

Curcumin attenuates EGF-induced AQP3 up-regulation and cell migration in human ovarian cancer cells

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Abstract

Objective Aquaporin (AQP) water channels are expressed in high-grade tumor cells of different tissue origins. Based on the involvement of AQPs in angiogenesis and cell migration as well as our previous studies which show that AQP3 is involved in human skin fibroblasts cell migration, in this study, we investigated whether AQP3 is expressed in cultured human ovarian cancer cell line CaOV3 cells, and whether AQP3 expression in these cells enhances cell migration and metastatic potential.

Methods Cultured CaOV3 cells were treated with EGF and/or various reagents and subjected to cell migration assay by phagokinetic track mobility assay or biochemical analysis for expression or activation of proteins by SDS-PAGE/Western blot analysis.

Results In this study, we demonstrate that AQP3 is expressed in CaOV3 cells. EGF induces CaOV3 migration

and up-regulates AQP3 expression. EGF-induced cell migration is inhibited by specific AQP3 siRNA knockdown or AQP3 water transport inhibitor CuSO_4 and NiCl_2 . We also find that curcumin, a well known anti-ovarian cancer drug, down-regulates AQP3 expression and reduces cell migration in CaOV3, and the effects of curcumin are mediated, at least in part, by its inhibitory effects on EGFR and downstream AKT/ERK activation.

Conclusions Collectively, our results provide evidence for AQP3-facilitated ovarian cancer cell migration, suggesting a novel function for AQP3 expression in high-grade tumors. The results that curcumin inhibits EGF-induced up-regulation of AQP3 and cell migration, provide a new explanation for the anticancer potential of curcumin.

Keywords EGFR · AQP3 · Ovarian cancer · Curcumin · AKT

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Introduction

The aquaporins (AQPs) are a family of small (~30 kDa/monomer), hydrophobic, integral membrane proteins that are expressed widely in the animal and plant kingdoms, with 13 members having been identified so far in mammals [30]. They are expressed in many epithelia and endothelia involved in fluid transport, as well as in cell types that are thought not to carry out fluid transport, such as skin and urinary bladder cells [30]. Several studies have reported AQPs expression in a variety of human tumors, which in some cases are correlated with tumor grade [9, 18, 31]. AQP expression in tumors has also been proposed to be of diagnostic and prognostic value [18, 31]. In vitro and in vivo evidence supports AQP-facilitated tumor cell migration and extravasation across blood vessels [9]. Our previous studies

also showed that AQP3 is involved in cell migration in human skin fibroblasts [4], suggesting that AQP3 expressed in cancer cells including ovarian cancer cells, may also facilitate cell migration and metastasis potential.

Growth factors control cell growth, proliferation, differentiation, survival and migration by activating receptors on specific target cells [2]. EGF and EGFR family are often over-expressed in various human cancers including ovarian cancer and are associated with poor prognosis [17, 19]. Aberrant activation of EGFR and EGF signal pathway are associated with neoplastic cell proliferation, migration, stromal invasion, resistance to apoptosis and angiogenesis [6]. EGF-induced cell migration has been proven to play important roles in ovarian cancer invasion and metastasis [7, 8]. In our previous study, we found that AQP3 is involved in EGF-induced human skin fibroblast cell migration [4]. We hypothesized that AQPs in ovarian cancer cells may also mediate EGF-induced cell migration and thus their metastatic potential.

A number of chemotherapy drugs have been developed for anti-ovarian cancer, of which curcumin is well studied [11, 14, 16, 24]. Curcumin is one of the most well-known naturally occurring compounds and has chemopreventive potentials against many types of cancerous cells [12]. Curcumin possesses anti-proliferative, antioxidant, anti-inflammatory, anti-angiogenic and anti-metastasis effects [5]. The anticancer potential of curcumin stems more specifically from its ability to suppress cell proliferation and to induce apoptosis of a wide variety of tumor cells, including ovarian cancer and other cancers [26, 32]. Recent studies have shown that curcumin inhibits human colon cancer cell growth by suppressing gene expression of EGFR [5, 13, 15]. Curcumin has a very potent inhibitory effect on the phosphorylation and activation of EGFR and AKT in prostate cancer [12]. However, underlying molecular mechanisms of its actions are yet to be defined.

Given that AQP3 is involved in EGFR-mediated cell migration and known effects of curcumin, we undertook this project to investigate whether water channel protein AQP3 is expressed in cultured human ovarian cancer cell CaOV3 cells, and if so, whether AQP3 is involved in EGF-induced cell migration, and whether curcumin has any effects on AQP3. Using Western blot and *in vitro* cell migration, we observed that AQP3 is expressed in CaOV3 cells. EGF, via EGFR signal transduction pathway, induces AQP3 expression which is involved in cell migration in CaOV3 cells. Furthermore, we found that curcumin treatment, which alone down-regulates AQP3 expression, blocks EGF-induced up-regulation of AQP3 and cell migration by inhibition of EGFR and its downstream AKT/ERK activation. Our results provide novel insights into understanding of the molecular mechanisms of curcumin's inhibition of ovarian cancer cell migration and may yield better therapeutic strategies for treatment of ovarian cancer.

Materials and methods

Cell culture

As previously reported [20, 22], cultured CaOV3 cells were maintained in a DMEM medium (Sigma, St. Louis, MO, USA) supplemented with a 10% fetal bovine serum (FBS), Penicillin/Streptomycin (1:100, Sigma, St. Louis, MO, USA) and 4 mM L-glutamine (1:100, Sigma, St. Louis, MO, USA), in a CO₂ incubator at 37°C. For Western blot, cells were reseeded in 6-well plates at a density of 0.2×10^6 cells/ml with fresh complete culture medium. Morphological changes were observed under phase contrast microscope.

Antibodies and reagents

Rabbit anti-aquaporin3 was obtained from Chemicon (Temecula, CA, USA), Rabbit anti-phospho-EGFR (Tyr1068), phospho-MEK and MEK, phospho-AKT and AKT, phospho-ERK and ERK were obtained from Cell Signaling Technology (Beverly, MA, USA). Rabbit anti-EGFR (1005), goat anti-rabbit IgG-HRP and goat anti-mouse IgG-HRP antibody were received from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Monoclonal mouse anti- β -actin was obtained from Sigma (St. Louis, MO, USA). PD153035, U0126, LY29400 and JNKi were from CalbioChem (San Diego, CA, USA). CuSO₄ and NiCl₂ were from Sigma (St. Louis, MO, USA).

Phagokinetic track motility assay

As described previously [4], 12-well plates were coated with coating medium of 20 μ g/ml fibronectin (Sigma, St. Louis, MO, USA) in PBS, and placed in CO₂ incubator at 37°C for at least 2 h. After removing the coating medium gently with a pasteur pipette, the wells were washed with PBS and 2.4 ml of microsphere suspension (86 μ l of stock microbeads solution in 30 ml PBS) was added to each well. The plates were then centrifuged at 1,200 rpm at 4°C for 20 min and carefully transferred to CO₂ incubator and incubated at 37°C for at least 1 h. From each well, 1.8 ml of supernatant was removed and finally 1,500 freshly trypsinized cells in 2 ml assay-medium (DMEM supplemented with a 0.05% FBS) were seeded per well. Cells with or without treatment were cultured for 24 h and photographed under a phase contrast microscope.

Western blot analysis

As reported previously [3, 4], cultured human CaOV3 cells with or without treatment were washed with cold

PBS and harvested by scraping into 150 μ l of RIPA buffer (containing 50 mM Tris–HCl, pH 7.4, 150 mM NaCl, 1% NP40, 1 mM EDTA, 0.25% Sodium Deoxycholate) with 1 mM NaF, 10 μ M Na₃VO₄, 1 mM phenylmethylsulfonyl fluoride, and protease inhibitor cocktail (10 μ g/ml leupeptin, 10 μ g/ml aprotinin, and 1 μ M pepstatin). Cell lysates were incubated in 4°C for 30 min. After centrifugation at 14,000 rpm for 10 min at 4°C, protein concentration was determined by a Bio-Rad protein assay (Bio-Rad, Hercules, CA, USA). Twenty micrograms of proteins (for AQP3, phospho-EGFR and EGFR, phospho-ERK and ERK, phospho-AKT and AKT, phospho-MEK and MEK) or 10 μ g of proteins (for β -actin) were denatured in 5 \times SDS-PAGE sample buffer for 5 min at 95°C. The proteins were separated by 10 or 7.5% SDS-PAGE and transferred onto PVDF membrane (Millipore, Bedford, MA, USA) for 2 h at 4°C. Nonspecific binding was blocked with 10% dry milk in TBST (20 mM Tris–HCl, 137 mM NaCl, 0.01% Tween 20, pH 7.4) for 1 h at room temperature. After blocking, membranes were incubated with specific antibodies against AQP3 (1:1,000), EGFR (1:1,000), phospho-EGFR (1:1,000), phospho-ERK and ERK, phospho-AKT and AKT, phospho-MEK and MEK (1:1,000), and β -actin (1:20,000) in dilution buffer (2% BSA in TBS) overnight at 4°C. The blots were incubated with horseradish peroxidase-conjugated anti-rabbit or anti-mouse IgG at appropriate dilutions and room temperature for 1 h. Antibody binding was detected using enhanced chemiluminescence (ECL) detection system following manufacturer's instructions and visualized by autoradiography with Hyperfilm.

RNA interference experiments

As described previously [4], custom SMART pool® RNA interference (RNAi) duplexes (more than four sequences) for AQP3 were chemically synthesized by Dharmacon Research (Lafayette, CO, USA). As described previously, human CaOV3 cells were cultured in complete medium that did not contain antibiotics for 4 days. One day prior to transfection, 50 \times 10⁴ cells were seeded into a 6-well plate and cultured to 60–70% confluence the following day. For RNAi experiments, 12 μ l of FuGene6 (Roche Diagnostics, Indianapolis, IN, USA) was diluted in 88 μ l of DMEM for 5 min in room temperature. Then, 10 μ l of 20 μ M Double-stranded RNAs for AQP3 RNAi was mixed with DMEM containing FuGene6 and incubated for 30 min at room temperature for complex formation. Finally, the complex was added to the well containing 2 ml medium with the final AQP3 siRNA concentration of 100 nM. AQP3 protein expression and cell migration was determined by Western blot and phagokinetic track motility assay, respectively, 24 h after treatment.

Statistical analysis

The values in the figures are expressed as the mean \pm standard error (SE). The figures in this study were representative of more than three different experiments. Statistical analysis of the data between the control and treated groups was performed by a Student's *t* test. Values of *P* < 0.05 were considered as statistically significant. For cell migration experiment, at least 100 cells' migration distance was counted for one experiment.

Results

EGF induces AQP3 expression in cultured human ovarian cancer cells

First, we examined whether AQP3 is expressed in CaOV3 cells, and whether EGF induces AQP3 expression. Cells were treated with EGF at concentrations of 10, 50 and 100 ng/ml, and cell lysates were analyzed for AQP3 by Western blot as described in methods. As shown in Fig. 1a, EGF induces AQP3 up-regulation in a dose dependent manner in CaOV3 cells. AQP3 expression begins to increase after cells are treated with 50 ng/ml of EGF and is most obvious at 100 ng/ml of EGF treatment. EGF also induces up-regulation of AQP3 in a time dependent manner (Fig. 1c). AQP3 begins to increase at 12 h and is most obvious at 24 h after being treated with 100 ng/ml of EGF. AQP3 expression is quantified in Fig. 1b and d for Fig. 1a and c, respectively. Figure 1e demonstrates that AQP3 is over-expressed in CaOV3 cells, compared with human skin keratinocytes (HaCaT cells) (quantified in Fig. 1f). AQP3 specific siRNA, as mentioned in methods, knocks down AQP3 expression in CaOV3, while control siRNA has no effects (Fig. 1e, f).

NiCl₂, CuSO₄ and AQP3 knockdown inhibit EGF-induced cell migration in human ovarian cancer cells

The data above clearly demonstrated that EGF induces AQP3 expression in CaOV3 cells. Our previous studies have shown that EGF induces CaOV3 cell migration [20, 22]. To further study whether AQP3 is involved in EGF-induced cell migration, water and glycerol transport inhibitors of AQP3, CuSO₄ and NiCl₂ [33, 34] were applied in the experiments. CaOV3 were treated with various concentrations of CuSO₄ and NiCl₂, together with 100 ng/ml of EGF for 24 h. Cell migration was measured by phagokinetic track motility assay as described above. The results showed that both CuSO₄ and NiCl₂ significantly inhibit cell migration induced by EGF in CaOV3 cells (Fig. 2a, b). To further examine the role of AQP3 in EGF-induced cell migration, RNAi experiments were performed. The results showed that AQP3 RNAi

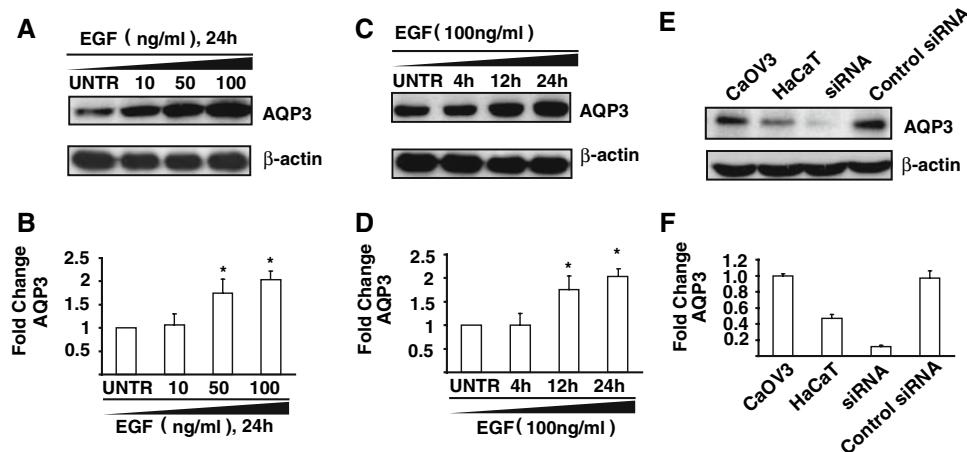


Fig. 1 EGF induces AQP3 expression in human ovarian cancer cell line CaOV3 cells. CaOV3 cells were treated with various doses of EGF (10, 50, and 100 ng/ml) and harvested at 24 h (a and quantified in b) or treated with EGF (100 ng/ml), harvested at different time points (4, 12 and 24 h) (c and quantified in d), AQP3 expression was analyzed by Western blot and its expression was quantified as normalized to beta actin. CaOV3 cells treated with or without specific AQP3 siRNA and

control siRNA as well as cultured skin keratinocytes cell line HaCaT cells were harvested. Twenty micrograms of total proteins were loaded for AQP3 expression detection using Western blot and its expression was quantified as normalized to beta actin (e and quantified in f). The data represent mean \pm SE of triplicate experiments. * $P < 0.05$ versus UNTR groups

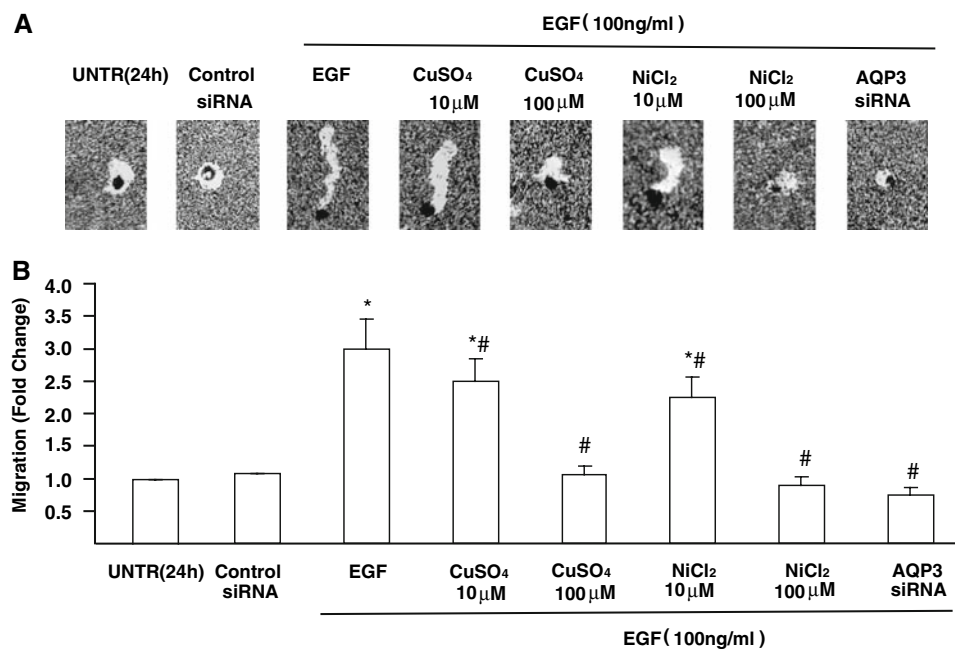


Fig. 2 NiCl₂, CuSO₄ and AQP3 knockdown inhibit EGF-induced cell migration in human ovarian cancer cells. CaOV3 cells were pre-treated with 10 or 100 μ M of CuSO₄ and NiCl₂ for 1 h, followed by treatment with 100 ng/ml of EGF for 24 h, CaOV3 cells were also treated with specific AQP3 siRNA, followed by treatment with EGF (100 ng/ml) for 24 h. In vitro cell migration was detected by “phagokinetic track

motility assay” (a, b). The data represent mean \pm SE of triplicate experiments. For phagokinetic track motility assay, at least 100 cells in ten random microscope fields for each group were counted for quantification of average migration distance. * $P < 0.05$ versus UNTR groups. # $P < 0.05$ versus EGF treated group

duplexes treatment significantly inhibits EGF-induced cell migration, while control siRNA has no effect (Fig. 2a, b). AQP3 siRNA, control siRNA, inhibitors CuSO₄ and NiCl₂ have no effects on CaOV3 viability as detected by MTT cell viability assay (data not shown).

EGFR mediates EGF-induced AQP3 expression and cell migration in human ovarian cancer cells

Next, we investigated the role of EGFR in EGF-induced AQP3 expression and cell migration in CaOV3 cells. The

results in Fig. 3a and b show that EGFR kinase inhibitor, PD153035, blocks EGF-induced AQP3 expression. Our previous studies have shown that EGFR is required for EGF-induced cell migration [10, 21, 22]. In this study, cell migration data show that pretreatment with PD153035 blocks EGF-induced cell migration in CaOV3 cells (Fig. 3c, d). These data indicate that activation of EGFR is important in EGF-induced AQP3 expression and cell migration in human ovarian cancer cells.

Curcumin down-regulates AQP3 and attenuates EGF-induced increase of AQP3 expression and cell migration in human ovarian cancer cells

Next, we examined whether curcumin affects AQP3 expression. As shown in Fig. 4a and b, curcumin induces AQP3 down-regulation in a dose dependent manner in CaOV3, and AQP3 expression begins to decrease after being treated with 30 μ M of curcumin and is most obvious at 40 μ M. Curcumin-induced down-regulation of AQP3 is also in a time dependent manner (Fig. 4c, d), AQP3 begins to decrease at 12 h and is most obvious at 24 h after being treated with 40 μ M of curcumin. Furthermore, as shown in Fig. 4e and f, curcumin also attenuates EGF (100 ng/ml)-induced AQP3 up-regulation in a dose dependent manner after being cultured for 24 h. Then, we investigated whether EGFR is involved in curcumin-attenuated EGF-induced AQP3 expression in CaOV3 cells. As shown in Fig. 4g and h, curcumin inhibits EGF-induced EGFR phosphorylation

in CaOV3 cells, which may explain the inhibitory effect of curcumin on EGF-induced AQP3 up-regulation. Cell migration data also show that pretreatment with curcumin blocks EGF-induced cell migration in CaOV3 cells (Fig. 4i, j).

Inhibition of PI3K and ERK activation is involved in curcumin's suppressive effect on EGF-induced cell migration and AQP3 expression in human ovarian cancer cells

Abundant studies have shown that activation of EGFR results in phosphorylation and activation of various effector proteins [29], among which are mitogen-activated protein kinase (MAPK) [5] and phosphatidylinositol-3-kinase (PI3K) [23, 25, 27, 28]. To further investigate the cell signaling pathways leading to EGF-induced AQP3 expression and cell migration, we used pharmacological inhibitors. Western blot analysis show that pretreatment of PI3K inhibitor, LY294002 or MEK/ERK inhibitor PD98059, U0126 partially inhibits EGF (100 ng/ml)-induced AQP3 expression while JNK inhibitor (JNKi) has no effect on EGF-induced AQP3 expression (Fig. 5a, b). As shown in Fig. 5c, e and g, EGF-induced phosphorylation of AKT, MEK and ERK is attenuated by curcumin treatment. The results are quantified in Fig. 5d, f and h, respectively. These data indicate that inhibition of PI3K and ERK activation is involved in curcumin's suppressive effect on EGF-induced cell migration and AQP3 expression in human ovarian cancer cells.

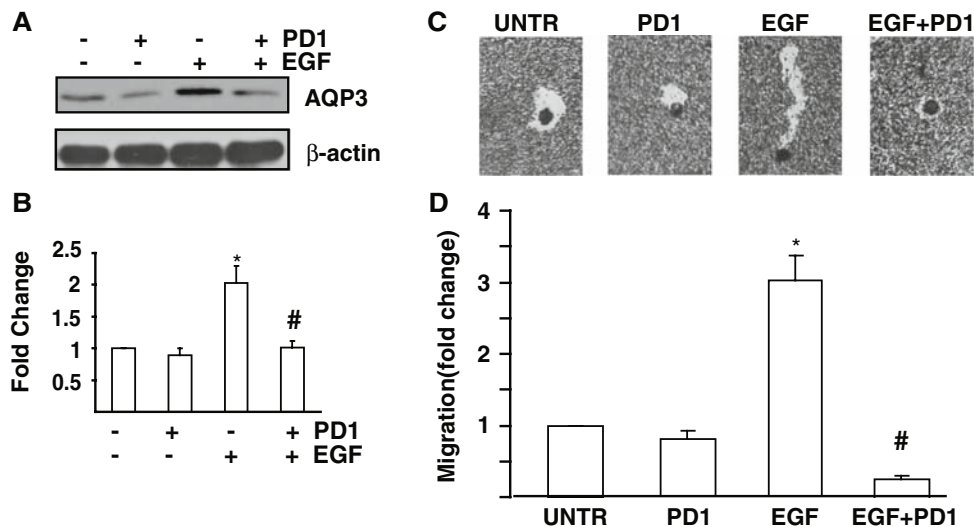


Fig. 3 EGFR mediates EGF-induced AQP3 expression and cell migration in human ovarian cancer cells. CaOV3 cells were treated with EGFR tyrosine kinase inhibitor PD153035 (PD1, 1 μ M) for 1 h, followed by EGF (100 ng/ml), and AQP3 expression were detected by Western blot (a) and quantified in (b). CaOV3 cells were treated with EGFR tyrosine kinase inhibitor PD153035 (PD1, 1 μ M) for 1 h, followed by treatment with or without EGF (100 ng/ml) for 24 h, in vitro

cell migration were detect by “phagokinetic track motility assay” (c) and quantified in (d). The data represent mean \pm SE of triplicate experiments. For phagokinetic track motility assay, at least 100 cells in ten random microscope fields for each group were counted for quantification of average migration distance. * $P < 0.05$ versus UNTR groups. # $P < 0.05$ versus EGF treated group

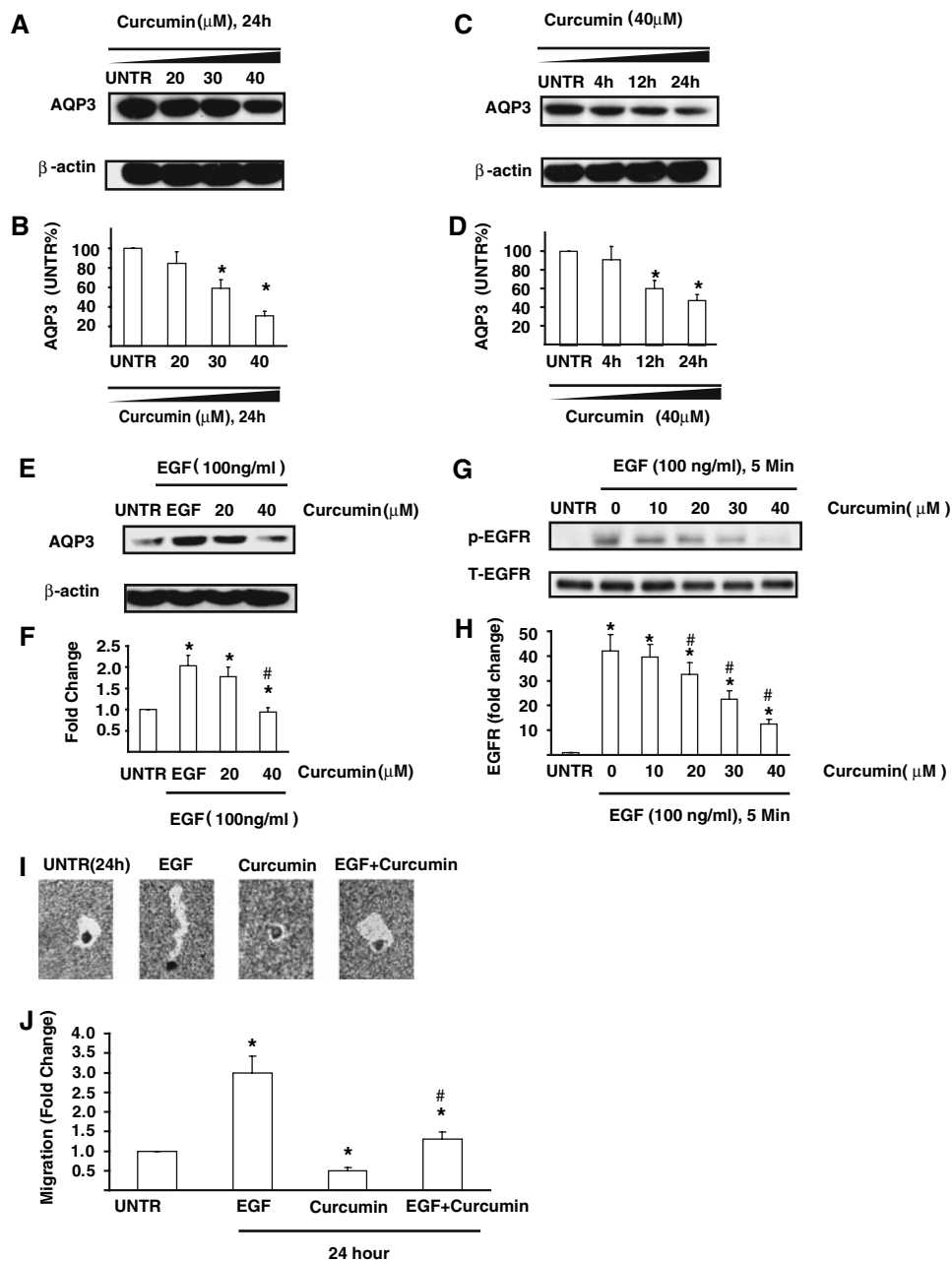


Fig. 4 Curcumin downregulates AQP3 and attenuates EGF-induced increase of AQP3 expression and cell migration in human ovarian cancer cells. CaOV3 cells were treated with various doses of curcumin (20, 30 and 40 μM) and harvested at 24 h (**a** and quantified in **b**) or treated with curcumin (40 μM), harvested at different time points (4, 12 and 24 h) (**c** and quantified in **d**), AQP3 expression was analyzed by Western blot and its expression was quantified as normalized to beta actin. CaOV3 cells were pre-treated with various doses of curcumin (20 and 40 μM) for 1 h, followed by EGF (100 ng/ml) treatment for 24 h, AQP3 expression was analyzed by Western blot and its expression was quantified as normalized to beta actin (**e** and quantified in **f**).

CaOV3 cells were pre-treated with various doses of curcumin (10, 20, 30 and 40 μM) for 1.5 h, followed by EGF (100 ng/ml) for 5 min, EGFR activation was analyzed by Western blot and was quantified as normalized to t-EGFR (**g** and quantified in **h**). CaOV3 cells were pre-treated with curcumin (40 μM) for 1.0 h, followed by EGF (100 ng/ml) treatment for 24 h, in vitro cell migration was detected by “phagokinetic track motility assay” (**i**, **j**). The data represent mean \pm SE of triplicate experiments. For phagokinetic track motility assay, at least 100 cells in ten random microscope fields for each group were counted for quantification of average migration distance. * $P < 0.05$ versus UNTR groups. # $P < 0.05$ versus EGF treated group

Discussion

The expression of various AQPs has been found in high grade tumors of different tissue origins [9, 18, 31]. Previous

studies have also demonstrated that AQP1-null mice exhibit greatly slowed tumor growth and improved survival (compared with wild-type mice) when implanted subcutaneously with tumor cells [9]. The tumors in AQP1-null

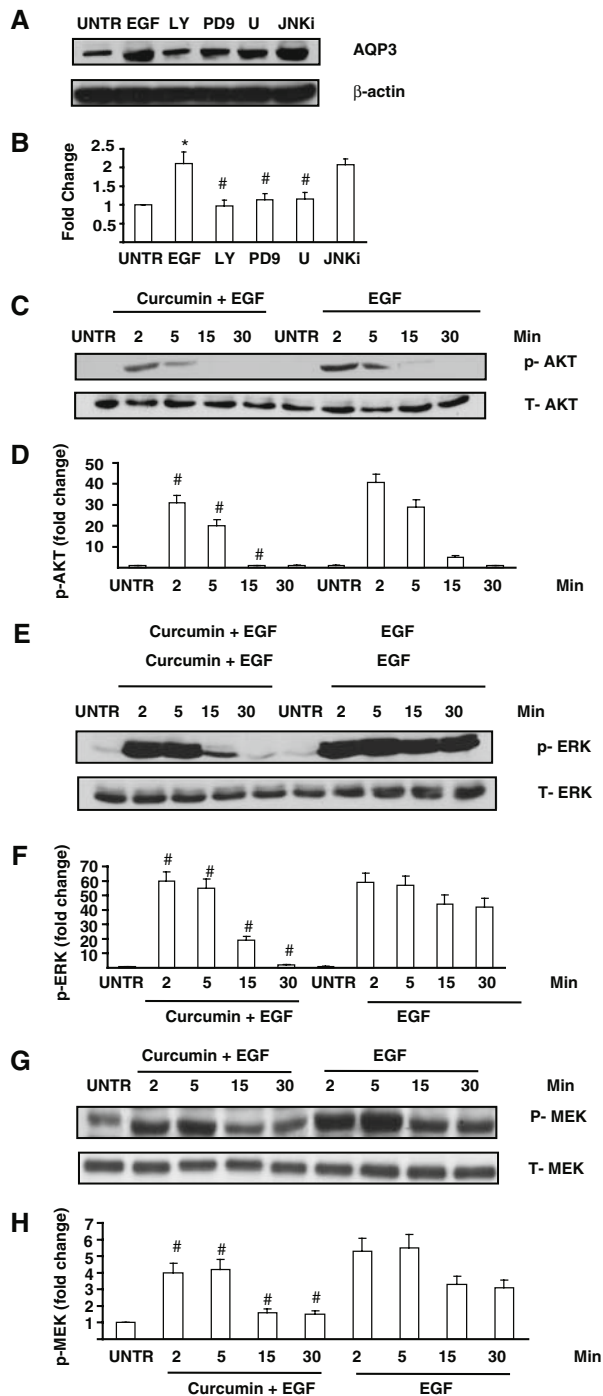


Fig. 5 Inhibition of PI3K/AKT and MEK/ERK activation is involved in Curcumin's suppressive effect on EGF-induced cell migration and AQP3 expression in human ovarian cancer cells. CaOV3 cells were treated with PI3K/AKT inhibitor LY294002 (LY, 1 μ M), MEK inhibitor PD 98059 (PD9, 1 μ M), ERK inhibitor U0126 (U, 1 μ M) and JNK inhibitor (JNKi, 1 μ M) for 1 h, followed by EGF (100 ng/ml) treatment for 24 h, AQP3 expression was detected by Western blot (a, b). CaOV3 cells were pre-treated with curcumin (40 μ M) for 1.5 h, followed by EGF (100 ng/ml) treatment for different time points (2, 5, 15 and 30 min), p-AKT (c, d), p-ERK (e, f) and p-MEK (g, h) were detected by Western blot. The data represent mean \pm SE of triplicate experiments. * $P < 0.05$ versus UNTR groups. # $P < 0.05$ versus EGF treated group

mice exhibit a much lower density of microvessels, and islands of viable tumor cells are surrounded by necrotic tissue [9]. The fact that AQP3 expression in tumor facilitates tumor cell migration and spread, suggests a novel function for AQP3 expression in high-grade tumors, and AQP3 inhibition may thus reduce the metastatic potential of some tumors [9, 18, 31]. We find that AQP3 is expressed in cultured human ovarian cancer cell line CaOV3 cells (Fig. 1). Using specific AQP3 knockdown and AQP3 water transport inhibitors, we also show that AQP3 is involved in EGF induced CaOV3 in vitro cell migration (Fig. 2).

Growth factors control cell growth, proliferation, differentiation, survival and migration by activating receptors on specific target cells. EGF and EGFR family are often over-expressed in various human cancers including ovarian cancer and are associated with poor patient prognosis. Aberrant activation of EGFR and the EGF signal pathway are associated with neoplastic cell proliferation, migration, stromal invasion, resistance to apoptosis and angiogenesis [1, 6–8]. In this study, we report that EGF induces AQP3 up-regulation which is involved in CaOV3 cell migration (Figs. 2, 3). We discover a new role of AQP3 in ovarian cancer cell migration and give a novel explanation for EGF-induced cancer cell migration. We also provide evidence that EGFR-mediated PI3K/AKT and MEK/ERK pathways are involved in EGF-induced AQP3 expression and cell migration in CaOV3 cells (Figs. 3, 5).

Curcumin is the principal bioactive curcuminoid found in the food spice, turmeric, which is produced commercially by processing the harvested rhizomes of the plant, *curcuma longa*. It is believed that curcumin is useful in preventing the development of many types of cancerous cells, including ovarian cancer [26, 32]. However, at the cellular level, it is unclear how curcumin works as a cancer chemopreventive agent. Our study demonstrates that curcumin alone down-regulates AQP3 in CaOV3 cells (Fig. 4). Curcumin also attenuates EGF-induced increase of AQP3 expression and cell migration in CaOV3 cells (Fig. 4). We conclude that the anti-cancer activities of curcumin may at least in part be due to its potent capacity of down-regulation of AQP3 in human ovarian cancer cells.

The results above have shown that curcumin attenuates EGF-induced increase of AQP3 expression and cell migration in CaOV3 cells (Fig. 4). However, the signal pathways involved has not been well studied. We find that EGFR inhibitor, PI3K inhibitor and MEK/ERK inhibitor inhibits AQP3 expression and cell migration induced by EGF in CaOV3 cells (Fig. 5). These results clearly demonstrate that EGF/EGFR, PI3K/AKT and MEK/ERK signaling pathways are involved in EGF-induced AQP3 expression and cell migration in CaOV3 cells. Furthermore, EGF-induced EGFR, ERK and AKT activation, which are responsible for AQP3 up-regulation and cell migration, are attenuated by

curcumin (Fig. 5). Thus, we conclude that inhibition of EGFR, PI3K/AKT and MEK/ERK activation is involved in curcumin's suppressive effect on EGF-induced cell migration and AQP3 expression in human ovarian cancer cells.

Collectively, we conclude that AQP3 is expressed in human ovarian cancer cells CaOV3 cells and AQP3 facilitates ovarian cancer cell migration. EGF up-regulates AQP3 expression and cell migration via EGFR signal transduction pathway in human ovarian cancer cells. Our results suggest a novel function for AQP3 in high-grade tumors. Furthermore, we for the first time find that curcumin down-regulates AQP3 and attenuates EGF-induced increase of AQP3 expression and cell migration in human ovarian cancer cells. Our findings also provide an explanation as to the molecular mechanisms of curcumin's inhibition of ovarian cancer cell migration and may contribute to potential therapeutic strategies for the treatment and prevention of ovarian cancer.

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