Supplementary Method:

Treatment of Autophagy Inhibitor 3-Methyladenine (3-MA)

3-MA (PI3K inhibitor) inhibits the formation of autophagosome, thus acts as a specific inhibitor of autophagic/lysosomal protein degradation. [1] It has been useful in defining the role of autophagy under various physiological conditions, such as cerebral ischemia. [2, 3] 3-MA at the concentration of 1 mM effectively blocked the activation of autophagy as evidenced by inhibiting the production of LC3-II. [4] Rat cortical neurons were incubated with 3-MA (1 mM) during OGD/RP treatment. Briefly, 3-MA (1 mM) was added into EBSS solution before OGD treatment and after 6 h OGD, fresh regular culture medium with 1 mM 3-MA was replaced when neurons underwent prolonged RP for 24, 48, or 72 h. Cell samples were collected and processed for western blot assays. The expression levels of autophagy markers as well as BNIP3/Bcl-2 were evaluated.

References

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