Sp1 Transcription Factor Interaction with Accumulated Prelamin A Impairs Adipose Lineage Differentiation in Human Mesenchymal Stem Cells: Essential Role of Sp1 in the Integrity of Lipid Vesicles

GARBÍNE RUIZ DE EGUINOA, ARANZA INFANTEA, KARIN SCHLANGENB, ANA M. ARANSAYB, ANE FULLAONDOB, MARIO SORIANOC, JOSÉ MANUEL GARCÍA-VERDUGOD, ÁNGEL G. MARTÍNEA, CLARA I. RODRÍGUEZA

Key Words. Mesenchymal stem cells • Adipogenesis • Experimental models • Transcription factor • Differentiation

ABSTRACT

Lamin A (LMNA)-linked lipodystrophies may be either genetic (associated with LMNA mutations) or acquired (associated with the use of human immunodeficiency virus protease inhibitors [Pis]), and in both cases they share clinical features such as anomalous distribution of body fat or generalized loss of adipose tissue, metabolic alterations, and early cardiovascular complications. Both LMNA-linked lipodystrophies are characterized by the accumulation of the lamin A precursor prelamin A. The pathological mechanism by which prelamin A accumulation induces the lipodystrophy associated phenotypes remains unclear. Since the affected tissues in these disorders are of mesenchymal origin, we have generated an LMNA-linked experimental model using human mesenchymal stem cells treated with a PI, which recapitulates the phenotypes observed in patient biopsies. This model has been demonstrated to be a useful tool to unravel the pathological mechanism of the LMNA-linked lipodystrophies, providing an ideal system to identify potential targets to generate new therapies for drug discovery screening. We report for the first time that impaired adipogenesis is a consequence of the interaction between accumulated prelamin A and Sp1 transcription factor, sequestration of which results in altered extracellular matrix gene expression. In fact, our study shows a novel, essential, and finely tuned role for Sp1 in adipose lineage differentiation in human mesenchymal stem cells. These findings define a new physiological experimental model to elucidate the pathological mechanisms LMNA-linked lipodystrophies, creating new opportunities for research and treatment not only of LMNA-linked lipodystrophies but also of other adipogenesis-associated metabolic diseases.

INTRODUCTION

Alterations in the lamin A/C gene (LMNA) are responsible for more than 10 different diseases called laminopathies, including cardiomyopathy, muscular dystrophy, progeria, and lipodystrophy [1, 2]. Lipodystrophies are a heterogeneous group of human disorders characterized by an anomalous distribution of body fat or a generalized loss of adipose tissue and variable degrees of resistance to insulin action, together with hypertriglyceridemia [3, 4]. Several types of lipodystrophies have been characterized at the molecular genetic level, such as mutations in the LMNA gene [5, 6]. Other lipodystrophies are acquired, such as the lipodystrophic syndrome associated with the use of human immunodeficiency virus (HIV) protease inhibitors (Pis), which leads to an accumulation of an immature, unprocessed form of lamin A called prelamin A [7].

The nuclear lamina, located primarily on the inner aspect of the nuclear membrane, is an intermediate filament meshwork composed of A- and B-type lamins [8, 9]. In mammalian somatic cells, A-type lamins are represented by lamins A and C, which originate from alternative splicing of the LMNA gene. Lamin A is initially generated as a precursor farnesylated prelamin A, which undergoes a series of post-translational modifications and endoproteolytic cleavages that ultimately result in the removal of the C-terminal farnesylated tail by ZMPSTE24 enzyme [10–13]. Regarding acquired lipodystrophies, Pis interfere with the processing of lamin A [7] by inhibiting ZMPSTE24 [14]. This inhibition leads to a significant accumulation of farnesyl- prelamin A relative to mature lamin A. In addition to the role of A-type lamins in maintaining the
for the first time that Sp1 transcription factor activity is indis-

cessaryprogramofPI-treated hMSCs. Importantly, our results show

ical interaction between accumulated prelamin A and Sp1 as a

nanceofadiposetissue[27].Wedemonstratehereanovelphys-

[26], which are indispensable for the homeostasis and mainte-

ration capacity of hMSCs by altering the normal expression of

thatPI-inducedprelaminAaccumulationreducesthedifferenti-

ation,orgeneexpressionprofiling)asaconsequenceofprelamin

AaccumulationprelaminA and lamin A/B bands. Western blots for prelamin A and lamin A/B were

formed for 4 hours at 200 V to achieve separation between

prelaminA and lamin A/B. Western blots for prelamin A and

lamin A/C were carried out using primary antibody (sc-6215;

Santa Cruz Biotechnology Inc., Santa Cruz, CA, http://www.scbt.

com) at a 1:100 dilution and appropriate secondary antibody
coupled to horseradish peroxidase (1:5,000 dilution). SuperSig-

nal West Pico chemiluminescent substrate developing kit

(Thermo Scientific Pierce, Rockford, IL, http://www.piercenet.

com) was used to develop the signal.

Immunofluorescence

For immunofluorescence experiments, hMSCs grown on glass
coverslips were fixed in 4% paraformaldehyde (Sigma) for 15 min-
utes at room temperature. The samples were incubated with

phosphate-buffered saline containing 5% FBS to saturate non-
specific binding and permeabilized with 0.1% Triton X-100
(Sigma) for 1 hour at room temperature. The dilutions of

the primary and secondary antibodies (Jackson Immunoresearch

Laboratories, West Grove, PA, http://www.jacksonimmuno.

com) were made in a similar solution and incubated for 1 hour

each at room temperature. Slides were mounted with an anti-
fade reagent (Invitrogen). BODIPY 493/503 (Invitrogen) was used
to stain lipid droplets following the manufacturer’s instructions.
The in situ proximity ligation assay (PLA) technique was used

with anti-prelamin A (sc-6214; Santa Cruz Biotechnology) and

Mechanical stability of the nucleus, it is becoming increasingly evi-
dent that A-type lamins are scaffolds for proteins that regulate DNA

synthesis, DNA damage responses, chromatin organization, gene

transcription, cell cycle progression, cell migration, and cell differen-
tiation[15, 16]. However, the manner in which these different func-
tions of lamins relate to disease pathophysiology remains to be elu-
cidated. Thus, despite the fact that there is a link between

accumulated prelamin A and LMNA-linked lipodystrophies[17–20], the

mechanism responsible for this pathology remains to be estab-

lished.

LMNA-linked lipodystrophic syndromes primarily affect tis-

sues from mesenchymal origin, such as the adipose lineage, which

arises from a multipotent stem cell population of meso-

dermal origin[21]. Thus, we hypothesized that prelamin A accu-
mulation in human mesenchymal stem cells (hMSCs) would com-

promise their homeostasis, preventing them from completing the

adipogenic program correctly. Given that adipocyte differenti-

ation involves a temporally regulated set of gene expression

events[22] that could be altered in prelamin A accumulating

hMSCs, the adipose lineage differentiation capacity of these cells is

one of the best candidates for a biological function altered

[21]. Therefore, tipranavir (TPV)-treated hMSCs would not dif-

ferentiate in the same way as control cells do (for example, in
terms of adipogenesis differentiation rate, lipid droplet forma-

tion, or gene expression profiling) as a consequence of prelamin

A accumulation.

Our results show that prelamin A accumulation in hMSCs

causes decreased proliferation, triggering premature cellular se-
nescence and an altered gene expression profile related to lipid

metabolism. By using hMSC-derived adipocytes, we also show

that PI-induced prelamin A accumulation reduces the differenti-

ation capacity of hMSCs by altering the normal expression of
genes involved in extracellular matrix and cell adhesion. Strik-

ingly, recent studies have pointed out that Sp1 transcription fac-
tor, besides its known function in the maintenance of basal tran-
scription[23], plays an essential role in the transcription of genes

related to fatty acid synthesis[24, 25] and extracellular matrix

[26], which are indispensable for the homeostasis and mainte-
nance of adipose tissue[27]. We demonstrate here a novel phys-
ical interaction between accumulated prelamin A and Sp1 as a

contributing factor responsible for the compromised adipogen-
esis program of PI-treated hMSCs. Importantly, our results show

for the first time that Sp1 transcription factor activity is indis-

pensable to the integrity of lipid vesicles in normal adipogenesis.

Materials and Methods

Cell Culture, Differentiation, and Drug Treatment of

hMSCs

Human bone marrow-derived (BM)-MSCs and adipose tissue-

derived (AT)-MSCs, respectively, were obtained from altruistic
donations from cadaveric brain-dead donors (hMSCs) or from

patients undergoing cosmetic liposuction from the Inbiobank

node of the Spanish National Stem Cell Bank (Fundación In-

biomed) (http://www.inbiobank.org). The cells were cultured in

low-glucose Dulbecco’s modified Eagle’s medium (Sigma, St.

Louis, http://www.sigmaaldrich.com) supplemented with 10%
fetal bovine serum (FBS; Sigma), penicillin, and GlutaMAX (Gibco,

Grand Island, NY, http://www.invitrogen.com). Donors’ ages and

sexes are given in supplemental online Table 1.

Prelamin A accumulation was induced by treating cells with

the HIV PI TPV at the indicated concentrations. Control cells were

incubated with the vehicle dimethyl sulfoxide (DMSO). TPV was

kindly provided by the pharmacy service of the Hospital Univer-
sitario Cruces (Barakaldo, Spain). For adipogenic differentiation,
hMSCs were seeded at confluence and the following day induced
to differentiate by culturing for 7 (early adipogenesis) or 21 days
(late adipogenesis) in the presence of adipogenic factors: 1 μM
dexamethasone, 500 μM 3-isobutyl-1-methylxantine, and 200
μM indomethacin (Sigma).

Oil Red O (Sigma) staining of hMSC-derived adipocytes was

performed to stain lipid droplets following a standard protocol

with some modifications[28]. Photographs were obtained under

a stereoscope (SMZ1000; Nikon, Tokyo, http://www.nikon.com)

using a digital camera (Nikon E8400).

In the case of WP631 (methanesulfonate, a specific inhibitor

of Sp1 transcription factor activity; Sigma), hMSCs were induced
to differentiate to adipose lineage, and 10 or 19 days after induc-
tion, WP631 was added to the medium at different concentra-
tions for 24 h, followed by another 24 h without the treatment.
Afterward (day 12 or 21), immunofluorescence confocal micro-
scopy was performed with BODIPY staining as described below.

Population Doubling Level

hMSCs were seeded and cultured under treatment with TPV or

DMSO. After 5 days in culture, the cells were detached, counted,

and reseeded at the initial concentration. This process was re-

peated until passage 11. The population doubling level (PDL) was

calculated as previously reported[20].

Western Blot

Eighty micrograms of total cellular extracts was loaded into 4%–

12% NuPAGE Novex 2-bis-(hydroxyethyl)aminol-2-(hydroxym-

ethyl)propane-1,3-dioli mini gels (Invitrogen, Carlsbad, CA,

http://www.invitrogen.com), and electrophoresis was performed

for 4 hours at 200 V to achieve separation between

prelaminA and lamin A/B bands. Western blots for prelamin A and

lamin A/C were carried out using primary antibody (sc-6215;

Santa Cruz Biotechnology Inc., Santa Cruz, CA, http://www.scbt.

com) at a 1:100 dilution and appropriate secondary antibody

coupled to horseradish peroxidase (1:5,000 dilution). SuperSig-
nal West Pico chemiluminescent substrate developing kit

(Thermo Scientific Pierce, Rockford, IL, http://www.piercenet.

com) was used to develop the signal.
anti-Sp1 (sc-14027; Santa Cruz Biotechnology) as primary antibodies and using the anti-goat PLUS and anti-rabbit MINUS Duolink in situ PLA kits (Olink Bioscience, Uppsala, Sweden, http://www.olink.com) according to the manufacturer’s protocol.

**Electron Microscopy**

The cells were seeded in chamber slides (Lab-Tek; Nunc, Rochester, NY, http://www.nuncbrand.com) and processed as described [29]. Photomicrographs were obtained under a transmission electron microscope (Tecnai Spirit G2; FEI, Eindhoven, The Netherlands, http://www.fei.com) using a digital camera (Morada Soft Imaging System; Olympus, Tokyo, http://www.olympus-global.com). Three independent measurements of at least 100 cells of each treatment were used to calculate the average length of heterochromatin aggregates.

**Senescence-Associated β-Galactosidase Assay**

Senescence-associated β-galactosidase activity was evaluated at passage 9 using a senescence β-galactosidase staining kit (Cell Signaling Technology, Beverly, MA, http://www.cellsignal.com) following the manufacturer’s instructions.

**Annexin V-FITC Propidium Iodide Staining**

At the end of the cell culture, hMSCs were washed, resuspended in staining buffer, and examined with the FITC Annexin V Apoptosis Detection Kit I (Becton, Dickinson and Company, Franklin Lakes, NJ, http://www.bd.com). Stained cells were analyzed by FACSCalibur (Becton Dickinson).

**Microarray Hybridization and Data Analysis**

Microarray experiments were performed from two independent hMSC cell lines derived from two independent bone marrow donors. Two biological replicates and one technical replicate were characterized per each treatment: thus, three independent hybridizations per treatment were performed. Total RNA was isolated using the High Pure RNA isolation kit (Roche Diagnostics, Mannheim, Germany, http://www.roche-applied-science.com) following the manufacturer’s instructions and quantified with a NanoDrop 2000 spectrophotometer (Fisher Scientific International, Hampton, NH, http://www.fisherscientific.com). RNA quality was assessed in a Bioanalyzer with RNA 6000 Nano Kits (Agilent Technologies, Palo Alto, CA, http://www.agilent.com). A whole-genome gene-expression characterization was conducted using HumanHT-12 v4 Expression BeadChips (Illumina Inc., San Diego, http://www.illumina.com), which allow interrogation of 47,000 gene targets covering more than 25,000 human annotated genes. cRNA synthesis was done with Epicenter’s TargetAmp Nanog Biotin-aRNA labeling kit for the Illumina system, and subsequent amplification, labeling, and hybridization were performed according to the manufacturer’s protocols.

**Gene Expression Profiling and Functional Annotation Analyses**

Expression data were analyzed with the R/Bioconductor statistical computing environment (http://www.r-project.org, http://www.bioconductor.org). Using the “lumi” package [30], the raw expression data were log2-transformed and quantile-normalized. For the detection of differentially expressed genes, a linear model was fitted to the microarrays, and empirical Bayes moderated T-statistics were calculated using the “limma” package [31] from Bioconductor. The microarray data were deposited at ArrayExpress (http://www.ebi.ac.uk/arrayexpress) with accession number E-MEXP-3289.

For automated functional annotation and classification of genes of interest, the dysregulated genes were analyzed using the annotation Database, Visualization, and Integrated Discovery (DAVID) Gene Ontology (GO) database (http://david.abcc.ncifcrf.gov) [32], taking into account a background that contained all genes present on the human HT12 v4 Illumina microarray. For this analysis, genes with a fold change more than 1.4 or less than −1.4 (<0.05) were considered to be regulated. Probe sets without annotation were removed from the analysis. Statistically over-represented GO terms were identified by selecting those with an Expression Analysis Systematic Explorer (EASE) score [33] (a modified Fisher exact probability p value) of <.05.

To test a possible enrichment for transcription factor-binding sites within the promoters of dysregulated genes, the DiRE server (http://dire.dcode.org) was used [34]. The dysregulated gene list contained sufficient annotated genes to accurately assess the number of regulatory elements present. The complete human microarray gene list was used as the background. The “occurrence” represented the fraction of putative regulatory elements that contain a particular transcription factor-binding site, whereas the “importance” was defined as the product between the occurrence and the weight assigned to each transcription factor.

**Luciferase Reporter Assay**

hMSCs were transiently transfected using the NucleoFector (Lonza, Basel, Switzerland, http://www.lonza.com) with pGL3-RARE-Luc reporter plasmid containing retinoic acid response elements (Addgene, Cambridge, MA, http://www.addgene.org). NF3TK-Luc plasmid containing a 3× nuclear factor-κB (NF-κB) enhancer, or pSp1 luciferase reporter plasmids. Transfection efficiency was determined cotransfecting with Renilla luciferase control vector (pRL-TK; Promega, Madison, WI, http://www.promega.com). Luciferase activity was measured in duplicate using the Dual-Glo luciferase assay system (Promega) in a GloMax 20/20 luminometer (Promega), and the results were normalized for protein content and expressed as fold induction above control levels.

**Statistical Analysis**

All the experiments were performed in triplicate in at least two different bone marrow- or adipose tissue-derived hMSCs as indicated. All of the data are expressed as the means ± SD. For the experiments carried out in two biological replicates, the statistical analyses were performed using n = 3 technical replicates. For the experiments performed in three or four biological replicates, n indicates the number of the biological replicates. Each treatment was compared with the control, and significant differences among the two groups were determined using the nonparametrical Mann-Whitney U test with Bonferroni correction. A value of p < .025 was taken as an indication of statistical significance.

**RESULTS**

TPV Treatment Leads to an Accumulation of Farnesylated Prelamin A and Altered Chromatin Organization in hMSCs

In order to confirm that farnesylated prelamin A is accumulated under TPV treatment in our experimental model (as reported in
fibroblasts [20]), hMSCs were treated with elevated, nonphysiological concentrations of TPV (50 and 100 μM). The presence of prelamin A was determined by Western blot; whereas prelamin A was nearly undetectable in control cells (vehicle) and in samples treated with 50 μM TPV, significant prelamin A accumulation was observed after the 100 μM TPV treatment, suggesting a TPV dose-dependent accumulation of prelamin A (Fig. 1A). The electrophoretic mobility of prelamin A in the TPV-treated cells was faster than that of the nonfarnesylated prelamin A, which accumulated in cells treated with a farnesyl transferase inhibitor (positive control lane), suggesting that this higher prelamin A band was farnesylated, as has been previously described for human and mouse fibroblasts [14]. The accumulation of prelamin A in cells treated with a lower TPV concentration (30 μM) was confirmed by immunofluorescence (Fig. 1B). Although it has been previously demonstrated that fibroblasts with LMNA mutations or treated with PIs show nuclear shape abnormalities [20], the nuclei of TPV-treated hMSCs showed no significant alterations (data not shown).

The heterochromatin protein 1γ (HP1γ) is one of the adaptors between the nuclear lamina and chromatin [35]. Since fibroblasts from individuals with a severe genetic laminopathy, such as progeria, show reduced expression of HP1γ, we wanted to test whether TPV treatment would show a similar nuclear phenotype in hMSCs. As shown in supplemental online Figure 1, TPV treatment caused a loss of HP1γ foci in

**Figure 1.** Prelamin A accumulation and altered chromatin organization in TPV-treated human mesenchymal stem cells (hMSCs). (A): Western blot of prelamin A. Farnesyl transferase inhibitor treatment is shown as positive control. Lamin C was used as a loading control. (B): Prelamin A staining by immunofluorescence. Scale bar = 50 μm. (C, D): Electron micrographs showing heterochromatin aggregates (small arrows) and lipid bilayer (large arrows) carried out in three different BM-hMSCs. Aberrant condensation of heterochromatin is shown in the lower panels in (D). Scale bars = 2 nm (C) and 500 nm (D). Abbreviations: BM, bone marrow; cont, control; DAPI, 4',6-diamidino-2-phenylindole; TPV, tipranavir.
hMSCs in a concentration-dependent manner (loss of 20% at 50 μM TPV and 12% at 20 μM TPV vs. 6% in control cells). This altered HP1γ expression prompted us to analyze the inner nuclear membrane (INM) of hMSCs treated with TPV by transmission electronic microscopy. In control cells, the chromatin layout thickness was homogeneous throughout the lipid bilayer in the INM (35.23 ± 2.28 nm wide; large arrows in Fig. 1D), except in the nuclear pore complexes, where it was interrupted, as shown in Figure 1C and 1D. However, in TPV-treated cells, we observed zones in the INM where chromatin disappeared, as well as several regions with heterochromatin aggregate patterns, reaching 650 nm of length (Fig. 1C, 1D, small arrows). The average length of heterochromatin aggregates was 300.67 ± 17.54 nm (Fig. 1C). Seventy-five percent of treated cells showed these aberrations versus 30% of control cells. Ablant condensation of heterochromatin was observed in the nucleoplasm of treated cells but not in cells treated only with vehicle (Fig. 1D). These results are consistent with previous studies carried out in fibroblasts of patients suffering laminopathies [36–39].

hMSCs with Prelamin A Accumulation Show a Reduced Proliferative Capacity and a Premature Senescent Phenotype

The proliferation capacity of hMSCs under TPV treatment was analyzed, since PI treatment has been shown to dramatically reduce the proliferative capacity of primary fibroblasts in culture [20]. Two TPV concentrations, a physiological 20 μM [14] and a slightly higher concentration of 30 μM, were used. The PDL of control hMSCs remained stable up to passage 6, when it began to decrease, although some proliferation was observed until passage 11 (Fig. 2A). In contrast, the proliferation rate of hMSCs treated with TPV showed a slight but significant decrease, reaching arrested cell division (PDL < 1.0) at passage 11. This effect was more pronounced with 30 μM TPV treatment (between passages 5 and 7), although both TPV concentrations induced a similar arrest of proliferation at passage 11. Apoptotic cells identified by flow cytometry represented less than 10% of total cells in all conditions, and there were no significant differences between vehicle and TPV treatments (Fig. 2B), suggesting that TPV-treated hMSCs could enter senescence more prematurely than control cells. Upon senescence, cells become flattened and enlarged, showing biochemical changes, such as increased perinuclear activity of senescence-associated β-galactosidase [40, 41]. We stained hMSCs at passage 9 with 5-bromo-4-chloro-3-indolyl-β-D-galactoside and observed a higher proportion of senescent cells in TPV-treated samples (40.89% and 51.00% with 20 μM TPV and 30 μM TPV treatments, respectively, vs. 28.6% in control BM-hMSCs; and 50.46% and 52.40% vs. 30% in different bone marrow-derived hMSCs) (Fig. 2C).

hMSCs with Accumulated Prelamin A Show an Altered Gene Expression Profile of Genes Related to Lipid Metabolism

The cellular phenotypes shown by accumulated prelamin A in hMSCs prompted us to investigate the transcriptional profiles of these cells in an attempt to find a molecular mechanism that could account for these phenotypes. For that purpose, we performed a whole-genome, microarray-based gene expression analysis of RNA extracted from hMSCs treated for seven passages with TPV, at which time a decrease in cell proliferation and an increase in cellular senescence begins to occur (Fig. 2A, 2C). hMSCs were treated with TPV at a physiological concentration, that is, at 20 μM, and the corresponding control cells were treated with DMSO. Significant changes (fold change ± 1.4, p < .05) in the expression of 111 transcripts (7.7% downregulated, 92.3% upregulated) encoding 80 annotated genes were identified (supplemental online Table 2). Surprisingly, GO analysis of the regulated transcript list using the DAVID bioinformatics resource [32] revealed “lipid biosynthetic process” and “steroid metabolic process” as two of the most significantly enriched categories within biological processes (EASE scores of <0.023 and 0.019, respectively) (Table 1), suggesting that the accumulation of prelamin A in hMSCs alters lipid homeostasis.

We wanted to confirm this suggested alteration of lipid homeostasis in hMSCs caused by the accumulation of prelamin A by a functional assay. For that purpose, we studied the effect of TPV treatment on a pathway involved in lipid metabolism, such as the retinoic acid pathway, which regulates the transcription of target genes controlling lipid metabolism [42, 43]. A known relationship between PI treatment and an alteration of the retinoic acid signaling pathway further supports the choice of the retinoic acid pathway for study [44]. hMSCs were transfected with a luciferase reporter vector containing retinoic acid response elements (RAREs) in its promoter, and, after transfection, the cells were treated for 48 hours with TPV. In the presence of TPV, RARE activity was significantly lower than in control cells (Fig. 2D), suggesting that prelamin A accumulation induced the repression of the transcriptional activity of the retinoic acid pathway. Furthermore, when hMSCs were treated for 24 hours with TPV, followed by another 24 hours without the treatment (TPV recovery), the RARE activity was restored to the level seen in the control cells, confirming that the downregulation observed after 48 hours of treatment with TPV is specific (Fig. 2D). As a negative control for TPV treatment, similar experiments were performed using an NF-κB reporter vector, because the microarray data showed no alteration in the expression of the main effectors of the NF-κB pathway. As shown in Figure 2D, this pathway was not altered by TPV treatment.

Prelamin A Accumulation Impairs hMSC Differentiation to Adipocytes

The gene expression regulation results encouraged us to investigate the adipose lineage differentiation capacity of hMSCs with accumulated prelamin A. Accordingly, hMSCs were treated for seven passages with TPV or DMSO and then induced to differentiate to adipocytes over 21 days, maintaining the TPV or DMSO treatment. Most TPV-treated hMSC-differentiated adipocytes showed an accumulation of prelamin A (Fig. 3A, 3C, red nuclei) and a reduced adipogenic differentiation capacity, as detected by Oil Red O staining (Fig. 3B). Lipid droplets were stained with the BODIPY fluorescent molecule, which specifically stains intracellular lipid droplets. TPV-treated hMSC-derived adipocytes with accumulated prelamin A showed a drastic reduction in their lipid droplet size (Fig. 3A, 3C, higher magnification). Likewise, as observed in Figure 3D, we detected a significant increase in nuclear abnormalities and blebbing in the TPV-treated hMSC-derived adipocytes at late adipogenesis (23.57% in TPV 30 μM vs. 9.78% in DMSO, p < .025). These results indicate a direct relationship between prelamin A accumulation and impaired adipogenesis in these hMSCs.
Prelamin A Accumulation in hMSC-Derived Adipocytes
Induces an Alteration in the Expression of Extracellular Matrix and Cell Adhesion Genes

To further elucidate the molecular mechanism for the reduced adipogenesis shown by hMSC-derived adipocytes with accumulation of prelamin A, a whole-genome gene-expression comparison was performed. hMSCs were treated for seven passages with the physiological concentration of 20 μM tipranavir or DMSO and then induced to differentiate to adipocytes over 7 (early adipogenesis) and 21 days (late adipogenesis), maintaining the TPV or DMSO treatment. Early adipogenesis samples treated with TPV showed significant changes, most of them downregulation, in the expression of 49 transcripts, encoding 44 annotated genes (fold change, ±1.4, p < .05) when compared with control cells (supplemental online Table 3). Surprisingly, GO analysis of these regulated genes showed a significant overrepresentation of “extracellular matrix” and...
regulation of cell adhesion categories (\(p < .001\)), but there were no enriched categories related to lipid metabolism (supplemental online Table 4). In the case of TPV-treated cells in late adipogenesis, significant changes in the expression of 105 transcripts, encoding 87 annotated genes (fold change \(\pm 1.4, p < .05\)) were identified (supplemental online Table 5), and as with the early adipogenesis gene regulation, the categories of “extracellular matrix” and “biological adhesion” were significantly enriched \((p < .01)\) (Table 2). These results were validated by quantitative reverse transcription-polymerase chain reaction analysis on a subset of genes from these categories, confirming their significantly altered expression in the TPV-treated hMSCs and hMSC-derived adipocytes (supplemental online Fig. 2).

**Prelamin A Colocalizes with Sp1 Transcription Factor in Adipocytes Derived from hMSCs, Resulting in an Altered Sp1 Transcription Factor Activity**

The in silico analysis of the dysregulated genes was extended using the comparative genomic tool DiRE, which predicts the enrichment for transcription factor-binding sites within the promoters of coexpressed genes [34]. Interestingly, we found that the Sp1 transcription factor was predicted to be one of the most important transcription factors within the promoters of the dysregulated genes in hMSC-derived adipocytes with accumulated prelamin A in late adipogenesis (importance, 0.2; occurrence, 26%). This result suggested that the alteration of gene expression in TPV-treated hMSC-derived adipocytes could be mediated in part by Sp1. Interestingly, Sp1 is known to be involved in the maintenance of the basal levels of extracellular matrix genes in 3T3-L1 adipocytes, and its activity must be regulated for the progression of the adipogenesis process [26]. Therefore, this in silico analysis indicated that prelamin A accumulation itself could be preventing normal Sp1 transcription factor activity. Since a previous study reported a sequestration of SREBP1 transcription factor (related with lipid metabolism) by accumulated prelamin A [17], we performed in situ PLA to explore a possible interaction between Sp1 and prelamin A. For this technique, the distance between the primary antibodies used (anti-Sp1 and anti-prelamin A in our case) must be less than 40 nm for the PLA to
generate a signal, making the methodology highly specific for physically interacting protein-protein complexes [45–47]. As shown in Figure 4A, a stable interaction between Sp1 and prelamin A was detected by PLA assay in adipocytes differentiated from hMSCs for 21 days. We failed to detect any interaction between these two proteins in vehicle cells (Fig. 4A).

To investigate whether this interaction between Sp1 transcription factor and prelamin A could be responsible for an alteration in Sp1 transcriptional activity, we performed a luciferase reporter vector assay with the Sp1-Luc reporter vector (Fig. 4B). As expected, Sp1 transcriptional activity was reduced in TPV-treated adipocytes, possibly because of the Sp1 retention by prelamin A.

Table 2. Prelamin A accumulation impairs human mesenchymal stem cell (hMSC) differentiation into adipocytes

| GO accession number | GO term                        | EASE score |
|---------------------|--------------------------------|------------|
| GO:0007155          | Cell adhesion                  | 2.5 × 10^{-3} |
| GO:0005578          | Proteinaceous extracellular matrix | 6.0 × 10^{-3} |
| GO:0031988          | Membrane-bound vesicle         | 9.0 × 10^{-3} |

Biological process and cellular component categories were identified by GO analysis as being significantly enriched among the dysregulated genes of hMSC-derived adipocytes in late adipogenesis treated with 20 μM tipranavir. Abbreviations: EASE, Expression Analysis Systematic Explorer; GO, Gene Ontology.

**Sp1 Activity Is Essential for Lipid Vesicle Integrity and for the Adipogenesis Program in hMSC-Derived Adipocytes**

In order to check whether the alteration in Sp1 transcription factor activity could be responsible for the impaired adipogenesis observed in prelamin A-accumulated hMSCs, we used the Sp1 specific inhibitor WP631, a bisintercalating anthracycline drug, which specifically inhibits Sp1-dependent transcription [26, 48, 49]. We wanted to determine whether the alteration of the Sp1 transcription factor activity could affect the adipose lineage differentiation process of hMSCs to a greater or lesser extent.

**Figure 4.** Prelamin A colocalizes with Sp1 in late adipogenesis, affecting Sp1 transcription factor activity. (A): Proximity ligation assay for prelamin A and Sp1. Red dots correspond to a protein-protein physical interaction. Scale bar = 20 μm. (B): Sp1 reporter vector assay performed in triplicate in bone marrow-derived human mesenchymal stem cells (hMSCs) and in duplicate in different bone marrow-derived hMSCs. hMSCs were nucleofected; 24 hours later, adipogenesis was induced for 72 hours, and then firefly and Renilla activities were measured. Abbreviations: BM, bone marrow; TPV, tipranavir.
depending on the time point of the adipogenesis process when the Sp1 specific inhibitor WP631 was added. As shown in Figure 5, at day 10 of differentiation in hMSC-derived adipocytes (when lipid droplets begin to appear), WP631 treatment reduced their lipid vesicle size in a dose-dependent manner. When WP631 was added near the end of the differentiation period (day 19), we observed a reduction in the lipid vesicle size, in a dose-dependent manner too, although this reduction was not as pronounced (Fig. 5). Interestingly, we analyzed the adipogenesis recovery after WP631 treatment by Oil Red O staining at the end of differentiation process (day 21) and found that adipogenesis could not be restored after adding WP631 at high concentrations at day 10 of differentiation (supplemental online Fig. 3). However, when WP631 was added at day 19, this inhibitor had practically no effect in adipogenesis (supplemental online Fig. 3). Altogether, these results suggested a requirement of Sp1 transcription factor for the maintenance and integrity of the lipid vesicles and indicated that Sp1 activity is fundamental to carry out a proper adipogenesis program.

**Discussion**

Adipogenesis involves a sequential and ordered cascade of gene expression events coordinated by transcription factors that simultaneously induce tissue-specific gene expression and repress alternate cell fates [22]. This process of adipogenesis involves a significant remodeling of the extracellular matrix since extracellular matrix progresses from a fibrillar to a laminar structure as cells move from the commitment phase to the growth phase, which is characterized by storage of enormous amounts of triglycerides [27]. LMNA-linked lipodystrophies may be either genetic (associated with LMNA mutations) or acquired (as a consequence of HIV antiretroviral treatment), and in both cases they share clinical features, such as anomalous distribution of body fat or generalized loss of adipose tissue; metabolic alterations, including insulin resistance and hypertriglyceridemia; and early cardiovascular complications [50, 51]. They share biological features as seen in previous studies using HIV protease inhibitor treatment performed in control fibroblasts, as well as in LMNA-linked lipo dystrophy patient cells—accumulation of prelamin A, nuclear shape abnormalities, and reduced proliferative activity—due to defective processing of lamin A [14, 17, 20]. The mechanism responsible for these lipodystrophic phenotypes upon the accumulation of prelamin A remains unclear.

Both the genetic and acquired LMNA-linked lipodystrophic syndromes mainly affect tissues from mesenchymal origin; therefore, our experimental model is based on hMSCs treated with an HIV protease inhibitor that has been previously demonstrated to inhibit mouse ZMPSTE24 in vitro and to induce mouse
prelamin A accumulation [14]. This experimental model avoids any interspecies differences that could arise using murine cells such as 3T3-L1 preadipocyte cells and provides all of the advantages of primary culture cells over established cell lines. In addition, although there is some controversy in the literature concerning whether BM- and AT-hMSCs have the same potential to differentiate into adipocytes, we focused on the comparative studies, which report the similarities between these cells in their adipogenic differentiation capacity [52, 53]. However, some studies report that subcutaneous adipose depots of obese animals show a significant upregulation in lipid metabolism-related genes [54]. Thus, the principal experiments that we performed have been done with bone marrow-derived hMSCs to avoid the possibility that some AT-hMSCs could be primed for altered lipid metabolism. Nevertheless, we have corroborated that accumulation of prelamin A induces a reduction of adipogenic capacity of the PI-treated AT-hMSC in a similar way as observed in the bone marrow-derived hMSCs (supplemental online Fig. 4). Moreover, since hMSCs are primary cells whose proliferation and differentiation capacity could be influenced by genotype, donor age, and health conditions [52, 55], we have performed all experiments in the same cell passage number and in hMSCs derived from at least two independent bone marrow donors and in three independent experiments to account for this challenge and to verify our results.

Accumulation of prelamin A as a consequence of the PI treatment in hMSCs was observed just as has been described specifically in lipodystrophy-linked laminopathies [17–20]. Furthermore, our proposed model recapitulates the features previously observed in fibroblasts treated with PI, as well as in LMNA-linked lipodystrophy-associated patient fibroblasts [20]; treated hMSCs showed a concentration-dependent reduction in proliferation capacity and an increase in cellular senescence without any alteration in the level of cellular apoptosis. Moreover, hMSCs with accumulated prelamin A showed a significant percentage of nuclei with altered distribution of peripheral heterochromatin in agreement with previous report of fibroblasts from familial partial lipodystrophy patient (LMNA-linked lipodystrophy) [26, 56], as well as in other laminopathies characterized by lipodystrophic phenotypes, namely the accelerated aging disorder Hutchinson-Gilford progeria syndrome (HPGS) [38, 57, 58] and mandibulofacial dysplasia type A [37, 39]. These results validate PI-treated hMSCs as an experimental model of LMNA-linked lipodystrophy.

The gene expression pattern studies done on undifferentiated hMSCs TPV-treated cells showed an alteration in genes involved in lipid homeostasis, a finding that was confirmed by the downregulation of the retinoic acid signaling pathway reporter. Thigh and abdomen biopsies of LMNA-linked lipodystrophy patients (type 2 familial partial lipodystrophy) have shown not only prelamin A accumulation but also a reduced expression of several genes involved in adipogenesis [56]. Moreover, abnormal expression patterns of genes involved in lipid metabolism have been described in murine 3T3 cells subjected to PI treatment [59]. These reports underscore our results and suggest a potential effect on hMSC adipose lineage differentiation when prelamin A is accumulated.

As expected, the adipose lineage differentiation capacity of the prelamin A-accumulated hMSCs was altered. These results are in agreement with the LMNA-linked disease progeria (HPGS), where hMSCs expressing the mutated prelamin A called progerin showed a reduced adipogenesis potential [60]. This lethal disorder is caused by the accumulation of the mutated prelamin A progerin and seems to affect mainly mesenchymal lineages [2, 18, 61]. In detail, our experimental model suggested that prelamin A accumulation affects the integrity of lipid vesicles, since we detected fewer differentiated cells and smaller lipid vesicles in differentiated TPV-treated hMSCs than in control hMSCs.

The gene expression patterns altered in differentiated prelamin A-accumulated hMSCs pointed to extracellular matrix genes. Noticeably, the extracellular matrix plays a crucial role in adipocyte development and function and, by implication, in lipid metabolism [27]. Additionally, a previous study suggested that HIV-Pis may suppress adipogenic differentiation by altering the extracellular matrix composition [62]. Moreover, this category of extracellular matrix genes has been found to be altered in progeria studies that include cultured HGPS cells [63], progerin-expressing hMSCs [60], a progeria mouse model [64], and progeria induced pluripotent stem cell derived hMSC model [65]. Two of these studies account for the observed altered expression patterns by two different signaling pathways, the Wnt and Notch pathways [60, 64]. However, neither downstream effectors of the Notch signaling pathway nor Wnt signaling genes showed altered expression in our experimental model.

Our in silico studies point out that the ubiquitous transcription factor Sp1, which has been demonstrated to play a role in regulating extracellular matrix [26, 66, 67] and lipid metabolism [24, 25] genes, is a potential regulator of several of the dysregulated genes obtained herein. Indeed, some of the lipid metabolism-related genes, such as SCD [68], SULT1A1 [69], and ABCA1 [70], are known targets of Sp1. Moreover, the in silico analysis that we performed with the Toucan program revealed that many of the dysregulated extracellular matrix genes found in the microarray data analysis (CTHRC1, LAMA3, PODNL1, LAMC2, and TIMP2) showed Sp1 binding sites in their promoters. Overall, these results directly implicate Sp1 in the dysregulation of the expression of extracellular matrix and lipid metabolism genes, which are essential for carrying out the adipogenesis program. We describe for the first time a novel interaction between accumulated prelamin A and the transcription factor Sp1, with functional consequences, as has been demonstrated by the reduction of Sp1 activity in prelamin A-accumulated hMSCs. We showed that the Sp1 specific inhibitor reproduces the phenotype observed in the adipogenesis program carried out in prelamin A-accumulated hMSCs, where differentiation is reduced and adipocytes show smaller lipid vesicles in a TPV dose-dependent manner. This result clearly demonstrates the physiological relevance of the interaction between prelamin A and Sp1. The proposed mechanism leading to LMNA-linked lipodystrophy includes the interaction of accumulated prelamin A and transcription factors with an essential role in the process of adipogenesis through the alteration of the extracellular matrix. In this mechanism, the interaction of transcription factors with accumulated lamin A precursor could restrict the access of the transcription factors to their target genes, thereby impairing their function. In addition to the results described here, this theory is supported by the previously reported interaction between accumulated prelamin A and SREBP1, a transcription factor that plays a major role in the control of genes involved in adipocyte differentiation [17]. Regarding this issue, it is known that SREBP1 transcription factor is a weak transcriptional activator on its own and that it interacts with additional regulators in its target promoters in cooperation with proteins such as NFY and
Sp1 [71–73]. Moreover, a genome-wide occupancy analysis in human HepG2 cells revealed the promoter occupancy of SREBP1, NFY, and Sp1 in a set of lipid metabolism-related genes [74], suggesting that a combination of these factors regulates this functional pathway. Taking into account these findings, it is plausible that Sp1 sequestration by prelamin A in adipogenesis could be responsible in part for an alteration of SREBP1 function. However, we did not observe any enrichment for SREBP1 target genes in the dysregulated genes obtained from microarray data. This might indicate that Sp1 sequestration by prelamin A in hMSCs is not sufficient to induce an alteration of the transcription of SREBP1 target genes.

Finally, we have described a novel role for Sp1 during hMSC adipose lineage differentiation, and this Sp1 function seems to be finely tuned, given that the time at which the Sp1 inhibitor is added during adipose lineage differentiation is crucial to the impairment of adipogenesis. The same concentrations of Sp1 specific inhibitor at different time points produced some mild and some severe phenotypes, suggesting a precise and time-sensitive regulation of Sp1 during hMSC adipogenesis. In fact, it is conceivable that Sp1 plays a crucial role in the early formation of lipid droplets through the regulation of the extracellular matrix composition. Of note, in adipocytes derived from hMSCs that accumulated prelamin A, we did not observe any dysregulation in the mRNA levels of vimentin or adipocyte differentiation-related protein, genes known to play an important role in intracellular lipid droplet formation [75, 76] and that are regulated by Sp1 [77, 78]. This could be due to functional redundancy between Sp1 and other transcription factors in the transcription of these genes. Our results suggest that Sp1 has a specific role in the transcriptional control of extracellular matrix genes, which influence the correct execution of the adipogenesis program. In fact, the two phases of adipogenesis, adipocyte commitment to the storage of triglycerides and the accumulation of high levels of triglycerides, are associated with a biphasic production of extracellular matrix components consistent with the constant turnover of the extracellular matrix during adipogenesis [27].

**CONCLUSION**

PI (TPV)-treated hMSCs provide a physiological experimental model for LMNA-linked lipodystrophies recapitulating the different phenotypes observed in patient biopsies. This model has been demonstrated to be a significant tool to unravel the mechanism of the LMNA-linked lipodystrophies. Also, this model provides an ideal system to identify potential targets to generate new therapies and to carry out drug discovery screening. The present study for the first time reports the mechanism of impaired adipogenesis as a consequence of the interaction of Sp1 transcription factor and accumulated prelamin A. In fact, our study has shown a novel, essential, and finely tuned role for Sp1 in the adipose lineage differentiation of human mesenchymal stem cells. In conclusion, these findings render a new physiological experimental model to elucidate the pathological mechanisms of LMNA-linked lipodystrophies, additionally offering new opportunities for research and treatment not only of LMNA-linked lipodystrophies but also of other adipogenesis-associated metabolic diseases.

**ACKNOWLEDGMENTS**

We are very grateful to Dr. M.A. iñiguez (Centro de Biología Molecular “Severo Ochoa,” Madrid, Spain) and Dr. Frank Peiretti (Université de la Méditerranée, Marseille, France) for providing us the pNF3TK-Luc and pRL-TK reporter vectors and the psp1-Luc reporter vector, respectively. We thank Dr. Catherine Shanahan (King’s College London) for her help with prelamin A antibody. We thank Dr. Ricardo Andrade for help and expertise from the University of the Basque Country General Research Services (SGIKER) confocal microscopy service. Special thanks go to Dr. Leslie Mounkes for critical revision of the manuscript. This work was supported by grants from the European Union; Marie Curie International Reintegration Grant MIRG-CT-2006-044914; Fondo de Investigación Sanitaria (Instituto de Salud Carlos III, Madrid, Spain) Grant PI06-1335; the Department of Industry, Tourism and Trade of the Government of the Autonomous Community of the Basque Country (Etorket Research Programs 2009/2011); and the Innovation Technology Department of Bizkaia County. G.R.d.E. is a predoctoral fellow supported by FPI Grant BFI09-135 from the Basque Department of Education, Universities and Research.

**AUTHOR CONTRIBUTIONS**

G.R.d.E. and A.I.: experimental design, assembly, analysis, writing; K.S.: assembly, analysis and interpretation of data; A.M.A. and J.M.G.-V.: data analysis and interpretation; A.F. and M.S.: experimental assembly; A.M.A. and J.M.G.-V.: data analysis and interpretation; A.F. and M.S.: experimental assembly; A.G.M.: experimental design, assembly, analysis, writing; C.I.R.: conception and design, assembly, analysis and interpretation of data, manuscript writing, final approval of manuscript.

**DISCLOSURE OF POTENTIAL CONFLICTS OF INTEREST**

The authors indicate no potential conflicts of interest.

**REFERENCES**

1. Mounkes LC, Stewart CL. Aging and nuclear organization: Lamins and progeria. Curr Opin Cell Biol 2004;16:322–327.
2. Burke B, Stewart CL. The laminopathies: The functional architecture of the nucleus and its contribution to disease. Annu Rev Genomics Hum Genet 2006;7:369–405.
3. Hegele RA. Monogenic forms of insulin resistance: Apertures that expose the common metabolic syndrome. Trends Endocrinol Metab 2003;14:371–377.
4. Mattout A, Dechat T, Adam SA et al. Nuclear lamins, diseases and aging. Curr Opin Cell Biol 2006;18:335–341.
5. Shackleton S, Lloyd DJ, Jackson SN et al. LMNA, encoding lamin A/C, is mutated in partial lipodystrophy. Nat Genet 2000;24:153–156.
6. Novelli G, Muchir A, Sanguolo F et al. Mandibuloacral dysplasia is caused by a mutation in LMNA-encoding lamin A/C. Am J Hum Genet 2002;71:426–431.
7. Caron M, Auclair M, Sterlingt H et al. Some HIV protease inhibitors alter lamin A/C maturation and stability, SREBP-1 nuclear localization and adipocyte differentiation. AIDS 2003;17:2437–2444.
8. Aebi U, Cohn J, Buhle L et al. The nuclear lamina is a meshwork of intermediate-type filaments. Nature 1986;323:560–564.
9. McKeon F, Kirschner MW, Caput D. Homologies in both primary and secondary structure between nuclear envelope and intermediate filament proteins. Nature 1986;319:463–468.
10. Weber K, Plessmann U, Traub P. Maturation of nuclear lamin A involves a specific carboxy-terminal trimming, which removes the
polysineprenylation site from the precursor; implications for the structure of the nuclear lamina. FEBS Lett 1989;257:411–414.
21 Beck LA, Hosick TJ, Sinensky M. Isoprenylation is required for the processing of the lamin A precursor. J Cell Biol 1990;110:1489–1499.
22 Corrigan DP, Kuszczak D, Rusinol AE et al. Prelamin A endoproteolytic processing in vitro by recombinant Zmpste24. Biochem J 2005;387:129–138.
23 Young SG, Feng LG, Michaelis S. Prelamin A, Zmpste24, misshapen cell nuclei, and progeria: New evidence suggesting that protein farnesylation could be important for disease pathogenesis. J Lipid Res 2005;46:2531–2558.
24 Coffinier C, Hudon SE, Farber EA et al. HIV protease inhibitors block the zinc metalloproteinase ZMPSTE24 and lead to an accumulation of prelamin A in cells. Proc Natl Acad Sci USA 2007;104:13432–13437.
25 Broers JL, Ramaekers FC, Bonne G et al. Nuclear lamins: Laminoapathies and their role in premature ageing. Physiol Rev 2006;86:967–1008.
26 Verstraeten VL, Broers JL, Ramaekers FC et al. The nuclear envelope, a key structure in cellular integrity and gene expression. Curr Med Chem 2007;14:1231–1248.
27 Capanni C, Mattioli E, Columbaro M et al. Altered pre-lamin A processing is a common mechanism leading to lipodyrophy. Hum Mol Genet 2005;14:1489–1502.
28 De Sandre-Giovannoli A, Bernard R, Cau P et al. Lamin A truncation in Hutchinson-Gilford progeria. Science 2003;300:2055.
29 Mounkes LC, Kozlo S, Hernandez L et al. A progeroid syndrome in mice is caused by defects in A-type lamins. Nature 2003;423:298–301.
30 Caron M, Alcuair M, Donadille B et al. Human lipodystrophies linked to mutations in A-type lamins and to HIV protease inhibitor therapy are both associated with prelamin A accumulation. oxidative stress and premature cellular senescence. Cell Death Differ 2007;14:1759–1767.
31 Otto TC, Lane MD. Adipose development: From stem cell to adipocyte. Crit Rev Biochem Mol Biol 2005;40:229–242.
32 Rosen ED, MacDougall OA. Adipocyte differentiation from the inside out. Nat Rev Mol Cell Biol 2006;7:885–896.
33 Black AR, Black JD, Azizkhan-Clifford J. Sp1 and Krüppel-like family of transcription factors in cell growth regulation and cancer. J Cell Physiol 2001;188:143–160.
34 Moon YA, Kim KS, Cho UH et al. Characterization of regulatory elements on the promoter region of human ATP-citrate lyase. Exp Mol Med 1999;31:108–114.
35 Xiong S, Chirala SS, Wakil SJ. Sterol regulation of human fatty acid synthase promoter I requires nuclear factor-Y- and Sp-l-binding sites. Proc Natl Acad Sci USA 2000;97:3948–3953.
36 Bernt B, Barruet E, Poggi M et al. Downregulation of tissue inhibitor of metalloproteinase-3 (TIMP-3) expression is necessary for adipocyte differentiation. J Biol Chem 2010;285:6508–6514.
37 Mariman EC, Wang P. Adipocyte extra-cellular matrix composition, dynamics and role in obesity. Cell Mol Life Sci 2010;67:1277–1292.
38 Ramirez-Zacarias JL, Castro-Muñozledo F, Kuri-Harcuch W. Quantitation of adipose conversion and triglycerides by staining intracellular lipids with Oil red O. Histochemistry 1992;97:493–497.
39 de Luna N, Gallardo E, Soriano M et al. Absence of dysferlin alters myogenin expression and delays human muscle differentiation in vitro. J Biol Chem 2006;281:17092–17098.
40 Du P, Kibble WA, Lin SM. lumi: A pipeline for processing Illumina microarray. Bioinformatics 2008;24:1547–1548.
41 Smyth GK. Linear models and empirical bayes methods for assessing differential expression in microarray experiments. Stat Appl Genet Mol Biol 2004;3:Article 3.
42 Huang dW, Sherman BT, Lempicki RA. Bioinformatics enrichment tools: paths toward the comprehensive functional analysis of large gene lists. Nucleic Acids Res 2009;37:1–13.
43 Hosack DA, Dennis G, Sherman BT et al. Identifying biological themes within lists of genes with EASE. Genome Biol 2003;4:R70.
44 Gotea V, Ovcharenko I. DIRe: Identifying distant regulatory elements of co-expressed genes. Nucleic Acids Res 2008;36:W133–W139.
45 Ye Q, Callebaut I, Pehazman A et al. Domain-specific interactions of human HP1-type chromodomain proteins and inner nuclear membrane protein LBR. J Biol Chem 1997;272:14983–14989.
46 Capanni C, Cenni V, Mattioli E et al. Failure of lamination of A/Co to functionally assemble in R482I mutated familial partial lipodystrophy fibroblasts: Altered intermolecular interaction with emerin and implications for gene transcription. Exp Cell Res 2003;291:122–134.
47 Fries I, Giullotta F, Lattanzi G et al. Alterations of nuclear envelope and chromatin organization in mandibuloacral dysplasia, a rare form of laminopathy. Physiol Genomics 2006;23:150–158.
48 Columbaro M, Capanni C, Mattioli E et al. Rescue of heterochromatin organization in Hutchinson-Gilford progeria by drug treatment. Cell Mol Life Sci 2006;62:2669–2678.
49 Lombardi F, Giullotta F, Columbaro M et al. Compound heterozygosity for mutations in LMNA in a patient with a myopathic and lipidemic mandibuloacral dysplasia type A phenotype. J Clin Endocrinol Metab 2007;92:4467–4471.
50 Dimri GP, Lee X, Basile G et al. A biomarker that identifies senescent human cells in culture and in aging skin in vivo. Proc Natl Acad Sci USA 1995;92:9363–9367.
51 Narita M, Núñez S, Heard E et al. Rb-dependent inhibition of gene transcription by the retinoblastoma protein complex in situ by proximity ligation. Nat Methods 2006;3:995–1000.
52 Mansilla S, Priebe W, Portugal J. Sp1-targeted gene expression through cell transcription. AIDs 2000;50:1063–1068.
53 Coste I, Le Corf F, Kfouri A et al. Dual function of MyD88 in RAS signaling and inflammation, leading to mouse and human cell transformation. J Clin Invest 2010;120:3663–3675.
54 Chamcheu JC, Pihl-Lundin I, Mouyobo CE et al. Immortalized keratinocytes derived from patients with epidermolysis ichthyosis reproduce the disease phenotype: A useful in vitro model for testing new treatments. Br J Dermatol 2011;164:263–272.
55 Soderberg O, Gullberg M, Jarvis M et al. Direct observation of individual endogenous protein complexes in situ by proximity ligation. Nat Methods 2006;3:995–1000.
56 Grinspoon S, Carr A. Cardiovascular risk and body-fat abnormalities in HIV-infected adults. N Engl J Med 2005;352:48–62.
57 Viguéroux C, Magré J, Vantyghem MC et al. Lamin A/C gene: Sex-determined expression of mutations in Dunnigan-type familial partial lipodystrophy and absence of coding mutations in congenital and acquired generalized lipodystrophy. Diabetes 2000;49:1958–1962.
58 Ryder M, Dicker A, Götterström C et al. Functional characterization of human mesenchymal stem-cell-derived adipocytes. Biochem Biophys Res Commun 2003;311:391–397.
59 Kern S, Eichler H, Stoeve J et al. Comparative analysis of mesenchymal stem cells from bone marrow, umbilical cord blood, or adipose tissue. STEM CELLS 2006;24:1294–1301.
60 Madhira S, Nappanveethi G, Kodavalla V et al. Comparison of adipocyte-specific gene expression from WNI/Nob mutant obese rats, lean control, and parental control. Mol Cell Biochem 2011;357:217–225.
61 Leskelä H, Ollku A, Lehtonen S et al. Estrogen receptor α genotype confers interindividual variability of response to estrogen and testosterone in mesenchymal-stem-cell-derived osteoblasts. Bone 2006;39:1026–1034.
62 Araujo-Vilar D, Lattanzi G, González-Méndez B et al. Site-dependent differences in both prelamin A and adipogenic genes in subcutaneous adipose tissue of patients with type 2 familial partial lipodystrophy. J Med Genet 2009;46:40–48.
63 Goldman RD, Shumaker DK, Erdos MR et al. Accumulation of mutant lamin A causes progressive changes in nuclear architecture in Hutchinson-Gilford progeria syndrome. Proc Natl Acad Sci USA 2004;101:8963–8968.
64 Ragnauth CD, Warren DT, Liu Y et al. Prelamin A acts to accelerate smooth muscle cell senescence and is a novel biomarker of human vascular aging. Circulation 2010;121:2200–2210.
65 Pacenti M, Barzon L, Favaretto F et al. Microarray analysis during adipogenesis identifies new genes altered by antiretroviral drugs. AIDS 2006;20:1691–1705.
60 Scaffidi P, Misteli T. Lamin A-dependent misregulation of adult stem cells associated with accelerated ageing. Nat Cell Biol 2008;10:452–459.
61 Capell BC, Collins FS. Human laminopathies: Nuclei gone genetically awry. Nat Rev Genet 2006;7:940–952.
62 Mondal D, Larussa VF, Agrawal KC. Synergistic antiadipogenic effects of HIV type 1 protease inhibitors with tumor necrosis factor α: Suppression of extracellular insulin action mediated by extracellular matrix-degrading proteases. AIDS Res Hum Retroviruses 2001;17:1569–1584.
63 Csoka AB, English SB, Simkevich CP et al. Genome-scale expression profiling of Hutchinson-Gilford progeria syndrome reveals widespread transcriptional misregulation leading to mesodermal/mesenchymal defects and accelerated atherosclerosis. Aging Cell 2004;3:235–243.
64 Hernandez L, Roux KJ, Wong ES et al. Functional coupling between the extracellular matrix and nuclear lamina by Wnt signaling in progeria. Dev Cell 2010;19:413–425.
65 Zhang J, Lian Q, Zhu G et al. A human iPSC model of Hutchinson Gilford progeria reveals vascular smooth muscle and mesenchymal stem cell defects. Cell Stem Cell 2011;8:31–45.
66 Verrecchia F, Rossert J, Mauviel A. Blocking Sp1 transcription factor broadly inhibits extracellular matrix gene expression in vitro and in vivo: Implications for the treatment of tissue fibrosis. J Invest Dermatol 2001;116:755–763.
67 Mukhopadhyay A, Khoo A, Cheong HH et al. Targeting of Sp1 transcription factor: A novel therapeutic approach for keloids, an in vitro analysis. Exp Dermatol 2007;16:1023–1031.
68 Mauvoisin D, Prévost M, Ducheix S et al. Key role of the ERK1/2 MAPK pathway in the transcriptional regulation of the stearoyl-CoA desaturase (SCD1) gene expression in response to leptin. Mol Cell Endocrinol 2010;319:116–128.
69 Hempel N, Wang H, LeCluyse EL et al. The human sulfotransferase SULT1A1 gene is regulated in a synergistic manner by Sp1 and GA binding protein. Mol Pharmacol 2004;66:1690–1701.
70 Zhao Y, Chen X, Yang H et al. A novel function of apolipoprotein E: Upregulation of ATP-binding cassette transporter A1 expression. PloS One 2011;6:e21453.
71 Sanchez HB, Yieh L, Osborne TF. Cooperation by sterol regulatory element-binding protein and Sp1 in sterol regulation of low density lipoprotein receptor gene. J Biol Chem 1995;270:1161–1169.
72 Jackson SM, Ericsson J, Mantovani R et al. Synergistic activation of transcription by nuclear factor Y and sterol regulatory element binding protein. J Lipid Res 1998;39:767–776.
73 Dooley KA, Millinder S, Osborne TF. Sterol regulation of 3-hydroxy-3-methylglutaryl-coenzyme A synthase gene through a direct interaction between sterol regulatory element binding protein and the trimeric CCAAT-binding factor/nuclear factor Y. J Biol Chem 1998;273:1349–1356.
74 Reed BD, Charos AE, Szekely AM et al. Genome-wide occupancy of SREBP1 and its partners NPY and SP1 reveals novel functional roles and combinatorial regulation of distinct classes of genes. PLoS Genet 2008;4: e1000133.
75 Marchesan D, Rutberg M, Andersson L et al. A phospholipase D-dependent process forms lipid droplets containing caveolin, adipocyte differentiation-related protein, and vimentin in a cell-free system. J Biol Chem 2003;278:27293–27300.
76 Heid HW, Schnölzer M, Keenan TW. Adipocyte differentiation-related protein is secreted into milk as a constituent of milk lipid globule membrane. Biochem J 1996;320:1025–1030.
77 Wu Y, Zhang X, Zehner ZE. c-Jun and the dominant-negative mutant, TAM67, induce vimentin gene expression by interacting with the activator Sp1. Oncogene 2003;22:8891–8901.
78 Corsini E, Zancanella O, Lucchi L et al. Role of SP-1 in SDS-induced adipose differentiation related protein synthesis in human keratinocytes. Gene Regul Syst Bio 2007;1: 207–215.

See www.StemCellsTM.com for supporting information available online.