Mechanistic insight into endothelial cell dysfunction linked to cardiovascular disease in Progeria

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Abstract

Aging-related cardiovascular disease (CVD) is one of the leading causes of death that is closely linked to endothelial cell (EC) dysfunction. We utilized the Hutchinson Gilford Progeria Syndrome (HGPS) disease mouse model to study the role of a dysfunctional endothelium in the development of CVDs. HGPS is caused by accumulation of a mutated version of the LMNA protein, termed progerin. Importantly, progerin accumulates not only in the endothelium of HGPS patients, but also in that of healthy aged individuals indicating a specific role of progerin in the CVD development.

We could show that a conditional EC specific progerin transgenic mouse model (Prog-Tg) showed to develop a CVD phenotype. Our working model proposes that progerin accumulation at the nuclear rim leads to defective nucleocytoskeletal coupling with elevated F-actin levels and mislocalization of mechano-responsive MRTF-A complexes. We believe that these deregulations lead to an extrinsic pro-fibrotic effect through downregulation of atheroprotective *eNOS* mediated from the Prog-Tg ECs to their surrounding tissues.

Results from mRNA and miRNA profiling in Prog-Tg ECs revealed a pro-inflammatory, pro-fibrotic and pro-senescent response in Prog-Tg ECs. Profiling of circulating plasma miRNA from Prog-Tg mice nicely reflected these results. Strikingly, miR-31 and miR-34 were found upregulated in both Prog-Tg ECs and plasma of Prog-Tg mice indicating a systemic effect. In particular, miR-31 is a EC specific senescence marker shown to be linked to pro-fibrotic and pro-inflammatory responses, while miR-34 is an established biomarker and mediator of heart fibrosis and heart failure. Therefore, these results are highly interesting and pave the ground for future identification of pro-inflammatory and pro-fibrotic effects exerted from Prog-Tg ECs to surrounding cells. Importantly, they open new aspects of miRNA based therapies for HGPS patients.

1

Working model

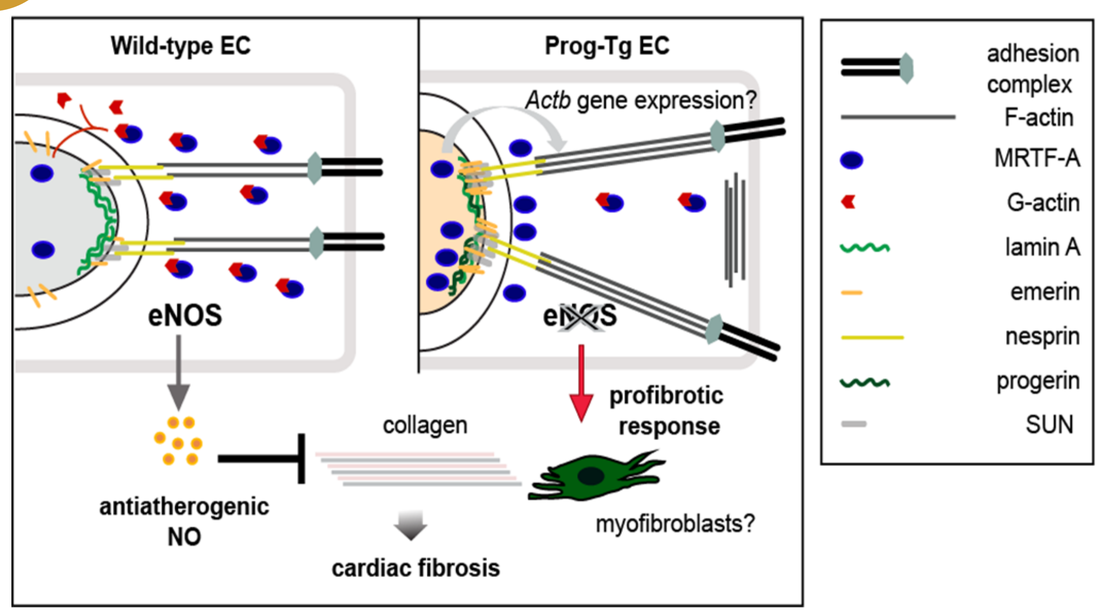
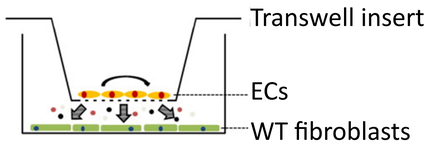


Figure 1: Progerin accumulation at the nuclear rim leads to defective nucleocytoskeletal coupling with a subsequent downregulation of atheroprotective *eNOS*. This in turn triggers a pro-fibrotic extrinsic effect mediated from the Pro-Tg ECs to the surrounding fibroblasts.

2

In vitro co-culture assay



WT fibroblast in co-culture with:

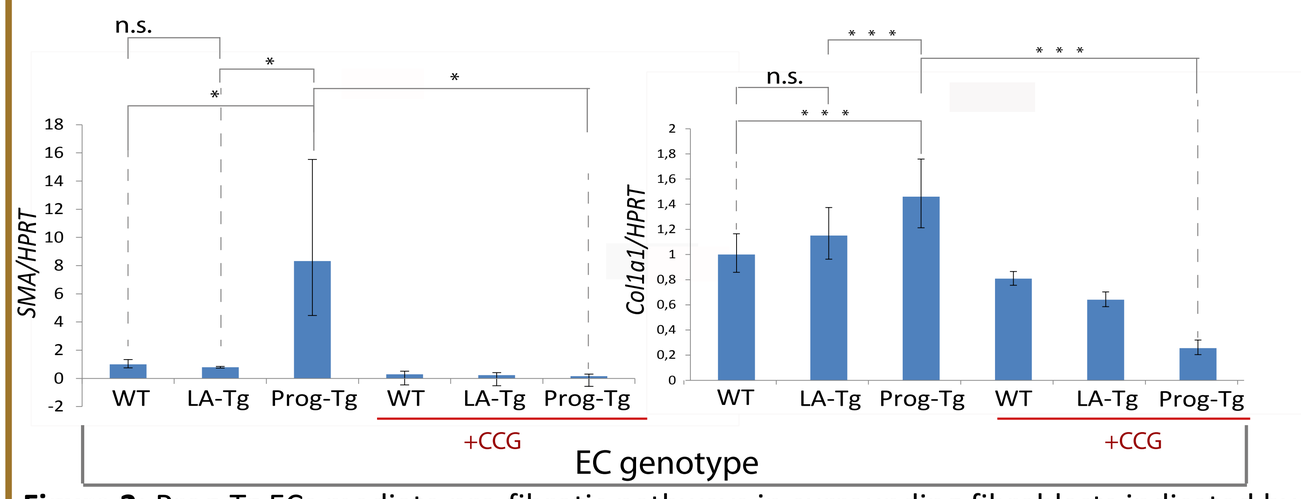
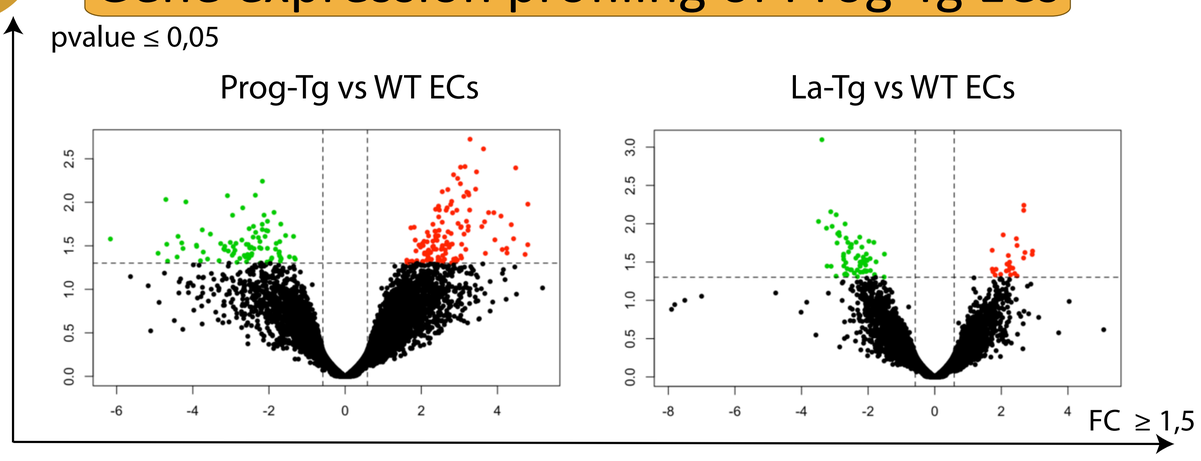


Figure 2: Prog-Tg ECs mediate pro-fibrotic pathways in surrounding fibroblasts indicated by upregulation of expression levels of *a-SMA* and *Col1a1* in an in vitro co-culture model. Specific inhibition of MRTF-A signaling axis by addition of the translocation inhibitor CCG abolished this extrinsic effect, indicating a specific role of MRTF-A signaling in mediation of the extrinsic effect.

3

Gene expression profiling of Prog-Tg ECs

A.



B. GO analysis for differentially expressed genes

Adhesion and ECM organization
- MMPs
- Col2a1
- Nox1

Pro-fibrotic mediators
- TGFb2
- CTGF
- BMP2
- EDN1

Pro-Inflammatory mediators
- Interleukins
- Chemokines

+ SASP members

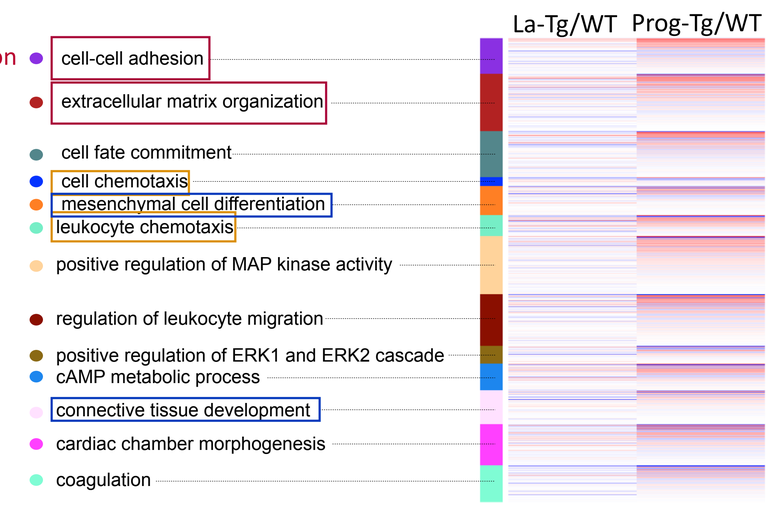


Figure 3: A. Gene profiling of Prog-Tg ECs reveals a unique set of deregulated miRNAs, B. GO analysis of deregulated genes in Prog-Tg ECs shows a pro-fibrotic, pro-inflammatory and pro-senescent effect.

4

miRNA profiling in ECs and plasma of Prog-Tg mice

miRNAs in ECs — miRNAs in plasma

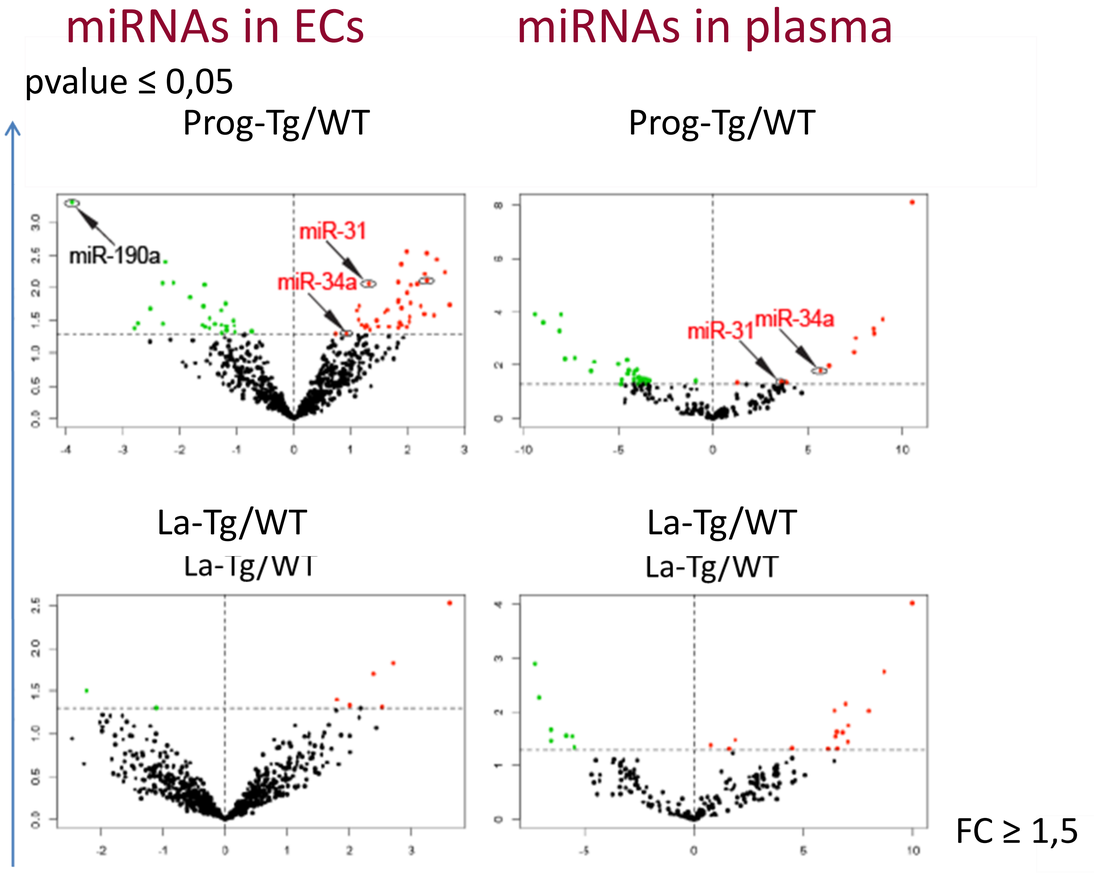


Figure 4: Comparison of miRNA-seq of Prog-Tg ECs and of circulating plasma miRNAs from Prog-Tg animals reveals systemic upregulation of EC senescence marker miR-31 and CVD mediator and biomarker miR-34. Anti-fibrotic miR-190 and also let-7 miRNA (not shown) were strongly downregulated in Prog-Tg ECs.

Conclusions

- Prog-Tg ECs exert pro-fibrotic effect on surrounding fibroblasts
- Pro-fibrotic effect is mediated through MRTF-A signaling axis
- Progerin expression in ECs leads to unique sets of deregulated genes and miRNAs
- Prog-Tg mice show a unique set of circulating plasma miRNAs
- Overlaping deregulated plasma and EC miRNAs are implicated in CVD development and senescence

Strong physiological relevance of deregulated miR-31 and miR-34 opens new perspectives for miRNA based therapies

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