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Defective Autophagy Leads to the Resistance to PP2 in ATG5 Knockout Cells Generated by CRISPR-Cas9 Endonuclease

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Abstract : Upregulated Src activity has been implicated in a variety of cancers. Thus, Src family tyrosine kinase (SFK) inhibitors are often effective cancer treatments. Here, we investigate the role of autophagy in ATG5 knockout cell lines generated by the Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)/Cas mediated genome editing. The CRISPR-associated protein Cas9 is an RNA-guided DNA endonuclease that uses RNA–DNA complementarity to identify target sites for sequence specific double-stranded DNA (dsDNA) cleavage. Interestingly, ATG5 KO cells clearly showed a greater proliferation rate than WT NIH 3T3 cells, implying that autophagy induction is cytotoxic. Also, the clonogenic survival of ATG5 KO cells was greater than WT cells. The MTT assay revealed that the cytotoxic effect of PP2 was weaker on ATG5 knockout cells than that WT cells. The conversion of non-autophagic LC3-I to autophagic LC3-II and RT-PCR confirmed the functional gene knockout. Furthermore, Cyto-ID autophagy assay also revealed that PP2 failed to induce autophagy in ATG5 knockout cells. Together, our findings suggest that the resistance to PP2 in ATG5 knockout cells is associated with defective autophagy.

Keywords : ATG5 knockout, Autophagy, CRISPR/Cas9, PP2
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