

Erratum - Autophagic degradation of farnesylated prelamin A as a therapeutic approach to lamin-linked progeria

V. Cenni,¹ C. Capanni,¹ M. Columbaro,² M. Ortolani,¹ M.R. D'Apice,³ G. Novelli,⁴ M. Fini,⁵ S. Marmiroli,⁶ E. Scarano,⁷ N.M. Maraldi,² S. Squarzone,¹ S. Prencipe,⁵ G. Lattanzi¹

¹National Research Council of Italy, Institute for Molecular Genetics, IGM-CNR, Unit of Bologna c/o IOR, Bologna

²Laboratory of Musculoskeletal Cell Biology, Rizzoli Orthopedic Institute, Bologna

³Department of Biopathology and Diagnostic Imaging Tor Vergata University, Rome

⁴National Agency for the Evaluation of Universities and Research, ANVUR, Rome Italy and San Pietro Fatebenefratelli Hospital, Rome

⁵Laboratory of Preclinical and Surgical Studies, Rizzoli Orthopedic Institute and BITTA, RIT, IOR, Bologna

⁶Department of Histology, University of Modena and Reggio Emilia, Modena

⁷Department of Pediatrics, S. Orsola-Malpighi Hospital, University of Bologna, Italy

We refer to our article by Vittoria Cenni *et al.* published in the *European Journal of Histochemistry*.¹ We have observed that Figure 1 A and Figure 2 A were not adequate to clearly represent the results we report in the paper. Therefore, we have replaced the pictures with better versions of images reporting the same experiments. In Figure 1A we show that degradation of the mutated prelamin A form called progerin in Hutchinson-Gilford progeria fibroblasts is obtained using rapamycin and it is driven through the lysosomal pathway, as demonstrated by LC3 2B activation. In Figure 2A, we support the data showing that the whole platform of nuclear envelope/lamina partners is positively affected by reduction of progerin elicited by rapamycin treatment. The results reported in our paper are consistent with those shown in 2011 by Cao and co-workers on Science Translational Medicine.² The efficacy of rapamycin in progeria cells prompted a group of clinicians in the American Progeria Research Foundation to include rapamycin in an upcoming clinical trial. Our paper further shows that the efficacy of rapamycin in progeria cells is due to its ability to restore key chromatin partners of lamin A such as LAP2 α and BAF and to rescue histone H3K9 trimethylation. A recent paper by Das *et al.*³ clearly shows that even in animal models of progeria like the delta9/delta9 Lmna mouse, restoring H3K9 trimethylation is a key point to obtain rescue of the pathological phenotype. Further, it has been reported that rapamycin improves

Correspondence: Dr. Giovanna Lattanzi, C.N.R., Institute of Molecular Genetics, Unit of Bologna, c/o IOR, via di Barbiano 1/10, 40136 Bologna, Italy.
Tel. +39.051.6366394 – Fax: +39.051.583593.
E-mail: lattanzi@area.bo.cnr.it

Received for publication: 06 November 2013.

Accepted for publication: 07 November 2013.

This work is licensed under a Creative Commons Attribution NonCommercial 3.0 License (CC BY-NC 3.0).

©Copyright V. Cenni *et al.*, 2013

Licensee PAGEPress, Italy

European Journal of Histochemistry 2013; 57:e42

doi:10.4081/ejh.2013.e42

recruitment of DNA repair factors mimicking a situation observed in cells from long-lived subjects (Lattanzi *et al.*, 2013).⁴

We are proud of giving a contribution through the *European Journal of Histochemistry* to the understanding of therapeutic approaches for the severe premature aging disease that affects Hutchinson-Gilford progeria patients. We thank you for allowing us to improve the quality and clearness of images in the Cenni's 2011 paper, so that a better support to the available evidence of rapamycin efficacy in Hutchinson-Gilford progeria and related progeroid disorders may be offered.

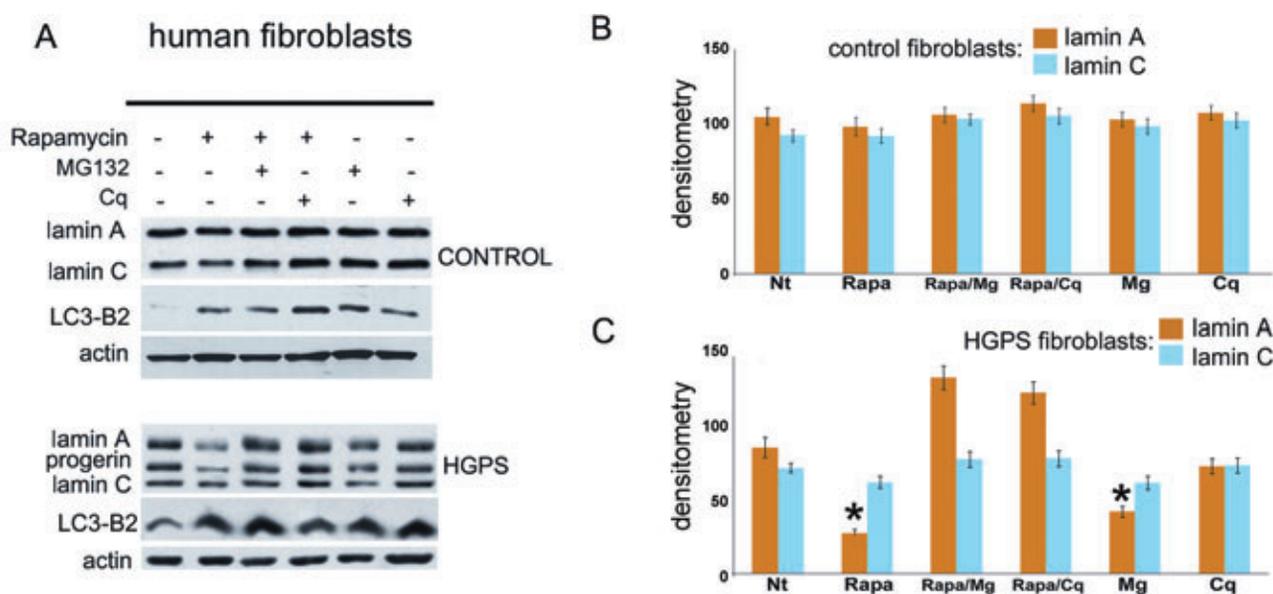


Figure 1. Rapamycin reduces progerin level in HGPS cells. A) Western blotting evaluation of lamin A/C and progerin in control (CONTROL) and Hutchinson Gilford progeria cells (HGPS). Whole lysates from control and HGPS cells untreated (-) or treated (+) with rapamycin (Rapamycin), MG132 or chloroquine-diphosphate (Cq) were subjected to lamin A/C, LC3-B2 and actin antibodies detection; B) densitometric analysis of lamin A and C immunolabeled bands detected in control fibroblasts; C) densitometric analysis of lamin A and C immunolabeled bands detected in HGPS fibroblasts; P=0.0358 for lamin A (rapa), P=0.0298 for lamin A (Mg).

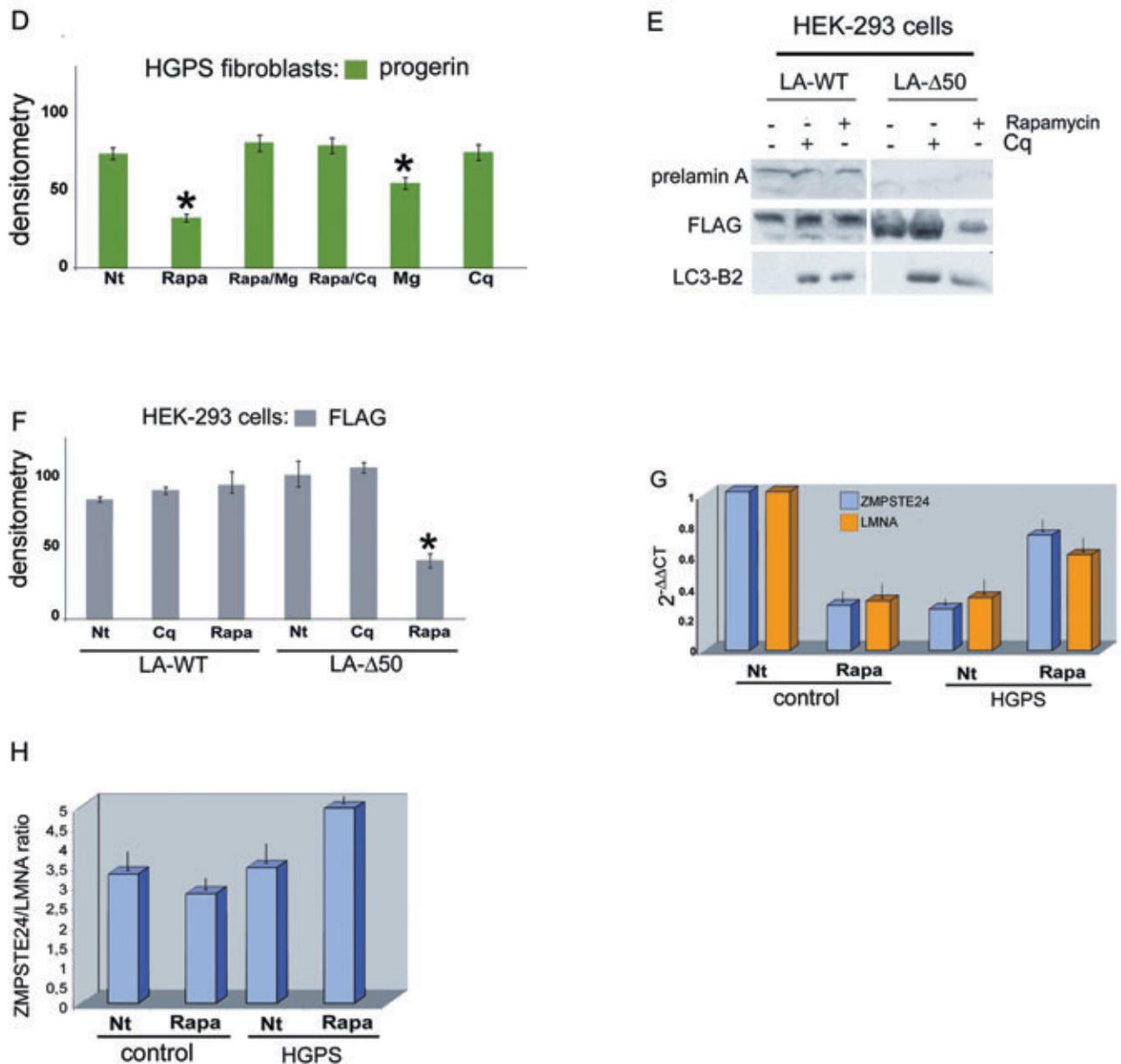


Figure 1. (Continued) Rapamycin reduces progerin level in HGPS cells. D) densitometric analysis of progerin immunoblotted bands; P=0.0390 for progerin (rapa); P=0.0458 for progerin (Mg); E) prelamin A, FLAG and LC3-B2 protein levels evaluation in HEK-293 cells expressing FLAG-tagged wild type prelamin A (LA-WT) or progerin (LA-Δ50). Immunolabeled bands observed in untreated (-) or rapamycin (Rapamycin) and chloroquine-diphosphate (Cq) treated (+) cells are shown; F) densitometric of FLAG immunoblotted bands; G) RT-PCR analysis of ZMPSTE24 and LMNA mRNA expression in untreated (Nt) and rapamycin-treated HGPS cells (Rapa) and control (control); 2^{-ΔΔCT} values are reported relative to untreated control samples. P=0.0236 for LA-Δ50 (rapa); H) ratio between ZMPSTE24 and LMNA mRNA expression. Values are means of duplicate experiments ± S.D. In B, C, D and F densitometric analysis of triplicate experiments was performed, and the mean values ± S.D. are reported; asterisk indicates statistically significant difference with respect to lamin A or progerin densitometry in untreated samples; statistical significance was calculated by the Mann-Whitney test vs untreated HGPS samples, or cells expressing LMNA constructs.

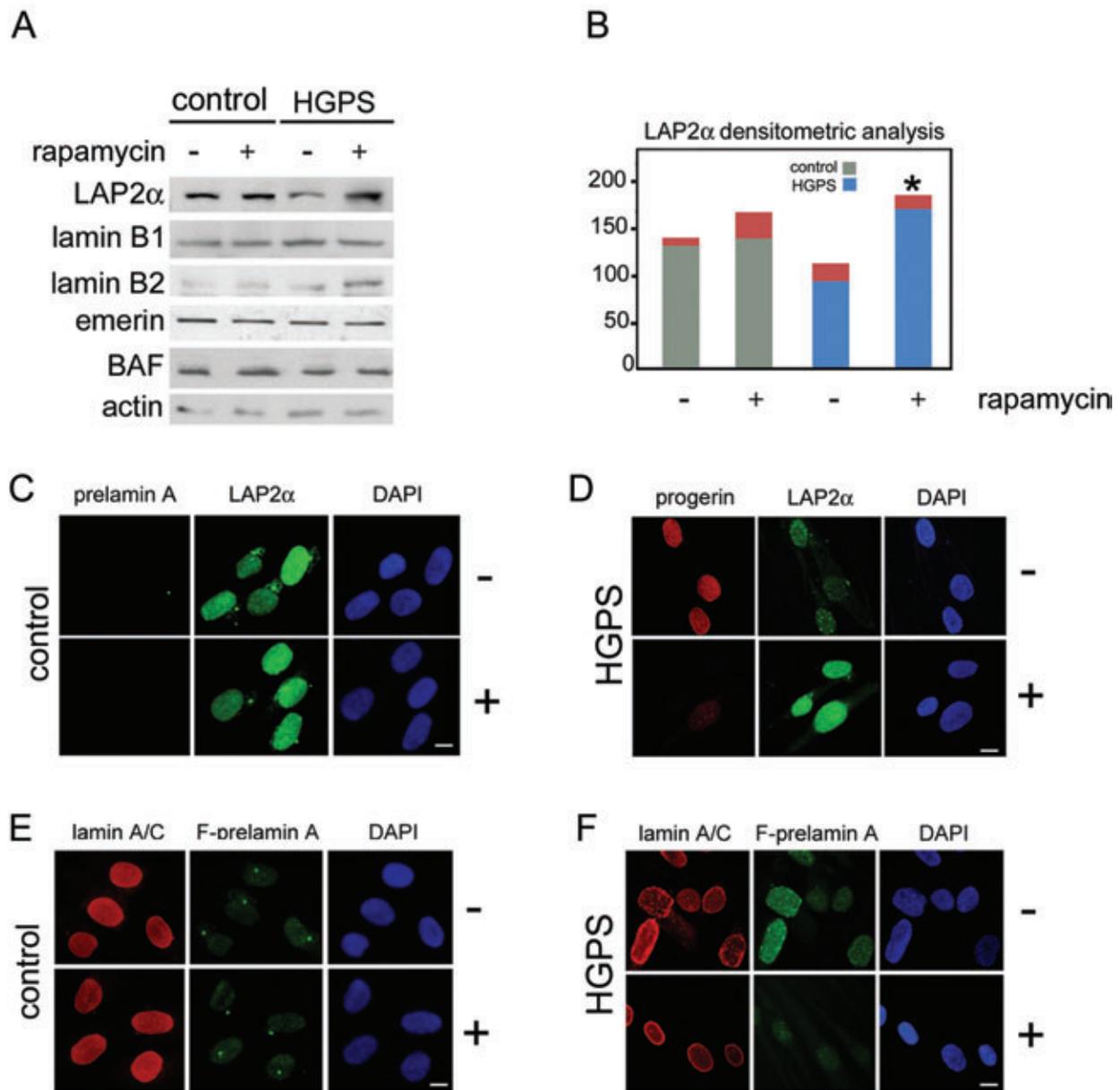


Figure 2. Nuclear envelope/lamina proteins in rapamycin treated cells. **A)** Western Blotting analysis of LAP2 α , lamin B1, lamin B2, emerin and Barrier-to-autointegration factor (BAF) in control and HGPS cells untreated (-) or treated (+) with rapamycin. Actin was detected as protein loading control. Immunolabeled bands are shown; **B)** densitometric analysis of LAP2 α immunolabeled bands detected in Western blotting analysis performed in control and HGPS untreated (-) or rapamycin treated cells (+). Asterisk indicates statistically significant difference, $P=0.0319$ for HGPS + rapamycin (+) vs untreated HGPS (-); **C)** prelamin A and LAP2 α immunofluorescence labeling performed in untreated (-) or rapamycin treated (+) control cells. Prelamin A was evaluated using a goat-polyclonal antibody visualized by TRITC-conjugated secondary antibody (red). LAP2 α distribution was evaluated using a rabbit-polyclonal antibody visualized by FITC-conjugated secondary antibody (green); **D)** progerin and LAP2 α immunolabeling detection performed in untreated (-) or rapamycin treated (+) HGPS cells. Progerin (progerin) detection was performed using a mouse-monoclonal antibody visualized by Cy3-conjugated secondary antibody (red). LAP2 α distribution was evaluated using a rabbit-polyclonal antibody visualized by FITC-conjugated secondary antibody (green). In untreated HGPS cells progerin staining was observed, while LAP2 α labeling was decreased. Rapamycin treatment dramatically reduced progerin labeling and restored LAP2 α staining levels; **E)** Lamin A/C and farnesylated-prelamin A detection performed in untreated (-) or rapamycin treated (+) control cells. Lamin A/C (lamin A/C) was evaluated using a goat-polyclonal antibody visualized by TRITC-conjugated secondary antibody (red). Farnesylated-prelamin A (F-prelamin A) staining was performed using a rabbit-polyclonal antibody visualized by FITC-conjugated secondary antibody (green). Lamin A/C was detected in untreated or treated cells at the same levels. F-prelamin A was undetected in both samples; **F)** Lamin A/C and farnesylated-prelamin A detection performed in untreated (-) or rapamycin treated (+) HGPS cells. Lamin A/C (lamin A/C) was evaluated using a goat-polyclonal antibody visualized by TRITC-conjugated secondary antibody (red). Farnesylated-prelamin A (F-prelamin A) staining was performed using a rabbit-polyclonal antibody visualized by FITC-conjugated secondary antibody (green). Lamin A/C staining was observed at the nuclear lamina of both untreated and treated cells. F-prelamin A staining observed in untreated cells was strongly reduced by rapamycin treatment.

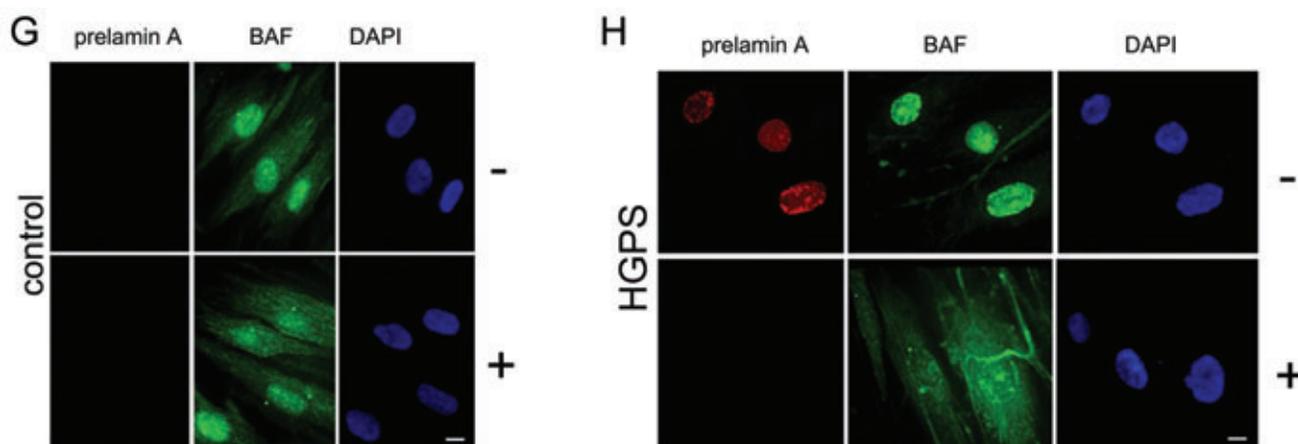


Figure 2. (Continued) Nuclear envelope/lamina proteins in rapamycin treated cells. G) prelamin A BAF detection performed in untreated (-) or rapamycin treated (+) control cells. Prelamin A was stained by a goat-polyclonal antibody visualized by TRITC-conjugated secondary antibody (red). BAF immunolabeling detection was performed by a rabbit-polyclonal antibody visualized by FITC-conjugated secondary antibody (green). Prelamin A was undetectable in untreated or rapamycin treated cells. BAF showed a normal cellular distribution in both samples; H) prelamin A and Barrier-to-autointegration (BAF) detection was performed in untreated (-) or rapamycin treated (+) HGPS cells. Prelamin A was stained by a goat-polyclonal antibody visualized by TRITC-conjugated secondary antibody (red). BAF was labeled by rabbit-polyclonal antibody and visualized by FITC-conjugated secondary antibody (green). Prelamin A staining was present at the nuclear lamina of untreated cells while in rapamycin treated cells lamin A precursor was undetectable. BAF nuclear localization was observed in HGPS untreated cells. The normal BAF nucleo-cytoplasmic cellular distribution was recovered by rapamycin treatment. In panel C, D, E, F, G and H nuclei were counterstained with DAPI. Scale bar, 10 μm .

References

1. Cenni V, Capanni C, Columbaro M, Ortolani M, D'Apice MR, Novelli G, et al. Autophagic degradation of farnesylated prelamin A as a therapeutic approach to lamin-linked progeria. *Eur J Histochem* 2011;55:e36.
2. Cao K, Graziotto JJ, Blair CD, Mazzulli JR, Erdos MR, Krainc D, et al. Rapamycin reverses cellular phenotypes and enhances mutant protein clearance in Hutchinson-Gilford progeria syndrome cells. *Sci Transl Med* 2011;3:89ra58.
3. Das A, Grotzky DA, Neumann MA, Kreienkamp R, Gonzalez-Suarez I, Redwood AB, et al. Lamin A Δ exon9 mutation leads to telomere and chromatin defects but not genomic instability. *Nucleus* 2013;4 [Epub ahead of print].
4. Lattanzi G, Ortolani M, Columbaro M, Prencipe S, Mattioli E, Lanzarini C, et al. Centenarian lamins: rapamycin targets in longevity. *J Cell Sci* 2013 [Epub ahead of print].