TRAM MB-PhD Project Summary

PhD project Title
A dual role for NBAS in Golgi-to-ER retrograde transport and NMD and its influence on osteogenesis imperfecta

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Background
Nonsense-mediated decay (NMD) is an RNA quality control mechanism that degrades mRNAs that contain premature termination codons (PTCs), but also regulates the stability of a large number of cellular RNAs[1]. NMD modulates the phenotypic outcome of genetic disorders caused by nonsense mutations that generate PTCs[2]. We identified a novel NMD factor, NBAS *(*Neuroblastoma amplified sequence) that localises to the endoplasmic reticulum (ER), and showed that is part of **a novel ER-localised NMD pathway, termed ER-NMD**. NBAS, is independently involved in the secretory pathway, as part of the Syntaxin 18 complex that functions in **Golgi-to-ER retrograde transport[3]**. Loss of function mutations in *NBAS* have been found in several human conditions, including the SOPH syndrome (short stature, optic nerve atrophy and Pelger-Huët anomaly of granulocytes); a multisystem disease affecting bone, connective tissue, immune system and retina; and in recurrent acute liver failure (RALF)[4]. With Meena Balasubramanian (Sheffield), we identified compound heterozygous variants in *NBAS* as a cause of atypical **osteogenesis imperfecta** (OI), an inherited disorder commonly caused by mutations in the COL1A1/2 genes that encode the chains of type I collagen[5]. The broad spectrum of phenotypes observed in these patients may be a result of a compromised NMD response and/or defects in Golgi-to-ER transport. Bi-allelic variations in RINT1, another component of the Syntaxin 18 complex, cause RALF and skeletal abnormalities[6], suggesting that defects in retrograde transport can lead to these clinical features. **However, the role of NMD in these diseases remains unclear.** The Unfolded Protein Response (UPR) senses and responds to excessive amounts of misfolded proteins in the ER and is regulated by NMD, ensuring appropriate activation of the UPR[1].

Aims
We aim to address the biological role of NMD at the ER and its impact on the stress response in relation to bone disease. We will identify RNAs that are misregulated upon defective ER-NMD caused by NBAS mutations. Profiling of transcripts regulated at the NMD level by NBAS in HeLa cells revealed an enrichment for genes involved in the stress response and identified MGP (matrix Gla protein) gene, an inhibitor of bone formation[7]. We propose that modulation of NMD and/or ER-NMD could be crucial to regulate ER stress[3], and this might be of particular importance in bone related diseases that lead to an increased ER stress response.

Aim 1- RNA-binding and RNA degradation. We will define the cellular transcriptome translated at the ER that is bound and regulated by NBAS in bone-derived cell lines, including U2OS osteosarcoma cells and SAOS2 cells. We will use individual-nucleotide resolution UV crosslinking and immunoprecipitation (**iCLIP**) to identify endogenous RNA targets directly bound by NBAS[8]. This will be complemented by metabolic labelling of newly transcribed RNAs that allows resolution of RNA synthesis degradation kinetics (SLAM-seq)[9]. This aim will unveil those transcripts that are regulated at the NMD level by NBAS in bone-derived cell lines.

Aim 2- OI and ER stress. In an OI mouse model with a dominant mutation in the terminal C-propeptide domain of *Col1a*, termed *Aga2* (abnormal gait 2), heterozygous animals develop severe-to-lethal phenotypes. Abnormal proa1(I) chains accumulate intracellularly in *Aga2/-* dermal fibroblasts and are poorly secreted extracellularly leading to ER stress induction with upregulation of UPR markers, and apoptosis of osteoblasts[10]. We will use CRISPR/Cas9-mediated genome editing to generate the *Aga2* mutation[10] in U2OS osteosarcoma cells. **We will drive overexpression of NBAS and core NMD factors to test the hypothesis that increasing NMD and/or ER-NMD activity has a protective effect in OI by limiting excessive UPR signalling.** The *Aga2 COL1A1* mutation leads to ex vivo osteoblast differentiation defect[10]. We will recapitulate the *Aga2* mutation in a MC3T3 mouse osteoblast precursor cell line and will assess whether ER-NMD activation has a protective effect on osteogenic differentiation in this system.

Aim 3- Mouse models. We will extend the studies described above by recreating the Aga2 mouse model. This will be crossed with transgenic mice with an increased expression of the core NMD factor UPF1 or the ER-NMD factor, NBAS.

Training and experience provided

This project will provide multidisciplinary training

- Contribute towards a mechanistic understanding of genetic disorders caused by defects in the NMD pathway
- Extensive training in RNA biology, biochemistry and molecular biology (Caceres lab)
- Focus on the molecular and genetic basis of OI and other bone diseases (Ralston lab)

Expected outcomes

We will address fundamental questions in the fields of RNA quality control, related to the NMD pathway and its relation to human disease. This project will reveal the biological consequences of manipulating NMD activity in relation to cellular stress and its impact on bone disease.

References

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