Inhibition of myogenesis enables adipogenic trans-differentiation in the C2C12 myogenic cell line

Karen Yeow[a], Blaine Phillips[b], Christian Dani[b], Candice Cabane[a], Ez Zoubir Amri[b], Benoît Dérijard[a,*]

[a] *CNRS UMR 6548, Laboratory of Cellular and Molecular Physiology, Faculty of Sciences, University of Nice-Sophia Antipolis, Parc Valrose, 06108 Nice Cedex 2, France*
[b] *CNRS UMR 6543, Centre de Biochimie, Faculty of Sciences, University of Nice-Sophia Antipolis, Parc Valrose, 06108 Nice Cedex 2, France*

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Abstract C2C12 cells are a well-established model system for studying myogenesis. We examined whether inhibiting the process of myogenesis via expression of dominant negative (DN) mitogen-activated protein kinase kinase-3 (MKK3) facilitated the trans-differentiation of these cells into adipocytes. Cells expressing DN MKK3 respond to rosiglitazone, resulting in adipocyte formation. The effects of rosiglitazone appear to be potentiated through peroxisome proliferator activating receptor-γ. This trans-differentiation is inhibited by the use of the phosphoinositide-3 (PI3) kinase inhibitor, LY294002. These results indicate that preventing myogenesis through expression of DN MKK3 facilitates adipocytic trans-differentiation, and involves PI3 kinase signalling.

Key words: Trans-differentiation; Adipogenesis; Myogenesis; C2C12; Mitogen-activated protein kinase kinase-3; Peroxisome proliferator activating receptor-γ

1. Introduction

The formation of muscle, bone, and adipose tissue are complex processes involving the determination of common precursor cells towards specific differentiation pathways. A current area of interest is the determination of factors that are able to promote the transition between one differentiated lineage to another, in particular, the trans-differentiation between muscle and adipose tissue. The imbalance between adipose and muscle mass is a hallmark of many disorders, including type 2 diabetes, obesity, and sarcopenia [1–3]. Skeletal muscle and adipose tissue are key players in the development of insulin resistance, manifested by decreased insulin-stimulated glucose transport and metabolism in these tissues [1]. It is known that myogenic cell lines and muscle satellite cells are capable of trans-differentiating into adipocytes, among other cell types [4,5]. Thus, the study of trans-differentiation of muscle tissue into fat is highly applicable to the study of disorders where muscle atrophy and increase in adipose tissue mass are correlated.

The development of skeletal muscle is controlled by the muscle regulatory factors, such as myf-5 [6], myogenin [7], and MyoD [8]. Fusion of myocytes into multinucleated myotubes is the terminal step of muscle differentiation. In vitro, the major steps in muscle differentiation can be reproduced with myoblastic cell lines such as C2C12 murine myoblast cells [9]. Differentiation of adipocytes appears to be controlled by peroxisome proliferator activating receptor-γ (PPARγ) and the C/EBP families of transcription factors [10]. PPARγ, a nuclear hormone receptor, is expressed primarily in adipose tissue, and is induced early in the process of adipose differentiation [10]. Fatty acids, as well as the insulin-sensitizing agents thiazolidinediones, are known to promote adipogenesis by activating PPARγ [11,12]. This results in the upregulation of adipocyte-specific genes such as adipocyte lipid-binding protein 2 (aP2), Glut4, and fatty acid transporter [10,13].

The mitogen-activated protein (MAP) kinase signalling pathways are crucial for the processes of myogenesis and adipogenesis, in particular, the MAP kinase kinase-3 (MKK3)/p38 pathway [14,15]. However, little is known regarding the roles of these signalling pathways in the trans-differentiation of myoblasts to adipocytes. In this study, we report that blocking myotube formation in C2C12 cells via the expression of dominant negative (DN) MKK3 enables these cells to trans-differentiate into adipocytes in response to rosiglitazone. In addition, we show that the process of trans-differentiation in these cells is characterized by upregulation of PPARγ, and involves the kinase phosphoinositide-3 (PI3) kinase pathway.

2. Materials and methods

2.1. Chemicals and materials

Cell culture reagents were purchased from Life Technologies (Cergy Pontoise, France). SB203580 was obtained from Calbiochem (La Jolla, CA, USA), and LY294002 and insulin from Sigma (Saint Quentin Fallavier, France). Rosiglitazone (CD3145 or BRL49653) was obtained from Galderma (France). The mouse monoclonal anti-myogenin antibody was purchased from Pharmingen (Le Pont de Claix, France). The anti-aP2 rabbit polyclonal antibody was a gift of Dr. David Bernlohr (University of Minnesota, St. Paul, MN, USA).

2.2. Cell culture and rosiglitazone treatment

Mouse C2C12 myoblasts (ATCC number CRL-1772) were cultured at 37°C in an atmosphere of 5% CO_2 in growth medium consisting of Dulbecco's modified Eagle's medium supplemented with 10% fetal calf

*Corresponding author. Fax: (33)-4-92 07 68 50.
E-mail address: derijard@unice.fr (B. Dérijard).

Abbreviations: aP2, adipocyte lipid-binding protein 2; CA, constitutively active; DN, dominant negative; MAP, mitogen-activated protein; MKK3, mitogen-activated protein kinase kinase-3; PI3 kinase, phosphoinositide-3 kinase; PKC, protein kinase C; PPAR, peroxisome proliferator activating receptor

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serum and 1% v/v penicillin/streptomycin. The DN MKK3 C2C12 cell line has been previously described [16]. To induce trans-differentiation, cells were placed in medium containing rosiglitazone (concentrations stated in figure legends) 2 days before confluence, and maintained up to 5 days post-confluence. All experiments were performed using three separate clones of each cell line.

2.3. Western blots

Cells were treated as indicated in the figures, rinsed, and proteins were solubilized in ice-cold lysis buffer (50 mM HEPES, pH 7.4, 150 mM NaCl, 100 mM NaF, 10 mM EDTA, 10 mM $Na_4P_2O_7$, 2 mM Na_3VO_4, 1% Triton X-100, and supplemented with protease inhibitors: aprotinin (2 μg/ml), leupeptin (10 μM) and AEBSF (1 mM)). Equal amounts of protein were separated by SDS–PAGE on a 10% acrylamide gel. Proteins were transferred to Hybond-C Extra membrane (Amersham), and the membranes were blocked for 1 h at room temperature in TBS, 0.1% Tween 20, 5% bovine serum albumin (BSA). The membranes were washed in wash buffer (TBS, 0.1% Tween 20) for 30 min, probed with the primary antibody and then secondary antibody for 1 h in TBS, 0.1% Tween 20, 1% BSA. After each incubation, membranes were washed for 30 min with three changes of wash buffer. Proteins were visualized using the Amersham ECL® system. Blots were scanned and quantification was performed using PCBas®.

2.4. Northern blots

RNA was isolated using the Tri-Insta Pure® kit (Eurogentec, France), according to manufacturer's directions. Northern blots were performed as previously described [17]. The filters were probed using a labelled, full-length PPARγ cDNA, as described in [18].

2.5. Oil red O staining

Oil red O staining was performed as previously described [17]. Cells were photographed using a Nikon Coolpix 990 digital camera mounted on a Leitz Wetzlar light microscope.

3. Results

3.1. C2C12 cells expressing DN MKK3 trans-differentiate into adipocytes upon treatment with rosiglitazone

We have previously shown that expression of DN MKK3 inhibits myotube formation and results in significantly decreased p38 activity [16]. We were interested in determining whether blocking myogenesis in C2C12 cells in this manner enabled the trans-differentiation of these cells into other lineages. Control experiments were performed using wild-type untransfected cells, as well as the empty vector control cell line. All experiments were performed using three separate clones of each cell line, including the DN MKK3 C2C12 line. Representative results are shown in the figures. To induce adipogenesis, we used rosiglitazone (also known as CD3145 or BRL49653), a specific PPARγ ligand able to induce adipogenesis in a variety of cell types, including myocytes [19]. The glitazones are known agonists of PPARγ, a master gene involved in the regulation of adipocyte-specific gene expression [11].

Treatment of DN MKK3 C2C12 cells with rosiglitazone resulted in the differentiation of adipocytes after 5 days (Fig. 1). In keeping with previous results on this cell line, the DN MKK3 C2C12 cells did not differentiate into myo-

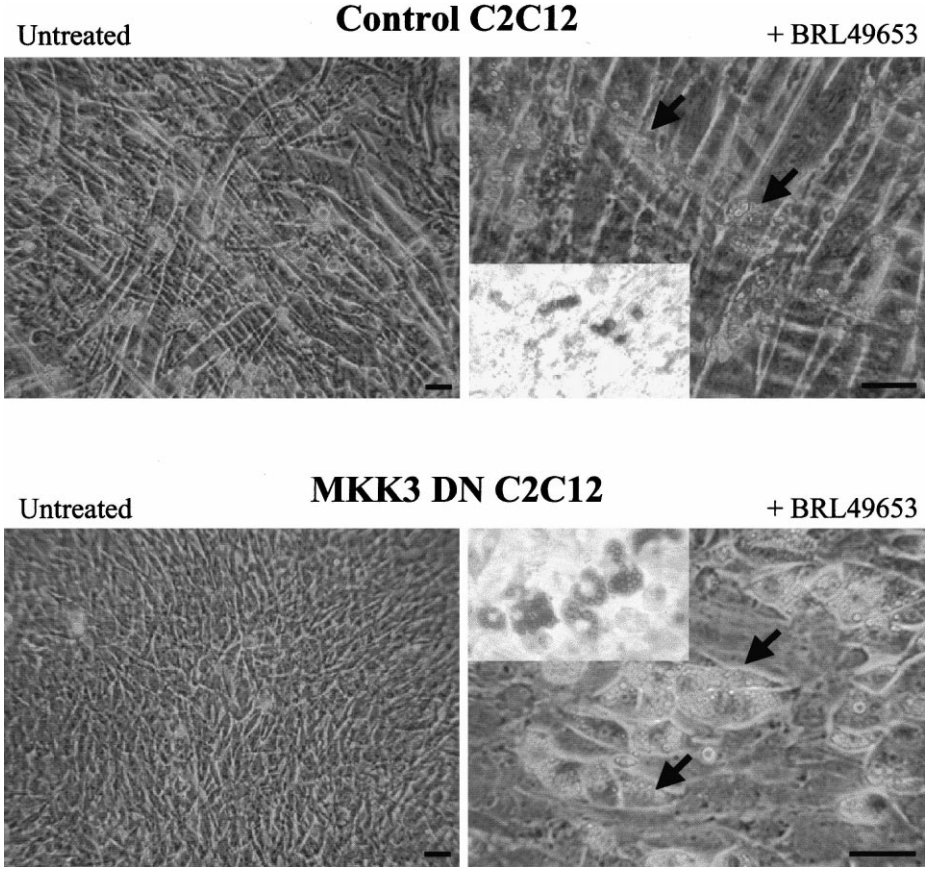

Fig. 1. MKK3 DN C2C12 cells differentiate into adipocytes upon treatment with rosiglitazone (BRL). Control and MKK3 DN C2C12 cells were grown in growth media, and treated with 5 μM rosiglitazone 2 days before confluence. The cells were maintained in medium containing rosiglitazone for up to 5 days post-confluence. Insets depict Oil red O staining at 5 days post-confluence. Arrows indicate the location of oil droplets. Scale bar = 10 μm.

tubes [14,16]. Control cells differentiated mainly into myotubes, whereas the DN MKK3 line displayed areas of rounded cells that contained lipid droplets. The rounded, lipid-containing cells appeared in clusters throughout the confluent monolayer of DN MKK3 cells beginning at 3 days post-confluence, with the number of adipocytic cells increasing over time. Oil red O staining for neutral triglycerides revealed only a few oil droplets in the control cells, whereas the DN MKK3 cells exhibited large regions of cells containing oil droplets. Representative fields are depicted in Fig. 1.

3.2. *Upregulation of PPARγ mRNA and the aP2 protein in the DN MKK3 cells upon rosiglitazone treatment*

Since PPARγ is known to play a major role in regulating adipogenesis, we examined whether this gene was upregulated in the DN MKK3 C2C12 cells. We performed Northern blots using RNA harvested from control and DN MKK3 cells treated with rosiglitazone. Both the control and DN MKK3 cells exhibited low basal levels of PPARγ mRNA, as seen in the untreated lanes (Fig. 2). The control C2C12 cells did not show an increase in PPARγ mRNA upon treatment with rosiglitazone. In contrast, a dramatic increase in PPARγ mRNA was seen in the DN MKK3 cells. There appeared to be two closely migrating bands in the rosiglitazone-treated lane, corresponding to the mRNA of the two different isoforms, PPARγ1 and PPARγ2. The high levels of PPARγ mRNA may reflect the large numbers of adipocytes present in the DN MKK3 cells after rosiglitazone treatment.

Analysis of protein extracts from the control and DN MKK3 cells by Western blotting revealed the expression of the adipocyte-specific marker aP2 in the DN MKK3 cells after 5 days treatment with rosiglitazone (Fig. 3A). In addition, levels of aP2 expression appeared to increase in a dose-responsive manner with increasing rosiglitazone concentration (Fig. 3B). No aP2 protein was detected in protein extracts from control cells. In contrast, the control extracts contained high levels of myogenin, reflecting their myogenic phenotype (Fig. 3A). The levels of myogenin were the same with and without rosiglitazone treatment, suggesting that treatment with rosiglitazone had no effect on the expression of this myogenic marker. The DN MKK3 cells expressed negligible amounts of myogenin, in keeping with their lack of ability to differentiate into myotubes [16].

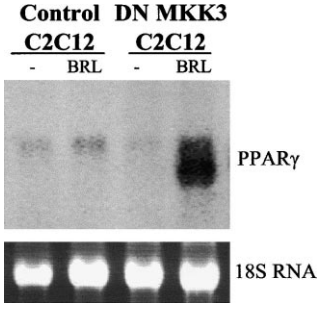

Fig. 2. Expression of PPARγ mRNA in C2C12 control and MKK3 DN cells. RNA was harvested from untreated cells, and cells treated with 5 μM rosiglitazone (BRL) from 2 days before confluence to 5 days post-confluence, and analyzed by Northern blotting using a PPARγ insert as a probe. RNA was harvested at day 5 post-confluence.

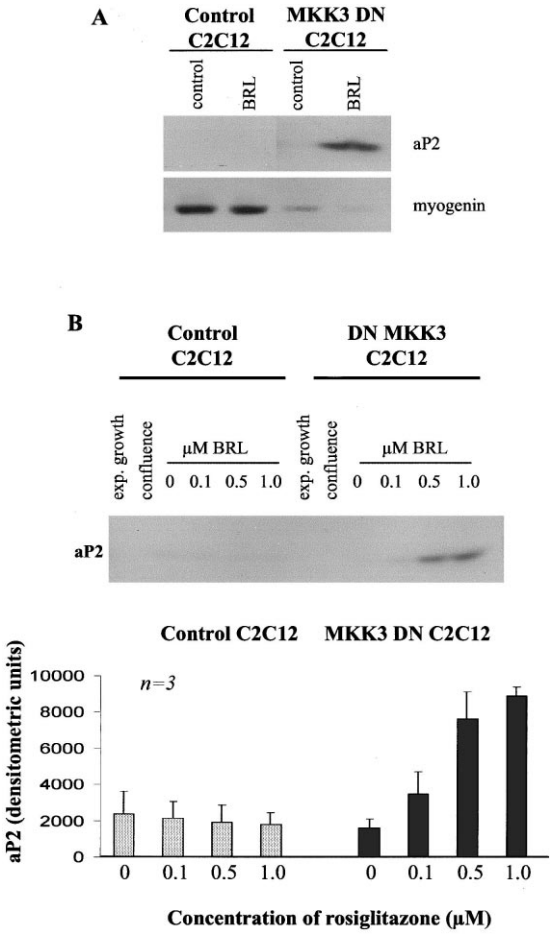

Fig. 3. Expression of adipogenic markers by MKK3 DN C2C12 cells in response to rosiglitazone treatment. A: Protein extracts were harvested from cells after treatment with 5 μM rosiglitazone for up to 5 days post-confluence, and analyzed by immunoblotting using antibodies against the adipogenic protein aP2 and the myogenic protein myogenin. B: The dose-dependence of aP2 protein expression upon rosiglitazone concentration. Control and MKK3 DN C2C12 cells were treated with increasing concentrations of rosiglitazone from 2 days before confluence to 5 days post-confluence. Protein extracts were harvested at day 5, and analyzed by immunoblotting with a monoclonal anti-aP2 antibody. The intensity of the bands were quantified using PCBas®, and expressed as arbitrary units. Error bars represent the standard deviation of three independent experiments with three separate clones of each cell line.

3.3. *Use of the inhibitors SB203580 and PD98059 do not affect the ability of C2C12 cells to differentiate into adipocytes*

In light of the potential role played by the MKK3/p38 pathway in the trans-differentiation of C2C12 cells into adipocytes, we decided to test the effects of the p38 inhibitor SB203580 on these cell lines. The extracellular signal-regulated kinase inhibitor PD98059 was included for comparison. We initially verified the effectiveness of the SB203580 compound in inhibiting p38 activity through the use of kinase assays (results not shown). We used the inhibitors SB203580 and PD98059 with and without rosiglitazone treatment of the cells. The inclusion of SB203580 in the medium along with rosiglitazone prevented myotube formation in control cells, but did not ameliorate adipogenesis. The addition of PD98059 plus rosiglitazone did not affect myotube formation or adipogenesis in control cells. The presence or absence of adipocytes was reflected by aP2 protein expression levels (Fig. 4). In the DN

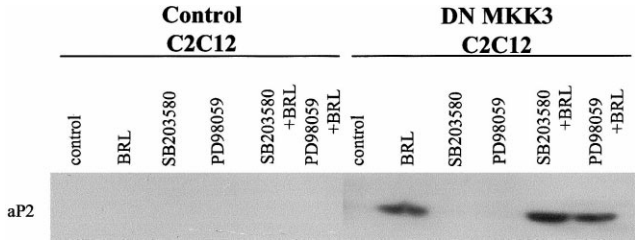

Fig. 4. Treatment of control and DN MKK3 C2C12 cells with different MAP kinase inhibitors does not affect their abilities to trans-differentiate into adipocytes upon treatment with rosiglitazone. Cells were grown in the presence of rosiglitazone alone, or in combination with the inhibitors SB203580 and PD098059 from 2 days before confluence up to 5 days post-confluence. Protein extracts were harvested at day 5, and analyzed for expression of aP2.

MKK3 cells, addition of either SB203580 or PD98059 along with rosiglitazone did not affect the ability of these cells to trans-differentiate (not shown). Similar amounts of aP2 protein were expressed in DN MKK3 cells treated with rosiglitazone, with and without SB203580 and PD98059 (Fig. 4).

3.4. Treatment of the DN MKK3 cells with the PI3 kinase inhibitor, LY294002, inhibits adipogenic trans-differentiation of the DN MKK3 cells

We next asked whether the PI3 kinase pathway was involved in the trans-differentiation of the DN MKK3 cells into adipocytes, as this pathway is known to be important in adipogenesis [20]. As previously shown, control cells failed to differentiate into myotubes upon treatment with the PI3 kinase inhibitor LY294002 [21]. No adipocytes or lipid droplets were present in control cells upon treatment with rosiglitazone plus LY294002 (not shown). In contrast, treatment of the DN MKK3 cells with LY294002, along with rosiglitazone, caused a significant decrease in the number of adipocytes after 5 days post-confluence (Fig. 5). The decrease in the level of adipocytes in the DN MKK3 cells is also reflected by a marked reduction in aP2 expression levels (Fig. 5).

Since the protein kinase Akt is known to act downstream of PI3 kinase, we tested C2C12 cells stably overexpressing DN or constitutively active (CA) Akt [21] for their ability to trans-differentiate into adipocytes (results not shown). However, treatment of these cell lines with rosiglitazone did not result in adipocytic trans-differentiation. No increase in the levels of PPARγ mRNA or aP2 protein were detected (results not shown). This suggests that the ability of C2C12 cells to trans-differentiate into adipocytes requires a functional PI3 kinase pathway, but the downstream kinase Akt appears not to be involved.

A general summary of our findings is depicted in Fig. 6.

4. Discussion

We demonstrate that the inhibition of myogenesis, via the expression of a DN MKK3, enhances the trans-differentiation of C2C12 cells into adipocytes in response to rosiglitazone treatment. In addition, the adipogenic effects of rosiglitazone on DN MKK3 cells are reflected by an increase in PPARγ. Adipocyte formation was demonstrated by the presence of clusters of rounded cells, containing lipid droplets that stained with Oil red O, and the expression of the adipocyte lipid binding protein aP2. All results were verified using three separate clones of each cell line.

Several reports have been published on the capacity of the C2C12 cell line to trans-differentiate into various lineages. Treatment of a naturally G418-resistant subclone of this cell line, C2C12N, with thiazolidinediones and fatty acids results in their conversion into adipose-like cells [22]. In contrast to

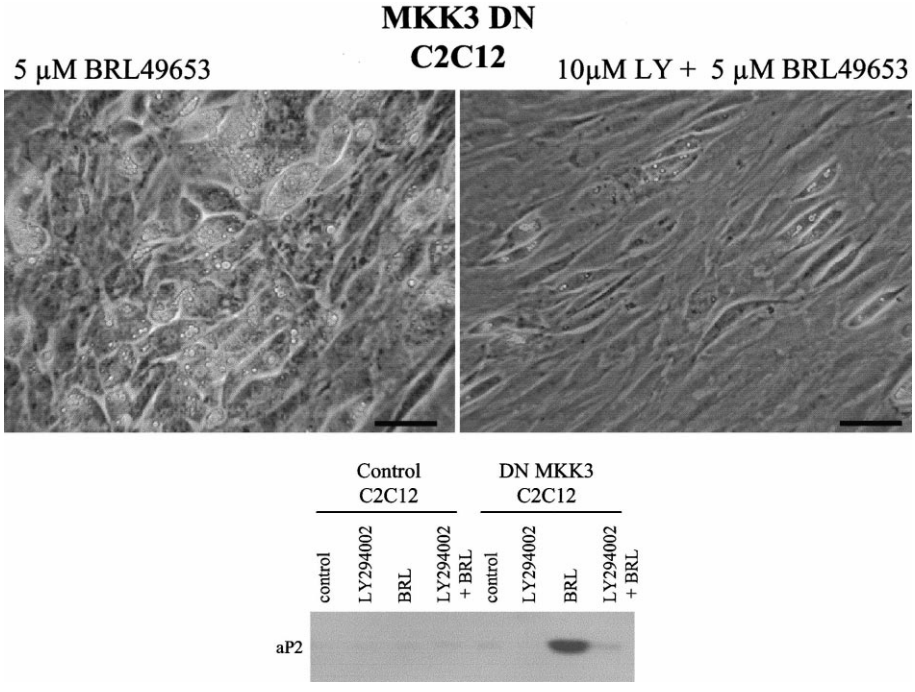

Fig. 5. The PI3 kinase inhibitor LY294002 impedes the trans-differentiation of the MKK3 DN C2C12 cells into adipocytes upon rosiglitazone treatment. Upper part: DN MKK3 cells were treated with either 5 μM rosiglitazone alone, or in combination with 10 μM LY294002, for up to 5 days post-confluence. Lower part: Protein extracts were harvested from control and DN MKK3 cells at day 5 and analyzed for aP2 protein expression by immunoblotting. Scale bar = 10 μm.

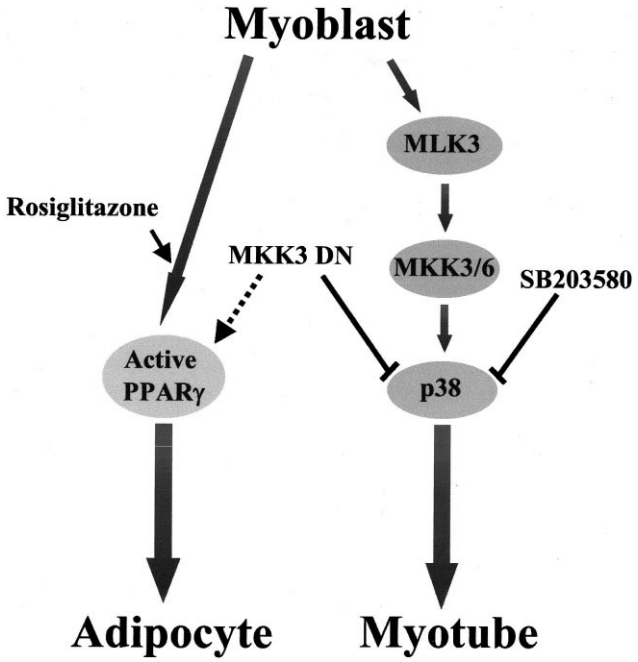

Fig. 6. This diagram summarizes the effect of rosiglitazone treatment of DN MKK3 C2C12 cells.

this report on adipogenic trans-differentiation of the C2C12N cell line, our study demonstrates that wild-type and control C2C12 cells stably transfected with the empty vector do not respond efficiently to rosiglitazone treatment. It is important to note that our study was based on wild-type C2C12 cells; the C2C12N cell line was originally isolated as a G418-resistant subclone of C2C12 cells. Thus, the difference in the ability of C2C12N versus C2C12 cells to form adipocytes in response to rosiglitazone treatment could be due to inherent genetic differences between these two cell lines.

Interestingly, treatment of the control C2C12 cells with the p38 inhibitor SB203580 followed by rosiglitazone stimulation, did not result in increased numbers of adipocytes. There are several possible explanations for this. First, the SB203580 inhibitor is thought to directly block the activation of p38 [23], which is the downstream target of MKK3. Thus, should MKK3 affect adipogenesis independently of p38, the use of this inhibitor would have no obvious effects on adipogenic trans-differentiation. In addition, short-term treatment of cells with an inhibitor cannot be compared to long-term, stable overexpression of a DN construct. Therefore, although expression of DN MKK3 and treatment of C2C12 cells with SB203580 have similar outcomes on myogenesis [14,24], one cannot assume that the use of SB203580 has actions equivalent to that of stable expression of DN MKK3 in the context of trans-differentiation into adipocytes.

The expression of DN MKK3 is known to block myogenesis, in particular, the process of myotube formation [16]. It is possible that inhibiting myogenesis in these cells facilitates adipocytic trans-differentiation by enabling them to respond to other differentiation agents. Ectopic expression of msx1, a nuclear protein thought to play a role in urodele cellular dedifferentiation, in differentiated C2C12 myotubes abrogates the differentiated phenotype and results in a dedifferentiated population of cells capable of expressing chondrocytic, osteogenic, myogenic, and adipogenic markers [25]. Our results with the DN MKK3 cells extends previous work on the plasticity of this myogenic cell line, in that expression of a kinase-inactive form of MKK3 is sufficient to enable adipogenic trans-differentiation, possibly as an indirect result of its ability to inhibit the normal process of myogenesis.

The adipocytic trans-differentiation of the DN MKK3 cells involves the upregulation of PPARγ mRNA. In fibroblastic cell lines, ectopic expression of PPARγ is sufficient to initiate adipogenesis [10]. Thiazolidinediones can act as ligands by directly binding and activating PPARγ [26], which then leads to the induction of genes involved in fatty acid uptake, storage, and metabolism, including aP2 [13,27–29]. This increase in PPARγ can be perpetuated via C/EBPα, which in turn further activates PPARγ expression [30]. In the case of the DN MKK3 C2C12 cells, activation of the low basal levels of PPARγ could lead to adipocytic trans-differentiation, in turn leading to elevated levels of PPARγ in a positive-feedback manner.

Interestingly, the ability of the DN MKK3 cells to form adipocytes in response to rosiglitazone treatment was drastically inhibited by the PI3 kinase inhibitor LY294002. The PI3 kinase [20,31] pathway is known to be extremely important for adipocytic differentiation. Thus, it is not surprising that LY294002 inhibited the formation of adipocytes in the DN MKK3 cells. However, treatment of C2C12 cells stably overexpressing DN Akt and CA Akt with rosiglitazone did not result in the formation of adipocytes, precluding the involvement of Akt. It is possible that this process of trans-differentiation involves other effectors of PI3 kinase besides Akt, such as the atypical protein kinase C (PKC) molecules, PKCλ and PKCζ [32,33]. It is known that insulin is able to activate the atypical PKCs through PI3 kinase-dependent increases in phosphatidylinositol-3,4,5-phosphate [34], and that thiazolidinedione treatment can enhance these effects [35]. Further work will be necessary in order to unravel the inter-relationships between these two signalling pathways in the trans-differentiation of myoblasts into adipocytes.

In summary, we show that inhibition of myogenesis via expression of DN MKK3 enables C2C12 myoblastic cells to trans-differentiate into adipocytes upon treatment with rosiglitazone. This process involves the upregulation of PPARγ, and may involve signalling via the PI3 kinase pathway.

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References

[1] Kahn, B.B. and Flier, J.S. (2000) J. Clin. Invest. 106, 473–481.
[2] Brink, M., Price, S., Chrast, J., Bailey, J., Anwar, A., Mitch, W. and Delafontaine, P. (2001) Endocrinology 142, 1489–1496.
[3] Pahor, M. and Kritchevsky, S. (1998) J. Nutr. Health Aging 2, 97–100.
[4] Lee, K. et al. (2000) Mol. Cell. Biol. 20, 8783–8792.
[5] Hu, E., Tontonoz, P. and Spiegelman, B.M. (1995) Proc. Natl. Acad. Sci. USA 92, 9856–9860.
[6] Buckingham, M. and Tajbakhsh, S. (1993) C. R. Acad. Sci. Ser. III 316, 1032–1046.
[7] Yee, S. and Rigby, P. (1993) Genes Dev. 7, 1277–1289.
[8] Cusella-De Angelis, M. et al. (1992) J. Cell. Biol. 116, 1243–1255.

[9] Yaffe, D. and Saxel, O. (1977) Differentiation 7, 159–166.
[10] Tontonoz, P., Hu, E. and Spiegelman, B.M. (1994) Cell 79, 1147–1156.
[11] Tontonoz, P. et al. (1997) Proc. Natl. Acad. Sci. USA 94, 237–241.
[12] Hertzel, A.V. and Bernlohr, D.A. (1998) Mol. Cell. Biochem. 188, 33–39.
[13] Tontonoz, P., Hu, E. and Spiegelman, B.M. (1995) Curr. Opin. Genet. Dev. 5, 571–576.
[14] Cuenda, A. and Cohen, P. (1999) J. Biol. Chem. 274, 4341–4346.
[15] Engelman, J.A., Lisanti, M.P. and Scherer, P.E. (1998) J. Biol. Chem. 273, 32111–32120.
[16] Englaro, W., Cabane, C., Yeow, K., Ragno, M. and Dérijard, B. (manuscript in preparation).
[17] Aubert, J. et al. (1999) J. Biol. Chem. 274, 24965–24972.
[18] Amri, E.Z., Bonino, F., Ailhaud, G., Abumrad, N.A. and Grimaldi, P.A. (1995) J. Biol. Chem. 270, 2367–2371.
[19] Grimaldi, P.A., Teboul, L., Inadera, H., Gaillard, D. and Amri, E.Z. (1997) Prostaglandins Leukotrienes Essent. Fatty Acids 57, 71–75.
[20] Sakaue, H., Ogawa, W., Matsumoto, M., Kuroda, S., Takata, M., Sugimoto, T., Spiegelman, B.M. and Kasuga, M. (1998) J. Biol. Chem. 273, 28945–28952.
[21] Cabane, C., Yeow, K. and Dérijard, B. J. Biol. Chem., submitted.
[22] Teboul, L., Gaillard, D., Staccini, L., Inadera, H., Amri, E.Z. and Grimaldi, P.A. (1995) J. Biol. Chem. 270, 28183–28187.
[23] Lali, F.V., Hunt, A.E., Turner, S.J. and Foxwell, B.M. (2000) J. Biol. Chem. 275, 7395–7402.
[24] Li, Y., Jiang, B., Ensign, W.Y., Vogt, P.K. and Han, J. (2000) Cell Signal 12, 751–757.
[25] Odelberg, S.J., Kollhoff, A. and Keating, M.T. (2000) Cell 103, 1099–1109.
[26] Lehmann, J.M., Moore, L.B., Smith-Oliver, T.A., Wilkison, W.O., Willson, T.M. and Kliewer, S.A. (1995) J. Biol. Chem. 270, 12953–12956.
[27] Hamm, J.K., Park, B.H. and Farmer, S.R. (2001) J. Biol. Chem. 27, 27–35.
[28] Tontonoz, P., Hu, E., Devine, J., Beale, E.G. and Spiegelman, B.M. (1995) Mol. Cell. Biol. 15, 351–357.
[29] Tontonoz, P., Nagy, L., Alvarez, J.G., Thomazy, V.A. and Evans, R.M. (1998) Cell 93, 241–252.
[30] Rosen, E.D., Walkey, C.J., Puigserver, P. and Spiegelman, B.M. (2000) Genes Dev. 14, 1293–1307.
[31] Xia, X. and Serrero, G. (1999) J. Cell. Physiol. 178, 9–16.
[32] Kanoh, Y., Bandyopadhyay, G., Sajan, M.P., Standaert, M.L. and Farese, R.V. (2001) Endocrinology 142, 1595–1605.
[33] Ikeda, Y., Olsen, G.S., Ziv, E., Hansen, L.L., Busch, A.K., Hansen, B.F., Shafrir, E. and Mosthaf-Seedorf, L. (2001) Diabetes 50, 584–592.
[34] Standaert, M.L., Bandyopadhyay, G., Kanoh, Y., Sajan, M.P. and Farese, R.V. (2001) Biochemistry 40, 249–255.
[35] Kanoh, Y., Bandyopadhyay, G., Sajan, M.P., Standaert, M.L. and Farese, R.V. (2000) J. Biol. Chem. 275, 16690–16696.