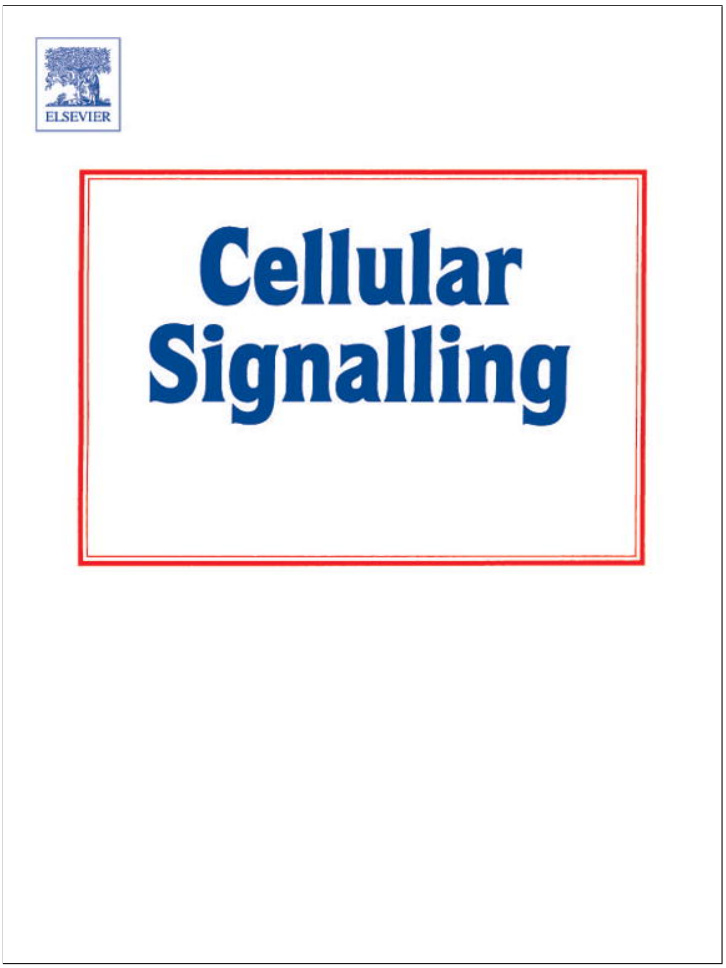


(This is a sample cover image for this issue. The actual cover is not yet available at this time.)

Cellular Signalling 25 (2013) 66–73

Contents lists available at SciVerse ScienceDirect

Cellular Signalling

journal homepage: www.elsevier.com/locate/cellsig

# Rapid activation of FAK/mTOR/p70S6K/PAK1-signaling controls the early testosterone-induced actin reorganization in colon cancer cells

Shuchen Gu [a], Michalis Kounenidakis [b], Eva-Maria Schmidt [a], Divija Deshpande [a], Saad Alkahtani [c], Saud Alarifi [d], Michael Föller [a,e], Konstantinos Alevizopoulos [b], Florian Lang [a], Christos Stournaras [a,b,*]

[a] Department of Physiology, University of Tübingen, Germany
[b] Department of Biochemistry, University of Crete Medical School, Heraklion Greece
[c] Department of Biology, Teachers College, King Saud University, Riyadh, Saudi Arabia
[d] Department of Zoology, Science College, King Saud University, Riyadh, Saudi Arabia
[e] Campell Family Institute for Breast Cancer Research, Ontario Cancer Institute, University Health Network (UHN), Toronto, Ontario, Canada

ARTICLE INFO

*Article history:*
Received 25 May 2012
Received in revised form 29 July 2012
Accepted 14 August 2012
Available online 23 August 2012

*Keywords:*
Membrane androgen receptors
Actin reorganization
Actin signaling

ABSTRACT

Actin cytoskeleton reorganization initiated by testosterone conjugates through activation of membrane androgen receptors (mAR) has recently been reported in colon tumor cells. This mAR-induced actin reorganization was recognized as a critical initial event, controlling apoptosis and inhibiting cell migration. The present study addressed the molecular signaling regulating the rapid actin remodeling initiated upon testosterone-induced mAR activation in Caco2 colon tumor cells. We report early phosphorylation of the Focal Adhesion Kinase (FAK), followed by substantial early phosphorylation of mammalian target of rapamycin (mTOR), S6 kinase (p70S6K) and the actin regulating p21-activated kinase (PAK1). Pharmacological inhibition of FAK-sensitive phosphatidylinositide-3-kinase (PI-3K), a known element of mAR-signaling, fully abrogated the testosterone-induced actin reorganization and the activation of mTOR, p70S6K and PAK1. Similarly, inhibition of mTOR blocked p70S6K and PAK1 phosphorylation and actin remodeling. Pretreatment of the cells with the intracellular androgen receptor (iAR) antagonist flutamide or silencing iAR through siRNA did not influence mTOR phosphorylation and actin reorganization, indicating specific mAR-induced testosterone effects that are independent of iAR signaling. In conclusion, we demonstrate for the first time a new mAR-governed pathway involving FAK/PI-3K and mTOR/p70S6K/PAK1-cascade that regulates early actin reorganization in colon cancer cells.



## 1. Introduction

Activation of various cell membrane receptors with subsequent triggering of specific intracellular signaling cascades often involves actin cytoskeleton rearrangements. Actin restructuring modifies cell-substratum adhesion and has been reported to control many aspects of cellular physiology, including cell motility, secretion, apoptosis, cell proliferation and survival (For reviews see [1–4]). From these and many other reports it became widely accepted that modifications in microfilament dynamics triggered by membrane receptor signaling play a pivotal role in various cell responses and seem to be involved in a large number of human diseases [5–7].

Early actin cytoskeleton reorganization is an important initial step of the non-genomic steroid hormone actions of androgens in a variety of tumor cell models [8–11]. Moreover, actin reorganization has been recognized as a critical cellular response that controls the androgen-induced induction of apoptosis and inhibition of cell migration triggered by the activation of membrane androgen binding sites in prostate, breast and colon cancer cells [8–10,12,13]. In prostate and breast cancer cells the molecular mechanisms regulating the non-genomic, steroid hormone-induced actin rearrangements have been elucidated. They involve mainly two signaling pathways: The FAK/PI3K/Rac1/Cdc42 [9,11] and the RhoA/B/ROCK/LimK/Cofilin cascades [10]. However, in colon tumor cells the intracellular signaling that may control the early actin restructuring upon membrane androgen binding site stimulation, remained unknown. In the present study we report the activation of a novel signaling pathway, acting downstream of FAK/PI-3K and involving mTOR/p70S6K and PAK1 that regulates the androgen-induced early actin reorganization in colon cancer cells.

*Abbreviations:* DEX, Dexamethasone; FAK, Focal Adhesion Kinase; HSA, human serum albumin; iAR, intracellular androgen receptor; mTOR, mammalian target of rapamycin; mAR, membrane androgen receptor; PAK1, p21-activated kinase 1; p70S6K, S6 kinase; PFA, paraformaldehyde; PI3K, phosphatidylinositide-3-kinase; TAC, testosterone albumin conjugates.

* Corresponding author at: Department of Biochemistry, University of Crete Medical School, GR-71110 Heraklion, Greece. Tel.: +30 2810 394563; fax: +30 2810 394530.
*E-mail address:* cstourn@med.uoc.gr (C. Stournaras).

0898-6568/$ – see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.cellsig.2012.08.005

## 2. Materials and methods

### 2.1. Cell cultures

The Caco2 human colon cancer cell line was studied between passages 60 and 70. Based on previous titration experiments [8] we have used a $10^{-7}$ M testosterone-HSA concentration for mAR stimulation throughout this study.

### 2.2. Immunoprecipitation and Western blotting

Cells were incubated with $10^{-7}$ M testosterone-HSA in the presence or absence of $10^{-6}$ M flutamide (Sigma), $10^{-6}$ M wortmannin (CALBIOCHEM), or $10^{-7}$ M rapamycin (Sigma) for the indicated time periods, washed twice with ice-cold PBS and suspended in ice-cold lysis buffer (50 mM Tris/HCl, 1% TritonX-100 pH 7.4, 1% sodium deoxycholate, 0.1% SDS, 0.15% NaCl, 1 mM EDTA, 1 mM sodium orthovanadate) containing a protease inhibitor cocktail (Sigma). The protein concentration was determined using the Bradford assay (BioRad). Sixty microgram of protein was solubilized in sample buffer at 95 °C for 5 min and resolved by 10% SDS-PAGE. For immunoblotting proteins were electro-transferred onto a PVDF membrane and blocked with 5% nonfat milk in TBS-0.10% Tween 20 at room temperature for 1 h. Then, the membrane was incubated with anti-phospho-FAK (Tyr397; 1:1000, Cell Signaling, USA), anti-FAK (1:1000, Cell Signaling), anti-phospho-mTOR (Ser2448; 1:1000, Cell Signaling), anti-mTOR (1:1000, Cell Signaling), anti-phospho-p70S6K (Thr389; 1:1000, Cell Signaling), anti-phospho-PAK1 (Thr423)/PAK2 (Thr402) (1:1000, Cell Signaling), anti-PAK1 (1:1000, Cell Signaling), or anti-GAPDH (1:1000, Cell Signaling) at 4 °C overnight. After washing (TBST) and subsequent blocking the blot was incubated with secondary anti rabbit antibody (1:2000, Cell Signaling) for 1 h at room temperature. After washing, antibody binding was detected with the ECL detection reagent (Amersham, Germany).

### 2.3. Measurement of G/total actin ratio by Triton X-100 fractionation

The Triton X-100 soluble G-actin- and total-actin-containing fractions of cells exposed to testosterone-HSA (15 min to 1 h pretreatment) were prepared and analyzed by Western blotting as previously described [14]. A decrease of the triton-soluble (G-) to the total (T-) actin ratio is indicative of actin polymerization.

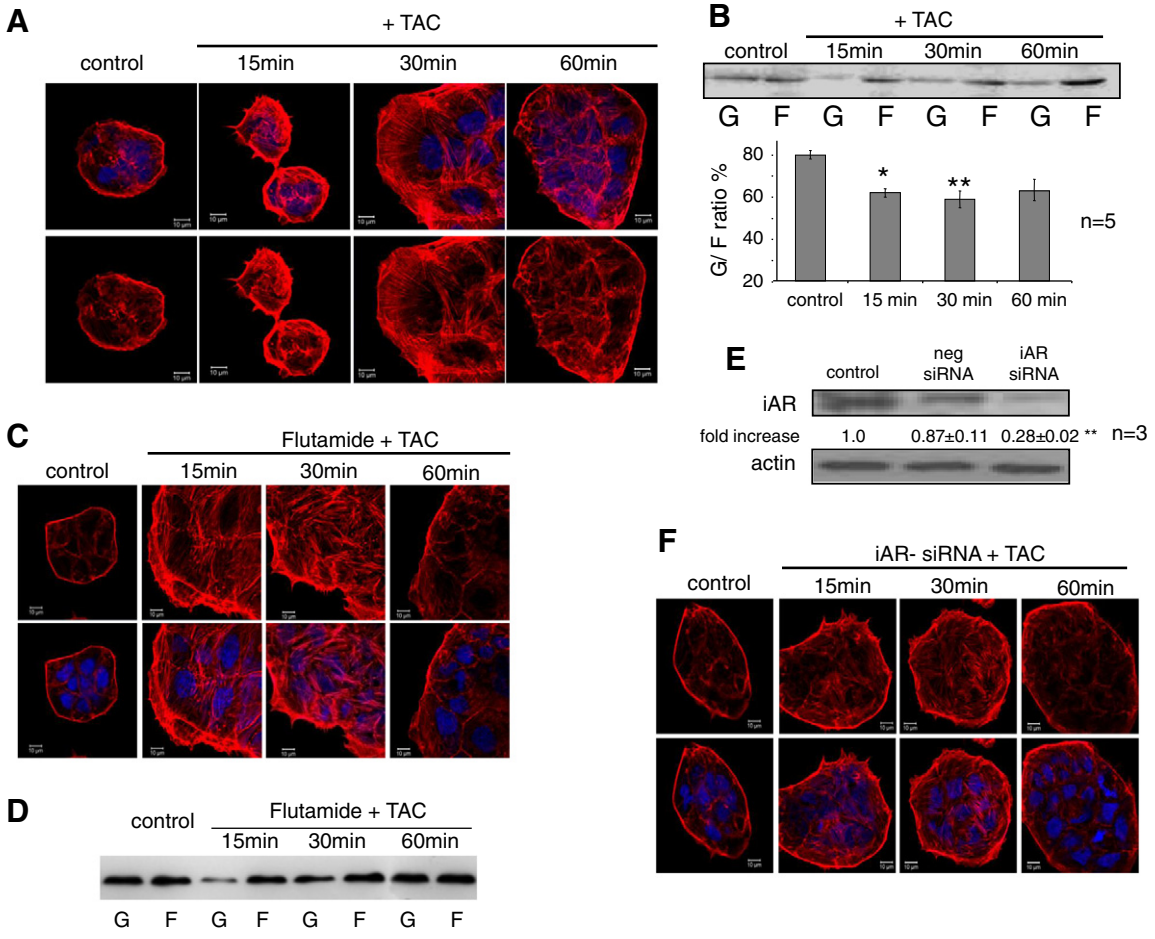


**Fig. 1.** Modulation of the dynamic equilibrium between G- and total actin in testosterone-HSA-stimulated Caco2 cells. 24 h serum-starved cells were stimulated with $10^{-7}$ M testosterone-HSA in the presence or absence of $10^{-6}$ M flutamide or silenced with or without siRNA against iAR for the indicated time. (A, C, F) Cells were stained with rhodamine-phalloidin for filamentous actin and DRAQ5™ for nuclei and subsequently analyzed by confocal laser scanning microscopy. Magnification ×100. (B) G- and F-actin were measured by quantitative immunoblot analysis after Triton X-100 subcellular fractionation. Bars present the G/F actin mean value ± SE of n = 5 independent duplicate experiments (**P<0.01, *P<0.05). (D) Representative G/F actin blot measured by quantitative immunoblot analysis after Triton X-100 subcellular fractionation from n = 2 experiments showing the TAC induced actin polymerization in the presence of flutamide. (E) Control western blot analysis showing the efficacy of iAR silencing by the specific siRNA and the unrelated control siRNA in Caco2 cells. The relative fold increases are indicated as mean values ± SE from n = 3 independent experiments of iAR silencing with that of control cells taken as 1. (**P<0.01).

*2.4. Immunofluorescence analysis and confocal laser scanning microscopy*

To determine the phosphorylation of FAK, cells were cultured on glass cover slips with testosterone-HSA or controls without testosterone-HSA for different time periods as indicated in the figure legends. After washing twice with PBS, cells were incubated with 4% PFA for 15 min and then incubated with 5% normal goat serum/1× PBS/0.3% Triton for 1 h at room temperature. Then, the cells were exposed to anti-phospho-FAK (Tyr397; 1:1000, Cell Signaling) at 4 °C overnight. The cells were rinsed three times with PBS and incubated with secondary FITC goat anti-rabbit antibody (1:500, Invitrogen, UK) for 1.5 h at room temperature.

For F-actin staining, cells were incubated with rhodamine-phalloidin (1:100, Molecular Probes, Eugene, OR) for 40 min in the dark. After three washing steps the nuclei were stained with DRAQ-5 dye (1:1000, Biostatus, Leicestershire, UK) for 10 min at room temperature. All the slides and coverslips were mounted with ProLong Gold antifade reagent (Invitrogen).

To determine the expression of mAR, cells were cultured on glass cover slips with testosterone-HSA-FITC or control HSA-FITC using the concentrations and the time points indicated in the figure legends. For testosterone-HSA-FITC staining, cells or specimens were washed twice with PBS 1.5% FBS for 1.5 min and incubated for 1 h with 1% BSA in PBS at room temperature. After two washes with PBS 1.5% FBS cells were exposed to testosterone-HSA-FITC $10^{-7}$ M while control cells were incubated with HSA-FITC $4\times10^{-7}$ M for 1 h at room temperature. Nuclei were stained with DRAQ5™ or TO-PRO-3 (Biostatus Limited). After two washes with PBS 1.5% FBS and fixation with 0.5% paraformaldehyde for 30 min, cells were washed twice with PBS 1.5% FBS for 3 min and mounted with slow anti-fade. Images were taken on a Zeiss LSM 5 EXCITER Confocal Laser Scanning Microscope (Carl Zeiss MicroImaging GmbH, Germany) with a water immersion Plan-Neofluar 40_/1.3 NA DIC. Images were analyzed with the instrument's software.

*2.5. siRNA experiments*

Caco2 cells were grown in DMEM medium containing 10% fetal calf serum under standard culture conditions (37 °C, 5% $CO^2$). $4\times10^4$ cells were seeded in 24 well plates and cultivated with fresh culture medium for 8 h. The cells were subsequently transfected with validated siRNA for iAR (ID: 1538, Ambion, Darmstadt, Germany) or with a negative control siRNA using siPORT Amine (Ambion) transfection agent according to the manufacturer's protocol.

*2.6. Statistical analysis*

Data are provided as means ± SEM, and *n* represents the number of independent experiments. Data were tested for significance using unpaired student's *t*-test or ANOVA as appropriate. Differences were considered statistically significant when *p*-values were $<0.05$. Statistical analysis was performed with GraphPad InStat version 3.00 for Windows 95, GraphPad Software, San Diego California USA, www.graphpad.com.

## 3. Results

*3.1. Early testosterone-induced actin reorganization is not dependent on iAR signaling*

In line with previous observations [8], treatment of Caco2 colon tumor cells with testosterone albumin conjugates (TAC) induced rapid (within 15 min) actin cytoskeleton restructuring. This was

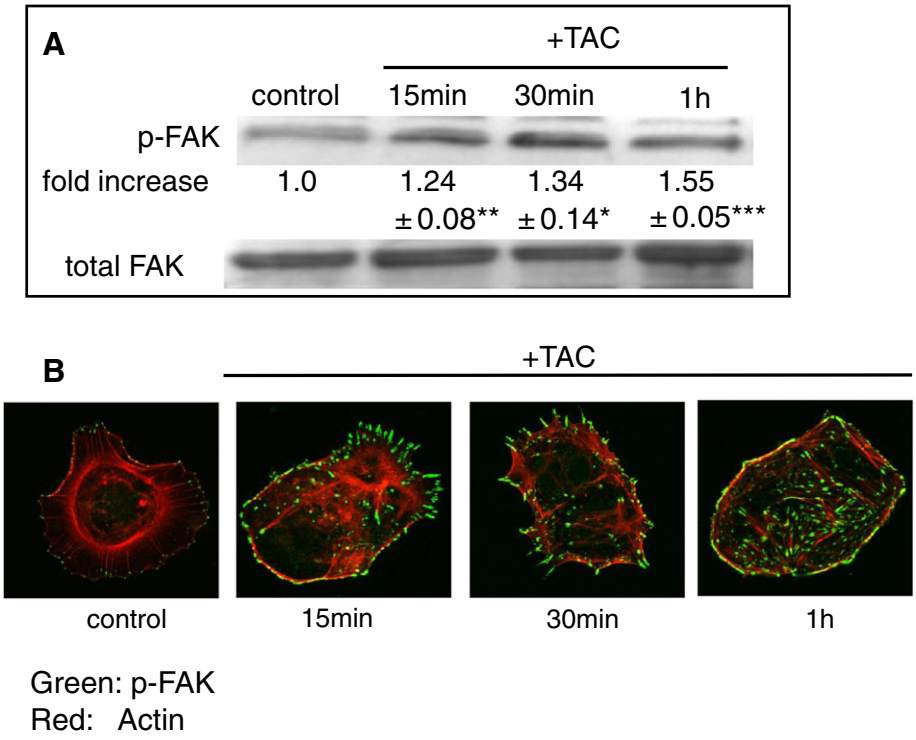


**Fig. 2.** Effects of testosterone-HSA on FAK phosphorylation and Caco2 cell morphology. (A) Caco2 cells were stimulated with $10^{-7}$ M testosterone-HSA for the indicated time periods. Following cell lysis equal amounts of total lysates were immunoblotted (IB) with a specific antibody against phospho-FAK and total FAK. Immunoblots were analyzed by densitometry. The intensity of phospho-FAK was normalized to the intensity of the corresponding total FAK band. Blots are from a representative experiment, whereas the relative fold increases are indicated as mean values ± SE from n = 3 independent experiments of FAK phosphorylation with that of untreated cells taken as 1. (*P<0.05; **P<0.01 ***P<0.001). (B) Confocal laser scanning microscopic analysis of Caco2 cells, untreated or treated with $10^{-7}$ M testosterone-HSA for different time periods. Cells were cultured in coverslips, fixed and stained with rabbit anti-phosphory-FAK. Anti-rabbit-FITC was used as secondary antibody; DRAQ5™ was used for nuclei staining and rhodamine-phalloidin for filamentous actin staining. Magnification ×100.

evident from confocal laser microscopic analysis showing clear formation of newly synthesized actin filaments (Fig. 1A), while quantitative determinations of the G to F-actin ratio fully confirmed the rapid actin polymerization (Fig. 1B). To identify the potential involvement of the classical androgen receptor (iAR) in this early event, the same experiments were performed in the presence of the specific nonsteroidal antiandrogen flutamide, or in cells transfected with a specific siRNA targeting iAR that resulted in reduced expression of iAR (Fig 1D). As shown in Fig. 1C and E, upon blocking of iAR either by flutamide or by siRNA silencing actin polymerization was still induced in Caco2 cells treated by testosterone conjugates, indicating that iAR is not involved in the early actin reorganization. These findings are in line with previous reports, which established clearly that neither flutamide treatment nor iAR silencing inhibited any of the mAR responses tested in prostate or colon cancer cells [8,11–13]. On the other hand mAR expression of Caco2 cells remained unaffected both, in flutamide-pretreated and in iAR-siRNA-transfected cells (Fig. S1A).

### 3.2. mAR stimulation triggers rapid activation of FAK as well as the downstream effectors mTOR/p70S6K/PAK1

We further addressed the molecular signaling that may be involved in the early mAR-induced actin restructuring in colon tumor cells. Fig. 2A shows the rapid activation via phosphorylation of FAK. This finding was fully supported by the visualization of the characteristic p-FAK staining in confocal microscopic analysis of TAC-treated Caco2 cells (Fig. 2B). It is well established that FAK activation may target PI-3K/Akt [11,15], which in turn regulates mTOR signaling [16]. Since we have recently established a role of PI-3K and Akt for testosterone-induced mAR stimulation of Caco2 cells [12], we further assessed the involvement of mTOR signaling acting downstream of FAK in these cells. Fig. 3A shows the early phosphorylation of mTOR in Caco2 cells treated with TAC for 15, 30 and 60 min respectively, while in the presence of flutamide the mTOR phosphorylation remained unchanged (Fig. 1SB), pointing to a iAR-unspecific effect. Fig. 3B depicts the phosphorylation and thus activation of the mTOR downstream effector p70S6K, showing similar kinetics to mTOR. Finally, we demonstrate early activation via phosphorylation of the actin cytoskeleton-regulating kinase PAK1 (Fig. 3C) upon TAC activation of the cells, showing kinetics similar to mTOR and p70S6K phosphorylation.

### 3.3. The FAK-downstream mTOR/p70S6K-signaling and PAK1 activation are directly implicated in early mAR-induced actin reorganization

To analyze the involvement of the FAK/mTOR/p70S6K/PAK1 signaling in testosterone-induced rapid actin polymerization, we further assessed both actin cytoskeleton dynamics and the activation of the

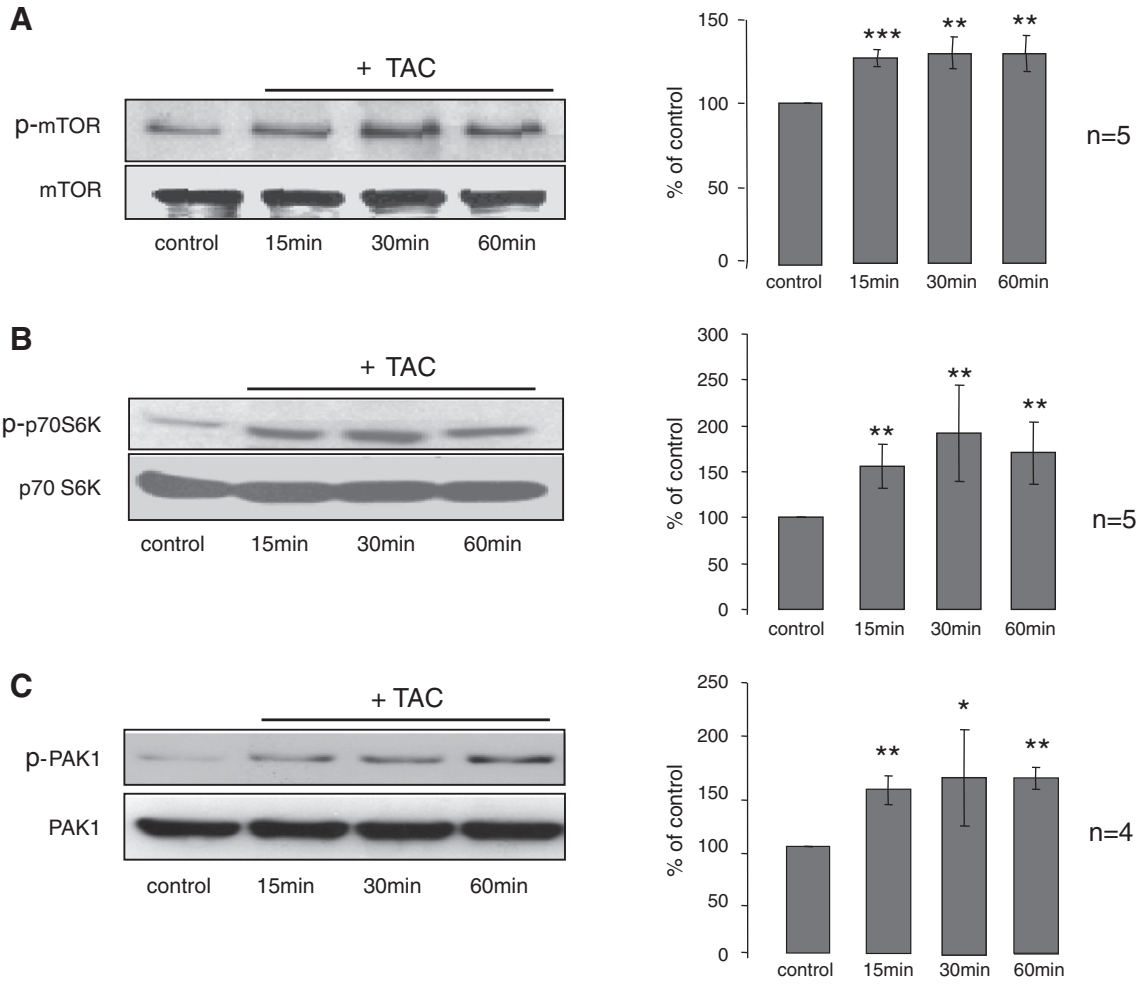


**Fig. 3.** Phosphorylation of mTOR, p70S6K and PAK1 in testosterone-HSA-treated Caco2 cells. Caco2 cells exposed to $10^{-7}$ M testosterone-HSA for the indicated time periods. The ratio of the cellular content of the phosphorylated residue (Ser2448) versus the total isoform of mTOR (A), the phosphorylated residue (Thr389) p-p70S6K versus GAPDH (B) and the phosphorylated residue (Thr423) versus total PAK1 (C) was measured in cell lysates by Western blotting using specific antibodies for each form and normalized to the corresponding control. Blots show a representative experiment, and the bar diagrams depict the mean values ± SE of the fold increase in the phosphorylation level for the indicated time points normalized to the controls from n = 5 or n = 4 independent experiments (*P<0.05; **P<0.01).

newly identified signaling effectors in the presence of specific inhibitors. First, since PI-3K and Akt are known to act downstream of FAK [11,15] and they are implicated in testosterone-induced mAR stimulation in Caco2 cells [12], we initially preincubated Caco2 cells with the PI-3K inhibitor wortmannin before stimulating mAR by TAC. As shown in Fig. 4A and B confocal laser scanning microscopy and G/F actin ratio determinations revealed clear abolishment of actin reorganization in the presence of wortmannin. Interestingly, TAC-induced phosphorylation of mTOR, p70S6K and PAK1 was also inhibited (Fig. 4C), indicating that this cascade is acting downstream of FAK/PI-3K and controls the rapid actin rearrangements. These results were further supported in a second series of experiments by using rapamycin. As shown in Fig. 5A, B and C, both, activation of mTOR, p70S6K and PAK1 as well as actin polymerization were clearly abolished in the presence of rapamycin in TAC-treated Caco2 cells. From these data we further conclude that mTOR/p70S6K signaling regulates PAK1, which in turn controls actin polymerization.

## 4. Discussion

Actin cytoskeleton reorganization triggered by the activation of mARs was shown to control specific cell responses in colon cancer cells, including induction of apoptosis and inhibition of cell migration [8,12]. However, the molecular mechanism that governs the actin restructuring remained unknown. Here we report the identification of a novel mAR-induced signaling cascade regulating the early actin reorganization in colon tumor cells. This pathway comprises rapid activation of FAK, the downstream signaling effectors mTOR/p70S6K and the actin regulating protein PAK1. Control experiments using either the anti-androgen flutamide or appropriate siRNAs to silence iAR underlined that the rapid actin polymerization triggered by testosterone albumin conjugates (TAC) is not correlated with the classical androgen receptor signaling, implying direct stimulation of this newly identified pathway by membrane androgen binding sites. In addition, by using specific inhibitors it was clearly shown that this signaling cascade is directly involved in the early, mAR-induced actin restructuring.

The implication of mTOR/p70S6K signaling and of PAK1 in regulating the rapid, mAR-induced actin reorganization indicates that the molecular mechanism controlling the non-genomic, steroid hormone-induced actin rearrangements in colon cancer cells is distinct from the mechanisms already described for prostate and breast cancer cells. In these tumor cells two major signaling cascades have been described; the FAK/PI3K/Rac1/Cdc42 and the RhoA/B/ROCK/LimK/Cofilin cascades [9–11,17]. Taken together these findings imply that mAR activation may orchestrate differential signaling mechanisms towards the rapid actin rearrangements in various tumor cells. This may be related to the significant regulatory role of

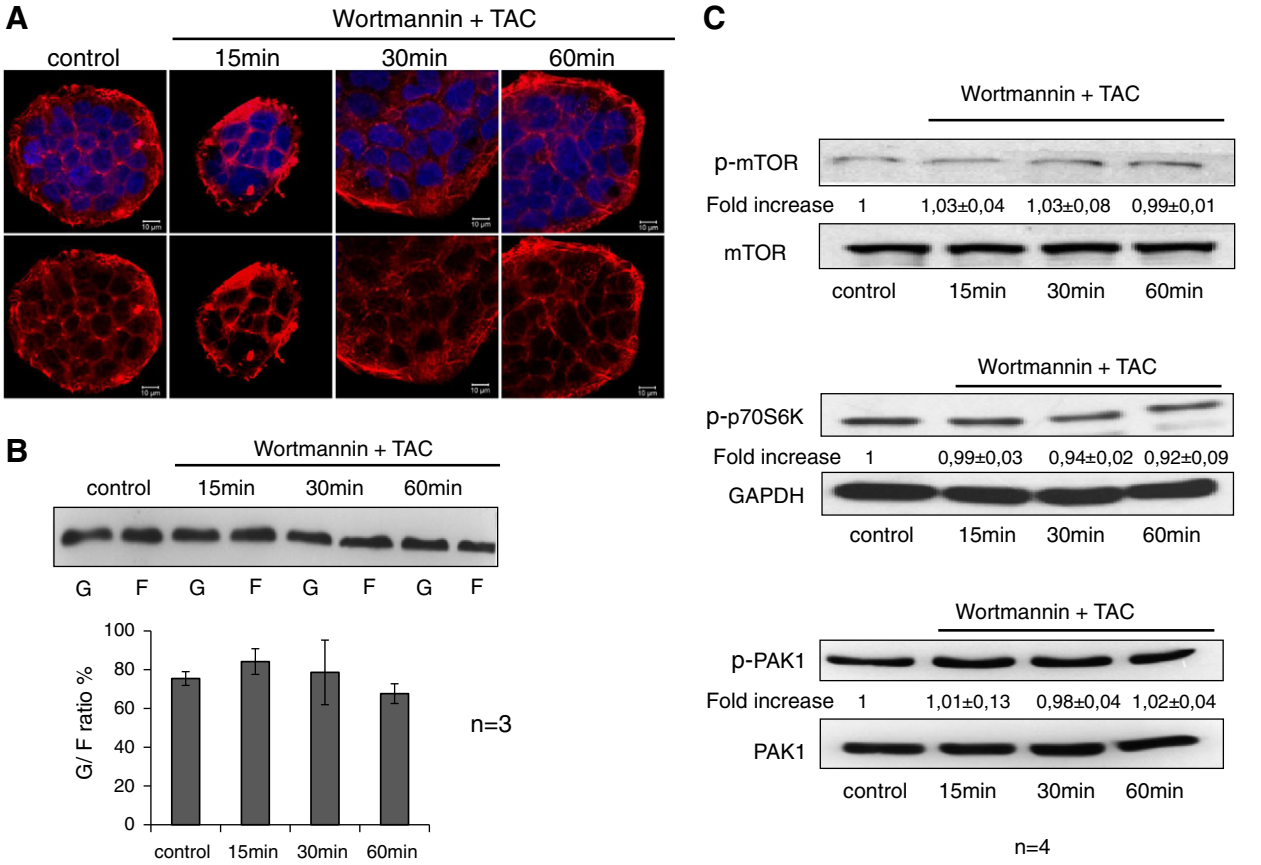


**Fig. 4.** Modulation of the dynamic equilibrium between G- and F actin and de-phosphorylation of mTOR, p70S6K and PAK in testosterone-HSA -treated Caco2 cells in the presence of wortmannin. 24 h serum-starved cells were stimulated with $10^{-7}$ M testosterone-HSA in the presence or absence of $10^{-6}$ M wortmannin for the indicated time periods. (A) Cells were stained with rhodamine-phalloidin for filamentous actin and DRAQ5™ for nuclei. Confocal laser scanning microscopy analyzed samples. Magnification ×100. (B) G- and F-actin content were measured in wortmannin pretreated cells followed by testosterone-HSA stimulation. Blot represents a representative experiment of the quantitative immunoblot analysis after Triton X-100 subcellular fractionation of G- and F-actin levels. Bars present the G/F actin mean value ± SE of n = 3 independent duplicate experiments. (C) The cellular content of the phosphorylated residue (Ser2448) versus total mTOR, the phosphorylated residue (Thr389) p-p70S6K versus GAPDH and the phosphorylated residue (Thr423) versus total PAK was measured by Western blotting using specific antibodies for each form in cell lysates. Cells were pre-treated with wortmannin followed by testosterone-HSA stimulation. Representative blots from n = 4 distinct experiments for every single molecule tested. Relative fold increases are indicated as mean values ± SE from n = 4 independent experiments with that of control cells taken as 1.

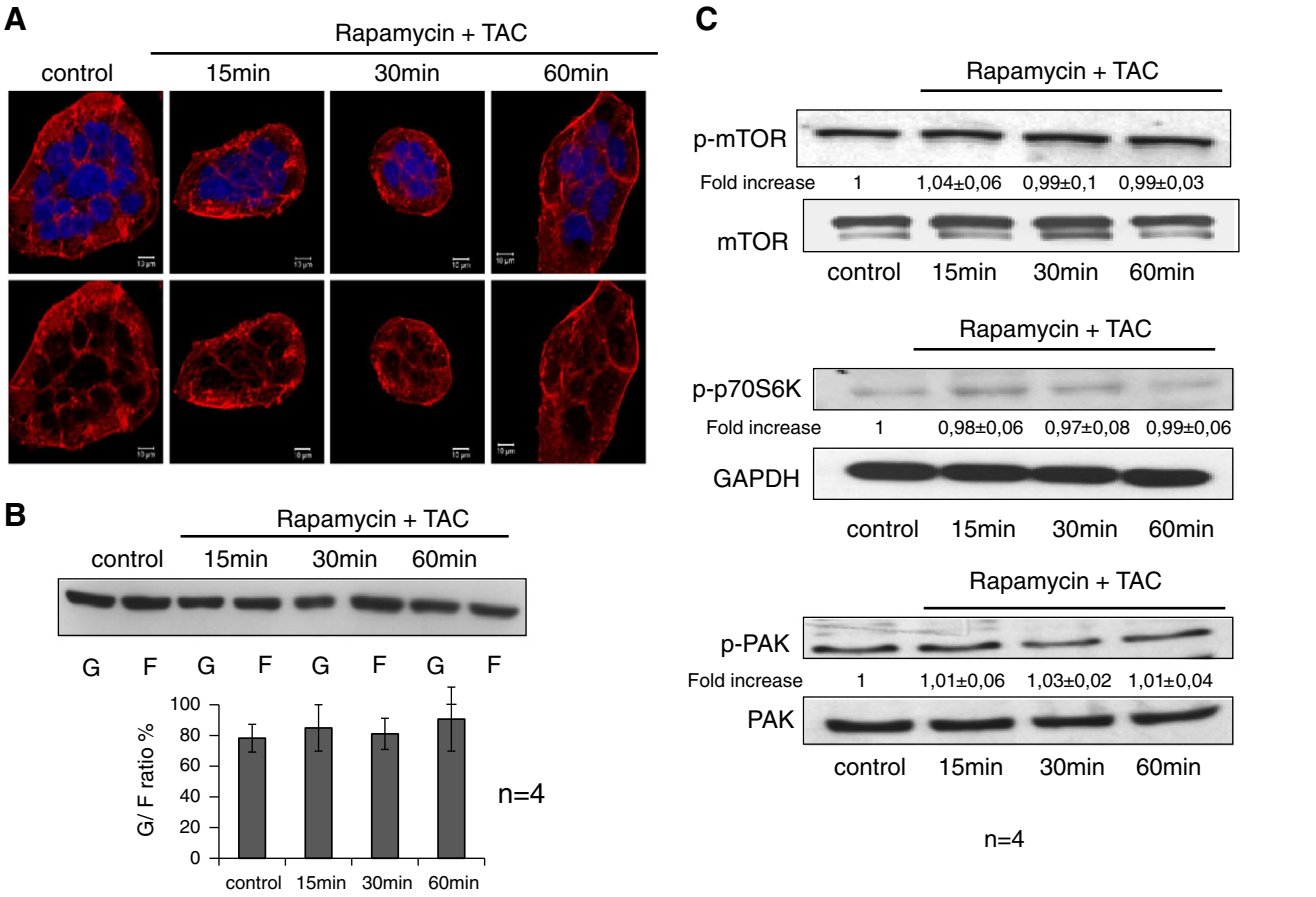


**Fig. 5.** Modulation of the dynamic equilibrium between G- and total actin and de-phosphorylation/inactivation of mTOR, p70S6K and PAK in testosterone-HSA-treated Caco2 cells in the presence of rapamycin. 24 h serum starved cells were stimulated with $10^{-7}$ M testosterone-HSA in the presence or absence of $10^{-6}$ M rapamycin for the indicated time periods. (A) Cells were stained with rhodamine-phalloidin for filamentous actin and DRAQ5™ for nuclei. Confocal laser scanning microscopy analyzed samples. Magnification ×100. (B) G- and F-actin content were measured in rapamycin pretreated cells followed by testosterone-HSA stimulation. Blot represents a representative experiment of the quantitative immunoblot analysis after Triton X-100 subcellular fractionation of G- and F-actin levels. Bars present the G/F actin mean value ± SE of four independent duplicate experiments. (C) The cellular content of the phosphorylated residue (Ser2448) versus total mTOR, the phosphorylated residue (Thr389) p-p70S6K versus GAPDH and the phosphorylated residue (Thr423) versus total PAK was measured by Western blotting using specific antibodies for each form in cell lysates. Cells were pre-treated with rapamycin followed by testosterone-HSA stimulation. Representative blots from n = 4 distinct experiments for every single molecule tested. Relative fold increases are indicated as mean values ± SE from n = 4 independent experiments with that of control cells taken as 1.

actin cytoskeleton reorganization in controlling mAR-induced cellular responses such as apoptotic regression and inhibition of the migratory capacity in tumor cells [8,10,12,17,18]. According to this hypothesis, the recruitment of differential alternative signaling molecules acting downstream of mAR towards actin restructuring aims to ensure the initiation of this crucial step in distinct tumor cells.

Early actin cytoskeleton reorganization seems to be a common target during non-genomic steroid hormone actions. Indeed, glucocorticoids have also been reported to generate cytoskeleton reorganization [19–21]. In these studies dexamethasone (DEX) was shown to stimulate rapid polymerization of actin and stabilization of microfilaments in human endometrial adenocarcinoma cells [19]. As the content of total cellular actin and the concentration of actin transcript levels did not change, it was concluded that polymerization of actin by glucocorticoids triggers non-genomic mechanisms [20], involving both, tyrosine phosphorylation of FAK and paxillin and up-regulation of Rho-B levels [21]. Interestingly, in TAC-stimulated colon tumor cells, although FAK phosphorylation was clearly evident, paxillin activation was not observed (Fig. S2A). Moreover, pre-treatment of the cells with Y27632, a specific inhibitor of the RhoA/RhoB downstream effector ROCK, did not significantly influence mAR-induced actin redistribution (Fig. S2B) and actin polymerization (Fig. S2C), implying that the Rho/ROCK signaling may not be involved in colon tumor cells. Although additional work is needed to further analyze this hypothesis, our data clearly identified the involvement of FAK/PI-3K signaling in the regulation of the mAR-induced early actin polymerization in colon tumor cells through the activation of PAK1 either directly, as has been reported previously [22,23], or following mTOR/p70S6K phosphorylation.

It should also be noted that among the results presented so far, FAK may represent one common, initial signaling effector acting downstream of membrane steroid binding sites in various tumor cells including endometrium, prostate, breast and colon. FAK localizes to sites of transmembrane integrin receptor clustering and facilitates intracellular signaling events [24]. Although the molecular link of the rapid FAK phosphorylation upon mAR stimulation still remains unknown, one may hypothesize that mAR activation is associated with integrin-mediated signaling that in turn regulates FAK [25]. On the other hand FAK is activated by a variety of stimuli including growth factors, steroid hormones, cytokines and neuropeptides [11,21,26–28]. FAK transmits the extracellular signals by activating downstream signaling molecules such as PI-3K [11,15] and/or paxillin [29,30], which have been implicated in cell survival cell migration and actin reorganization [1,31]. Our previous observations point to activation via phosphorylation of PI-3K/Akt signaling during the early phase (15 to 120 min) of TAC treatment in colon tumor cells and to PI-3K/Akt dephosphorylation in the late phase (6–12 h) [8]. Since early FAK activation failed to induce paxillin stimulation (Fig. S2A), we

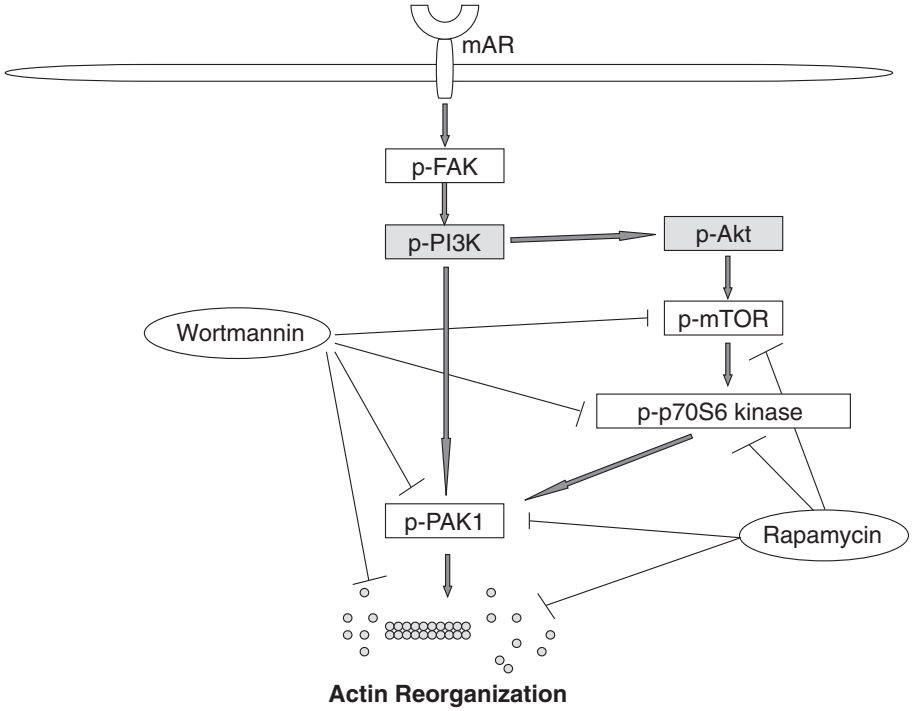


**Fig. 6.** A model summarizing the current findings on mAR-induced signaling regulating early actin reorganization in Caco2 cells.

conclude that the reported mAR-induced early PI-3K/Akt activation is most probably the FAK-governed, downstream signaling cascade triggering mTOR/p70S6K and PAK1 stimulation that regulates rapid actin restructuring. Our results using the specific PI-3K inhibitor wortmannin fully supported this assumption.

## 5. Conclusions

Based on the findings presented in this study we propose a novel signaling cascade (Fig. 6) acting downstream of the TAC-stimulated membrane androgen binding sites in colon cancer cells.

This cascade involves FAK/PI-3K activation that may govern the early actin restructuring through PAK1 phosphorylation either directly, or via activation of the mTOR/p70S6K signaling.

Paxillin, acting downstream of FAK and the Rho/ROCK signaling pathway seem to be not involved in the novel cascade regulating early mAR-induced actin reorganization in colon cancer cells.

## Competing interests

The authors declare that they have no competing interests towards any aspect of the work described in this paper.

## Author's contributions

SG and MK carried out the G/F actin determinations and the signaling effector analysis. E.M.S performed the mAR staining in colon cell lines and paxillin analysis. D.D. performed the siRNA control experiments. SA and SA carried out the flutamide and Y27632 control experiments and IF analysis of actin reorganization. MF participated in the design of the study and performed the statistical analysis. KA participated in the design of the study and drafting of the manuscript. FL participated of the coordination of the study and evaluation of the results. CS conceived of the study, participated in the design coordination and drafting of the manuscript.

All authors read and approved the final manuscript.

## Acknowledgments

This work was supported by grants from Deutsche Forschungsgemeinschaft (GRK 1302; SFB773; Mercator program), the “National Plan for Science Technology and Innovation”, King Saud University (11-MED-1765-02) and the grants KA1562 and KA3452 from the University of Crete Research Committee.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.cellsig.2012.08.005.

## References

[1] K. Burridge, C.E. Turner, L.H. Romer, The Journal of Cell Biology 119 (1992) 893–903.
[2] C. Janetopoulos, R.A. Firtel, FEBS Letters 582 (2008) 2075–2085.
[3] L.M. Machesky, FEBS Letters 582 (2008) 2102–2111.
[4] E.A. Papakonstanti, C. Stournaras, FEBS Letters 582 (2008) 2120–2127.
[5] B. Bugyi, C. Le Clainche, G. Romet-Lemonne, M.F. Carlier, FEBS Letters 582 (2008) 2086–2092.
[6] C.L. Carpenter, Critical Care Medicine 28 (2000) N94–N99.
[7] F.M. Vega, A.J. Ridley, FEBS Letters 582 (2008) 2093–2101.
[8] S. Gu, N. Papadopoulou, E.M. Gehring, O. Nasir, K. Dimas, S.K. Bhavsar, M. Foller, K. Alevizopoulos, F. Lang, C. Stournaras, Molecular Cancer 8 (2009) 114.
[9] G. Kallergi, S. Agelaki, H. Markomanolaki, V. Georgoulias, C. Stournaras, Cellular Physiology and Biochemistry 20 (2007) 977–986.
[10] N. Papadopoulou, I. Charalampopoulos, K. Alevizopoulos, A. Gravanis, C. Stournaras, Experimental Cell Research 314 (2008) 3162–3174.
[11] E.A. Papakonstanti, M. Kampa, E. Castanas, C. Stournaras, Molecular Endocrinology 17 (2003) 870–881.
[12] S. Gu, N. Papadopoulou, O. Nasir, M. Foller, K. Alevizopoulos, F. Lang, C. Stournaras, Molecular Medicine 17 (2011) 48–58.
[13] A. Hatzoglou, M. Kampa, C. Kogia, I. Charalampopoulos, P.A. Theodoropoulos, P. Anezinis, C. Dambaki, E.A. Papakonstanti, E.N. Stathopoulos, C. Stournaras, A. Gravanis, E. Castanas, Journal of Clinical Endocrinology and Metabolism 90 (2005) 893–903.
[14] E.A. Papakonstanti, C. Stournaras, Methods in Enzymology 428 (2007) 227–240.
[15] H.C. Chen, P.A. Appeddu, H. Isoda, J.L. Guan, Journal of Biological Chemistry 271 (1996) 26329–26334.
[16] A.M. Martelli, F. Chiarini, C. Evangelisti, C. Grimaldi, A. Ognibene, L. Manzoli, A.M. Billi, J.A. McCubrey, Histology and Histopathology 25 (2010) 669–680.

[17] N. Papadopoulou, E.A. Papakonstanti, G. Kallergi, K. Alevizopoulos, C. Stournaras, IUBMB Life 61 (2009) 56–61.
[18] E.M. Schmidt, S. Gu, V. Anagnostopoulou, K. Alevizopoulos, M. Foller, F. Lang, C. Stournaras, FEBS Journal 279 (2012) 1231–1242.
[19] S.B. Koukouritaki, P.A. Theodoropoulos, A.N. Margioris, A. Gravanis, C. Stournaras, Journal of Cellular Biochemistry 62 (1996) 251–261.
[20] S.B. Koukouritaki, A.N. Margioris, A. Gravanis, R. Hartig, C. Stournaras, Journal of Cellular Biochemistry 65 (1997) 492–500.
[21] S.B. Koukouritaki, A. Gravanis, C. Stournaras, Molecular Medicine 5 (1999) 731–742.
[22] M. Foller, A. Hermann, S. Gu, I. Alesutan, S.M. Qadri, O. Borst, E.M. Schmidt, F. Schiele, H.J. Muller Vom, C. Saft, L. Schols, H. Lerche, C. Stournaras, A. Storch, F. Lang, FASEB Journal, 2012.
[23] E.A. Papakonstanti, C. Stournaras, Molecular Biology of the Cell 13 (2002) 2946–2962.
[24] D.J. Sieg, C.R. Hauck, D.D. Schlaepfer, Journal of Cell Science 112 (Pt 16) (1999) 2677–2691.
[25] A. Richardson, T. Parsons, Nature 380 (1996) 538–540.
[26] S.B. Koukouritaki, E.A. Vardaki, E.A. Papakonstanti, E. Lianos, C. Stournaras, D.S. Emmanouel, Molecular Medicine 5 (1999) 382–392.
[27] J.L. Rodriguez-Fernandez, Bioessays 21 (1999) 1069–1075.
[28] M.D. Schaller, Biochimica et Biophysica Acta 1540 (2001) 1–21.
[29] S.L. Bellis, J.T. Miller, C.E. Turner, Journal of Biological Chemistry 270 (1995) 17437–17441.
[30] C.E. Turner, M.D. Schaller, J.T. Parsons, Journal of Cell Science 105 (Pt 3) (1993) 637–645.
[31] C. Jimenez, R.A. Portela, M. Mellado, J.M. Rodriguez-Frade, J. Collard, A. Serrano, A. Martinez, J. Avila, A.C. Carrera, The Journal of Cell Biology 151 (2000) 249–262.