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Bortezomib activation of mTORC1 impairs autophagic flux resulting in DRG neuronal apoptosis

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ABSTRACT

Introduction： Peripheral neurotoxicity is the most common side-effect of bortezomib (Bort). Autophagy, documented to be negatively regulated by mTORC1, plays an important role in controlling cell survival/death. However, how autophagy and mTORC1 contribute to Bort's peripheral neurotoxicity remains enigmatic. **Material and methods：** DRG neurons or DRG neurons infected with lentivirus-Raptor-shRNA were treated with/without Bort for 18 or 24 h after treatment with/without Rapamycin (Rap) for 2 h. Protein levels were analyzed by western blot. Accumulation of autophagosomes was assessed by GFP-LC3 assay. Autophagic flux was monitored by co-localization assay for autophagosomes/lysosomes and RFP-p62 assay. Apopotosis was evaluated by FACS using annexin-V-FITC/PI staining and DAPI staining. **Results：** Bort treatment resulted in elevation of LC3-II, p62 and Cleaved-caspase-3 and apoptotic cell death in DRG neurons. Also, Bort decreased fusion of autophagosomes with lysosomes and degradation of autophagosomes leading to accumulation of autophagosomes in the cells, suggesting an impairment of autophagic flux. Furthermore, Bort activated mTORC1 and its target S6K1. Inhibition of mTORC1 with Rap or down-regulation of Raptor with shRNA dramatically alleviated Bort-elicited blockage of autophagic flux and apoptosis in the cells. **Conclusions:** Bort activation of mTORC1 impairs autophagic flux leading to accumulated autophagosomes-dependent apoptotic cell death in DRG neurons. Manipulation of mTORC1 activity to improve autophagic flux is a promising strategy against Bort-induced peripheral neurotoxicity.

RESULTS

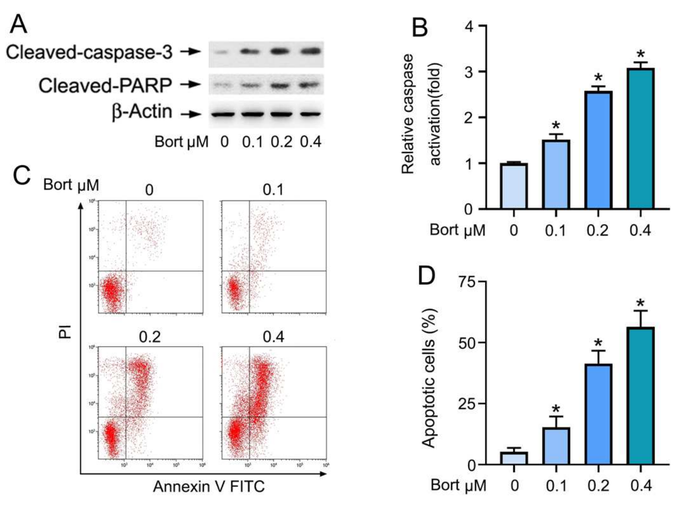

Fig. 1. Bort induces DRG neuronal apoptosis in a dose-dependent manner.

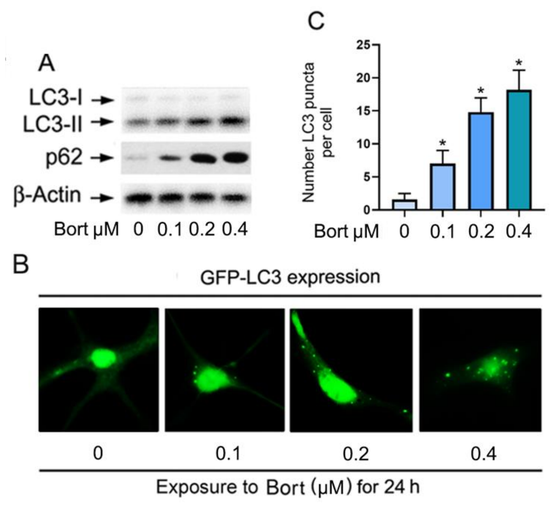

Fig. 2. Bort impairs autophagic flux resulting in accumulation of autophagosomes in DRG neurons.

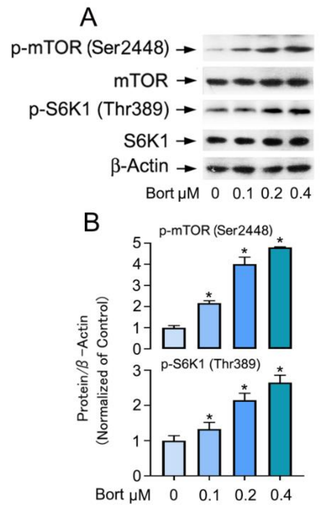

Fig. 3. Bort activates mTORC1 and its target S6K1 in DRG neurons.

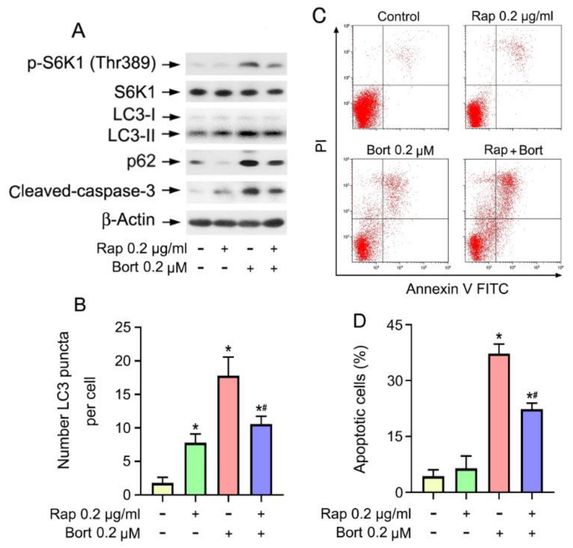

Fig. 4. Inhibition of mTORC1 with rapamycin diminishes Bort-induced impairment of autophagic flux and apoptosis in DRG neurons.

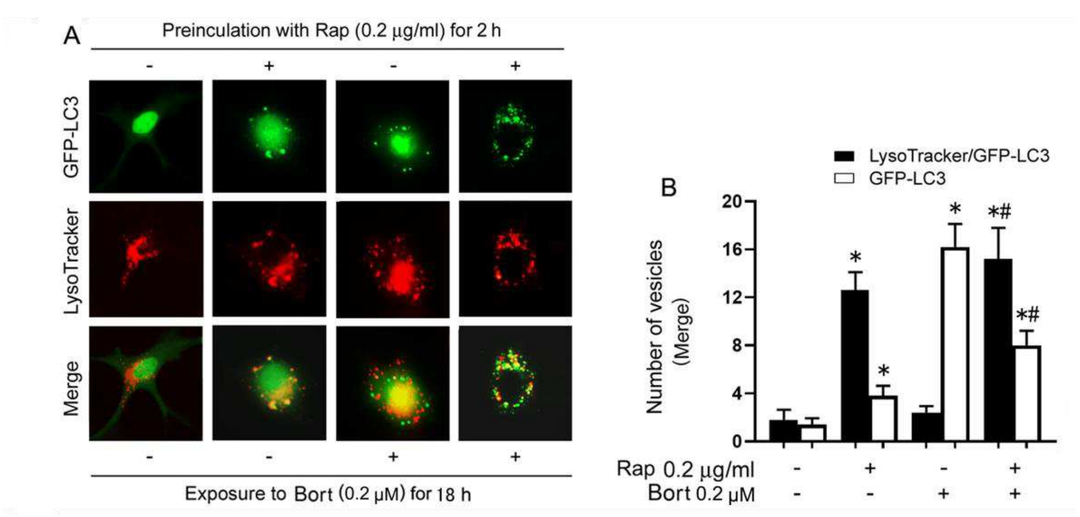

Fig. 5. Inhibition of mTORC1 with rapamycin rescues Cd-impaired autophagic flux by promoting autophagosome-lysosome fusion in DRG neurons.

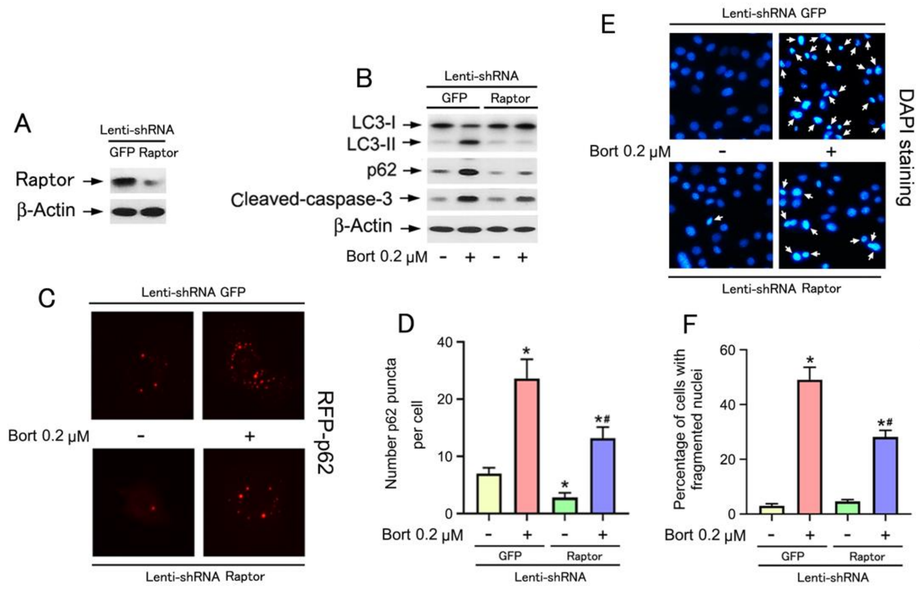

Fig. 6. Knockdown of Raptor alleviates Bort-elicited impairment of autophagic flux and apoptosis in DRG neurons.

CONCLUSION

mTORC1 plays a critical role in Bort-induced apoptotic cell death through impairing autophagic flux in DRG neurons. Manipulation of mTORC1 activity to improve autophagic flux is a promising strategy against Bort-induced peripheral neurotoxicity.

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