Dynamic complexes of A-type lamins and emerin influence adipogenic capacity of the cell via nucleocytoplasmic distribution of β-catenin

Katarzyna Tilgner*, Kamila Wojciechowicz*, Colin Jahoda, Christopher Hutchison and Ewa Markiewicz‡

The School of Biological and Biomedical Sciences, The University of Durham, South Road, Durham DH1 3LE, UK

*These authors contributed equally to this work

‡Author for correspondence (e-mail: e.m.markiewicz@durham.ac.uk)

Accepted 22 October 2008
Journal of Cell Science 122, 401-413 Published by The Company of Biologists 2009
doi:10.1242/jcs.026179

Summary

It is well documented that adipogenic differentiation of the cell is associated with downregulation of Wnt/β-catenin signalling. Using preadipocytes and dermal fibroblasts, we have found that activation of the adipogenic program was associated with marked changes in the expression of nuclear β-catenin-interacting partners, emerin and lamins A/C, to influence expression and activation of peroxisome proliferators-activated receptors γ (PPARγ). In addition, silencing of protein expression with siRNA revealed that β-catenin and emerin influenced each other's levels of expression and the onset of adipogenesis, suggesting that changes in the expression of nuclear lamina proteins were intimately linked to the stability of β-catenin. By contrast, dermal fibroblasts, which are emerin null, demonstrated increased nuclear accumulation of stable β-catenin and constant lamin expression. This was also associated with an unusual adipogenic capacity of the cells, with adipogenesis occurring in the presence of activated β-catenin but declining upon silencing of the protein expression with siRNA. We propose that the process of adipogenesis is affected by a dynamic link between complexes of emerin and lamins A/C at the nuclear envelope and nucleocytoplasmic distribution of β-catenin, to influence cellular plasticity and differentiation.

Key words: Nuclear lamina, β-catenin, Adipogenesis

Introduction

The nuclear envelope (NE) constitutes a major structure of eukaryotic cells that functionally separates the cytoplasm from the nucleoplasm. Recent findings have demonstrated that proteins of the NE are involved in the maintenance of tissue homeostasis, and mutations in NE proteins have been implicated in a wide range of serious degenerative diseases (reviewed by Broers et al., 2006). The best-characterised proteins of the NE are the type V intermediate filament proteins, lamins A and C. Lamins A/C form nuclear complexes with other key proteins of the inner nuclear membrane to influence signalling pathways that are crucial for cellular proliferation and differentiation (Markiewicz et al., 2002b; Johnson et al., 2004; Favreau et al., 2004; Capanni et al., 2005; Lin et al., 2005; Pan et al., 2005; van Berlo et al., 2005; Markiewicz et al., 2005; Dorner et al., 2006; Ivorra et al., 2006). A consensus view is emerging that lamins A/C can orchestrate these mechanisms via their binding partners at multiple points in cell-signalling pathways that are known to be important for adult stem-cell self-renewal and differentiation (Hutchison and Worman, 2004; Gotzman and Foisner, 2006).

Emerin is a type II integral membrane protein that is anchored at the inner nuclear membrane through interaction with lamins A/C (Sullivan et al., 1999; Vaughan et al., 2001). Recently, we have shown that emerin binds to and regulates the nuclear accumulation of the canonical Wnt-signalling effector β-catenin in a lamin A-dependent manner and that fibroblasts from patients with X-linked Emery–Dreifuss muscular dystrophy (X-EDMD), which lack emerin, have an autostimulatory growth phenotype (Markiewicz et al., 2006). In the canonical Wnt-signalling pathway, the ability of cytoplasmic β-catenin to relocate to the nucleus and activate target genes via TCF/LEF transcription factors depends on its capacity to escape the proteasomal degradation via the complex containing scaffolding protein axin, the glycogen synthase kinase 3β (GSK3β) and adenomatous polyposis coli (APC) (Behrens et at., 1996; Huber et al., 1996; Molenaar et al., 1996; Peifer and Polakis, 2000; Fukumoto et al., 2001; Nelson and Nusse, 2004). Several studies have shown that Wnt/β-catenin signalling has a central role in specifying the fate of mesenchymal progenitor cells (Cossu and Borello, 1999) and that suppression of this pathway causes spontaneous adipogenic conversion (Ross et al., 2000). The process of adipogenesis is associated with the expression of a cascade of transcriptional factors that involve CCAAT-enhancer-binding protein β (C/EBPβ) and C/EBPδ, which induce expression of peroxisome proliferators-activated receptors γ (PPARγ) and C/EBPα. These adipogenic factors regulate the differentiation program leading to the formation of mature fat cells (Rosen et al., 2000). It has been demonstrated that the suppression of Wnt/β-catenin signalling during adipogenesis involves PPARγ-associated targeting of β-catenin for degradation by proteasome in a GSK3β-dependent manner (Moldes et al., 2003; Liu and Farmer, 2004). Several reports have also shown that there is a balance between PPARγ and β-catenin signalling, as overexpression of a stable form of β-catenin, which is defective for phosphorylation by GSK3β and degradation, blocks adipogenesis partly by inhibiting PPARγ expression (Ross et al., 2000; Benett et al., 2002; Moldes et al., 2003). Moreover, as the presence of Wnt-mimicking factors [lithium chloride (LiCl)] or persistent overexpression of Wnt 1 or Wnt 10b block terminal differentiation in preadipocytes, it has been suggested that for adipogenesis to occur Wnt signalling must be actively suppressed (Bennett et al., 2002; Moldes et al., 2003).

The aim of this study was to determine how the loss of emerin expression would affect the β-catenin signalling during

adipogenesis. Here we report that the balance between β-catenin and PPARγ signalling to control the adipogenic capacity of the cell is intimately linked to the expression of NE proteins, lamins A/C and emerin. In agreement with these findings, we propose that the loss of these mechanisms might contribute to some tissue degeneration phenotypes seen in laminopathies.

Results

Adipogenesis in the presence of activated β-catenin can occur in dermal fibroblasts that are emerin null

Previously, we have shown that emerin-null fibroblasts accumulate active β-catenin in the nucleus (Markiewicz et al., 2006). As one of the effects of nuclear accumulation and sustained activation of β-catenin is partial inhibition of PPARγ expression (Moldes et al., 2003), we investigated the levels of PPARγ in control and emerin-null fibroblasts. When analysed by PCR, the PPARγ expression was low in both cell types. In addition, emerin-null cells demonstrated consistent downregulation of PPARγ at the mRNA level, which was decreased by ~40% compared with controls (Fig. 1A). Unexpectedly, reduced amount of PPARγ mRNA in emerin-null fibroblasts was accompanied by increased nuclear accumulation of the protein, suggesting its increased stabilisation (Fig. 1B). To measure transcriptional activity of PPARγ, the cells were transfected with TK-PPRE-luciferase reporters. Consistent with increased nuclear accumulation of the protein, activity of PPARγ in growth medium (GM) was increased by ~70% in emerin-null compared with control fibroblasts (Fig. 1C, GM). To investigate this further, the TK-PPRE-luciferase was measured in GM supplemented with troglitazone (TDZ), a ligand and activator of PPARγ transcriptional activity. In control fibroblasts, stimulation with TDZ resulted in a twofold increase of PPARγ activity. Unexpectedly, the presence of TDZ led to marked decrease in luciferase activity in emerin-null cells, which was reduced by ~60% compared with GM alone, indicating an impaired response of PPARγ to its ligand when the emerin is absent (Fig. 1C, TDZ). As activation of PPARγ has been ultimately linked to the ability to downregulate β-catenin protein levels and execution of the adipogenic program (Moldes et al., 2003; Liu and Farmer, 2004), we wondered how this signalling would be coupled in emerin-null fibroblasts in response to adipogenic signals. Initially, and to stimulate early adipogenic changes, control and emerin-null fibroblasts were grown in GM or exposed to serum-free, preadipocyte differentiation medium (PRE) containing dexamethasone (DEX), isobutylmethylxanthine (IBMX) and insulin as well as the PPARγ activator ciglitazone for 72 hours. Importantly, culturing in this medium did not lead to lipid accumulation in fibroblasts (data not shown) and 3T3-F442A preadipocytes (see Fig. 8). The cells were analysed for PPARγ expression by PCR and immunoblotting, together with β-actin as a loading control (Fig. 1D-F). Initially, both control and emerin-null fibroblasts responded to PRE by marked upregulation of PPARγ, which was increased by ~90% and 80% in control and emerin-null cells, respectively (Fig. 1D). Increased expression of mRNA in PRE corresponded to upregulated PPARγ protein levels. In addition, emerin-null cells also expressed higher levels of PPARγ protein when cultured in GM (Fig. 1F). To measure the transcriptional activity of PPARγ, cells were transfected with TK-PPRE-luciferase reporters in both growth and preadipogenic media. Strikingly, when compared with GM, the activity of TK-PPRE-luciferase in PRE was actually reduced by ~60% in both cell types, despite upregulated PPARγ protein levels. Nevertheless, in the absence of emerin the activity of PPARγ in PRE was still almost threefold higher compared with control fibroblasts (Fig. 1G). This indicated that exposure of skin fibroblasts

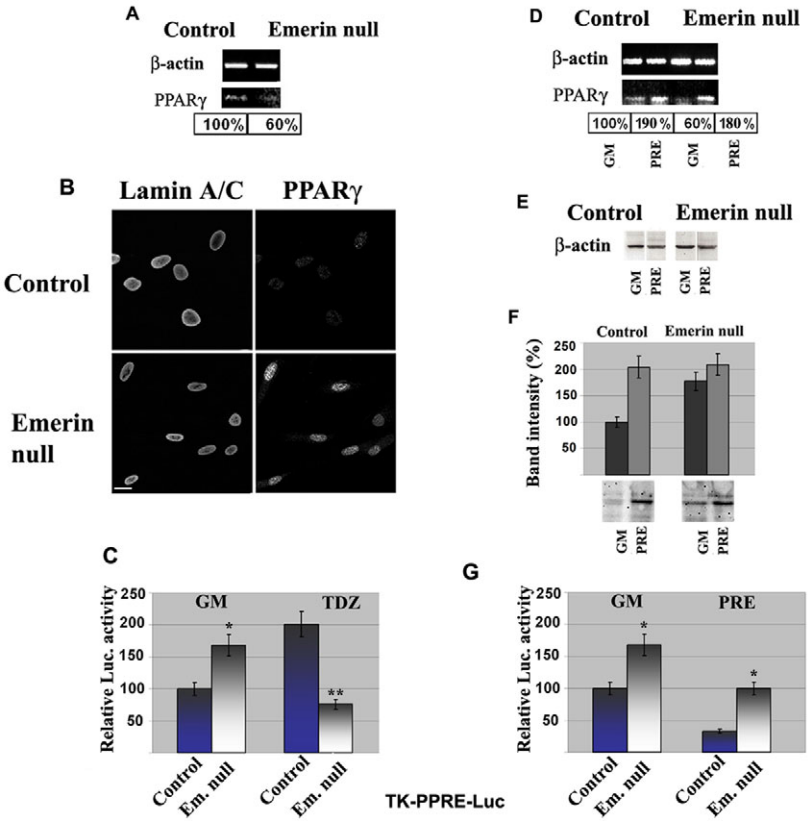

Fig. 1. Expression of PPARγ in skin dermal fibroblasts. (A) PCR with primers specific for β-actin and PPARγ. (B) Cells were co-stained with antibodies against lamin A/C and PPARγ. (C) PPARγ activity in control and emerin-null fibroblasts. Luciferase activity of control cells in GM was assigned a value of 100%. (D-G) Control and emerin-null fibroblasts were cultured in growth medium or preadipocyte differentiation medium. (D) mRNA levels of β-actin and PPARγ analysed by PCR. (E,F) Immunoblotting with antibodies against β-actin and PPARγ. (G) Cells were transfected with TK-PPRE-luciferase reporters. Bars: 10 μm; **P*<0.05; ***P*<0.01.

to the factors attenuating Wnt/β-catenin signalling initially led to a marked increase of PPARγ expression without increase in its transcriptional activity, and this 'silencing' of PPARγ activity was more prominent in the cells expressing emerin.

To investigate the relationship between emerin, PPARγ and β-catenin further, we examined the expression, activity and localisation of β-catenin in growth and preadipogenic media conditions. Interestingly, culture in PRE was associated with ~50% increase in β-catenin expression on the mRNA level in both cell types (Fig. 2A). Initially, we measured the transcriptional activity of β-catenin in the cells transfected with TOP FLASH reporters. As described previously, in GM β-catenin activity was almost threefold higher in emerin-null fibroblasts compared with controls (Markiewicz et al., 2006). Upon transfer to PRE the luciferase activity was markedly reduced, indicating that β-catenin signalling was inhibited upon upregulation of PPARγ protein in both cell types. However, the activity of β-catenin in emerin-null fibroblasts in PRE was still almost twofold higher compared with control fibroblasts (Fig. 2B). The changes in β-catenin activity upon transfer from GM to PRE were not due to reduced protein levels, which remained relatively constant in both cell types (Fig. 2C,D). By contrast, when the relative abundance of β-catenin was examined in cytoplasmic versus nuclear fractions, it was evident that growth in preadipogenic medium led to an increase in the cytoplasmic protein, from ~45% in GM to ~60% in PRE (Fig. 2E). It was also associated with significantly decreased levels of active (unphosphorylated) β-catenin, which reduced by ~70% and ~60% in control and emerin-null fibroblasts, respectively (Fig. 2F). When subcellular fractions were analysed by immunoblotting, active β-catenin was detected mostly in the nucleus, and upon transfer from GM to PRE the levels were decreased by ~60% in both cell types (Fig. 2G). Despite reduced levels, active β-catenin was still prominent and almost four times higher in emerin-null cells cultured in PRE compared with controls (Fig. 2F,G). To extend these observations further, we examined the localisation of active β-catenin by immunofluorescence in growth and preadipogenic media (Fig. 2H). In agreement with previous findings, in GM the levels of active β-catenin were low in the nucleus of control fibroblasts. By contrast, in emerin-null fibroblasts the active β-catenin was almost exclusively nuclear and partially co-localised with PPARγ. Upon transfer to PRE, the PPARγ was markedly upregulated in both cell types, together with decreased expression of active β-catenin, which

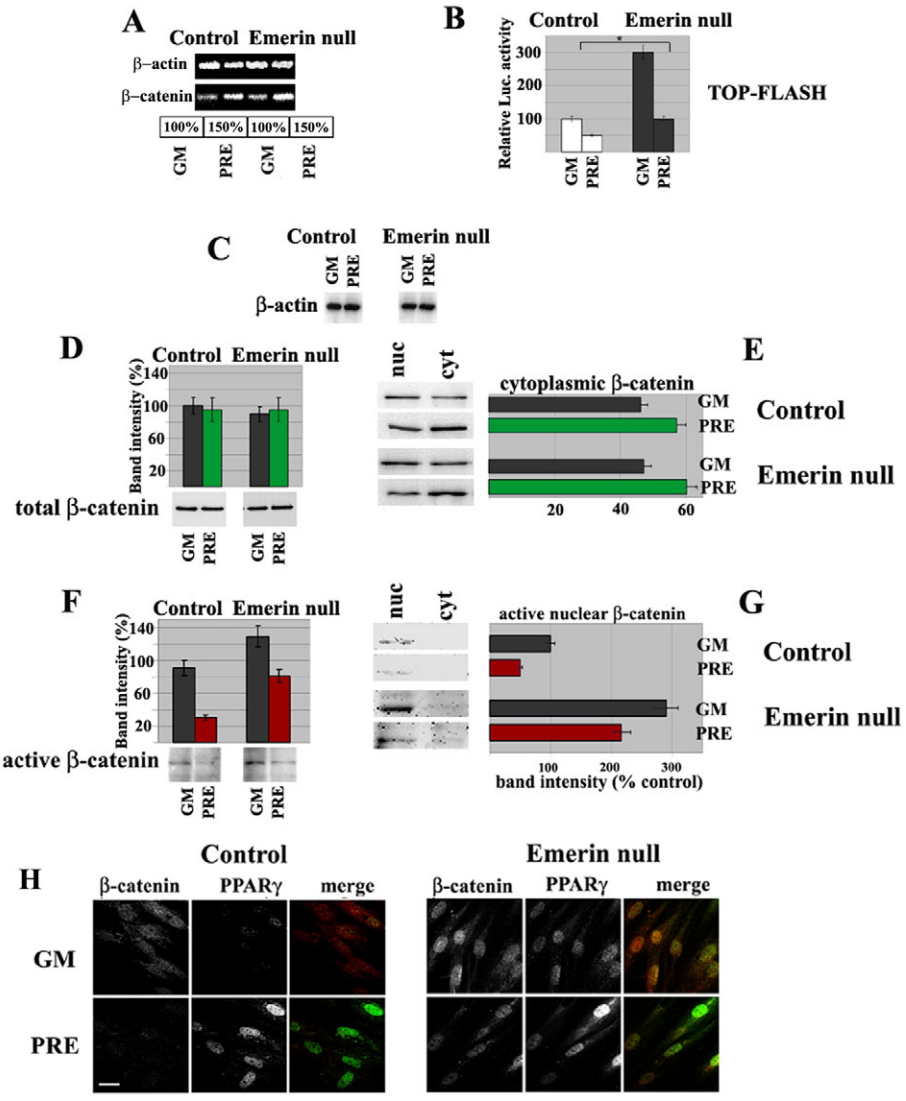

Fig. 2. Emerin-dependent changes in β-catenin activity and localisation in preadipogenic medium conditions. Control and emerin-null fibroblasts were cultured in growth medium or preadipocyte differentiation medium for 72 hours. (A) PCR with primers specific for β-actin and β-catenin. (B) β-catenin activity measured with TOP-FLASH reporters. (C,D,F) Immunoblots of whole-cell extracts with β-actin, total β-catenin and active β-catenin antibodies. (E,G) Cytoplasmic (cyt) and nuclear (nuc) fractions probed for total and active β-catenin. (H) Cells were co-stained with antibodies against active β-catenin and PPARγ. Bars: 10 μm; **P*<0.05.

was almost absent from control fibroblasts. However, the protein could still be detected in the nuclei of emerin-null fibroblasts (Fig. 2H). These results indicated that at the initial stages of adipogenic activation in fibroblasts, upon upregulation of PPARγ expression β-catenin accumulated in the cytoplasm and both proteins were inactive. Whereas downregulation of PPARγ and β-catenin activity were independent of emerin expression, it was also evident that in the absence of emerin both proteins maintained significantly enhanced nuclear accumulation, suggesting the impaired balance between PPARγ and β-catenin signalling in preadipogenic conditions.

As activation of PPARγ is associated with suppression of β-catenin signalling by proteasomal degradation of the protein and this mechanism is central to the differentiation of mesenchymal cells into adipocytes and accumulation of lipids (Ross et al., 2000; Moldes et al., 2003; Liu and Farmer, 2004), we wondered how absence of emerin would affect this process. To induce the final stages of adipogenesis and lipid-laden cells, the cultures were grown in adipogenic differentiation medium (AM), which has adipogenic capacity towards adult dermal cells as described previously (Jahoda et al., 2003). The cells were harvested after 120 hours and mRNA was examined by PCR or extracts analysed by immunoblotting using antibodies against total and active β-catenin (Fig. 3A-D). Upon transfer from GM to AM, the mRNA levels of β-catenin remained unchanged in both cell types (Fig. 3A). However, culture of the control fibroblasts in AM did result in a significant decrease in both total and active β-catenin, (~50% and ~70%, respectively), compared with GM (Fig. 3C,D, Control). This was also associated with a corresponding decrease in β-catenin activity (~50%, as measured by TOP FLASH reporter assays) (Fig. 3E, Control) and highly increased PPARγ activity (~400%) (Fig. 3F, Control). The increase in TK-PPRE-luciferase activity in AM corresponded to enhanced expression of PPARγ at the mRNA and the protein levels (Fig. 3G,H, Control). Downregulation of β-catenin protein levels

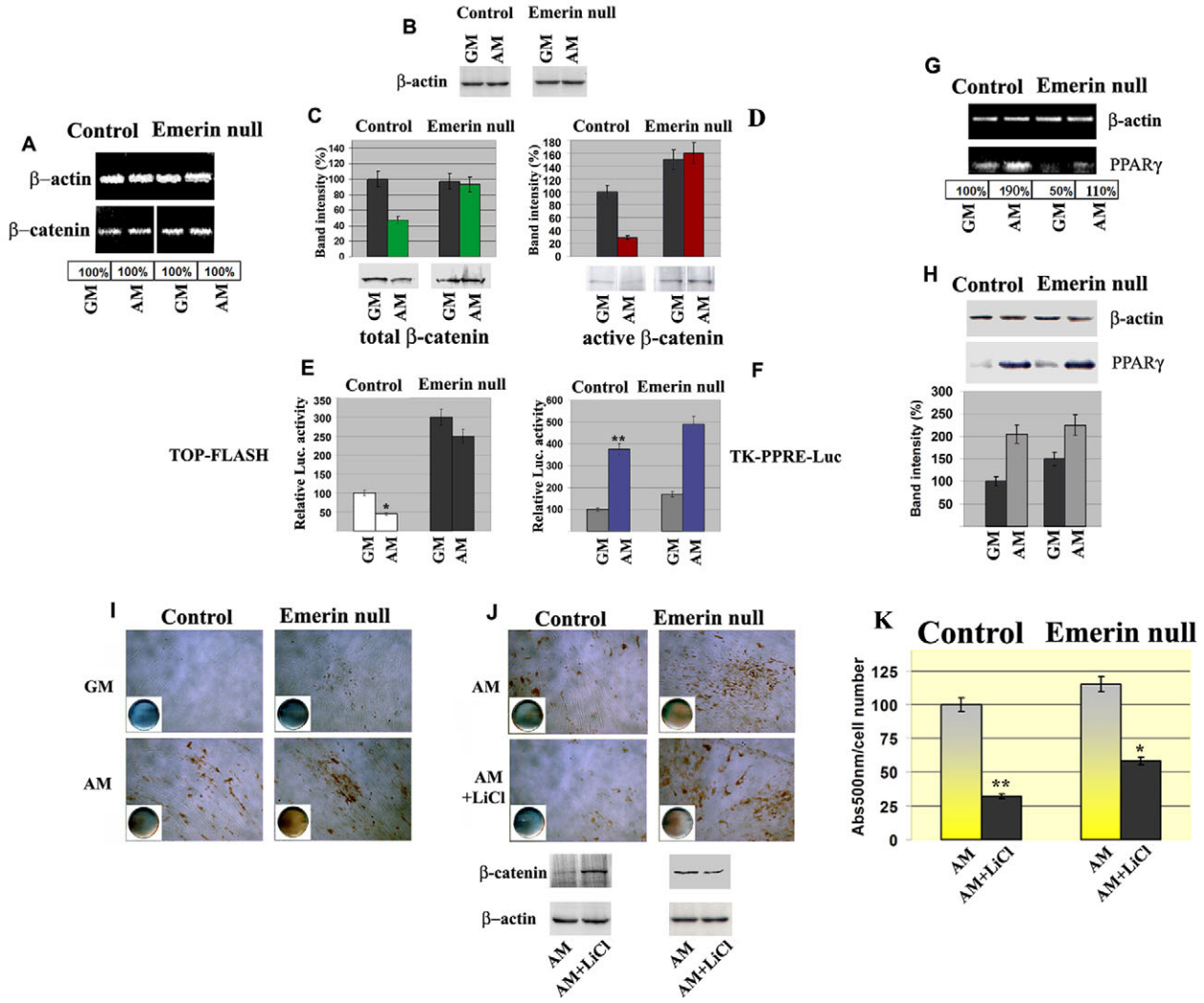

Fig. 3. Enhanced adipogenesis in emerin-null fibroblasts occurs in the presence of activated β-catenin. Fibroblasts were cultured in growth medium or adipogenic medium for 120 hours. (A) PCR with primers specific for β-actin and β-catenin. (B,C,D) Immunoblotting with antibodies against β-actin, total β-catenin and active β-catenin. (E,F) Cells were transfected with TOP FLASH or TK-PPRE-luciferase reporters. (G) PCR with primers specific for β-actin and PPARγ. (H) Immunoblotting with antibodies against β-actin and PPARγ. (I) Fibroblasts were stained with Oil-red O. (J) Cells were grown in adipogenic medium with 10 mM LiCl (AM+LiCl) and stained with Oil-red O or probed with antibodies against β-catenin and β-actin. (K) Oil-red O was extracted with 100% isopropanol and quantified in spectrophotometer at 500 nm. *P<0.05, **P<0.01.

and increase in PPARγ activity were also accompanied by accumulation of lipid droplets (Fig. 3I, Control). Moreover, the accumulation of lipid was inhibited by the presence of 10 mM LiCl, an inhibitor of GSK3β activity, demonstrating that adipogenic conversion of control fibroblasts was dependent upon destruction of β-catenin (Fig. 3J, Control).

In stark contrast to control cells, the levels of total and active β-catenin did not change significantly in emerin-null fibroblasts cultured in AM (Fig. 3C,D, Emerin null). More importantly and in contrast to their behaviour in PRE, there was only a slight reduction in β-catenin activity when emerin-null fibroblasts were cultured in AM (Fig. 3E, Emerin null). We therefore investigated PPARγ activity, expecting it to be inhibited. Surprisingly, we found that PPARγ was activated to even higher levels than controls when emerin-null fibroblasts were cultured in AM (Fig. 3F), together with increased mRNA and protein levels (Fig. 3G,H, Emerin null). Strikingly, the PPARγ mRNA levels in AM were reduced by ~40% compared with control fibroblasts in AM, suggesting that protein accumulation and enhanced activity of PPARγ were only partially due to increased mRNA expression. Changes in PPARγ were associated with accumulation of fat droplets in emerin-null fibroblasts (Fig. 3I, Emerin null). Interestingly, when the cells were cultured in AM supplemented with 10 mM LiCl, it was evident that inhibition of lipid accumulation was less efficient in emerin-null compared with control fibroblasts (Fig. 3J, Emerin null). Quantification of Oil-Red O revealed that the presence of LiCl in AM led to ~70% reduction in lipid accumulation in controls, compared with only a 50% reduction in emerin-null fibroblasts, respectively (Fig. 3K). Our results showed that, as expected, inhibition of β-catenin signalling was required for adipogenic conversion of normal skin fibroblasts but that unexpectedly in cells lacking emerin, increased PPARγ activity and enhanced lipid accumulation occurred in the presence of activated β-catenin.

Downregulation of β-catenin expression in the absence of emerin prevents lamina remodelling and adipogenesis

Our data suggested that in emerin-null fibroblasts β-catenin was protected from degradation despite adipogenic conversion. To investigate this further, control and emerin-null fibroblasts were cultured in the presence of cycloheximide or proteasome inhibitor MG-132 and the amount of active β-catenin was analysed in protein extracts of cells harvested at regular intervals between 1 and 4 hours (Fig. 4A-C). In the cells expressing emerin, active β-catenin declined rapidly and remained at only ~10% of initial levels 4 hours after exposure to cycloheximide (Fig. 4A, left panel). Downregulation of β-catenin in control fibroblasts was due to proteasomal degradation, as the presence of proteasome inhibitor in GM resulted in a rapid accumulation of the protein, with an approximate threefold increase compared with initial levels 4 hours after exposure to MG-132 (Fig. 4B, left panel). By contrast, in the absence of emerin the levels of active β-catenin remained relatively constant, with the protein downregulated by only ~20% in the presence of cycloheximide and increased by only ~10% in the presence of MG-132 (Fig. 4A,B, right panel). In addition, the mRNA levels in control and emerin-null cells were examined by PCR using primers specific for β-catenin, and demonstrated equal levels of β-catenin expression (Fig. 4D). These data indicated that increased levels of active β-catenin in emerin-null cells resulted from reduced degradation of the protein and not from increased expression. These results also suggested that in the absence of emerin, the balance between cytoplasmic and nuclear β-catenin shifted toward the nucleus and the protein escaped proteasomal degradation because of its enhanced nuclear accumulation.

To further extend these findings and to use a more specific approach, control and emerin-null fibroblasts were transfected with specific siRNA to transiently downregulate the β-catenin (β-cat siRNA). RNA was prepared 72 hours post-transfection, and the levels of β-catenin mRNA were analysed by PCR. In both cell types, transfection with β-cat siRNA resulted in efficient downregulation of mRNA, which was reduced by ~80% in control and ~90% in emerin-null fibroblasts, compared with siRNA-scrambled controls (Fig. 5A). As treatment with β-cat siRNA resulted in relatively more efficient downregulation of β-catenin protein levels in control fibroblasts (data not shown), we wondered whether there were also any changes in emerin expression associated with β-catenin knockdown. To this end, mRNA was isolated from the cells transfected with scrambled and β-cat siRNA and emerin levels were analysed by RT-PCR. This revealed that downregulation of β-catenin with siRNA in control fibroblasts also led to a decrease in emerin expression on the mRNA level, which was reduced by ~30% compared with scrambled controls (Fig. 5B). When the cells were examined by immunoblotting, the emerin in the nuclear fractions was clearly reduced compared with scrambled controls (Fig. 5C). Additionally, when examined by immunofluorescence, the emerin signal diminished slightly at the NEs of control cells treated with β-cat siRNA (Fig. 5D). This suggested that downregulation of β-catenin expression also triggered the downregulation of expression and nuclear accumulation of emerin.

To investigate whether changes in β-catenin and emerin expression might also lead to the changes in the expression of emerin-interacting partner lamins A/C, mRNA isolated from control

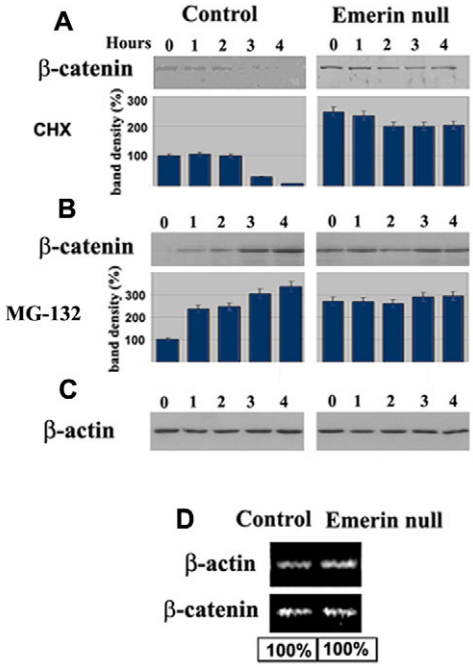

Fig. 4. Enhanced stability of nuclear β-catenin in the absence of emerin. (A-C) 10 μM of cycloheximide or 10 μM of MG-132 was added to the medium. Cells were harvested at intervals of 1 hour and analysed for active β-catenin by immunoblotting. (D) PCR with primers specific for β-actin and β-catenin.

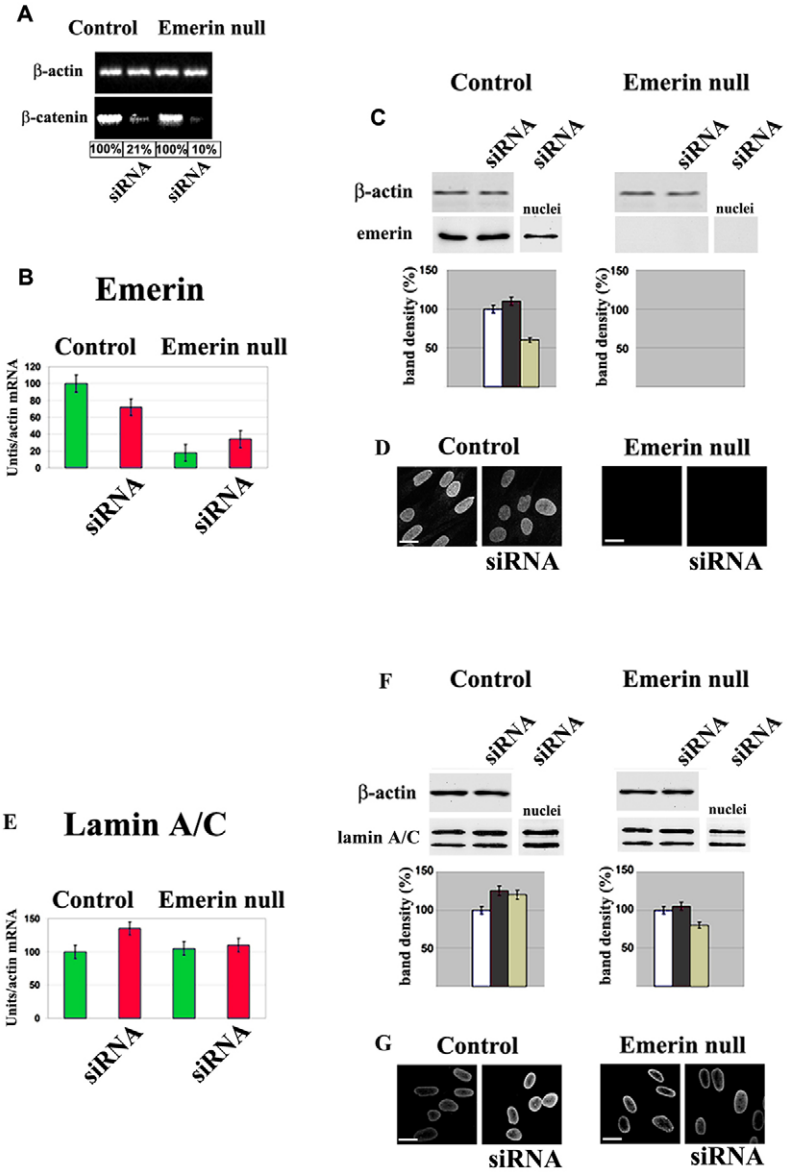

Fig. 5. Changes in emerin and lamins A/C expression upon downregulation of β-catenin with siRNA. (A) PCR with primers specific for β-actin and β-catenin. (B,E) Emerin and lamins A/C expression by RT-PCR against β-actin. (C,F) Immunoblotting of whole-cell extracts or nuclei with emerin and lamins A/C antibody, together with β-actin loading control. (D,G) Cells were stained with emerin or lamins A/C antibodies. Bars: 10 μm.

and emerin-null fibroblasts transfected with scrambled and β-cat siRNA was analysed for lamins A/C by RT-PCR. In control fibroblasts and in contrast to emerin, transfection with β-cat siRNA led to ~40% increase in lamins A/C expression at the mRNA level (Fig. 5E). It was also associated with increased protein levels, in both whole-cell extracts and in the nuclear fractions. In whole-cell extracts, lamins A/C was ~30% higher compared with scrambled controls. In nuclear fractions, the protein was maintained at similar levels (Fig. 5F). When examined by immunofluorescence, the lamins A/C signal was also increased at the NEs of control cells treated with β-cat siRNA (Fig. 5G).

In striking contrast, levels of lamins A/C mRNA remained unchanged in emerin-null fibroblasts treated with β-cat siRNA (Fig. 5E). Similarly, when the cells were examined by immunoblotting, levels of lamins A/C remained unchanged in whole-cell extracts. In addition, there was a clear reduction (~20%) of lamins A/C in the nuclear fractions (Fig. 5F). Using immunofluorescence, the lamins A/C signal also remained unchanged at the NEs of emerin-null cells treated with β-cat siRNA (Fig. 5G).

These results suggested that not only was emerin required for degradation of β-catenin but also that downregulation of β-catenin promoted changes of emerin and lamins A/C expression in control fibroblasts but not in emerin-null fibroblasts.

As efficient downregulation of β-catenin is central to the activation of the adipogenic program, we wondered whether treatment of the fibroblasts with β-cat siRNA would also affect PPARγ expression. To this end, mRNA was isolated from control and emerin-null cells, treated with scrambled and β-cat siRNA, and then analysed by PCR for PPARγ expression. Initially, transfection of β-cat siRNA in control fibroblast resulted in a marked upregulation in PPARγ, which increased by ~60% compared with scrambled controls. By contrast, PPARγ mRNA remained low in both scrambled and β-cat siRNA-treated emerin-null fibroblasts (Fig. 6A). Increase in PPARγ mRNA upon β-catenin knockdown also resulted in an approximate twofold increase in PPARγ protein levels in control fibroblasts. In emerin-null fibroblasts, the levels of PPARγ protein were already upregulated and did not increase substantially when the cells were treated with β-cat siRNA (Fig.

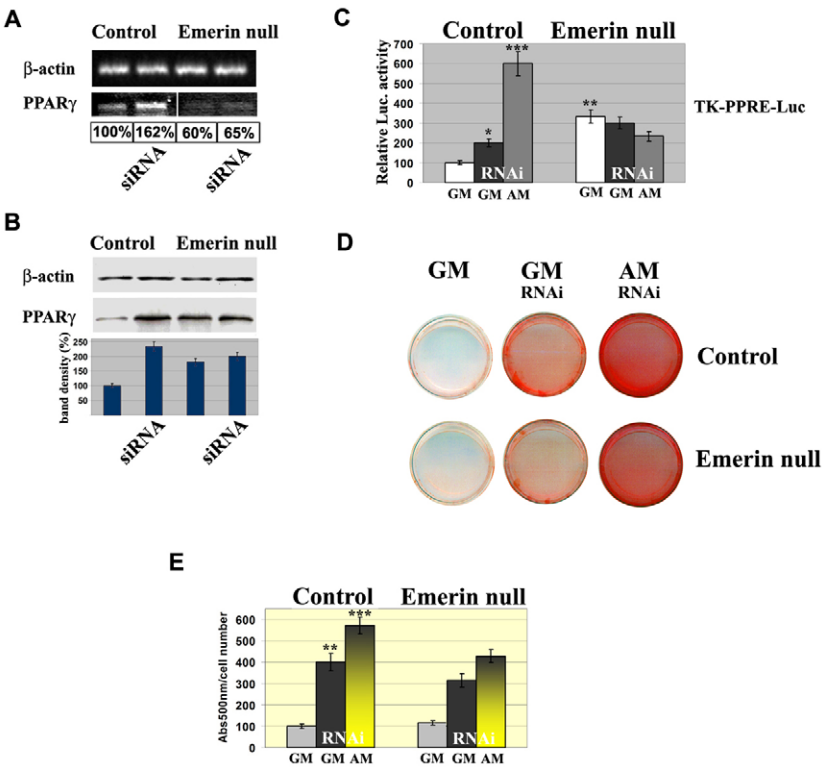

Fig. 6. Transfection with β-catenin siRNA rescues adipogenesis in emerin-null cells. (A) PCR with primers specific for β-actin and PPARγ. (B) Immunoblotting with β-actin and PPARγ antibodies. (C) Activity of PPARγ in GM and AM upon β-catenin knockdown. (D,E) siRNA-transfected cultures were stained with Oil-red O. **P*<0.05, ***P*<0.01, ****P*<0.001.

6B). To investigate how the β-catenin knockdown would influence PPARγ activity and adipogenesis, control and emerin-null cells that had been treated with scrambled and β-cat siRNA were cultured in GM or AM and transfected with TK-PPRE-luciferase. Consistently, upon transfection with scrambled siRNA in GM, the PPARγ activity was approximately threefold higher in emerin-null fibroblasts compared with control fibroblasts. In the cells transfected with β-cat siRNA, the TK-PPRE-luciferase activity increased approximately twofold in control cells but was slightly reduced in the emerin-null cells. When the TK-PPRE-luciferase reporters were measured in AM, downregulation of β-catenin with siRNA resulted in an approximate sixfold increase of PPARγ activity in control fibroblasts. By contrast, treatment of emerin-null fibroblasts with β-cat siRNA in AM led to a further decrease in PPARγ activity, which was reduced by ~60% compared with control fibroblasts in AM (Fig. 6C). To determine whether the differences in PPARγ expression and activity would lead to different extents of lipid accumulation, control and emerin-null fibroblasts that had been treated with scrambled and β-cat siRNA were cultured in GM and AM and stained with Oil-red O. In GM, downregulation of β-catenin with siRNA clearly resulted in spontaneous accumulation of the lipids in both cell types, which was further enhanced by the presence of AM, though adipogenesis was significantly lower in emerin-null fibroblasts. Quantification of Oil-red O revealed that the lipid accumulation upon β-catenin knockdown in control cells increased approximately fourfold in GM and approximately sixfold in AM. By contrast, treatment of emerin-null fibroblasts with β-catenin siRNA resulted in less efficient accumulation of lipids, which was ~25% and ~40% lower in GM and AM, respectively, compared with control cells (Fig. 6D,E).

These results indicated that downregulation of β-catenin expression led to efficient downregulation of nuclear β-catenin in control fibroblasts and subsequent increase in PPARγ activity and activation of the adipogenic program in control but not emerin-null fibroblasts. These changes were also associated with downregulation of emerin expression and increased expression of lamins A/C in control fibroblasts.

Expression of both lamins A/C and emerin is regulated differentially in early and late stages of adipogenesis in skin fibroblasts

These data demonstrate that changes in the levels and subcellular distribution of β-catenin upon siRNA treatment to affect the adipogenic program are closely followed by changes in emerin and lamins A/C expression. Our results also indicated that the initial adipogenic response, preceding accumulation of the lipids in the preadipogenic medium, was accompanied by cytoplasmic distribution of β-catenin and downregulation of its activity without changes in the overall protein levels and that this response was more pronounced in emerin-expressing fibroblasts. As subsequent lipid accumulation was associated with a downregulation of β-catenin protein levels in control but not emerin-null fibroblasts, we wondered whether these early and late adipogenic events might also be intimately linked to the changes in NE architecture. To this end, control and emerin-null fibroblasts were cultured in GM and transferred either to PRE for 72 hours or to AM for 120 hours. The cells were analysed for expression of emerin and lamins A/C at the level of mRNA by PCR and at the protein level by immunoblotting or stained for confocal microscopy (Fig. 7A-G). The changing balance between β-catenin and PPARγ expression in PRE and AM was apparently linked to the changes in emerin and lamins A/C expression. The culture of control fibroblasts in PRE resulted in a twofold increase in emerin at the mRNA level (Fig. 7B, Control), with slight changes at the protein level, as revealed by immunoblotting and immunofluorescence (Fig. 7C,D, Control). By contrast, transfer from GM to AM led to ~50% decrease in the

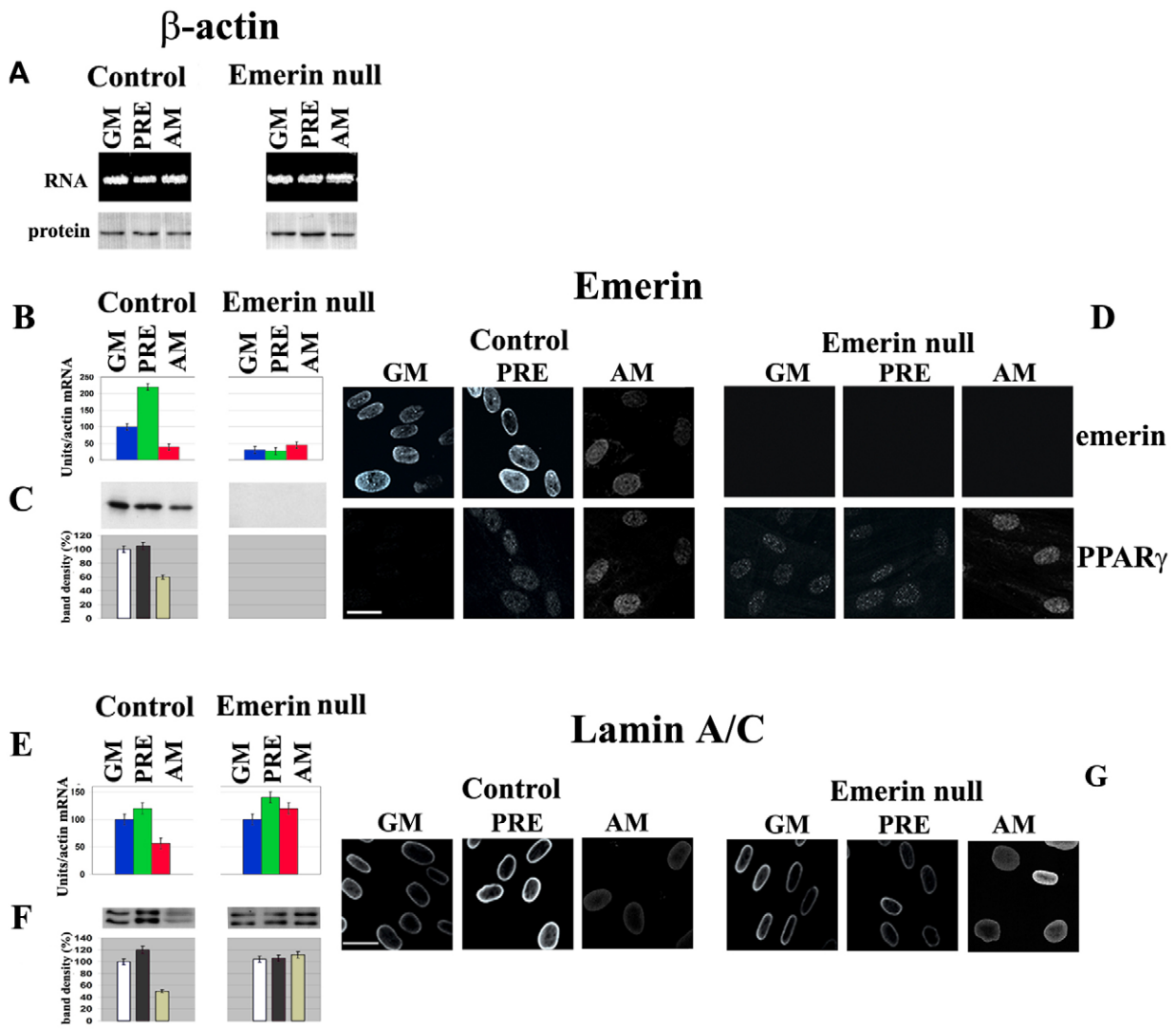

Fig. 7. Dynamic changes in lamins A/C and emerin expression in different stages of adipogenesis. Control and emerin-null fibroblasts were cultured in growth medium (GM), then preadipocyte differentiation medium (PRE) for 72 hours or adipogenic medium (AM) for 120 hours. (A) β-actin loading controls by PCR and immunoblotting. (B,C) Emerin expression by RT-PCR and immunoblotting. (D) Cells were co-stained with PPARγ and emerin antibodies. (E,F) Lamins A/C expression by RT-PCR and immunoblotting. (G) Cells were stained with lamins A/C antibodies. Bars: 10 μm.

emerin mRNA (Fig. 7B, Control), closely followed by decreased expression of the protein on immunoblotting and significantly decreased staining at the NE (Fig. 7C,D, Control). By contrast, expression levels of emerin mRNA were consistently low in emerin-null fibroblasts, and at only ~20% of the levels in control fibroblasts in PRE (Fig. 7B, Emerin null).

Changes in emerin expression in PRE and AM were also accompanied by corresponding changes in the expression of lamins A/C. In PRE, there was ~20% increase in lamins A/C expression on the mRNA (Fig. 7E, Control) and protein level (Fig. 7F, Control). In AM, expression of lamins A/C decreased by ~60%, at both the mRNA (Fig. 7E, Control) and protein levels (Fig. 7F, Control). These changing levels of expression were associated with significantly enhanced staining at the NE in PRE and decreased staining in AM (Fig. 7G). Emerin-null fibroblasts did respond to growth in PRE by upregulation (~30%) of lamins A/C mRNA (Fig. 7E, Emerin null). Despite the upregulated mRNA levels, accumulation of the protein at the NE remained relatively constant in PRE (Fig. 7F,G, Emerin null). Upon transfer to AM and in contrast to control fibroblasts, expression of lamins A/C at the mRNA level remained upregulated (Fig. 7E, Emerin null), and there was no evidence for decreased expression of the protein on immunoblotting and immunofluorescence (Fig. 7F,G, Emerin null). These results suggested that in normal fibroblasts increased accumulation of lamins A/C at the NE precedes adipogenesis and lipid accumulation and may be, in concert with emerin, responsible for downregulation of β-catenin and PPARγ activity. Upon adipogenic conversion, levels of both emerin and lamins A/C declined, presumably because β-catenin was no longer present and PPARγ no longer activated. In emerin-null fibroblasts, there were no significant changes in the nuclear accumulation of lamins A/C protein despite adipogenic conversion, suggesting that emerin could be the sensor that responds to changes in levels of β-catenin and lamins A/C expression.

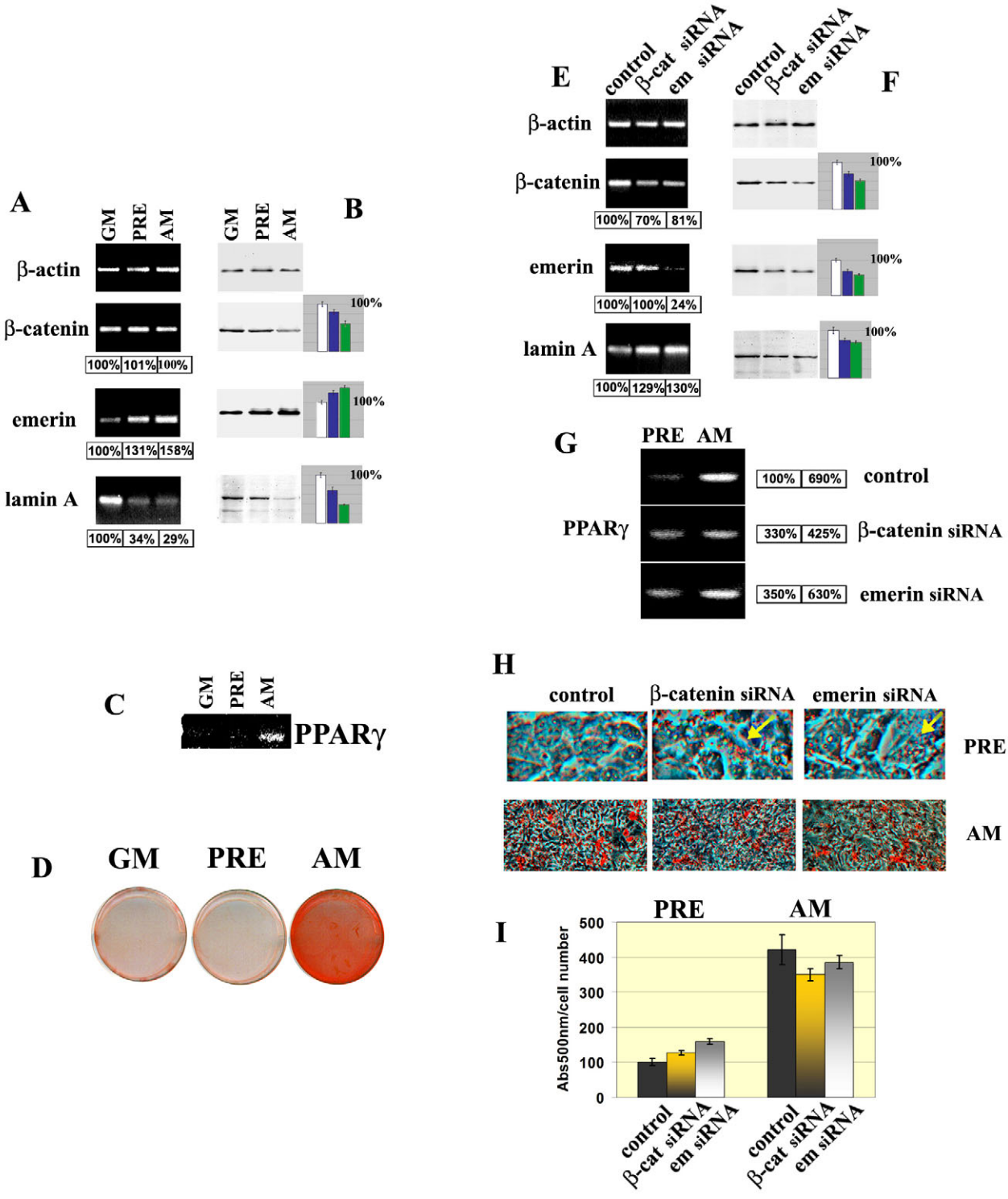

Fig. 8. Adipogenesis in committed preadipocytes is accompanied by changes in emerin and lamin A expression. 3T3-F442A preadipocytes were cultured in growth medium (GM), preadipocyte differentiation medium (PRE) for 72 hours or adipogenic medium (AM) for 120 hours or transfected with scrambled, emerin-specific or β-catenin-specific siRNA. (A,E) PCR with β-actin, β-catenin, emerin and lamins A/C-specific primers. (B,F) Immunoblotting with β-actin, β-catenin, emerin and lamins A/C antibodies. (C,G) PCR with PPARγ-specific primers. (D,H) Oil-red O staining of the cultures. (I) Oil-red O was extracted with 100% isopropanol and quantified in spectrophotometer at 500 nm.

In 3T3-F442A preadipocytes, emerin and β-catenin influence each other's expression and the onset of adipogenesis

Our data indicated that skin fibroblasts have a great capacity to accumulate the lipids, which rely on both the sequential changes in the expression and activity of β-catenin and PPARγ and the integrity of NE proteins. As adipogenesis is an intrinsic feature that underlies the function of preadipocytes and has been extensively studied in this system, we wondered whether changes

in the expression of NE proteins to influence execution of adipogenic program were also specific for this cellular model. To this end, we chose mouse 3T3-F442A preadipocytes, a committed cell line that undergoes adipogenic differentiation in vitro upon stimulation with insulin by clearly defined and timely events of clonal expansion, cell-cycle exit and terminal differentiation. For clarity of the system and comparison with fibroblasts, we cultured them in growth (GM), preadipogenic (PRE, 72 hours) and adipogenic media (AM, 120 hours). Initially, mRNA and protein extracts were prepared and the cells were analysed by PCR and immunoblotting for β-catenin, emerin and lamins A/C, together with β-actin as a loading control (Fig. 8A,B). Culture of 3T3-F442A preadipocytes in PRE did not lead to changes in β-catenin mRNA (Fig. 8A) and resulted in only slight (~10%) downregulation in β-catenin protein levels. In AM, β-catenin protein decreased by a further ~40% compared with GM (Fig. 8B). Downregulation of β-catenin in 3T3-F442A, in contrast to fibroblasts, was accompanied by significant upregulation of emerin. In both PRE and AM, emerin was increased at the mRNA and protein levels by ~30% and ~50%, respectively (Fig. 8A,B). Upregulation of emerin expression was associated with profound downregulation of lamin A. Culture in PRE and AM resulted in ~70% decrease in lamin A at the mRNA level (Fig. 8A), closely followed by progressively reduced protein levels, by ~30% and ~60% in PRE and AM, respectively (Fig. 8B). These results indicated some important differences in the mechanisms by which the adipogenic program is activated in 3T3-F442A preadipocytes and skin fibroblasts. First, growth in PRE was associated with immediate downregulation of lamin A. Secondly, and in contrast to fibroblasts, growth in AM did not lead to downregulation of emerin but instead its progressive accumulation.

Subsequent analysis revealed that growth in AM stimulated upregulation of PPARγ at the mRNA level and, as expected, simultaneous adipogenesis in the conditions of this medium. By contrast, expression of PPARγ was low in PRE conditions, as was lipid accumulation (Fig. 8C,D). This suggested that in preadipocytes, the early upregulation of emerin and downregulation of lamin A expression, evident by cell culture in PRE, would be enhanced further by AM leading to the established events of β-catenin degradation, expression of PPARγ and adipogenesis.

Next we wanted to investigate what effect silencing of β-catenin and emerin could have on 3T3-F442A preadipocytes. To this end, we transfected 3T3-F442A preadipocytes with scrambled control and β-catenin or emerin-specific siRNA. Cells were harvested after 72 hours and analysed by PCR and immunoblotting for β-catenin, emerin and lamins A/C, together with β-actin as a loading control (Fig. 8E,F). Transfection of preadipocytes with β-catenin siRNA led to ~30% downregulation of β-catenin mRNA. Strikingly, the β-catenin mRNA also decreased by ~20% in the cells treated with emerin siRNA (Fig. 8E). Transfections with β-catenin siRNA and emerin siRNA also both led to between 20% to 40% reduction of β-catenin at the protein level (Fig. 8F). Transfection of preadipocytes with emerin siRNA resulted in ~75% and ~40% downregulation of emerin at the mRNA (Fig. 8E) and protein levels (Fig. 8F), respectively. In addition, emerin expression was influenced by β-catenin siRNA, with effective downregulation of emerin protein levels by ~30% (Fig. 8F). These data suggested that both β-catenin and emerin could influence each other's levels of expression in 3T3-F442A preadipocytes. Interestingly, treatment of preadipocytes with β-catenin siRNA and emerin siRNA also led to a marked increase in lamin A mRNA (Fig. 8E).

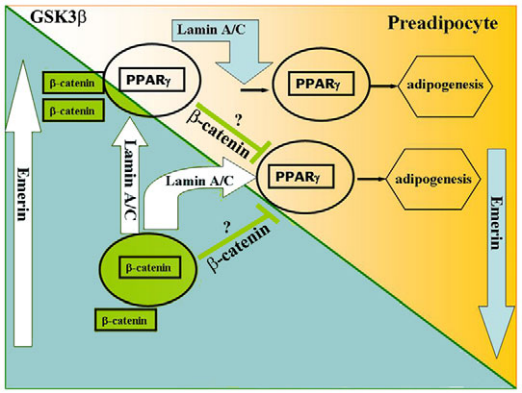

Fig. 9. Proper execution of adipogenesis requires emerin and lamina remodelling. In early stages of adipogenic activation, upregulation of emerin expression could contribute to efficient re-distribution of β-catenin from nucleus to the cytoplasm. In skin fibroblasts, this is additionally associated with increased expression of lamins A/C. These changes could facilitate the proteasomal degradation of β-catenin, activation of PPARγ and decreased expression of lamins A/C in later stages of adipogenesis. In addition to proteasomal degradation of β-catenin, downregulation of both β-catenin and emerin expression, accompanied by increased expression of lamins A/C, could also result in early onset of PPARγ activation and adipogenesis. This feedback mechanism would ensure correct response to the environmental signals influencing extent of adipogenesis.

Because in control preadipocytes the significant increase in emerin expression in preadipogenic media preceded the downregulation of β-catenin protein levels and lipid accumulation in AM, we wondered how the siRNA treatment would affect the adipogenic program. To this end, cells transfected with β-catenin siRNA and emerin siRNA were cultured in the preadipogenic or adipogenic medium. Transfection with both siRNAs resulted in a clear upregulation of PPARγ expression in PRE, which was increased approximately threefold compared with scrambled control (Fig. 8G). Upregulation of PPARγ was accompanied by an increased accumulation of lipid droplets in PRE, which suggested premature activation of the adipogenic program (Fig. 8H, PRE). By contrast, when the preadipocytes transfected with β-catenin siRNA and emerin siRNA where cultured in AM, the lipid accumulation did not increase (Fig. 8H, AM). Quantification of Oil-red O revealed that silencing of β-catenin and emerin expression in preadipocytes led to ~30% and ~60% increase in lipid accumulation in PRE, respectively. By contrast, treatment of preadipocytes with specific siRNAs in AM did not result in enhanced adipogenesis but accumulation of lipids was slightly reduced (Fig. 8I). This suggested that in preadipogenic conditions, emerin could have an inhibitory effect on the onset of adipogenesis but its expression could be important for the final stages of differentiation.

Discussion

The stability of β-catenin is linked to altered levels of expression of lamins A/C and emerin

In this study, we have shown that the cellular levels and distribution of β-catenin, a central regulatory molecule of the Wnt-signalling pathway, are affected by NE proteins, emerin and lamins A/C, to influence cellular differentiation. One of the very important aspects of Wnt/β-catenin signalling is regulation of stem-cell function, with roles in the maintenance of stem cells (Korinek et al., 1998; Batlle

et al., 2002) as well as cell fate determination (Ross et al., 2000; Lako et al., 2002; Kubo et al., 2003). In the canonical Wnt pathway, inhibition of the destruction complex by Wnt signalling triggers the subsequent translocation of β-catenin to the nucleus and transcriptional activation of TCF-4 (Behrens et at., 1996; Huber et al., 1996; Molenaar et al., 1996; Fukumoto et al., 2001). The relation between differentiation and Wnt signalling, which is apparent in multipotent stem cells, is thought to be controlled by dosage of β-catenin signalling through altered degradation (Alonso and Fuchs, 2003). In this study, by blocking protein synthesis and using a proteasome inhibitor, we have demonstrated that the stability of β-catenin dramatically increased when emerin was not present. Further attempts to downregulate β-catenin using siRNA were less effective in emerin-null fibroblasts because β-catenin accumulated in the nucleus where it was apparently protected from degradation. Subsequently, we have shown that mechanisms controlling the stability of β-catenin were intimately linked to the accumulation of emerin and A-type lamins at the NE. In control fibroblasts, downregulation of β-catenin expression with siRNA was associated with downregulation of emerin expression and increased accumulation of lamins A/C. Strikingly, this upregulation of lamins A/C was not observed in emerin-null cells that were treated with β-catenin siRNA, suggesting that emerin is the key mediator that links levels of β-catenin to NE remodelling. Based on these findings, we suggest that the stability and accumulation of β-catenin are linked to those of emerin and lamins A/C, via an intrinsic feedback mechanism ensuring that not only is downregulation of β-catenin signalling required for nuclear remodelling, but also in the absence of this remodelling β-catenin is extremely stable.

Emerin is essential for linking inactivation of β-catenin to adipogenesis

As sustained activation of wnt/β-catenin signalling has been shown to inhibit the process of adipogenesis and adipogenic transdifferentiation of mesenchymal stem cells (Ross et al., 2000; Vertino et al., 2005), it was of interest to investigate the response of emerin-null cells to the presence of adipogenic factors. Central to the adipogenic program is initial upregulation and activation of the adipogenic transcriptional factor, PPARγ. It has been reported that sustained activation of β-catenin signalling blocks this process partly by inhibiting PPARγ expression in preadipocytes (Ross et al., 2000; Benett et al., 2002; Moldes et al., 2003). Consistent with these findings, in the work presented here we have observed that PPARγ expression at the mRNA level was significantly lower in the fibroblasts that lacked emerin. Intriguingly though, both protein accumulation and activity were greatly enhanced in the emerin-null fibroblasts, suggesting that in the absence of emerin, PPARγ protein was stabilised despite reduced mRNA expression.

Transient upregulation of PPARγ, without upregulation of its transcriptional activity, could constitute part of the mechanism influencing transdifferentiation of the fibroblast towards preadipocytes. Upregulation of PPARγ at the mRNA and protein levels, observed in this study as an initial response of fibroblasts to preadipogenic medium conditions, was indeed associated with inhibition of β-catenin activity. This was due to apparent re-distribution of the protein to the cytoplasm, together with upregulation of emerin expression and accumulation of lamins A/C at the NE. Importantly, preadipogenic differentiation of emerin-null cells was associated with much higher activity of β-catenin. One interpretation of these data is that increased expression of PPARγ, triggered by the preadipogenic conditions, is counteracted by the remodelling of the nuclear lamina to stabilise the cytoplasmic pool of β-catenin and to influence a balance between PPARγ and β-catenin signalling in regulating the cellular fate choice. Failure of this mechanism in emerin-null fibroblasts would limit the capacity of these cells to undergo adipogenesis.

It has been well documented that activation of the adipogenic program in preadipocytes leads to a loss of free cytoplasmic β-catenin by PPARγ-associated targeting of β-catenin for GSK3β-dependent degradation by the proteasome (Ross et al., 2000; Bennett et al., 2002; Moldes et al., 2003; Liu and Farmer, 2004). In this study, we have shown that a decrease in β-catenin protein levels in control fibroblasts accompanied activation of PPARγ and lipid accumulation in later stages of adipogenesis. Moreover, adipogenesis could be prevented by the stabilisation of β-catenin in the presence of LiCl, a specific inhibitor of GSK3β, indicating that as in preadipocytes, adipogenesis in fibroblasts depends on downregulated β-catenin levels. Intriguingly, when emerin-null fibroblasts were cultured in the adipogenic medium, they underwent enhanced adipogenic conversion despite the presence of activated β-catenin. In addition, inhibition of β-catenin degradation by LiCl resulted in less efficient inhibition of lipid accumulation. Therefore, it appears that the interplay between β-catenin signalling and PPARγ signalling could be abrogated in the absence of emerin. The finding that stimulation of PPARγ activity with TDZ was also ineffective in emerin-null cells could indicate that the mechanisms linking the adipogenic program to the proteasomal degradation of β-catenin via activation of PPARγ could be limited in the absence of emerin.

Importantly and in contrast to emerin-null fibroblasts, control fibroblasts demonstrated enhanced activation of PPARγ and adipogenesis upon downregulation of β-catenin levels with siRNA. This suggests that emerin and β-catenin could act on the same pathway to regulate adipogenesis and that the absence of emerin would limit plasticity and adipogenic potential of the cells by impairing the balance between β-catenin and PPARγ signalling. In the absence of emerin, increased stability and enhanced nuclear accumulation of β-catenin would initially lead to stabilisation of PPARγ but also to its decreased expression and effectively to impaired adipogenesis.

In this study, using models of differentiating 3T3-F442A preadipocytes, we have found that in the cells committed to adipogenesis, decrease in β-catenin levels was accompanied by immediate downregulation of lamin A expression and, in contrast to human fibroblasts, marked increase in emerin expression. Upregulation of emerin during adipogenesis could be a rate-limiting factor and reflect important differences between human and mouse systems. Silencing of emerin expression in 3T3-F442A cells led to downregulation of β-catenin at both the mRNA and protein levels. In addition, silencing of β-catenin was associated with reduced emerin levels, most probably reflecting changes in the protein stability. These results suggest that emerin and β-catenin could act on the same pathway to regulate the onset and execution of adipogenesis in preadipocytes. As silencing of emerin and β-catenin expression led to increased accumulation of lipids in preadipogenic media but not in adipogenic media, high levels of β-catenin could be important in preventing premature entry into adipogenesis and high levels of emerin could be important in securing β-catenin degradation events in the late stages.

Based on the data presented in this manuscript, we propose that proper execution of adipogenesis relies on the upregulation of emerin expression to influence the balance between β-catenin and PPARγ signalling in the early stages of adipogenic activation of skin

fibroblasts and committed preadipocytes. Upregulation of emerin could contribute to the efficient re-distribution of β-catenin from the nucleus to the cytoplasm in preadipogenic conditions and facilitate the proteasomal degradation of β-catenin, expression of PPARγ and decreased expression of lamins A/C in later stages of adipogenesis. In addition to proteasomal degradation of β-catenin, downregulation of both β-catenin and emerin expression levels, accompanied by increased expression of lamins A/C, could also result in early onset of adipogenesis. This feedback mechanism would ensure the correct response of different cell types to the environmental signals influencing the extent of adipogenesis (Fig. 9).

Implications for disease

Mutations in proteins of the NE are associated with a range of serious degenerative diseases, including Emery–Dreifuss muscular dystrophy (EDMD) and Dunnigan-type familial partial lipodystrophy, which affect adipose tissue (Bonne et al., 1999; Cao and Hegele, 2000; Shackleton et al., 2000). Loss of emerin function leads to the X-linked form of EDMD, which is characterized by progressive muscle wasting and cardiomyopathy through progressive replacement of skeletal muscle fibres and cardiomyocytes with fatty fibrotic tissue. It has been proposed that these phenotypes arise because mutations affect cell-signalling pathways that are important for adult stem-cell self-renewal and differentiation (Hutchison and Worman, 2004; Gotzman and Foisner, 2006). Here we have shown that emerin-null fibroblasts are predisposed to transdifferentiation events and undergo enhanced adipogenic conversion due to abnormal PPARγ signalling that is not inactivated by β-catenin. Therefore, the phenotype might arise because of the expansion and transdifferentiation of populations of fibroblasts in the heart and skeletal muscle to fat cells. The finding that emerin-dependent lamina remodelling was evident during adipogenic differentiation of normal fibroblasts and preadipocytes could constitute an important regulator of cell fate determination in adult tissues and reflect critical differences between subcutaneous and visceral fat.

Materials and Methods

Cell cultures and media

Fibroblast cultures have been described previously (Markiewicz et al., 2002a). Cultures were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% newborn calf serum (NCS) plus antibiotics. 3T3-F442A preadipocytes were purchased from the European Collection of Cell Cultures (ECACC).

Adipogenic assay

Cells were transferred to preadipocyte differentiation medium for 72 hours [PromoCell; 0.5 μg/ml of insulin, 400 ng/ml dexamethasone (DEX), 44 μg/ml isobutyl-methylxanthine (IBMX), 8 μg/ml d-Biotin, 9 ng/ml L-thyroxine, 3 μg/ml ciglitazone] or to adipogenic medium for 120 hours [15% rabbit serum, 0.45 mM of IBMX, 2.07 mM of insulin and 100 nM of DEX (for details, see Jahoda et al., 2003)].

Lipid detection

Cultured cells were stained with Oil-red O as described previously (Jahoda et al., 2003). For lipid quantification, Oil-red O was extracted with 100% isopropanol and absorbance of extracts was measured in a spectrophotometer at 500 nm. Cells from the parallel dish were harvested and counted in a haemocytometer. Adipogenic units are expressed as Abs 500 nm/cell number.

Analysis of RNA expression

Total RNA was extracted from human or mouse cultures using the RNeasy Mini Kit (Qiagen) according to the manufacture's protocol. 1 μg of RNA was converted into cDNA using Promega Reverse Transcription System and analysed with primers specific for β-actin, human β-catenin, human PPARγ, mouse β-catenin, mouse PPARγ, mouse emerin and mouse lamins A/C. The primers used for RT-PCR were: human emerin; Inventoried Hs00609152-g1 and human lamin A; Inventoried Hs00153462-m1 were purchased from Applied Biosystems. Values against β-actin obtained from 7500 Fast System Software are presented in Microsoft Excel and assigned 100% for control fibroblasts in GM and control fibroblasts transfected with scrambled siRNA.

Indirect immunofluorescence microscopy

Cells growing on coverslips were prepared as described previously (Vaughan et al., 2001). Cells were stained with the following antibodies: monoclonal anti-active β-catenin (1:1000; clone 8E7; Upstate Biochemicals), monoclonal anti-lamins A/C (1:50; JoL2–Serotec), monoclonal anti-emerin (1:100; NCL, Novocastra), rabbit polyclonal anti-PPARγ (1:100, Cell Signaling). Secondary antibodies were TRITC-anti-mouse or FITC-anti-rabbit (1:100, Jackson ImmunoResearch). Stained cells were mounted in MOWIOL containing 1 μg/ml DAPI.

Preparation of cells extracts and Western blotting experiments

Cell pellets were washed with ice-cold PBS and lysed in 0.1 ml ice-cold hypotonic buffer (10 mM Tris pH 7.4; 10 mM KCl; 3 mM $MgCl_2$ and 0.1% Triton X-100) containing protease inhibitor cocktail and 100 U/ml of DNase I (Sigma) for 10 minutes on ice. For cytoplasmic and nuclear fractions, cells were incubated in ice-cold hypotonic buffer, homogenised, and the fractions separated by centrifugation at 1000 ***g***. Extracts were probed with monoclonal anti-β-catenin (1:1000, BD Bioscience), monoclonal anti-active β-catenin (1:1000, clone 8E7, Upstate Biochemicals), monoclonal anti-lamins A/C (1:100; JoL2–Serotec), monoclonal anti-lamin A (1:100, JoL4-Serotec), monoclonal anti-emerin (1:200; NCL, Novocastra), monoclonal anti-PPARγ (1:500, Invitrogen), or anti-β-actin (1:1000, Sigma) using chemiluminescence. Densitometry was performed using UVI Bandmap software (UVItec Ltd).

β-catenin/TCF and PPARγ transactivation assays

Cells growing in DMEM/10% NCS (GM), preadipocyte differentiation medium (PRE) or adipogenic medium (AM) were transfected with mixtures of 4 μg of TOP FLASH or FOP FLASH plasmid DNA, 0.5 μg TK *Renilla* and 0.5 μg TCF4 using Lipofectamine 2000 (Invitrogen). For PPARγ transactivation assay, cells were transfected with mixtures of 4 μg of TK-PPRE-luciferase plasmid and 0.5 μg TK Renilla. Luciferase and Renilla activities were assayed using a luciferase assay system (Promega) and luminescence was measured using a Lumat LB 9507 luminometer (Berthold Technologies).

Transfection of siRNA

Human-validated β-catenin-specific siRNA duplexes and scrambled negative controls were obtained from Ambion. Mouse-validated β-catenin siRNA and emerin siRNA were purchased from Santa Cruz. Cells were seeded at 5×10^4 cells/well in 6-well plates and transfected in tandem using Oligofectamine reagent (Life Technologies).

This work was supported by MRC (Stem Cell Career Development Fellowship) to E.M., Muscular Dystrophy Campaign and Euro-Laminopathies to C.H., and BBSRC to C.J. E.M. would like to thank Ronald Evans, Salk Institute, La Jolla for the kind gift of TK-PPRE-luciferase plasmid and Vanja Pekovic for critical reading of the manuscript. Deposited in PMC for release after 6 months.

References

Alonso, L. and Fuchs, E. (2003). Stem cells in the skin: waste not, Wnt not. *Genes Dev.* **15**, 1189-2000.

Batlle, E., Henderson, J. T., Beghtel, H., van den Born, M. M. W., Sancho, E., Huls, G., Meeldijk, J., Robertson, J., van de Wetering, M., Pawson, T. et al. (2002). β-Catenin and TCF mediate cell positioning in the intestinal epithelium by controlling the expression of EphB/EphrinB. *Cell* **111**, 251-263.

Behrens, J., von Kries, J. P., Kuhl, M., Bruhn, L., Wedlich, D., Grosschedl, R. and Birchmeier, W. (1996). Functional interaction of beta-catenin with the transcription factor LEF-1. *Nature* **382**, 638-642.

Bennett, C. N., Ross, S. E., Longo, K. A., Bajnok, L., Hemati, N., Johnson, K. W., Harrison, S. D. and MacDougald, O. A. (2002). Regulation of Wnt signaling during adipogenesis. *J. Biol. Chem.* **277**, 30998-31004.

Bonne, G., Di Barletta, M. R., Varnous, S., Becane, H. M., Hammouda, E. H., Merlini, L., Muntoni, F., Greenberg, C. R., Gary, F., Urtizberea, J. A. et al. (1999). Mutations in the gene encoding lamin A/C cause autosomal dominant Emery-Dreifuss muscular dystrophy. *Nat. Genet.* **21**, 285-288.

Broers, J. L. V., Ramaekers, F. C. S., Bonne, G., Ben Yaou, R. and Hutchison, C. J. (2006). Laminopathies and their role in premature ageing. *Physiol. Rev.* **86**, 967-1008.

Cao, H. and Hegele, R. A. (2000). Nuclear lamin A/C R482Q mutation in canadian kindreds with Dunnigan-type familial partial lipodystrophy. *Hum. Mol. Genet.* **1**, 109-112.

Capanni, C., Mattioli, E., Columbaro, M., Lucarelli, E. K., Parnaik, V. K., Novelli, G., Wehnert, M., Cenni, V., Maraldi, N. M., Squarzoni, S. et al. (2005). Altered pre-lamin A processing is a common mechanism leading to lipodystrophy. *Hum. Mol. Genet.* **14**, 1489-1502.

Cossu, G. and Borello, U. (1999). Wnt signaling and the activation of myogenesis in mammals. *EMBO J.* **15**, 6867-6872.

Dorner, D., Vlcek, S., Foeger, N., Gajewski, A., Makolm, C., Gotzmann, J., Hutchison, C. J. and Foisner, R. (2006). Lamina-associated polypeptide 2α regulates cell cycle progression and differentiation via the retinoblastoma-E2F pathway. *J. Cell Biol.* **173**, 83-93.

Favreau, C., Higuet, D., Courvalin, J. C. and Buendia, B. (2004). Expression of a mutant lamin A that causes emery-dreifuss muscular dystrophy inhibits *in vitro* differentiation of C2C12 myoblasts. *Mol. Cell. Biol.* **24**, 1481-1492.

Fukumoto, S., Hsieh, C. M., Maemura, K., Layne, M. D., Yet, S. F., Lee, K. H., Matsui, T., Rosenzweig, A., Taylor, W. G., Rubin, J. S. et al. (2001). Akt participation in the Wnt signaling pathway through Dishevelled. *J. Biol. Chem.* **276**, 17479-17483.

Gotzman, J. and Foisner, R. (2006). A-type lamin complexes and regenerative potential: a step towards understanding laminopathic diseases? *Histochem. Cell Biol.* **125**, 33-41.

Huber, O., Korn, R., McLaughlin, J., Ohsugi, M., Herrmann, B. G. and Kemler, R. (1996). Nuclear localization of β-catenin by interaction with transcription factor LEF-1. *Mech. Dev.* **59**, 3-10.

Hutchison, C. J. and Worman, H. J. (2004). A-type lamins: guardians of the soma? *Nat. Cell Biol.* **6**, 1062-1067.

Ivorra, C., Kubicek, M., González, J. M., Sanz-González, S. M., Álvarez-Barrientos, A., O'Connor, J. E., Burke, B. and Andrés, V. (2006). A mechanism of AP-1 suppression through interaction of c-Fos with lamin A/C. *Genes Dev.* **20**, 307-320.

Jahoda, C. A. B., Whitehouse, C. J., Reynolds, A. J. and Hole, N. (2003). Hair follicle dermalcells differentiate into adipogenic and osteogenic lineages. *Exp. Dermatol.* **12**, 849-859.

Johnson, B. R., Nitta, R. T., Frock, R. L., Mounkes, L., Barbie, D. A., Stewart, C. L., Harlow, E. and Kennedy, B. K. (2004). A-type lamins regulate retinoblastoma protein function by promoting subnuclear localization and preventing proteasomal degradation. *Proc. Natl. Acad. Sci. USA* **101**, 9677-9682.

Korinek, V., Barker, N., Moerer, P., van Donselaar, E., Huls, G., Peter, J., Peters, P. J. and Clevers, H. (1998). Depletion of epithelial stem-cell compartments in the small intestine of mice lacking Tcf-4. *Nat. Genet.* **19**, 379-383.

Kubo, F., Takeichi, M. and Nakagawa, S. (2003). Wnt2b controls retinal cell differentiation at the ciliary marginal zone. *Development* **130**, 587-598.

Lako, M., Armstrong, L., Cairns, P. M., Harris, S., Hole, N. and Jahoda, C. A. B. (2002). Hair follicle dermal cells repopulate the mouse haematopoietic system. *J. Cell Sci.* **115**, 3967-3974.

Lin, F., Morrison, J. M., Wu, W. and Worman, H. J. (2005). MAN1, an integral protein of the inner nuclear membrane, binds Smad2 and Smad3 and antagonizes transforming growth factor-β signaling. *Hum. Mol. Genet.* **14**, 437-445.

Liu, J. and Farmer, S. R. (2004). Regulating the balance between peroxisome proliferator-activated receptor γ and β-catenin signaling during adipogenesis, *J. Biol. Chem*. **279**, 45020-45027.

Markiewicz, E., Venables, R., Alvarez-reyes, M., Quinlan, R., Dorobek, R., Hausmanowa-Petrusewicz, I. and Hutchison, C. J. (2002a). Increased solubility of lamins and redistribution of lamin C in X-linked Emery-Dreifuss muscular dystrophy fibroblasts. *J. Struct. Biol.* **140**, 241-253.

Markiewicz, E., Dechat, T., Foisner, R., Quinlan, R. A. and Hutchison, C. J. (2002b). Lamin A/C binding protein LAP2alpha is required for nuclear anchorage of retinoblastoma protein. *Mol. Biol. Cell* **13**, 4401-4413.

Markiewicz, E., Ledran, M. and Hutchison, C. J. (2005). Remodelling of the nucleoskeleton is required for skeletal muscle differentiation *in vitro*. *J. Cell Sci.* **188**, 409-420.

Markiewicz, E., Tilgner, K., Barker, N., van de Wetering, M., Clevers, H., Dorobek, M., Hausmanowa-Petrusewicz, I., Ramaekers, F. C. S., Broers, J. L. V., Blankesteijn, W. M. et al. (2006). The inner nuclear membrane protein Emerin regulates β-catenin activity by restricting its accumulation in the nucleus. *EMBO J.* **25**, 3275-3285.

Moldes, M., Zuo, Y., Morrison, R. F., Silva, D., Park, B. H., Liu, J. and Farmer, S. R. (2003). Peroxisome-proliferator-activated receptor γ suppresses Wnt/β-catenin signalling during adipogenesis. *Biochem. J.* **376**, 607-613.

Molenaar, M., van der Wetering, M., Oosterwegel, M., Petersib-Maduro, J., Godsave, S., Korinek, V., Roose, J., Destree, O. and Clevers, H. (1996). Xtcf-3 transcription factor mediates beta-catenin-induced axis formation in Xenopus embryos. *Cell* **86**, 391-399.

Nelson, W. J. and Nusse, R. (2004). Convergence of Wnt, beta-catenin, and cadherin pathways. *Science* **5**, 1483-1487.

Pan, D., Estevez-Salmeron, L. D., Stroschein, S. L., Zhu, X., He, J., Zhou, S. and Luo, K. (2005). The integral inner nuclear membrane protein MAN1 physically interacts with the R-Smad proteins to repress signaling by the transforming growth factor-{beta} superfamily of cytokines. *J Biol. Chem.* **280**, 15992-16001.

Peifer, M. and Polakis, P. (2000). Wnt signaling in oncogenesis and embryogenesis: a look outside the nucleus**.** *Science* **287**, 1606-1609.

Ross, S. E., Hemati, N., Longo, K. A., Bennett, C. N., Lucas, P. C., Erickson, R. L. and MacDougald, O. A. (2000). Inhibition of adipogenesis by Wnt signaling. *Science* **289**, 950-953.

Shackleton, S., Lloyd, D. J., Jackson, S. N., Evans, R., Niermeijer, M. F., Singh, B. M., Schmidt, H., Brabant, G., Kumar, S., Durrington, P. N. et al. (2000). LMNA, encoding lamin A/C, is mutated in partial lipodystrophy. *Nat. Genet.* **24**, 153-156.

Sullivan, T., Escalante-Alcalde, D., Bhatt, H., Anver, M., Bhat, N., Nagashima, K., Stewart, C. L. and Burke, B. (1999). Loss of A-type lamin expression compromises nuclear envelope integrity leading to muscular dystrophy. *J. Cell Biol.* **147**, 913-920.

van Berlo, J. H., Voncken, J. W., Kubben, N., Broers, J. L. V., Duisters, R., van Leeuwen, R. E., Crijns, H. J., Ramaekers, F. C. S., Hutchison, C. J. and Pinto, Y. M. (2005). A-type lamins are essential for TGF-{beta}1 induced PP2A to dephosphorylate transcription factors. *Hum. Mol. Genet.* **14**, 2839-2849.

Vaughan, A., Alvarez-Reyes, M., Bridger, J. M., Broers, J. L. V., Ramaekers, F. C. S., Wehnert, M., Morris, G. E., Whitfield, W. G. F. and Hutchison, C. J. (2001). Both emerin and lamin C depend on lamin A for localization at the nuclear envelope. *J. Cell Sci.* **114**, 2577-2590.

Vertino, A. M., Taylor-Jones, J. M., Kenneth, A., Longo, K. A., Bearden, E. D., Lane, T. F., McGehee, R. E., Jr, MacDougald, O. A. and Peterson, C. A. (2005). Wnt10b deficiency promotes coexpression of myogenic and adipogenic programs in myoblasts. *Mol. Biol. Cell* **16**, 2039-2048.

Journal of Cell Science