Lamin A and microtubules collaborate to maintain nuclear morphology

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ABSTRACT

Lamin A (LA) is a critical structural component of the nuclear lamina. Mutations within the LA gene (LMNA) lead to several human disorders, most striking of which is Hutchinson-Gilford Progeria Syndrome (HGPS), a premature aging disorder. HGPS cells are best characterized by an abnormal nuclear morphology known as nuclear blebbing, which arises due to the accumulation of progerin, a dominant mutant form of LA. The microtubule (MT) network is known to mediate changes in nuclear morphology in the context of specific events such as mitosis, cell polarization, nucleus positioning and cellular migration. What is less understood is the role of the microtubule network in determining nuclear morphology during interphase. In this study, we elucidate the role of the cytoskeleton in regulation and misregulation of nuclear morphology through perturbations of both the lamina and the microtubule network. We found that LA knockout cells exhibit a crescent shape morphology associated with the microtubule-organizing center. Furthermore, this crescent shape ameliorates upon treatment with MT drugs, Nocodazole or Taxol. Expression of progerin, in LA knockout cells also rescues the crescent shape, although the response to Nocodazole or Taxol treatment is altered in comparison to cells expressing LA. Together these results describe a collaborative effort between LA and the MT network to maintain nuclear morphology.

KEYWORDS

HGPS; lamin A; nuclear shape; microtubule; progerin

Introduction

Lamin A (LA), a type V intermediate filament encoded by the LMNA gene, is a key component of the nuclear lamina. The lamina supports the nuclear envelope, allowing the nucleus to resist mechanical perturbations. LA directly interacts with chromosomes and nuclear regulatory proteins, implicating the nuclear lamina in key cellular processes such as apoptosis, chromatin organization, and gene expression.

Mutations in the LMNA gene have been associated with a heterogeneous, rare group of hereditary diseases collectively termed laminopathies that include Emery–Dreifuss Muscular Dystrophy, Dunnigan-type familial partial lipodystrophy, dilated cardiomyopathy, and Hutchinson-Gilford Progeria Syndrome (HGPS). Among these laminopathies, HGPS has been extensively studied due to its striking clinical phenotypes including osteoporosis, loss of subcutaneous fat, alopecia, and joint stiffening after 12 months of age. HGPS arises due to the accumulation of a mutant LA isoform termed progerin which anchors to the nuclear membrane and leads to thickening of the nuclear lamina, loss of heterochromatin, alterations in histone methylation, gene misregulation, and genomic instability. Most Evidently, the accumulation of progerin results in an abnormal nuclear morphology termed nuclear blebbing. Nuclear blebbing appears to drive the pathology of HGPS, as treatments that improve blebbing have been associated with ameliorated HGPS cellular phenotypes. A change in nuclear shape is also suggested to be a hallmark of lamin mutations that lead to other laminopathies.

The nucleus is not an isolated system. Prior studies have shown that the cytoskeleton directly influences nuclear shape in fundamental cellular processes. The most extreme example is the Microtubule (MT) facilitated nuclear breakdown during mitosis. Furthermore, during cell migration, a filamentous actin...
structure known as the actin cap compresses and supports the nucleus.32 Studies investigating granulopoiesis found that the MT network mediates nuclear lobulation.34 Other studies have focused on MT influences during migration/positioning. In Drosophila follicle cells, individual MT fibers push the nucleus creating local indentations resulting in nuclear “wriggling”.35 In Drosophila oocytes, the dorsal-ventral axis is polarized by nucleus migration. This process is mediated by the MTOC which pushes the nucleus causing an indentation.36 A direct connection between the nuclear lamina and the cytoskeleton has been established through LINC complexes, transmembrane proteins that span the nuclear membrane and bind to both.37 These studies, as well as others, have focused on event specific microtubule-nucleus interactions.33,34,37-41 In comparison, relatively little is known about how the equilibrium nuclear morphology is affected by the cytoskeleton, especially in laminopathies. Interestingly, NAT10 inhibitor, remodelin has been associated with improved nuclear morphology and cellular fitness in HGPS cells through a mechanism targeting the MT organizing center (MTOC),42 which suggests a role for the cytoskeleton in the nuclear blebbing phenotype in HGPS.

In this study, we investigate the interplay between microtubules and the nuclear lamina to elucidate the role of the cytoskeleton in maintaining normal nuclear morphology. We find that the MT network in the presence of LA is necessary for appropriate nuclear morphology. The absence of LA led to MT-driven nuclear abnormalities. Furthermore, the presence of progerin led to the altered MT-nucleus interactions. Together, these results suggest that normal nuclear shape is dependent upon balanced interactions between both cytoskeletal and lamin networks.

Results

Loss of LA results in the MTOC-associated crescent-shaped nuclei

The MT network is dynamic, with MTs growing and pushing, while also pulling on their surrounding via molecular motors.43 LA has previously been described as a rigid material.31,44 We, therefore, hypothesized that if MT network dynamicity and LA rigidity exhibit some interplay, then LA removal would result in MT driven abnormalities. To test this hypothesis, we characterized LA knockout (LA−/−) fibroblast nuclei and compared them to LA+/+ fibroblast nuclei.

To accurately and quantitatively determine the nuclear shape, we applied a described previously method for nuclear measurements.45,46 This program reports nuclear morphology as a measure of local invaginations typically associated with blebs and nuclear deformations. Mean negative curvature (MNC), one of the reported measures, is defined as the absolute value of the average negative curvature (negative when measured relative to the center of the nucleus) excluding all positive curvature values, i.e. all regions where the nucleus bulges outward. While it is counterintuitive to not measure outward bulges, our previous work clearly showed that blebs are best identified from their surrounding inward invaginations.45,46 Indeed, nuclei we would identify as more abnormal by visual inspection exhibit higher MNC.

Using lamin B1 (LB1) immunofluorescence staining, we observed that LA−/− cells more frequently exhibited a crescent shaped nuclear morphology compared with LA+/+ cells (Fig. 1A). We then counted over 100 randomly selected nuclei and found that the frequency of crescent-shaped nuclei was significantly higher in LA−/− cells than LA+/+ cells (Fig. 1B). The crescent shape of the nucleus was associated withgamma tubulin localization to the arc of the crescent (Fig. 1C and D), suggesting that the MTOC may be involved in this nuclear abnormality in the absence of LA.

Nuclear shape analysis showed that LA−/− nuclei exhibited increased mean negative curvature (Fig. 1E) and reduced nuclear area (Fig. 1F), suggesting that LA−/− nuclei displayed worsened morphology. The differences were significant enough allowing for robust identification of a boundary between the 2 conditions (Fig. 1G). When using all the 5 metrics of nuclear shape (i.e., MNC, area, eccentricity, solidity, and tortuosity) for classification, the classification accuracy is quite high at 88%, with a 10% likelihood of classifying a single LA+/+ cell as LA−/−, and a 13% chance of classifying a single LA−/− cell as LA+/+. These results show that LA is necessary for appropriate nuclear morphology, and further suggests that the MTOC drives this change in nuclear shape.

Disruption of the MT network improves LA−/− nuclear morphology

To test whether the MT network facilitated abnormal nuclear morphology observed in LA−/− cells, we treated LA−/− and LA+/+ cells with classical MT
drugs Nocodazole (NOC) and Taxol (TX). We used NOC to depolymerize MTs and used TX to stabilize and polymerize MTs. LA−/− and LA+/+ fibroblasts were treated with increasing concentrations of either NOC or TX for 2 hours and then stained for MTs. Appropriate concentrations (4μM NOC and 2μM)

were identified by changes in MT organization in LA−/− and LA+/+ fibroblasts (Fig S1 and S2).

We observed improvement of LA−/− nuclear morphology following either NOC or TX treatment (Fig. 2A). Quantitative image analysis showed no change in MNC for LA+/+ cells (Fig. 2B), indicating

Figure 1. Loss of LA results in an MTOC associated crescent shape. (A) Representative confocal immunofluorescence images of LA−/− and LA+/+ cells. Green = lamin B1; Blue = DAPI. (B) Frequency of crescent-shaped nuclei in LA−/− and LA+/+ cells. N > 100 nuclei per population. *** p < 0.001. Counted by 3 independent observers. Error bars are the standard deviation. (C) Representative confocal images of MTOC colocalization with crescent invagination in LA−/− cells. Green = gamma tubulin; Blue = DAPI. (D) Frequency of LA−/− and LA+/+ crescent-shaped nuclei colocalized with gamma tubulin. N > 100 nuclei per population. Counted by 3 independent observers. Error bars are standard deviation. Error bars are the standard deviation. (E) Distribution of LA−/− and LA+/+ nuclear morphology as quantified by mean negative curvature. Density denotes cell count. N > 150. (F) Distribution of LA−/− and LA+/+ nuclear morphology as quantified by nuclear area. The area measurements are normalized to LA−/−. (G) Clustering of LA−/− and LA+/+ nuclei with a dotted line denoting the boundary between the 2 populations. Both metrics are normalized to LA−/−.
Figure 2. Disruption of the microtubule network improves abnormal LA−/− nuclear morphology. (A) Representative confocal images of LA−/− and LA+/+ cells treated with Mock, 4μM Nocodazole (NOC), or 2μM Taxol (TX). Green = Lamin B1; Blue = DAPI. (B-D) Nuclear morphology quantifications of LA−/− and LA+/+ cells treated with Mock, 4μM NOC, and 2μM TX. (B) Mean negative curvature, (C) Area, (D) Volume measurements. N > 300 nuclei per population. Data is represented as mean with 95% confidence intervals. All values are normalized to LA−/− mock treated. *** p < 0.001. (E-G) Mean negative curvature by area contour plots. (E) LA−/− treated nuclei. (F) LA+/+ treated nuclei. (G) Both LA−/− and LA+/+ nuclei excluding mock treated. N > 200 nuclei per population. Both metrics are normalized to LA−/− Mock. Dotted line depicts boundary between LA−/− and LA+/+. (H) Representative confocal images of LA−/− and LA+/+ cells treated with Mock, 4μM Nocodazole (NOC), or 2μM Taxol (TX). Red = Gamma-tubulin; Blue = DAPI.
under the experimental condition, either of these treatments significantly affects the nuclear shape in healthy cells. Interestingly, a decrease in mean negative curvature for LA-/− cells was observed in NOC or TX treatments. This result further suggests that the MT network is not needed to maintain the rounded morphology of healthy cells, but plays a role in facilitating LA-/− nuclear abnormalities.

Image analysis further showed that nuclear area decreased for both LA-/− and LA++/+ cells following NOC treatment and that the area increased following TX treatment of LA++/+ cells (Fig. 2C). To determine whether MT affected the projected area or the volume of the nucleus, we took Z-stack images of each nucleus and measured nuclear volume using a built-in function in Volocity (Perkin Elmer, Waltham, MA). We analyzed over 300 nuclei per condition and found that in these experiments, differences in the nuclear area are associated with changes in nuclear volume (Fig. 2D). Using our previously identified boundary (Fig. 1G), we asked whether NOC and TX shifted LA-/− nuclei toward LA++/+ morphology in all of the parameters we measure. The analysis agrees with the observed changes in MNC alone; LA-/− cells treated with NOC or TX become more LA++/+ like while LA++/+ treated cells exhibit little change (Fig. 2E–G).

We were curious about whether any of these drug treatments caused a disassociation between the nucleus and MTOC allowing for the rescue of the crescent shape. Staining with anti-gamma tubulin antibodies revealed no such disassociation (Fig. 2H). Altogether, these results show that the MTs play a role in driving LA-/− nuclear abnormality and furthermore demonstrate that LA plays a role in mediating nucleus-MT interactions.

**Expression of LA and progerin in LA-/− cells partially rescues the crescent shape**

To further elucidate the role of LA in the nucleus-MT interactions, we conducted a rescue experiment by transducing LA-/− cells with LA-GFP through lentivirus infection (Fig S3). As we previously reported,28,47,48 the expression level of LA-GFP from lentiviruses is moderate and comparable to the endogeneous LA/C (Fig S3). Interestingly, we found that LA-GFP expression in LA-/− cells quickly and effectively rescued nuclear morphology (Fig. 3A and B) and GFP-alone control did not improve the crescent nuclear shape (Fig. 3A and B). As LA-/− cells may have considerable perturbations in the organization of actin, vimentin and other structural components,49 this rescue experiment validated the direct causal relationship between lack of LA and the crescent shape nuclear morphology.

HGPS is a well-studied laminopathy that arises due to a dominant mutant LA isoform termed progerin. Nuclear blebbing is considered a hallmark phenotype in HGPS cells.5,16,50-52 To test whether progerin expression alone is sufficient for inducing nuclear blebbing, we also transduced LA-/− cells with progerin-GFP expressing lentivirus and quantified changes in nuclear morphology. Our initial observations unexpectedly revealed that progerin-GFP over-expression partially rescued the LA-/− morphological phenotype (Fig. 3A). These observations were consistent with automated analysis, which showed decreased MNC (Fig. 3B and D) in progerin-GFP expressing cells. Considering that progerin is a truncated form of LA, this rescue is likely due to progerin retaining some LA functionality. Additionally, it is important to note that our transfected nuclei did not contain LA (Fig S3). It is possible that nuclear blebs arise due to interaction between LA and progerin rather than just progerin accumulation (see discussion).

No significant changes in the nuclear area were observed in either LA-GFP or progerin-GFP expressing LA-/− cells in comparison to GFP alone control cells (Fig. 3C and E). Neither did the lentiviral transduction cause any observable changes with MT organization and MTOC position (Fig S4).

**Progerin-GFP alters responses to NOC and TX drug treatments**

We then tested whether progerin-GFP expressing cells responded to NOC and TX treatment in a similar fashion as LA-GFP expressing cells. As expected, GFP expressing LA-/− cells showed improved nuclear morphology in response to NOC and TX treatments (Fig. 4A and D). Interestingly, LA-GFP expressing cells exhibited similar behavior to drug treatment as LA++/+ cells (Fig. 4B and D), reiterating that both the MT network and LA are necessary for appropriate nuclear morphology. Surprisingly, progerin-GFP cells did not mimic LA-GFP cells’ response to NOC and
TX. LB1 immunofluorescence staining showed that progerin-GFP cells had worsened nuclear morphology in response to NOC and further improved morphology following TX (Fig. 4C). These visual impressions were further confirmed by nuclear quantitative analysis. MNC increased in progerin-GFP cells in response to NOC and decreased in response to TX, supporting our immunofluorescence images (Fig. 4D). Notably, both LA-GFP and progerin-GFP expressing nuclei exhibited decreased area following NOC treatment and increased area in response to TX (Fig 4E and F), reminiscent of the LA+/+ cells (Fig. 2C). We confirmed that all of these models responded to drug treatments as expected (Fig S5) and observed that the MTOC remained associated positioning (Fig S6). Together, these results suggest that progerin, despite partially rescuing LA−/− nuclear morphology, alters nucleus-MT interactions.

Discussion

In this study, we investigated the relationship between the MT network and the nuclear lamina in an attempt to better understand how the cytoskeleton modulates nuclear shape. We applied high-throughput automated nuclear shape analysis program to determine changes in nuclear morphology after MT and LA perturbations. We used

Figure 3. Ectopic expression of LA-GFP or Progerin-GFP partially rescues abnormal LA−/− nuclear morphology. (A) Representative confocal immunofluorescence images of LA−/− nuclei transfected with GFP, LA-GFP, or progerin-GFP. Green = GFP; Blue = DAPI. (B-C) Nuclear morphology quantification of GFP-expressing LA−/− nuclei. (B) Mean negative curvature, and (C) Area. N > 300 nuclei per population. Data is normalized to GFP-expressing LA−/− samples. *** p < 0.001. (D-E) Distribution of nuclear morphology quantification in LA−/− cells expressing GFP, LA-GFP or progerin-GFP.
progerin as a disease model to understand how altered lamina impacted cytoskeletal influence on the nucleus. Understanding how the cytoskeleton and nuclear lamina interact to sustain nuclear shape, as well as the impact of progerin on this interplay provides insights into HGPS pathology and treatments from a mechanical perspective.

**Local MT influence on nuclear morphology**

Loss of LA resulted in an abnormal nuclear morphology, with crescent-shaped nuclei where the center of the crescent is associated with the MTOC. The MTOC’s position provides insights into the mechanical balance within the cell as the MTOC location is
based upon balancing forces of the emanating MT fibers. When these forces are balanced, the MTOC is at equilibrium. If the MTOC is not at this point, the forces are unbalanced, and the MTOC will move back to the balanced state. Thus, the MTOC is said to have a “center seeking” behavior (Fig. 5).

Since the nucleus prevents such centering of the MTOC, it is likely that the MTOC locally pushes against the nucleus, but that the nucleus is still enough to resist the MTOC yet preserve normal nuclear shape. The paucity of lamins leading to nuclear invagination centered about the MTOC was proposed in one model of HL-60 cell differentiation. Specifically, it was observed that retinoic acid differentiation of HL-60 cells leads to invagination/lobulation of the nucleus in a MT-dependent mechanism. Our LA knockout and LA-GFP rescue (Fig. 3) experiments suggest that it is the LA layer that retains a concave nuclear shape against a pushing MTOC. The importance of LA in providing nuclear stiffness against MTOC driven deformations is further supported by the change in nuclear morphology during differentiation of

Figure 5. Local Microtubule-Nucleus interaction. We hypothesize that the microtubule-organizing center (MTOC) locally deforms the nucleus mediated through LINC complexes. Force produced by the MTOC is normally resisted by the nucleus, specifically LA. Removal of LA leads to the invagination which can be rescued by progerin or LA expression. Nuclear morphology can also be rescued by Nocodazole and Taxol treatments which reduce MTOC force production.
monocytes into macrophages. Monocytes normally lack LA and exhibit crescent-shaped nuclei, however upon differentiation into macrophages they express LA and return to a circular nuclear morphology. It is worth noting that our results disagree with findings in another publication, in which a disassociation between MTOC and the nucleus in LA−/− cells was documented, and the authors pointed to an emerin dimer as the anchor of these 2 together. We confirmed that our cells are completely negative for LA (Fig S3). Since emerin requires LA to target to the nucleus, it would appear unlikely to be involved in anchoring the MTOC within our system.

Rather than weaken and strengthen the nuclear rigidity, we can also alter the pushing of the microtubule network. Perturbations of the MT fibers via NOC and TX resulted in improved nuclear morphology (Fig. 2), even for LA−/− cells. This indicates a reduced force about the MTOC (Fig. 5). There is some concern with whether the usage of MT drugs could inhibit mitosis impinging on our analysis. Considering the shortness of our treatment (2 hours) in comparison to the cell doubling time (18–24 hours) we do not expect significant biasing of results.

Progerin is traditionally associated with an abnormal nuclear morphology. However, when we transfected progerin-GFP into LA−/− nuclei, we found rescue of the crescent morphology rather than the presence of nuclear blebs (Figs. 3 and 4). Considering that progerin is a truncated form of LA, this rescue is likely due to progerin retaining some LA functionality. Additionally, it is important to note that our transfected nuclei did not contain LA (Fig S3). It is possible that nuclear blebs arise due to interaction between LA and progerin rather than just progerin accumulation. Lamin interactions leading to nuclear blebs is not an unprecedented idea. Prior work has mathematically modeled nuclear blebbing as a mechanical phenomenon arising due to LA and LB interactions. A recent study provides experimental supports to this notion by identifying compounds that efficiently blocked progerin-lamin A/C binding and alleviated nuclear deformation and HGPS phenotypes in animal models.

A working model

We summarize these findings with a schematic explained below (Fig. 5). The microtubule network is anchored to the MTOC, and thus the MTOC experiences forces due to microtubule polymerization, as well as forces due to molecular motors attached to microtubules that pull on the surrounding. Application of NOC and TX affect the MT and their force production. NOC depolymerizes MT and TX “freezes” them both of which reduces the forces pushing onto the MTOC (Fig S7).

The nucleus also applies a force onto the MTOC, and we approximate this interaction as a spring whose stiffness is a function of lamin composition. Removal of LA decreases nucleus stiffness, changing the equilibrium resulting in a local concavity/crescent shaped nucleus. This shape can be rescued by altering the force balance. If the MTs are perturbed via NOC or TX, the force about the MTOC pushing into the nucleus is vastly reduced or near zero resulting inamelioration of shape. If the spring is stiffened via LA-GFP or progerin-GFP transfection, the MTOC faces a stronger opposing force resulting in more rounded nuclear shape (Fig. 3). One open question is whether this more rounded nuclear shape may go hand in hand with amelioration of the disease phenotypes of laminopathies.

Global MT influence on nuclear morphology

The MT cytoskeleton is composed of more than just the MTOC: it is a global network found throughout the cell. Therefore, the MT network should influence nuclear morphology beyond the large deformations seen near the MTOC. We found that NOC treatment always resulted in smaller nuclei (Fig. 2C, D, and Fig. 4E) and that TX resulted in increased nuclear size for LA+/+, LA-GFP, or progerin-GFP nuclei (Fig. 2C, D, and Fig. 4F). These findings suggest that while the MTOC pushes against the nucleus, other MT may also pull on the nucleus, with the overall effect that the MT network expands the nucleoskeleton, thereby helping to maintain its shape.

To pull on the nucleus, the MT cytoskeleton must be anchored to it. However, the MT cytoskeleton does not directly interact with the nuclear membrane or lamina, but rather is connected to the lamina by transmembrane LINC complexes. LINC complexes SUN1-Nesprin3α along with BPAG1 connect LA to the MT network and are stabilized when interacting with LA. Thus, LA−/− cells provide at best a reduced anchoring for the MT network to the nucleus. Indeed,
the size of LA−/− nuclei did not increase after TX treatment (Fig. 2C, D and Fig. 4F).

Rather than removal of the LA, its structure can also be altered, specifically in HGPS. LA is released from the inner nuclear membrane following ZMPSTE24-mediated removal of its farnesyl group, while progerin remains anchored to the inner membrane. Thus a LA-dominant nuclear lamina is organized differently than a progerin-dominant nuclear lamina within the nucleus. Normally the LA forms a spherical mesh along with Lamin B, another intranuclear lamin. The meshes are rather homogenous with some interconnectedness. Progerin creates a disrupted network that disrupts the LB network and blends these 2 networks together. Previous studies show that distribution of the nuclear lamina plays a role in HGPS pathology, emphasizing the importance of lamina organization in preserving nuclear morphology. Indeed, we find that Progerin-GFP nuclei responded differently than LA-GFP nuclei following NOC and TX treatments. NOC treatment resulted in less rounded nuclear morphology while TX improved MNC (Fig. 4D). Our study suggests that appropriate nuclear lamina organization is necessary for proper MT-mediated forces on the nucleus and thereby nuclear morphology.

Materials and methods

Cell lines and drug treatments

LA knockout (LA −/−) along with control LA (LA+/+) mouse embryonic fibroblasts (MEFs) were obtained from Dr. Colin L. Stewart and cultured in Dulbecco’s Modified Eagle’s Medium (Lonza) supplemented with 10% FBS (Gemini- Bio-Products) under 5% CO2 at 37°C. Nocodazole, Taxol or DMSO was diluted to specified concentrations in 10% FBS DMEM media and then co-cultured with cells for 2 hours. Following treatment, cells were immediately fixed with 4% paraformaldehyde/PBS.

Immunofluorescence staining

For immunofluorescence, cells were grown in glass-bottom dishes, fixed with 4% paraformaldehyde/PBS for 25min at room temperature, permeabilized with 0.5% Triton X-100/PBS for 5 min and then incubated in blocking solution (4% BSA/TBS) overnight at 4°C. Cells were then incubated with primary antibodies overnight at 4°C, followed by incubation with fluorescently conjugated secondary antibody for 1h at room temperature. Fluorescence was visualized by either a Nikon Spinning Disc Confocal or a Leica SP5-X system.

Antibodies

Antibodies used in this study included: goat anti-Lamin B antibody (M20, Santa Cruz), mouse anti-α-tubulin antibody (DM1a, Santa Cruz) and mouse anti-γ-tubulin antibody (019K4794, Sigma).

Lentivirus production

LA-GFP and progerin-GFP expressing lentiviral plasmids were constructed as previously reported. In short, LA or progerin cDNA was subcloned into a pHr-SIN-CSGW lentiviral vector. For lentiviral production pHr-LA-GFP-SIN-CSGW or pHr-progerin-GFP-SIN-CSGW vectors was transfected into 293T cells together with both pHr-CMV-8.2 DcR, and pCMV-VSVG via Fugene 6 (Promega, E2692). The viruses were collected 48 hours after transfection, filtered through 0.45 μm filters, tittered through FACS and ultimately stored at −80°C.

Image analysis

Images were analyzed by a custom in house MATLAB program described previously. In short, the program outlines nuclear boundaries and extracts shape measures, such as boundary curvature thus allowing for a more sensitive, controlled analysis as compared with traditional hand counting.

Nuclei morphology was characterized through 4 different metrics. Mean negative curvature is defined as the average negative curvature on the boundary of each nuclei. Area is measured as the number of pixels enclosed by a nucleus boundary and then converted to μm using image resolution. Eccentricity is defined as 1 – the ratio of the nucleus’ major axis over the minor axis. Solidity is defined as the ratio of the area over convex hull area.

Statistical analysis

Data was analyzed by a 2-tailed Student’s t-test assuming unequal variance, a p-value less than 0.05 was considered significant. Multiple comparison corrections were applied where appropriate. A chi-square test was conducted to compare the number of crescent-shaped nuclei in LA −/− and LA +/- fibroblasts.
Abbreviations

- HGPS: Hutchinson-Gilford Progeria Syndrome
- LA: Lamin A
- LA −/−: Lamin A Knockout
- LBI: Lamin B1
- MNC: Mean Negative Curvature
- MT: Microtubule
- MTOC: Microtubule Organizing Center
- NOC: Nocodazole
- TX: Taxol

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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