Multiple degradation mechanisms ensure disposal of an Angelman-syndrome associated NHE6 deletion protein

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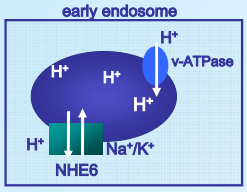
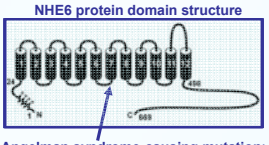


Figure 1

Background:

- NHE6 is a member of the family of Na^+/H^+ exchangers (NHE) that mediate electro neutral exchange of H^+ for Na^+ or K^+ across membranes
- NHE6 is localized to early endosomes where it has been proposed to regulate endosomal pH (Figure 1)
- Mutations in *SLC9A6*, the gene encoding NHE6, have recently been found to cause the mental retardation syndrome Angelman syndrome (Figure 2)



Angelman syndrome-causing mutation: 2 amino acid deletion in transmembrane domain 7

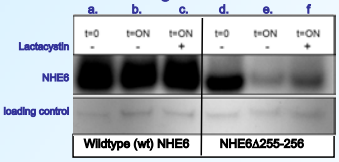
Figure 2

Project aim:
characterization of the Angelman syndrome-causing NHE6Δ255-256 deletion protein

Results:

1.

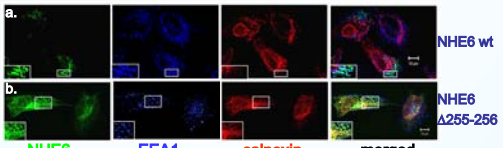
NHE6Δ255-256 is unstable and rapidly degraded, partly by proteasomal degradation

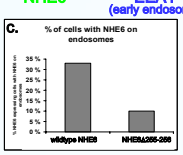


HeLa cells transiently expressing NHE6 wt (a-c) or Δ255-256 (d-f) were incubated in the presence or absence of the proteasomal inhibitor Lactacystin over night (ON). The results reveal that NHE6Δ255-256 is largely degraded (e) and this degradation is partly abolished by inhibiting the proteasome (f).

2.

NHE6Δ255-256 displays a disturbed localization pattern

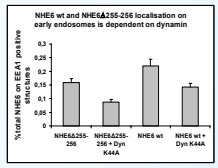




Transiently expressed NHE6 wildtype localizes to early endosomes (a) while NHE6Δ255-256 mainly accumulates in the ER (b). Additionally a small pool of NHE6Δ255-256 localizes to early endosomes, although significantly less than wt NHE6 (b-c).

3.

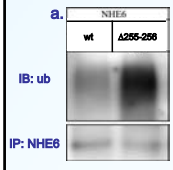
NHE6Δ255-256 is targeted to endosomes via the plasma membrane



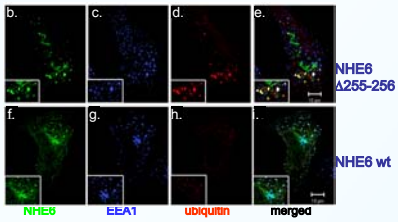
Inhibition of endocytosis by the use of a dominant negative dynamin (DynK44A) reduces the endosomal localization of NHE6 wt and NHE6Δ255-256, indicating that both proteins are transported to endosomes via the plasma membrane.

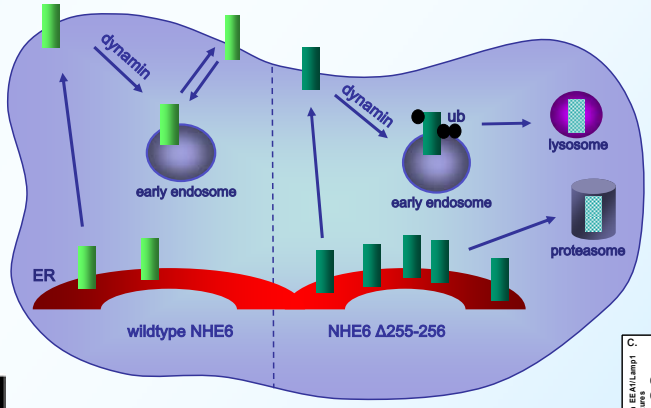
4.

NHE6Δ255-256 on endosomes is ubiquitinated



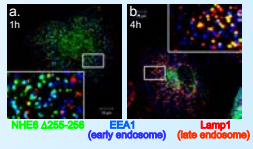
Immunoprecipitation experiments show that NHE6Δ255-256 is ubiquitinated to a larger extent than wt NHE6 (a). Additionally ubiquitin accumulates on early endosomes containing NHE6Δ255-256 (b-e), but not on endosomes positive for NHE6 wt (f-i).

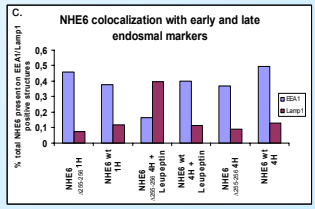




5.

NHE6Δ255-256 is trafficked through the endocytic pathway and is degraded in lysosomes





NHE6Δ255-256 is transported from early (a,c) to late (b,c) endosomes and degraded in lysosomes (c), while wildtype NHE6 is stably associated with early endosomes (c).

Conclusions

Our results indicate that the disease-causing NHE6Δ255-256 deletion protein is unstable in the cell and is degraded by two separate degradation mechanisms: -ER-associated degradation by the proteasome

-lysosomal degradation by transport through the endocytic pathway