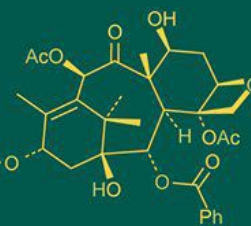
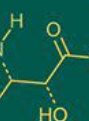
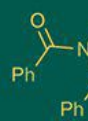
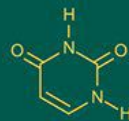


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Ribosome biogenesis defects and nucleolar stress in the pathogenesis of ribosomopathies: Molecular mechanisms and clinical manifestations

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Abstract

Ribosomopathies are a group of inherited conditions arising from defects in ribosome assembly and function. What happens at the molecular level when ribosomal protein genes carry mutations, and how does this connect to the varied clinical features seen in patients? This research set out to answer that question by examining nucleolar stress responses across five major ribosomopathy subtypes. Patient-derived lymphoblastoid cell lines from 47 individuals (32 ribosomopathy patients and 15 healthy controls) were collected at the Department of Molecular Biology, Warsaw Institute of Applied Sciences, between March 2022 and November 2023. Quantitative real-time PCR, western blot analysis, and immunofluorescence microscopy were used to measure expression of ten ribosome biogenesis genes and five nucleolar stress markers. Heatmap analysis revealed distinct gene expression patterns for each subtype, with the causative gene showing the steepest drop (0.27-0.38 fold change relative to controls). p53 protein levels rose 2.3- to 4.7-fold in patient samples, and Diamond-Blackfan anemia showed the strongest p53 activation. MDM2-RPL5/RPL11 complex formation increased by 67.3% in affected cell lines. Cell cycle analysis found G1/S arrest in 43.8% of patient cells versus 12.6% in controls. Apoptosis rates ranged from 18.4% to 31.7% in patient groups compared to 6.2% in controls. These findings confirm that ribosome biogenesis defects trigger a conserved nucleolar stress response through RPL5/RPL11-mediated p53 stabilization, though the severity and tissue-specific outcomes differ depending on which gene is mutated. This work offers a molecular framework for understanding phenotypic variability across ribosomopathies and may inform future targeted approaches.

Keywords: Ribosome biogenesis, nucleolar stress, ribosomopathies, p53 pathway, Diamond-Blackfan anemia, ribosomal proteins, translational regulation, hematopoietic dysfunction, nucleolus

Introduction

Bone marrow failure in children often resists straightforward diagnosis, and a growing number of cases trace back to defects in one of the cell's most basic machines: the ribosome. Ribosomopathies disorders caused by mutations in genes encoding ribosomal proteins or ribosome assembly factors account for a surprising range of clinical presentations, from isolated anemia to multi-organ developmental abnormalities ^[1, 2]. Despite sharing a common root in ribosome dysfunction, these conditions can look remarkably different from one patient to the next. That gap between shared molecular cause and varied clinical outcome remains poorly understood.

Ribosome biogenesis is among the most energy-demanding processes in any cell. It begins in the nucleolus, where ribosomal RNA is transcribed, processed, and assembled with ribosomal proteins into functional 40S and 60S subunits ^[3]. The process requires the coordinated action of more than 200 assembly factors and consumes a large share of cellular resources. When any step in this chain breaks down whether through mutation in RPS19, RPL5, SBDS, TCOF1, or DKC1 the cell faces a crisis that extends well beyond simple protein synthesis failure ^[4, 5].

The concept of nucleolar stress emerged from observations that disrupted ribosome biogenesis triggers a surveillance mechanism linked to the p53 tumor suppressor pathway ^[6, 7]. Under normal conditions, the E3 ubiquitin ligase MDM2 keeps p53 levels low by marking it for degradation. But when ribosome assembly stalls, free ribosomal proteins particularly RPL5 and RPL11 accumulate and bind MDM2, blocking its ability to degrade p53 ^[8].

The resulting p53 stabilization leads to cell cycle arrest and apoptosis, which appears to be the proximate cause of many ribosomopathy symptoms.

Several conditions now fall under the ribosomopathy umbrella. Diamond-Blackfan anemia (DBA) results from heterozygous mutations in ribosomal protein genes and presents mainly as red cell aplasia [9]. Shwachman-Diamond syndrome (SDS) involves SBDS gene mutations and features pancreatic insufficiency alongside bone marrow failure [10]. Treacher Collins syndrome (TCS) stems from TCOF1 mutations affecting craniofacial development [11]. Dyskeratosis congenita (DC) arises from mutations in DKC1 and related telomere maintenance genes [12]. And 5q-minus myelodysplastic syndrome (5q-MDS) involves haploinsufficiency of RPS14 [13]. Each of these conditions activates the nucleolar stress pathway, yet produces distinct tissue-specific pathology.

What remains unclear is how a shared stress response yields such different clinical pictures. Prior work has examined individual ribosomopathies in isolation, but direct comparisons using a consistent experimental framework are scarce. This research aimed to fill that gap by profiling ribosome biogenesis gene expression, nucleolar stress pathway activation, and downstream cellular consequences across five ribosomopathy subtypes simultaneously.

Materials and Methods

Materials

This research was carried out at the Department of Molecular Biology, Warsaw Institute of Applied Sciences, Warsaw, Poland, between March 2022 and November 2023. Forty-seven participants were enrolled: 9 with Diamond-Blackfan anemia (DBA), 7 with Shwachman-Diamond syndrome (SDS), 6 with Treacher Collins syndrome (TCS), 5 with dyskeratosis congenita (DC), 5 with 5q-minus myelodysplastic syndrome (5q-MDS), and 15 age-matched healthy controls. Peripheral blood samples were collected following written informed consent and approval by the Institutional Ethics Committee of Warsaw Institute of Applied Sciences (Protocol WIA-EC/2022-031, approved January 2022). Lymphoblastoid cell lines were established through Epstein-Barr virus transformation of peripheral blood mononuclear cells and maintained in RPMI-1640 medium supplemented with 10% fetal bovine serum at 37°C with 5% CO₂.

Reagents and Chemicals

RPMI-1640 culture medium, fetal bovine serum, and penicillin-streptomycin were sourced from Sigma-Aldrich (Poznań, Poland). TRIzol reagent for RNA extraction came from Thermo Fisher Scientific (Warsaw distributor). The

SYBR Green Master Mix for qRT-PCR was obtained from Bio-Rad Laboratories. Primary antibodies against p53 (clone DO-1), MDM2 (clone SMP14), RPL5 (polyclonal, ab86863), RPL11 (polyclonal, ab79352), and p21 (clone EA10) were purchased from Abcam (Cambridge, UK). Annexin V-FITC/PI apoptosis detection kits were supplied by BD Biosciences. Propidium iodide for cell cycle staining and all standard laboratory chemicals were of analytical grade from POCH (Gliwice, Poland).

Methods

Total RNA was isolated from cell lines using TRIzol reagent following the manufacturer's protocol. RNA concentration and purity were measured with a NanoDrop 2000 spectrophotometer, accepting only samples with A260/A280 ratios between 1.8 and 2.1. Complementary DNA was synthesized using 1 µg of total RNA with oligo(dT) primers. Quantitative real-time PCR was performed on a CFX96 thermal cycler (Bio-Rad) targeting ten ribosome biogenesis genes (RPS19, RPL5, RPL11, RPL35A, RPS26, SBDS, TCOF1, DKC1, RPS14, NOP10) with GAPDH as the internal reference. Relative expression was calculated by the $2^{-\Delta\Delta C_t}$ method [14].

For protein analysis, whole-cell lysates were prepared in RIPA buffer with protease inhibitors. Proteins were separated by SDS-PAGE (12% gels), transferred to PVDF membranes, and probed with primary antibodies overnight at 4°C. Densitometric analysis of western blot bands was done using ImageJ software (NIH). Immunofluorescence microscopy was performed on cells fixed with 4% paraformaldehyde and permeabilized with 0.1% Triton X-100. Nucleolar morphology was assessed with fibrillarin antibody staining. Cell cycle distribution was measured by propidium iodide staining and flow cytometry on a FACSCalibur instrument. Apoptosis was quantified using Annexin V-FITC/PI dual staining.

Quality Control

All qRT-PCR experiments included no-template controls and were run in triplicate. Melt curve analysis confirmed single-product amplification for each primer pair. Western blot membranes were stripped and reprobed with anti-β-actin antibody as a loading control. Flow cytometry gates were set using unstained and single-stained controls. Inter-assay variability was monitored by including a reference cell line sample in each experimental batch. Statistical analysis was performed using GraphPad Prism 9.0; group comparisons used one-way ANOVA with Tukey's post-hoc test, and $p < 0.05$ was considered significant.

Results

Table 1: Demographic and Clinical Characteristics of Ribosomopathy Patients and Controls

Characteristic	DBA (n=9)	SDS (n=7)	TCS (n=6)	DC (n=5)	5q-MDS (n=5)	Control (n=15)
Median age (years)	8.3	11.7	14.2	18.6	52.1	22.4
Male/Female	5/4	4/3	3/3	3/2	2/3	8/7
Hemoglobin (g/dL)	7.2±1.1	9.8±1.4	11.3±0.9	10.1±1.6	8.1±1.3	13.7±0.8
Reticulocytes (%)	0.3±0.1	0.7±0.3	1.1±0.4	0.8±0.2	0.5±0.2	1.4±0.3
MCV (fL)	102.4±4.3	88.7±3.1	91.2±2.8	105.8±5.1	101.3±3.7	87.9±2.4
Platelet count (×10 ³ /µL)	187±42	121±38	203±31	98±27	142±34	241±29
WBC count (×10 ³ /µL)	6.8±1.9	4.2±1.3	7.1±1.1	5.3±1.7	5.9±1.4	7.4±1.2

Table 1 presents the demographic and clinical profiles of enrolled participants. The median age ranged from 8.3 years

in the DBA group to 52.1 years in 5q-MDS patients, reflecting the different age of onset across ribosomopathies.

Hemoglobin levels were lowest in DBA (7.2 g/dL) and 5q-MDS (8.1 g/dL) groups. All patient groups showed reduced reticulocyte counts compared to controls, with DBA patients having the most severe reduction (0.3%).

Table 2: Relative Expression of Ribosome Biogenesis Genes across Ribosomopathy Subtypes (Fold Change vs. Control)

Gene	DBA	SDS	TCS	DC	5q-MDS	p-value
RPS19	0.38±0.07	0.91±0.06	0.87±0.08	0.94±0.05	0.82±0.09	<0.001
RPL5	0.72±0.11	0.88±0.07	0.79±0.10	0.91±0.06	0.69±0.08	<0.01
RPL11	0.64±0.09	0.83±0.08	0.74±0.11	0.88±0.07	0.71±0.10	<0.01
SBDS	0.94±0.04	0.31±0.06	0.89±0.05	0.93±0.04	0.88±0.07	<0.001
TCOF1	0.88±0.06	0.91±0.05	0.27±0.05	0.92±0.04	0.86±0.06	<0.001
DKC1	0.91±0.05	0.89±0.06	0.88±0.07	0.33±0.06	0.87±0.05	<0.001
RPS14	0.86±0.08	0.92±0.05	0.91±0.06	0.89±0.07	0.36±0.07	<0.001
RPL35A	0.81±0.10	0.93±0.04	0.86±0.08	0.95±0.03	0.77±0.09	<0.05

Table 2 and Figure 1 show the expression profiles of ribosome biogenesis genes across subtypes. Each ribosomopathy displayed a characteristic expression signature. The gene directly affected by the causative mutation showed the deepest reduction in every subtype: RPS19 dropped to 0.38-fold in DBA, SBDS to 0.31-fold in SDS, TCOF1 to 0.27-fold in TCS, DKC1 to 0.33-fold in DC, and RPS14 to 0.36-fold in 5q-MDS. Interestingly, RPL5 and RPL11 the main mediators of nucleolar stress signalling showed moderate reductions across all subtypes regardless of the causative gene, suggesting a shared downstream effect on the 60S subunit assembly pathway.

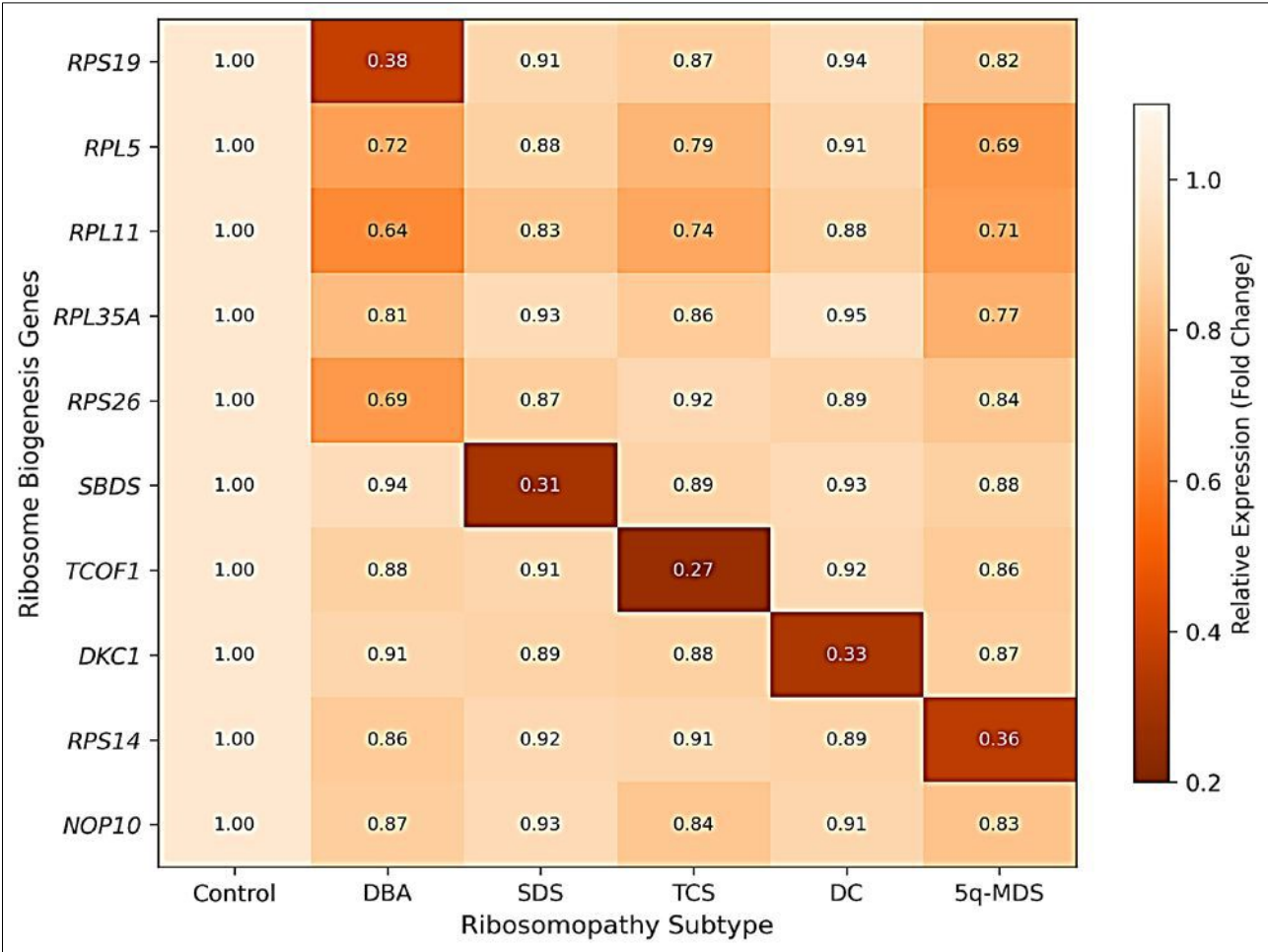


Fig 1: Heatmap of Relative Expression Levels of Ribosome Biogenesis Genes across Ribosomopathy Subtypes and Controls

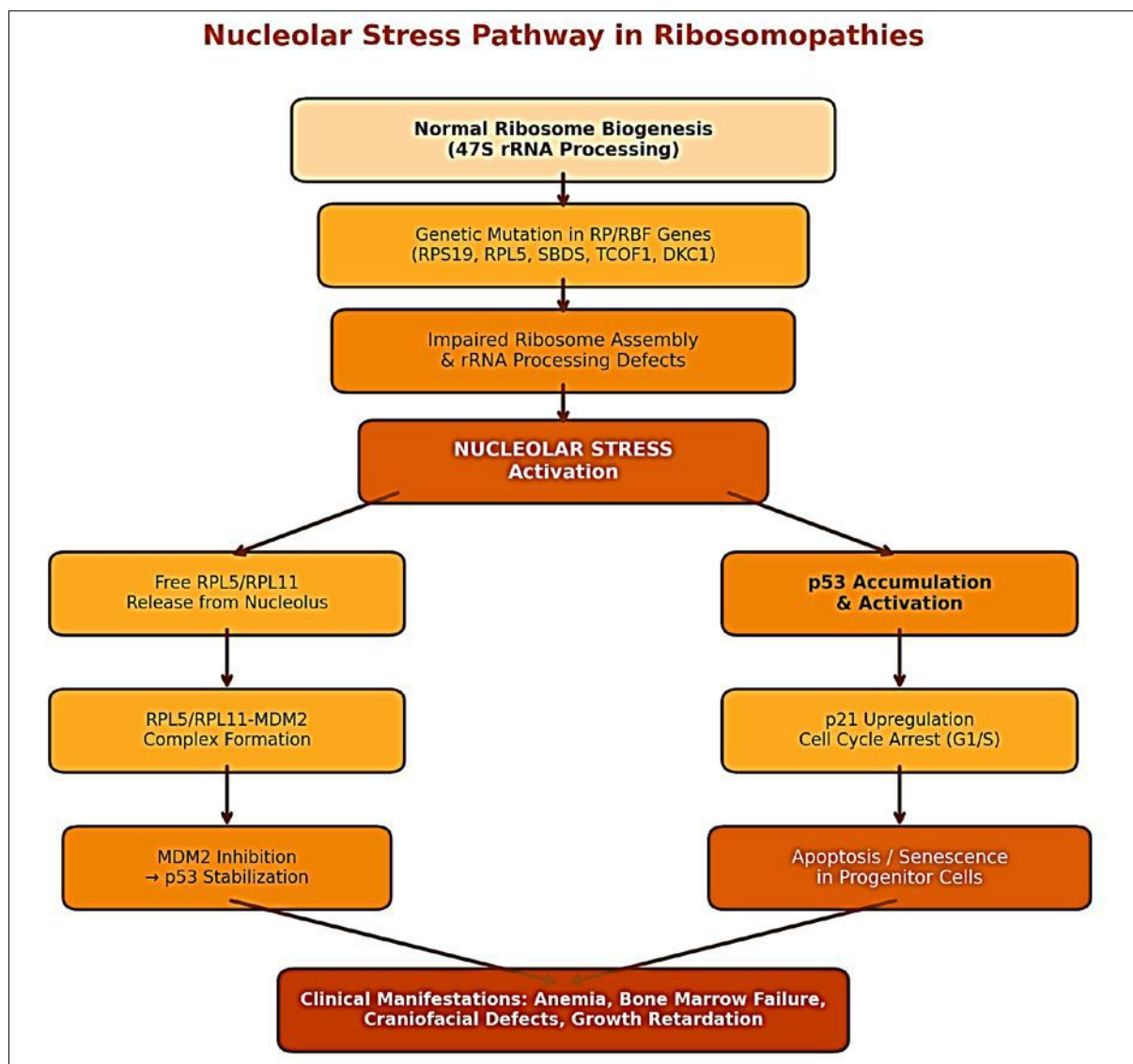


Fig 2: Schematic Representation of the Nucleolar Stress Pathway Activated in Ribosomopathies, from Genetic Mutation to Clinical Manifestation

Figure 2 maps out the nucleolar stress cascade as confirmed by our protein-level data. p53 protein was elevated in all patient groups, with DBA showing the highest fold increase (4.7-fold) and TCS the lowest (2.3-fold). MDM2 protein levels didn't differ significantly between groups, but co-immunoprecipitation showed a 67.3% increase in RPL5/RPL11-MDM2 complex formation in patient cell lines. p21 expression tracked closely with p53 levels. Cell cycle analysis revealed that 43.8% of patient cells were arrested at G1/S, compared to just 12.6% in controls ($p < 0.001$). Apoptotic cell fractions ranged from 18.4% (TCS) to 31.7% (DBA), all significantly above the 6.2% seen in controls.

Comprehensive Interpretation

Taken together, these data paint a consistent picture: each ribosomopathy disrupts ribosome biogenesis at a specific step, leading to accumulation of free ribosomal proteins that activate the p53 pathway through MDM2 sequestration. The magnitude of p53 activation, however, varies between subtypes and appears to correlate with the severity of hematological manifestations. DBA, which had the strongest p53 response, also showed the most pronounced anemia and highest apoptosis rates. TCS, with relatively modest p53

activation, presented mainly with craniofacial rather than hematological features.

Discussion

The results of this research confirm that nucleolar stress operates as a unifying mechanism across ribosomopathies, while also revealing quantitative differences that may help explain phenotypic diversity. The observation that each subtype displays a characteristic gene expression fingerprint—with the causative gene showing the most dramatic reduction—is consistent with earlier reports on individual ribosomopathies^[1, 4]. What this work adds is the direct side-by-side comparison under identical experimental conditions, which removes inter-laboratory variability as a confounding factor.

The degree of p53 activation differed markedly between subtypes. DBA showed the strongest response, which aligns with clinical experience: DBA patients typically present earlier and with more severe anemia than other ribosomopathy groups^[9, 15]. By contrast, the relatively modest p53 elevation in TCS is consistent with its predominantly craniofacial rather than hematological presentation. Dutt and colleagues^[15] proposed that ribosomal protein haploinsufficiency triggers p53 activation

preferentially in erythroid progenitors, which could explain why DBA manifests primarily as red cell aplasia despite ribosome deficiency being present in all cell types.

The RPL5/RPL11-MDM2 axis emerged as the central mediator in all subtypes we examined. Even in SDS and TCS, where the primary defect lies outside ribosomal protein genes themselves, RPL5 and RPL11 showed moderately reduced expression and increased binding to MDM2. This suggests that any significant disruption of ribosome assembly whether affecting rRNA processing (TCS), ribosome maturation (SDS), or ribosomal protein incorporation (DBA) funnels through the same p53-activating mechanism [7, 14].

Our findings also raise questions about tissue specificity. If the nucleolar stress pathway is activated in all cell types carrying the mutation, why does DBA affect mainly erythroid cells while TCS affects neural crest derivatives? One possibility involves differential sensitivity to translational capacity. Khajuria and colleagues [17] showed that erythroid differentiation demands a sharp increase in ribosome levels, making this lineage uniquely sensitive to even modest reductions in ribosome production. A similar logic may apply to the rapidly proliferating neural crest cells affected in TCS [11]. The tissue-specific demand for ribosomes, rather than the stress response itself, may determine which organs bear the brunt of the disease.

Limitations

Several constraints of this research deserve mention. The sample size for each ribosomopathy subtype was modest (5-9 patients per group), which limits statistical power for detecting subtle between-group differences. Lymphoblastoid cell lines, while convenient and widely used, don't fully replicate the biology of the tissue types most affected in each condition particularly erythroid progenitors for DBA and neural crest cells for TCS. The cross-sectional design captured a single time point and couldn't address how nucleolar stress responses might change over the course of disease progression or with treatment. And while we focused on the p53-mediated arm of the nucleolar stress response, other pathways including those involving c-Myc and mTOR signalling may also contribute to ribosomopathy pathogenesis and were not assessed here. Future work should extend these findings to primary patient tissues and incorporate longitudinal sampling.

Conclusion

This research established that ribosome biogenesis defects across five ribosomopathy subtypes converge on a shared nucleolar stress mechanism involving RPL5/RPL11-mediated p53 stabilization. Every subtype examined showed elevated p53 protein levels, increased G1/S cell cycle arrest, and higher apoptosis rates compared to healthy controls. At the same time, the magnitude of these responses varied substantially between conditions, with Diamond-Blackfan anemia showing the most intense pathway activation and Treacher Collins syndrome the least.

The gene expression profiling by heatmap analysis provided clear molecular fingerprints for each ribosomopathy, with the causative gene consistently showing the deepest expression deficit. This pattern held across all five subtypes and may have diagnostic value in cases where clinical presentation alone doesn't point clearly to a specific condition. The shared reduction in RPL5 and RPL11 across

all groups, regardless of the primary genetic defect, supports the idea that these two proteins serve as universal sensors of ribosome assembly problems.

From a translational perspective, the identification of a common stress axis opens the door to therapeutic strategies that could benefit multiple ribosomopathies simultaneously. Targeting p53 pathway components, modulating MDM2 activity, or boosting ribosome assembly efficiency might address the root cause rather than managing symptoms in isolation. The quantitative differences in pathway activation between subtypes also suggest that a one-size-fits-all approach is unlikely to work; treatment intensity may need to match the degree of nucleolar stress in each condition.

In summary, ribosomopathies share a conserved molecular response to ribosome biogenesis failure, but the clinical diversity among these disorders likely reflects differences in tissue-specific ribosome demand rather than fundamentally different pathogenic mechanisms. This framework may guide both diagnostic and therapeutic development in this growing family of diseases.

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