AUTOPHAGY SUPPRESSION POTENTIATES THE SUNITINIB-DEPENDENT INHIBITION OF CELL GROWTH AND MIGRATION IN KIDNEY CARCINOMA CELLS

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BACKGROUND

Renal cell carcinoma (RCC) is a heterogeneous group of cancers arising from renal tubular epithelial cells. The most common subtype of RCC is the clear cell RCC (ccRCC) that accounts for 70-80% of cases. The thirty percent of patients with RCC show metastasis at diagnosis and about 30% of cases develop disease recurrence after surgical resection. Many RCC patients treated with first-line vascular endothelial growth factor receptor (VEGFR) inhibitors such as sunitinib and pazopanib develop disease recurrence and require second-line therapy. The resistance to pharmacological treatment could be due to the activation of autophagy. In fact, the treatment with chemotherapeutic agents may cause metabolic stress that might induce autophagy, limiting the antitumor efficacy of these drugs. Therefore, the suppression of this process could enhance the effectiveness of conventional therapy.

MATERIAL AND METHODS

This study was performed by using kidney carcinoma cells (KJ29 and Caki-2) and SW-40 transformed normal epithelial kidney cells (4/5). The inhibition of autophagy was conducted infecting normal and ccRCC cells with lentiviruses containing the empty pLKO vector, used as control and the 394 and 395 recombinant vectors expressing specific shRNAs able to silence the autophagic ATG7 gene. The efficiency of ATG7 gene silencing as well as the inhibition of autophagy were evaluated by Western blotting using specific antibodies for ATG7 and LC3II proteins, respectively. Cell growth was investigated by CellTiter cell proliferation assay [3]. Briefly, normal (4/5) and ccRCC (KJ29 and Caki-2) cells were infected with either pLKO or 395 lentivirus for 24 h in DMEM/F12 medium with 1% FBS. Next, cells were cultured for further 48 h in presence of 10 μM sunitinib or DMSO (vehicle) and processed following the manufacturer's protocol. Cell migration was evaluated in Caki-2 ccRCC cells, treated as described above. Before the treatment with sunitinib or DMSO, pLKO and 395 transduced cells were grown at confluence in 24 well plates and a groove between the cells was generated using a sterile tip. Cell migration (groove filling) was analyzed after 48 h of culture with a phase contrast microscope equipped with a CCD camera and processed through the ImageJ software. Apoptosis was analyzed by the Hoechst method. Apoptotic nuclei were visualized by a fluorescence microscope after Hoechst staining of Caki-2 ccRCC cells treated with pLKO and 395 lentiviruses alone or in combination with sunitinib. Images were acquired by a CCD camera and processed using the ImageJ software. Statistical analysis was performed by ANOVA test.

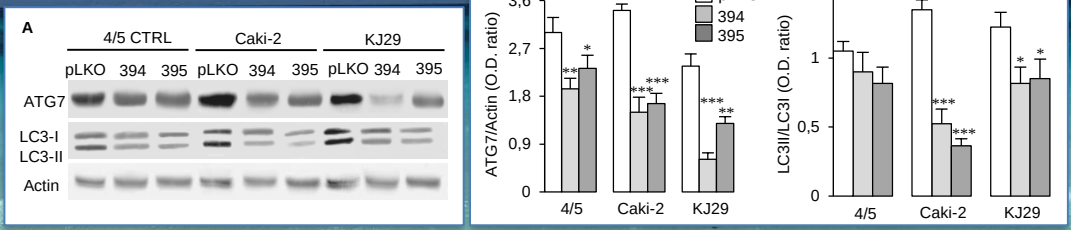

Figure 1. ATG7 gene silencing reduces autophagy in normal and ccRCC cells.
(A-B) Western blot analysis of normal (4/5) and ccRCC (Caki-2 and KJ29) cells shows that the expression of shRNAs for the silencing of the autophagic ATG7 gene (394 and 395) causes the reduction of ATG7 protein compared with control (pLKO). Consistently, also the levels of the autophagy marker LC3II are decreased (A-C). The statistical significance is: **p*<0.05, ***p*<0.01 and ****p*<0.001.

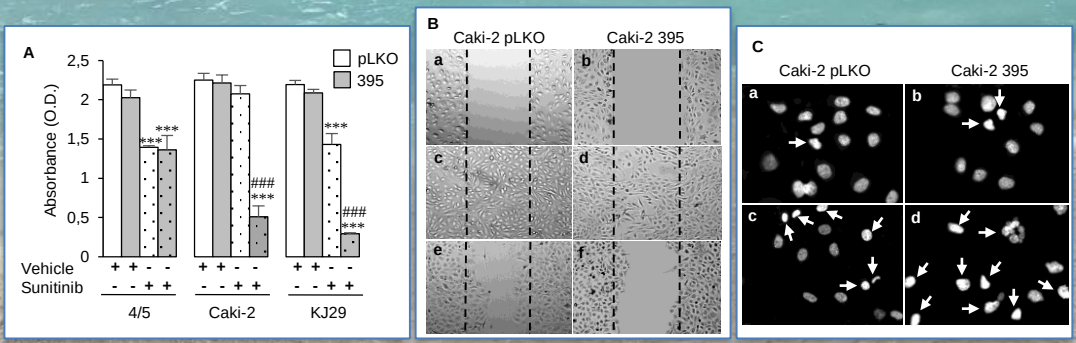

Figure 2. Inhibition of autophagy potentiates the sunitinib treatment reducing cell growth and migration as well as increasing apoptosis.
(A) Normal and ccRCC cells were infected with pLKO and 395 lentiviruses and cultured in DMEM/F12 1% FBS in presence of vehicle (DMSO) or sunitinib (10 μM) and analyzed by CellTiter method. The treatment with sunitinib significantly reduced cell proliferation in all cell types except for Caki-2 cells (****p*<0.001). The combined treatment with 395 shRNA and sunitinib strongly reduces cell growth in both Caki-2 and KJ29 ccRCC cells, but not in 4/5 control cells (###*p*<0.001). (B) Cell migration is arrested by autophagy inhibition and sunitinib application. Cell migration was analyzed in Caki-2 cells treated with pLKO and 395 lentiviruses and cultured at 100% confluence. Before the treatment, Caki-2 pLKO and 395 transduced cells were detached in order to generate a groove (boxes a and b). Next, cells were cultured for further 48 h in DMEM/F12 1% FBS medium in presence of DMSO (boxes c and d) and 10 μM sunitinib (boxes e and f). Images were acquired at 10X magnification. (C) The autophagy suppression combined with sunitinib treatment induces apoptosis in ccRCC cells. Caki-2 cells were treated with pLKO and 395, as described above, and cultured on glass coverslips in 6 well plates for 48 h in DMEM/F12 0.4% BSA in presence of DMSO (boxes a and b) or with 10 μM sunitinib (boxes c and d). Images were acquired at 40X magnification. The white arrows indicate the apoptotic nuclei.

CONCLUSION

Data here reported suggest that the inhibition of autophagy improves the efficacy of sunitinib potentiating the reduction of cell proliferation and migration and increasing apoptosis. Thus, the suppression of autophagy combined with the administration of tyrosine kinase inhibitors could lead to new opportunities for the treatment of metastatic kidney carcinoma.