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Effect of keratinocyte growth factor on growth and transdifferentiation of primary alveolar epithelial type II cells

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Background/aim: To investigate the effects of keratinocyte growth factor (KGF) on the growth and transdifferentiation of primary alveolar epithelial type II cells (AECIIs).

Materials and methods: The number of primary AECIIs, their viability, and cell cycle and apoptosis were studied under KGF treatment using a hemocytometer, trypan blue exclusion, and flow cytometry, respectively. Positive expressions of surfactant protein C (SP-C), aquaporin 5 (AQP5), and thyroid transcription factor 1(TTF-1) were examined with indirect immunofluorescence; mRNA levels of *SP-C*, *AQP5*, and *TTF-1* were determined by polymerase chain reaction.

Results: In response to KGF treatment, cell numbers were significantly increased, more cells were blocked in the S phase, and fewer cells were apoptotic or necrotic on days 2 and 4, but there was little effect on cell viability. In addition, KGF treatment resulted in higher levels of *SP-C* on days 2 and 8 while lowering them on day 4, higher levels of *AQP5* on day 4 while lowering them on day 8, and higher levels of *TTF-1* on days 2, 6, and 8.

Conclusion: KGF treatment promoted proliferation of AECIIs, inhibited cell apoptosis, promoted transdifferentiation of AECIIs, and induced alveolar epithelial type I cells to revert to AECIIs.

Key words: Keratinocyte growth factor, alveolar epithelial type II cells, transdifferentiation, surfactant protein C, aquaporin 5, thyroid transcription factor-1

1. Introduction

Keratinocyte growth factor (KGF) is a paracrine mitogenic agent produced by stromal cells (1). It mediates the interaction between mesenchymal and epithelial cells. It is crucially important in normal lung development and acts as a protective agent after many different types of lung injury. KGF enhances alveolar epithelial tightness by altering the actin cytoskeleton to protect the lung from acute injury (2). It also can activate transepithelial ion transport in alveoli and preserves expression of water channels to alleviate high-altitude pulmonary edema (3). An increasing number of studies suggest that KGF protects the lungs from a variety of damage via different mechanisms (4), as well as regulating the development of bronchi during maturation of the fetal lung (5). Nevertheless, the underlying protective mechanism of KGF remains elusive.

It is well established that alveolar epithelial type II cells (AECIIs) serve to defend the alveoli, and their functions include synthesis and secretion of pulmonary surfactant, activation of ion transport, and maintenance and repair of alveolar epithelium after lung injury (6). Primary AECIIs cultured over a period of 3 to 4 days gradually transdifferentiate to alveolar epithelial type I cells (AECIs). Studies have shown that KGF promotes the proliferation, differentiation, and maturation of AECIIs through a KGF paracrine loop (7) and efficiently prevents the damage of intracellular injured DNA (3). However, the physiological links among KGF, AECIIs, and pulmonary development need to be further investigated. In consideration of the multiple effects of KGF on lung development and the healing of injuries, the present study investigated the effects of KGF on AECII proliferation and differentiation, which may reveal the physiological roles of KGF in the prevention of pulmonary damage.

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2. Materials and methods

2.1. Rat preparation

Adult Sprague Dawley rats were obtained from the Beijing Academy of Military Medical Sciences Animal Research Center. The Animal Ethics Committee of Binzhou Medical University Affiliated Hospital approved the animal experiments. The study was performed from January to July in 2012.

Female rats (230–260 g) and male rats (300–350 g) were housed in the same cages at a female-to-male ratio of 2:1. Vaginal smears from the females were examined at 0800 hours for the presence of sperm, which indicated positive mating. The morning on which evidence of mating was found was considered day zero (D0) of pregnancy.

2.2. AECII isolation and culture

On day 19 of gestation the pregnant rats were anesthetized and pups were removed by cesarean section. The AECIIs were isolated from fetal rats as previously described (8).

The AECIIs were randomly divided into a control group and a KGF group. The KGF group cells were cultured in a cell-culture medium at a final KGF concentration of 10 ng/mL. The control group was cultured in an identical cell culture medium but without KGF. The cells of both groups were maintained in an incubator with a humidified atmosphere and 5% CO_2. Each of the 2 groups was further divided into 2-, 4-, 6-, and 8-day subgroups according to the culture incubation period.

2.3. Assessment of cell number

The filtered and purified AECIIs were suspended and adjusted to a cell confluency of approximately 4×10^5/mL. Cell suspension (0.5 mL) was plated into individual wells of 24-well plates. The cells in 5 wells were harvested and contents were digested with 0.25% trypsin every 48 h, and the cell number was counted with a hemocytometer under an inverted microscope. The mean number of cells in the 5 wells was considered the cell number for each sample.

2.4. Assay of cell viability

Cell viability was determined via trypan blue exclusion at each time point. Briefly, 9 drops of cell suspension were mixed with 1 drop of 0.4% trypan blue. Living and dead cells were counted with a blood counting plate within 3 min. The percentage of the total number of cells that were living was calculated. Each sample was counted 5 times and the mean was calculated from these.

2.5. Assay of cell cycle

At each culture time point, 1×10^5 cells were harvested and washed twice with cold phosphate buffered saline (PBS). Cells were resuspended with 250 µL of trypsin buffer and kept at room temperature for 10 min, then mixed with 200 µL of buffer B (trypsin inhibitor and RNase buffer) and kept at room temperature for another 10 min. Propidium iodide (PI) dye (200 µL) was added and cells were kept at 2–8 °C for 10 min. The cell suspension was finally filtered through a 200-mesh sieve and the distribution of cell cycle phases was examined with a FACScan flow cytometer (BD Biosciences, USA) using a Cycletest Plus DNA staining kit in accordance with the manufacturer's instructions (BD Biosciences, 340242). The percentage of cells in each phase of the cell cycle was calculated.

2.6. Analysis of apoptosis

Rates of apoptosis were analyzed by flow cytometry. An annexin V-fluorescein isothiocyanate (FITC) kit (CX1001) was used in accordance with the manufacturer's instructions (Beijing Biosea Biotechnology, China). Results were analyzed using CellQuest and Motifit software. Stained cells were separated into 3 subgroups: 1) viable cells with intact cell membranes without externalized phosphatidylserine (lower left quadrant, annexin V^-/PI^-); 2) early apoptotic cells with intact cell membranes and with externalized phosphatidylserine (lower right quadrant, annexin V^+/PI^-); 3) late apoptotic cells and necrotic cells with lost membrane integrity and with externalized phosphatidylserine (upper right quadrant, annexin V^+/PI^+).

2.7. Immunofluorescence

The fluorescence expressions of surfactant protein C (SP-C), aquaporin 5 (AQP5), and thyroid transcription factor 1 (TTF-1) were observed with confocal laser scanning microscopy (C1-SHS, Nikon, Japan) at each time point. AECIIs were kept in special laser confocal dishes. Cells in these dishes were fixed with 4% paraformaldehyde for 10 min and washed 3 times with PBS. The primary antibodies sheep anti-SP-C, rabbit anti-AQP5, and sheep anti TTF-1 (Santa Cruz Biotechnology, USA) were added in serial procedures and the dishes were kept in a wet box at 4 °C for 24 h and washed 3 times with PBS. Antisheep IgG marked by FITC or antisheep IgG marked by Texas Red (Sigma, USA) was added and cells were kept in the incubator at 37 °C for 2 h, followed by washing 3 times with PBS. One milliliter of 10 ng/mL Hoechst 33342 (Sigma, USA) was added and the cells in the dishes were kept at room temperature for 20 min. After washing with PBS, cells were mounted in Vectashield mounting medium. Finally, cells were observed with a C1-SHS laser confocal microscope (Nikon, Japan).

2.8. Detection of mRNA expression of SP-C and *AQP5*

SP-C and *AQP5* mRNAs were detected via reverse transcription (RT)-PCR (ABI 9700, Applied Biosystems, USA). The amplified PCR products were resolved in agarose and visualized at ultraviolet wavelength 260 nm. The amount of *SP-C* or *AQP5* was calculated relative to glyceraldehyde-3-phosphate dehydrogenase (GAPDH).

2.9. Detection of mRNA expression of TTF-1

The expression of *TTF-1* was examined via quantitative fluorescent PCR, performed in accordance with the manufacturer's instructions (RG6000, Corbett).

Relative changes in gene expression were determined using the relative quantitative ($2^{-\Delta\Delta Ct}$) method, where $F = 2^{-\Delta\Delta Ct}$, and Ct is the threshold cycle (i.e. the cycle number at which fluorescence reached the set threshold). ΔCt was calculated by subtracting the Ct value of the GAPDH reference from the Ct values of the gene expression of interest (that is, the sample): $\Delta Ct_{sample} = Ct_{sample} - Ct_{GAPDH}$. ΔΔCt was calculated by subtracting the ΔCt of the control from the ΔCt of the sample: $\Delta\Delta Ct = \Delta Ct_{sample} - \Delta Ct_{control}$. The $2^{-\Delta\Delta Ct}$ value (F) represents the relative fold changes in expressed quantity between the control and the target gene (9).

2.10. Statistical analysis

All data were presented as mean ± standard deviation (SD) and analysis was performed using SPSS 11.5. The interaction effect of incubation time and culture group was analyzed by factorial analysis of variance (ANOVA). Differences between groups were analyzed using Student's t-test while differences among multiple groups were analyzed by one-way ANOVA. The Bonferroni method was used to correct for multiple comparisons. $P < 0.05$ was considered statistically significant.

3. Results

3.1. KGF treatment significantly facilitated AECII proliferation

With the isolated AECIIs, we initially investigated the effects of KGF on cell growth (Figure 1). The cells in the KGF group showed regular morphology with clear nucleoli. The cells in the KGF group grew more quickly and had more dikaryocytes on days 2 and 4. The number of cells in the KGF group was significantly higher than that of the control group on days 2, 4, 6, and 8 ($P < 0.05$; Table 1). In total, these results indicated that the KGF treatment facilitated AECII proliferation.

3.2. KGF treatment did not affect cell viability

We then examined the effect of KGF on cell viability. Compared with cells in the control group, KGF treatment did not significantly change the viability of AECIIs at any measured time point (Table 2). Altogether, these results indicated that the KGF treatment had very little effect on cell viability.

3.3. KGF treatment caused alteration of the cell cycle in AECIIs

To examine the effect of KGF on the AECII cell cycle, we analyzed the cell phase distribution after KGF treatment.

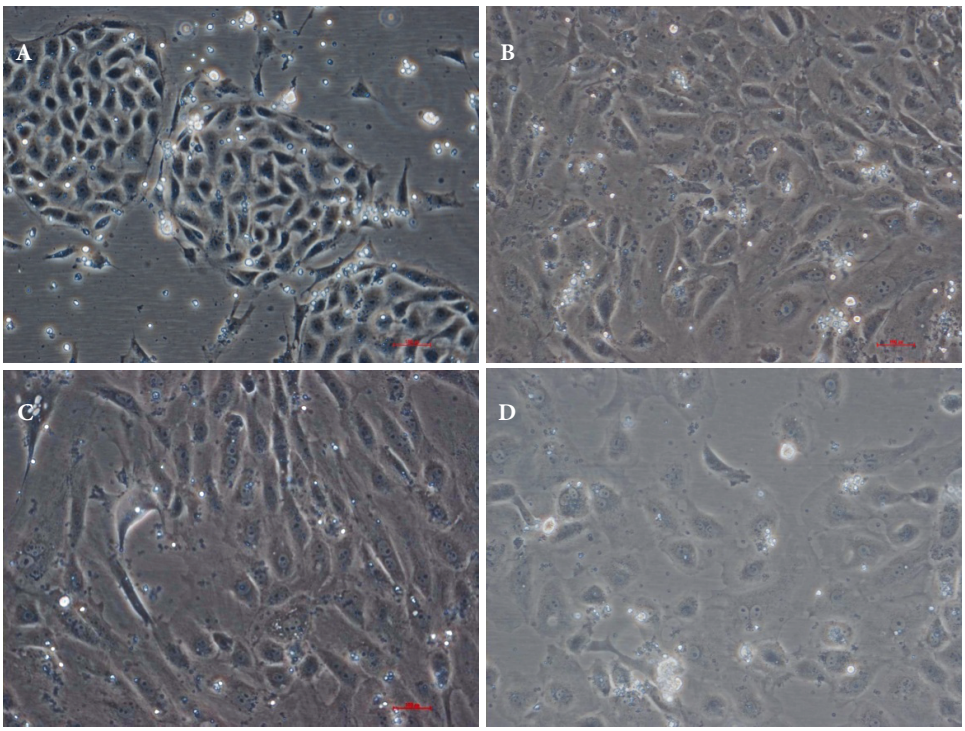

Figure 1. The number of AECIIs in the KGF group. The number of AECII cells increased on days 2 and 4, and then decreased in a time-dependent fashion (200×). **A)** Day 2. The number of cells was greater on day 2 than at day 0. The cells attached to the dish tightly, and there were some dikaryocytes. **B)** Day 4. The cell number was significantly increased at the culture time point of day 4 in the KGF group. **C)** Day 6. The number of cells gradually decreased on day 6. **D)** Day 8. The number of cells gradually decreased on day 8.

Table 1. Comparison of cell population size ($\times 10^5$/mL) between the control and KGF groups[a] (mean ± SD).

Time (days)	Control group	KGF group	t/F	P
0	4.02 ± 0.99	4.00 ± 0.09	0.391	0.701
2	10.41 ± 0.24 [b]	16.00 ± 0.93 [b]	14.104	0.000
4	28.13 ± 1.93 [b]	37.49 ± 1.50 [b]	11.505	0.000
6	27.12 ± 0.85	36.19 ± 0.86	22.159	0.000
8	26.29 ± 1.59	35.23 ± 1.22	13.374	0.000
F	748.852	1879.459	57.065 [c]	0.000 [c]
P	0.000	0.000		

[a] n = 5.
[b] P < 0.05 compared with preceding culture time point.
[c] Interaction effect.

Table 2. Comparison of cell viability between control and KGF groups[a] (mean ± SD%).

Time (days)	Control group	KGF group	t/F	P
2	97.00 ± 0.71	97.60 ± 1.14	1.000	0.347
4	97.20 ± 0.84	97.60 ± 1.14	0.632	0.545
6	95.00 ± 0.71 [b]	95.60 ± 0.89 [b]	1.177	0.273
8	92.80 ± 1.30 [b]	93.20 ± 0.84 [b]	0.577	0.580
F	24.863	21.333	0.036 [c]	0.991 [c]
P	0.000	0.000		

[a] n = 5.
[b] P < 0.05 compared with preceding culture time point.
[c] Interaction effect.

Compared with cells in the control group, those in the KGF group were markedly more concentrated in the S phase but less concentrated in the G1 phase (Table 3; Figure 2), suggesting that KGF treatment arrested cells in the S phase.

3.4. KGF treatment inhibited AECII apoptosis

To analyze the effect of KGF on AECII apoptosis, we determined the percentage of apoptotic cells after KGF treatment. On days 2 and 4, compared with the control group, the percentage of early apoptotic/necrotic cells in the KGF group was significantly less (P < 0.05), while the difference was not significant on days 6 and 8 (P > 0.05). On day 2, compared with the control group, the percentage of late apoptotic cells and necrotic cells of the KGF group was significantly less (P < 0.05), but the difference was not significant on days 4, 6, and 8 (P > 0.05; Table 4; Figure 3). Altogether, these results indicated that KGF treatment inhibited apoptosis of AECIIs.

3.5. Effect of KGF on the expression of SP-C, AQP5, and TTF-1 and their mRNA levels

SP-C is the only identified active protein that is expressed in AECIIs, and SP-C expression can be used to identify AECIIs (10). AQP5 is localized in the apical membrane of AECIs, and AQP5 expression can be used to identify AECIs (6). TTF-1 is a transcription factor characteristic of AECIIs (11).

The fluorescent intensity of SP-C (green fluorescence), AQP5 (red fluorescence), and TTF-1 are consistent with the expressions of *SP-C* mRNA, *AQP5* mRNA, and *TTF-1* mRNA (Figure 4).

Shown by this simple effect analysis, the expression of *SP-C* mRNA in the KGF group was greater than that of the control group on days 2 and 8 (P = 0.008 and P = 0.000, respectively) and was less than that of the control group on day 4 (P = 0.000), while the difference was not significant at day 6 (P = 0.709; Table 5). The expressions of *AQP5* mRNA

Table 3. Comparison of the percent of cells in G1 and S between the control and KGF groups[a] (mean ± SD%).

Time (days)	Cycle phase	Control group	KGF group	t/F	P
2	G1	64.03 ± 1.24	60.43 ± 1.60	3.973	0.004
	S	22.15 ± 0.85	31.14 ± 1.23	13.457	0.000
4	G1	64.82 ± 1.29	50.90 ± 1.34 [b]	16.696	0.000
	S	23.02 ± 1.09	37.91 ± 1.15 [b]	21.021	0.000
6	G1	64.40 ± 1.85	54.11 ± 1.24 [b]	10.324	0.000
	S	22.20 ± 1.27	34.01 ± 1.22 [b]	15.020	0.000
8	G1	65.68 ± 1.09	56.79 ± 1.28 [b]	11.789	0.000
	S	22.01 ± 1.07	30.92 ± 1.25 [b]	12.082	0.000
F	G1	1.282	43.221	23.830 [c]	–
	S	0.891	36.114	15.250 [c]	–
P	G1	0.314	0.000	–	0.000 [c]
	S	0.467	0.000	–	0.000 [c]

[a] n = 5.
[b] P < 0.05 compared with preceding culture time point.
[c] Interaction effect.

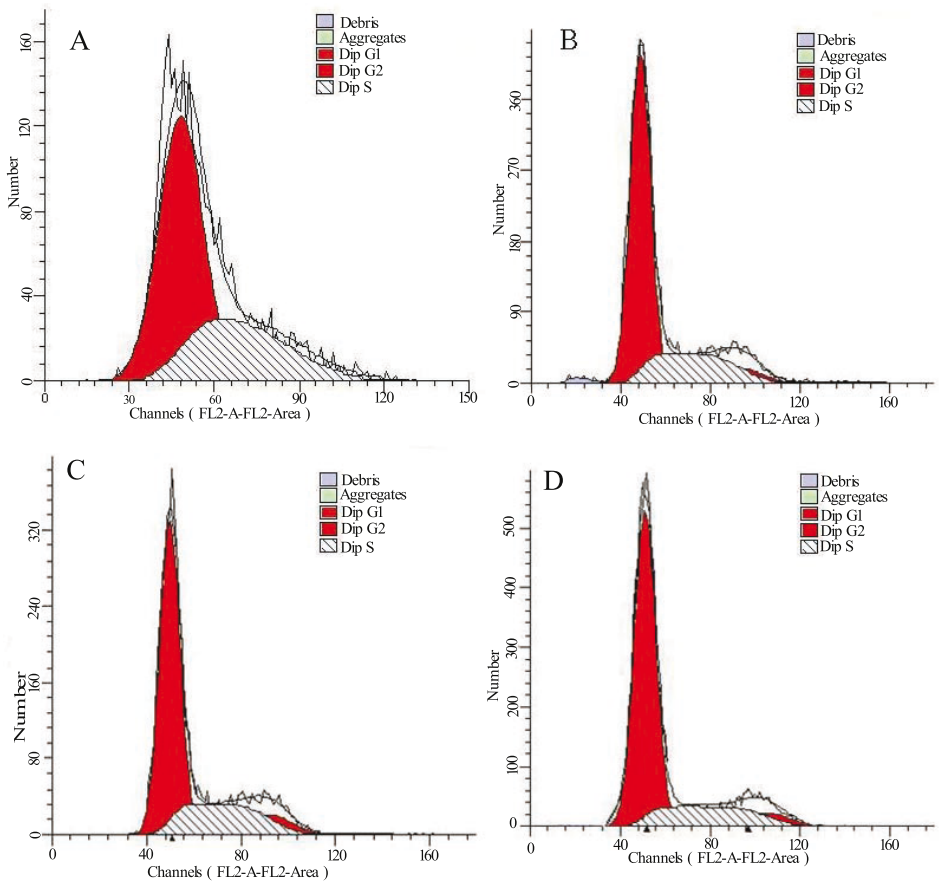

Figure 2. Cell cycle in the KGF group. The proportion of cells in G1 phase cells declined sharply on day 4, then rose gradually on days 6 and 8. The proportion of cells in the S phase was highest on day 4. **A)** Day 2. **B)** Day 4. **C)** Day 6. **D)** Day 8.

Table 4. Comparison of the percentage of apoptotic cells between the control group and KGF group[a] (mean ± SD%).

Time (days)		Control group	KGF group	t/F	P
2	Annexin V^+/PI^-	7.78 ± 0.57	6.78 ± 0.52	2.900	0.020
	Annexin V^+/PI^+	7.88 ± 0.71	3.55 ± 0.64	10.104	0.000
4	Annexin V^+/PI^-	14.11 ± 1.06 [b]	12.15 ± 1.44 [b]	2.442	0.040
	Annexin V^+/PI^+	6.93 ± 1.10	6.77 ± 0.64 [b]	0.278	0.788
6	Annexin V^+/PI^-	11.86 ± 0.83 [b]	12.06 ± 0.99	0.339	0.743
	Annexin V^+/PI^+	12.16 ± 1.61 [b]	11.46 ± 0.47 [b]	0.937	0.376
8	Annexin V^+/PI^-	11.85 ± 0.85	11.47 ± 1.20	0.569	0.585
	Annexin V^+/PI^+	12.29 ± 0.68	11.39 ± 0.71	2.061	0.073
F	Annexin V^+/PI^-	48.723	27.737	2.225 [c]	–
	Annexin V^+/PI^+	33.038	192.347	11.400 [c]	–
P	Annexin V^+/PI^-	0.000	0.000	–	0.104 [c]
	Annexin V^+/PI^+	0.000	0.000	–	0.000 [c]

[a] n = 5.
[b] $P < 0.05$ compared with preceding culture time point.
[c] Interaction effect.

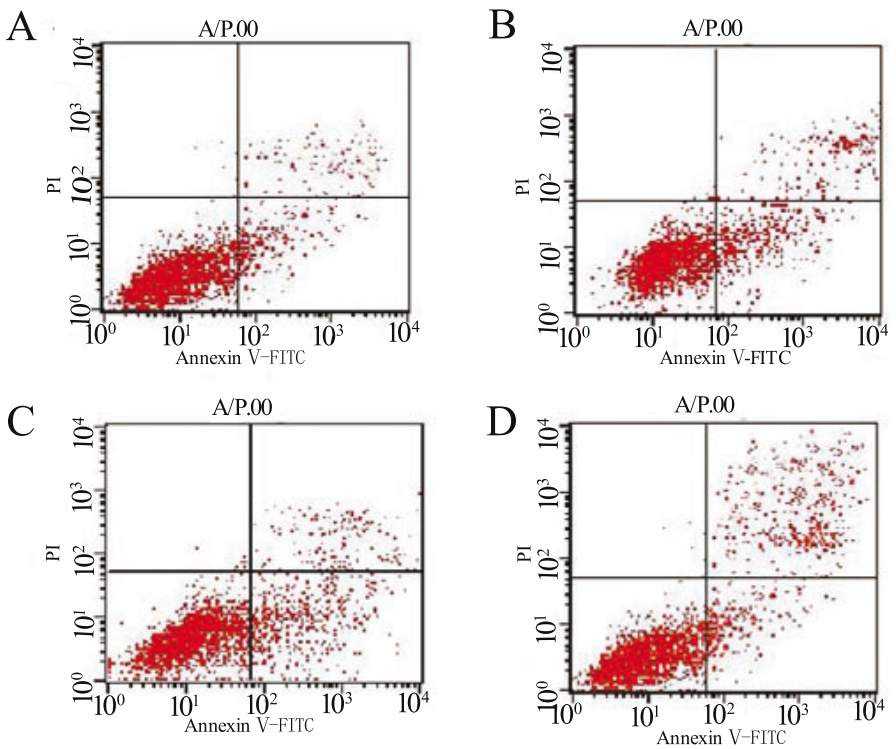

Figure 3. Apoptosis in the KGF group. The proportion of annexin V^+/PI^- and annexin V^+/PI^+ cells declined obviously on day 2. The proportion of annexin V^+/PI^- cells was highest on day 4, and the proportion of annexin V^+/PI^+ cells was highest on day 8. **A)** Day 2. **B)** Day 4. **C)** Day 6. **D)** Day 8.

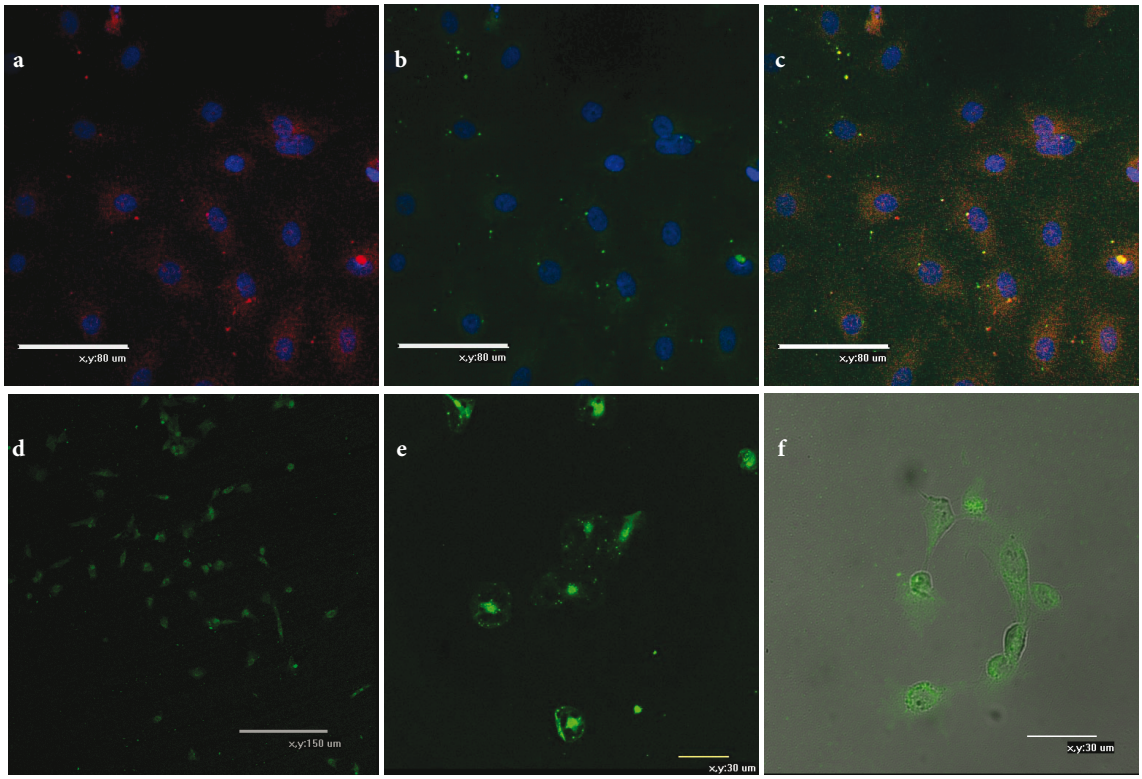

Figure 4. Fluorescence expression of AQP5, SP-C, and TTF-1 (200×). **A)** Red punctiform expression of AQP5 on surface of AECIIs. **B)** Green fluorescence showing SP-C in cytoplasm of AECIIs. **C)** Expression of SP-C in cytoplasm and AQP5 on the surface. **D)** Expression of TTF-1 in nucleus after 2 days of culture; control group. **E)** Expression of TTF-1 in nucleus after 4 days of culture; control group. **F)** The expression of TTF-1 in nucleus of AECIIs after 2 days of culture; KGF group.

Table 5. Comparison of mRNA expressions of SP-C and AQP5 between the control group and KGF group[a] (mean ± SD A_{260} value).

Time (days)	mRNA	Control group	KGF group	t/F	P
2	SP-C	0.74 ± 0.07	0.94 ± 0.10	3.483	0.008
	AQP5	0.20 ± 0.04	0.25 ± 0.04	2.169	0.062
4	SP-C	1.22 ± 0.12 [b]	0.53 ± 0.05 [b]	12.034	0.000
	AQP5	0.33 ± 0.05 [b]	0.71 ± 0.07 [b]	9.558	0.000
6	SP-C	0.70 ± 0.08 [b]	0.71 ± 0.07 [b]	0.387	0.709
	AQP5	0.55 ± 0.09 [b]	0.52 ± 0.05 [b]	0.669	0.522
8	SP-C	0.30 ± 0.06 [b]	0.86 ± 0.06 [b]	15.193	0.000
	AQP5	0.68 ± 0.08 [b]	0.33 ± 0.05 [b]	8.299	0.000
F	SP-C	102.804	29.298	110.086 [c]	–
	AQP5	49.751	76.522	60.745 [c]	–
P	SP-C	0.000	0.000	–	0.000 [c]
	AQP5	0.000	0.000	–	0.000 [c]

[a] n = 5.
[b] $P < 0.05$ compared with preceding culture time point.
[c] Interaction effect.

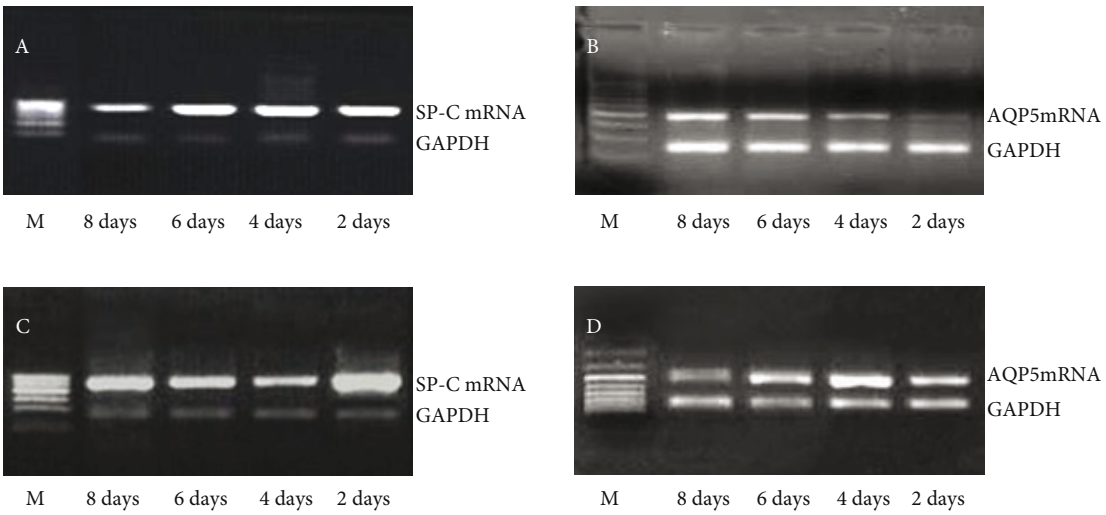

Figure 5. RT-PCR of *SP-C* mRNA and *AQP5* mRNA. **A)** RT-PCR of *SP-C* mRNA in control group. **B)** RT-PCR of *AQP5* mRNA in control group. **C)** RT-PCR of *SP-C* mRNA in KGF group. **D)** RT-PCR of *AQP5* mRNA in KGF group.

was greater than that of the control group on day 4 (P = 0.000) and was less than that of the control group on day 8 (P = 0.000), while the difference was not significant on days 2 and 6 (P = 0.062 and P = 0.522; Table 5). The mRNA level of *TTF-1* was greater than that of the control group on days 2, 6, and 8 (P < 0.05, all), while the difference was not significant on day 4 (P > 0.05; Table 6). These results suggest that KGF treatment promoted transdifferentiation of AECIIs, and made the AECI cell-like phenotype revert to that of AECII.

4. Discussion

KGF is a member of the fibroblast growth factor family, which was originally isolated and purified from cultured human embryonic lung fibroblasts, and is expressed in various organs including the lungs (12). KGF is the only member in its family that is expressed in epithelial cells but not in fibroblasts and endothelial cells. It has been documented that KGF promoted rapid repair of transplanted lung trachea and increased the number of cycled progenitor epithelial cells in the airway (13). Although KGF is not the only effective mitogen, it is an effective inducer of the differentiation of AECIIs (14). Nevertheless, the protective mechanism is not clear.

In this study, we found that the expression of SP-C in AECIIs after KGF treatment was significantly higher compared with the untreated control group. This may be related to a KGF-induced increase of the surfactant content in epithelial cells. Sadovski et al. (15) found that, compared with donor lung tissue treated with KGF, the eluates of transplanted lungs without KGF treatment contained less protein but comparable phospholipids, while SP-C

Table 6. Comparison of TTF-1 expression between the control group and KGF group[a] (mean ± SD × E-04).

Time (days)	Control group	KGF group	t/F	P
2	11.83 ± 1.05	14.53 ± 0.91	4.347	0.002
4	14.23 ± 1.20 [b]	12.67 ± 0.98	2.255	0.054
6	7.85 ± 1.22 [b]	15.39 ± 1.14 [b]	10.097	0.000
8	5.54 ± 0.70 [b]	15.46 ± 0.70	22.470	0.000
F	67.572	9.440	64.688 [c]	0.000 [c]
P	0.000	0.001		

[a] n = 5.
[b] P < 0.05 compared with preceding culture time point.
[c] Interaction effect.

in the lung tissue treated with KGF increased, as well as pulmonary surfactant. Our observations are consistent with these results, suggesting that higher SP-C levels induced by KGF treatment may be an intrinsic protective mechanism in pulmonary injury.

Earlier studies showed that AECIIs are the progenitors of alveolar epithelial cells (16). After lung injury and AECI damage, AECIIs can proliferate and repair the epithelium. The proliferation of AECIIs is the normal reaction to alveolar injury, which can be observed in acute lung injury and interstitial lung disease. KGF is an effective AECII mitogen both in vivo and in vitro (17). KGF could induce the hyperplasia of AECIIs. In the present study we showed that KGF treatment can facilitate AECII proliferation, implying that KGF treatment has a central role in AECII proliferation-mediated protection of lung injury.

KGF promotion of cell proliferation may be via the influencing of cell-phase transition. It is well known that cell-cycle arrest occurs at one of two well-defined checkpoints: the G1/S transition or the G2/M transition. The cell cycle is the final pathway for cell proliferation, and it is regulated at various checkpoints, of which the G1–S phase represents the most important step (18). At this checkpoint, extra- and intracellular signals are integrated and transmitted to cells, and they determine whether cells begin cell division, become apoptotic, or enter the quiescent G0 phase. In our study, when treated with KGF, most cells entered the cell division phase and consequently the cells began to proliferate. We found that KGF treatment arrested cells in the S phase. A similar phenomenon was found in other cells by Lin et al. (19). In that study, KGF could promote oral squamous carcinoma cell proliferation by increasing the viability of carcinoma cells and inducing more of them to enter the G2 and S phases (19). However, regarding cell viability, we did not find in the present study any significant difference between the KGF treatment group and the control group. However, the mechanism underlying the effect of KGF on the cell cycle is not well elucidated. One possible mechanism is that stimulation with KGF may upregulate the expression of cyclin *D1* and downregulate *p21*, and thus induce cells to pass the G1–S phase checkpoint (20) and begin automatic division.

Moreover, some studies have shown that KGF could inhibit the apoptosis of intestinal epithelial cells (21,22). The inhibition of the apoptosis of AECIIs was also observed in our present research. However, the mechanism involved in this process is still not clear.

Previous studies have shown that AQP5 is a marker of AECI, and SP-C is a marker of AECII (6,10). In the present study, our evaluation showed that KGF promoted transdifferentiation of AECIIs, as well as making AECIs revert to AECIIs. This suggests that the promotion effect of KGF was bidirectional. However, little is known about the signal transduction pathways that regulate phenotypic transitions between AECIIs and AECIs. Qiao et al. (6) found that c-Jun activation is a crucial pathway through which KGF mediates phenotypic transitions in AECs. It is likely that KGF made AECIs retrotransdifferentiate to AECIIs via this mechanism and then mediated protective effects after injury and during repair of the lung.

The *TTF-1* gene is essential to lung development (23). To date, the investigation of KGF effects on the expression of TTF-1 are limited. In this study we found that the expression of *TTF-1* mRNA in KGF-treated AECIIs was significantly greater compared with the control group. Tichelaar et al. (24) demonstrated that KGF not only induced the proliferation of AECIIs, but also increased the expression of TTF-1. They also showed that the expression of TTF-1 increased at first and then activated the promoter Ccsp. The research found that there were two consistent TTF-1 sites between the 197 and 158 DNA in SP-C. Mutagenesis in one of the two sites could completely block the activation of TTF-1. This showed that the two sites were essential in the transcription activation of TTF-1 to SP-C. As KGF promoted the proliferation of AECII and the expression of SP-C, and TTF-1 promoted the expression of SP-C, it seems that KGF activated the expression of TTF-1 at first and then TTF-1 stimulated the expression of SP-C, which indicated that it promoted the proliferation of AECIIs.

In conclusion, according to our cytological study, KGF administration significantly affected the growth and transdifferentiation of AECIIs. The proliferation effect of KGF on AECIIs acted through the promotion of cells into the S phase, but not by affecting cell viability. In addition, KGF treatment resulted in fewer apoptotic and necrotic cells and changed the expression patterns of the genes *SP-C*, AQP5, and *TTF-1*. However, the association between KGF and TTF-1 and the mechanism underlying the inhibition of apoptosis and promotion of differentiation by KGF will need to be studied further.

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