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Selinexor, a CRM1 inhibitor with anti-senescence activity, modulates the non- coding transcriptome of Hutchinson–Gilford progeria syndrome fibroblasts

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Abstract

Background Hutchinson-Gilford progeria syndrome (HGPS) is characterized by premature and accelerated aging due to progerin expression, a lamin A mutant variant. Progerin disrupts a variety of cellular processes leading to premature cellular senescence. The senescent phenotype of HGPS cells is functionally linked to the altered gene expression mediated by progerin; nonetheless, the contribution of the noncoding transcriptome to this phenomenon remains to be elucidated.

Methods The coding and non-coding transcriptome of HGPS fibroblasts was characterized by bioinformatic analysis of RNA sequencing data. Selected long non-coding RNAs (lncRNAs) were validated by reverse transcription-polymerase chain reaction (RT-PCR) assays

Results Altered expression profiling of lncRNAs and enriched cellular pathways were found in HGPS fibroblasts compared with WT fibroblasts. Furthermore, the analysis demonstrated that HGPS fibroblasts treated with selinexor, a CRM1-mediated nuclear export inhibitor with antisenescence activity, reversed missregulation of the lncRNA profile. Finally, four differentially expressed lncRNAs (UB2R2-AS1, MSC-AS1, MBNL1-AS1, and H19) that were predicted to regulate mTOR and senesce pathways and respond to selinexor treatment were validated by RT-PCR assays in HGPS fibroblasts, and in a progerin-inducible cell model, under treatment with selinexor or the vehicle.

Conclusion The influence of lncRNAs in the transcriptome profile and the enriched cellular pathways of HGPS fibroblasts was established. Interestingly selinexor treatment restores the expression of specific lncRNAs altered by progerin, targeting the aging-associated pathways mTOR and senescence in HGPS fibroblasts. Overall, these results show that the analysis of the noncoding transcriptome can serve to monitor treatment efficacy and support the use of selinexor as anti-senescent compound in HGPS.

Introduction

Aging is a universal irreversible process characterized by a progressive deterioration of the physiological and cognitive integrity of the individual [1]. This process manifests as fragility and the appearance of chronic degenerative diseases, including obesity, diabetes, cardiovascular diseases, and cancer, ultimately leading to death [2]. In the recent decade, there has been an increasing research effort aimed at elucidating the cellular mechanisms underlying the process of aging. A seminal advancement in this field has emerged from the study of a rare condition known as Hutchinson-Gilford progeria syndrome (HGPS), which is characterized by premature and accelerated aging [3]. HGPS is the result of a de novo point mutation in the LMNA gene, which codes for lamin A and C, structural proteins of the nuclear membrane. Although the mutation is silent, it causes an aberrant splicing in exon 11 of the LMNA gene, which subsequently results in the deletion of 150 nucleotides in the mRNA and 50 amino acids in the corresponding protein sequence [4, 5]. The absence of this sequence of amino impedes the maturation of prelamin A. Conventionally, the C-terminal CaaX motif pof prelaima A becomes farnesylated, followed by endoproteolytic trimming of the final three residues and methylation of the carboxyl-terminal farnesyl-cysteine [3]. This series of reactions facilitates its transit from the endoplasmic reticulum to the inner

nuclear membrane. Finally, the last 15 amino acids of the protein, including the farnesylcysteine residue, are cleaved off by zinc metalloprotease ZMPSTE24 to generate mature lamin A [4, 5]. The deleted sequence in prelamin A contains the ZMPSTE24 recognition site, thereby preventing carboxyl-terminal processing and causing the protein to remain farnesylated [3]. This mutant variant of lamina A is referred to as progerin.

Progerin exerts its negative dominant effect through its misassembly in the nuclear envelope (NE), which consequently affects the proper conformation of the various protein complexes that regulate nuclear structure and function [6]. A plethora of cellular processes controlled by the NE are altered by progerin, namely DNA damage response, telomere stability, mitochondrial function, autophagy, and the nucleolar function [7, 8]. The impact of progerin on gene expression is of particular interest. It has been observed that progerin interferes with gene expression by altering the nuclear traffic of proteins [9–14] and mislocalizing certain transcription factors [15, 16], resulting in a substantial number of differentially expressed genes (DEGs), as shown by RNA sequencing analyses in different studies [17–22]. It is worth noting that none of these studies have examined the expression of non-coding RNAs (ncRNAs), the majority of transcribed RNAs in eucaryotic genomes (~ 80). Based on the transcription length, ncRNAs are divided into two groups: small ncRNAs (< 200 nucleotides), which include microRNAs, small interfering RNAs, piRNAs, small nucleolar RNA (snoRNA), small nuclear RNA (snRNA), and transfer RNA-derived fragments (tRFs); and long ncRNAs (lncRNAs; > 200 nucleotides), including RNA molecules, synthesized by RNA polymerase I (Pol I), RNA polymerase II (Pol II) and RNA polymerase III (Pol III), as well as processed introns [23–27]. LncRNAs have the ability to interact with DNA, RNA, or proteins, thereby modulating gene expression at virtually all levels, including RNA processing, translation, and post-translational modifications [27–29]. Interestingly, lncRNAs act as regulators of aging by mediating age-related cellular processes, namely telomerase stability, genomic instability, intracellular communication, cell proliferation, epigenetic regulation, senescence and proteostasis [30–33]. The implication of lncRNAs in the pathogenesis of HGPS was revealed by the identification of telomeric lncRNAs, whose expression is induced following progerin-induced telomere dysfunction [34]. Importantly, the downregulation of these lncRNAs using telomeric antisense oligonucleotides ameliorated HGPS cell senescence and extended the lifespan of an HGPS mice model [34].

In a recent work, we showed that pharmacological modulation of exportin CRM1 with the first-in-class selective inhibitor of CRM1, termed selinexor, alleviated the senescent phenotype of HGPS fibroblasts, and the coding transcriptome profile underlying the geroprotective action of Selinexor was obtained by RNA sequencing (RNA-seq) [14]. Interestingly, selinexor treatment restored the expression of numerous DEG and rescued various aging-associated cellular processes in HGPS fibroblasts [14]. Given the evident contribution of the non-coding transcriptome to HGPS pathogenesis, we analyzed herein the ncRNAs profile of HGPS fibroblasts and determined the impact of selinexor on this transcriptome. A set of lncRNAs functionally linked to the mTOR pathway was found to be dysregulated in HGPS fibroblasts. Furthermore, we found that the expression of these lncRNAs was altered by the induction of progerin expression but subsequently restored by selinexor treatment in an HGPS-inducible cell model.

Materials and Methods

Differential expression and functional network analysis of WT and HGPS fibroblast transcriptomes

Row RNA-seq reads (FASTQ) from WT and HGPS fibroblasts treated with Selinexor or DMSO were retrieved from the NCBI Gene Expression Omnibus (GEO) database under the accession number GSE134448 [14] and used sed to analyze coding and non-coding transcriptome profiler. RNA-seq FASTQ files were evaluated for quality using FastQC (v0.11.9). Adapter sequences and low-quality bases (Phred score <30) were subsequently trimmed using trimmomatic (v0.39). The High-quality reads were mapped to the reference human genome (GRCh38, GENCODE v35) using STAR Aligner (v2.7.10a) to build the count matrix. Parallel, the matrix was normalized in counts per million (CPM) and used them to graphic the expression of interest genes. Independent, count matrix was filtered and normalized to identify differential expressed genes (DEGs) using R/Bioconductor DESeq2 v1.34.0 algorithm applying threshold criteria of Log2Fold Change (log2FC) ≥ 1 and FDR ≤ 0.05 (Benjamini–Hochberg correction) [35].

Hierarchical heatmaps and interactive volcano plots were generated using R-studio with ggplot2, selected the DEG significative and complex-Heatmap packages following the developer criteria. Over-representation analysis (ORA) was conducted in NcPath against KEGG and Gene Ontology (GO) databases using a significance cutoff of $p < 0.05$ and a minimum fold enrichment of 2 times. Signaling network interactions re-construction was done with Biorender, using the lncRNAs and downstream target genes mapped within Pathview online tool (https://pathview.uncc.edu/analysis).

Fibroblast culturing and treatments

Primary human dermal fibroblasts derived from an HGPS patient (AG11513, an 8-year-old female donor) and from a healthy subject (WT; GM03440, a 20-year-old donor) were acquired from Coriell Institute for Medical Research (Camden, NJ). Fibroblasts were cultured at 37°C in a humidified, 5% CO2 atmosphere in Minimal Essential Medium Eagle (MEM; Invitrogen, Carlsbad, CA) supplemented with 15% (v/v) fetal bovine serum (Invitrogen, USA), 100 U/mL penicillin, 10 µg/mL streptomycin, and 1 mM sodium pyruvate (Sigma-Aldrich, Saint Louis, MO). When indicated, fibroblasts were treated for 6 days with 60 nM Selinexor (Karyopharm Therapeutics Inc.), diluted in DMSO (vehicle) to a 0.1% final concentration in the culture medium, or with the vehicle alone. Human dermal fibroblasts (HDFs) harboring the doxycycline-inducible GFP-progerin (Tet-On system) were cultured at 37 ^{o}C in a humidified CO^2 incubator in Dulbecco's modified Eagle's medium (DMEM), with 10% fetal bovine serum (FBS) and 1% antibiotics/antimycotics, as previously outlined [36]. The cells were incubated with 1 µg/mL doxycycline or vehicle (DMSO) for 6 days to induce GFP-progerin and the medium was replenished every 48 h.

Indirect Immunofluorescence and confocal microscope analysis

The fibroblasts grown on coverslips were fixed with 4% paraformaldehyde for 10 min in PBS, permeabilized, and blocked for 10 min at room temperature with immunofluorescence (IF) buffer (0.1% Triton X-100, 0.02% SDS, and 10 mg/ml BSA), and then incubated overnight at 4°C with anti-progeria

primary antibodies (Santa Cruz Biotechnology, CA, USA [sc-81611]). The following day, the fibroblasts were washed prior to being incubated for 1 h at room temperature with the corresponding fluorochrome-conjugated secondary antibodies (Jackson ImmunoResearch Laboratories). Where indicated F-actin was labeled using TRITC-conjugated Phalloidin (Sigma-Aldrich, St. Louis, MO, USA) diluted 1:150 in IF buffer for 15 min at room temperature. Then, cells were incubated for 15 min at room temperature with 0.2 μg/ml Diamino-2-phenylindole (DAPI; Sigma-Aldrich, St. Louis, MO, USA) in PBS for nuclei labeling. After washing, coverslips were mounted on microscope slides with VectaShield (Vector Laboratories, Inc. Burlingame, CA, USA) and examined on a confocal laser scanning microscope (CLSM; Eclipse Ti-E inverted confocal laser scanning microscope (NiKon, Japan).

Immunoblotting

The fibroblast lysates were subjected to electrophoresis on 12% SDS-polyacrylamide gels and transferred onto nitrocellulose membranes (Bio-Rad Laboratories, Hercules, CA) for 1 h. The membranes were blocked in TBST and incubated at 4°C overnight with the proper primary antibodies; anti-progerin (Santa Cruz Biotechnology, CA, USA [sc-81611]) and anti-β-actin (and β-actin (a gift from Dr. Manuel Hernández, CINVESTAV, Mexico). The protein signal was developed using the corresponding horseradish peroxidase-conjugate secondary antibodies and the enhanced chemiluminescence Western blotting detection system (ECL™), according to the manufacturer's instructions. Blot images were acquired using a C-Digit® Blot Scanner (LI-COR Biosciences Lincoln, NE, USA).

Total RNA isolation and validation of lncRNAs expression by qRT-PCR assays

Total RNA was isolated from cultured fibroblast using TRIzol Reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer’s instructions. Total RNA concentration and purity were evaluated by spectrophotometry (NanoDrop™ 2000, Thermo), and the integrity was confirmed by 1% agarose gel electrophoresis. Relative expression levels of four differentially expressed lncRNAs (H19, UB2R2-AS1, MSC-AS1 and MBNL1-AS1) were quantified by reverse transcription quantitative real-time PCR. Assays were performed on a StepOnePlus Real-Time PCR System (Applied Biosystems, Cat. No. 4379704) using the KAPA SYBR FAST One-Step qRT-PCR Universal Kit (Roche Diagnostics / KAPA Biosystems, Cat. No. KK4650), following the manufacturer’s instructions. For each reaction, **80 ng of total RNA** was used as a template. Amplification and quantification were carried out on a **Rotor-Gene real-time PCR cycler (Qiagen, Hilden, Germany)**.Specific primer sequences were as follow: H19: forward, 5’-CAGGAGTGATGACGGGTGGA-3’ and reverse, 5’-CAGCTGCCACGTCCTGTAA-3’. UB2R2-AS1: forward, 5’-GTCTGGGTAGTCAGCTGTGAGG-3´ and reverse, 5´-TCTCCAGAGGCAGTGTTCCTC-3´. MSC-AS1: forward, 5´-AAGCAACAACTGTCTGGCCT-3´ and reverse 5´-TGATGCCAGCAAATTGGTGC-3´. MBNL1-AS1: forward, 5´-TGGATAAGACAGTCCCTACA-3´ and reverse 5´-ATTGGATTGCTTCCCACATA-3´. TBP) forward, 5´-AGTTCTGGGATTGTACCGCA-3´ and reverse 5´-TGTGCACACCATTTTCCCAG-3´. Amplicon specificity was confirmed by melting curve, and the RNA relative expression of these transcripts was measured using the 2−ΔΔCt method, and the values were normalized to TBP expression, to posterior statics analysis the batch effect between experiments were considerate.

Statical analysis

The analysis and graphics were performed with Prism software (v11.05) and non-parametric tests with unequal variances Kruskal-Willis in tree pared independents experiments were employed. The significance of each result was determined by the $P \leq 0.05$. All data are expressed as the mean ± standard deviation.

Results

Non-Coding RNA Profiling Uncovers Dysregulated mTOR and MAPK Signaling in HGPS Fibroblast

To delineate the molecular alterations underlying Hutchinson–Gilford progeria syndrome (HGPS), we contrasted the expression landscape of HGPS fibroblasts against wild-type fibroblasts (WT; control), evaluating both protein-coding and non-coding transcripts. HGPS fibroblasts showed a broad and directionally asymmetric transcriptional phenotype. The class-level summary identified 1,121 differentially expressed transcripts (Fig. 1A; Table S1): protein-coding genes were predominantly upregulated (403 upregulated and 241 downregulated), whereas non-protein-coding transcripts were predominantly downregulated (184 upregulated and 293 downregulated). Biotype decomposition confirmed that the response comprised 644 protein-coding genes, 163 lncRNAs, 35 pseudogenes, 3 other non-coding RNAs, and 276 other or unclassified transcripts (Fig. 1B; Table S1). The volcano plot resolved the magnitude of the disease-associated shifts and placed all four validation candidates among threshold-positive genes (Fig. 1C; Table S3): H19, UBE2R2-AS1, and MSC-AS1 increased by log2 fold changes of 7.71, 4.75, and 1.77, respectively, whereas MBNL1-AS1 decreased by −1.29.

Pathway analysis showed that the disease signature was concentrated in signaling and stress-response programs rather than distributed randomly across the transcriptome (Fig. 1D; Table S2). mTOR signaling was the highest-ranked term (enrichment score 14.33; FDR 1.9 × 10−13; 143 genes), closely followed by MAPK signaling (14.32; 255 genes); focal adhesion (12.19; 179 genes) and cellular senescence (9.65; 141 genes) were also prominent. The lncRNA heatmap demonstrated that the selected transcripts belonged to coordinated expression modules that were reorganized after selinexor exposure (Fig. 1E). Consistently, the NcPath network connected the non-coding signature to disease-associated coding nodes, including PIK3CD (+1.18), RPTOR (+2.07), SLC7A5 (+2.52), STK11 (+1.57), and IGFBP3 (−1.42) (Fig. 1F; Table S3). Together, these panels identify an HGPS expression program in which lncRNA dysregulation converges on mTOR and cellular-senescence circuitry, providing a mechanistic basis for the subsequent treatment analyses.

Selinexor Modulates Non-Coding RNAs to Target Cellular Senescence, MAPK, and mTOR Pathways in HGPS fibroblast

As previously described, the treatment of HGPS fibroblasts with selinexor alleviates the senescence phenotype of HGPS cells [14]; therefore, it is expected that such therapy influences their transcriptome profile. Selinexor elicited a large, predominantly suppressive response among protein-coding genes in

HGPS fibroblasts. A total of 1,423 treatment-responsive transcripts were identified (Fig. 2A; Table S1): 639 protein-coding genes were downregulated and 191 were upregulated, while non-protein-coding transcripts showed 265 decreases and 328 increases. Biotype analysis assigned these transcripts to 830 protein-coding genes, 205 lncRNAs, 55 pseudogenes, 5 other non-coding RNAs, and 328 other or unclassified transcripts (Fig. 2B; Table S1). The volcano plot showed that selinexor reversed the direction of all four validation candidates relative to their HGPS-versus-WT behavior (Fig. 2C; Table S3): H19, UBE2R2-AS1, and MSC-AS1 decreased by log2 fold changes of −7.06, −2.27, and −2.62, whereas MBNL1-AS1 increased by +2.03.

The treatment-responsive interaction network linked these lncRNA shifts to coding components of both mTOR and cellular-senescence programs (Fig. 2D; Table S3). Notable changes included downregulation of DDIT4 (−2.44), PIK3CD (−1.01), CHEK1 (−2.96), RAD9A (−1.32), ITPR1 (−1.79), and IGFBP3 (−1.11), together with upregulation of SKP2 (+1.22). Across WT, untreated HGPS, and selinexor-treated HGPS samples, the lncRNA heatmap showed a treatment-dependent reorganization in which several HGPS-associated expression patterns shifted toward the WT range (Fig. 2E). Thus, selinexor did not produce a uniform global reversal; instead, it selectively remodeled a connected coding–non-coding network centered on growth control, DNA-damage responses, mTOR and senescence.

A pathway enrichment analysis predicts the regulation of senescence and mTOR signaling by lncRNAs in HGPS fibroblasts

The integrated heatmap assembled 17 protein-coding genes and 13 lncRNAs across 12 RNA-seq samples, allowing disease- and treatment-associated changes to be evaluated within the same expression space (Fig. 3A). The four validated lncRNAs occupied the same coordinated modules as pathway-linked coding transcripts, supporting their interpretation as components of the broader HGPS response rather than isolated biomarkers. Mapping the HGPS-versus-WT contrast onto mTOR signaling highlighted increased DDIT4 (+2.58), PIK3CD (+1.18), RAPTOR (+2.07), SLC7A5 (+2.52), and STK11 (+1.57) expression (Fig. 3B; Table S3). The corresponding cellular-senescence map showed a distinct configuration that included reduced IGFBP3 (−1.42) together with shared pathway nodes such as PIK3CD (Fig. 3C; Table S3).

The treatment maps revealed a coordinated but pathway-specific response. Within the mTOR module, selinexor decreased DDIT4 (−2.44) and PIK3CD (−1.01) while increasing SKP2 (+1.22) (Fig. 3D; Table S3). Within the cellular-senescence module, the drug decreased CHEK1 (−2.96), RAD9A (−1.32), ITPR1 (−1.79), and IGFBP3 (−1.11) (Fig. 3E; Table S3). In parallel, H19, UBE2R2-AS1, and MSC-AS1 shifted downward and MBNL1-AS1 shifted upward after treatment (Table S3). The concordant movement of coding and non-coding nodes indicates that selinexor engages a structured regulatory program spanning nutrient sensing, cell-cycle control, and stress signaling, rather than altering the selected lncRNAs independently of pathway context.

Validation of differentially expressed lncRNAs in HGPS fibroblasts by quantitative real time reverse transcription polymerase chain reaction (qRT-PCR)

RNA-seq quantitatively confirmed the disease-associated and treatment-responsive behavior of the four selected lncRNAs (Fig. 4A; Table S4). Mean H19 expression increased from 0.54 in WT cells to 11.24 in HGPS cells and returned to 0.72 after selinexor (P = 0.0022 for both indicated comparisons). UBE2R2-AS1 increased from 1.12 to 24.53 and decreased to 4.28 after treatment (P = 0.0002 and P = 0.0003, respectively). MSC-AS1 increased from 55.22 to 188.64 and decreased to 25.88 after treatment (P < 0.0001 for both comparisons). Conversely, MBNL1-AS1 decreased from 76.62 in WT cells to 31.36 in HGPS cells and increased to 107.45 after selinexor (P = 0.0034 and P = 0.0002). These reciprocal shifts reproduce the directions observed in the global analyses and establish a coherent four-lncRNA treatment signature.

RT-qPCR in primary fibroblasts provided orthogonal support for this signature (Fig. 4B; Table S4). In HGPS cells, selinexor reduced H19 from 0.73 to 0.13 (P = 0.0315) and MSC-AS1 from 1.79 to 0.71 (P = 0.0415), while increasing MBNL1-AS1 from 0.38 to 1.40 (P = 0.0312). UBE2R2-AS1 decreased from 2.30 to 1.51, although this comparison was marginal (P = 0.0542). The basal H19 difference between WT and HGPS was significant by RT-qPCR (P = 0.0127), whereas the basal differences for the other three lncRNAs did not reach the displayed significance threshold. Collectively, Fig. 4A and Fig. 4B show that the treatment direction is reproducible across sequencing and targeted amplification, with the strongest cross-platform agreement for H19, MSC-AS1, and MBNL1-AS1.

The induction of progerin expression upregulates the expression of UB2R2-AS1 and H19 lncRNAs in human dermal fibroblasts, but selinexor treatment prevents this effect

To ascertain whether progerin directly influences the expression of selected lncRNAs (UB2R2-AS1, MSC-AS1, MBNL1-AS1, and H19), human dermal fibroblasts (HDFs) containing green fluorescent protein (GFP)-progerin or GFP-lamin A under the control of a doxycycline-inducible (Tet-on) promoter were utilized. Upon incubation of HDF with Doxicycline the expression of GFP-progerin (~70 kDa) was evidenced by immunoblotting (Fig. 5A). Immunofluorescence/confocal microscopy analysis independently verified GFP-progerin induction. The presence of progerin in these cells caused an aberrant nuclear morphology, a hallmark of HGPS fibroblasts (Fig. 5B), which validated the cell model for the subsequent experiments. The inducible model also revealed transcript- and context-dependent responses to selinexor (Fig. 5C; Table S5). H19 increased in WT cells from 0.33 to 0.75 (P = 0.0193), and progerin induction was associated with a higher H19 level than the WT vehicle condition (0.74 versus 0.33; P = 0.0267). UBE2R2-AS1 increased from 3.02 in WT cells to 8.52 in HGPS-model cells (P = 0.0489) and decreased to 2.38 after selinexor (P = 0.0097), reproducing the treatment direction observed in primary cells and RNA-seq. By contrast, MSC-AS1 changed from 1.28 to 1.44 after treatment (P = 0.7831), and MBNL1-AS1 changed from 1.09 to 1.33 (P = 0.2956), indicating no significant treatment response in this experiment. The HGPS + selinexor group contained two available biological values, whereas the other groups contained three. Overall, the inducible system supports UBE2R2-AS1 as the most reproducible treatment-responsive lncRNA across cellular contexts and highlights that the remaining lncRNA effects depend on the model used.

Discussion

In this study, a bioinformatic analysis of the non-coding transcriptome of HGPS fibroblasts was conducted, with the aim of identifying differentially expressed lncRNAs and predicting the impact of this profile on downstream cellular pathways. Furthermore, the effect of selinexor, a selective nuclear export inhibitor with antisenescence activity, on the transcriptomic profile of lncRNAs and their associated pathways was examined. A significant misregulation of lncRNAs was identified in HGPS fibroblasts, which resulted in the enrichment of multiple pathways when both coding and non-coding transcripts were considered, with the underscoring of mTORC1 and senescence signaling pathways. Remarkably selinexor treatment reversed the altered expression of numerous lncRNas, including those involved in mTORC1 and senescence pathways. Finally, the differential expression of four selected lncRNAs and their response to selinexor treatment were validated in fibroblast cultures of HGPS patients and in a progerin inducible cell system.

The pathogenesis of HGPS is initiated by the expression of progerin, a mutant variant of lamin A, which anchors abnormally to the nuclear envelope (NE). This aberrant attachment of progerin disrupts a multitude of critical cellular processes, including DNA replication, mitochondrial function, cell cycle regulation, chromatin organization, and gene expression [7, 8]. Altered gene expressions in HGPS are the result of progerin-mediated sequestering at the nuclear envelope of transcription factors (nuclear factor erythroid 2-related 2; Nrf2) and global loss of epigenetic marks (H3K27me3 and H3K9me3) [16, 37, 38]. These changes further alter the activation or repression of gene expression, respectively. The transcriptome characterization of HGPS fibroblasts is a necessary step to identify downstream effects of progerin on crucial cellular processes, which in turn would facilitate the identification of therapeutic targets. In line with this, the transcriptome profile of HGP fibroblasts has been examined in various studies, unveiling widespread gene misregulation that may alter a variety of processes/pathways, including genome instability (DNA replication, repair, and recombination pathways), cell death (p53 and Rb pathways), cell growth and cytoskeletal organization (mTORC1 signaling: TNFα signaling via NF-κB pathways), inflammation (TNFα signaling and mTORC1 signaling), gene expression (transcriptional and epigenetic regulation pathways), proteostasis (ubiquitin-proteasomal degradation and autophagy pathways), and mesodermal and mesenchymal cell fate (bone and muscle development, fat deposition) [17–22, 39, 40]. However, the aforementioned studies overlooked the impact of non-coding RNAs on the altered transcriptome of HGPS fibroblasts. For instance, lncRNAs have the ability to interact with DNA, RNA, or proteins to modulate gene expression, thereby affecting aging-related cellular processes, [30–33]. Specifically, previous study demonstrated that telomeric lncRNAs play a role in the progerin-induced telomere dysfunction [34]. Therefore, this study focused on characterizing the expression profile of lncRNAs, as well as on determining the response of differentially expressed lncRNAs to selinexor treatment.

A significant switch in the expression profile of lncRNA was found in HGPS fibroblasts compared to WT fibroblasts, with 77 upregulated and 120 downregulated RNAs. Interestingly, treatment with selinexor reversed the altered expression of numerous lncRNAs, implying their role in the anti-senescence activity

exhibited by the drug. In line with this, GO term analysis encompassing coding- and non-coding transcripts showed the enrichment of two aging-associated pathways, senescence and mTOR. The expression of four selected lncRNas which were predicted to be involved in cellular senescence (UB2R2-AS1, NEAT1 and H19) and mTOR (MSC-AS1, UB2R2-AS1, NEAT1, and H19) pathways, were validated in both HGPS fibroblasts and in a progerin inducible cell system. Three of the four lncRNAs, UB2R2-AS1, MSC-AS1 and MBNL1-AS1 have previously been implicated in the development of cancer [41–43]. All three function by acting as sponges for microRNAs, thereby preventing the degradation of target mRNAs and consequently altering the survival, growth, and migration of cancer cells. Specifically, the oncogenic function of MSC-AS1 has been demonstrated in stomach, liver, and lung cancers [42], while the role of UB2R2-AS1 is dependent on the type of cancer, with the potential to act either as a suppressor or promoter of tumors (41). Finally, MSC-AS1 has been demonstrated to have oncogenic function in several cancer types, including gastric cancer [43].

Specifically, the regulation of mTOR pathway by H19 in HGPS fibroblast would be relevant because mTORC1 is a key regulator of cell growth and proliferation in response to nutrient signaling, which concurrently inhibits autophagy initiation through the phosphorylation of diverse components of the ULK complex, including autophagy related gene 13 (ATG13), Unc-51-like autophagy-activating kinase 1(ULK1) and Autophagy/Beclin 1 regulator 1 (AMBRA1) [44, 45]. It has been shown that H19 directly binds to 4E-BP1, impeding Raptor from binding and phosphorylating 4E-BP1 an essential scaffolding protein that recruits substrates (S6K1 and 4E-BP1) to mTORC1 for phosphorylation [44, 45]. Thus, the increased expression of H19 in HGPS fibroblasts, which is reversed by selinexor treatment, is anticipated to release the activity of mTOR counteracting thereby senescence. Likewise, since H19 has been linked to senescence [46, 47], its overexpression in HGPS fibroblasts is predicted to inhibit the function of E2F3, an essential S-phase transcription factor that drives cell proliferation [48]. Consequently, selinexor-mediated downregulation of H19 might impede its inhibitory action on E2F3, thereby hindering the progression of senescence. Overall, these results suggest that the antisenescence effect of selinexor is, at least in part, mediated by downregulation of H19.

Limitations

Further experiments are required to determine the precise role of lncRNAs in HGPS fibroblasts, as well as to validate the hypothesis that the expression of H19 is a driver of the senescent phenotype and its implication in the anti-senescence action of selinexor in HGPS fibroblasts.

Conclusion

The expression profile of lncRNAs in HGPS fibroblasts was determined, as well as the effect of selinexor treatment on this landscape. Furthermore, mTOR and senescence, two main processes enriched in HGPS fibroblasts, were found to be targeted by selinexor. Finally, the expression of four specific long non-coding RNAs (lncRNAs) that were predicted to be implicated in senescence and mTOR pathways, were validated in HGPS fibroblasts and in a progerin-inducible model.

Abbreviations

HGPS Hutchinson-Gilford Progeria Syndrome

RNA-seq RNA sequencing

lncRNAs long non-coding RNAs

WT wild type

NE nuclear envelope

DEGs differentially expressed genes

ncRNAs non-coding RNAs

snoRNA small nucleolar RNA

snRNA small nuclear RNA

tRFs transfer RNA-derived fragments

Pol I RNA polymerase I

Pol II RNA polymerase II

Pol III RNA polymerase III

FASTQ Row RNA-seq reads

GEO Gene Expression Omnibus

CPM counts per million

log2FC Log2Fold Change

ORA Over-representation análisis

GO Gene Ontology

MEM Minimal Essential Medium Eagle

HDFs Human dermal fibroblasts

DMEM Dulbecco's modified Eagle's médium

FBS fetal bovine serum

IF immunofluorescence

DAPI Diamino-2-phenylindole

CLSM confocal laser scanning microscope

qRT-PCR quantitative real time reverse transcription polymerase chain reaction

GFP green fluorescent protein

Nrf2 nuclear factor erythroid 2-related 2

ATG13 autophagy related gene 13

ULK1 Unc-51-like autophagy-activating kinase 1

AMBRA1 Autophagy/Beclin 1 regulator 1

Declarations

Supplementary Information The online version contains supplementary material available at

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Data availability Data will be made available by the corresponding author on request.

Competing interests The authors declare no competing interests.

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