Correspondence on 'Role of AMPK/mTOR-independent autophagy in clear cell renal cell carcinoma' by Radovanovic *et al*

Shahrokh Lorzadeh,[1] Saeid Ghavami [2,3,4]

[1]Department of Human Anatomy and Cell Science, University of Manitoba, Winnipeg, Manitoba, Canada
[2]University of Manitoba College of Medicine, Winnipeg, Manitoba, Canada
[3]Research Institute of Oncology and Hematology, Cancer Care Manitoba-University of Manitoba, Winnipeg, Manitoba, Canada
[4]Biology of Breathing Theme, Children Hospital Research Institute of Manitoba, University of Manitoba, Winnipeg, Manitoba, Canada

Correspondence to
Professor Saeid Ghavami, Department of Human Anatomy and Cell Sciecne, University of Manitoba College of Medicine, Winnipeg, MB R3E 0J9, Canada;
saeid.ghavami@umanitoba.ca

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Dear Editor,

We have read this article published in the *Journal of Investigative Medicine* with great interest. In this paper, Radovanovic *et al*[1] investigated macroautophagy (hereafter autophagy) and apoptosis focusing on the regulatory roles of 5'-adenosine monophosphate-activated protein kinase (AMPK) and mechanistic target of rapamycin (mTOR) in clear cell renal cell carcinoma (ccRCC). They showed autophagy induction (uncoordinated protein 51-like kinase 1 (ULK1) phosphorylation, LC3 lipidation and p62 degradation) in ccRCC compared with normal renal cells. Interestingly, the active form of AMPK was shown to be lower in ccRCC whereas the phosphorylated form of

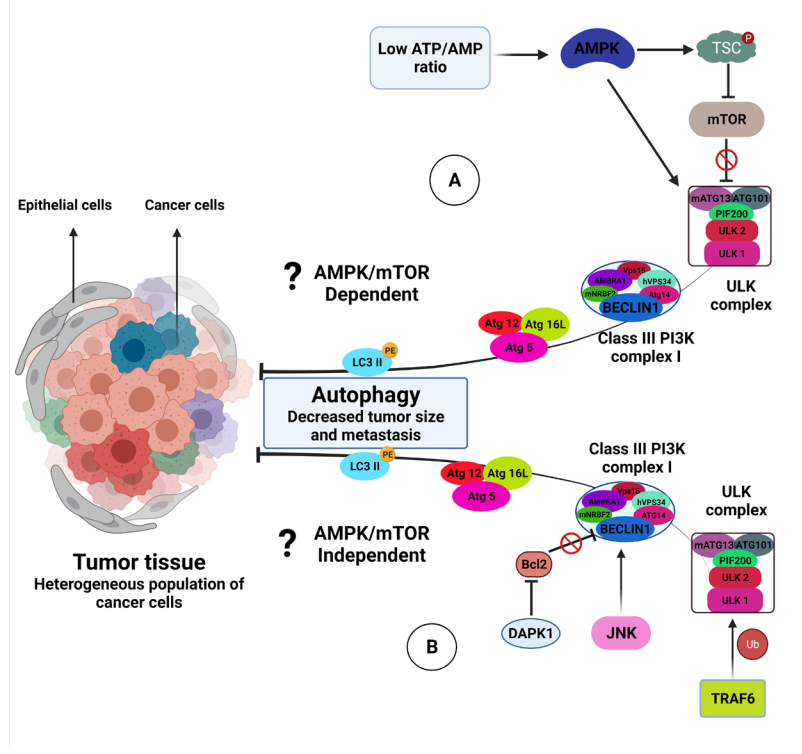

Figure 1 Autophagy induction decreases tumor size and metastasis in clear cell renal cell tumors either through AMPK/mTOR-dependent or AMPK/mTOR-independent pathways. This might be related to a heterogeneous population of the tumor specimens. (A) AMPK/mTOR-dependent autophagy. mTOR negatively regulates autophagy by inhibiting the autophagy initiation complex, ULK complex. When the ATP/AMP ratio is low, AMPK activates the TSC complex by phosphorylation leading to mTOR inhibition and relief of the inhibitory effect of mTOR on the ULK complex. Alternatively, AMPK can also directly activate the ULK complex. Activation of ULK triggers the hierarchical activation autophagy machinery including class III PI3K complex I, consisting of BECLIN1 and hVPS34 proteins, ATG12-ATG5-ATG16L and phosphatidylethanolamine (PE) conjugated LC3 (LC3II). (B) AMPK/mTOR-independent autophagy. TRAF6 enhances ULK activity through ULK1 ubiquitination (Ub). The JNK pathway can directly activate BECLIN1. Also, DAPK1 can inhibit Bcl2, the inhibitory partner of BECLIN1. Activation of BECLIN1 triggers activation of class III PI3K complex I and therefore autophagy initiation, independent of the AMPK/mTOR pathway. AMPK, 5'-adenosine monophosphate-activated protein kinase; DAPK, death associated protein kinase 1; JNK, c-Jun N-terminal kinase; mTOR, mechanistic target of rapamycin; TRAF6, tumor necrosis factor receptor-associated factor 6; TSC, tuberous sclerosis complex; ULK, uncoordinated protein 51-like kinase. (The scheme was prepared by BioRender software).

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initiation factor 4E-binding protein 1 (4EBP1), the substrate of mTOR, was increased. Therefore, they concluded that autophagy was executed independent of the AMPK and mTOR pathway.

Autophagy is responsible for degradation and recycling of impaired organelles and cytosolic misfolded proteins to maintain cellular function.[2] The autophagy process is executed by autophagy related (ATG) proteins. mTOR is one of the key regulators of autophagy by negatively controlling the autophagy initiation complex, ULK, under normal conditions and energy abundance.[3] The AMPK pathway is energy sensitive which leads to mTOR inhibition and autophagy induction.[4]

Autophagy components are subject to various post-translational regulatory modifications, such as phosphorylation, ubiquitination and acetylation. For instance, autophagy can be stimulated by death associated protein kinase 1 (DAPK1)- and c-Jun N-terminal kinase (JNK)-mediated phosphorylation of BECLIN1 and its inhibitory binding partner, Bcl2, respectively.[5] Tumor necrosis factor receptor (TNFR)-associated factor 6 (TRAF6) causes ULK1 ubiquitination during autophagy induction, facilitating its kinase activity. TRAF6 is also involved in the regulatory mechanism between the Wnt/β-catenin signaling pathway and autophagy.[6 7] Moreover, ATG proteins have been shown to play roles in cellular processes other than autophagy including autophagy-independent apoptosis induction.[5 8]

The dual role of autophagy as a pro- and anti-cancer pathway has been investigated extensively. Autophagy can support the energy requirement of cancer cells for growth and metastasis.[5 9] Conversely, induction of autophagy has been shown to restrain the growth and progression of tumor cells in several cancers, including breast cancer, hepatocellular carcinoma and RCC.[10–12] In line with this, a study by Li *et al*[13] showed the anti-metastatic effect of autophagy induction in an adenocarcinoma; renal cell (ACNH) and 786-O RCC cell model-based study. In contrast to the work by Radovanovic and colleagues, this study demonstrated that the AMPK/mTOR pathway was involved in autophagy induction.[13] This discrepancy might be due to the fact that, in the study by Radovanovic *et al*, whole tumor tissue was used for total cell lysate preparation, which may also contain a heterogeneous population of cancer cells and renal epithelial cells (figure 1).[14 15] Therefore, the AMPK/mTOR axis in autophagy activation requires further investigation and we propose that assessing and fully understanding the functional impact of non-canonical autophagy regulators in ccRCC might provide novel cancer treatment strategies and discover the roles of unidentified regulatory proteins in the autophagy pathway.

Twitter Saeid Ghavami @cellAutophagy

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ORCID iD
Saeid Ghavami http://orcid.org/0000-0001-5948-508X

REFERENCES

1 Radovanovic M, Vidicevic S, Tasic J, *et al*. Role of AMPK/mTOR-independent autophagy in clear cell renal cell carcinoma. *J Investig Med* 2020;68:1386–93.
2 Eshraghi M, Adlimoghaddam A, Mahmoodzadeh A, *et al*. Alzheimer's disease pathogenesis: role of autophagy and mitophagy focusing in microglia. *Int J Mol Sci* 2021;22. doi:10.3390/ijms22073330. [Epub ahead of print: 24 Mar 2021].
3 Behrouj H, Seghatoleslam A, Mokarram P, *et al*. Effect of casein kinase 1α inhibition on autophagy flux and the AKT/phospho-β-catenin (S552) axis in HCT116, a Ras-mutated colorectal cancer cell line. *Can J Physiol Pharmacol* 2021;99:284–93.
4 Mokarram P, Albokashy M, Zarghooni M, *et al*. New frontiers in the treatment of colorectal cancer: autophagy and the unfolded protein response as promising targets. *Autophagy* 2017;13:781–819.
5 Boya P, Reggiori F, Codogno P. Emerging regulation and functions of autophagy. *Nat Cell Biol* 2013;15:713–20.
6 Lorzadeh S, Kohan L, Ghavami S, *et al*. Autophagy and the Wnt signaling pathway: a focus on Wnt/β-catenin signaling. *Biochim Biophys Acta Mol Cell Res* 2021;1868:118926.
7 Nazio F, Strappazzon F, Antonioli M, *et al*. mTOR inhibits autophagy by controlling ULK1 ubiquitylation, self-association and function through AMBRA1 and TRAF6. *Nat Cell Biol* 2013;15:406–16.
8 Bestebroer J, V'kovski P, Mauthe M, *et al*. Hidden behind autophagy: the unconventional roles of ATG proteins. *Traffic* 2013;14:1029–41.
9 Bhatt V, Khayati K, Hu ZS, *et al*. Autophagy modulates lipid metabolism to maintain metabolic flexibility for *Lkb1*-deficient *Kras*-driven lung tumorigenesis. *Genes Dev* 2019;33:150–65.
10 Fan Y, Hou T, Dan W, *et al*. Silibinin inhibits epithelial-mesenchymal transition of renal cell carcinoma through autophagy-dependent Wnt/β-catenin signaling. *Int J Mol Med* 2020;45:1341–50.
11 Ji Y, Li L, Ma Y-X, *et al*. Quercetin inhibits growth of hepatocellular carcinoma by apoptosis induction in part via autophagy stimulation in mice. *J Nutr Biochem* 2019;69:108–19.
12 Zhang Y, Fan Y, Huang S, *et al*. Thymoquinone inhibits the metastasis of renal cell cancer cells by inducing autophagy via AMPK/mTOR signaling pathway. *Cancer Sci* 2018;109:3865–73.
13 Li F, Ma Z, Guan Z, *et al*. Autophagy induction by silibinin positively contributes to its anti-metastatic capacity via AMPK/mTOR pathway in renal cell carcinoma. *Int J Mol Sci* 2015;16:8415–29.
14 Cairns P. Renal cell carcinoma. *Cancer Biomarkers* 2011;9:461–73.
15 Hsieh J, Purdue M, Signoretti S. Renal cell carcinoma (RCC) encompasses a heterogeneous group of cancers derived from renal tubular epithelial cells. *Nat Publ Group* 2017;3:1–19.