Biochemical and Biophysical Research Communications 426 (2012) 659–663

Contents lists available at SciVerse ScienceDirect

Biochemical and Biophysical Research Communications

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

Involvement of the transcription factor FoxM1 in contact inhibition

Dagmar Faust, Firas Al-Butmeh, Berenike Linz, Cornelia Dietrich *

Institute of Toxicology, Medical Center of the Johannes Gutenberg-University, Obere Zahlbacherstr. 67, 55131 Mainz, Germany

ARTICLE INFO

Article history:
Received 28 August 2012
Available online 12 September 2012

Keywords:
Contact inhibition
FoxM1
Fibroblasts

ABSTRACT

Contact inhibition is a crucial mechanism regulating proliferation *in vitro* and *in vivo*. Although it is generally accepted that contact inhibition plays a pivotal role in maintaining tissue homeostasis, the molecular mechanisms of contact inhibition are still not fully understood. FoxM1 is known as a proliferation-associated transcription factor and is upregulated in many cancer types. Vice versa, anti-proliferative signals, such as TGF-β and differentiation signals decrease FoxM1 expression. Here we investigated the role of FoxM1 in contact inhibition in fibroblasts. We show that protein expression of FoxM1 is severely and rapidly downregulated upon contact inhibition, probably by inhibition of ERK activity, which then leads to decreased expression of cyclin A and polo-like kinase 1. Vice versa, ectopic expression of FoxM1 prevents the decrease in cyclin A and polo-like kinase 1 and causes a two-fold increase in saturation density indicating loss of contact inhibition. Hence, we show that downregulation of FoxM1 is required for contact inhibition by regulating expression of cyclin A and polo-like kinase 1.

1. Introduction

Cell–cell contact is known to be a critical regulator of cellular proliferation, differentiation and motility. Inhibition of proliferation by cell–cell contact is generally referred to as contact-dependent inhibition of growth or contact inhibition [1]. *In vitro*, non-transformed cells are arrested in G0/G1-phase at a critical cell density forming a confluent monolayer. In adult tissues, contact inhibition is thought to be continuously active, playing a critical role in the repression of somatic cell proliferation and probably organ size control [2]. The importance of contact inhibition for tissue homeostasis is demonstrated, for instance, by the fact that hypersensitivity to contact inhibition in the naked mole-rat provides cancer resistance in these animals [3]. Vice versa, release from contact inhibition *in vivo* and *in vitro* is associated with abnormal cellular proliferation and tumorigenesis [4]. In line, transformed cells are characterized by a loss of contact inhibition which is manifested by a higher saturation density and the emergence of multi-layered foci *in vitro*. Despite its importance for cell cycle control, knowledge about the signaling cascade mediating contact inhibition is still scarce [5].

One central regulator for G1 cell cycle progression and transition into S-phase is the ERK-Cyclin D/Cdk4-pRB-pathway [6]. Activation of ERK, for instance by growth factors, leads to expression of cyclin D1, which in association with Cdk4 (or Cdk6) phosphorylates the retinoblastoma protein (pRB), the gate keeper of G1-S-transition [7]. Downstream, pRB is phosphorylated by the cyclin E/Cdk2 complex thereby allowing dissociation from the transcription factor EF2 which then activates transcription of S-phase specific genes, such as cyclin A [6,8]. Upon contact inhibition in fibroblasts, phosphorylation of pRB is blocked by inhibition of ERK activity, subsequent downregulation of cyclin D1 as well as upregulation of the cdk4 inhibitor p16 and the cdk2 inhibitor p27 [9–13].

Gene expression studies comparing confluent versus proliferating cultures of mouse fibroblasts revealed differential expression of genes which are involved e.g. in proliferation, signal transduction, transcriptional regulation, cell adhesion and communication [12]. One interesting observation was downregulation of the transcription factor FoxM1. FoxM1 has been described as Trident, WIN (winged helix from INS-1 cells), FKHL 16 (forkhead drosophila homolog-like 16), MPP2 (MPM2-reactive phosphoprotein2, M-phase phosphoprotein 2), and HFH-11 [HNF-3(hepatocyte nuclear factor 3)/forkhead homolog 11] reviewed in [14]. It belongs to the forkhead/winged helix transcription factors and is known to be a typical proliferation-associated transcription factor which is downregulated upon anti-proliferative signals, such as serum-depletion, differentiation, aging or TGF-β [14]. In adult tissues, expression of FoxM1 is limited to self-renewing epithelia, e.g. small intestine or colon, or organs with a large fraction of proliferating cells, such as thymus or testis [14]. In non-proliferating tissues, such as lung or liver, expression is very low. However, expression is re-induced during regenerative proliferation, for instance due to injury or partial hepatectomy [14]. In line, upregulation of FoxM1 has been described in several cancer types and FoxM1 is known to be one of the few genes which are upregulated during early cancer development [14,15]. Its dominant role during

* Corresponding author. Fax: +49 6131 230506.
E-mail address: cdietric@uni-mainz.de (C. Dietrich).

0006-291X/$ - see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bbrc.2012.09.013

proliferation is demonstrated by regulation of genes being involved in G1/S and G2/M transition, such as Skp2 and Cks, which are subunits of the Skp1/cullin/F-box protein (SCF) complex regulating degradation of the cyclin-dependent kinase inhibitors p21 and p27, as well as expression of cyclin D1, Cdc25B, cyclin A, cyclin B, Aurora B kinase, survivin, polo-like kinase 1 (Plk1) and centromere protein A (CENPA), CENPB, and CENPF [14]. Interestingly, to date nothing was known about its regulation upon contact inhibition. Here we show that downregulation of FoxM1 is crucial for contact inhibition. In confluent cultures, expression of FoxM1 is diminished leading to a decrease in cyclin A and Plk1. Vice versa, ectopic expression of FoxM1 causes a loss of contact inhibition by preventing downregulation of these proteins. Time course analysis revealed early decrease in FoxM1 upon contact inhibition, very likely as a result of decreased ERK activity. We provide a model in which the decrease in ERK activity upon contact inhibition not only blocks the cyclin D/Cdk4-pRB-pathway, but additionally induces downregulation of FoxM1 which is essential to prevent G1/S transition and further cell cycle progression.

2. Materials and methods

2.1. Cell culture

Non-transformed NIH3T3 cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) (PAA) supplemented with 10% fetal calf serum (FCS) (PAA), 4 mM glutamine, penicillin and streptomycin (100 U/ml).

2.2. Flow cytometry

Cells were either seeded to a low density of $1.4 \times 10^4/cm^2$ (60% confluence) or to a high density of $1.8 \times 10^5/cm^2$ (100% confluence) and cultured for 24 and 48 h. Cells were trypsinized and washed twice with phosphate-buffered saline (PBS). $1–2 \times 10^6$ cells were vortexed in 200 μl of PBS and fixed with 2 ml of ice-cold 70% ethanol for 30 min at 4 °C. Cells were then permeabilized by incubation with 1 ml of 0.2% Tween 20/PBS for 15 min at 37 °C. Cells were resuspended in 2% FCS/PBS in the presence of RNAse A (11.25 kU/sample) and incubated with propidium iodide (50 μg/sample, Applichem) for 30 min at room temperature in the dark. Finally, the cells were resuspended in 800 μl of PBS and flow cytometric analysis was performed by a FACSCalibur (Becton Dickinson).

2.3. Western blot

Cells were either seeded to a low density of $1.4 \times 10^4/cm^2$ (60% confluence) or to a high density of $1.8 \times 10^5/cm^2$ (100% confluence), cultured and treated as described in the figure legend. Total cell extracts were prepared by lysing the cells in hot Laemmli sample buffer and protein concentration was determined according to Smith et al. [16]. Equal amounts of protein (20–50 μg protein/lane) were separated by SDS-PAGE (7.5–12.5%) and electroblotted overnight onto Immobilon membrane (Millipore). The membranes were blocked for 1 h with 5% low-fat milk-powder in TBS (50 mM Tris–HCl, pH 7.5, 150 mM NaCl) containing 0.05% Tween 20 and then incubated for 1.5 h at room temperature with anti-FoxM1-, anti-cyclin A-, anti-Plk-1-antibody (1:1000, Santa Cruz) or anti-phospho-ERK1/2 (1:1000, Cell Signaling) followed by incubation with horseradish-peroxidase-conjugated secondary antibody and ECL-detection according to the manufacturer's instructions. To control equal loading, the blots were stripped and reprobed with anti-ERK2- or anti-ERK1/2-antibody (1:2000, Santa Cruz).

2.4. Transient transfection of siRNA

NIH3T3 cells were transfected with FoxM1 siRNA (MWG Biotech AG) or control siRNA (Sigma), respectively, using RNAiMAX (Invitrogen). In 24-well-plates, 28 pmol FoxM1siRNA or control siRNA, respectively, was diluted in 100 μl of Opti-MEM I reduced medium. 1.4 μl of RNAiMAX reagent were added and incubated for 20 min at room temperature. 2×10^4 NIH3T3 cells were diluted in 500 μl of DMEM/10% FCS without antibiotics, added to each well (final concentration of siRNA 47 nM) and incubated for another 5 h at 37 °C. Medium was changed to DMEM/10% FCS containing antibiotics. Cells were cultured for another 48 h at 37 °C. FoxM1 siRNA: GGACCACUUCCCUUACUU UTT (sense), AAAGUAAGGGAAGUGGUCC (antisense).

2.5. RT-PCR

RNA was isolated by RNeasy Mini Kit (Qiagen) and reverse-transcribed by Advantage RT-for-PCR Kit (Clontech). Primers, PCR conditions and fragment sizes are listed in Supplementary Table 1.

2.6. Stable transfection with pCMV-FoxM1

NIH3T3 cells were stably transfected with pCMV-Foxm1b (generously provided by Pradip Raychaudhuri, Illinois) [17] or empty vector and pcDNA6/TR (Invitrogen) carrying the blasticidin resistence gene using Lipofectamine (Invitrogen). NIH3T3 cells were grown in DMEM/10% FCS to 80% confluence (100 mm culture dishes). Before transfection, medium was replaced by 3.2 ml DMEM without FCS and without antibiotics. For transfection, 7 μg of pCMV-FoxM1 and 1.5 μg of pcDNA6/TR were diluted in 800 μl of Opti-MEM I reduced medium and incubated with 50 μl of Lipofectamine, also diluted to 800 μl of Opti-MEM I reduced medium, for 30 min at room temperature. 1.6 ml of Opti-MEM I reduced medium was added to the complex solution (total volume 3.2 ml) which was then added to the cells. After 5 h incubation at 37 °C, 6.4 ml of DMEM/20% FCS (no antibiotics) was added. After another 24 h, cells were passaged in DMEM/10% FCS, antibiotics and 3 μg/ml blasticidin (Invitrogen). After 8–10 days colonies were picked.

3. Results

Cultures of NIH3T3 cells undergo contact inhibition. When they are seeded to confluence, about 75% of the cells are arrested in G0/G1-phase after 24 h, and more than 90% are arrested in G0/G1 after 48 h, respectively (Fig. 1A, [12,13]). Simultaneously, we detected a strong decrease in the expression of the transcription factor FoxM1 and of its downstream-targets cyclin A and Plk1 (Fig. 1B). Downregulation of FoxM1 upon contact inhibition was not restricted to NIH3T3 cells, but was observed in all cell lines tested including rat liver oval cells (WB-F344), mouse C3H10T1/2 fibroblasts and human colon epithelial cells (Caco-2) indicating that FoxM1 levels generally decrease upon contact inhibition (Fig. 1C and unpublished observation).

To investigate whether the observed downregulation of cyclin A and Plk1 was caused by the decrease in FoxM1 expression, we knocked-down FoxM1 by transient transfection of siRNA targeted against FoxM1 and performed RT-PCR and Western blot analysis after 48 h. Fig. 2A and B clearly shows that downregulation of FoxM1 results in decreased mRNA and protein expression of cyclin A and Plk1. In line with the work of others reviewed in [14], we also observed upregulation of the cdk inhibitor p27 (data not shown). As a result, proliferation was blocked which was demon-

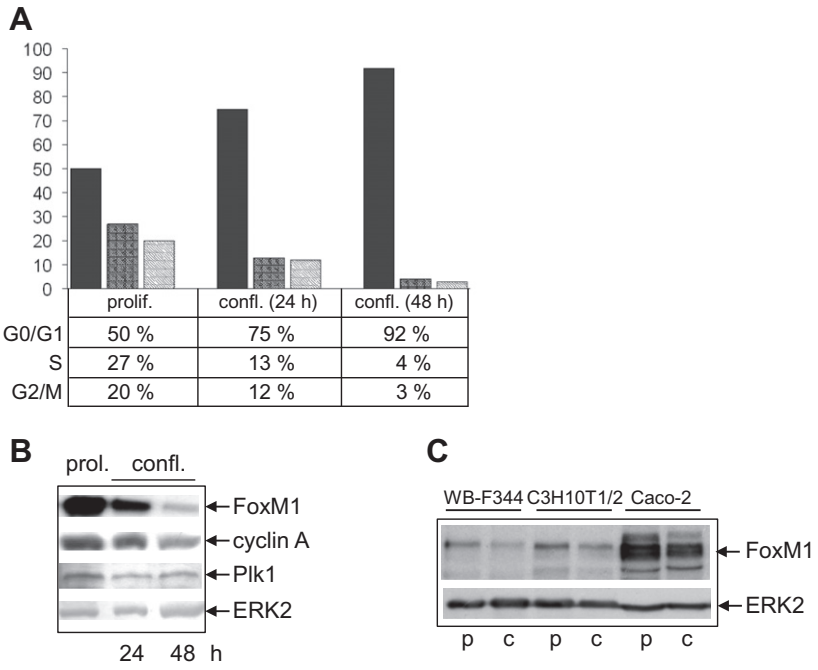

Fig. 1. G0/G1 arrest upon contact inhibition in NIH3T3 cells and downregulation of FoxM1. (A, B) NIH3T3 cells were either seeded at a low density (60% confluence = prol.) or high density (100% confluence = confl.) and cultured for 24 or 48 h in the presence of 10% FCS. (A) Cell cycle distribution was assessed by flow cytometric analysis after propidium iodide staining. (B) Western blot analysis was performed using anti-FoxM1-, anti-cyclin A- or anti-Plk1-antibodies. The blots were stripped and reprobed with anti-ERK2-antibody to control equal loading. (C) Different cell lines were seeded at a low density (p) or to confluence (c) and cultured for 48 h. Western blot analysis was performed using anti-FoxM-antibody. The blots were stripped and reprobed with anti-ERK2-antibody to control equal loading.

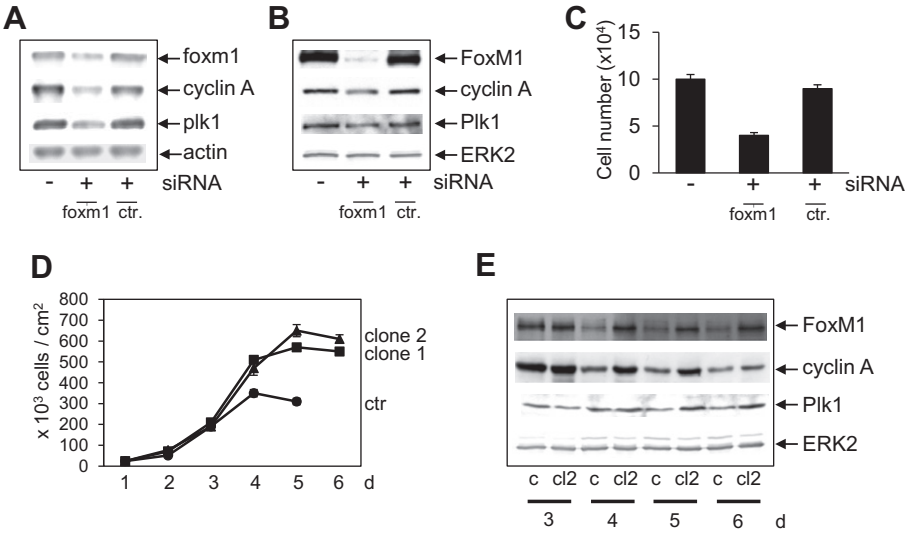

Fig. 2. Requirement of downregulation of FoxM1 for contact inhibition. (A–C) NIH3T3 cells were transiently transfected with siRNA targeted against FoxM1 or control siRNA and cultured for 48 h. (A) Semi-quantitative RT-PCR was performed to analyze downregulation of foxm1-, cyclin A- and plk1-mRNA. (B) Western blot analysis was performed using anti-phospho-FoxM1-, anti-cyclin A-, or anti-Plk1-antibodies. The blots were stripped and reprobed with anti-ERK2-antibody to control equal loading. (C) Cell number was determined by counting after Trypan blue exclusion. (D, E) NIH3T3 cells were stably transfected with pCMV-FoxM1 expression vector according to the Material and methods section. Controls were transfected with empty vector. Cells (mock-transfected control = ctr) were seeded to a density of 2.4×10^4 cells/cm^2, cultured for 6 days. (D) Cell number was determined at the indicated time points. (E) Cell extracts were prepared (c = mock-transfected control, cl2 = clone 2) at the indicated time points and Western blot analysis was performed using anti-phospho-FoxM1-, anti-cyclin A-, or anti-Plk1-antibodies. The blots were stripped and reprobed with anti-ERK2-antibody to control equal loading.

strated by a strong decrease in cell number 72 h after transfection (Fig. 2C).

In order to show the causal role of FoxM1 in contact inhibition, FoxM1 was overexpressed by stable transfection of pCMV-FoxM1 [17]. Controls were transfected with empty vector. There was no difference in proliferation between mock-transfected and non-transfected cultures (data not shown). Several clones were analyzed for their growth properties and all of them showed increased saturation density compared to mock-transfected cells. Fig. 2D shows the example of two representative clones. Although ectopic expression of FoxM1 was hardly detectable in exponentially growing cultures, Fig. 2D clearly demonstrates that

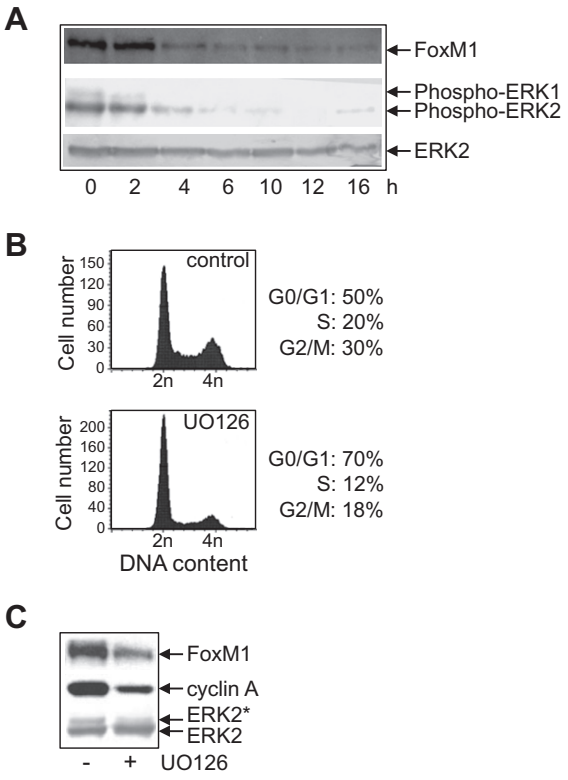

Fig. 3. Inhibition of ERK activity leads to downregulation of FoxM1. (A) NIH3T3 cells were seeded to a high density (100% confluence) in the presence of 10% FCS. The cells were harvested at the indicated time points after adherence to the culture dish (4 h after seeding). Western blot analysis was performed using anti-FoxM1- or anti-phospho-ERK1/2-antibodies. The blots were stripped and reprobed with anti-ERK2-antibody to control equal loading. (B) NIH3T3 cells were not treated or treated with U0126 (50 μM) for 24 h. Cell cycle distribution was assessed by flow cytometric analysis after propidium iodide staining. (C) NIH3T3 cells were not treated or treated with U0126 (50 μM) for 24 h. Western blot analysis was performed using anti-FoxM1-, anti-cyclin A- or anti-ERK1/2-antibody. ERK* = phosphorylated ERK2 [11].

downregulation of FoxM1 at increasing cell densities was abolished in the FoxM1 expressing clones. In line with the knock-down-experiments described above, downregulation of cyclin A and of Plk1 was prevented in the FoxM1 expressing clones (Fig. 2E).

It has been reported that FoxM1 protein stability is dependent on ERK in breast carcinoma cells [18]. We had shown in our previous work that ERK activity rapidly decreases upon contact inhibition [13]. We therefore hypothesized that ERK might also influence protein stability of FoxM1 upon contact inhibition. To this end, we performed time course experiments and revealed a rapid downregulation of FoxM1 protein which was detectable already 4 h after the establishment of cell–cell contacts (Fig 3A). Phosphorylation of ERK1/2 diminished concomitantly (Fig. 3A and [13]). Moreover, exposure to the MEK1/2-inhibitor UO126, which blocks activation of ERK1/2 thereby causing G0/G1 arrest in NIH3T3 cultures, resulted in a decreased FoxM1 protein expression (Fig. 3B and C). This shows that ERK activity regulates FoxM1 protein levels in NIH3T3 fibroblasts. We propose a model (Fig. 4) in which the decrease in ERK activity leads (i) to inhibition of the cyclin D1/Cdk4–pRB pathway [6,9–13] and (ii) to a rapid downregulation of FoxM1 protein causing inhibition of transcription of cyclin A and Plk1 and thereby preventing progression into and through S- and M-phase. We conclude that inhibition of both transcription factors, EF2 and FoxM1, is required for downregulating cyclin A and preventing G1/S transition.

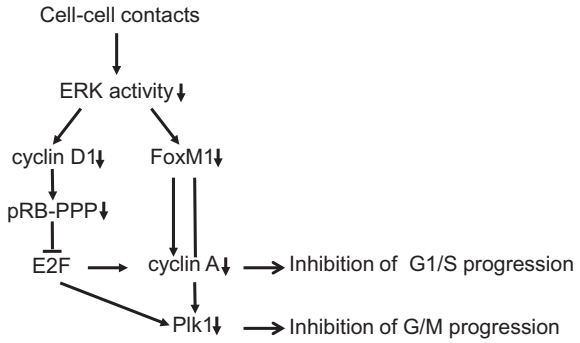

Fig. 4. Proposed model of intracellular signaling upon contact inhibition. Contact inhibition is induced by rapid inhibition of ERK activity which leads a decreased expression of cyclin D1 and thereby reduced cyclin D1/Cdk4 activity. As a result, pRB remains in its hypophosphorylated form and inhibits the transcription factor E2F. Hence expression of cyclin A and Plk1 is reduced. In a parallel pathway, inhibition of ERK activity results in downregulation of FoxM1 which also contributes to the decrease in cyclin A and Plk1 expression. As a result, bypass of contact inhibition by FoxM1 is prevented.

4. Discussion

Contact inhibition is a crucial mechanism of regulating proliferation *in vivo* and *in vitro*. Although some important players, such as $p16^{INK}$, $p27^{KIP1}$, PKCδ, and p38α MAPK could be identified ([9–13,19] for review see [5,8]), the molecular mechanisms are still not fully understood.

It has become clear that the ERK-Cyclin D/Cdk4-pRB-pathway is blocked upon contact inhibition ([6,9–13], Fig. 4). As a consequence, the transcription factor E2F is inhibited by binding to hypophosphorylated pRB and thereby transcription of S-phase-specific genes, such as cyclin A, is prevented [7,20]. Here we show that downregulation of the transcription factor FoxM1 is further required for contact inhibition. The observation that (i) knock-down of FoxM1 by RNA interference techniques significantly blocks cyclin A transcription and (ii) ectopic expression of FoxM1 prevents downregulation of cyclin A at increasing cell densities indicates that FoxM1 plays a pivotal role in regulating transcription of cyclin A. Hence, inhibition of both transcription factors, E2F and FoxM1, is required for contact inhibition. To note, ectopic expression of FoxM1 leads to an increased saturation density indicating that FoxM1 is sufficient to overcome contact inhibition. We propose that loss of contact inhibition is mediated by cyclin A since overexpression of cyclin A leads to a release from G0/G1 arrest and progression into S-phase [21,22].

We have previously shown that expression of Plk1, a key player of mitotic entry and progression is reduced upon contact inhibition [12]. According to cyclin A, expression of Plk1 is regulated (amongst others) by E2F and FoxM1 [23]. Here we show that the decrease in Plk1 upon contact inhibition is at least partially mediated by downregulation of FoxM1. Interestingly, expression of cyclin D1 was not regulated by FoxM1 in NIH3T3 cells (unpublished observation). Whether additional well-known FoxM1 target genes, such as cyclin B, Cdc25B or Aurora B kinase, are also deregulated upon contact inhibition remains to be elucidated.

We further show that FoxM1 is rapidly decreased after establishment of cell–cell contacts. Two observations argue for a role of ERK in destabilization of FoxM1 protein: (i) the rapid decline in FoxM1 protein parallels the decline of ERK-phosphorylation, (ii) inhibition of ERK activity by the MEK1/2-inhibitor UO126 also induces a decrease in FoxM1 protein levels. A similar role of ERK in regulating FoxM1 protein levels has been reported by Madureira and coworkers in breast carcinoma cells [18]. To note, in their work the decrease in FoxM1 protein occurs as rapid as 1 h after

inhibition of ERK supporting our suggestions that ERK functions upstream of FoxM1 and is responsible for the observed rapid downregulation of FoxM1 upon contact inhibition. Possibly, regulation of transcription also contributes to FoxM1 downregulation at later time points. For instance, a functional E2F-binding site has been identified in the human FoxM1 promoter [24]. Moreover, direct binding of pRB to the FoxM1 promoter thereby inhibiting expression of FoxM1 has been described [25]. Whether a similar crosstalk of the pRb/E2F axis with FoxM1 transcriptional regulation exists upon contact inhibition has to be determined.

FoxM1 is a typical proliferation-associated transcription factor whose expression is high in proliferating cells *in vitro* and *in vivo* with a peak at G2/M [26,27] for review see [14]. It is known that FoxM1 regulates transcription of proteins involved in G1/S transition and S-phase progression, such as cyclin D1, Skp2, cyclin A and of proteins required for G2/M progression, such as cyclin B, Aurora B kinase, survivin, polo-like kinase 1 (Plk1) and centromere protein A (CENPA), CENPB, and CENPF [15]. However, studies in knock-out mice revealed a major role of FoxM1 in normal coupling of DNA replication (S-phase) and proper mitotic progression including genome stability [28] which has been confirmed in numerous *in vitro* studies [26,29,30], for review see [14,31]. Accordingly, knock-down of FoxM1 by siRNA in U2OS cells or FoxM1-deficieny in mouse embryonal fibroblasts results in G2/M arrest [26]. This raises the question about the exact role of FoxM1 in contact inhibition since confluent cells are arrested in G0/G1-phase. It has been reported that when cells are arrested in G0/G1 due to serum-depletion, downregulation of FoxM1 causes inhibition of serum-induced S-phase entry [26]. On the other hand, we here show that overexpression of FoxM1 is sufficient to overcome contact inhibition. These observations suggest that the decrease in FoxM1 is not responsible for induction of G0/G1 arrest, but is required to inhibit bypass of contact inhibition by FoxM1. As presented in our model (Fig. 4), G0/G1 arrest is mediated by downregulation of the cyclin D/Cdk4-pRB-pathway and upstream ERK-phosphorylation is reduced. Here we show that the decrease in ERK activity additionally leads to downregulation of FoxM1. Hence, expression of cyclin A and Plk1 is blocked thereby preventing S-phase entry and further cell cycle progression.

Acknowledgments

This work is part of the MD thesis of FA-B and BL. We thank Beate Köberle for critical reading of the manuscript. The technical support by Julia Altmaier, FACS and Array Core Facility, is greatfully acknowledged.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2012.09.013.

References

[1] H. Eagle, E. Levine, Growth regulatory effects of cellular interaction, Nature 213 (1967) 1102–1106.
[2] Q. Zeng, W. Hong, The emerging role of the hippo pathway in cell contact inhibition, organ size control, and cancer development in mammals, Cancer Cell 13 (2008) 188–192.
[3] A. Seluanov, C. Hine, J. Azpurua, M. Feigenson, M. Bozzella, Z. Mao, K.C. Catania, V. Gorbunova, Hypersensitivity to contact inhibition provides a clue to cancer resistance of naked mole-rat, Proc. Natl. Acad. Sci. USA 106 (2010) 19352–19357.
[4] M. Abercrombie, Contact inhibition and malignancy, Nature 281 (1979) 259–262.
[5] P.J. Nelson, T.O. Daniel, Emerging targets: molecular mechanisms of cell contact-mediated growth control, Kidney Int. 61 (Suppl. 1) (2002) S99–S105.
[6] R. Assoian, M.A. Schwartz, Coordinate signaling by integrins and receptor tyrosine kinases in the regulation of G1 phase cell-cycle progression, Curr. Opin. Genet. Dev. 11 (2001) 48–53.
[7] R. Weinberg, The retinoblastoma protein and cell cycle control, Cell 81 (1995) 323–330.
[8] C. Dietrich, B. Kaina, The aryl hydrocarbon receptor (AhR) in the regulation of cell–cell contact and tumor growth, Carcinogenesis 31 (2010) 1319–1328.
[9] C. Dietrich, K. Wallenfang, F. Oesch, R. Wieser, Differences in the mechanisms of growth control in contact-inhibited and serum-deprived human fibroblasts, Oncogene 15 (1997) 2743–2747.
[10] R. Wieser, D. Faust, C. Dietrich, F. Oesch, $P16^{INK4}$ mediates contact inhibition of growth, Oncogene 18 (1999) 277–281.
[11] D. Faust, I. Dolado, A. Cuadrado, F. Oesch, C. Weiss, A.R. Nebreda, C. Dietrich, p38α MAPK is required for contact inhibition, Oncogene 24 (2005) 7941–7945.
[12] M. Küppers, C. Ittrich, D. Faust, C. Dietrich, The transcriptional programme of contact- inhibition, J. Cell Biochem. 110 (2010) 1234–1243.
[13] M. Küppers, D. Faust, B. Linz, C. Dietrich, Regulation of ERK1/2 activity upon contact inhibition of fibroblasts, Biochem. Biophys. Res. Commun. 406 (2011) 483–487.
[14] I. Wierstra, J. Alwes, FOXM1, a typical proliferation-associated transcription factor, Biol. Chem. 388 (2007) 1257–1274.
[15] C.-Y. Koo, K.W. Muir, E.W.-F. Lam, FOXM1: from cancer initiation to progression and treatment, Biochim. Biophys. Acta 2012 (1819) 28–37.
[16] P.K. Smith, R.I. Krohn, G.T. Hermanson, A.K. Mallia, F.H. Gartner, M.D. Provenzano, E.K. Fujimoto, N.M. Goeke, B.J. Olson, D.C. Klenk, Measurement of protein using bicinchoninic acid, Anal. Biochem. 150 (1985) 76–85.
[17] L. Pani, D.G. Overdier, A. Porcella, X. Qian, E. Lai, R.H. Costa, Hepatocyte nuclear factor 3b contains two transcriptional activation domains, one of which is novel and conserved with the *drosophila* fork head protein, Mol. Cell Biol. 12 (1992) 3723–3732.
[18] P. Madureira, R. Varshochi, D. Constantinidou, R.E. Francis, R.C. Coombes, K.-M. Yao, E.W.-F. Lam, The forkheadbox M1 protein regulates the transcription of the Estrogen receptor a in breast cancer cells, J. Biol. Chem. 281 (2006) 25167–25176.
[19] I. Heit, R. Wieser, T. Herget, D. Faust, M. Borchert-Stuhlträger, F. Oesch, C. Dietrich, Involvement of PKCδ in contact-dependent inhibition of growth in human and murine fibroblasts, Oncogene 20 (2001) 5143–5154.
[20] M. Malumbres, M. Barbacid, Mammalian cyclin-dependent kinases, Trends Biochem. Sci. 30 (2005) 630–641.
[21] C. Weiss, D. Faust, I. Schreck, A. Ruff, T. Farwerck, A. Melenberg, S. Schneider, B. Oesch-Bartlomowicz, J. Zatloukalová, J. Vondrácek, F. Oesch, C. Dietrich, TCDD deregulated contact inhibition in rat liver oval cells via Ah receptor, JunD and cyclin A, Oncogene 27 (2008) 2198–2207.
[22] M.W. Strobeck, A.F. Fribourg, A. Puga, E.S. Knudsen, Restoration of retinoblastoma mediated signaling to Cdk2 results in cell cycle arrest, Oncogene 19 (2000) 1857–1867.
[23] B.T. Martin, K. Strebhardt, Polo-like kinase 1, target and regulator of transcriptional control, Cell Cycle 5 (2006) 2881–2885.
[24] J. Millour, N. de Olando, Y. Horimoto, L.J. Monteiro, J.K. Langer, R. Alique, N. Hajji, E.W. Lam, ATM and p53 regulate FOXM1 expression via E2F in breast cancer epirubicin treatment and resistance, Mol. Cancer Ther. 10 (2011) 1046–1058.
[25] I. Wierstra, J. Alves, Transcription factor FOXM1c is repressed by RB and activated by cyclin D1/Cdk4, Biol. Chem. 387 (2006) 949–962.
[26] I.-C. Wang, Y.-J. Chen, D. Hughes, V. Petrovic, M.L. Major, H.J. Park, Y. Tan, T. Ackerson, R.H. Costa, Forkhead box M1 regulates the transcriptional network of genes essential for mitotic progression and genes encoding the SCF (Skp2-Cks1) ubiquitin ligase, Mol. Cell Biol. 25 (2005) 10875–10894.
[27] W. Korver, J. Roose, H. Clevers, The winged-helix transcription factor Trident is expressed in cycling cells, Nucleic. Acids Res. 25 (1997) 1715–1719.
[28] W. Korver, M.W. Schilham, P. Moerer, M.J. van den Hoff, K. Dam, W.H. Lamers, R.H. Medema, H. Clevers, Uncoupling of S-phase and mitosis in cardiomyocytes and hepatocytes lacking the winged-helix transcription factor Trident, Curr. Biol. 8 (1998) 1327–1330.
[29] J. Laoukili, W.R.H. Kooistra, A. Brás, J. Kauw, R.M. Kerkhoven, A. Morrison, H. Clevers, R.H. Medema, FoxM1 is required for execution of the mitotic programme and chromosome stability, Nat. Cell Biol. 7 (2005) 126–136.
[30] Z. Fu, L. Malureanu, J. Huang, W. Wang, H-. Li, J.M. van Deursen, D.J. Tindall, J. Chen, Plk1-dependent phosphorylation of FoxM1 regulates a transcriptional programme required for mitotic progression, Nat. Cell Biol. 10 (2008) 1076–1082.
[31] R.H. Costa, FoxM1 dances with mitosis, Nat. Cell Biol. 7 (2005) 108–110.