

Investigating the localisation of PEX10 in-line with patient mutations

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Lay Summary:

Peroxisomes are essential organelles found in all eukaryotic cells. They are membrane bound compartments that play a critical role in lipid metabolism and detoxifying toxic products, such as hydrogen peroxide. Peroxisome Biogenesis Disorders (PBDs) are inherited metabolic diseases that affect children and are caused by a disruption in function of peroxisomes. There is a current lack of understanding surrounding the contribution of individual mutations and how this results in clinical symptoms for patients, so this project investigated a compound mutation in the PEX10 gene of a patient with PBD. PEX10 is a peroxin that resides on the peroxisomal membrane and is part of the ubiquitin ligase complex (PEX2, PEX10, PEX12), which is involved in protein import into peroxisomes. The mutation we studied is in-line with the patient's missense mutation at residue 290, where histidine replaces aspartic acid. We explored the cellular localisation of this mutated PEX10 protein to determine whether it remains in the peroxisomal membrane or mislocalises to the cytosol, and to begin to explain the loss of peroxisomal function observed in the patient.

Methods

To quantify the localisation of PEX10, we used a conventional GFP reporter as our negative control, and PXMP2-mCherry was used as a spatial and quantitative reference for peroxisome identification.

Plasmids were amplified in DH5 α E.coli, plated on LB agar plates, colonies transferred to secondary ampicillin LB plates, plasmids isolated and measured using Thermo Scientific™ NanoDrop™ spectrophotometer.

HEK293T cell lines were co-transfected with PXMP2-mCherry and either, GFP, GFP-PEX10 or GFP-PEX10-H290D (1 μ g of plasmids). These were fixed (4% PFA), visualised using the Leica SP8 confocal microscope and images were processed using Fiji (Image J). The co-expression of plasmids, allowed for visualisation and comparison of fluorescent intensity for GFP and mCherry channels

at regions of interest (peroxisomes). For each cell line fluorescence data for 30 cells per experiment were collected across at least three biological repeats. Statistical significance was assessed using Kruskal-Wallis tests for non-parametric data, which enabled us to determine differences in localisation between constructs.

Results

Based on previous work by members of our lab, we hypothesised that the H290D variant of PEX10 would not localise to peroxisomes. Our aim was to determine whether PEX10-H290D co-localises with peroxisomes to gain insight into the mechanism underlying this loss of import.

Visualisation of PEX10-H290D mutation by confocal microscopy

To test this hypothesis, we performed co-expression

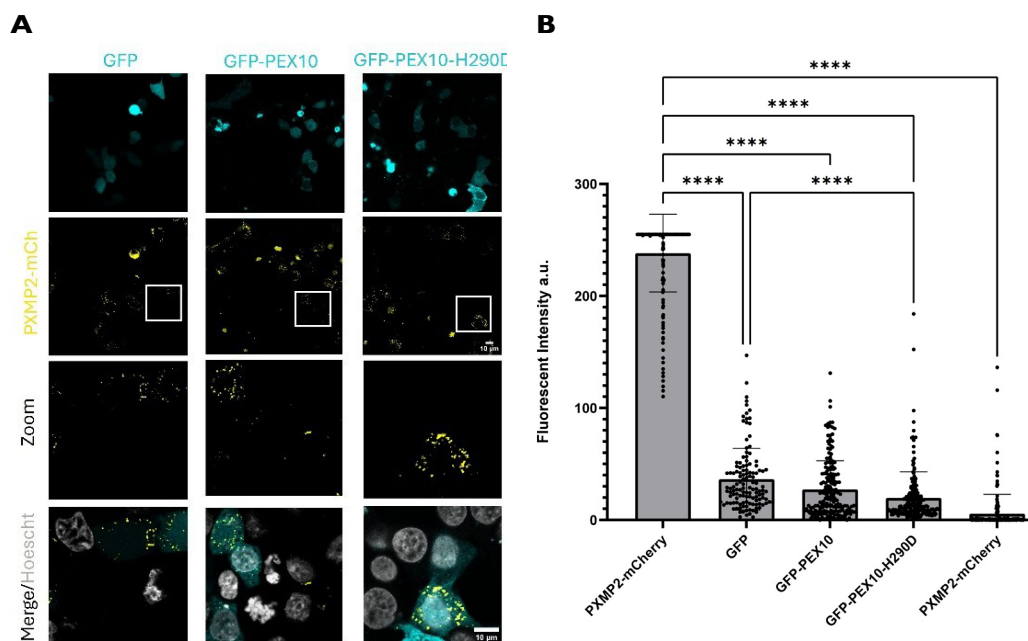


Figure 1. A. Confocal microscopy of fixed wild-type Human Embryonic Kidney (HEK293T) cells co-expressing PXMP2-mCherry (yellow, 1 μ g/ml) and either GFP, GFP-PEX10, or GFP-PEX10-H290D (cyan, 1 μ g/ml). Nuclei were stained with Hoechst (10 μ g/ml). Scale bar, 10 μ m. B. Quantification shows average fluorescent intensity of the same region of interest (peroxisomes) between the red channel (mCherry) and the green channel (GFP), mean $\pm$ SEM, N= 30 cells, Kruskal-Wallis (**** = <0.0001).

experiments in HEK293T cells co-expressing PXMP2-mCherry, as a peroxisomal marker, with either GFP, GFP-PEX10 or GFP-PEX10-H290D. This allowed for comparison of the fluorescent intensity between channels and visualisation of the distribution PEX10 variants between the cytosol and peroxisomes (Figure 1A).

Quantification of co-expression experiment shows a significant reduction in fluorescence intensity from PEX10-H290D to control

Statistical analysis revealed a significant difference in fluorescent intensity between PXMP2-mCherry and GFP signals, consistent with GFP being cytosolic (Figure 1B, $p < 0.0001$). Similarly, a significant difference between PXMP2-mCherry and GFP-PEX10-H290D, as well as between GFP and GFP-PEX10-H290D ($p < 0.0001$) indicated that the mutated PEX10 variant is predominantly cytosolic (Figure 1B).

However, the data does show that GFP-PEX10 also differed significantly from PXMP2-mCherry

(Figure 1B, $p < 0.0001$), this is unexpected as it suggests PEX10 fusion localisation may not be strictly peroxisomal, and means there may be a methodological or construct related error. This was also supported by the lack of significant difference between GFP-PEX10 and GFP-PEX10-H290D (Figure 1B). Future experiments will include establishing the baseline localisation of GFP-PEX10, colocalisation analysis and performing fusion assays to independently confirm subcellular localisation of PEX10-H290D.

What did I learn from participating in this project and how has it affected my long-term goals?

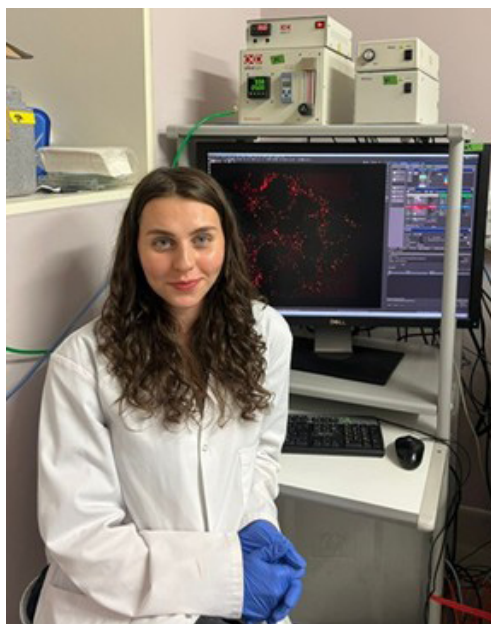
Participating in this project significantly strengthened my technical and analytical abilities. I gained hands-on experience using the Leica SP8 confocal microscope, which deepened my understanding of high-resolution microscopy. Through experimental planning and data interpretation, this experience helped to refine my critical thinking and problem-solving skills.

This project has reinforced my passion for cell biology and microscopy, as well enhancing my ambition to pursue a PhD in this field. My experience using the Leica SP8 confocal system motivated me to explore other imaging techniques to advance our understanding of this field, and I am particularly inspired by the application of advanced imaging techniques to explore model systems in relation to disease.

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Charlotte Howman.

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