Autophagy Induction Promotes Aristolochic Acid-I-Induced Renal Injury *in vivo* and *in vitro*

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Introduction

Aristolochic acid (AA), one of the most common nephrotoxic agents, induces Chinese herb nephropathy. Ingestion of AA causes AA nephropathy first by inducing apoptosis. Autophagy involves a bulk degradation pathway to maintain cellular homeostasis when cell undergo stresses. we have demonstrated that autophagy is involved in AA-induced acute kidney injury in vivo and that high doses of AA activate Atg5-dependent autophagy and promote renal tubular apoptosis.

Materials & Methods

All rats were subcutaneously injected with AA (10 mg/kg body weight) (AA group) or with phosphate buffer solution (PBS; control group). Four rats per group were sacrificed following 1, 3, 5 and 7 d of injections for differential analysis of apoptosis and autophagy activation. Normal rat renal proximal tubular epithelial cells (NRK52E) were cultured with Dulbecco's modified Eagle's medium and 5% fetal calf serum. Cells were treated with AA, 3-MA, and shRNA of ATG5 at the indicated dosages and time points. Autophagy phenomenon was detected by analysis of western blotting, immunofluorescence staining, and transmission electron microscopy. AA induced apoptosis was assayed by flow cytometry, Hoechst staining, and western blotting.

Figures

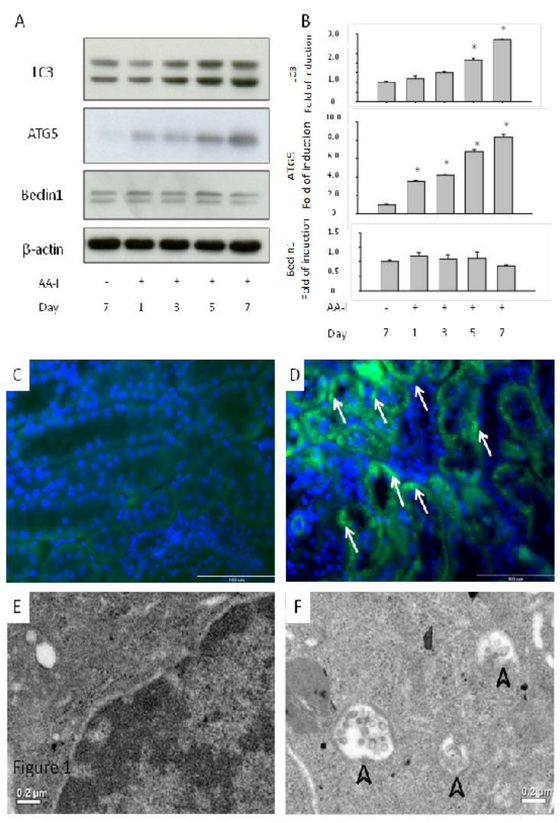

Figure 1. AA-I-induced autophagy associated with Atg5 induction in vivo.

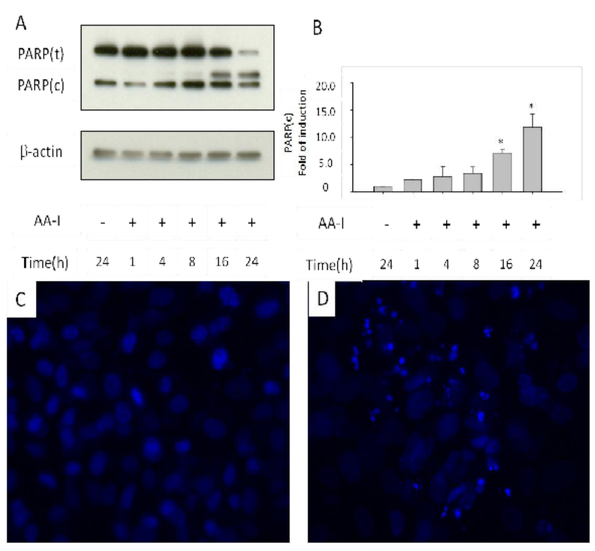

Figure 2. Effects of AA-I on cell apoptosis in NRK52E cells.

Results

AA induced autophagy phenomenon both in vivo and in vitro assessed by western blot, Immunofluorescence staining, and transmission electron microscopy. Marker of autophagy activity, LC3II accumulated in tubular cells after exposure to AA, and autophagy regulate protein 5 (ATG5) expression also significantly enhanced following AA exposure in vivo and in vitro.

Results

Attenuated autophagy phenomenon with autophagy inhibitor, 3-methyl adenine (3MA), not only suppress cell undergo autophagy, but also protect cell from apoptosis. Knock down expression of ATG5 protein by shRNA also attenuate autophagy accumulation, and prevent cell from apoptosis (all $P<0.01$). These findings clearly demonstrated that Atg5-dependent autophagy plays important roles in AA induced renal tubular cell death.

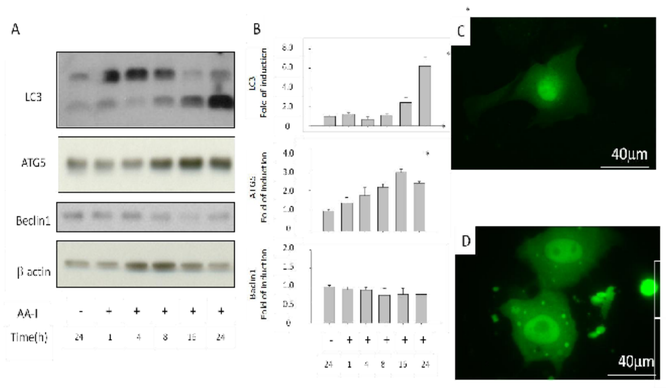

Figure 3. AA-I-induced autophagy accompanied by upregulation of Atg5 in NRK52E cells.

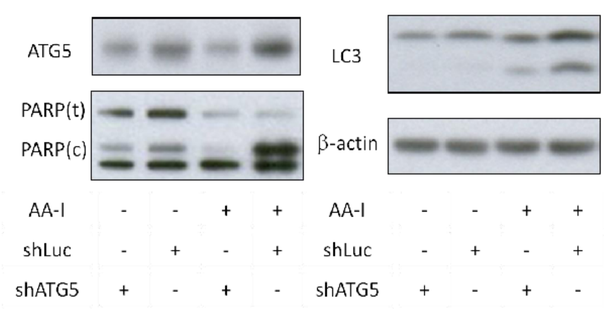

Figure 6. Atg5 knockdown extenuated PARP cleavage in AA-treated NRK52E cells.

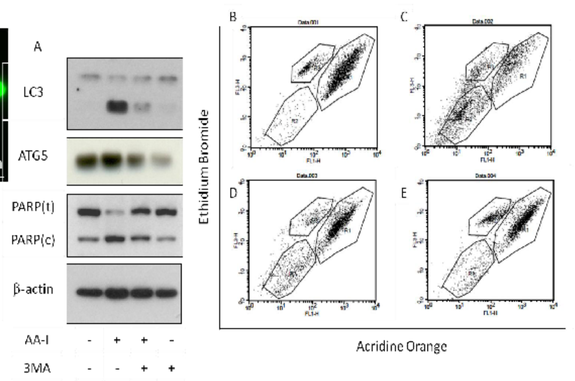

Figure 4. 3-MA attenuated AA-I-induced PARP cleavage , Atg5 expression and apoptosis in NRK52E cells.

Summary

In conclusion, AA induced autophagy both in vivo and intro, and ATG5 expression significantly enhanced following AA exposure. Inhibitor of autophagy and shRNA of ATG5 could not only attenuate accumulation of autophagy but also protect cells from death.

Discussion

Our study demonstrated that high dose AA exposure led to the induction of autophagy both in vivo and in vitro. Inhibition of autophagy attenuated AA induced apoptosis, at least partially, through an Atg5-dependent pathway. These data suggest a possible causative role for autophagy in the development of AA induced acute kidney injury.

References

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