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Short communication

Andrographolide reduces proliferation and migration of lens epithelial cells by modulating PI3K/Akt pathway

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ABSTRACT

Lens epithelial cell proliferation, migration, and transdifferentiation are involved in the development of subcapsular cataracts and postoperative capsular opacification (PCO). PI3K/Akt pathway is involved in the proliferation and migration of lens epithelial cells. Andrographolide is the main bioactive component of *Andrographis paniculata* and is known to possess anti-proliferative and anti-migratory activities. The purpose of this study is to evaluate the effect of andrographolide on proliferation and migration induced by growth factors (TGF-β and bFGF) in the lens epithelial cell line, FHL 124. We have also evaluated the role of the PI3K/Akt pathway and its alteration by andrographolide during proliferation and migration of lens epithelial cells. The results showed that andrographolide significantly inhibited proliferation in a dose and time dependent manner. The growth factors, TGF-β and bFGF, induced migration of lens epithelial cells, which was lowered by andrographolide. The growth factors also up regulated phosphorylated Akt (Ser473) and Akt (Thr308), which was abolished by simultaneous treatment of andrographolide. Similar changes were also observed with the PI3K inhibitor, LY290042. Our findings suggest that andrographolide reduces proliferation, migration, and phosphorylated Akt levels in lens epithelial cells. Hence andrographolide can be utilized for the prevention of PCO.

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The lens epithelial cells (LECs) line the anterior surface of the lens up to the lens equator. Rest of the lens is made up of lens fiber cells resulted due to terminal differentiation of the LECs at lens equator (Gupta et al., 2004). Cataract is a leading cause of blindness worldwide (WHO, 2010). Although most age-related cataracts are associated with oxidative stress, some types such as subcapsular cataracts are caused by abnormal proliferation and differentiation of LECs (Johar et al., 2007). After the surgical removal of cataract using phacoemulsification, the residual LECs also undergo similar types of changes as those seen in subcapsular cataracts. This leads to posterior capsule opacification (PCO). PCO affects about 20%–50% of adults and almost 100% of children undergoing cataract surgery (Raj et al., 2007). The abnormal differentiation of LECs involves their transdifferentiation into myofibroblast-like cells through a process called epithelial mesenchymal transition (EMT) and deposition of fibrous extracellular matrix (Lovicu et al., 2002). Previous studies have shown that growth factors, especially the transforming growth factorβ2 (TGF-β2) and the basic fibroblastic growth factor (bFGF), play important roles in the development of PCO (Dawes et al., 2009; Symonds et al., 2006; Yang et al., 2013).

Although several approaches have been developed to prevent PCO by chemical means, none of them have been applied in a clinical setting (Biswas et al., 1999; Chandler et al., 2007; Rabsilber and Auffarth, 2006). Andrographolide (andro) is a labdane diterpenoid, the main bioactive component of the medicinal plant *Andrographis paniculata*. Andro is known to have anti-fibrotic, anti-inflammatory, and anti-cancerous properties (Akbarsha and Murugaian, 2000; Lee et al., 2010; Li et al., 2007; Rana and Avadhoot, 1991; Shen et al., 2009, 2000). Therefore, we hypothesize that andro can inhibit proliferation and migration of the lens epithelial cell line (FHL 124). Earlier studies have shown that the PI3K/Akt pathway plays an important role in the growth factor executed migration and proliferation of LECs (Liegl et al., 2014; Xiong et al., 2010; Yao et al., 2008). Therefore, we have evaluated the role played by andro in modulating the PI3K/Akt signaling pathway.

FHL 124 (a kind gift from Dr. John R. Reddan, Oakland University, Michigan, USA) is a non-virally transformed cell line generated from human capsule-epithelial explants (Reddan et al., 1999a). These cells show 99.5% homology (in transcript profile) with the native lens epithelium (Wormstone et al., 2004). The FHL 124 cell

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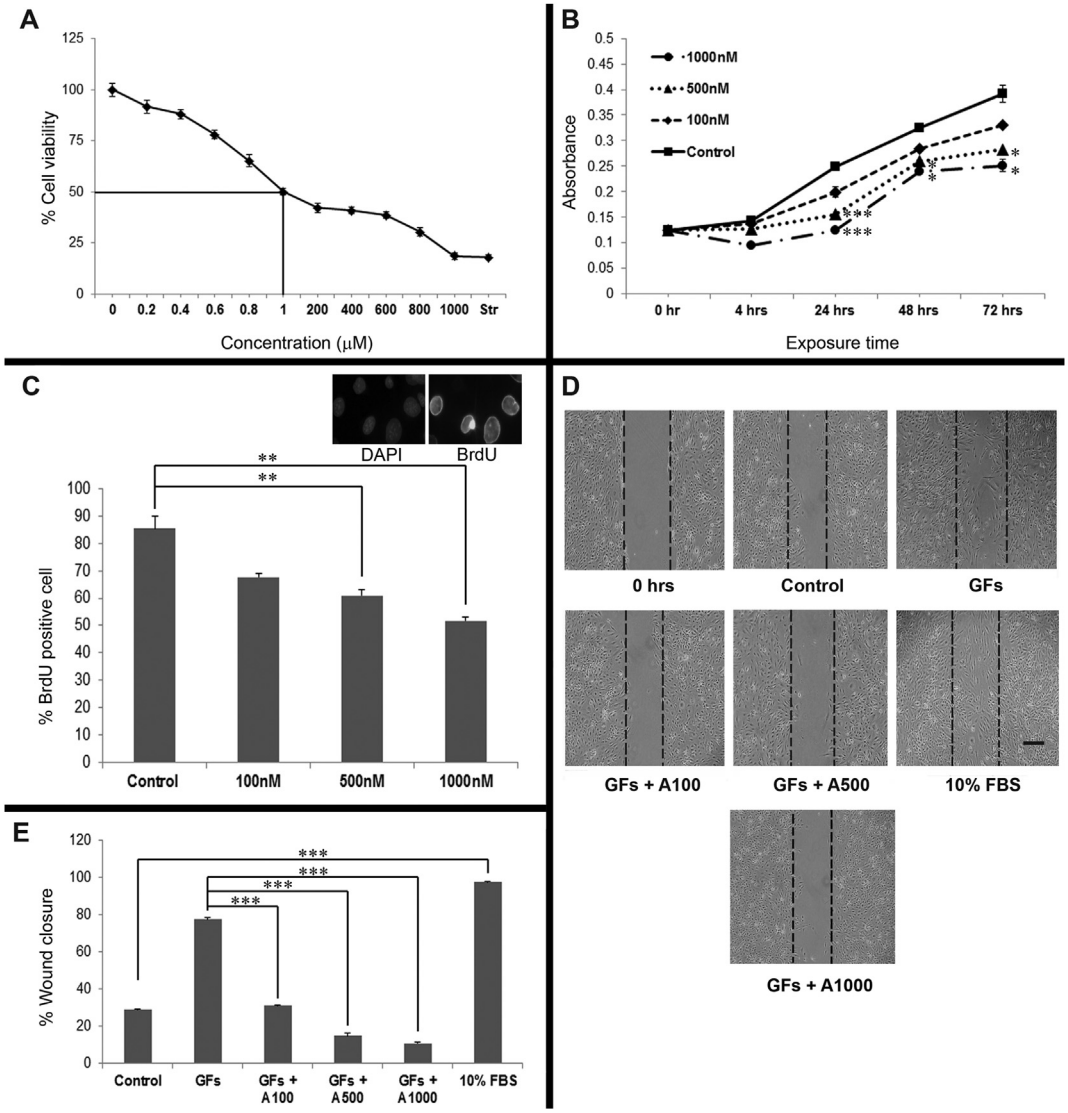

Fig. 1. Effect of andrographolide on viability, proliferation, and migration of FHL 124 cells. (A) Cell viability was determined by MTT assay. The cells were exposed to 0.1–1000 μM of andro for 24 h ($n = 3$). The IC50 value for andro was 1 μM. (B) Cell proliferation was determined by MTT assay. After serum deprivation for 24 h, the cells were exposed to 100, 500, and 1000 nM of andro for an additional 4, 24, 48, and 72 h. Exposure to andro reduced cell proliferation in all groups. A significant difference was obtained between the controls versus the 500 nM andro group as well as between control and the 100 nM andro group after 24, 48 and 72 h. $^{***}p < 0.001$, $^{*}p < 0.05$ ($n = 5$). (C) Cell proliferation was determined by BrdU incorporation assay. Cells were exposed to 100, 500, and 1000 nM andro for 24 h. For BrdU incorporation, cells of all groups were further incubated with 30 μM BrdU for 5 h and labeled cells were detected by immunofluorescence localization of BrdU using anti-BrdU antibody (Abcam, MA, USA). Cells were counter-stained with DAPI. Labeled cells in a given area were counted using BIOVIS Image plus v4. Exposure to andro reduced cell proliferation in all groups. $^{**}p < 0.001$. (D) Cell migration was determined by scratch assay. After confluence, cells in the line were scraped off using a 200 μl microtip and were exposed to 100, 500 and 1000 nM of andro with and without growth factors for an additional 24 h ($n = 5$). The initial wound margin was denoted using dashed lines and bar = 100 μm. (E) The percentage of wound area was determined using Image J software. Ten per cent FBS and growth factor exposure increased migration while andro treatment along with growth factor exposure reduced migration in all groups.$^{***}p < 0.001$.

line was authenticated using a short tandem repeat (STR) analysis and our findings corroborate with the original report (Reddan et al., 1999b). Cells were cultured in Eagle's minimal essential medium (EMEM) with 10% fetal bovine serum (FBS) and 50 μg/ml gentamycin at 35.5 °C. For the viability and proliferation assay, FHL 124 cells were exposed to a range of andro concentrations and the effects were evaluated using the MTT assay. The study population was divided into the following groups: control (no treatment), GFs [TGF-β (10 ng/ml) + bFGF (40 ng/ml)], GFs + A100 [GFs + andro (100 nM)], GFs + A500 [GFs + andro (500 nM)] and GFs + A1000 [GFs + andro (1000 nM)]. Some cultures were also exposed to andro (100 nM) alone and the PI3K inhibitor (LY294002) (30 μM) along with GFs. LY294002 was added 1 h prior to addition of the growth factors (Yang et al., 2014). All the experiments were performed at least in triplicates or as mentioned otherwise. A non-parametric Kruskal–Wallis test was carried out for statistical analysis. The effect of andro on FHL 124 cells was both dose and time-dependent. Based on the dose–response curve, the IC50 value was 1 μM (Fig. 1A). The following were selected for further study: 50% (500 nM) and 10% (100 nM) of andro to that of the IC50 value. Cell proliferation decreased with increasing concentration of andro. A significant difference was observed after 24, 48, and 72 h in 500 and 1000 nM of andro compared to that of the control group (Fig. 1B). Cell proliferation was also analyzed after 24 h using the BrdU incorporation assay. Andro reduced proliferation and a significant difference was observed between the control group and the 500 nM andro group ($p < 0.01$) as well as between the control group and the 1000 nM andro group ($p < 0.01$) (Fig. 1C).

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