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Molecular mechanisms underlying neuroepithelial/ependymal denudation in the hydrocephalic *hyh* mutant: spatial and temporal expression of alpha-SNAP and N-cadherin

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Background

The *hyh* mutant mouse develops fetal-onset neuroepithelial/ependymal denudation that precedes cerebral aqueduct obliteration and hydrocephalus [1,2]. A hypomorphic point mutation (M105I) in alpha-SNAP protein has been identified as responsible of the *hyh* phenotype [3]. Alpha-SNAP is widely distributed in all mammalian tissues and cell types [4]. It is a key component of the SNARE machinery for membrane fusion and participates at different levels of vesicular traffic of proteins, including transport to plasma membrane [5]. But, why does a mutation in such an ubiquitous protein lead to selective developmental disorders of the central nervous system? How is alpha-SNAP mutation involved in neuroepithelial/ependymal denudation? Considering that (i) the pattern of ependymal denudation matches that of ependymal differentiation [1], and (ii) the ependyma of circumventricular organs, endowed with a special set of junctions, never detach; it is proposed that alpha-SNAP mutation could result in a failure in the adhesion/junction proteins physiology during brain development leading to neuroepithelial/ependymal denudation. The aim of the present investigation was two fold: (a) to study the temporal and spatial expression of alpha-SNAP, NSF, and some proteins involved in intercellular junctions, and (b) to evaluate the importance of these proteins on ependymal physiology and stability.

Materials and methods

(i) Brain samples of non-hydrocephalic (wild type) and hydrocephalic (mutant) mice from the hyh strain (B6C3Fe-*a*/*a*-*hyh*) were studied by immunocytochemistry (IMC) and transmission electron microscopy (TEM) at various developmental stages. Protein homogenates from telencephalum, mesencephalum/brain stem and cerebellum were analyzed by Western blot. The expression levels of mRNA encoding for alpha-SNAP and NSF were analyzed by semi-quantitative PCR. (ii) Ependymal explants obtained from adult bovine Sylvius aqueduct were cultured for 24 hours and used to evaluate the role of adherens junctions and N-cadherin in ependymal stability. Basically, after validation of this *ex-vivo* model, N-cadherin functional blocking assays in 1DIV explants using specific antibodies and competitive peptides were performed. The effect of N-cadherin blockage was evaluated by light microscopy (quantitative analysis), IMC, and TEM.

Results

(1) alpha-SNAP and NSF are preferentially expressed in the CNS and at early developmental stages; (2) alpha-SNAP is preferentially expressed at ventricular lining; (3) in mutant animals, the decrease of alpha-SNAP protein varies at different stages and at different brain regions; (4) hyh mutant mice present an increase in NSF protein,

probably due to its overexpression; (5) ependymal cells express N-cadherin but not E-cadherin; (6) different ependymal subpopulations showed a differential expression of alpha-SNAP and N-cadherin; (5) functional blocking of N-cadherin led to (i) changes in N-cadherin immunocytochemical pattern, (ii) ultrastructural modifications of adherens junctions, (iii) increase of the intercellular space, and (iv) detachment of the ependyma leading to large denuded areas of the explants.

Conclusion

The selective expression of alpha-SNAP in the brain, and its differential expression at distinct brain regions and cell types may contribute to the understanding of the molecular mechanisms underlying hyh phenotype. N-cadherin-dependent adherens junctions play a key role in ependymal stability. An alteration in the physiology (traffic?) of N-cadherin appears to be the one of the mechanisms operating in the ependymal denudation of hyh mice.

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