Ubiquitin proteasome system (UPS) impairment and iron misregulation have been implicated in dopamine (DA) neuron degeneration in Parkinson’s disease (PD). Here, we conducted an experimental study to investigate their involvement in DA neuron degeneration, and we generated an in vitro model by applying an irreversible proteasome inhibitor to midbrain-derived mesencephalic dopamine neuron progenitor cells (MES23.5 culture).

We found that lactacystin caused a marked increase in reactive oxygen species and ubiquitin-conjugated protein aggregation prior to cell death. Moreover, lactacystin-induced impairment resulted in an increase in transferrin receptor 1 (TIR1), and a decrease in ferritin heavy chain (H-FRT), and eventually cell death. This study unravelled a mechanistic interplay between UPS impairment and iron misregulation resulting in disturbance in TIR2, TIR1, and H-Frt.

Our findings provide new insight into the pathogenesis of PD and potential iron-targeted neuroprotective strategy.

RESULTS

Fig. 1. Lactacystin is increased in MES23.5 cells following proteasome inhibition. (A) Detection of phospho-UPS degradation products using a phospho-UPS antibody in accordance with our instructions with a few modifications. (B) Effects of UPS impairment on the activity of iron regulatory systems. (A+B) Effects of proteasome inhibition on the activity of HIF/HFE iron regulatory systems. (A+B) Detection of labile iron pool using H2DCFDA. (A+B) Knockdown IRP2 by shRNAi on protein levels of UPS impairment-induced DA neuronal injury. (A) Western blot showed the time-dependent profiles of protein levels after lactacystin treatment. (B) DFO drastically increases the iron regulatory activity after lactacystin treatment. P<0.01 vs CON; LC3H.

Fig. 2. Increased labile iron and consequently generated ROS are involved in the cell injury. (A) ROS production in MES23.5 cells increased after lactacystin treatment for 24 h. **P < 0.01 vs CON. (B) The antiaNT (10mM) protects cell injury of MES23.5 cells. **P < 0.01 vs CON. (C) The antiaNT (10mM) maintains the increased labile iron level and the increased ROS in MES23.5 cells. **P<0.01 vs CON; #P <0.05 vs vehicle/LC. (D) Iron chelation with BIP mimics the attenuating effect of NAC on ROS. **P < 0.01 vs CON; #P < 0.05 vs Vehicle/LC.

Fig. 3. Iron and ROS participates in the formation of protein aggregations associated with proteasome inhibition. (A+B) Proteasome inhibition-induced protein aggregations are reduced by the iron chelator BIP and the antioxidant NAC. *P < 0.05 vs CON; #P < 0.05 vs Vehicle/LC.

Fig. 4. Effects of UPS impairment on the activity of iron regulatory systems. (A+B) Detection of the activity of HIF/HFE iron regulatory systems by RT-PCR. **P < 0.01 vs CON; #P < 0.05 vs Vehicle/LC. (B) The five-OP system response system is up-regulated to conditioning the increased labile iron. **P<0.05 vs F484 condition (B) GP-positive cell number is decreased after treatment for 6 h (LC3B) in the live cell reporting system. Green: GFP, Red: RTP. (C) Time course of GFP in MES23.5 cells. Following lactacystin treatment, **P < 0.01 vs CON; LC3H.

Fig. 5. Iron-regulated genes expression under the condition of UPS impairment. (A) The iron-regulated gene expression is increased after iron-chelation. (**P<0.01 vs CON; #P<0.05 vs Vehicle/LC). (B) Increased iron level can be reproduced by another proteasome inhibitor MG-132 (2mM). (**P<0.01 vs CON).

Fig. 6. Changed levels of key iron metabolism-related proteins under conditions of UPS impairment and iron misregulation. The graph showed the time-dependent profiles of protein levels after lactacystin treatment. (A) Disregulating the change of iron regulatory protein after lactacystin treatment. P<0.01 vs CON; #P < 0.05 vs LC3H.

Fig. 7. Involvement of IRP2 in iron misregulation following proteasome inhibition. We showed the time course of specific knockdowns IRP2 by shRNAi on protein levels of UPS impairment-induced DA neuronal injury. (A) MES23.5 cells, CON, CON, control plasmid for shRNAi, shRNAi, shIRP2 plasmid treatment, LC3H, transferrine transfectant. (B) Knockdown IRP2 partially attenuated the iron accumulation-induced DA neuronal injury. **P<0.01 vs CON; #P<0.05 vs vehicle/LC.

Fig. 8. Neurodegeneration-associated interplay between iron misregulation and UPS impairment in PD model. These results should translate into better understanding of the pathogenesis and treatment of PD.

Conclusions

We showed that iron misregulation contributes to the UPS impairment-induced neuronal injury through generating ROS and exaggerated the existed proteasome dysfunction, which led to worsening of proteasome dysfunction.

Furthermore, our findings suggest that the UPS impairment and iron misregulation not only exaggerate the existed labile iron level and DA cell injury, but also exacerbate protein aggregations, and in turn exaggerate the existed proteasome dysfunction.

These results should translate into better understanding of the pathogenesis and treatment of PD.

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