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Supplemental Information

A Cell-Intrinsic Interferon-like Response Links Replication Stress to Cellular Aging Caused by Progerin

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Supplemental experimental procedures

Immunoblotting and Immunofluorescence

Immunoblotting. Cells were lysed in UREA buffer (8 M Urea, 40 mM Tris pH7.5, 1% NP40) for 20 minutes on ice, and 60-120 µg of total protein was loaded on 4-15% Criterion-TGX-Gel (Bio-Rad). Subcellular fractionation was performed using a fractionation kit (Thermo, 78840). We started with an equal number of cells, and an equal volume of lysate was loaded for westerns. Densitometry analysis was performed using Image-J software.

Immunofluorescence. $3x10^5$ cells were fixed in 3.7% formaldehyde+0.2% Triton-X100 in PBS for 10 minutes, washed 3X in PBS, and blocked 1 hour at 37°C in 10% BSA/PBS. Incubations with antibodies were performed for 1 hour at 37°C, in a humid chamber. After washes in PBS, cells were counterstained with DAPI in Vectashield. Microscopy and photo capture was performed on a Leica DM5000 B microscope using 40x or 63x oil objective lenses with a Leica DFC350FX digital camera and the Leica Application Suite.

Quantitative Reverse-Transcription PCR

cDNA was generated by reverse transcription of 1 µg total RNA using GeneAmp® RNA PCR kit. qRT-PCR was performed using the 7500HT Fast Real-Time PCR system with the Taqman® Universal PCR Master Mix or Universal SYBR Green Supermix. Reactions were carried out in triplicate and target gene and endogenous controls amplified in the same plate. Relative quantitative measurements of target genes were determined by comparing cycle thresholds. Table 3 in SI lists oligos used.

DNA Fiber Assays

Fiber assays were performed on retinal pigment epithelial cells (RPE cells) expressing EV or progerin. Asynchronous RPE cells were labeled for 25 min each with two thymidine analogs: 20 µM iododeoxyuridine (IdU) followed by 200 µM chlorodeoxyuridine (CIdU). Cells were collected by trypsinization, washed and resuspended in 100 µl of PBS. Then, 2 µl cell suspension was dropped on a polarized slide (Denville Ultraclear) and cell lysis was performed in situ by adding

8 µl of lysis buffer (200 mM Tris-HCl pH7.5; 50 mM EDTA; 0.5% SDS). Stretching of high-molecular weight DNA was achieved by tilting the slides at 15-45°. The resulting DNA spreads were air dried and fixed for 5 min in 3:1 Methanol:Acetic acid, and refrigerated overnight. For immunostaining, stretched DNA fibers were denatured with 2.5 N HCl for 60 min, washed 3X in PBS, then blocked with 5% BSA in PBS for 30 min at 37°C. Rat anti-CldU/BrdU (Abcam, ab6326) (1:100), chicken anti-rat Alexa 488 (Invitrogen, A21470) (1:100), mouse anti-IdU/BrdU (BD Biosciences, 347580) (1:20) and goat anti-mouse IgG1 Alexa 547 (Invitrogen, A21123) (1:100) antibodies were used to reveal CldU- and IdU-labeled tracts, respectively. A Leica SP5X confocal microscope was used to visualize the labeled tracts, and tract lengths were measured using ImageJ (http://rsbweb.nih.gov.beckerproxy.wustl.edu/ij/). Statistical analysis of the tract length was performed using GraphPad Prism (http://www.graphpad.com/scientific-software/prism/).

RNAseq

Normal and HGPS fibroblasts supplemented with 10^{-7} M calcitriol (1α,25-dihydroxyvitamin D_3; Sigma-Aldrich) or FBS for different periods of time (NF for 4 days and HGPS for 4 and 90 days) were utilized to extract total RNA using Aurum Total RNA Mini Kit (Bio-Rad) with DNaseI treatment following the manufacturer's protocol. Ribosomal RNA depletion was performed with 500 ng of total RNA with the Ribominus Eukaryotic System v2 (Life Technologies) according to the manufacturer's directions. The rRNA-depleted RNA was used to construct barcoded libraries using the Ion Total RNA-Seq Kit v2 (Life Technologies), following the manufacturer's instructions. The libraries were sequenced on an Ion Torrent Proton with a mean read length of ~100 nucleotides.

RNA-seq quantification was performed using a method similar to that described previously [1]. Sequencing reads were aligned to the hg19 genome sequence using the map4 algorithm in the TMAP aligner (https://github.com/iontorrent/TMAP/blob/master/doc/tmap-book.pdf), allowing soft clipping of both the 5' and 3' ends (option -g 0) and a minimum seed length of 20. The

resulting bam files were sorted using SAMtools [2] and used to generate strand-specific bedgraph files of the base pair coverage using BEDTools [3]. A custom R (R Core Team; http://www.R-project.org) script was used to convert the strand-specific bedgraph files to sgr files, which were used to sum the strand-specific base pair coverage of non-redundant sets of plus strand and minus strand exons using a custom R script. The nucleotide coverage values of all exons for each gene were summed using an R script to generate nucleotide coverage values for all annotated genes. The coverage values for each gene were normalized to the total coverage for all genes for each library to allow quantitative comparisons.

All RNA-seq files were submitted to the GEO repository, and the following accession number was assigned: GSE97986.

Gene Ontology analysis and heat-map generation

DAVID (http://david.niaid.nih.gov) bioinformatics resource was used to obtain functional interpretation of gene expression changes in HGPS compared to NF and in HGPS treated with calcitriol versus vehicle. We uploaded gene lists and via functional annotation clustering, we obtained gene-term enrichment analysis and biological mechanisms associated with the gene lists. We used gene-enrichment scores to generate heat-maps in order to represent biological data. Heat-maps were generated using a custom R script using the g-plots package. Values represent log2 values of relative gene expression as determined by RNA sequencing.

Resource Table

Cells (Skin fibroblasts) from Progeria Research Foundation	**Description**
HGPS2-HGADFN167	Fibroblasts from HGPS patient with classic mutation
HGADFN003	Fibroblasts from HGPS patient with classic mutation
HGPS3-PSADFN392	Fibroblasts from HGPS patient with non-classic mutation
PSADFN257	Fibroblasts from HGPS patient with non-classic mutation
PSFDFN327	Fibroblasts from parent of HGPS patient
HGMDFN090	Fibroblasts from parent of HGPS patient
PSMDFN346	Fibroblasts from parent of HGPS patient

Antibodies used for immunoblotting	Antibody (Dilution)
γH2AX	#2577, Cell Signaling (1:1000)
53BP1	sc-22760, SCBT (1:1000)
Lamin A	ab26300, Abcam (1:1000)
Progerin	SAB4200272, Sigma-Aldrich (1:1000)
Lamin A/C	sc-20681, SCBT (1:2000)
Vinculin	sc-7649, SCBT (1:1000)
VDR	sc-13133, SCBT (1:200)
β-Tubulin	T8238, Sigma Aldrich (1:2000)
STAT	#9172, Cell Signaling (1:1000)
P-STAT (Y701)	#7649, Cell Signaling (1:1000)
P-STAT (S727)	#9177, Cell Signaling (1:1000)
IRF3	#11904, Cell Signaling (1:1000)
P-RPA	A300-245A, Bethyl Laboratories (1:1000)
RPA	NA18, EMD Millipore (1:1000)
ISG15	sc-166755, SCBT (1:1000)
MDA5	#5321, Cell Signaling (1:1000)
RIG-I	#3743, Cell Signaling (1:1000)
cGAS	#D1D3G, Cell Signaling (1:300)
STING	#D2P2F, Cell Signaling (1:1000)

Antibodies used for immunofluorescence	Antibody
γH2AX	#2577, Cell Signaling
Lamin A	ab26300, Abcam
STAT	#9172, Cell Signaling
P-STAT (Y701)	#7649, Cell Signaling
P-STAT (S727)	#9177, Cell Signaling
Goat anti-Mouse and Goat anti-Rabbit Alexa Fluor 594/488-labeled secondary antibodies	Invitrogen

Primers used for SYBR Green qRT-PCR	Primers (5'-3')
18S (H)	F-GGTAACCCGTTGAACCCCATT
	R-CCATCCAATCGGTAGTAGCG
APOBEC3G (H)	F-GTCCTCTGACCCAAGGTTCA
	R-AGCACTGTTGGAGCAAGTTC
GAPDH (H)	F-GCATGGCCTTCGGTGTCC

	R-AATGCCAGCCCCAGCGTCAAA
IFITM1 (H)	F-CCAAGGTCCACCGTGATTAAC
	R-ACCAGTTCAAGAAGAGGGTGTT
LMNA (H)	F–GAGGAGGGCAAGTTTGTCCG
	R–CATTCTGGCGCTTGATCTGC
Lamin A Only (H)	F-GGACAATCTGGTCACCCGC
	R-GCGTCAGGAGCCCTGAGC
MX1 (H)	F-CTGCACAGGTTGTTCTCAGC
	R-GTTTCCGAAGTGGACATCGCA
Progerin (H)	F-GCGTCAGGAGCCCTGAGC
	R-GACGCAGGAAGCCTCCAC
STAT1 (H)	F-ATCAGGCTCAGTCGGGGAATA
	R-TGGTCTCGTGTTCTCTGTTCT
18S (M)	F-CATTCGAACGTCTGCCCTATC
	R-CCTGCTGCCTTCCTTGGA
Gapdh (M)	F-GTGCAGTGCCAGCCTCGTCC
	R-GCCACTGCAAATGGCAGCCC
Lmna (M)	F-ACCCCGCTGAGTACAACCT
	R-TTCGAGTGACTGTGACACTGG

References:

1. Wu, Y. *et al.* Drosophila Nipped-B Mutants Model Cornelia de Lange Syndrome in Growth and Behavior. *PLoS Genet* **11**, e1005655 (2015).
2. Li, H. *et al.* The Sequence Alignment/Map format and SAMtools. *Bioinformatics* **25**, 2078-2079 (2009).
3. Quinlan, A.R. & Hall, I.M. BEDTools: a flexible suite of utilities for comparing genomic features. *Bioinformatics* **26**, 841-842 (2010).

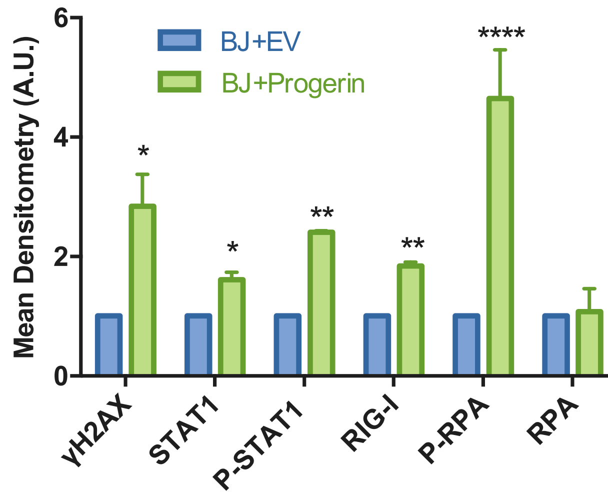

Figure S1. Related to Figure 3A; Replication stress and activation of STAT1 pathway upon progerin expression in normal cells. Total cell lysates from BJ fibroblasts expressing progerin or EV control were processed for immunoblotting and densitometry analysis performed in three biological repeats. Note the increased levels of DNA damage (γH2AX), replication stress (P-RPA), and STAT1 phosphorylation in progerin-expressing cells.