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The Hypertrophic Effect of Transforming Growth Factor-β is Reduced in the Absence of Cyclin-Dependent Kinase-Inhibitors p21 and p27

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Abstract. Transforming growth factor-β (TGF-β) has both antiproliferative and hypertrophic effects on mesangial cells (MC). However, it is not known if these processes are independent or if they share common signaling pathways. Proliferation and hypertrophy are regulated by specific cell-cycle regulatory proteins, where the cyclin-dependent kinase (CDK) inhibitors inhibit target cyclin-CDK complexes. This study examined whether the growth regulatory effects of TGF-β were determined by the CDK inhibitors p21 and p27. Accordingly, cultured MC from wild type (+/+) and single and double null (−/−) p21 and p27 mice were grown in 5% serum in the presence or absence of TGF-β1 (2 ng/ml). Proliferation ([^{3}H]-thymidine incorporation, cell number, cell cycle) and hypertrophy ([^{3}H]-leucine incorporation, total protein content, forward light scatter) were measured after 24 h, 48 h, and 96 h. TGF-β inhibited proliferation in +/+ and p21/p27 double −/− MC to a similar extent. TGF-β induced hypertrophy in +/+ MC (18.0% increase at 48 h), and to lesser extent in p21 −/− (12.8%) and p27 −/− MC (11.5%) measured by forward light scatter analysis. In p21/p27 double −/−, the hypertrophic effects of TGF-β were significantly reduced (3.9% at 48 h). Similar results were obtained by measuring hypertrophy by total protein and [^{3}H]-leucine incorporation. In conclusion, the CDK inhibitors p21 and p27 are not required for the antiproliferative effects of TGF-β. However, the hypertrophic growth effects of TGF-β are reduced in the absence of both p21 and p27. These data suggest that the regulation of the antiproliferative and hypertrophic effects of TGF-β may be distinct processes.

Transforming growth factor-β (TGF-β) is an important cytokine in renal disease (1,2). TGF-β mediates extracellular matrix protein accumulation, cell proliferation, and hypertrophy, and it also regulates the immune response. TGF-β induces growth arrest in many cell types including mesangial cells (3,4). Cell proliferation is regulated at the level of the cell cycle (5), where progression through the cell cycle requires that cyclin-dependent kinases (CDK) be activated by partner cyclins. Cyclin-CDK complexes are negatively regulated by cyclin-kinase inhibitors (CKI), which inhibit cell proliferation by causing cell-cycle arrest. Two families of CKI have been identified on the basis of sequence homology and their target cyclin-CDK complexes that are inhibited. The INK4 family of CKI, $p15^{INK4b}$ (p15), $p16^{INK4a}$, and $p18^{INK4c}$, specifically inhibit G_1 CDK. The CIP/KIP family of CKI, which includes $p21^{CIP1/WAF1}$ (p21), $p27^{KIP1}$ (p27), and $p57^{KIP2}$, share homology at the amino terminus and inhibit G_1- and S-phase cyclin-CDK complexes. Cell-cycle arrest induced by TGF-β is mediated by several cell-cycle proteins. TGF-β decreases the levels of cyclins D, E, and A, and it also decreases the activity of CDK4 and CDK2 (6). p15 and cdc25A participate in the antiproliferative effects of TGF-β (7,8). Although TGF-β increases the levels of p21 and p27 (9,10), the importance of these CKI in this process remains to be fully elucidated.

In contrast to an increase in cell number during proliferation, an increase in cell size (not cell number) characterizes hypertrophy. Glomerular cell hypertrophy occurs during many forms of chronic renal disease, including diabetic nephropathy (11), and may antecede the development of glomerulosclerosis (12–14). TGF-β induces cell hypertrophy in mesangial (15), vascular smooth muscle (16), and renal epithelial cells (17). Cell hypertrophy induced by glucose is also partially mediated by TGF-β (17,18). There is a growing body of literature showing that cell hypertrophy is regulated by specific cell-cycle proteins (5,19). We have shown that high glucose induces hypertrophy in normal mesangial cells but not in mesangial cells lacking p27 and that high glucose induced cell hypertrophy when p27 levels were restored into p27 null cells (20). Experimental diabetic nephropathy is associated with glomerular hypertrophy in normal mice but not in p21 null mice (21). Terada *et al.* (22) showed that the forced overexpression of p21 and p27 in tubular LLC-PK1 cells stimulated the *de novo* protein synthesis in the presence of epidermal growth factor and increased cell

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size. Overexpression of p21 induced hypertrophy in vascular smooth muscle cells (23), and Wolf and Stahl (24) and Braun-Dullaeus *et al.* (25) have demonstrated that angiotensin II–induced cell hypertrophy requires p27 accumulation. The effect of TGF-β on hypertrophy has also been linked to the cell cycle (17). However, the role of p21 and p27 in TGF-β–mediated cell hypertrophy remains to be elucidated.

It has been suggested that these CKI may have redundant roles. Thus, to clarify the function of p21 and p27 in mediation of the antiproliferative and hypertrophic effects of TGF-β, we used mesangial cells lacking both p21 and p27. Our results showed that the antiproliferative effect of TGF-β was independent of p21 and p27. In contrast, the hypertrophic growth effects of TGF-β was reduced in the absence of both p21 and p27. Taken together, our data shows that the role of these CKI are distinct in the growth effects of TGF-β in mesangial cells.

Materials and Methods

Cell Culture

p21 null (−/−) mice (originally provided by Tyler Jacks, Massachusetts Institute of Technology, Cambridge, MA) and p27 −/− mice (originally provided by James M. Roberts, Fred Hutchinson Cancer Research Center, Seattle, WA) were developed by gene-targeting. p21/p27 double −/− mice were produced by intercrossing the individual null strains (provided by Matthew Fero, Fred Hutchinson Cancer Research Center, Seattle, WA). Mesangial cells were derived from wild type (+/+) mice, single p21 −/−, single p27 −/−, and p21/p27 double −/− mice by the standard sieving method as described previously (26). In brief, kidneys from six to eight mice from each strain and genotype were pooled for glomerular isolation. Mesangial cells were identified by well-characterized methods, and several early passage cells were isolated from each mouse strain and frozen away for future study. For all experiments, we typically performed studies on three separately frozen cell stocks, which we referred to as "clones," each given the arbitrary numbers 1, 2, and 3. Cells were cultured in 2:1 mixtures of Dulbecco's modified Eagle's medium (DMEM) and Ham's F-12 medium, containing 20% fetal calf serum (FCS), L-glutamine (0.68 mM), penicillin (100 U/ml), streptomycin (100 μg/ml), and 10 mM Hepes, pH 7.4. Cell cultures were incubated at 37°C in a humidified atmosphere of 5% CO_2/95% air with media changes every 2 to 3 d. Cells were grown to subconfluence and then detached by trypsinization (0.05% trypsin/0.02% ethylenediaminetetraacetic acid).

Assessment of Proliferation and Hypertrophy

For all experiments, cells were synchronized in low-serum (0.2%) for 24 h to prevent apoptosis. To determine the role of p21 and p27, mesangial cells were incubated with medium containing 5% FCS in the presence or absence of 2 ng/ml (80 p.m.) of TGF-β1 (R&D System Inc., Minneapolis, MN). Proliferation was measured by cell counts, [^{3}H]-thymidine incorporation, and flow cytometry (see below). Hypertrophy was measured by total protein content and [^{3}H]-leucine incorporation, and cell size was assessed by forward light scatter on flow cytometry (see below). Proliferation and hypertrophy were measured at 24 h, 48 h, and 96 h. Each experiment was performed a minimum of four times.

Cell Counts and Total Protein Content

For the determination of cell number, mesangial cells were plated onto 24-well culture dishes at a density of 4×10^3 cells/well and were synchronized in low serum (0.2%) for 24 h. The media was changed to 5% FCS with or without the presence of 2 ng/ml of TGF-β. Cells were detached by trypsinization at 24 h, 48 h, and 96 h, and cell number was determined by Coulter counter (Beckman Coulter, Inc., Fullerton, CA). Total protein content was determined by the bicinchoninic acid (BCA) method according to the manufacture's instructions after cells on the dishes were hydrolyzed by 0.4 N NaOH and neutralized with 0.2 N acetic acid. Protein content was corrected for cell number. Quadruplicate wells were counted for each group at every time point.

[^{3}H]-Thymidine Incorporation and [^{3}H]-Leucine Incorporation

Relative rates of DNA synthesis and protein synthesis were assessed by measuring [^{3}H]-thymidine (27) and [^{3}H]-leucine incorporation (20), respectively. Mesangial cells were plated onto 24-well culture dishes at 4×10^3 cells/well and synchronized in low serum (0.2%) for 24 h and then switched to media containing 5% FCS with or without the presence of 2 ng/ml of TGF-β. Cells were pulsed with [^{3}H]-thymidine (2 μCi/ml) or [^{3}H]-leucine (5 μCi/ml) for the last 4 h at each time point studied. The supernatant was removed, and cells were washed three times with phosphate-buffered saline and precipitated with ice-cold 10% trichloroacetic acid (TCA). Cells were allowed to dry, then they were solubilized in 0.4 N NaOH. After incubation at 37°C for 20 min, cells were neutralized with 0.2 N acetic acid. The solubilized material was counted in a liquid scintillation counter for [^{3}H]-thymidine incorporation and [^{3}H]-leucine incorporation. Incorporation was corrected for cell number. Quadruplicate wells were counted for each group at every time point.

Flow Cytometry

Mesangial cells were plated onto 10-cm culture dishes at a density of 1×10^4 cells/well and synchronized in low-serum (0.2%) for 24 h, then switched to media containing 5% FCS with or without the presence of 2 ng/ml of TGF-β. Cells were harvested by trypsinization at 24 h, 48 h, or 96 h and fixed in iced 70% ethanol and kept at −20°C until analysis. Fixed cells were suspended in 1 ml of propidium iodide staining solution (50 μg/ml propidium iodide, 30 units/ml RNase A, 0.1% Triton X-100, 4 mM sodium citrate) and incubated at 37°C for 10 min before adding NaCl at a final concentration of 138 mM NaCl. Typically, 10,000 gated events were collected on a FACscan machine (Beckton Dickinson, Franklin Lakes, NJ) and analyzed by using CELLQUEST software (Beckton Dickinson). Cell-cycle analyses were performed by Flowjo Software (Tree Star, Inc., San Carlos, CA).

Assessment of Apoptosis

Apoptosis was measured by staining with Hoechst 33342 (Sigma Bioscience, St. Louis, MO), as we have previously described (28).

Statistical Analyses

Statistical significance was determined by *t* test. $P < 0.05$ was considered statistically significant.

Results

The Antiproliferative Effect of TGF-β Does Not Require p21 or p27

To evaluate the contribution of p21 and p27 to the antiproliferative effects of TGF-β, we studied mesangial cells derived from +/+ and p21/p27 double −/− mice. The effect of TGF-β on DNA synthesis was measured by [^{3}H]-thymidine incorpo-

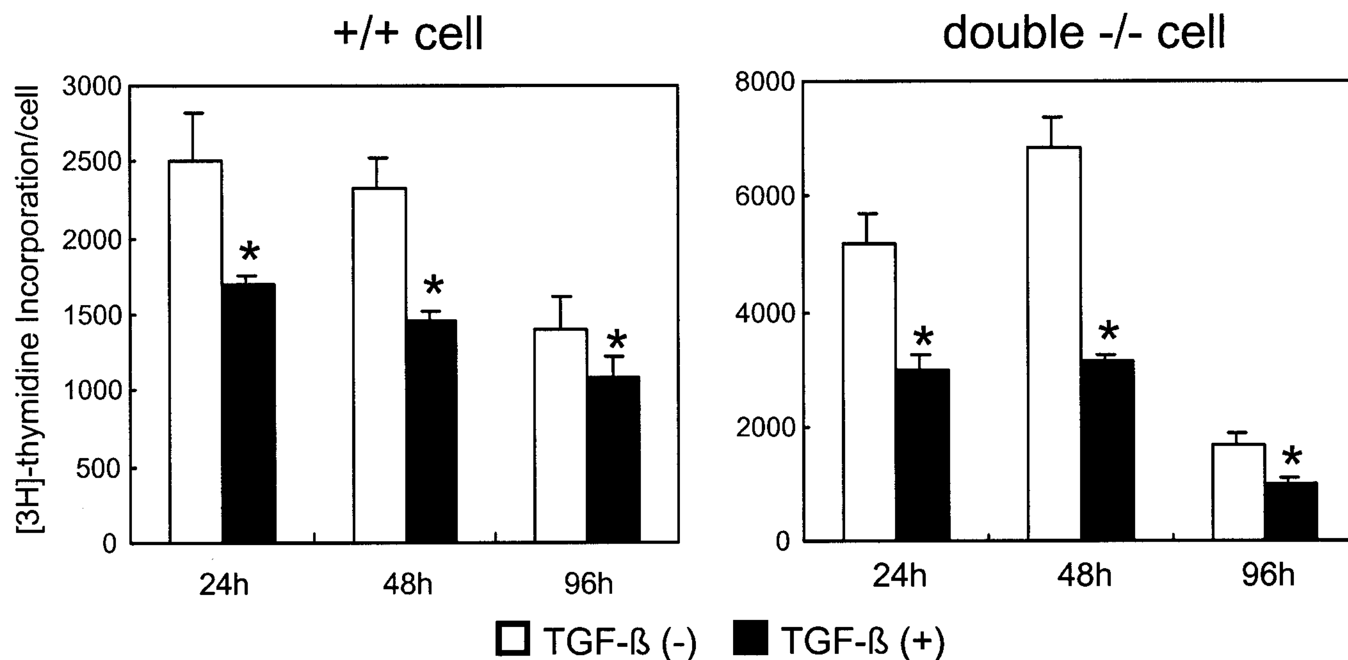

Figure 1. The effect of transforming growth factor–β (TGF-β) on [^{3}H]-thymidine incorporation. TGF-β decreased [^{3}H]-thymidine incorporation, a marker of DNA synthesis, in wild-type mesangial cells (+/+ cell) exposed to 5% fetal calf serum at all time points (24, 48, and 96 h). TGF-β also reduced [^{3}H]-thymidine incorporation in p21/p27 double null mesangial cells (double −/− cell) at all time points studied. The values are the mean of four experiments; [^{3}H]-thymidine incorporation was corrected for cell number. □, not treated with TGF-β; ■, treated with 2 ng/ml of TGF-β. * $P < 0.05$.

ration (Figure 1). TGF-β decreased [^{3}H]-thymidine incorporation in +/+ cells at all time points (31.8% at 24 h, 37.3% at 48 h, and 21.4% at 96 h compared with control cells not exposed to TGF-β; $P < 0.05$ at all time points). TGF-β also reduced [^{3}H]-thymidine incorporation in p21/p27 double −/− cells at all time points studied (42.2% at 24 h, 54.4% at 48 h, and 40.9% at 96 h compared with cells not exposed to TGF-β; $P < 0.05$).

The effect of TGF-β on cell number of +/+ and p21/p27 double −/− mesangial cells is shown in Figure 2. TGF-β inhibited the increase in cell number induced by FCS (a source of growth factors) in +/+ mesangial cells at 48 h (20.8%) and 96 h (50.5%) ($P < 0.05$ *versus* no TGF-β). TGF-β also inhibited cell number in p21/p27 double −/− mesangial cells at 48 h (13.6%) and 96 h (37.8%) ($P < 0.05$ *versus* no TGF-β). Moreover, the decrease in cell number at 48 h and 96 h was similar in control (+/+) cells compared with p21/p27 double −/− cells.

The effect of TGF-β on cell-cycle progression at 24 h was also measured by flow cytometry. TGF-β increased the G1 subpopulation in +/+ cells from 53.1% to 61.4%. TGF-β also increased the G1 subpopulation from 51.2% to 64.1% in p21/p27 double -/- mesangial cells. These data suggests that TGF-β arrests mesangial cells at G1/S. To exclude the possibility that apoptosis induced by TGF-β accounted for these differences, apoptosis was quantified after a 48-h incubation with 2 ng/ml of TGF-β. The number of apoptotic cells was not significantly different in cells incubated with or without TGF-β in all the different types of mesangial cells studied (apoptosis rate: 0.6 ± 0.2% *versus* 0.8 ± 0.3% in +/+ cell, 0.4 ± 0.4% *versus* 0.9 ± 0.7% in p21 −/− cell, 0.8 ± 0.3% *versus* 0.5 ± 0.3% in p27 −/− cell, and 0.5 ± 0.2% *versus* 1.2 ± 0.9% in p21/p27 double −/− cell; $n = 4$). Analysis of the G2/M phase showed similar changes to that described for G1 phase (data not shown).

Hypertrophy Is Reduced in the Absence of Both p21 and p27

TGF-β induces hypertrophy in mesangial cells (15). Hypertrophy was measured by assessing cell size by forward light scatter analysis, [^{3}H]-leucine incorporation and total protein content. Cell size was measured in the G1 and G2/M phases of the cell cycle.

Figure 3 shows the cell size distribution in G1 phase measured by flow cytometry analysis in control and experimental mesangial cells exposed to TGF-β. At 24 h, the mean forward light scatter in wild-type (control) cells exposed to TGF-β was 12.3% higher than +/+ cells not exposed to TGF-β. In contrast, there was no significant increase in forward light scatter in single p21 −/−, single p27 −/−, and p21/p27 double −/− cells at 24 h. After exposure to TGF-β for 48 h, there was an increase in the forward light scatter in +/+ MC (18.0%), single p21 −/− MC (12.8%), and single p27 −/− (11.5%) MC ($P < 0.05$ in each cell type *versus* no TGF-β). In contrast, there was no significant increase in cell size measured by forward light scatter in p21/p27 double −/− cells after incubation with TGF-β for 48 h (Figure 3). At 96 h, TGF-β induced hypertrophy in +/+ cells (30.2%), single p21 −/− (25.5%), and single

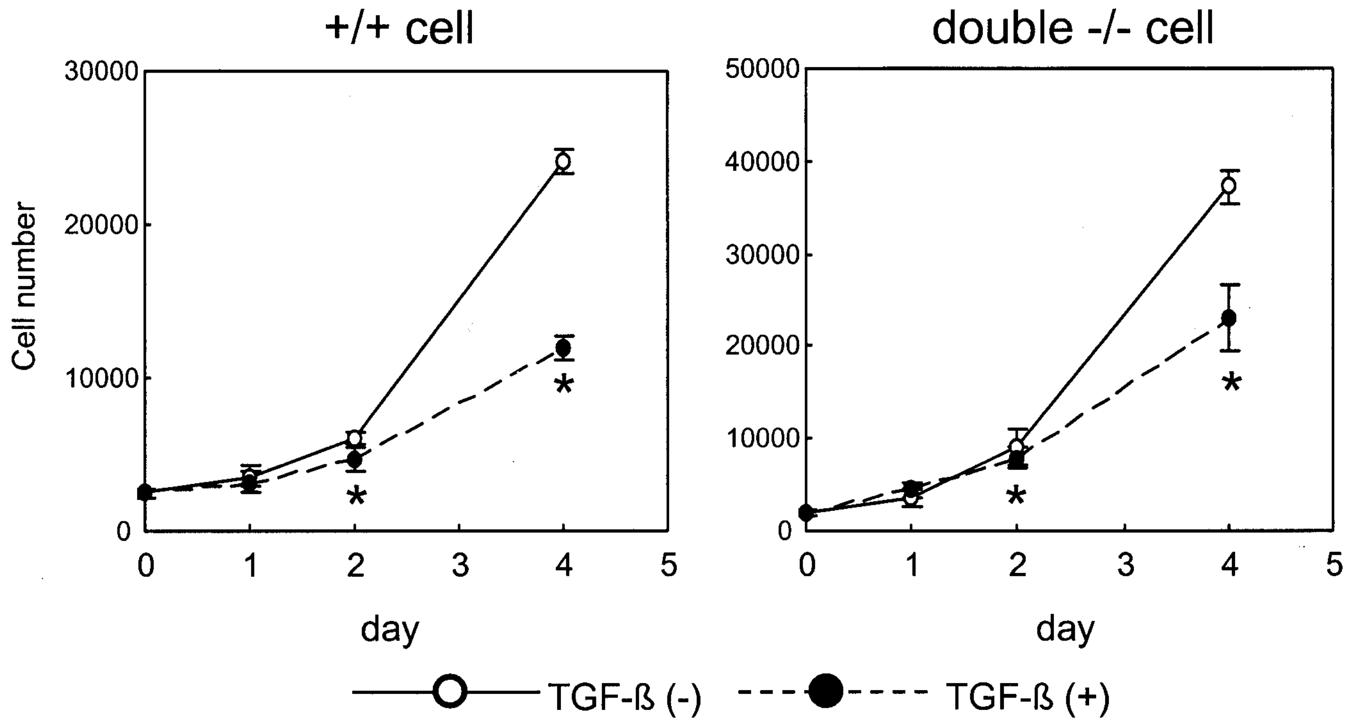

Figure 2. The effect of TGF-β on proliferation of mesangial cells. TGF-β inhibited the increase in cell number induced by 5% serum in wild-type mesangial cells (+/+ cell) at 48 h and 96 h. TGF-β also inhibited proliferation in p21/p27 double null mesangial cells (double −/− cell) at 48 h and 96 h. The values are the mean of four experiments. These data show that the absence of p21 and p27 did not alter the antiproliferative response to TGF-β. ○, not treated with TGF-β; ●, treated with 2 ng/ml of TGF-β. * $P < 0.05$.

p27 −/− (20.8%) cells ($P < 0.05$ in each cell type *versus* no TGF-β). In contrast, the increase in cell size was significantly reduced in p21/p27 double −/− cells (11.2%) at 96 h (Figure 3). To ensure that the reduction in hypertrophy in p21/p27 double −/− cells was not specific to one cell clone, similar studies were also performed in another two clones of p21/p27 double −/− mesangial cells. These three clones were isolated from the same pooled glomeruli derived from six p21/p27 double −/− mice. Hypertrophy measured by flow cytometry was also reduced in these clones of p21/p27 double −/− mesangial cells (data not shown). This was done to ensure that our results were not specific to a "clone" or "harvest" of cells.

Protein content is also used as a measure of hypertrophy (20). Accordingly, we also determined whether the increase in cell size induced by TGF-β correlated with an increase in protein content and protein biosynthetic rates in mesangial cells. Figure 4 shows that TGF-β increased the total protein content per cell in +/+ cells at 24 h (41.6%), 48 h (26.9%), and 96 h (70.8%) ($P < 0.05$ *versus* no TGF-β at all time points). New protein synthesis is another method to measure hypertrophy. Figure 5 shows that the increase in total protein content correlated with an increase in new protein synthesis measured by the incorporation of [^{3}H]-leucine per cell at 24 h (38.0%), 48 h (51.2%), and 96 h (20.1%) ($P < 0.05$ *versus* no TGF-β at each time point). In contrast, TGF-β increased neither total protein content (Figure 5) nor [^{3}H]-leucine incorporation (Figure 4) in p21/p27 double −/− cells during the early phase (24 h and 48 h) of TGF-β–induced hypertrophy.

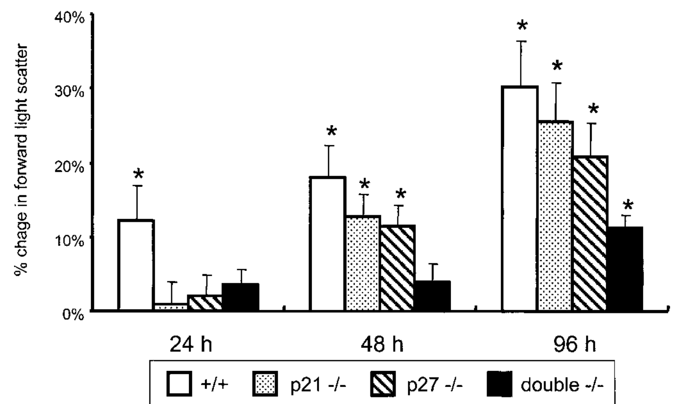

Figure 3. Effect of TGF-β on cell size. Cell size was measured by forward light scatter of the G1 subpopulation and is expressed as an increase compared with control cells not exposed to TGF-β. TGF-β increased cell size (+/+, □) at all time points compared with control +/+ cells not exposed to TGF-β. Cell size was not increased by TGF-β in p21 null (p21 −/−, ▨), p27 null (p27 −/−, ▧), and p21/p27 double null (double −/−, ■) at 24 h. TGF-β increased cell size in single p21 −/− and in single p27 −/− cells at 48 and 96 h, but the increase was not as marked as +/+ cells exposed to TGF-β. In contrast, cell size did not increase in p21/p27 double −/− cells at 24 h and 48 h. Cell size increased at 96 h, but the increase was significantly less compared with +/+, p21 −/−, and p27 −/− mesangial cells. These results show that hypertrophy was significantly reduced in the absence of p21 and p27, and this effect was more pronounced in the absence of both CDK inhibitors. * $P < 0.05$.

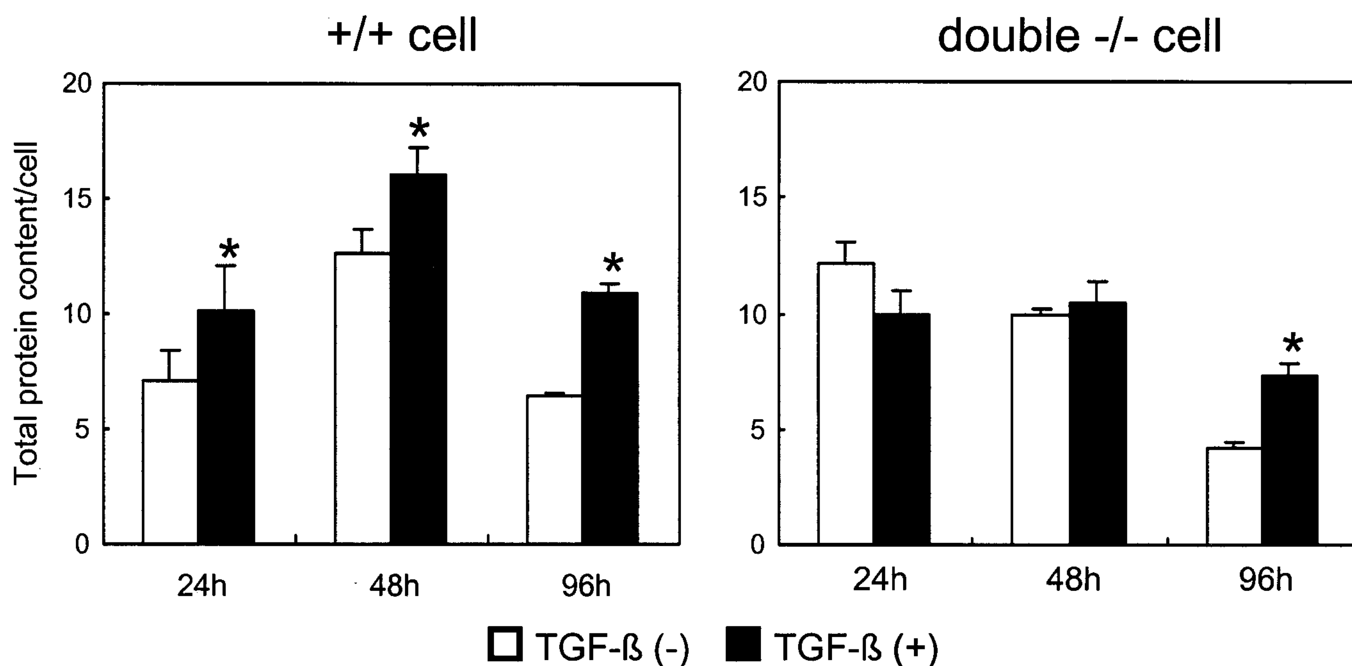

Figure 4. Effect of TGF-β on total protein content. TGF-β increased the total protein content, a marker of hypertrophy, in wild-type mesangial cells (+/+ cell) at all time points. In contrast, TGF-β did not increase the total protein content in p21/p27 double null mesangial cells (double −/− cell) during the early time course (24 h and 48 h). However, TGF-β increased total protein content at 96 h. The values shown are means of four experiments; total protein content was corrected for cell number. □, not treated with TGF-β; ■, treated with 2 ng/ml of TGF-β. * $P < 0.05$.

Discussion

Although TGF-β has been shown to regulate cell growth (proliferation and hypertrophy), it is not known whether similar pathways mediate the regulation of these biologic effects. Previous studies have shown that TGF-β decreases the levels of specific cyclins and CDK and increases the levels of certain CDK inhibitors. In the current study, we show that the CDK inhibitors p21 and p27 are not required for the antiproliferative effect of TGF-β on mesangial cells. In contrast, this is the first study to show that the hypertrophic effect of TGF-β is reduced in the absence of both p21 and p27.

Our results showed that p21 and p27 are not necessary for TGF-β–mediated antiproliferation in cultured mouse mesangial cells. TGF-β suppressed cell proliferation in mesangial cells lacking p21 and p27 to a similar extent as control wild-type cells. TGF-β is known to cause apoptosis in some types of cells including cultured mesangial cells (29). However, TGF-β–suppressed DNA synthesis measured by [^{3}H]-thymidine incorporation, and cell-cycle analysis on flow cytometry clearly showed that TGF-β induced G1 arrest. In addition, there was no significant increase in apoptosis induced by TGF-β in all the cell types used in this study.

Several studies have shown that the levels of p21 and p27 are increased by TGF-β during TGF-β–induced G1 arrest. These results lead the authors to conclude that p21 and p27 were required for TGF-β–induced antiproliferation (30). In contrast, in certain cell types, p21 or p27 protein levels do not change in response to TGF-β (31). In the current study, we demonstrate that antiproliferative effects of TGF-β are independent of p21 or p27. Thus, one interpretation could be that the increase in the expression levels of p21 and p27 by TGF-β might be secondary to cell-cycle arrest. A similar paradigm has been shown for the CDK-inhibitor p15, where p15 is increased by TGF-β (7), yet cells lacking p15 undergo TGF-β–induced G1 arrest (8,31). Taken together, these results suggest that the absolute increase in levels of cell-cycle proteins does not correlate with a role for that gene alone in mediating antiproliferation. These results also show the redundancy of many cell-cycle proteins.

The mechanisms underlying the hypertrophic effect of TGF-β are not fully understood. Accordingly, we were particularly interested in the role of the CDK inhibitors p21 and p27 in mediating the hypertrophic effects of TGF-β. Our data showed that hypertrophy induced by TGF-β was reduced in single p21 −/− and single p27 −/− mesangial cells compared with control +/+ mesangial cells. However, a second major finding in this study was that the initial maximal TGF-β–induced hypertrophy required the presence of both the Cip/Kip inhibitors, p21 and p27. This conclusion was based on the data showing that in the absence of both p21 and p27 in p21/p27 double −/− mesangial cells, hypertrophy was significantly reduced compared with single −/− cells. The reduction in hypertrophy was not due to an absent responsiveness of the p21/p27 double −/− cells, because as stated earlier, we showed that TGF-β inhibited their proliferation. Our data in single p21 −/− and p27 −/− cells also showed that the initial phase of hypertrophy was dependent on p21 or p27 and that in the absence of these CDK-inhibitors, hypertrophy induced by TGF-β was delayed compared with control cells expressing these genes. Finally, the

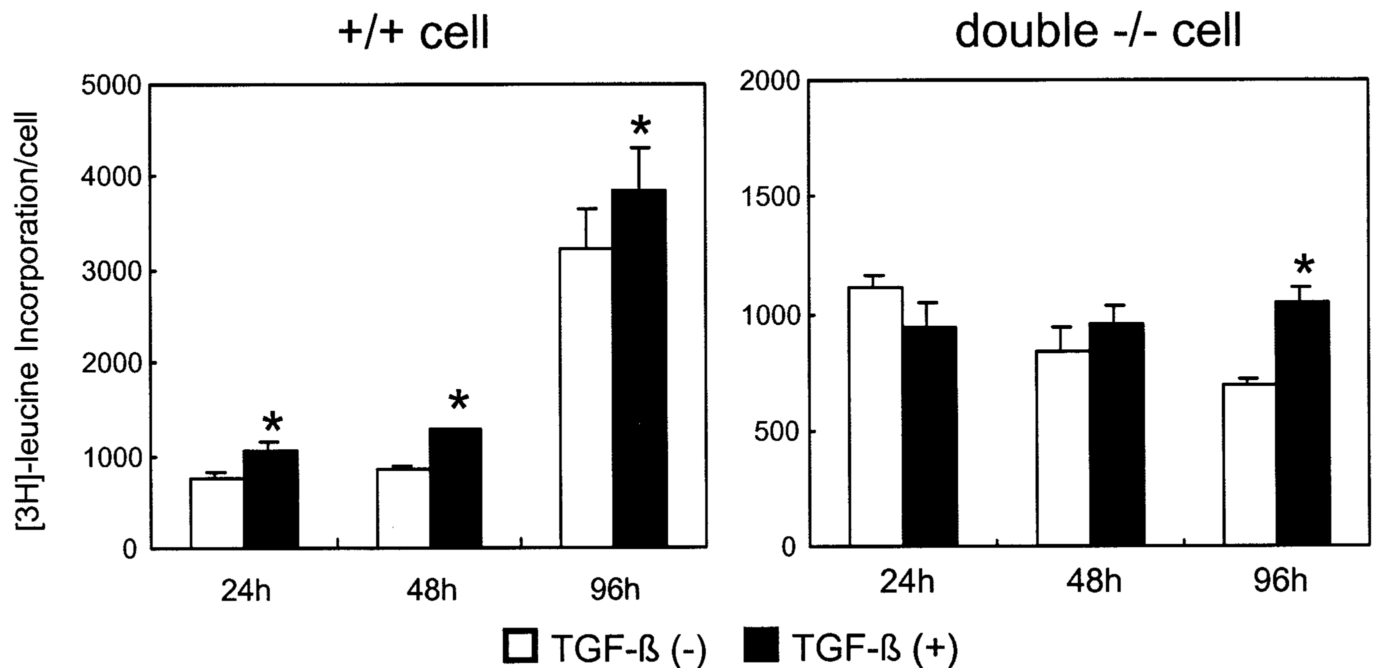

Figure 5. Effect of TGF-β on [^{3}H]-leucine incorporation. TGF-β increased new protein synthesis measured by the incorporation of [^{3}H]-leucine per cell in wild-type mesangial cells (+/+ cell) at all time points. In contrast, TGF-β did not increase [^{3}H]-leucine incorporation in p21/p27 double null mesangial cells (p21/p27 double −/− cell) at the early time course (24 h and 48 h). TGF-β increased protein synthesis at 96 h (* $P < 0.05$). The values shown are means of four experiments; [^{3}H]-leucine incorporation was corrected for cell number. □, not treated with TGF-β; ■, treated with 2 ng/ml of TGF-β. * $P < 0.05$.

finding that cell size increased at 96 h in p21/p27 double −/− cells suggests a cell-cycle–independent mechanism underlying the later phase of TGF-β–induced hypertrophy.

Several methods are used to measure cell hypertrophy, including protein:DNA content ratio and total protein content per cell. Forward light scatter (flow cytometry) is the most accurate measure of cell size. However, there are difficulties in evaluating the effect of TGF-β on cell size, because TGF-β also affects cell proliferation. During cell-cycle progression, cell size increases physiologically from G1 phase to M phase. Studies have shown that the mean cell size in G1 is smaller than the G2 subpopulation. In the current study, we accordingly measured forward light scatter specific for individual phases of cell cycle, G1 and G2/M. The results in both G1 phase and G2/M phase showed a similar increase in hypertrophy. The lack of hypertrophy (at 24 h and 48 h) in p21/p27 double −/− induced by TGF-β was also confirmed by measuring total protein content per cell and [^{3}H]-leucine incorporation per cell. However, there was a discrepancy at the later time point (96 h). Our results showed that TGF-β increased total protein content and [^{3}H]-leucine incorporation in p21/p27 double −/− cells to a similar magnitude compared with +/+ cells. The increase in forward light scatter in p21/27 double −/− cells was less at 96 h compared with +/+ cells. This discrepancy might be explained in several ways. First, the absence of these specific CDK inhibitors may be associated with a delay in TGF-β responsiveness rather than completely abolishing the hypertrophic effect of TGF-β. Second, the mechanisms of cell hypertrophy may be different in early and later time points. Third, because the total protein and [^{3}H]-leucine incorporation was measured without trypsinization, these measures could also reflect changes in extracellular proteins. Finally, because TGF-β increases the production of extracellular matrix proteins, the measurement of adherent cells could be affected.

Preisig proposed that cell hypertrophy is due to cell cycle–independent and cell cycle–dependent mechanisms (19). Most examples of hypertrophy are associated with growth arrest. Studies have shown that CDK-inhibitors are involved in growth arrest–dependent mechanisms of hypertrophy. Overexpressing the Cip/Kip inhibitors p21 or p27 induces hypertrophy (22). Mesangial cell hypertrophy caused by high glucose is associated with increased levels of p21 and p27 (32,33), and hypertrophy is reduced in p27 −/− mesangial cells exposed to high glucose (20). Moreover, glomerular hypertrophy is reduced in diabetic p21 null mice (21). However, in this study, TGF-β induced cell-cycle arrest but the hypertrophy induced by TGF-β was reduced in p21/p27 double −/− cells. This suggests that hypertrophy induced by TGF-β is not secondary to antiproliferation and that there is a mechanism of hypertrophy independent of growth arrest and dependent on p21 and p27. Owens has also shown that TGF-β–induced hypertrophy may not be a direct consequence of growth inhibition but rather occurs concurrently due to additional effects of TGF-β distinct from growth inhibition (16). Thus, we propose that cell hypertrophy is independent of cell-cycle arrest and is dependent on p21 and p27. It remains to be elucidated how p21 and p27 exert the hypertrophic function independently of regulation of cell cycle.

We conclude that p21 and p27 are not required for the antiproliferative effects of TGF-β. However, the hypertrophic growth effects of TGF-β were reduced in the absence of both p21 and p27. These results suggest that the regulation proliferation and hypertrophy by TGF-β are distinct processes.

Acknowledgments

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