Parkinson's disease

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Recycling tRNA fragment 'trash' into treasure

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Identification of early biomarkers of neurodegenerative disorders such as Parkinson's disease is a dire need for the timely management of disease. Previously regarded as cellular byproducts or waste, transfer RNA fragments have recently emerged from the trash as potential gold mines for distinguishing between individuals in the pre-symptomatic stage of Parkinson's disease and healthy control individuals.

A major goal of neurodegenerative disease research is the identification of affected individuals before the onset of symptoms and irreversible loss of brain cells occurs. This has been particularly challenging for Parkinson's disease (PD), for which sensitive and specific predictive biomarkers are few and far between. A recent study from Soreq and colleagues[1] reports that measuring levels of specific transfer RNA fragments (tRFs) from the peripheral blood might help to identify persons with PD as well as those who will soon develop PD. To accomplish this, the investigators first combed through a large body of RNA expression data obtained from cerebrospinal fluid samples from patients with PD or Alzheimer's disease, or healthy control individuals. They found increased levels of nuclear-encoded tRFs and reduced levels of mitochondrially encoded tRFs (mtRFs) specifically in the PD samples. After further examination, they determined that there were common stretches of seven-nucleotide-sequence motifs in these nuclear-encoded tRFs (specifically, A or G, followed by GTTC, then A or G, and A (abbreviated as RGTTCRA)). Examining post-mortem data from the substantia nigra, where most of the dopamine cells are lost in PD, showed the same result (increased levels of nuclear-encoded tRFs that contain the RGTTCRA motif and decreased levels of mtRFs), which lent further support to their findings. Drawing on these data, the investigators then designed an innovative quantitative PCR (qPCR) assay that could amplify numerous tRFs with the same motif of interest, as well as mtRFs, and created a differential test based on the ratio of these, which showed strong clinical relevance and possible associations with the cause, progression and treatment of PD. For example, symptomatic patients carrying known high-risk PD-related mutations presented higher blood RGTTCRA tRF:mtRF ratios than non-symptomatic control individuals carrying the same mutations. There were also higher ratios in brain tissue from individuals with PD who had higher Braak Lewy body scores, and RGTTCRA tRF levels were decreased in patients with PD who underwent deep brain stimulation surgery to alleviate their symptoms. Thus, the findings could help to serve as a biomarker of disease onset, progression and treatment, and thereby improve clinical management. Furthermore, the investigators showed that their qPCR assay could distinguish individuals with presymptomatic (prodromal) PD from control individuals at a level comparable to or slightly better than traditional clinical scoring methods based on the current gold-standard neurologic assessment, achieving an area under the receiver operating characteristic curve (ROC AUC) of 0.75 versus 0.71 in a naive holdout set that was not used to train their model. Finally, the investigators were able to demonstrate that RGTTCRA tRFs exhibited complementarity to ribosomal RNA, which might aggravate PD via translational inhibition, as reflected by disrupted ribosomal association of RGTTCRA tRFs in depolarized neuroblastoma cells.

Why is this work so noteworthy? One reason concerns the subject matter itself. The central dogma of molecular biology long ago established the essential role of transfer RNAs (tRNAs) in the translation of mRNA sequences into proteins. Each tRNA has a distinctive cloverleaf structure and anticodon loop that matches a specific amino acid that it can transport to the ribosome. In human cells, the nuclear genome contains more than 500 tRNA genes clustered throughout the genome, and the mitochondrial genome contains an additional 22 tRNA genes. The tRNAs produced by these genes decode 64 triplet codons that are present in mRNAs to promote cytoplasmic and mitochondrial protein synthesis, although the nuclear and mitochondrial interpretations are slightly different. Because cells continually need to synthesize proteins, the total cellular tRNA population is actually far more abundant than any other class of RNA; tRNAs account for approximately 10–15% of total cellular RNA mass, despite their small size[2].

In recent years, the roles of tRNAs have expanded well beyond simply functioning as amino acid transporters, owing at least in part to their extensive processing and binding activities. For example, tRFs and tRNA halves have emerged as functional small non-coding RNAs that derive from precursor and mature tRNA cleavage. Once thought of as merely degradation products (that is, cellular 'trash'), these 14–40-nucleotide fragments are now recognized as important regulatory molecules that participate in diverse cellular processes, including gene-expression regulation through transcriptional and post-transcriptional silencing, protein translation modulation, RNA stabilization, and cellular stress responses[3]. This represents an notable shift in our understanding of RNA biology, and new findings continue to suggest potentially important contributions for these tRNA fragments in health and disease – particularly in diseases associated with aging and neurodegeneration, in which cellular stress is very well documented[4].

When a cell experiences oxidative stress or amino acid starvation, it can trigger the activation of enzymes such as angiogenin to boost production of tRFs and tRNA halves, which can directly dampen gene expression either by binding to mRNA and promoting mRNA degradation (similar to microRNAs) or inhibiting protein translation by competing with mRNAs for ribosome access, binding to the smaller ribosomal complex, or interfering with translation initiation complex (eiF4) assembly. Interestingly, Soreq and colleagues[1] demonstrate that angiogenin overexpression significantly increases RGTTCRA tRFs and not mtRFs in cell lines, but that knockout of the angiogenin gene in mice has no effect on the production of these classes of tRFs. Thus, angiogenin could be boosting levels of the PD-associated tRF, but is probably not required for its expression.

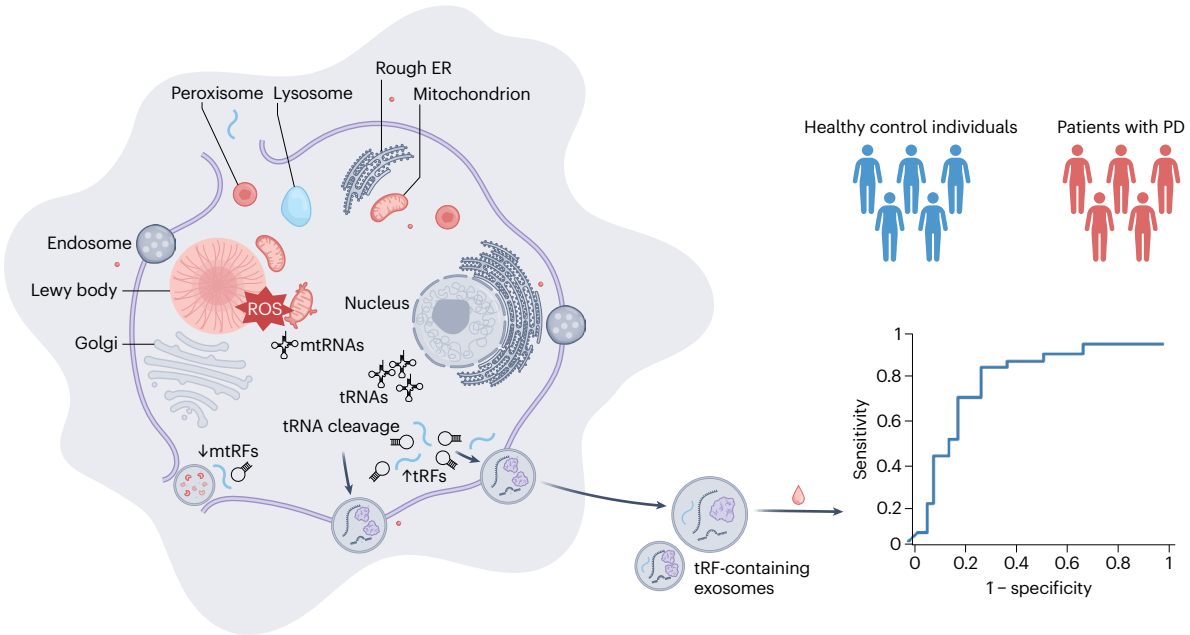

Fig. 1 | Dopamine neuron tRF changes in PD. A dopamine-synthesizing neuron affected by PD that contains a Lewy body (large red circular mass) and shows evidence of increased ROS in response to proteotoxicity and oxidative stress. The neuron subsequently shows increased cleavage of selected nuclear tRNAs into tRFs and reduced cleavage of mitochondrial tRNAs (mtRNAs) into mtRFs, which occurs in PD. These tRFs are released extracellularly in exosomes and other vesicles and measured in blood to train a diagnostic predictor that displays high sensitivity and specificity, according to the area under a receiver operating characteristic curve (bottom right). ER, endoplasmic reticulum.

Within the brain, evidence suggests tRFs can strongly influence normal neuronal function as well as the changes that occur during a neurodegenerative disease process. A notable example of this can be found in studies of patients with pontocerebellar hypoplasia type 10 (PCH10), a rare autosomal recessive neurodegenerative disorder caused by mutations in the *CLP1* gene that encodes a component of the tRNA splicing complex[5]. Studies in mice that carry the PCH10 mutation show accumulation of tRNA fragments in affected cells[6].

Similar to PCH10, mutations in the angiogenin gene have also been identified as a risk factor for the familial form of amyotrophic lateral sclerosis[7]. Moreover, the 5′ ValCAC tRNA fragment that is generated by angiogenin shows increased levels in spinal cord and blood serum in the SOD1G93A transgenic mouse model of amyotrophic lateral sclerosis at the time of symptom onset[8], and its levels are also correlated with slower disease progression and increased survival in patients with amyotrophic lateral sclerosis, which suggests that it might serve as a potential prognostic marker[8].

Beyond PCH10 and amyotrophic lateral sclerosis, tRNA metabolism disruptions are also linked to epilepsy. Specifically, mutations in tRNA biogenesis and modification genes increase seizure susceptibility, which underscores their likely influence on neuronal activity[4,9]. Moreover, studies have reported age-related changes in tRF expression that might contribute to cognitive decline. For example, in Alzheimer's disease brain tissue, there are reports of increased angiogenin and tRF expression in the hippocampus that correlate with age and disease progression[10]. Supporting these observations, recent studies have also identified age-related accumulations of a specific glutamate tRNA fragment (Glu–5′ tRNA-derived small RNA–CTC) in mitochondria from glutamatergic neurons in aged mice, which impaired mitochondrial function and accelerated cognitive decline[11]. Notably, specifically targeting these fragments with antisense oligonucleotides reversed memory deficits in aged mice, which suggests there might be potential therapeutic approaches of targeting some overexpressed tRFs.

Prior to the current report by Soreq and colleagues[1], work in the PD field had identified angiogenin gene variants in some patients, which suggests a possible genetic link between tRNA cleavage and disease development in these patients[12]. Moreover, a previous publication based on datasets available from the prefrontal cortex, cerebrospinal fluid and serum of patients with PD revealed their potential utility as diagnostic biomarkers[13]. However, despite the fact that the classification model developed in that study could distinguish patients from control individuals with high accuracy, it was never evaluated in a set of naive hold-out participants, unlike in Soreq and colleagues' study[1].

Taken together, the available data combined with the current article give a strong impression of disease-related changes in selected tRF expression in PD. Although the current article does have several limitations (such as reduced performance of their qPCR assay in some racial categories and the use of a relatively modest size holdout set), their findings nonetheless emphasize the need to pay more attention to what we have previously considered as cellular waste. Clearly, additional work will be needed to determine the extent to which changes in tRF levels are involved in the pathogenesis of PD, rather than simply its pathophysiology. The former is not hard to imagine, given that mtRNAs are indispensable for maintaining mitochondrial protein synthesis, which is required for several components of the electron transport chain involved in ATP generation. Without this, cellular respiration

falters, reactive oxygen species (ROS) levels are increased, and severe oxidative stress ensues. On the other hand, a hallmark of brain neurons affected in PD is the formation of protein inclusions known as Lewy bodies, which create a state of proteotoxic stress superimposed on oxidative mitochondrial stress[14]. Thus, the gate could easily swing both ways, and downregulation in mtRFs could reflect an adaptive response to reduce intracellular protein load (Fig. 1). In fact, Soreq and colleagues' study also noted the possibility that the identified overexpressed RGTTCRA motif is found on tRNAs that transport phenylalanine or cysteine (which are amino acids required for dopamine and glutathione synthesis, respectively), raising the possibility that the biomarker signature they detected could reflect an attempt to upregulate dopamine and antioxidant production. Looking ahead, these are just a few of the key questions that will need to be addressed. Larger-scale clinical studies will also be needed to validate the potential of specific tRNA fragments to serve as reliable diagnostic and prognostic biomarkers in patient cohorts that can determine disease specificity across a wide variety of brain disorders. Continued research into the biogenesis pathways of these tRFs in the brain, including the enzymes and regulatory factors involved, is also required to reveal new therapeutic targets for PD.

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Competing interests

The author declares no competing interests.