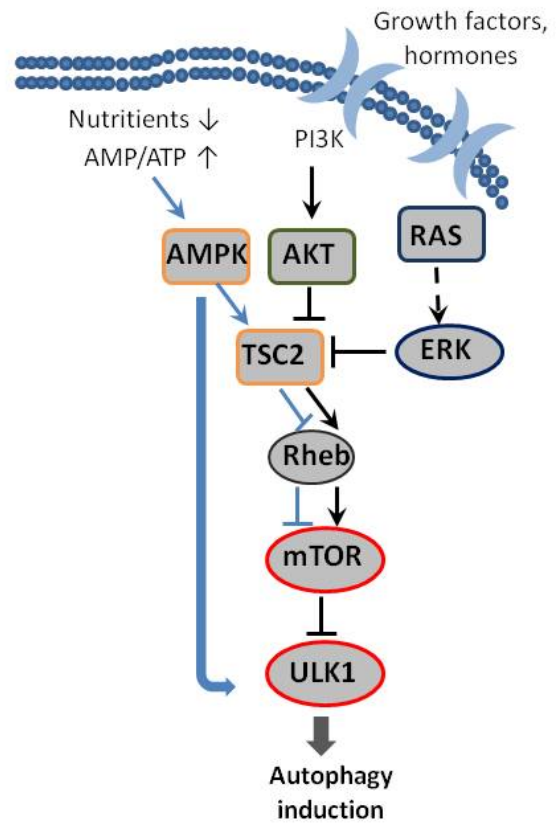
approaches in order to increase tumor sensitivity to chemotherapeutics by inducing specific cell death mechanism. Thus, in recent years, autophagy in RCC has been widely studied as an alternative pathway for cell death through which tumor cells can overcome resistance to apoptosis activation therapy.

## Autophagy mechanisms and major signaling pathways involved in autophagy control

Autophagy (macroautophagy) is a process of intracellular digestion, mediated by lysosomes, which removes various cellular components. Under physiological conditions, autophagy removes long-lived proteins and damaged organelles, but during the period of nutrient deprivation, autophagy becomes a process that allows the cell to survive, providing it with the necessary molecules to maintain metabolic processes, for example, the synthesis of adenosine three phosphates - ATP (16). In the following text, solely molecules significant for RCC autophagy will be discussed.

During autophagy, the part of the cytoplasm that contains the intracellular components that need to be removed is surrounded by a membrane and thus a phagophore is formed (17). Macroautophagy is realized in several steps (**figure 1**): Its induction is initiated by specific stress and is mediated by a complex containing the ULK1 protein; phagophore is formed through activation of complex containing VPS34 kinase, Beclin-1 and UV irradiation resistance-associated tumor suppressor gene (UVRAG); phagophore elongation is mediated by ATG4-dependent cleavage of microtubule-associated protein light chain 3 (LC3) to LC3-I and subsequent conjugation of phosphatidylethanolamine to LC3-I yielding to LC3-II formation (specific marker of autophagosome formation); once formed, the autophagosome containing a cytosolic cargo will fuse with the lysosome, which triggers the degradation of ubiquitinated cytoplasmic material bound to autophagic cargo receptor sequestosome 1 (SQSTM1/p62). Numerous genes included in the process of autophagy have been detected in yeast, collectively called ATG genes (AuTophagy-related Genes), and a significant part of them has their homologues in mammalian cells (18).

Autophagy is often metaphorically called a

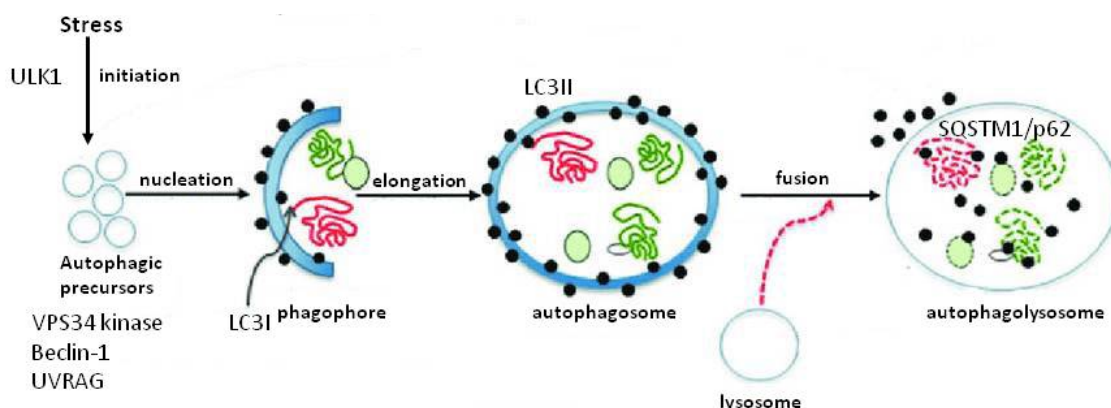


**Figure 2.** The process of autophagy

double-edged sword, since it can be a process that allows cells of cancer to survive and, on the other hand and under other conditions, it can be a cell dying mechanism, independent or closely related to other cell death modalities, like apoptosis and necrosis. The determining factors for the impact that the activation of autophagy will have on tumor cells include: the stage of carcinogenesis, cell type or tissue, and the nature of the stressor (19, 20).

The central role in the tempering of the process of autophagy, in general, belongs to the mTOR complex (mammalian target of rapamycin), which integrates numerous signals that affect autophagy, such as growth factors, nutrients, various stressors and the energy status of the cell (**figure 2**).

A major repressor of autophagy, mTOR kinase, is included in the control of cell growth and proliferation. One of the major activators of the mTOR pathway is the



**Figure 1.** Autophagolysosome formation. (Adapted from Nguyen TG, Honson NS, Arns S, Davis TL, Dhe-Paganon S, Kovacic S, et al. *Assay Drug Dev Technol.* 2014; 12(3):176-89)

Akt/protein kinase B signaling pathway, the activation of which is triggered by different hormones and growth factors. The Akt protein accomplishes its action by inhibiting the phosphorylation of the tumor suppressor gene TSC 1/2 (tuberous sclerosis complex 1/2) products. A major mTOR inhibitor is TSC 1/2, through which many mTOR activators exert their action (21). On the other hand, during nutrient deprivation, there is an increase in the intracellular concentration of adenosine monophosphate-AMP, which results in the activation of AMP-activated protein kinases (AMPK). It activates TSC 1/2, resulting in mTOR inhibition and induction of autophagy that, as previously mentioned, will contribute to renewal of ATP. Also, many intracellular signaling molecules are involved in autophagy regulation, such as: RAS, ERK, MEK, c-Jun aminoterminal kinase, etc. Furthermore, stressors can also initiate autophagy. For example, damage to the mitochondria or endoplasmic reticulum, represent triggers for the onset of autophagy (22, 23). In addition, increased production of free radicals may also initiate autophagy (24).

## Autophagy signaling pathways in renal cancer

At present, it is known that regulation of autophagy is in direct relation to the functioning of RCC cells, disease, pathogenesis and target therapy resistance. (25)

Autophagy signaling pathway in RCC is under control of numerous different molecules. The most important one is PI3K/AKT/mTOR signaling pathway (**figure 2**) and its activation leads to survival of tumor cells through mTOR activation and thus autophagy inhibition. (26) Moreover, PI3K/Akt/mTOR pathway is defined as the most important pathway in regulation, initiation and progression of autophagy in RCC (27). By examining the role of autophagy and its signaling pathway in ccRCC, transcriptional induction of autophagy in ccRCC was found, accompanied by AMPK/mTOR-independent increase in ULK1 activation and autophagic flux, which might slow tumor progression and metastasis independently of apoptosis (28).

Genes included in the mTOR pathway (genes encoding for phosphatidylinositol-3-kinase (PI3K), phosphatase and tensin homolog (PTEN), protein kinase B (AKT), and mTOR) are mutated in even 28% of RCC cases (29, 30). In this respect, a great importance was dedicated to PTEN, as a favorable prognostic factor in RCC. (31) Consequently, inhibiting PI3K signaling pathway leads to inhibition of RCC tumor growth and therefore can be considered a significant therapeutically target. That was confirmed by *in vitro* studies involving different lines of RCC cell that were treated with different inhibitors of PI3K/mTOR pathway. The positive results were obtained under the treatment with specific PI3K inhibitor LY294002 which increased tumor cell death. Furthermore, the more promising results were obtained in study of Seo et al. (32) by co-treatment with an inhibitor of mTORC1 and mTORC2 (PP242) and curcumin which induced down regulation of AKT and autophagy in RCC.

Moreover, it was shown that mutation of different intracellular signaling molecules, like tumor suppressor gene of phosphatase and tensin homolog (PTEN), tuberous sclerosis complex (TSC) 1 and TSC2, PI3K and AKT can inhibit autophagy (33). According to its role in cancerogenesis (34) as expected, expression of PTEN was significantly lower in ccRCC tumor tissue (35). Using immunohistochemical analysis, it was shown that PTEN expression is nearly lost during carcinogenesis of ccRCC and that this is an early event in the evolution of ccRCC (36). Besides that, the loss of PTEN is also associated with the worst reaction to therapy (37).

It is also worth mentioning that the existence of a close connection between the regulation of autophagy and apoptosis in RCC is achieved by p53 protein. This interconnection is very important since modulation of autophagy can help the cells overcome tumor resistance which usually arises from deficient apoptosis. Protein p53 is a product of p53 tumor suppressor gene. As presented in nucleus, it plays a pivotal role in regulating cell division and cell death. Its activity is promoted when DNA damage occurs. As a result, p53 induces cell cycle arrest providing damaged cell with quite enough time necessary for DNA repair. If the cell fails, the apoptotic cell death will be initiated. Speaking of its role in autophagy, in case of its overexpression, p53 can promote autophagy via transactivation of autophagy-inducing genes and inhibition of mTOR through AMPK and TSC1/TSC2 dependent pathways (38). It is interesting that the overexpression of p53 was demonstrated in RCC tissues in two clinical studies confirming its role in RCC tumor genesis (39, 40). Taking into consideration its physiological role, it was also shown that the promotion of tumor cell growth by p53 overexpression is possibly mediated via its role in DNA repair (41). Bearing in mind the critical role of p53 in tumor evolution, as well as in apoptosis vs autophagy interconnection, more than 30 studies analyzed the prognostic power of p53 in prediction of RCC (42), confirming that the positive p53 status can reliably serve as a predictive factor for tumor recurrence and shorter survival. There is also the evidence that p53 over expression is present in primary and metastatic RCC specimens (40).

## Autophagy markers as prognostic parameters in RCC

Up to now, it was found that Beclin-1 and LC3-II can be considered as prognostic markers for RCC since the high level of Beclin 1 was detected in tissues and cells of RCC (A498 and ACHN cell lines) and that cell mobility is increased by the induced expression of LC3 (43).

An additional interesting finding is related to connection between the Von Hippel-Lindau (VHL) tumor suppressor, as a well known risk factor associated with RCC, and autophagy. The loss of VHL promotes tumor growth via induction of the hypoxia inducible factor (HIF) (44). At the same time, VHL regulates autophagy in ccRCC providing VHL negative cells more dependent on autophagy

and therefore more vulnerable to its restriction (45). This finding makes an interesting view of targeted therapy based on autophagy modulation.

### Autophagy modulation as a mechanism of the anticancer drug action

Considering the general role of autophagy in the formation and progression of various tumors, autophagy as a mechanism of cell death can be induced in tumor cells using different cytotoxic drugs. For example, the antiestrogenic drug tamoxifen, which is applied in the therapy of breast cancer, but also for its prevention, induces autophagy in MCF-7 cell line (human breast cancer) and this effect appears necessary for definitive cell death (46). Arsenic trioxide induces glioma and leukemia cell death by apoptosis and autophagy processes, which are associated with increased expression of Beclin I (47). Rapamycin, a direct inhibitor of mTOR, is probably the most well-known inducer of autophagy and clinical trials of its potential use as an anticancer drug are currently underway (48). Some drugs that induce DNA damage, e.g. capotecin, etoposide, and temolozamide, can also cause autophagy, but such induced autophagy is not a mechanism of cell death, but may be a mechanism of drug cell resistance (49). Namely, autophagy induced by etoposide and temolozamide leads to the production of ATP, which protects tumor cells from mitotic catastrophe (cell death that occurs during cell division, most often as a consequence of inadequate chromosome segregation).

It is obvious that the modulation of autophagy with different agents has a very large therapeutic potential, however, it is necessary to analyze in details the type of tumor that is affected, the appropriate time of action and the type of agent which is used for autophagy modulation.

### Autophagy modulation as a possible therapeutic approach of the renal cell carcinoma treatment

Renal cell carcinoma localized neoplasms are usually successfully treated with a partial or radical nephrectomy. Systemic chemotherapy or immunotherapy treatment is used in cases of metastatic, relapsed, and surgically unresectable tumors. Therefore, the great interest has been focused on autophagy modulation as a novel modality in RCC treatment.

As previously discussed, since autophagy plays a major role in cell survival and preservation of their normal functioning, its role in tumor genesis may be bidirectional: tumor-suppressive and tumor-survival. Therefore, different methods based on stimulation or inhibition of autophagy have been investigated and described in RCC therapy.

The connection that exists between VHL and autophagy in RCC represents one of the potential target spots of therapeutic action. Great attention was focused on STF-62247, a compound, which selectively targets VHL deficient

cells in vitro and in vivo (50). It was shown that it exhibits a dual positive effect: by increasing autophagy and increasing radio sensitivity of these cells by inducing autophagy (51).

Since autophagy in RCC is under control of PI3K/Akt/mTOR signaling pathway, it should be safe to assume that stimulation of autophagy in RCC can be initiated by inhibition of the Akt / mTOR signaling pathway. This result can be achieved most commonly by the use of tyrosine kinase receptor inhibitors, such as Sunitinib (52). Also, direct inhibition of mTOR by AZD-2014 as a dual mTORC1/2 inhibitor can induce autophagy cell death of RCC (53). In addition to this therapeutic approach, there is also the possibility of stimulating autophagy through increased LC3 expression (Silybin) (54). This autophagy marker can be also affected with Ubenimex that upregulates LC3II expression and therefore induces cytotoxic autophagy in RCC cells (55).

There are also many different plant derived substances that can induce autophagy in RCC. Some of them acts also by enhancing LC3II expression (Goniothalamus, Silibinin), Beclin-1 and LC3-I/LC3-II up-regulation and p62 down-regulation (Sinomenine), or by p53-mediated AMPK/mTOR signaling (Rezveratrol). Furthermore, Silibinin also exhibited anti-metastatic capacity in human RCC cells (56).

Beside autophagy induction, autophagy inhibition can be also considered as a potential therapeutic approach in RCC treatment. The most prominent autophagy inhibitors, up to now, are Chloroquine, 5-Methyladenine and Bafilomycin A1 were investigated. Chloroquine and hydroxychloroquine inhibit autophagosome formation via deacidification of lysosomes thus preventing a formation of autophagolysosome. Usually, they are used in RCC treatment in combination with other autophagy inhibitors like everolimus and sunitinib (57).

Everolimus belongs to PIK3 family inhibitors, and it inhibits mTOR by blocking important downstream molecules in its signaling pathway. It is usually used as a second line of RCC therapy, but a significant problem for its application is a resistance that develops in RCC patients. While mTOR inhibitors activate autophagy, it can be assumed that the basis of this resistance to everolimus lies in autophagy activation. As already mentioned, everolimus can be also used in combination with chloroquine and hydroxychloroquine, a classical autophagy inhibitors (58). However, limited clinical studies have evaluated hydroxychloroquine, an autophagy inhibitor, in patients with RCC and they were usually based on its combination with other autophagy inhibitors (59, 60).

### Conclusion

Autophagy plays a pivotal role in RCC initiation and evolution, with a dual function in the activation, progression, treatment, and drug resistance of RCC. Therefore, both autophagy activators and autophagy inhibitors may exhibit beneficial effects by inhibiting a RCC growth and/or metastatic potential. Several autophagy markers can contribute

to a prediction of postoperative disease recurrence in patients with RCC, but they can also serve as target molecules for precise therapeutically approach. Therefore, a progress in RCC therapy can be expected from the development and synthesis of specific compounds targeting autophagy, as well as therapy based on their combination.

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