Joint Requirement for Rac and ERK Activities Underlies the Mid-G1 Phase Induction of Cyclin D1 and S Phase Entry in Both Epithelial and Mesenchymal Cells*

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Cyclin D1 gene induction is a key event in G1 phase progression. Our previous studies indicated that signaling to cyclin D1 is cell type-dependent because the timing of cyclin D1 gene expression in MCF10A mammary epithelial cells and mesenchymal cells such as fibroblasts and vascular smooth muscle cells is very different, with epithelial cells first expressing cyclin D1 in early rather than mid-G1 phase. In this report, we induced mesenchymal phenotype in MCF10A cells by long-term exposure to TGF-β and used the control and transitioned cells to examine cell type specificity of the signaling pathways that regulate cyclin D1 gene expression. We show that early-G1 phase cyclin D1 gene expression in MCF10A cells is under the control of Rac, whereas mid-G1 phase cyclin D1 induction requires parallel signaling from Rac and ERK, both in the control and transitioned cells. This combined requirement for Rac and ERK signaling is associated with an increased requirement for intracellular tension, Rb phosphorylation, and S phase entry. A similar co-regulation of cyclin D1 mRNA by Rac and ERK is seen in primary mesenchymal cells. Overall, our results reveal two mechanistically distinct phases of Rac-dependent cyclin D1 expression and emphasize that the acquisition of Rac/ERK co-dependence is required for the mid-G1 phase induction of cyclin D1 associated with S phase entry.

Cells control their rates of proliferation by sensing external cues in their microenvironment. Cell cycle progression is dependent on these external cues until a time in late G1 phase, called the restriction point, when cells enter the committed and intrinsically controlled portion of the cell cycle (1). The restriction point is thought to occur after activation of the two G1 phase cyclin-dependent kinases (cdks),2 cyclin D-cdk4 (or its homolog cdk6; hereafter called cdk4/6), and cyclin E-cdk2. A D-type cyclin (mostly cyclin D1 in fibroblastic and epithelial cells) is usually the first of the periodically expressed cyclins to be induced in G1 phase of the cell cycle after reentry from quiescence.

The levels of cyclin D1 mRNA and protein are low in quiescent cells and increase during progression through G1 phase (2–4). The increase in cyclin D1 mRNA requires cooperative signaling by growth factor receptors and adhesion receptors (5). In mesenchymal cells, activation of integrins by the extracellular matrix (ECM) transmits the adhesion signal to the cyclin D1 gene. Epithelial cell proliferation also requires signals from integrins, but these cells are also strongly influenced by cell-cell adhesion, which is mediated, at least in large part, by E-cadherin (4, 6).

The induction of cyclin D1 mRNA requires a sustained activation of extracellular signal-regulated kinases (ERK), ~5–6 h after mitogenic stimulation of quiescent cells, at least in fibroblasts (7–9). ERK activity has also been linked to cyclin D1 gene expression in several other cell types (10–13). Growth factor receptors and integrins collaboratively regulate the Ras-Raf-MEK-ERK cascade (14), and this allows for sustained ERK activity in cells that have active Rho (3). Activated ERK translocates to the nucleus where it stimulates cyclin D1 transcription in mid-G1 phase. The ERK-dependent immediate-early genes, Jun-B and Fra-1, have been implicated in the ERK-stimulated transcription of the cyclin D1 gene (8, 15).

Another Rho-family GTPase, Rac, has also been linked to the induction of cyclin D1 mRNA (16–20). Rac signaling to cyclin D1 mRNA is thought to be jointly regulated by growth factor receptors and integrins: both receptor systems can support GTP loading of Rac, and integrin-mediated adhesion is required for the coupling of Rac to its effectors (21). In fibroblasts, Rac-dependent induction of cyclin D1 mRNA is not seen unless Rho signaling is inhibited (3). Because Rho inhibition blocks stress fiber formation, Rac signaling to cyclin D1 does not require a high intracellular tensional environment. Rac-dependent cyclin D1 gene expression is readily detected in MCF10A mammary epithelial cells (4), and the absence of well-defined actin stress fibers indicate that these cells do not have the high levels of intracellular tension characteristic of mesenchymal cells.

Rac and ERK signaling have distinguishable effects on the timing of cyclin D1 gene expression within G1 phase, with Rac signaling resulting in an early G1 phase expression of cyclin D1 while ERK signaling results in the mid-G1 phase induction of cyclin D1 (3, 4, 7). However, most studies on ERK and Rac signaling to cyclin D1 have either been performed in different...
cell types (epithelial versus mesenchymal) or under conditions of Rac overexpression (3, 4, 16, 17). Thus, little is known about the potential interplay between endogenous ERK and Rac activities as it relates to expression of the cyclin D1 gene within a single cell type.

When MCF10A cells are treated with TGF-β for 3 days, they acquire a mesenchymal phenotype in a process that resembles an epithelial-mesenchymal transition (22). We exploited this approach to study the interplay between endogenous ERK and Rac signaling to cyclin D1 mRNA in a cell type-specific manner yet within cells of the same origin. Our results identify two effects of endogenous Rac signaling on cyclin D1 gene expression, which can be distinguished by cell type (epithelial versus mesenchymal), the dependence or independence from ERK activity, a requirement for intracellular tension, and the ability to support Rb phosphorylation and S phase entry.

**EXPERIMENTAL PROCEDURES**

**Cell Culture**—MCF10A mammary epithelial cells were maintained in 1:1 low glucose DMEM:Ham’s F12 nutrient media supplemented with 10 mM HEPES, pH 7.4, 5% horse serum (Invitrogen), growth factor mixture (20 ng/ml EGF (BD Biosciences), 10 μg/ml insulin (Sigma), 0.5 μg/ml hydrocortisone (Sigma), and 100 ng/ml cholera toxin (List Biologicals)), and 1% gentamicin (Invitrogen). Near confluent MCF10A cells were starved for 48 h in 1:1 DMEM:Ham’s F12 nutrient medium with 1 mg/ml heat-inactivated, fatty acid-free BSA. The quiescent cells were trypsinized and suspended in serum-free media for 30 min at 37 °C prior to reseeding (at 2–3 × 10⁶ cells per 100-mm dish) with mitogens comprised of 10% FBS plus 10% growth factor mixture unless noted otherwise. Primary mouse vascular smooth muscle cells (VSMCs) were collected from 2–3-month-old male C57BL/6 aortic explants, and used at passages 2–5. To synchronize cells in G0, VSMCs were grown to ~90% confluence and serum-starved for 48–72 h in DMEM with 1 mg/ml heat-inactivated, fatty acid-free bovine serum albumin. Unless pretreated with a pharmacological inhibitor, serum-starved VSMCs were directly stimulated with 10% FBS.

In some experiments, trypsinized MCF10A (2–3 × 10⁵ cells/ml) or VSMCs (2–3 × 10⁴ cells/ml) were suspended in starvation media and preincubated in suspension (30 min at 37 °C) with vehicle (DMSO) or 50 μM U0126 (Promega). MCF10A cells were also preincubated for a 30-min suspension with 1 μM cytochalasin D (CCD; EMD Biosciences), 100 ng/ml human Rac1, and mouse Rac1 have been previously reported (23). RNA interference and adenoviral infection—siRNA-medi-ated knockdown was performed as previously described (23). The siRNA oligonucleotide sequences for human E-cadherin, human Rac1, and mouse Rac1 have been previously reported (23), and those for p21 were: No. 1: AACAUACUGGCCCUGGACUGGt and No. 2: AUGCUGACGCACUCCUUt. All siRNA oligonucleotides were used at 150 nM. Adenoviruses were titered and used as described (23). RacN17 adenovirus was a generous gift from Anne Ridley (Ludwig Institute for Cancer Research, University College London, UK).

**Induction of a Mesenchymal Phenotype in MCF10A Cells**—MCF10A cells in maintenance medium were seeded at low confluence (3 × 10⁵ and 6 × 10⁵ cells for control and TGF-β-treated cells, respectively) in 100-mm dishes containing autoclaved glass coverslips. A mesenchymal phenotype was induced similar to Maeda et al. (22) by incubating the cells for 3 days in maintenance medium with 3 ng/ml human recombinant TGF-β1 (R&D Systems). The transitioned cells were serum-starved for 2 days in the continued presence of TGF-β. Duplicate cultures were incubated in parallel without TGF-β. Each dish of quiescent control and TGF-β-treated cells was then trypsinized, reseeded into two 100-mm dishes coated with 1.27 μg/cm² collagen (containing autoclaved coverslips), and stimulated with 10% FBS and growth factor mixture in DMEM:F12 in the continued absence or presence of TGF-β.

**Rho and Rac GTPase Activity Assays**—MCF10A cells were seeded in 6-well plates at 10⁴ cells/well (control cells) or 2 × 10⁴ cells/well (TGF-β-treated cells). Cells were treated for 3 days ± 3 ng/ml TGF-β and starved for 2 days. The cells were stimulated with 10% FBS and the growth factor mixture. Protein was collected and quantified, and active Rho and Rac GTPase levels were measured using G-LISA small G-protein activation assay kits (Cytoskeleton, Inc.) according to the manufacturer’s directions.

**Other Methods**—BrdU incorporation and E-cadherin surface expression was determined by epifluorescence microscopy performed as described (23). F-actin staining was performed using fluorescein isothiocyanate- or rhodamine-phalloidin (Invitrogen).

Total RNA was prepared from collected cells and analyzed by quantitative real-time RT-PCR (QPCR) as described (23). Taqman primer and probe sequences for human cyclin D1 mRNA and 18S rRNA have been listed previously (23). Immediate-early gene induction was analyzed by SYBR Green QPCR using the following primers (forward and reverse pairs, respectively): human Fra-1 CAGGGAGGACACTGAAACT and CTTCGACACCCGTCATTAG and mouse Fra-1 ACCGAAGAAAGGACTGAC and CTGGTCTCGACGCTTCTCAA. The expression of mesenchymal markers was determined using Taqman Assay-On-Demand primers and probes (Applied Biosystems): fibronectin, Hs00415006_m1; vimentin, Hs0018584_m1; snail, Hs00195591_m1; and slug, Hs00161904_m1. RNA expression was quantified by standard curve. QPCR results were normalized to 18S rRNA levels and show the mean ± S.D. of duplicate PCR reactions.

For Western blotting, cells were collected and extracted as described (3), and total cellular protein (~40 μg) was fractionated on reducing SDS-polyacrylamide gels, electrophoretically transferred to nitrocellulose filters, and blotted using standard procedures. Filters were probed with the following antibodies: cyclin D1 (sc-718, Santa Cruz Biotechnology), human E-cadherin, human Rac1, and mouse Rac1 have been previously reported (23), and those for p21 were: No. 1: AACAUACUGGCCCUGGACUGGt and No. 2: AUGCUGACGCACUCCUUt. All siRNA oligonucleotides were used at 150 nM. Adenoviruses were titered and used as described (23). RacN17 adenovirus was a generous gift from Anne Ridley (Ludwig Institute for Cancer Research, University College London, UK).
phospho-Rb S807/S811 (P-Rb S807/S811, ab4783, Abcam). Resolved proteins were visualized by enhanced chemiluminescence. Autoradiograms were digitized by scanning, and figures were assembled in Illustrator (Adobe).

RESULTS

Differential Expression of Cyclin D1 in MCF10A Cells before and after Acquisition of a Mesenchymal Phenotype—Our previous studies in MCF10A cells and fibroblasts indicated that the timing of cyclin D1 gene induction might be cell type-specific with an early-G1 phase induction in epithelial cells and a mid-G1 phase induction in mesenchymal cells (3, 4, 7). To address this issue without using distinct cell lines, we induced a mesenchymal phenotype in MCF10A mammary epithelial cells (MCF10A cells) by a 3-day treatment with TGF-β.

TGF-β efficiently induced a mesenchymal phenotype as assessed by loss of cell-cell contacts, appearance of actin stress fibers, and loss of membrane-localized E-cadherin (Fig. 1A). Consistent with previous reports (22, 24, 25), the transcription factors snail and slug, as well as their downstream targets, fibronectin (FN) and vimentin (vim), were also induced (Fig. 1B). Thus, this system allows us to explore cell type-specific signaling to cyclin D1 mRNA without use of overexpression or cells of different origins.

We serum-starved control and TGF-β-treated MCF10A cells and monitored the expression of cyclin D1 mRNA during a 12-h period (the time when cyclin D1 appears in fibroblastic cells). Interestingly, the early-G1 phase induction of cyclin D1 mRNA seen in control MCF10A cells (Fig. 2A, Control, 3 h) was lost after TGF-β treatment: cyclin D1 mRNA was now induced...
in mid-G1 phase (Fig. 2A; TGF-β, 9–12 h) as it is in fibroblasts. A similar shift in the kinetics of cyclin D1 protein expression was seen before and after TGF-β treatment (Fig. 2B). When control cells were treated with TGF-β for 12 h (which was an insufficient time to induce the mesenchymal phenotype; not shown), the early-G1 phase induction of cyclin D1 was retained (Fig. 2C). Thus, the change in cyclin D1 expression kinetics is associated with the transition to a mesenchymal phenotype rather than simple exposure to TGF-β.

Loss of E-cadherin and the appearance of actin stress fibers are two hallmarks of an epithelial-mesenchymal transition (22, 24, 25). To determine if the change in cyclin D1 expression kinetics in TGF-β-treated MCF10A cells was due to loss of E-cadherin, we transfected control MCF10A cells with E-cadherin siRNA. E-cadherin depletion led to the loss of cell-cell adhesions (Fig. 3A), and consistent with our previous report showing that E-cadherin-mediated adhesion contributes to cyclin D1 mRNA expression (4), MCF10A cells treated with E-cadherin siRNA had lower levels of cyclin D1 (Fig. 3C). However, E-cadherin knock-down was not sufficient to induce mesenchymal markers (Fig. 3B), nor did it generate mesenchymal expression kinetics of cyclin D1: near constitutive expression persisted between 3 and 9 h after mitogenic stimulation (Fig. 3C).

Cyclin D1 Gene Expression in MCF10A Cells Occurs in Two Distinct Phases with Differing Requirements for Cytoskeletal Integrity—We then tested the importance of actin polymerization and intracellular tension on early- and mid-G1 phase cyclin D1 gene expression by treating control and transitioned MCF10A cells with CCD. CCD treatment blocked mid-G1 phase cyclin D1 mRNA expression both before and after TGF-β treatment (Fig. 4A, 9 h). In contrast, the early G1 phase induction of cyclin D1 seen in control MCF10A cells was relatively resistant to CCD (Fig. 4A, 3 h).

Because our previous studies indicated that early-G1 phase cyclin D1 gene expression is mediated by Rac and repressed by Rho in fibroblasts (3), it was possible that our ability to detect early-G1 phase cyclin D1 mRNA in control MCF10A cells was due to an increased level of Rac signaling or a reduced level of Rho signaling relative to that found after TGF-β treatment. This potential mechanism would also be consistent with the increase in actin stress fibers seen after TGF-β treatment. However, we found that Rac and Rho GTP levels were similar in control and TGF-β-treated MCF10A cells (Fig. 4, B and C, respectively). Collectively, these data indicate that there are two distinct phases of cyclin D1 expression in MCF10A cells (early- and mid-G1 phase), which are resolvable on the basis of their requirements for an organized actin cytoskeleton. If changes in
Rac and Rho signaling differentially regulate these two phases, these changes must be imposed downstream of GTP loading.

The Two Phases of Cyclin D1 Gene Expression Require Rac Activity and Are Distinguished by Their Requirement for ERK Activity—We asked whether ERK activity is required for cyclin D1 induction in MCF10A cells both before and after transition to a mesenchymal phenotype by incubating control and TGF-β-treated MCF10A cells with the MEK inhibitor, U0126. U0126 either had little effect (not shown) or increased (Fig. 5A, 3 h) early-G1 phase cyclin D1 expression in MCF10A cells. In contrast, U0126 strongly inhibited cyclin D1 mRNA induction in mid-G1 phase in MCF10A cells both before (Fig. 5A, 12 h) and after (Fig. 5B, 12 h) TGF-β treatment. Intermediate time points showed an intermediate dependence on ERK activity (Fig. 5A, 9 h), likely reflecting the transition from ERK-independent to ERK-dependent cyclin D1 gene expression.

As opposed to ERK activity, we found that inhibition of Rac using either a dominant negative mutant (N17) or Rac1 siRNA efficiently blocked both the early (3 h) and mid (9–12 h) G1 phase cyclin D1 mRNA expression (Fig. 6, A and B). The Rac requirement was retained in MCF10A cells that had undergone TGF-β treatment (Fig. 6A). Thus, the early- and mid-G1 phase expression of cyclin D1 are both dependent on Rac but can be distinguished by their requirement for ERK activity.

We considered the possibility that Rac was required for cyclin D1 gene expression through an interaction with the ERK pathway mediated by the stimulatory effect of PAK on Raf and/or MEK (26). However, Western blotting with antibodies specific to dually phosphorylated (activated) ERK showed that Rac inhibition attenuated the expression of cyclin D1 mRNA without affecting ERK activity in control MCF10A cells (Fig. 6C and Ref. 4).

One previous study concluded that Rac activity is necessary for the translocation of ERK from the cytoplasm to the nucleus.
Joint Regulation of Cyclin D1 by Rac and ERK

In this scenario, Rac inhibition would prevent the mid-G1 phase induction of cyclin D1 mRNA by blocking ERK-dependent transcription. To examine this possibility, we asked whether Rac was required for the induction of Fra-1, an immediately-early gene that is strongly regulated by ERK activity and linked to the expression of cyclin D1 mRNA (8, 15). Consistent with previous reports (15), the induction of Fra-1 in MCF10A cells was strongly blocked by MEK/ERK inhibition with U0126 (Fig. 6D) but not by the knock-down of Rac1 (Fig. 6E). Thus, Rac and ERK signal through parallel pathways to stimulate the mid-G1 phase induction of cyclin D1 mRNA.

This joint Rac/ERK requirement for mid-G1 phase cyclin D1 appears to be a conserved feature of cells. Inhibition of either Rac or ERK prevented the mid-G1 phase induction of cyclin D1 mRNA in primary mouse vascular smooth muscle cells (Fig. 7A) and MEFs (not shown). As seen in MCF10A cells, Rac knock-down affected neither ERK activation (Fig. 7B) nor ERK-dependent Fra-1 gene induction (Fig. 7, C and D) in mouse vascular smooth muscle cells.

Rb Phosphorylation and S Phase Entry Linked to Co-regulation of Cyclin D1 Gene Expression by Rac and ERK—Inhibition of ERK activity with U0126 efficiently blocked S phase entry in MCF10A cells (Fig. 8A, NS) despite the fact that Rac-dependent cyclin D1 expression persists for 9 h (refer to Fig. 5A). This result implies either that the effects of ERK activity extend beyond cyclin D1 or that continued expression of cyclin D1 is required for mitogen-dependent S phase entry. Other studies have indicated that ERK activity can regulate the cip/kip family of cdk inhibitors (20, 28), and indeed we found that U0126 strongly increased p21cip1 (but not p27kip1) levels (Fig. 8B). While the suppressive effect of ERK activity on p21 levels likely contributes to G1 phase arrest in U0126-treated cells, S phase entry was effectively blocked by U0126 even when p21cip1 was knocked-down with siRNA (Fig. 8A). Consistent with this data, we found that despite expressing cyclin D1 in early-G1 phase, Rb was not phosphorylated on cyclin D1-cdk4/6 sites in MCF10As until ~12–15 h after mitogenic stimulation (Fig. 8C), the time at which cyclin D1 gene expression is strongly dependent on ERK activity (Fig. 5A).

These results indicate that joint Rac/ERK-dependent expression of cyclin D1 throughout mid-G1 phase is required for Rb phosphorylation and S phase entry.

DISCUSSION
Our previous studies have shown that cyclin D1 gene expression in mammary epithelial cells is regulated by an endogenous Rac signaling pathway (4), whereas most cell types rely on sustained ERK activity to induce cyclin D1 mRNA (29). We now reconcile these two results by showing that Rac-dependent, G1 phase cyclin D1 gene expression in mammary epithelial cells can be resolved into two phases on the basis of their requirements for an organized actin cytoskeleton and ERK activity. Early-G1 phase expression of cyclin D1 in MCF10A cells proceeds in the absence of an organized actin cytoskeleton, is independent of ERK activity, and is insufficient to support Rb phosphorylation or S phase entry (Fig. 9). This phase is either absent or repressed in mesenchymal cells such as fibroblasts (3), smooth muscle, or MCF10A cells after 3 days of exposure to TGF-β. In contrast, mid-G1 phase expression of cyclin D1 mRNA is jointly regulated by Rac and ERK activities, and this co-regulation is conserved among these cell types (Fig. 9). We did not observe this joint requirement in our previous studies on cyclin D1 gene expression using α5-overexpressing NIH-3T3 cells (3), mostly likely because the overexpression of α5β1 integrin in those cells (30) may have overcome the need for co-regulation of cyclin D1 mRNA by Rac and ERK.

The two phases of cyclin D1 expression (early- and mid-G1 phase) in MCF10A cells are reminiscent of the two phases of
ERK activity seen during G1 phase progression. In both cases, the early-G1 phase (also referred to as “transient activity” for ERK) component is dispensable for S phase entry. Moreover, early-G1 phase ERK activity is dispensable for cyclin D1 gene expression, and early-G1 phase cyclin D1 expression is dispensable for Rb phosphorylation. In fact, the timing of Rb phosphorylation in MCF10A cells is similar to that seen in mesenchymal cells, which do not express cyclin D1 until mid-G1 phase (2, 3).

One of the ways we approached the issue of cell type-specific signaling was to exploit the finding that long-term exposure to TGF-β allows MCF10A mammary epithelial cells to acquire a mesenchymal-like phenotype (22, 24, 25). Control MCF10A cells have the characteristic epithelial cobblestone appearance at confluence which is the result, at least in part, of robust cell-cell adhesions mediated by E-cadherin. Following TGF-β treatment, surface E-cadherin is lost, the cells dissociate, and the resulting single cells acquire mesenchymal features such as fibronectin and vimentin expression and prominent actin stress fibers. We found that post-TGF-β treatment, the timing of cyclin D1 expression was delayed to mid-G1 phase just as we have seen in fibroblasts and smooth muscle cells (3, 31). This cell type-specific change in cyclin D1 kinetics was not associated with changes in the activity profiles of Rac or Rho, but was associated with appearance of actin stress fibers as typical for mesenchymal cells in culture.
Joint Regulation of Cyclin D1 by Rac and ERK

FIGURE 9. Integration of Rac and ERK signaling to cyclin D1 mRNA throughout G1 phase. A working model shows the distinct requirements for Rac and ERK activities and F-actin for the induction of cyclin D1 mRNA, Rb phosphorylation, and S phase entry. The link between F-actin and ERK activity has been previously described (3).

Both E-cadherin and integrins can support Rac signaling to cyclin D1 (4). The results described here indicate that the early-G1 phase cyclin D1 expression seen in MCF10A cells is due, at least in part, to E-cadherin, because that phase of cyclin D1 expression is lost coincident with down-regulation of E-cadherin during TGF-β treatment. The absence of strong cadherin-mediated adhesion likely cooperates with Rho-mediated repression (3) to ensure efficient suppression of early-G1 phase cyclin D1 gene expression in mesenchymal cells. We do not yet know if the Rac-dependent signaling pathway(s) that leads to early-G1 phase cyclin D1 mRNA and mid-G1 phase cyclin D1 gene expression are the same or different. Evidence that they are different includes the observation that Rac-dependent induction of cyclin D1 in early-G1 phase requires concomitant signaling by NF-κB, whereas its induction of cyclin D1 in mid-G1 phase is independent of NF-κB (17).

Collectively, our data indicate that Rac and ERK are mutually co-dependent for functionally productive expression of cyclin D1 mRNA and mitogenesis. As a conserved feature of epithelial and mesenchymal cells, the transcriptional mechanism underlying this co-regulation will be an important matter for further study, and its elucidation should provide interesting insights into extracellular control of the cell cycle.

REFERENCES

1. Blagosklonny, M. V., and Pardee, A. B. (2002) Cell Cycle 1, 103–110
2. Bohmer, R. M., Scharf, E., and Assoian, R. K. (1996) Mol. Biol. Cell 7, 101–111
3. Welsh, C. F., Roovers, K., Villanueva, J., Liu, Y., Schwartz, M. A., and Assoian, R. K. (2001) Nat. Cell Biol. 3, 950–957
4. Fournier, A. K., Campbell, L. E., Castagnino, P., Liu, W. F., Chung, B. M., Weaver, V. M., Chen, C. S., and Assoian, R. K. (2008) J. Cell Sci. 121, 226–233
5. Assoian, R. K., and Schwartz, M. A. (2001) Curr. Opin. Genet. Dev. 11, 48–53
6. Braga, V. (2000) Exp. Cell Res. 261, 83–90
7. Villanueva, J., Yung, Y., Walker, J. L., and Assoian, R. K. (2007) Mol. Biol. Cell 18, 1457–1463
8. Balmanno, K., and Cook, S. J. (1999) Oncogene 18, 3085–3097
9. Weber, J. D., Raben, D. M., Phillips, P. J., and Baldassare, J. J. (1997) Biochem. J. 326, 61–68
10. Page, K., Li, J., and Hershenson, M. B. (1999) Am. J. Respir. Cell Mol. Biol. 20, 1294–1302
11. Ramakrishnan, M., Musa, N. L., Li, J., Liu, P. T., Pestell, R. G., and Hershenson, M. B. (1998) Am. J. Respir. Cell Mol. Biol. 18, 736–740
12. Talarmin, H., Rescan, C., Cariou, S., Glaise, D., Zanninelli, G., Baffet, G., Moyer, P., Guiguen-Guillouzo, C., and Baffet, G. (1999) Mol. Cell. Biol. 19, 6003–6011
13. Watanabe, G., Lee, R. J., Albanese, C., Rainey, W. E., Batlle, D., and Pestell, R. G. (1996) J. Biol. Chem. 271, 22570–22577
14. Aplin, A. E., Howe, A., Alahari, S. K., and Juliano, R. L. (1998) Pharmacol. Rev. 50, 197–263
15. Cook, S. J., Aziz, N., and McMahon, M. (1999) Mol. Cell. Biol. 19, 330–341
16. Joyce, D., Bouzahzah, B., Fu, M., Albanese, C., D’Amico, M., Steer, J., Klein, J. U., Lee, R. J., Segall, J. E., Westwick, J. K., Der, C. J., and Pestell, R. G. (1999) J. Biol. Chem. 274, 25245–25249
17. Klein, E. A., Yang, C., Kazanietz, M. G., and Assoian, R. K. (2007) Cell Cycle 6, 1115–1121
18. Page, K., Li, J., Hodge, J. A., Liu, P. T., Vanden Hoek, T. L., Becker, L. B., Pestell, R. G., Rosner, M. R., and Hershenson, M. B. (1999) J. Biol. Chem. 274, 22065–22071
19. Westwick, J. K., Lambert, Q. T., Clark, G. J., Symons, M., Van Aelst, L., Pestell, R. G., and Der, C. J. (1997) Mol. Cell. Biol. 17, 1324–1335
20. Yang, C., Klein, E. A., Assoian, R. K., and Kazanietz, M. G. (2008) Biochem. J. 410, 167–175
21. Del Pozo, M. A., Kiosses, W. B., Alderson, N. B., Meller, N., Hahn, K. M., and Schwartz, M. A. (2002) Nat. Cell Biol. 4, 232–239
22. Maeda, M., Johnson, K. R., and Wheelock, M. J. (2005) J. Cell Sci. 118, 873–887
23. Klein, E. A., Yung, Y., Castagnino, P., Kothapalli, D., and Assoian, R. K. (2007) Methods Enzymol. 426, 155–175
24. Peinado, H., Quintanilla, M., and Cano, A. (2003) J. Biol. Chem. 278, 21113–21123
25. Zavadil, J., and Bottinger, E. P. (2005) Oncogene 24, 5764–5774
26. Frost, J. A., Steen, H., Shapiao, P., Lewis, T., Ahn, N., Shaw, P. E., and Cobb, M. H. (1997) EMBO J. 16, 6426–6438
27. Hirsh, E., Barberis, L., Brancaccio, M., Azzolinno, O., Xu, D., Kyriakis, J. M., Silengo, L., Giancotti, F. G., Tarone, G., Fassler, R., and Altruda, F. (2002) J. Cell Biol. 157, 481–492
28. Neve, R. M., Holbro, T., and Hynes, N. E. (2002) Oncogene 21, 4567–4576
29. Roovers, K., and Assoian, R. K. (2000) Bioessays 22, 818–826
30. Roovers, K., Davey, G., Zhu, X., Bottazzi, M. E., and Assoian, R. K. (1999) Mol. Biol. Cell 10, 3197–3204
31. Kothapalli, D., Zhao, L., Hawthorne, E. A., Cheng, Y., Lee, E., Pure, E., and Assoian, R. K. (2007) J. Biol. Chem. 176, 535–544