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Cell Rounding in Cultured Human Astrocytes and Vascular Endothelial Cells Upon Inhibition of CK2 Is Mediated by Actomyosin Cytoskeleton Alterations

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ABSTRACT

Protein kinase CK2 participates in a wide range of cellular events, including the regulation of cellular morphology and migration, and may be an important mediator of angiogenesis. We previously showed that in the retina, CK2 immunolocalizes mostly to vascular endothelium and astrocytes in association with the cytoskeleton. Additionally, CK2 inhibitors significantly reduced retinal neovascularization and stem cell recruitment in the mouse model of oxygen-induced proliferative retinopathy. We have also shown that CK2 and F-actin co-localized in actin stress fibers in microvascular endothelial cells, and that highly specific CK2 inhibitors caused cell rounding in astrocytes and microvascular endothelial cells, which was alleviated by serum that promotes spreading by Rho/Rho-kinase (RhoK) activation of myosin II. Therefore, we examined a possible role of CK2 in the regulation of actin–myosin II-based contractility. Treatment with CK2 inhibitors correlated with disassembly of actomyosin stress fibers and cell shape changes, including cytoplasmic retraction and process formation that were similar to those occurring during astrocyte stellation. Low doses of specific inhibitors of kinases (RhoK and MLCK) that phosphorylate myosin light chain (MLC) enhanced the effect of suboptimal CK2 inhibition on cell shape. Such striking stellation-like alteration was accompanied by decreased level of phospho-MLC, thus implying a CK2 role in regulation of actomyosin cytoskeleton. Our results suggest an important role of CK2 in the control of cell contractility and motility, which may account for suppressing effect of CK2 inhibition on retinal neovascularization. Together, our data implicate protein kinase CK2 for the first time in stellation-like morphological transformation. J. Cell. Biochem. 113: 2948–2956, 2012.

KEY WORDS: PROTEIN KINASE CK2; TBB, TBCA; ACTIN STRESS FIBERS; MYOSIN LIGHT CHAIN; CELL SHAPE; CYTOSKELETON

Cell migration affects all morphogenetic processes and may be a contributing factor in many diseases, including cancer and cardiovascular disease. Cytoskeleton changes underlie all steps of cell migration, including plasma membrane protrusions, formation of new (at the cell front) and disassembly of old (at the rear end) adhesions, and generation of traction forces that move the cell forward. The migration cycle is driven by activation of Rho GTPases that regulate actin polymerization, myosin II activity, and adhesion dynamics [Ridley et al., 2003]. Changes in cell shape and cytoskeletal structure are not only critical for migration, but also crucial for control of growth and cell fate switching [Mammoto and Ingber, 2009].

Protein kinase CK2 is a multifunctional regulatory molecule that participates in a wide range of cellular events by phosphorylating

Abbreviations used: CK2, acronym for the former casein kinase II or 2; RhoK, Rho kinase; MLC, myosin light chain; MLCK, myosin light chain kinase; TBB, 4,5,6,7-tetrabromobenzotriazole; TBCA, 3-(2,3,4,5-tetrabromophenyl)acrylic acid or tetrabromocinnamic acid; LPA, 1-oleoyl-lysophosphatidic acid; HF, hydroxyfasudil; ML7, (5-iodonaphthalene-1-sulfonyl)homopiperazine; OIR, oxygen-induced proliferative retinopathy.

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and/or interacting with key signaling molecules, structural proteins, and transcription factors [Guerra and Issinger, 1999; Tawfic et al., 2001; Meggio and Pinna, 2003]. CK2 may be an important mediator of angiogenesis, as several key components of the signaling cascades triggered by angiogenic growth factors are substrates for CK2 [Di Maira et al., 2005; Ritt et al., 2007]. Additionally, CK2 is involved in the regulation of cellular morphology and cell polarity, and in the regulation of the actin and tubulin cytoskeleton networks [Faust et al., 1999; Canton and Litchfield, 2006]. CK2 phosphorylates membrane and cytoskeletal proteins including those involved in the regulation of the actin cytoskeleton [Ghosh et al., 2002; Cory et al., 2003] and microtubule dynamics [Lim et al., 2004; Carneiro et al., 2008].

We have previously shown that CK2 inhibitors significantly reduced retinal neovascularization and stem cell recruitment in the mouse model of oxygen-induced proliferative retinopathy (OIR) [Kramerov et al., 2006, 2008]. CK2 was immunolocalized in the retinal tissue, mostly in astrocytes and vascular endothelium, apparently in association with the cytoskeleton [Kramerov et al., 2006]. We showed immunohistochemically that CK2 and F-actin co-localized in stress fibers in cultured microvascular endothelial cells [Kramerov et al., 2011]. Importantly, highly specific CK2 inhibitors including 4,5,6,7-tetrabromobenzotriazole (TBB) and 3-(2,3,4,5-tetrabromophenyl)acrylic acid or tetrabromocinnamic acid (TBCA) caused dramatic shape changes (cytoplasmic retraction and cell rounding) in a number of cultured human cells, including astrocytes and microvascular endothelial cells [Kramerov et al., 2011]. Additionally, we found that serum alleviated CK2 inhibitor-induced cell rounding. The promoting effect of serum on cell spreading and adhesion is reportedly mediated by Rho/Rho-kinase (RhoK) activation of myosin II [Hall, 2005]. Therefore, we suggested that CK2 could play a role in the regulation of actin–myosin II-based contractility and cytoskeleton.

Here, we present data indicating that treatment with CK2 inhibitors correlated with disassembly of actomyosin stress fibers and cell shape changes, including cytoplasmic retraction and process formation. We also showed that suboptimal doses of specific inhibitors of RhoK and MLCK, kinases that phosphorylate myosin light chain (MLC) enhanced the effect of CK2 inhibition on cell shape, which was accompanied by decreased level of phospho-MLC, thus implying a CK2 role in regulation of actomyosin cytoskeleton.

METHODS

CK2 INHIBITOR TREATMENT AND IMMUNOSTAINING

Human optic nerve astrocytes (ONA), human brain microvascular (HBMVEC), and bovine retinal (BREC) endothelial cells were cultured as described elsewhere [Kramerov et al., 2011]. Rat bone marrow-derived late outgrowth endothelial cells (OEC) were a gift from Dr. M. Grant, and were obtained and cultured as described [Li Calzi et al., 2010]. Highly specific CK2 inhibitors TBB and TBCA (both from EMD Biosciences, San Diego, CA), dissolved in dimethylsulfoxide (DMSO; Sigma–Aldrich, St. Louis, MO) were added 1 day after passage of cultured cells at concentrations of 10–60 μM to the medium containing 0.1% fetal bovine serum (FBS). Two other protein kinase inhibitors (5-iodonaphthalene-1-sulfonyl)homopiperazine (ML7) and hydroxyfasudil (HF; both from EMD Biosciences) were added alone or simultaneously with the CK2 inhibitors, or in combination with 1-oleoyl-lysophosphatidic acid (Santa Cruz Biotechnology, Santa Cruz, CA). The cell shape changes were monitored using the inverted microscope (Nikon Diaphot) at 10× or 20× magnification, and images taken using Nikon digital camera D3100.

After 1–18 h of treatment at 37°C, cultured cells were fixed in 4% *p*-formaldehyde for 10 min, permeabilized in 0.1% Triton X-100 (Sigma–Aldrich), blocked in 5% normal goat serum, and incubated with mouse anti-CK2α/α′ antibody (D8E mAb, IgM) [Goueli et al., 1990], mouse anti-β-tubulin antibody (clone 2-28-33; Sigma–Aldrich), or rhodamine-phalloidin to visualize F-actin (Sigma–Aldrich) for 2 h, followed by cross-species adsorbed secondary antibodies conjugated with fluorescein (Millipore, Billerica, MA).

The images were captured with high-sensitivity 2-megapixel color digital MicroFire camera (Optronics, Goleta, CA) attached to a BX40 Olympus microscope (Olympus USA, Melville, NY) and were merged using MicroFire 2.1c software.

For Western blotting, cultured cells were extracted with lysis buffer containing proteinase and phosphatase inhibitors (1% SDS, 1% Triton X-100, 10 μg/ml aprotinin, 20 μM leupeptin, 1 mM E-64, 1 mM NaF, 200 μM sodium pervanadate, 1 mM dithiothreitol, 5 mM EDTA, 25 mM Tris, pH 6.8). Proteins were resolved by SDS–PAGE (8–16% gradient gels; Invitrogen, Carlsbad, CA) and transferred to nitrocellulose membranes (Invitrogen) for immunodetection as previously described [Kramerov et al., 2006, 2011]. Gel loading was normalized by β-actin and total MLC content using a monoclonal antibody (clone AC-74; Sigma–Aldrich) and rabbit polyclonal antibodies (#3672; Cell Signaling, Danvers, MA), respectively. For analyses of protein phosphorylation, rabbit polyclonal antibodies to phospho-Ser19-MLC (#3671; Cell Signaling) were used, and immune reaction was revealed with alkaline phosphatase-conjugated secondary antibodies (Millipore).

RESULTS

CK2 INHIBITOR-INDUCED CELL SHAPE CHANGE IS ACCOMPANIED BY DISASSEMBLY OF STRESS FIBERS

Previously, we observed dramatic shape changes (cytoplasmic retraction and cell rounding) caused by treatment of cultured cells with specific CK2 inhibitors [Kramerov et al., 2011]. We also have for the first time immunolocalized CK2 to F-actin-containing stress fibers in microvascular endothelial cells (HBMVEC), indicating that CK2 may play a role in actin cytoskeleton regulation. Thus, a connection of CK2 localization to stress fibers and normal cell shape may be proposed and tested by analyzing changes in this localization after CK2 inhibition. In cultured human ONA, CK2 localization to stress fibers (Fig. 1a–c) was affected by administration of TBB or TBCA. At the early stage of CK2 inhibitor treatment (1 h), we observed both a decrease of CK2 associated with stress fibers and their disorganization leading to punctate pattern of F-actin (Figs. 1d and 2a), whereas microtubules and cell morphology

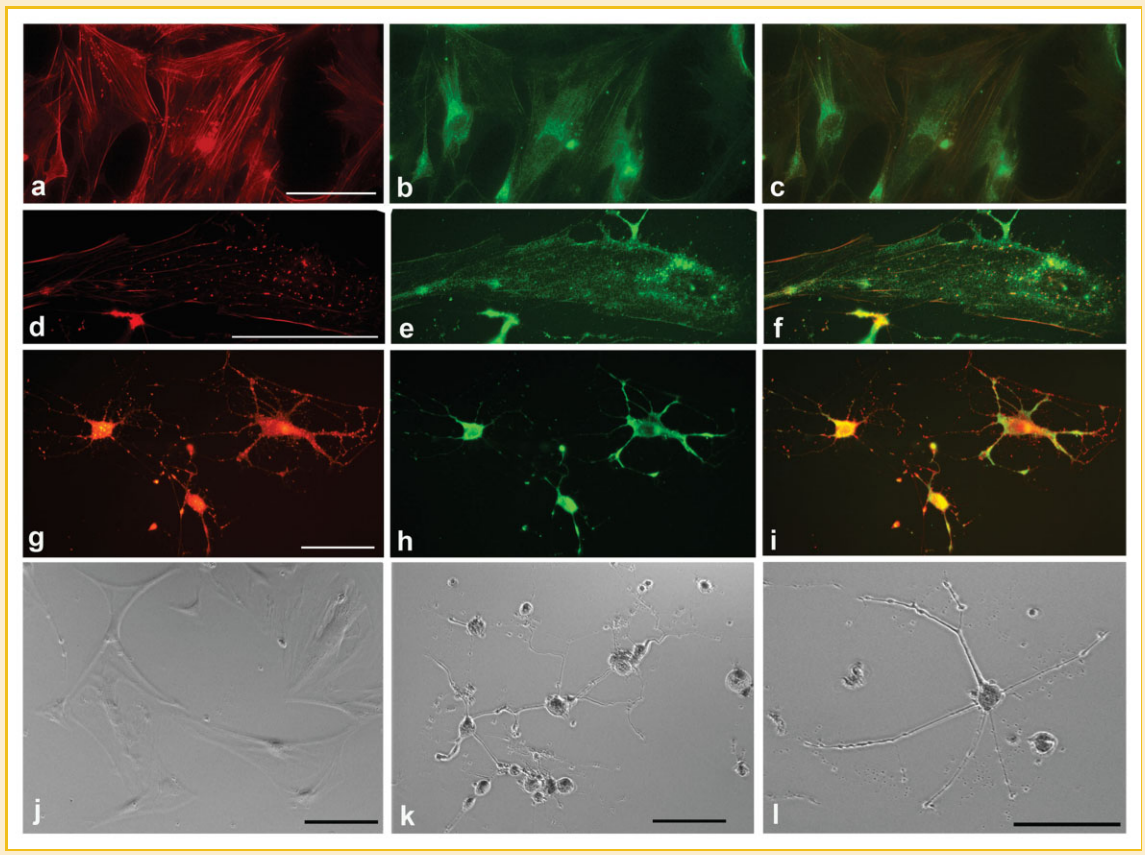

Fig. 1. Association of CK2 with F-actin in stress fibers (a–c) is disrupted after CK2 inhibition by TBCA (60 μM; d–i) and TBB (60 μM; k,l). Cultured ONA were immunostained by rhodamine-phalloidin for F-actin (a,d,g) or anti-CK2α/α′ antibody (b,e,h). Co-localization of CK2 and F-actin in control cells (c) is dramatically reduced even at the early stage of CK2 inhibitor treatment (f) when stress fibers are being dissolved (1 h). Longer TBCA or TBB treatment (3–6 h) caused severe contraction of cell bodies (g–i), followed by rounding of cells that retain long "varicose" processes (k,l). The bars correspond to 100 μm.

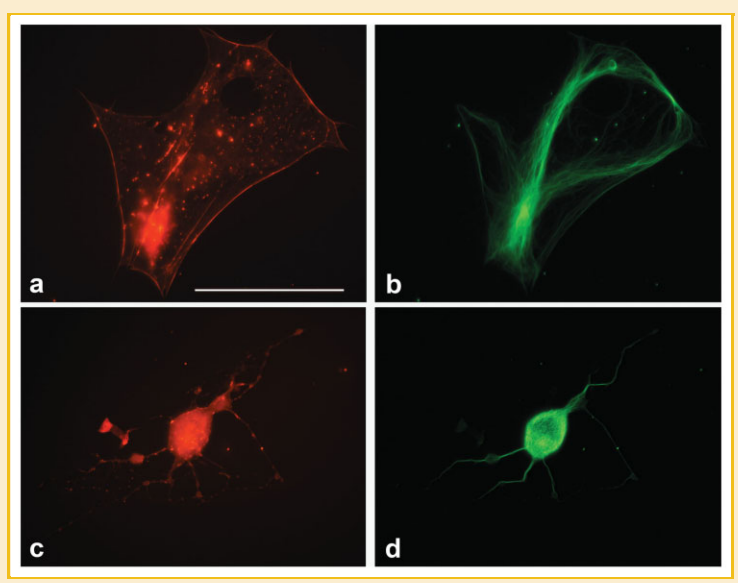

Fig. 2. Cytoskeleton changes induced by CK2 inhibition in ONA. At the early stage of TBCA (60 μM) treatment (1 h), cell shape has not yet changed, and actin filaments became disorganized and display a characteristic punctate staining with phalloidin (a), whereas microtubules appeared largely unaffected (b). At later stage (3–6 h), when cell dramatically retracted and became shrunk and rounded with remaining thin processes, both actin (c) and tubulin (d) cytoskeletal networks appeared collapsed and fully disorganized. The bar corresponds to 100 μm.

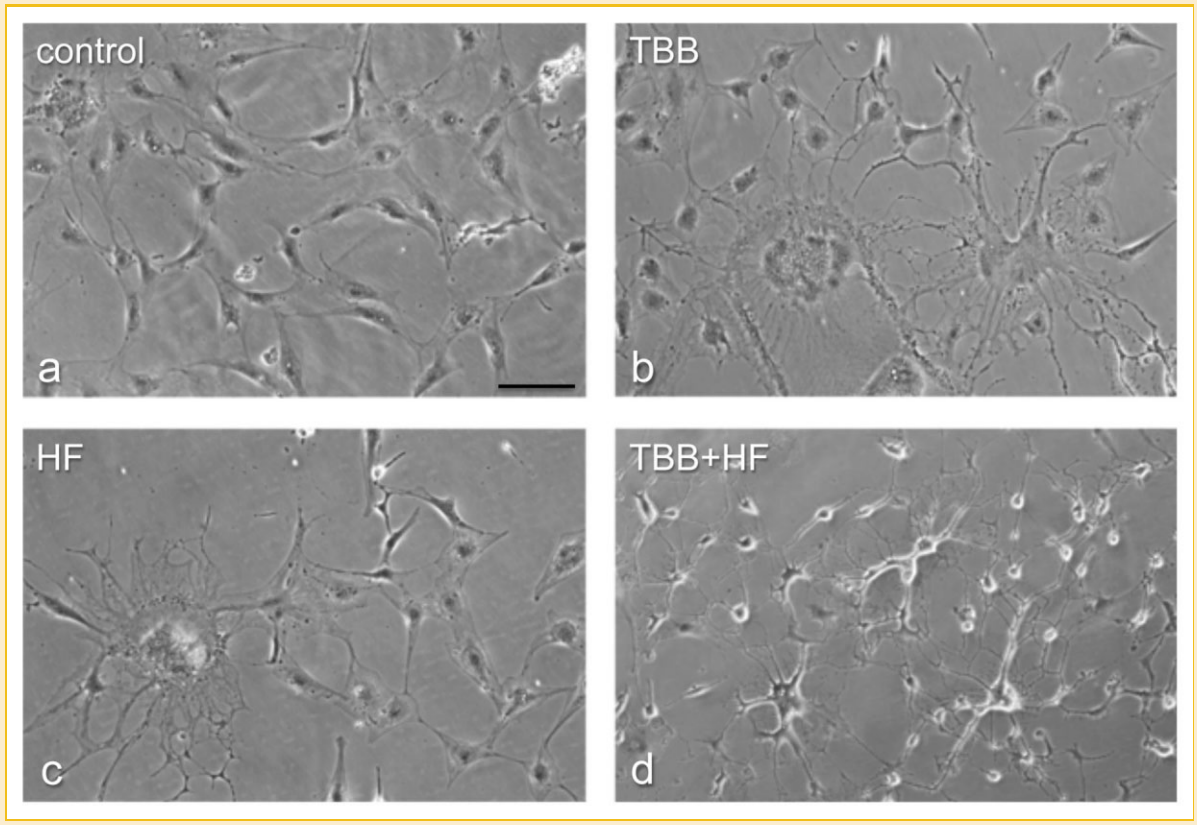

Fig. 3. Cell shape changes induced by combined action of sub-optimal doses of inhibitors of CK2 and RhoK in BREC: a, control (DMSO-treated) cells; b, cells treated with TBB (20 μM); c, HF-treated (10 μM); and d, combined treatment with TBB and HF. The bar corresponds to 100 μm.

remained mainly unaffected (Fig. 2b). After longer treatment (3–6 h), dramatic retraction of the cytoplasm occurred, whereas stress fibers were no longer detectable, as well as CK2 associated with them. Instead, both F-actin and CK2 displayed a punctate pattern of immunolocalization (Fig. 1g–i) in the contracted cell body and "varicose" processes formed due to cytoplasmic retraction. As retraction proceeded, cells with contracted bodies became rounded with collapsed cytoskeleton and thin processes (Figs. 1k,l and 2c,d). Similar results were obtained after TBCA (or TBB) treatment of cultured endothelial cells (HBMVEC, BREC) and OEC (not shown). These data indicate that F-actin, rather than microtubules, is primarily involved in CK2-inhibitor-induced cell shape changes, and suggest a correlation between CK2 localization to F-actin-containing stress fibers and normal cell morphology.

RHO- AND MLC-KINASE INHIBITORS ENHANCE MORPHOLOGICAL ALTERATIONS INDUCED BY SUB-OPTIMAL DOSE OF CK2 INHIBITORS

Stress fiber formation is mainly regulated by phosphorylation of MLC that leads to an increase in myosin II activity, which cross-links actin filaments and generates contractile force. MLC kinase directly phosphorylates MLC, whereas RhoK also phosphorylates and inactivates MLC phosphatase (MLCP), thus increasing phosphorylation of MLC [Tan and Leung, 2009]. To test for possible involvement of CK2 in the regulation of actomyosin contractility, a combined treatment with CK2 inhibitor TBCA and specific inhibitors of RhoK (HF) or MLC kinase (ML7) was used.

When used at suboptimal doses and at low serum concentration (0.1% or less), neither TBB (20 μM) nor HF (10 μM) caused a significant morphological effect in BREC, that is, cell body retraction (Fig. 3). Incomplete inhibition of protein kinases by low doses of the inhibitors may result in only partial decrease in MLC phosphorylation and limited disassembly of actomyosin stress fibers required for maintaining flattened cell morphology. As a result, cell shape was minimally affected. However, a combination of suboptimal doses of TBB and HF very rapidly (1–2 h) elicited a dramatic morphologic transformation of spread cells into cells with retracted body and extended processes (Fig. 3d), an effect very similar to a characteristic cell response to CK2 inhibition, or to high doses of either HF or ML7 (not shown). Longer treatment by combinations of TBB (or TBCA) with ML7 or HF (6–15 h) resulted in complete rounding of the cells (not shown). Similar additive effects of combined treatment with TBCA and HF or ML7 were observed for other cell types, including OEC (Fig. 4) and HBMVEC (Fig. 5), suggesting the general nature of this phenomenon. Such a potentiating effect may be interpreted either as complementation of CK2 inhibitor and the inhibitors of RhoK (or MLCK) in suppressing MLC phosphorylation, or as their targeting of non-overlapping pathways of cell shape regulation. Interestingly, lysophosphatidic acid (LPA) that stimulates Rho signaling and phosphorylation of MLC, when added together with

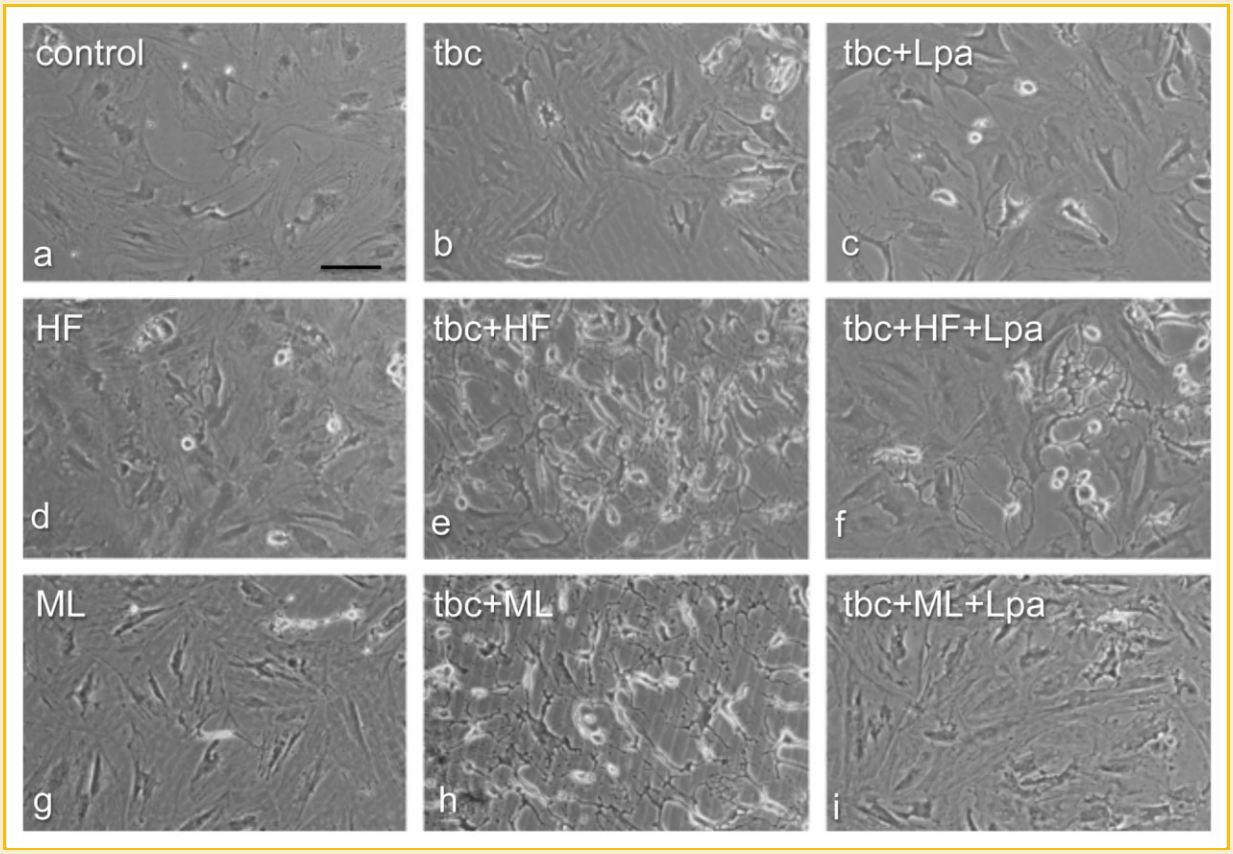

Fig. 4. Morphological alterations in OEC treated with: a, DMSO; b, TBCA (30 μM); c, TBCA + LPA; d, HF (10 μM); e, HF + TBCA; f, HF + TBCA + LPA; g, ML7 (15 μM); h, ML7 + TBCA; i, ML7 + TBCA + LPA. Sub-optimal concentrations of each inhibitor alone (b,d,g) did not elicit significant cell shape changes, whereas combined treatment lead to striking retraction of cytoplasm and process formation (e,h) that was counteracted by simultaneous treatment with Rho-activator LPA (40 μM; c,f,i). The bar corresponds to 100 μm.

TBCA and HF (or ML7), significantly suppressed the dramatic morphological changes as a result of the combined use of inhibitors (Figs. 4 and 5).

MORPHOLOGICAL EFFECT OF COMBINED SUB-OPTIMAL DOSES OF CK2 AND MLC KINASES INHIBITORS CORRELATES WITH DECREASED LEVEL OF MLC PHOSPHORYLATION

A possible link between the morphologic effect of the inhibitors and the level of MLC phosphorylation was confirmed by Western analysis with anti-pSer19-MLC antibody that binds to the main phosphorylation site in MLC (pMLC). Either inhibitor of MLC phosphorylation (HF or ML7) did not significantly reduce it when used at low sub-optimal concentrations (10 and 15 μM, respectively). However, simultaneous treatment by sub-optimal doses of HF, or ML7, and a CK2 inhibitor (20 μM TBCA or TBB) resulted in a significantly decreased level of pMLC (about half of the control, or of either inhibitor alone; Fig. 6). LPA, when added together with HF (or ML7) and TBCA (or TBB), not only reversed morphological changes caused by combined inhibitors in OEC (Fig. 4) or HBMVEC (Fig. 5), but also nearly restored the level of pMLC (OEC, Fig. 6) or HBMVEC (not shown). These data suggest an association between the changes in morphology and the level of pMLC, as well as CK2 involvement in the regulation of actomyosin contractility.

DISCUSSION

Morphological changes elicited by specific CK2 inhibitors comprise an essential part of the data presented in this study, and may need verification by other methods. Indeed, a significant number of studies using down-regulation of CK2, such as siRNA silencing or overexpression of dominant negative variants of CK2, played an important role in corroborating results obtained with pharmacological inhibitors [Guerra, 2006; Götz et al., 2007; Ponce et al., 2011]. However, most of the published papers in the field report on using only chemical inhibitors, indicating that specific CK2 inhibitors are widely considered as a reliable means to prove CK2 involvement in the regulation of various cellular functions.

In our study, which focused on cell shape alterations after CK2 inhibition, there appears to be an important distinction between using chemical inhibitors and CK2 down-regulation. We investigated CK2 inhibitor-induced cell shape change – an early effect that can be observed within 1–2 h after administration of the inhibitors. Short exposure to CK2 inhibitors (TBB and TBCA) capable to

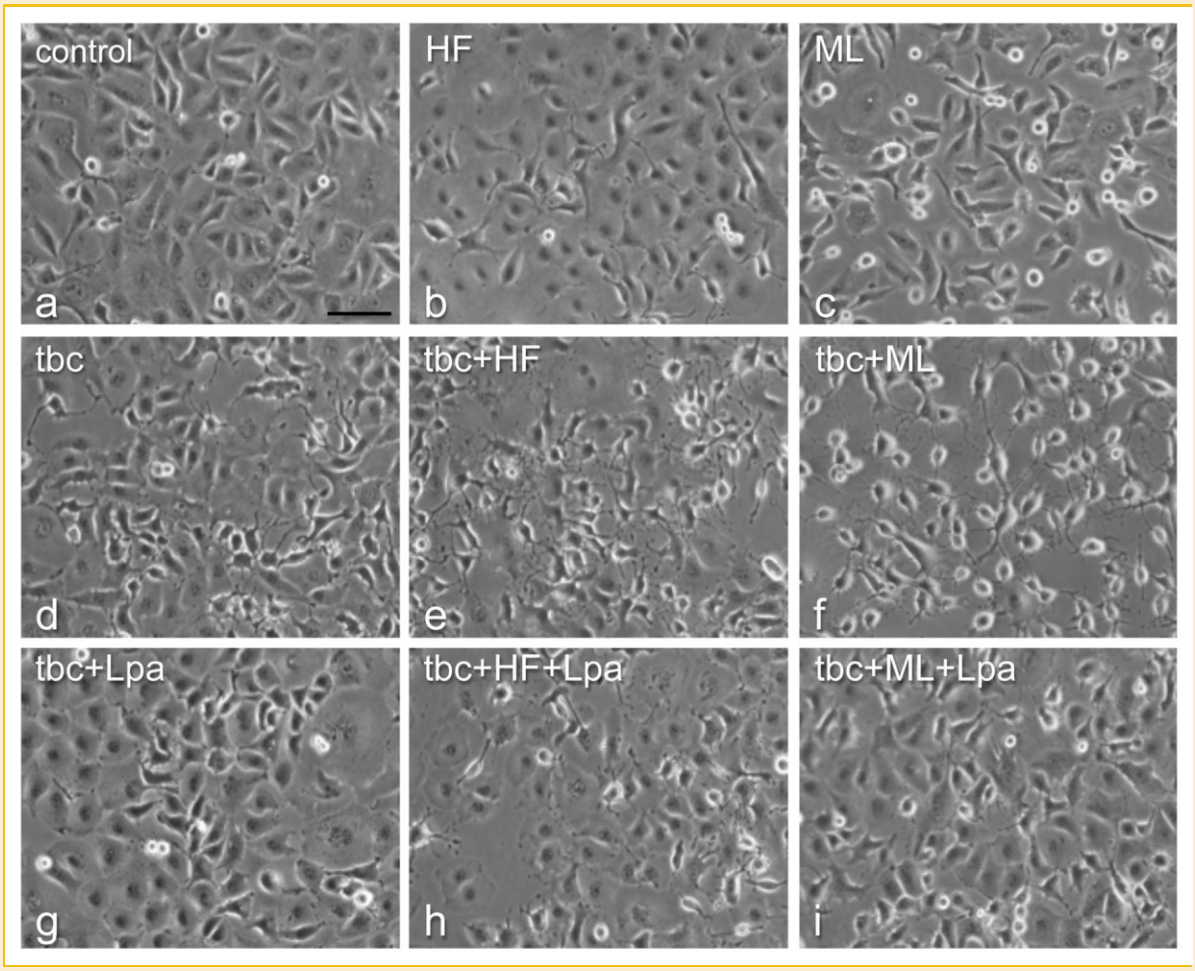

Fig. 5. Morphological alterations in HBMVEC treated with: a, DMSO; b, HF (10 μM); c, ML7 (15 μM); d, TBCA (20 μM); e, HF + TBCA; f, ML7 + TBCA; g, TBCA + LPA; h, HF + TBCA + LPA; i, ML7 + TBCA + LPA. Each inhibitor alone when added at sub-optimal dose (b–d) caused rather weak cell shape changes, whereas a combined treatment of each MLC kinase inhibitor with TBCA lead to striking stellation-like transformation (e,f) that was nearly abolished by LPA (40 μM, g–i). The bar corresponds to 100 μm.

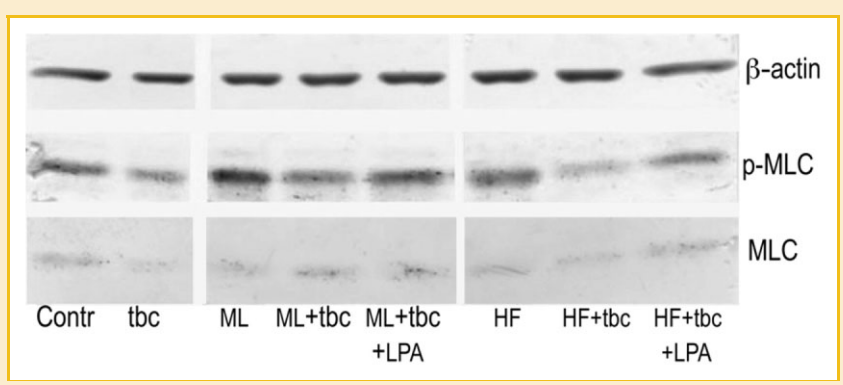

Fig. 6. Immunoblot analysis with anti-pSer19-MLC of OEC treated with inhibitors of RhoK (HF, 10 μM), MLCK (ML, 15 μM), and CK2 (TBC, 20 μM) or their combinations, and with activator of RhoA, LPA (40 μM). Antibodies to total MLC and β-actin were used to ensure equal lane loading. The Western blot is representative of three independent experiments.

promptly suppress up to 90% of CK2 activity (Pagano et al., 2007), was sufficient to elicit rapid morphological and cytoskeletal alterations. This result is most likely caused by modulation of the phosphorylation status of CK2 substrates rather than by influencing CK2-dependent transcriptional processes.

On the contrary, CK2 down-regulation using genetic means usually required a rather long time (3–6 days) to take effect (Smolock et al., 2007; Wang and Jang, 2009). Moreover, previous published work on dominant-negative down-regulation of CK2 catalytic subunits achieved only partial effect on CK2 activity. The residual CK2 activity in the cells with the kinase inactive mutants in an in vitro phosphorylation reaction turned out to be from 60% (Götz et al., 2007) to 70% [Schneider et al., 2011] of the original activity after 72 h of transfection, which may not be suitable for our study. Likewise, in our experiments on HBMVE cells with Morpholino antisense oligonucleotides, after 3 days of treatment we obtained a similarly moderate suppression of CK2 catalytic α- and α′-subunits (up to 30% decrease) that was not sufficient to induce cell shape changes (data not shown). Therefore, pharmacological inhibition appears to be indispensable while studying a possible role of CK2 in *early* morphological changes requiring rapid and nearly complete depletion of its activity.

Our data on human cultured astrocytes and vascular endothelial cells show that (a) CK2 associates with cytoskeleton, including stress fibers; (b) CK2 inhibition correlates with stress fiber disappearance and cell retraction with eventual rounding; and (c) CK2 may be involved in the control of cytoskeletal organization by regulating actomyosin interactions via MLC phosphorylation. These results suggest an important role of CK2 in cell motility, which may account for suppressing effect of CK2 inhibition on retinal neovascularization in the mouse OIR model.

Functional activity in vivo of various cells, such as astrocytes, pituicytes, or renal glomerular podocytes, is associated with "stellate" morphology characterized by the formation of branching extensions that allow for increased cell surface and numerous cell contacts. Astrocyte stellation represents one of the most striking examples of the importance of cytoskeletal organization for astrocyte function. In developing retinal vasculature, when astrocytes migrate away from the vessels and become hypoxic, they change from bipolar to stellate form, cease migration, and start to express VEGF causing blood vessel growth. The vessels bring oxygen and relieve the hypoxia stabilizing vessel growth and allowing migration of astrocytes that again transform from stellate to bipolar form [Zhang and Stone, 1997; Zhang et al., 1999]. Thus, hypoxia appears to be an important physiological factor regulating cell shape and migration of astrocytes during retinal vascularization.

Stellation can be induced in vitro by various factors. In all cases, it is accompanied by a loss of actomyosin stress fibers and focal adhesions and by cytoplasmic retraction, which is mainly driven by actin filaments depolymerization. Depending on stimuli used, many cultured cells, including astrocytes, pituicytes, and osteoblasts, alternate between a flat, fusiform morphology, and a stellate appearance characterized by a shrunk and rounded cell body with extended branching processes. In the presence of even small amount (0.02%) of serum, these cells adopt a flat polygonal shape, apparently due to serum component LPA that activates stress fiber-inducing RhoA GTPase [Kranenburg and Moolenaar, 2001]. Stellation then can be induced by adenosine, its derivative cAMP or agents that raise cAMP levels, such as β-adrenergic agonists [Rosso et al., 2002], through the inhibition of PI3K/RhoA pathway causing stress fiber depolymerization [Perez et al., 2005].

Our data for the first time implicate protein kinase CK2 in stellation-like morphological transformation. Moreover, CK2 inhibitor-induced "stellation" occurs not only in astrocytes, but also in other cell types, including microvascular endothelial and late outgrowth endothelial precursor cells. However, these changes occur by the retraction of cytoplasm leaving processes behind, rather than by the growth of new processes following initial cytoplasmic retraction, as was previously described for astrocytes in vivo and for cAMP-induced astrocyte stellation [Baorto et al., 1992]. Interestingly, preceding the TBB-induced retraction, a decrease of CK2 associated with stress fibers and their disorganization leading to punctate localization of F-actin occurs (Figs. 1d and 2a), whereas microtubules and cell morphology remain mainly unaffected. Upon dramatic retraction of the cytoplasm, stress fibers are no longer detectable, as well as CK2 associated with them, and the cells become round with collapsed cytoskeleton and thin processes (Figs. 1 and 2). These data indicate that F-actin, rather than microtubules, is primarily involved in TBB-induced cell shape changes, and suggest a correlation between CK2 localization to F-actin-containing stress fibers and normal cell morphology.

Stress fiber formation is mainly regulated by phosphorylation of the regulatory MLC that leads to cross-linking of actin filaments and generating of contractile force. Possible involvement of CK2 in actomyosin contractility was examined by combined treatment with CK2 inhibitor TBCA and specific inhibitors of RhoK or MLCK that phosphorylate MLC. We observed a striking cumulative effect (synergy) of the combined treatment by the inhibitors of CK2 and RhoK or MLCK during transformation to stellate morphology. This result suggests that CK2 may regulate cell shape by controlling MLC phosphorylation. That was corroborated by Western blot analyses that showed a correlation between the inhibitor-induced cell shape changes and decreased level of pMLC.

Previous reports on the important role of MLC phosphorylation in the "flat to stellate morphology" transition implicate three Rho-regulated MLC kinases—RhoK, MLCK, and MRCK [Tan and Leung, 2009]. MRCK (a myotonic dystrophy kinase-related Cdc42-binding kinase) is involved in phosphorylation of MLC responsible for reversal of the cAMP-induced stellate morphology in human neuroblastoma cells [Dong et al., 2002]. It has also been reported that cultured astrocytes can be induced to stellate by inhibitors of MLCK [Baorto et al., 1992; Miyata et al., 1999] and RhoK [Rosso et al., 2002; Abe and Misawa, 2003; Rosso et al., 2007], which is accompanied by a decrease in phosphorylation of MLC and activation of actin depolymerizing factor (ADF), resulting in the disappearance of stress fibers [Rosso et al., 2002] and actin depolymerization [Baorto et al., 1992; Padmanabhan and Shelanski, 1998]. These morphological changes are highly similar of those observed in our studies implicating CK2 in the regulation of actomyosin cytoskeleton.

It remains unclear whether there is a correlation between stellation and cell migration. Some data indicate that migrating

retinal astrocytes lose their stellate morphology during development of retinal vasculature [Zhang and Stone, 1997; Zhang et al., 1999]. However, inhibitors of RhoA (C3 transferase) and RhoK (Y27632) that prompted stellation of cultured astrocytes, were shown to accelerate wound closure due to enhanced polarized process formation and increased migratory activity into the lesion site [Höltje et al., 2005]. Also, RhoK inhibitors increased the migration of cultured human osteoblasts and induced a stellate appearance with poor assembly of stress fibers and focal adhesions [Zhang et al., 2011]. It is possible that weak or moderate stellate-inducing stimuli increase plasticity of the actomyosin cytoskeleton by reorganizing it from thick and stable stress fibers to thin and more dynamic ones, thus increasing cell migration, whereas the stimuli that strongly inhibit F-actin contractility and cause stress fibers to dissolve compromise cell motility.

Previously, it was shown that in the wound migration assay, growth factors increased BRECs migration more than threefold, and CK2 inhibitors significantly reduced this effect [Ljubimov et al., 2004]. The cell shape changes were not observed in those experiments, presumably, due to the presence of a fivefold higher concentration (0.5%) of fetal calf serum that promotes cell spreading and migration, and counteracts cell rounding, as we have recently published [Kramerov et al., 2011].

Our data indicate that despite their distinct roles in cell motility [Totsukawa et al., 2004], RhoK and MLCK similarly cooperate with CK2 to repress stellate morphology. Therefore, it will be interesting to study whether the effects of CK2 inhibition on cell migration [Ljubimov et al., 2004] might be modified by combined treatment with inhibitors of MLC phosphorylation.

In conclusion, CK2 inhibition in cultured human astrocytes and vascular endothelial cells causes dramatic stellation-like morphological alteration and reorganization of actomyosin cytoskeleton, which presumably may alter their adhesive properties and migratory ability. Similar changes in retinal astrocytes and/or vascular endothelial cells may underlie the previously reported anti-angiogenic effect of CK2 inhibition in vivo.

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