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Cytoskeleton-interacting proteins in brainstem development

Roles of KCC2 and Vangl2

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ABSTRACT

The brainstem is the most evolutionary conserved division of the brain. It develops from the hindbrain and midbrain regions of the neural tube and forms neural networks that regulate vital functions of the body. One of the most critical roles is to generate respiratory rhythm for the regulation of oxygen, carbon dioxide and pH levels. This is achieved by pacemaker neurons and neural networks in the medulla oblongata, controlled by different modulatory systems. The mechanisms whereby the respiratory rhythm is generated and regulated are not fully understood and have only recently started to be unveiled.

This thesis describes the importance of two different gene products, KCC2 and Vangl2, for proper development of the brainstem. We show that, while these genes act in separate phases of development, they share the common feature of regulating the integrity of the neuronal cytoskeleton necessary for maturation of the brainstem.

KCC2 is a neuronal K^+/Cl^- cotransporter that is responsible for the developmental shift in the postsynaptic response to GABA. A fundamental premise for this thesis is that we found KCC2 protein expression in the hindbrain region of mice already at embryonic day 9.5, although its ion transport activity does not become functional until late fetal age. We show that the depolarizing effect of GABA elicits increased activity of fetal respiration-related neurons. In addition, the developmental GABA shift is associated with plasma membrane targeting of KCC2 in respiration-related regions of rats around birth.

Overexpression of KCC2 in the mouse neural tube resulted in altered neuronal differentiation and neural crest migration. These effects were independent of the ion transport function of KCC2 and were shown to rely on a structural interaction with the cytoskeleton-associated protein 4.1N. Thus, transport-inactive KCC2 may regulate neuronal differentiation and migration during early development. We assessed the early importance of KCC2 further in mice knockout for this gene, which die at birth from respiratory failure. Brainstem organotypic cultures of these mice displayed a lower correlated network activity in the preBötzinger region. In addition, characterization of the respiration-related regions showed less glutamatergic synapses in the parafacial respiratory group of KCC2-deficient mice. This indicates that KCC2 is essential for the maturation of respiratory neural networks.

Finally, we show that the planar cell polarity gene Vangl2 regulates neural tube closure in the hindbrain region by promoting the formation of adherens junctions. Vangl2 was found to structurally interact with Rac1. Moreover, disruption of adherens junctions by a partial blockade of Rac1 could be rescued by Vangl2. This suggests that Vangl2 plays a critical role in the recruitment of Rac1 to the adherens junctions.

In conclusion, the results presented in this thesis increase our knowledge of brainstem development, from closure of the neural tube until the formation of functional neural networks. Our findings have potential implications for research and understanding of neural tube defects as well as breathing disorders, such as congenital central hypoventilation syndrome, that arise from aberrant formation of the neural networks constituting the central pattern generator for breathing.

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