FEBS Letters

FEBS PRESS science publishing by scientists

Fibroblast growth factor (Fgf) 23 gene transcription depends on actin cytoskeleton reorganization

Abul Fajol[1], Sabina Honisch[1], Bingbing Zhang[1], Sebastian Schmidt[1], Saad Alkahtani[2,3], Saud Alarifi[2,3], Florian Lang[1,#], Christos Stournaras[1,3,#] and Michael Föller[4,#]

1 Department of Physiology, University of Tübingen, Germany
2 Department of Zoology, Science College, King Saud University, Riyadh, Saudi Arabia
3 Department of Biochemistry, University of Crete Medical School, Heraklion, Greece
4 Institute of Agricultural and Nutritional Sciences, Martin-Luther University Halle-Wittenberg, Halle (Saale), Germany

Correspondence
M. Föller, Institute of Agricultural and Nutritional Sciences, Martin-Luther University Halle-Wittenberg, Von-Danckelmann-Platz 2, D-06120 Halle (Saale), Germany
Fax: +49 345 55 27 124
Tel: +49 345 55 22702
E-mail: michael.foeller@landw.uni-halle.de

[#]Contributed equally, thus share last authorship.

(Received 18 November 2015, revised 27 January 2016, accepted 11 February 2016)

doi:10.1002/1873-3468.12096

Edited by Lukas Alfons Huber

FGF23 regulates renal phosphate and vitamin D metabolism. Loss of FGF23 results in massive calcification and rapid aging. FGF23 production is stimulated by $1,25(OH)_2D_3$ and NFκB signaling. Here, we report that treatment of UMR106 osteoblast-like cells with $1,25(OH)_2D_3$, inducing Fgf23 transcription, resulted in actin polymerization which was blocked by NFκB inhibitor wogonin. Interestingly, $1,25(OH)_2D_3$-induced Fgf23 gene transcription was abolished by the actin microfilament-disrupting agent cytochalasin B, as well as by the inhibition of actin-regulating Rac1/PAK1 signaling. Our results provide strong evidence that actin redistribution regulated by the Rac1/PAK1 pathway participates in $1,25(OH)_2D_3$ -induced Fgf23 gene transcription.

Keywords: actin polymerization; cytochalasin B; fibroblast growth factor 23; PAK1; Rac1; Vitamin D

Fibroblast growth factor (FGF) 23 is a strong regulator of phosphate and vitamin D homeostasis [1–3]. It is mainly produced by bone osteoblasts and exerts major effects in the kidney: FGF23 inhibits phosphate transport in the proximal tubule as well as renal 25-hydroxyvitamin D 1α-hydroxylase (Cyp27b1) [1] and stimulates 25-hydroxyvitamin D 24-hydroxylase (Cyp24a1) [1]. The consequence is a decrease in the plasma concentration of phosphate and $1,25(OH)_2D_3$, the biologically active form of vitamin D. To mediate these renal effects, FGF23 requires αKlotho as a co-receptor [4,5].

In addition, FGF23 is a powerful counteracter of aging. Mice deficient for FGF23 or for αKlotho suffer from rapid aging with a life span of a few weeks only and numerous age-related diseases [5,6]. The rapid aging is directly or indirectly dependent on the hyperphosphatemia of the mice as feeding them a low-phosphate or low-vitamin D diet greatly expands their life span [7].

In contrast, excess FGF23 production due to FGF23-synthesizing tumors in men results in osteomalacia [8]. The regulation of FGF23 formation by bone cells has remained ill-defined. Among the known triggers of FGF23 expression are parathyroid hormone (PTH) [9–11], $1,25(OH)_2D_3$ [12,13] and increased dietary phosphorus intake [14,15]. Mutations of the dentin

Abbreviations
$1,25(OH)_2D_3$, calcitriol; Fgf, fibroblast growth factor; NFκB, nuclear factor kappa B; PI3, phosphoinositide-3; PTH, parathyroid hormone; PVDF, polyvinylidene fluoride; TNF, tumor necrosis factor; VDR, vitamin D receptor.

matrix protein (Dmp1) or PHEX gene result in excessive FGF23 synthesis in men, and Dmp1 has hence been shown to be a negative regulator of FGF23 production [16–21]. Other regulators are the sympathetic nervous system [22] and iron [23–27].

Early and/or late actin restructuring, following modifications of actin polymerization dynamics equilibrium, is a cellular response initiated by various signals including growth factors and cytokines [28–34], hormones [34–41], and ions [42,43]. Alterations of actin organization, in turn, govern crucial cellular functions and outcomes such as cell volume regulation [43–48], survival [31,49], migration and cell motility [50–53], secretion [54], cell growth and proliferation [55] as well as apoptosis [38,49,56,57]. Specific actin cytoskeleton signaling pathways have been reported to regulate both, early and late actin reorganization [58–62] and the resulting cellular outcomes.

$1,25(OH)_2D_3$ has been shown to initiate actin reorganization in various cell types including osteoblasts [63–66]. This effect was implicated in cellular responses, such as osteoblast maturation [67], organization of the mitotic spindle [64], or inhibition of cell proliferation [65]. Since FGF23 production is stimulated by $1,25(OH)_2D_3$ and NFκB signaling [12,68,69] and both, vitamin D [63] and NFκB signaling [31,39] are relevant for actin reorganization, we investigated the role of actin dynamics in Fgf23 gene regulation in osteoblast-like cells. Our results show that $1,25(OH)_2D_3$ as well as NFκB signaling induce a potent reorganization of actin cytoskeleton, an effect regulating Fgf23 gene transcription via Rac1/PAK1-dependent processes.

Materials and methods

Cell culture

UMR106 rat osteosarcoma cells were cultured in DMEM high glucose medium supplemented with 10% FCS and 1% penicillin/streptomycin under standard culture conditions. Cells were pretreated with 100 nM $1,25(OH)_2D_3$ (Sigma, Schnelldorf, Germany). After 42 h cells were in addition treated with 100 nM cytochalasin B (TOCRIS, Bristol, UK), 50 μM Rac1 inhibitor NSC23766 (TOCRIS), 10 μM IPA3 (TOCRIS), or with vehicle only for another 6 h and then harvested for qRT-PCR. For western blotting cells were treated with $1,25(OH)_2D_3$ for 15 min, 30 min, or 24 h. For some experiments, cells were incubated with or without 100 μM NFκB inhibitor wogonin (Enzo, Lörrach, Germany) for 24 h and in addition treated with 100 nM $1,25(OH)_2D_3$ or with vehicle for another 15 min.

Quantification of mRNA expression

Total RNA was isolated from the cells using Trifast reagent (Peqlab, Erlangen, Germany). Messenger RNA was transcribed with SuperScriptIII Reverse Transcriptase (Invitrogen, Darmstadt, Germany) using random hexamers (Invitrogen). 2 μg of RNA was used for cDNA synthesis. For qRT-PCR analysis, the final volume of the qRT-PCR reaction mixture was 15 μL and contained: 1 μL cDNA, 1 μM of each primer, 7.5 μL GoTaq Master Mix (Promega, Mannheim, Germany), and sterile water up to 15 μL. PCR conditions were set to 95 °C for 3 min, followed by 40 cycles of 95 °C for 10 s, 58 °C for 30 s, and 72 °C for 45 s. Quantitative RT-PCR was performed on a BioRad iCycler iQTM Real-Time PCR Detection System (Bio-Rad Laboratories, München, Germany).

The following primers were used:

Rat Tbp (TATA box-binding protein):
forward (5′–3′): ACTCCTGCCACACCAGCC
reverse (5′–3′): GGTCAAGTTTACAGCCAAGATTCA
Rat Fgf23
forward (5′–3′): TGGCCATGTAGACGGAACAC
reverse (5′–3′): GGCCCCTATTATCACTACGGAG

Calculated mRNA expression levels were referred to the expression levels of Tbp of the same cDNA sample. All qRT-PCRs were performed in duplicate. Relative quantification of gene expression was expressed as $2^{Ct(Tbp)-Ct(Fgf23)}$.

Measurement of F/G-actin ratio by triton X-fractionation

For measurement of monomeric (Triton soluble) and polymerized (Triton insoluble) actin, UMR106 cells were incubated for different periods with or without $1,25(OH)_2D_3$, wogonin, Rac1 inhibitor NSC 23766, or PAK1 inhibitor IPA3 as indicated. Then, cells were harvested. Actin cytoskeleton determination by Triton X-100 was performed as described previously [70] with minor modifications. Briefly, cells were incubated with 130 μL of Triton extraction buffer containing 0.3% TritonX-100, 5 mM Tris, pH 7.4, 2 mM EGTA, 300 mM sucrose, 2 μM phalloidin, 1 mM PMSF, 10 $\mu g \cdot mL^{-1}$ leupeptin, 20 $\mu g \cdot mL^{-1}$ aprotinin, 1 mM sodium orthovanadate, and 50 mM NaF for 5–7 min on ice. The supernatant (G-actin) containing soluble protein was removed. The Triton insoluble fraction remaining on the culture plate was scraped and lyzed with RIPA buffer (Cell Signaling, Frankfurt, Germany). An equal amount of protein of each fraction was subjected to 10% SDS/PAGE. The proteins were transferred onto PVDF membranes which were then blocked with 5% nonfat dry milk powder in TBS-T for 1 h at room temperature. Next, the membrane was incubated overnight at 4 °C with pan-actin primary antibody (1 : 1000 in 5% BSA with TBS-T; Cell Signaling) and washed 3–5 times with TBS-T. Then,

incubation with secondary anti-rabbit horseradish peroxidase-conjugated antibody was carried out for 1 h at room temperature (1 : 2000; Cell Signaling). Blots were developed by the ECL reagent (Amersham, Freiburg, Germany) and band intensities were quantified by CHEMIDOC QUANTITY one software (Bio-Rad).

Immunofluorescence

For fluorescence staining of actin, vitamin D receptor (VDR), and nuclei, UMR106 cells were cultured on glass chamber slides (BD biosciences, Heidelberg, Germany) for 24 h and treated with or without $1,25(OH)_2D_3$ (100 nM) and/or with cytochalasin B (500 nM; Sigma-Aldrich) as indicated in the figure legends. After washing twice with PBS, cells were fixed with 4% PFA for 15 min at room temperature, permeabilized with 0.1% *Triton*-X100 for 10 min and blocked with 3% BSA in PBST. For actin staining, the cells were incubated with rhodamine-phalloidin (1 : 200; Life Technologies, Darmstadt, Germany) and with DRAQ-5 dye (1 : 3000; Biostatus, Leicestershire, UK) for 30 min in the dark.

For another experiment, cells were incubated with anti-VDR primary antibody (1 : 100; Santa Cruz Biotechnology, Santa Cruz, CA, USA) at 4 °C overnight. Then, the cells were washed three times with PBS and incubated with CF™ 488A-labeled anti-rabbit secondary antibody (1 : 200, Sigma-Aldrich) and with DRAQ-5 dye (1 : 3000; Biostatus) for 1 h at room temperature.

All slides were washed with PBS and mounted with ProLong Gold antifade reagent (Life Technologies). Images were taken on a Zeiss LSM 5 EXCITER confocal laser scanning microscope (Carl Zeiss, Göttingen, Germany) with a water immersion Plan-Neofluar 40/1.3 NA DIC.

Statistics

Data are provided as means ± SEM, n represents the number of independent experiments. All data were tested for significance using Student's t-test or ANOVA. Only results with $P < 0.05$ were considered statistically significant.

Results

Our study addressed the role of the organization of the actin cytoskeleton in Fgf23 transcription in UMR106 osteoblast-like cells. First, we exposed the cells to $1,25(OH)_2D_3$, a well-characterized inducer of FGF23 formation, and determined the changes in the actin polymerization dynamics by western blotting. Figure 1A,B illustrates that treatment with $1,25(OH)_2D_3$ resulted in a polymerization of the actin cytoskeleton in UMR106 cells as apparent from an increase in the F- over G-actin ratio. This effect was evident after 15 or 30 min (Fig. 1A) and could be observed even after 24 h (Fig. 1B), indicating an early but persistent effect of $1,25(OH)_2D_3$ on actin reorganization. Confocal laser scaning microscopy fully supported these findings, demonstrating a pronounced actin cytoskeleton restructuring with the formation of stress fibers (Fig. 2).

Recently, it has been shown that transcription factor NFκB is involved in the signaling leading to the

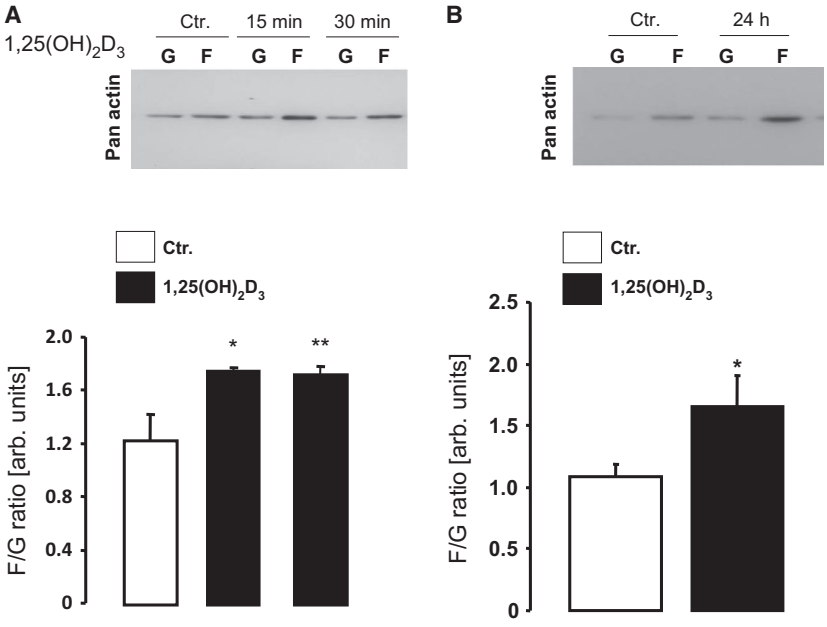

Fig. 1. $1,25(OH)_2D_3$ induced polymerization of the actin cytoskeleton in UMR106 cells. (A) Original western blot demonstrating G-actin and F-actin abundance in UMR106 cells left untreated (Ctr.) or treated for 15 or 30 min with $1,25(OH)_2D_3$ (100 nM). Lower panel: Bars showing arithmetic means ± SEM of the ratio of filamentous (F) over soluble (G) actin in UMR106 cells ($n = 6$, $^*P < 0.05$, $^{**}P < 0.01$). (B) Original western blot demonstrating G-actin and F-actin abundance in UMR106 cells left untreated (Ctr.) or treated for 24 h with $1,25(OH)_2D_3$ (100 nM). Lower panel: Bars showing arithmetic means ± SEM of the ratio of filamentous (F) over soluble (G) actin in UMR106 cells ($n = 15$ $^*P < 0.05$).

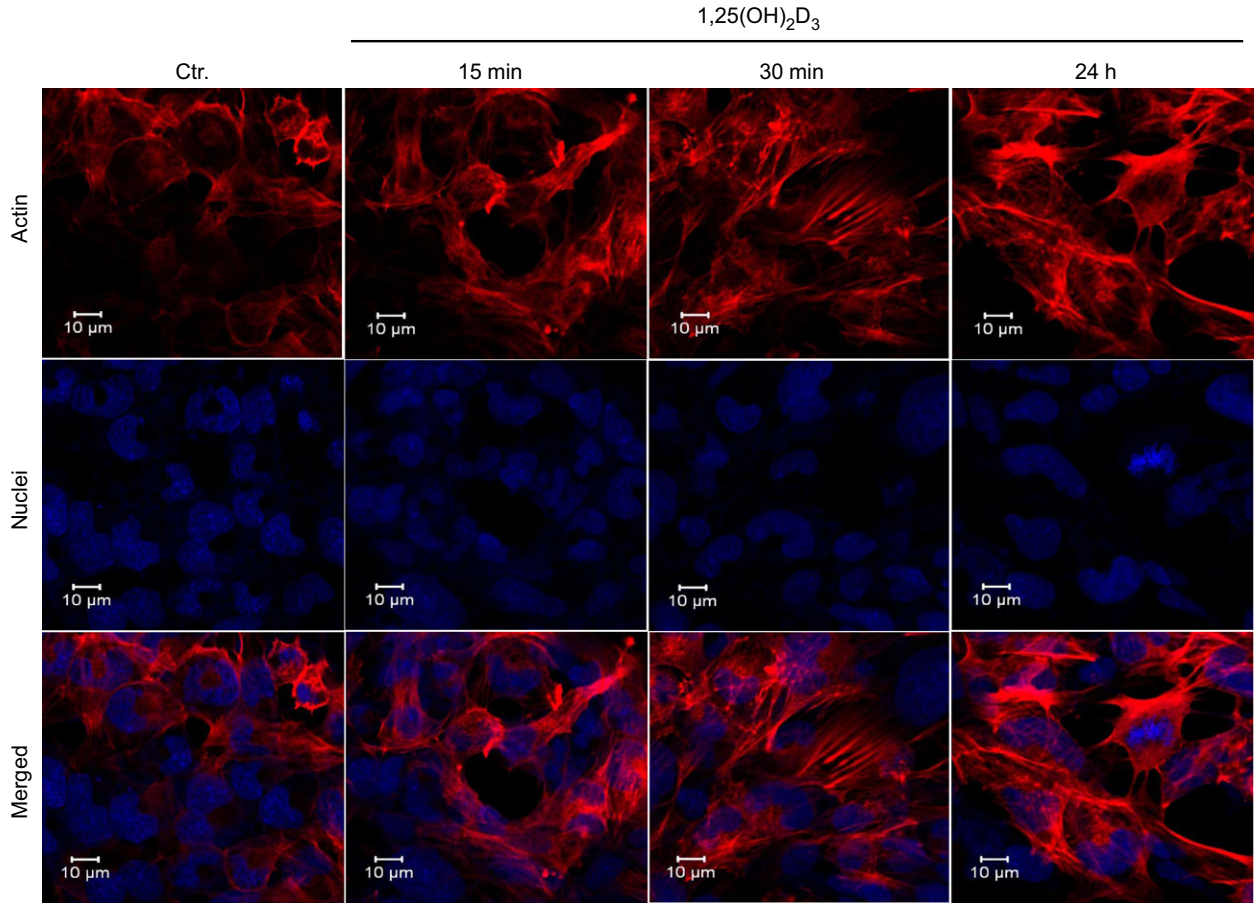

Fig. 2. Confocal microscopy of $1,25(OH)_2D_3$ -induced actin stress fiber formation in UMR106 cells. Confocal microscopy images demonstrating actin staining (upper images), nucleus staining (middle images), or the merged staining (lower images) in UMR106 cells left untreated or treated with $1,25(OH)_2D_3$ (100 nM) for 15 min, 30 min, or 24 h.

expression of FGF23 in bone cells [68]. Moreover, NFκB signaling is implicated in TNFα- [31], steroid hormones- [38] and IL8- [71] induced actin reorganization in various cells. Therefore, we studied whether the suppression of Fgf23 gene expression by NFκB inhibition also affects the actin cytoskeleton. As shown in Fig. 3, NFκB inhibitor wogonin totally blocked the polymerization of the actin cytoskeleton induced by $1,25(OH)_2D_3$.

Likewise, we studied whether the early but persistent $1,25(OH)_2D_3$-induced actin restructuring also impacts on Fgf23 transcription in UMR106 cells. As shown in Fig. 4, $1,25(OH)_2D_3$ significantly up-regulated Fgf23 gene transcription, an effect largely inhibited by cytochalasin B, a potent blocker of actin polymerization.

This result implies a role of actin reorganization in the regulation of Fgf23 transcription. In an effort to identify the underlying mechanism, we first tried to characterize the role of Rac1-governed actin cytoskeleton organization for Fgf23 expression. We treated UMR106 cells with the Rac1 inhibitor NSC 23766 to block Rac1-induced actin reorganization and determined $1,25(OH)_2D_3$-induced Fgf23 transcription. As shown in Fig. 5A, Fgf23 transcripts were significantly reduced upon treatment of the cells with Rac1 inhibitor NSC 23766, further supporting that Rac1-governed actin restructuring may be directly implicated in $1,25(OH)_2D_3$-induced modulation of the Fgf23 expression profile.

Rac1 has been shown to trigger downstream PAK1, which in turn may as well control actin polymerization [57,70]. Thus, we further analyzed Fgf23 gene transcription in the presence of the specific PAK1 inhibitor IPA3. As illustrated in Fig. 5B, $1,25(OH)_2D_3$-induced Fgf23 transcription was fully blocked in the presence of IPA3, indicating a dominant role of PAK1 signaling in Fgf23 expression. Importantly, the Rac1 inhibitor NSC 23766 blocked

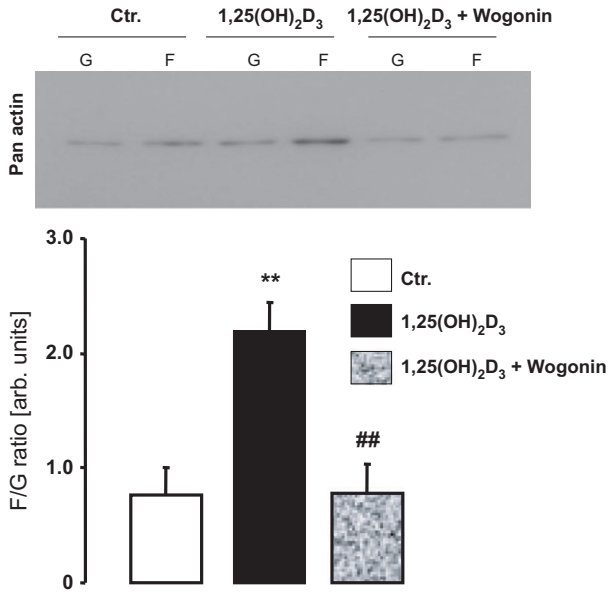

Fig. 3. 1,25(OH)$_2$D$_3$ -induced actin polymerization was abolished by NFκB inhibitor wogonin. Original western blot demonstrating G-actin and F-actin abundance in UMR106 cells left untreated (Ctr.) or treated for 15 min with 1,25(OH)$_2$D$_3$ (100 nM) in the presence or absence of NFκB inhibitor wogonin (100 μM). Lower panel: Bars showing arithmetic means ± SEM ($n = 6$) of the ratio of filamentous (F) over soluble (G) actin in UMR106 cells. $^{**}P < 0.01$. $^{\#\#}$Indicates significant difference from 1,25(OH)$_2$D$_3$ alone ($P < 0.01$).

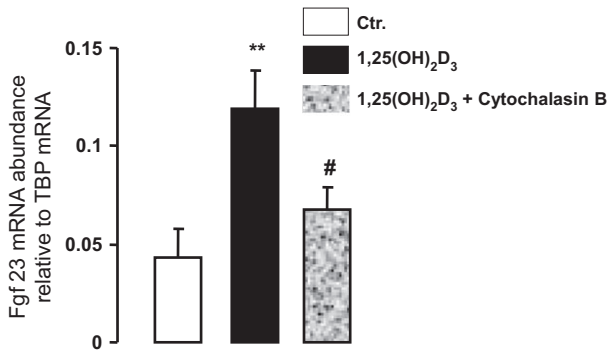

Fig. 4. Actin-depolymerizing agent cytochalasin B inhibited 1,25 (OH)$_2$D$_3$ –induced Fgf23 transcription. Arithmetic means ± SEM ($n = 6$) of the relative Fgf23 mRNA abundance in UMR106 cells following a 6 h treatment with vehicle alone or with 1,25(OH)$_2$D$_3$ (100 nM) without or with cytochalasin B (100 nM). $^{**}P < 0.01$. $^{\#}$Indicates significant difference from 1,25(OH)$_2$D$_3$ alone ($P < 0.05$).

as expected actin polymerization in UMR106 cells as well (Fig. 6), further supporting our conclusion that actin redistribution upon 1,25(OH)$_2$D$_3$ treatment governs Fgf23 gene transcription in these cells. However, PAK1 inhibitor IPA3 only moderately influenced actin polymerization in UMR106 cells (Fig. 6).

Possibly, blocking the actin cytoskeleton with cytochalasin B could impact on the expression and/or trafficking of the vitamin D receptor (VDR). To test this possibility, we analyzed the VDR localization in UMR106 cells treated with or without 1,25(OH)$_2$D$_3$ in the presence or absence of cytochalasin B by confocal microscopy. As shown in Fig. 7, enhanced VDR staining was obvious in 1,25(OH)$_2$D$_3$-treated cells for 24 h, an effect largely inhibited in the presence of the actin polymerization inhibitor cytochalasin B. This effect, however, was less evident at earlier time points.

Discussion

According to our study, the production of the hormone FGF23 was paralleled by polymerization of the actin cytoskeleton in UMR106 osteoblast-like cells. Moreover, reorganization of the actin network by actin polymerization is required for the production of FGF23 as blocking the actin polymerization with cytochalasin B stopped the transcription of the Fgf23 gene. Likewise, suppression of FGF23 production by inhibiting NFκB signaling with wogonin was paralleled by a blockade of actin network reorganization. Our study further provides novel mechanistic insight into the 1,25(OH)$_2$D$_3$-induced regulation of Fgf23 gene transcription by demonstrating the involvement of Rac1 and PAK1 signaling. Indeed, we demonstrate significant downregulation of Fgf23 expression up to control levels upon blocking Rac1 and PAK1 activities by specific inhibitors. Since the Rac1/PAK1 signaling pathway is a key regulatory mechanism of actin polymerization in various cell types [57,70,72,73], we conclude that the control of Fgf23 expression by 1,25(OH)$_2$D$_3$ is restricted to Rac1/PAK1-signaling via governing the 1,25(OH)$_2$D$_3$-induced actin cytoskeleton reorganization.

Actin cytoskeleton reorganization, induced by various signals and regulated by distinct signaling pathways, has been implicated in the control of gene transcription in recent studies [61,74]. Indeed, it has been reported that early initiation of actin polymerization by various signals may liberate G-actin-associated transcription cofactors, such as myocardin-related transcription factor (MRTF) [74–76]. This transcription factor may in turn stimulate transcriptional regulation of various genes (for a review see [74]). Similarly, previous reports link early actin reorganization by polymerization to the regulation of the RhoB-gene, facilitated by enhanced RhoB promoter activity [29,77], as well as the alpha Smooth Muscle Actin (alpha-SMA) gene [29,78] and various guanine nucleotide exchanger fac-

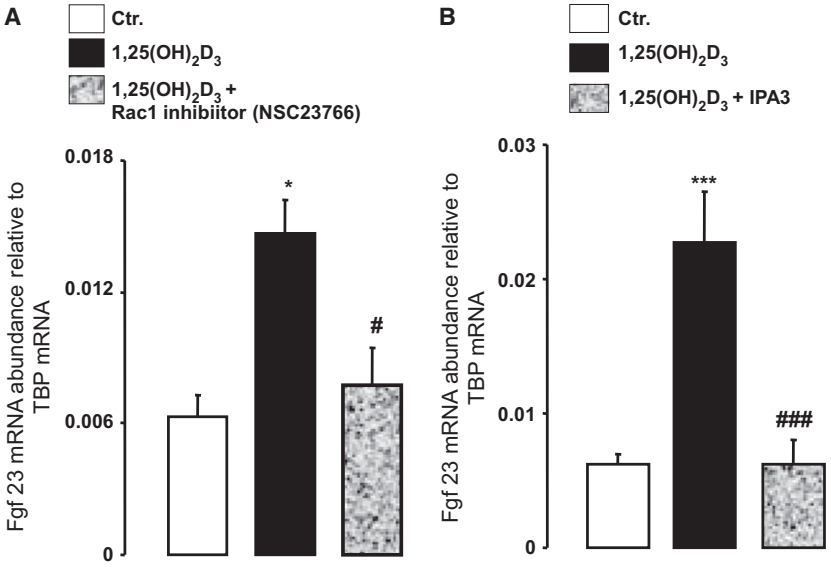

Fig. 5. Rac1 and PAK1 inhibitors blocked $1,25(OH)_2D_3$ –induced Fgf23 transcription. (A) Arithmetic means ± SEM ($n = 7$) of the relative Fgf23 mRNA abundance in UMR106 cells following a 6 h treatment with vehicle alone or with $1,25(OH)_2D_3$ (100 nM) without or with Rac1 inhibitor NSC 23766 (50 μM). (B) Arithmetic means ± SEM ($n = 6$) of the relative Fgf23 mRNA abundance in UMR106 cells following a 6 h treatment with vehicle alone or with $1,25(OH)_2D_3$ (100 nM) without or with PAK1 inhibitor IPA3 (10 μM). *,***$P < 0.05$, $P < 0.001$. #,### indicate significant difference from $1,25(OH)_2D_3$ alone ($P < 0.05$, $P < 0.001$).

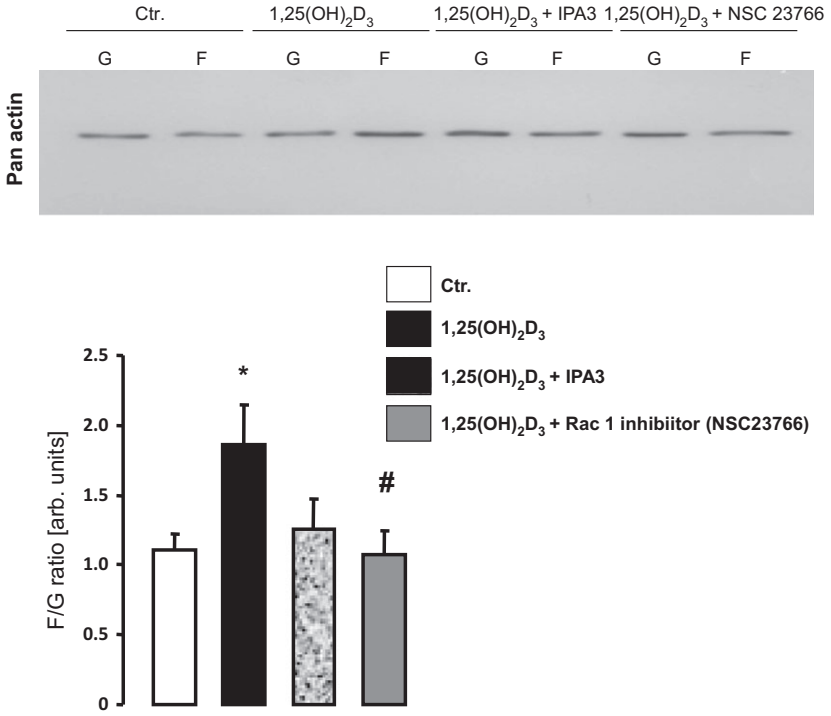

Fig. 6. $1,25(OH)_2D_3$ -induced actin polymerization was inhibited by Rac1 and PAK1 inhibitor. Original western blot demonstrating G-actin and F-actin abundance in UMR106 cells left untreated (Ctr.) or treated for 15 min with $1,25(OH)_2D_3$ (100 nM) without or with PAK1 inhibitor IPA3 (10 μM) or with Rac1 inhibitor NSC 23766 (50 μM). Lower panel: Bars showing arithmetic means ± SEM ($n = 6$) of the ratio of filamentous (F) over soluble (G) actin in UMR106 cells. *$P < 0.05$, #indicates significant difference from $1,25(OH)_2D_3$ alone ($P < 0.05$).

tors (GEF's), including Net1/Net1A [78]. These studies revealed that early actin reorganization may regulate the transcription of various genes encoding specific regulatory effectors [74,75,79] and may link early modifications of actin cytoskeleton dynamics to late cell responses controlled by gene activation (for reviews see [61,74]). Our results presented in this study provide further support for this hypothesis. Indeed, actin restructuring manifested by actin polymerization in 1,25 $(OH)_2D_3$-treated UMR106 osteoblast-like cells seems to control $1,25(OH)_2D_3$-induced Fgf23 gene transcription, since blockade of actin polymerization, either by the pharmacological inhibitor cytochalasin B, or by inhibition of specific actin signaling molecules that govern actin polymerization totally inhibited the transcription of the Fgf23 gene.

The Rac1 small GTPase is a major signaling effector of actin polymerization [80,81], acting mostly down-

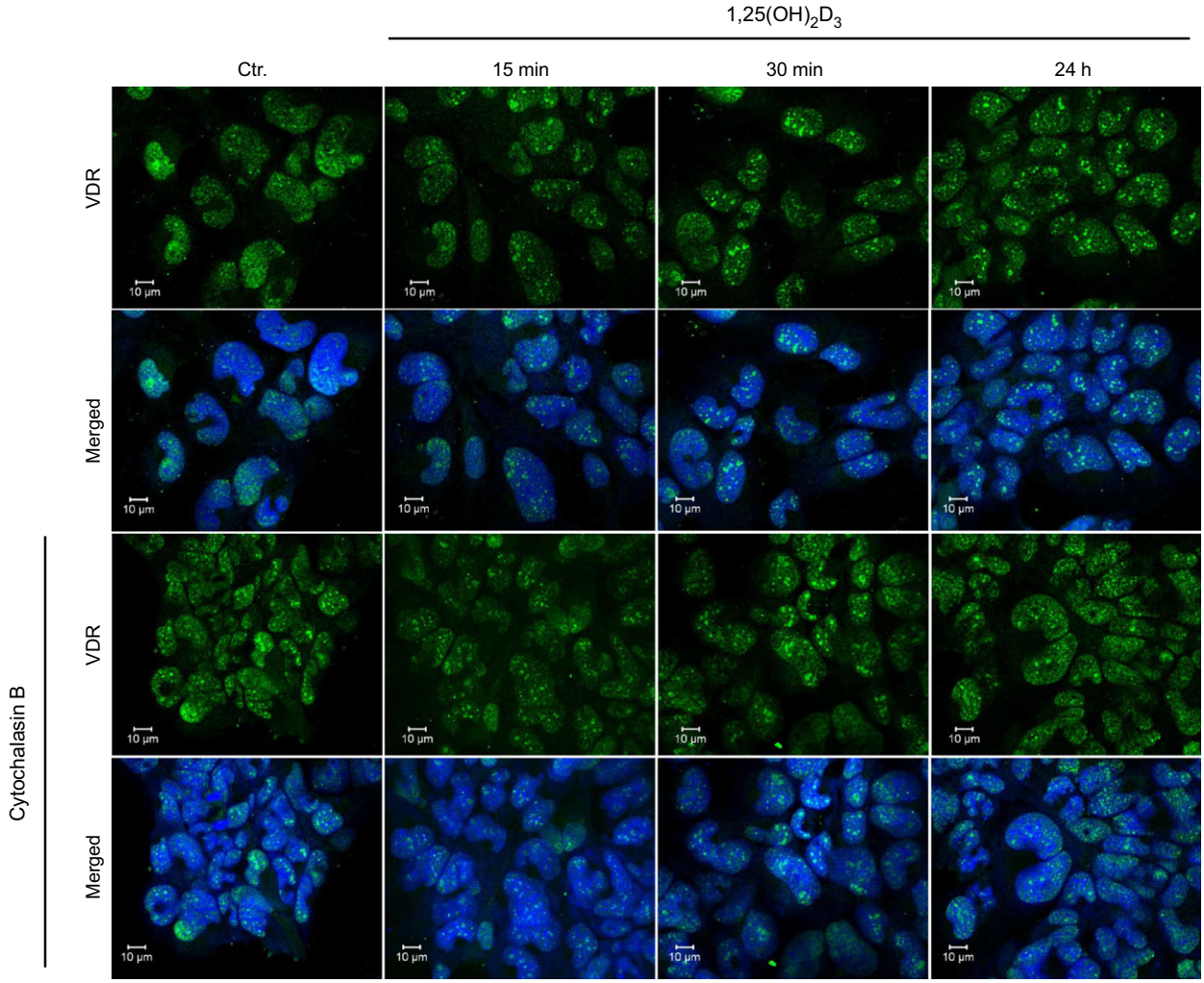

Fig. 7. Confocal microscopy of the cytochalasin B effect on the cellular localization of the vitamin D receptor (VDR). Confocal microscopy images demonstrating VDR staining or the combined staining of VDR and nuclei in UMR106 cells left untreated or treated with $1,25(OH)_2D_3$ (100 nM) for 15 min, 30 min, or 24 h in the absence (upper panels) or presence (lower panels) of cytochalasin B.

stream of the FAK/PI-3K signaling pathway. It may either directly stimulate actin polymerization, or it may interact with PAK1 that in turn is implicated in actin reorganization [70,81]. The data presented here provide strong evidence that Rac1 (and PAK1) are mainly involved in both, actin redistribution and Fgf23 gene transcription, supporting previous studies, which established an important role of Rac1 in osteoblastic cells [82]. Although at present, we cannot exclude that also other small Rho-GTPases, such as RhoA/B- signaling, may as well be involved, our findings strongly indicate that Rac1/PAK1-stimulated actin redistribution is required for the $1,25(OH)_2D_3$-induced production of FGF23 in UMR106 osteoblast-like cells. Furthermore, by controlling FGF23 formation through the regulation of the actin cytoskeleton, Rac1/PAK1 signaling may turn out to be a powerful regulator of renal phosphate and vitamin D metabolism. Further studies are needed to define its exact role. In addition, since suppression of FGF23 production by inhibiting NFκB signaling with wogonin resulted in a blockade of actin network reorganization, our findings further support previous observations postulating the involvement of NFκB signaling in actin cytoskeleton rearrangements [31,38,71]. In theory, trafficking of the VDR may be relevant for the effect of the reorganization of the actin cytoskeleton on Fgf23 transcription. Indeed, confocal microscopy showed an impact of cytochalasin B on the cellular VDR expression upon $1,25(OH)_2D_3$ treatment, implying a possible role of actin restructuring in VDR expression and relocalization, which may in turn regulate Fgf23 transcription. However, additional studies

are now needed to address in more detail the possible cross-talk between actin reorganization and VDR expression/translocation.

From the results reported in this study, we conclude that the $1,25(OH)_2D_3$-induced production of FGF23 in UMR106 osteoblast-like cells requires reorganization of the actin network a mechanism involving NFκB and Rac1/PAK1 signaling. These findings support the notion that actin cytoskeleton reorganization may be implicated in the control of Fgf23-gene transcription. Further studies are now needed to identify potential factors that may regulate the transcription of Fgf23 via modification of the cytoskeletal structure.

Acknowledgements

This study was supported by the Deutsche Forschungsgemeinschaft (Fo 695/1-1, Fo 695/1-2) and by the Deanship of Scientific Research at King Saud University, Riyadh Saudi Arabia (KSU-RGP-018 Program).

Author contributions

AF, SH, BZ, SS, SA, and SA performed experiments. FL, CS, and MF designed the study. AF, CS, and MF wrote the paper. AF, SH, BY, and MF analyzed the data.

References

1 Shimada T, Hasegawa H, Yamazaki Y, Muto T, Hino R, Takeuchi Y, Fujita T, Nakahara K, Fukumoto S and Yamashita T (2004) FGF-23 is a potent regulator of vitamin D metabolism and phosphate homeostasis. *J Bone Miner Res* **19**, 429–435.

2 Hori M, Shimizu Y and Fukumoto S (2011) Minireview: fibroblast growth factor 23 in phosphate homeostasis and bone metabolism. *Endocrinology* **152**, 4–10.

3 Marsell R and Jonsson KB (2010) The phosphate regulating hormone fibroblast growth factor-23. *Acta Physiol (Oxf)* **200**, 97–106.

4 Hu MC, Shiizaki K, Kuro-o M and Moe OW (2013) Fibroblast growth factor 23 and Klotho: physiology and pathophysiology of an endocrine network of mineral metabolism. *Annu Rev Physiol* **75**, 503–533.

5 Kuro-o M (2010) Overview of the FGF23-Klotho axis. *Pediatr Nephrol* **25**, 583–590.

6 Shimada T, Kakitani M, Yamazaki Y, Hasegawa H, Takeuchi Y, Fujita T, Fukumoto S, Tomizuka K and Yamashita T (2004) Targeted ablation of Fgf23 demonstrates an essential physiological role of FGF23 in phosphate and vitamin D metabolism. *J Clin Invest* **113**, 561–568.

7 Kuro-o M (2010) Klotho. *Pflugers Arch* **459**, 333–343.

8 de Jongh RT, Vervloet MG, Bravenboer N, Heijboer AC, den Heijer M and Lips P (2013) Chronic bone pain due to raised FGF23 production? The importance of determining phosphate levels. *Ned Tijdschr Geneeskd* **157**, A5908.

9 Lavi-Moshayoff V, Wasserman G, Meir T, Silver J and Naveh-Many T (2010) PTH increases FGF23 gene expression and mediates the high-FGF23 levels of experimental kidney failure: a bone parathyroid feedback loop. *Am J Physiol Renal Physiol* **299**, F882–F889.

10 López I, Rodríguez-Ortiz ME, Almadén Y, Guerrero F, de Oca AM, Pineda C, Shalhoub V, Rodríguez M and Aguilera-Tejero E (2011) Direct and indirect effects of parathyroid hormone on circulating levels of fibroblast growth factor 23 in vivo. *Kidney Int* **80**, 475–482.

11 Rhee Y, Bivi N, Farrow E, Lezcano V, Plotkin LI, White KE and Bellido T (2011) Parathyroid hormone receptor signaling in osteocytes increases the expression of fibroblast growth factor-23 in vitro and in vivo. *Bone* **49**, 636–643.

12 Masuyama R, Stockmans I, Torrekens S, Van Looveren R, Maes C, Carmeliet P, Bouillon R and Carmeliet G (2006) Vitamin D receptor in chondrocytes promotes osteoclastogenesis and regulates FGF23 production in osteoblasts. *J Clin Invest* **116**, 3150–3159.

13 Saini RK, Kaneko I, Jurutka PW, Forster R, Hsieh A, Hsieh JC, Haussler MR and Whitfield GK (2013) 1,25-dihydroxyvitamin D(3) regulation of fibroblast growth factor-23 expression in bone cells: evidence for primary and secondary mechanisms modulated by leptin and interleukin-6. *Calcif Tissue Int* **92**, 339–353.

14 Ferrari SL, Bonjour JP and Rizzoli R (2005) Fibroblast growth factor-23 relationship to dietary phosphate and renal phosphate handling in healthy young men. *J Clin Endocrinol Metab* **90**, 1519–1524.

15 Perwad F, Azam N, Zhang MY, Yamashita T, Tenenhouse HS and Portale AA (2005) Dietary and serum phosphorus regulate fibroblast growth factor 23 expression and 1,25-dihydroxyvitamin D metabolism in mice. *Endocrinology* **146**, 5358–5364.

16 Inoue Y, Segawa H, Kaneko I, Yamanaka S, Kusano K, Kawakami E, Furutani J, Ito M, Kuwahata M, Saito H *et al.* (2005) Role of the vitamin D receptor in FGF23 action on phosphate metabolism. *Biochem J* **390**, 325–331.

17 Martin A, Liu S, David V, Li H, Karydis A, Feng JQ and Quarles LD (2011) Bone proteins PHEX and DMP1 regulate fibroblastic growth factor Fgf23 expression in osteocytes through a common pathway involving FGF receptor (FGFR) signaling. *FASEB J* **25**, 2551–2562.

18 Quarles LD (2003) FGF23, PHEX, and MEPE regulation of phosphate homeostasis and skeletal mineralization. *Am J Physiol Endocrinol Metab* **285**, E1–E9.
19 Tenenhouse HS and Sabbagh Y (2002) Novel phosphate-regulating genes in the pathogenesis of renal phosphate wasting disorders. *Pflugers Arch* **444**, 317–326.
20 Consortium, A (2000) Autosomal dominant hypophosphataemic rickets is associated with mutations in FGF23. *Nat Genet* **26**, 345–348.
21 White KE, Jonsson KB, Carn G, Hampson G, Spector TD, Mannstadt M, Lorenz-Depiereux B, Miyauchi A, Yang IM, Ljunggren O *et al.* (2001) The autosomal dominant hypophosphatemic rickets (ADHR) gene is a secreted polypeptide overexpressed by tumors that cause phosphate wasting. *J Clin Endocrinol Metab* **86**, 497–500.
22 Kawai M, Kinoshita S, Shimba S, Ozono K and Michigami T (2014) Sympathetic activation induces skeletal Fgf23 expression in a circadian rhythm-dependent manner. *J Biol Chem* **289**, 1457–1466.
23 Takashi Y and Fukumoto S (2015) [Bone and Nutrition. The relationship between iron and phosphate metabolism]. *Clin Calcium* **25**, 1037–1042.
24 Clinkenbeard EL, Farrow EG, Summers LJ, Cass TA, Roberts JL, Bayt CA, Lahm T, Albrecht M, Allen MR, Peacock M *et al.* (2014) Neonatal iron deficiency causes abnormal phosphate metabolism by elevating FGF23 in normal and ADHR mice. *J Bone Miner Res* **29**, 361–369.
25 Wolf M, Koch TA and Bregman DB (2013) Effects of iron deficiency anemia and its treatment on fibroblast growth factor 23 and phosphate homeostasis in women. *J Bone Miner Res* **28**, 1793–1803.
26 Imel EA, Peacock M, Gray AK, Padgett LR, Hui SL and Econs MJ (2011) Iron modifies plasma FGF23 differently in autosomal dominant hypophosphatemic rickets and healthy humans. *J Clin Endocrinol Metab* **96**, 3541–3549.
27 Farrow EG, Yu X, Summers LJ, Davis SI, Fleet JC, Allen MR, Robling AG, Stayrook KR, Jideonwo V, Magers MJ *et al.* (2011) Iron deficiency drives an autosomal dominant hypophosphatemic rickets (ADHR) phenotype in fibroblast growth factor-23 (Fgf23) knock-in mice. *Proc Natl Acad Sci USA* **108**, E1146–E1155.
28 Vardouli L, Moustakas A and Stournaras C (2005) LIM-kinase 2 and cofilin phosphorylation mediate actin cytoskeleton reorganization induced by transforming growth factor-beta. *J Biol Chem* **280**, 11448–11457.
29 Vardouli L, Vasilaki E, Papadimitriou E, Kardassis D and Stournaras C (2008) A novel mechanism of TGFbeta-induced actin reorganization mediated by Smad proteins and Rho GTPases. *FEBS J* **275**, 4074–4087.
30 Moustakas A and Stournaras C (1999) Regulation of actin organisation by TGF-beta in H-ras-transformed fibroblasts. *J Cell Sci* **112** (Pt 8), 1169–1179.
31 Papakonstanti EA and Stournaras C (2004) Tumor necrosis factor-alpha promotes survival of opossum kidney cells via Cdc42-induced phospholipase C-gamma1 activation and actin filament redistribution. *Mol Biol Cell* **15**, 1273–1286.
32 Moustakas A and Heldin CH (2008) Dynamic control of TGF-beta signaling and its links to the cytoskeleton. *FEBS Lett* **582**, 2051–2065.
33 Konstantinidis G, Moustakas A and Stournaras C (2011) Regulation of myosin light chain function by BMP signaling controls actin cytoskeleton remodeling. *Cell Physiol Biochem* **28**, 1031–1044.
34 Koukouritaki SB, Gravanis A and Stournaras C (1999) Tyrosine phosphorylation of focal adhesion kinase and paxillin regulates the signaling mechanism of the rapid nongenomic action of dexamethasone on actin cytoskeleton. *Mol Med* **5**, 731–742.
35 Papakonstanti EA, Kampa M, Castanas E and Stournaras C (2003) A rapid, nongenomic, signaling pathway regulates the actin reorganization induced by activation of membrane testosterone receptors. *Mol Endocrinol* **17**, 870–881.
36 Castoria G, Lombardi M, Barone MV, Bilancio A, Di Domenico M, De Falco A, Varricchio L, Bottero D, Nanayakkara M, Migliaccio A *et al.* (2004) Rapid signalling pathway activation by androgens in epithelial and stromal cells. *Steroids* **69**, 517–522.
37 Clark AF, Brotchie D, Read AT, Hellberg P, English-Wright S, Pang IH, Ethier CR and Grierson I (2005) Dexamethasone alters F-actin architecture and promotes cross-linked actin network formation in human trabecular meshwork tissue. *Cell Motil Cytoskeleton* **60**, 83–95.
38 Papadopoulou N, Charalampopoulos I, Alevizopoulos K, Gravanis A and Stournaras C (2008) Rho/ROCK/actin signaling regulates membrane androgen receptor induced apoptosis in prostate cancer cells. *Exp Cell Res* **314**, 3162–3174.
39 Papadopoulou N, Charalampopoulos I, Anagnostopoulou V, Konstantinidis G, Föller M, Gravanis A, Alevizopoulos K, Lang F and Stournaras C (2008) Membrane androgen receptor activation triggers down-regulation of PI-3K/Akt/NF-kappaB activity and induces apoptotic responses via Bad, FasL and caspase-3 in DU145 prostate cancer cells. *Mol Cancer* **7**, 88.
40 Gu S, Papadopoulou N, Gehring EM, Nasir O, Dimas K, Bhavsar SK, Föller M, Alevizopoulos K, Lang F and Stournaras C (2009) Functional membrane androgen receptors in colon tumors trigger

pro-apoptotic responses in vitro and reduce drastically tumor incidence in vivo. *Mol Cancer* **8**, 114.
41 Kirsch T, Beese M, Wyss K, Klinge U, Haller H, Haubitz M and Fiebeler A (2013) Aldosterone modulates endothelial permeability and endothelial nitric oxide synthase activity by rearrangement of the actin cytoskeleton. *Hypertension* **61**, 501–508.
42 Papakonstanti EA, Emmanouel DS, Gravanis A and Stournaras C (1996) Na+/Pi co-transport alters rapidly cytoskeletal protein polymerization dynamics in opossum kidney cells. *Biochem J* **315** (Pt 1), 241–247.
43 Theodoropoulos PA, Stournaras C, Stoll B, Markogiannakis E, Lang F, Gravanis A and Haussinger D (1992) Hepatocyte swelling leads to rapid decrease of the G-/total actin ratio and increases actin mRNA levels. *FEBS Lett* **311**, 241–245.
44 Moustakas A, Theodoropoulos PA, Gravanis A, Haussinger D and Stournaras C (1998) The cytoskeleton in cell volume regulation. *Contrib Nephrol* **123**, 121–134.
45 Papakonstanti EA, Vardaki EA and Stournaras C (2000) Actin cytoskeleton: a signaling sensor in cell volume regulation. *Cell Physiol Biochem* **10**, 257–264.
46 Lang F, Shumilina E, Ritter M, Gulbins E, Vereninov A and Huber SM (2006) Ion channels and cell volume in regulation of cell proliferation and apoptotic cell death. *Contrib Nephrol* **152**, 142–160.
47 Gaspar P and Tapon N (2014) Sensing the local environment: actin architecture and Hippo signalling. *Curr Opin Cell Biol* **31**, 74–83.
48 Lambert IH, Hoffmann EK and Pedersen SF (2008) Cell volume regulation: physiology and pathophysiology. *Acta Physiol (Oxf)* **194**, 255–282.
49 Leadsham JE, Kotiadis VN, Tarrant DJ and Gourlay CW (2010) Apoptosis and the yeast actin cytoskeleton. *Cell Death Differ* **17**, 754–762.
50 Kallergi G, Agelaki S, Markomanolaki H, Georgoulias V and Stournaras C (2007) Activation of FAK/PI3K/Rac1 signaling controls actin reorganization and inhibits cell motility in human cancer cells. *Cell Physiol Biochem* **20**, 977–986.
51 Pollard TD and Cooper JA (2009) Actin, a central player in cell shape and movement. *Science* **326**, 1208–1212.
52 Gu S, Papadopoulou N, Nasir O, Foller M, Alevizopoulos K, Lang F and Stournaras C (2011) Activation of membrane androgen receptors in colon cancer inhibits the prosurvival signals Akt/bad in vitro and in vivo and blocks migration via vinculin/actin signaling. *Mol Med* **17**, 48–58.
53 Fu XD, Goglia L, Sanchez AM, Flamini M, Giretti MS, Tosi V, Genazzani AR and Simoncini T (2010) Progesterone receptor enhances breast cancer cell motility and invasion via extranuclear activation of focal adhesion kinase. *Endocr Relat Cancer* **17**, 431–443.
54 Schmidt EM, Schmid E, Münzer P, Hermann A, Eyrich AK, Russo A, Walker B, Gu S, vom Hagen JM, Faggio C *et al.* (2013) Chorein sensitivity of cytoskeletal organization and degranulation of platelets. *FASEB J* **27**, 2799–2806.
55 Hatzoglou A, Kampa M, Kogia C, Charalampopoulos I, Theodoropoulos PA, Anezinis P, Dambaki C, Papakonstanti EA, Stathopoulos EN, Stournaras C *et al.* (2005) Membrane androgen receptor activation induces apoptotic regression of human prostate cancer cells in vitro and in vivo. *J Clin Endocrinol Metab* **90**, 893–903.
56 Kampa M, Kogia C, Theodoropoulos PA, Anezinis P, Charalampopoulos I, Papakonstanti EA, Stathopoulos EN, Hatzoglou A, Stournaras C, Gravanis A *et al.* (2006) Activation of membrane androgen receptors potentiates the antiproliferative effects of paclitaxel on human prostate cancer cells. *Mol Cancer Ther* **5**, 1342–1351.
57 Föller M, Hermann A, Gu S, Alesutan I, Qadri SM, Borst O, Schmidt EM, Schiele F, vom Hagen JM, Saft C *et al.* (2012) Chorein-sensitive polymerization of cortical actin and suicidal cell death in chorea-acanthocytosis. *FASEB J* **26**, 1526–1534.
58 Papakonstanti EA and Stournaras C (2008) Cell responses regulated by early reorganization of actin cytoskeleton. *FEBS Lett* **582**, 2120–2127.
59 Lang F, Alevizopoulos K and Stournaras C (2013) Targeting membrane androgen receptors in tumors. *Expert Opin Ther Targets* **17**, 951–963.
60 Schmid E, Gu S, Yang W, Munzer P, Schaller M, Lang F, Stournaras C and Shumilina E (2013) Serum- and glucocorticoid-inducible kinase SGK1 regulates reorganization of actin cytoskeleton in mast cells upon degranulation. *Am J Physiol Cell Physiol* **304**, C49–C55.
61 Stournaras C, Gravanis A, Margioris AN and Lang F (2014) The actin cytoskeleton in rapid steroid hormone actions. *Cytoskeleton (Hoboken)* **71**, 285–293.
62 Gu S, Honisch S, Kounenidakis M, Alkahtani S, Alarifi S, Alevizopoulos K, Stournaras C and Lang F (2014) Membrane androgen receptor down-regulates c-src-activity and beta-catenin transcription and triggers GSK-3beta-phosphorylation in colon tumor cells. *Cell Physiol Biochem* **34**, 1402–1412.
63 Mansell JP, Farrar D, Jones S and Nowghani M (2009) Cytoskeletal reorganisation, 1alpha,25-dihydroxy vitamin D3 and human MG63 osteoblast maturation. *Mol Cell Endocrinol* **305**, 38–46.
64 Meijerman I, Blom WM, de Bont HJ, Mulder GJ and Nagelkerke JF (1999) Changes of G-actin localisation in the mitotic spindle region or nucleus during mitosis and after heat shock: a histochemical study of G-actin in various cell lines with fluorescent labelled vitamin D-binding protein. *Biochim Biophys Acta* **1452**, 12–24.

65 Brackman D, Trydal T, Lillehaug JR and Aarskog D (1992) Reorganization of the cytoskeleton and morphological changes induced by 1,25-dihydroxyvitamin D3 in C3H/10T1/2 mouse embryo fibroblasts: relation to inhibition of proliferation. *Exp Cell Res* **201**, 485–493.
66 Gronowicz G, Egan JJ and Rodan GA (1986) The effect of 1,25-dihydroxyvitamin D3 on the cytoskeleton of rat calvaria and rat osteosarcoma (ROS 17/2.8) osteoblastic cells. *J Bone Miner Res* **1**, 441–455.
67 Gidley J, Openshaw S, Pring ET, Sale S and Mansell JP (2006) Lysophosphatidic acid cooperates with 1alpha,25(OH)2D3 in stimulating human MG63 osteoblast maturation. *Prostaglandins Other Lipid Mediat* **80**, 46–61.
68 Ito N, Wijenayaka AR, Prideaux M, Kogawa M, Ormsby RT, Evdokiou A, Bonewald LF, Findlay DM and Atkins GJ (2015) Regulation of FGF23 expression in IDG-SW3 osteocytes and human bone by pro-inflammatory stimuli. *Mol Cell Endocrinol* **399**, 208–218.
69 Zhang B, Yan J, Umbach AT, Fakhri H, Fajol A, Schmidt S, Salker MS, Chen H, Alexander D, Spichtig D *et al.* (2015) NFκB-sensitive Orai1 expression in the regulation of FGF23 release. *J Mol Med (Berl)*; PMID: 26631141.
70 Papakonstanti EA and Stournaras C (2002) Association of PI-3 kinase with PAK1 leads to actin phosphorylation and cytoskeletal reorganization. *Mol Biol Cell* **13**, 2946–2962.
71 Zhang B, Shi L, Lu S, Sun X, Liu Y, Li H, Wang X, Zhao C, Zhang H and Wang Y (2015) Autocrine IL-8 promotes F-actin polymerization and mediate mesenchymal transition via ELMO1-NF-kappaB-Snail signaling in glioma. *Cancer Biol Ther* **16**, 898–911.
72 Wang SE, Shin I, Wu FY, Friedman DB and Arteaga CL (2006) HER2/Neu (ErbB2) signaling to Rac1-Pak1 is temporally and spatially modulated by transforming growth factor beta. *Cancer Res* **66**, 9591–9600.
73 Gu S, Kounenidakis M, Schmidt EM, Deshpande D, Alkahtani S, Alarifi S, Föller M, Alevizopoulos K, Lang F and Stournaras C (2013) Rapid activation of FAK/mTOR/p70S6K/PAK1-signaling controls the early testosterone-induced actin reorganization in colon cancer cells. *Cell Signal* **25**, 66–73.
74 Olson EN and Nordheim A (2010) Linking actin dynamics and gene transcription to drive cellular motile functions. *Nat Rev Mol Cell Biol* **11**, 353–365.
75 Small EM (2012) The actin-MRTF-SRF gene regulatory axis and myofibroblast differentiation. *J Cardiovasc Transl Res* **5**, 794–804.
76 Esnault C, Stewart A, Gualdrini F, East P, Horswell S, Matthews N and Treisman R (2014) Rho-actin signaling to the MRTF coactivators dominates the immediate transcriptional response to serum in fibroblasts. *Genes Dev* **28**, 943–958.
77 Vasilaki E, Papadimitriou E, Tajadura V, Ridley AJ, Stournaras C and Kardassis D (2010) Transcriptional regulation of the small GTPase RhoB gene by TGF {beta}-induced signaling pathways. *FASEB J* **24**, 891–905.
78 Papadimitriou E, Vasilaki E, Vorvis C, Iliopoulos D, Moustakas A, Kardassis D and Stournaras C (2012) Differential regulation of the two RhoA-specific GEF isoforms Net1/Net1A by TGF-beta and miR-24: role in epithelial-to-mesenchymal transition. *Oncogene* **31**, 2862–2875.
79 Kardassis D, Murphy C, Fotsis T, Moustakas A and Stournaras C (2009) Control of transforming growth factor beta signal transduction by small GTPases. *FEBS J* **276**, 2947–2965.
80 Kaibuchi K, Kuroda S and Amano M (1999) Regulation of the cytoskeleton and cell adhesion by the Rho family GTPases in mammalian cells. *Annu Rev Biochem* **68**, 459–486.
81 Ridley AJ (2006) Rho GTPases and actin dynamics in membrane protrusions and vesicle trafficking. *Trends Cell Biol* **16**, 522–529.
82 Guignandon A, Faure C, Neutelings T, Rattner A, Mineur P, Linossier MT, Laroche N, Lambert C, Deroanne C, Nusgens B *et al.* (2014) Rac1 GTPase silencing counteracts microgravity-induced effects on osteoblastic cells. *FASEB J* **28**, 4077–4087.