Molecular mechanisms of *Alpinia oxyphylla* Miq.fruits extract on the nerve regeneraion

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Abstract

Nerve regeneration is a complex physiological response that takes place after injury. Neurons can be separated into central and peripheral nervous systems, which have different anatomical structures and regeneration ability. Schwann cells play a crucial role in endogenous repair of peripheral nerves due to their ability to proliferate and migrate. *Alpinia oxyphylla* Miq. is an important traditional Chinese medicinal herb whose fruits are widely used as a tonic, aphrodisiac, anti-salivation, and anti-diarrhea. The aim of the present study was to investigate the molecular mechanisms by which *Alpinia oxyphylla* Miq.fruits (AOF) promote neuron regeneration. Here, we investigated the molecular signaling pathways, which include: (1) Survival signaling, IGFs-IGFIR-Akt-BCl_2 and proliferative signaling, mitogen- activated protein kinase (MAPK) pathways, (2) migrating signaling, uPA-MMPs. Our results show that treatment with extract of AOF induces the phosphorylation of the insulin-like growth factor-I (IGF-I)-mediated phosphatidylinositol 3-kinase/serine-threonine kinase (PI3K/Akt) pathway. Moreover, AOF induced phosphorylation of ERK1/2 , JNK and p38 activate the downstream signaling expression of PAs and MMPs. AOF-stimulated ERK1/2 and p38 phosphorylation was attenuated by pretreatment with U0126 and SB203580, resulting in migration and uPA-related signal pathway inhibition. Taken together, our data suggests the MAPKs (ERK1/2, p38)-, PAs (uPA,tPA)-, MMP (MMP2, MMP9) and IGFs-IGFIR-Akt- BCl_2 signaling pathway of Schwann cells regulated by AOF might plays a major role in Schwann cell migration and proliferation.

Materials&Methods

Cell Culture and Treatments

RSC96 cells were purchased from American Type Culture Collection (ATCC) and cultured in Dulbecco's modified Eagle's medium (DMEM) supplementes with 10% fetal bovine serum (FBS), 4mM L-glutamate, 1.5 g/l sodium bicarbonate and 1% nonessential amino acids (NEAAs) in a humidified atmosphere of 5% CO_2.

Western Blotting

Cultured RSC96 cells were scraped and washed once with PBS. The cell pellets were lysed for 30 min in the lysis buffer and then centrifuged at 12000g for 10 min. Proteins from the RSC96 cells were separated in 12% gradient SDS-PAGE and transferred on nitrocellulose membranes. Nonspectific protein binding was blocked in the blocking buffer at room temperature for 1h. The membranes were incubated in 4℃ blocking buffer overnight with specific antibodies.

Migration Assay

We use a Boyden chamber and polyvinyl-pyrrolidone-free polycarbonate membranes with 8μm pores (Neuro Probes, Inc.) for the migration assay .The bottom wells of the chamber were filled with 10% FBS DMEM medium. The wells were covered with the membrane sheet, which had been serum free, were added into the top chamber. Membrane were stain with Giemsa stain (Sigma).Cells that migrated through the membrane were counted using a counting grid, which was fitted into an eyepiece of a phase contrast microscope.

Wound healing

Cells were initially seeded in 60-mm culture plates with an artificial "wound" carefully created at 0 h, using a sterile P-200 pipette tip to scratch on the subconfluent cell monolayer to make an approximately 1.0 mm gap. After 24-h of culture with different AOF concentrations, the cell migration was calculated by counting cell numbers that had advanced into the cell-free space randomly selected from the area of the initial wound border. Photographs were taken of the wounded regions using an inverted Olympus microscope.

Results

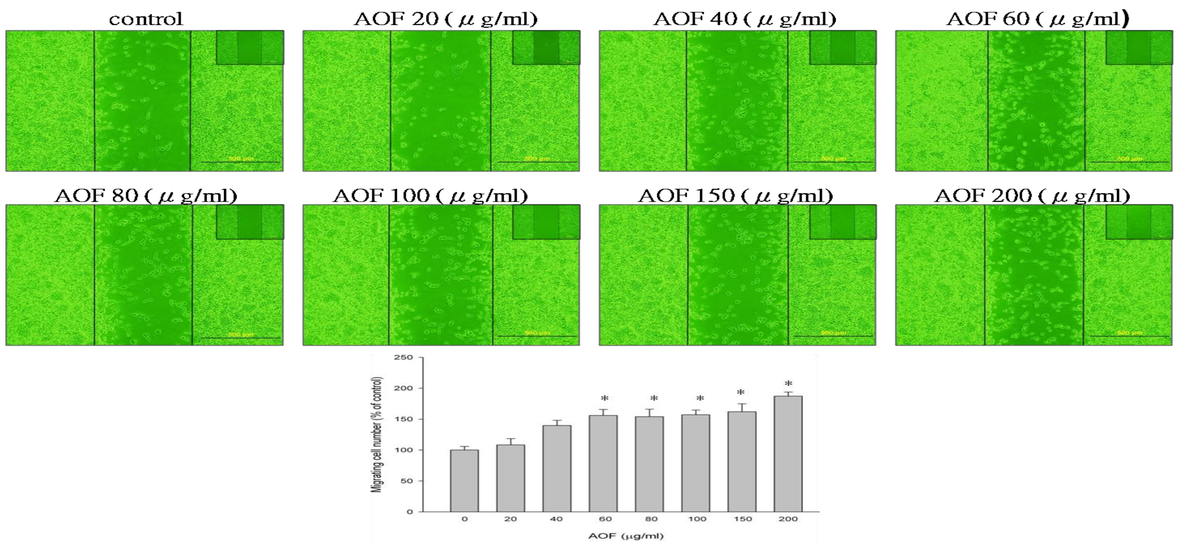

Figure 1. The migrative effect of AOF extract on RSC96 cell. Schwann cells were incubated with different doses of AOF as indicated. Data are shown as the mean of three independent experiments ± SE. * denote significant differences from control values with $p<0.05$.

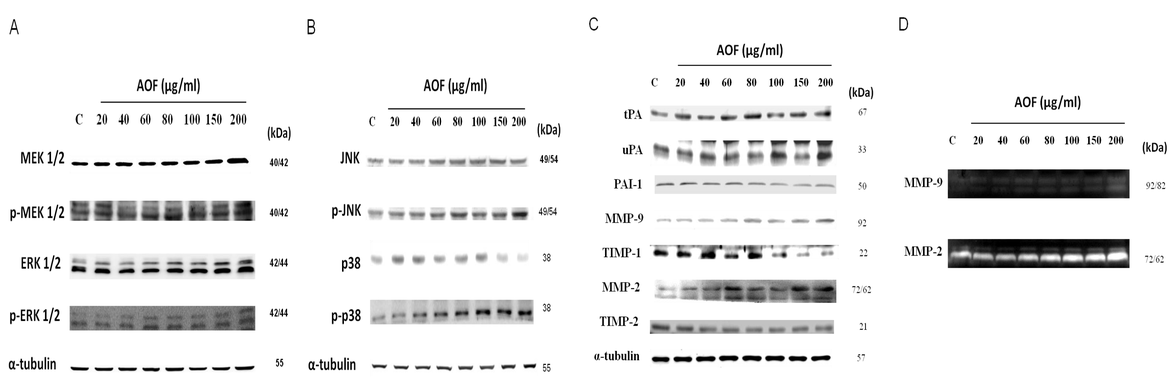

Figure 2. The migrative effect of AOF on RSC96 cell viability was mediated by MAPK signaling. The RSC96 cells were treated with various doses of AOF. The MAPK-signaling activities were measured by western blot (A-C). α-tubulin was used as a loading control. Further to confirm the MMP-9 and MMP-2 activity by gelatin zymopraphy (D).

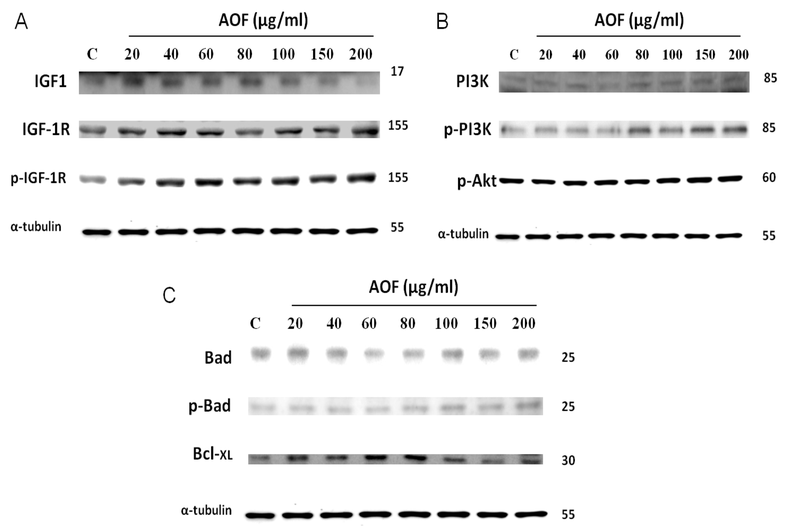

Figure 3. IGF-1 mediated PI3K / Akt signal pathway activation dose course for RSC96 cell treated with AOF extract. The protein expression of IGF-1was determined by western blot. α-tubulin was used as a loading control (A-C).

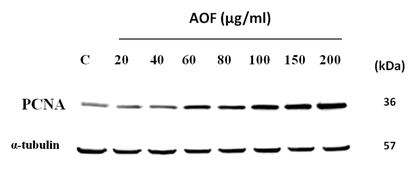

Figure 4. AOF extract induce the expression of PCNA. RSC96 cells were incubated with different doses of AOF as indicated. The protein expression of PCNA was determined by western blot. α-tubulin was used as a loading control.

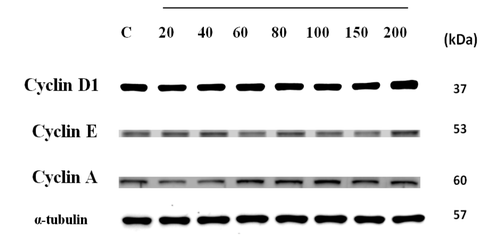

Figure 5. AOF extract induces the expression of proteins involved in the cell cycle in a dose-dependent manner. RSC96 cells were stimulated for 24h. The protein expression of cell cycle regulatory proteins cyclin A, cyclin D1 and cyclin E were determined by western blot. α-tubulin wasused as a loading control.

Conclusion

MAPKs (ERK1/2, p38, JNK)-, PAs (uPA, tPA)-, MMP (MMP2, MMP9) and IGFs-IGFIR-Akt- Bcl_2 signaling pathway of Schwann cells regulated by AOF might plays a major role in Schwann cell migration and proliferation.