Disease-associated mutations abolish palmitoylation and membrane association of syntaxin 11 in natural killer cells

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Introduction

Natural killer (NK) cells are an important component of the innate arm of the immune system, killing infected and tumorigenic cells. NK cell recognition of target cells promotes the formation of an immunological synapse at the junction of the two cells. The polarised exocytosis of secretory lysosomes releases cytotoxic molecules at the immunological synapse. Perforin, a pore forming protein, facilitates the entry of granzymes into the target cell cytoplasm triggering apoptosis of the target cell. Subjects with Familial Haemophagocytic Lymphohistiocytosis (FHL), a rare familial disorder, display low NK cell cytotoxicity and a corresponding defect in secretory lysosome exocytosis. The SNARE protein syntaxin 11 is mutated in type 4 FHL (FHL-4). SNARE proteins mediate membrane fusion reactions, however the precise role of syntaxin 11 in secretory lysosome exocytosis is unknown. To better understand the function of this SNARE protein and why mutations in the protein result in FHL-4 we characterised the role of disease-associated mutations on the palmitoylation, membrane association and subcellular localisation of syntaxin 11.

Results

The C-terminal region of STX11 is necessary for palmitoylation and membrane association

SNARE proteins typically associate with membranes via a transmembrane domain; syntaxin 11, however, is an atypical SNARE in that it lacks a transmembrane domain. Instead syntaxin 11 is predicted to be palmitoylated at its cysteine rich C-terminus. We have demonstrated experimentally that syntaxin 11 is palmitoylated using the biotin switch assay (Figure 1A). This is an assay which switches palmitate groups on proteins for biotin such that they can then be pulled down from cell lysates on avidin beads. The FHL-4-associated mutant of syntaxin 11, Gln268X, which lacks the cysteine rich C-terminal region was not palmitoylated when expressed in the NK cell line YTS (Figure 1A). Moreover, syntaxin 11 Gln268X was not present in the membrane fraction of YTS NK cells (Figure 1B) and was instead in the cytosolic fraction. Thus the Gln268X mutation abolishes membrane palmitoylation and membrane association of syntaxin 11.

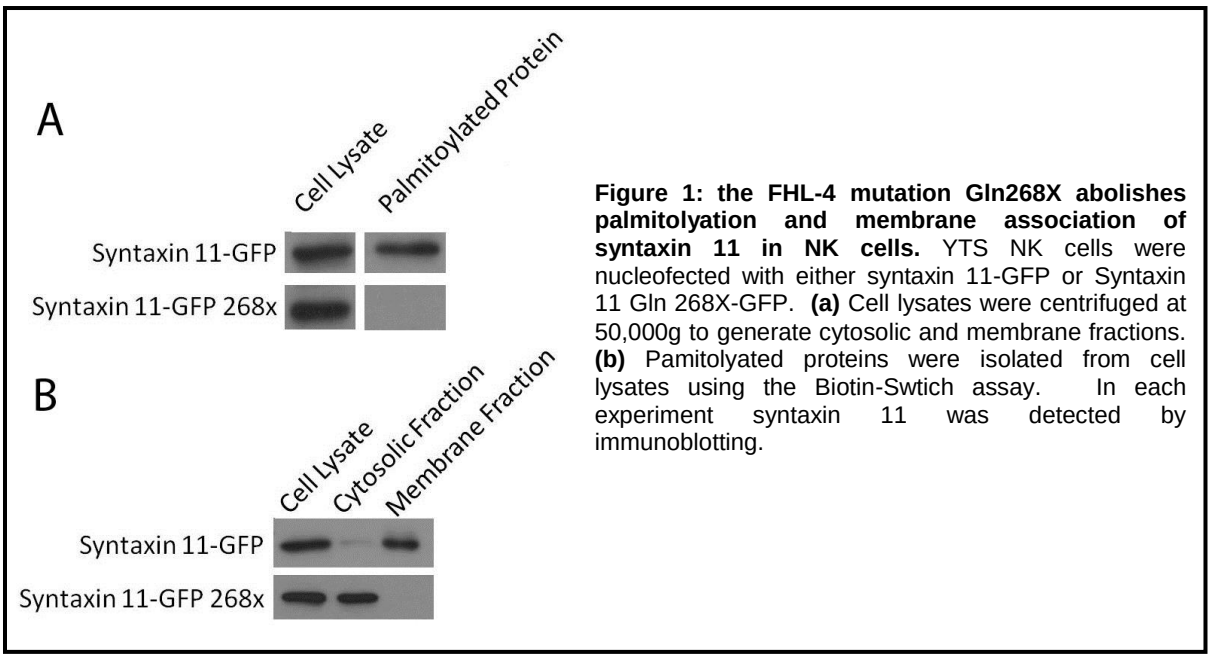

Figure 1: the FHL-4 mutation Gln268X abolishes palmitolyation and membrane association of syntaxin 11 in NK cells. YTS NK cells were nucleofected with either syntaxin 11-GFP or Syntaxin 11 Gln 268X-GFP. **(a)** Cell lysates were centrifuged at 50,000g to generate cytosolic and membrane fractions. **(b)** Pamitolyated proteins were isolated from cell lysates using the Biotin-Swtich assay. In each experiment syntaxin 11 was detected by immunoblotting.

Palmitoylation and membrane association are necessary for trafficking to the immunological synapse

During natural killer cell exocytosis, syntaxin 11 has been shown to traffic to the immunological synapse and to co-localise with secretory lysosomes. Using live-cell confocal microscopy we observed that in resting YTS cells, syntaxin 11 is localised to cytoplasmic puncta that are distinct from lysosomes (stained with Lysotracker), during conjugation with a target cell however, syntaxin 11 displays increased co-localisation with lysosomes at the immunological synapse. However, we found that syntaxin 11 Gln268X, exhibited a diffuse cytoplasmic localisation and did not traffic to the immunological synapse in activated YTS NK cells.

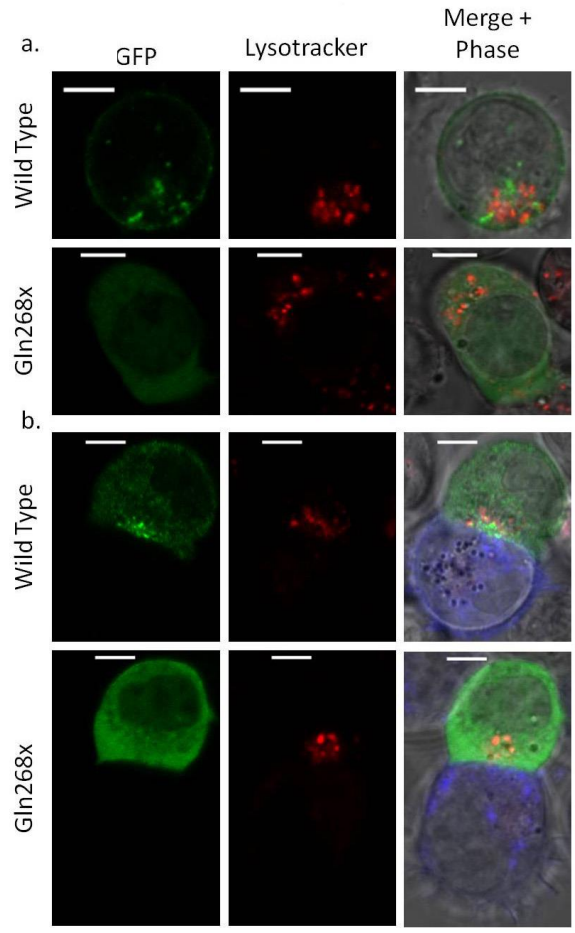

Figure 2: the FHL-4 mutation Gln268X prevents the recruitment of syntaxin 11 to the immunological synapse in activated NK cells. YTS NK cells were nucleofected with either syntaxin 11-GFP or Syntaxin 11 Gln 268X-GFP and incubated in the absence (A) or presence of the target cell line 721.221 (B) that had been labelled with Cell Trace (blue). Cells were stained with LysoTracker (red) to visualise secretory lysosomes and imaged by live cell confocal microscopy. Scale bar is 5 μm

Summary

Analysis of the FHL-4-associated mutant syntaxin 11 Gln268X reveals that palmitoylation is required for membrane association and recruitment of synatxin 11 to the immunological synapse in NK cells.

Funding

This work is supported by the Wellcome Trust.