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ROLE OF THE SMALL GTPase RhoE IN MYELINATION AND AXONAL TRACTS DEVELOPMENT

Presentada por

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I. SUMMARY.

The Rho family of small GTPases is a group of proteins with important roles in actin cytoskeleton organization. In the last years, mounting evidences suggest that these proteins have key roles in the development and function of the central nervous system (CNS). In particular, Rnd proteins are a subfamily of Rho GTPases characterized by their constitutive activity. Rnd3/RhoE is a member of this subfamily, whose specific functions during brain development are still not well defined. Thus, the main aim of the present work is determine its role during the development of CNS by studying the anatomical and morphological consequences present in a transgenic mouse lacking the RhoE expression ($RhoE^{gt/gt}$).

The $RhoE^{gt/gt}$ mice display severe alterations in the brain. On the one hand, $RhoE^{gt/gt}$ mice exhibit hypomyelination in the brain. The mutants show a decreased expression of several myelin proteins, reduction in the number of myelinated axons and those that are myelinated display thinner myelin sheaths. The analysis of the RhoE expression showed that it is expressed in oligodendrocyte precursor cells and also in mature oligodendrocytes *in vivo* and *in vitro*. In fact, in the mutant, the striatum and the corpus callosum, showed a reduction in the number of mature and total oligodendrocytes. These results together with the *in vitro* cultures studies suggested that in $RhoE^{gt/gt}$ mutants the differentiation of the oligodendrocyte precursor cells is altered. Altogether indicates that RhoE is directly required for the differentiation of oligodendrocytes and in consequence for the correct myelination of the CNS.
On the other hand, $RhoE^{gt/gt}$ mice show aberrant axonal projections in the forebrain. Specifically, the anterior branch of the anterior commissure growths from the anterior olfactory nucleus but is missprojecting ventrally without reach or cross the midline. Moreover, the thalamocortical projection is also altered, and only few axons enter the telencephalon. During the development of these axonal tracts, RhoE is expressed both in origin (anterior olfactory nucleus and thalamus) and in the surrounding regions that the axons have to cross to reach to their targets. Hence, these data suggest that RhoE is key for the CNS development by controlling the extension of these axonal systems and/or the establishment of the territories essential for their axon pathfinding.

In summary, the results of this work highlight the relevance of RhoE in multiple and essential processes occurring during the development of the forebrain, in particular the myelination and pathfinding of some of the major axonal tract in this brain region.

VIII. CONCLUSIONS.

1. The absence of RhoE expression results in a decrease of the amount of several essential myelin proteins in the mouse brain.
2. The number of myelinated axons and the thickness of the myelin sheath of myelinated axons are smaller in the corpus callosum and striatum when RhoE is absent.
3. RhoE is expressed in oligodendrocyte precursor cells and in mature oligodendrocytes, both *in vivo* and *in vitro*.
4. Mice lacking RhoE expression show a decrease in the total number of oligodendrocytes and a delay in the differentiation of the oligodendrocyte precursor cells to mature oligodendrocytes.
5. The absence of RhoE produces a delay in the development of the connections between the thalamus and the cortex. During development, RhoE is expressed in the thalamus and also around the thalamocortical pathway; thus, the defects present in absence of RhoE could be due to a cell autonomous and /or non-cell autonomus function of RhoE.
6. Lack of RhoE expression causes a defective anterior branch of the anterior commissure, which ventrally migrates from the anterior olfactory nucleus, but these axons do no reach and cross the midline. However, the other forebrain commissures (corpus callosum and hippocampal commissure) are properly formed.
7. The defect of the anterior branch of the anterior commissure is seen early at embryonic stages, indicating a developmental problem in this tract which is never completely formed.
8. RhoE is expressed in the anterior olfactory nucleus and in the pathway of the anterior commissure during developmental stages. Thus, the AC phenotype could be the result of both, cell autonomous and non-cell autonomous functions of RhoE.
9. Altogether, these results indicate that RhoE is an essential protein in the central nervous system development, controlling diverse events as oligodendrocyte differentiation and axonal tracts development.