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Research Article

Rho GTPase function in development: How in vivo models change our view

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ABSTRACT

Rho GTPase functions have been carefully investigated for many years using cell biological models. In recent years, mouse models with targeted mutations in Rho GTPase genes enabled the study of Rho GTPase function in vivo, partially confirming and partially contradicting expectations based on earlier in vitro experiments. This review sums up recent findings on the role of Rho GTPases in development, underlining the importance of in vivo research for our understanding of Rho GTPases in living organisms, and describing challenges for the future.

Introduction

In mammalians, Rho GTPases are a family of 20 proteins belonging to the small GTPases. Binding of GTP induces a conformational shift and allows interaction with effector molecules, which mediate the biological effect [1–3]. Hydrolysis of GTP to GDP by the GTPases themselves inactivates the Rho GTPases. Regulatory molecules promote activation and inactivation, respectively: Guanine nucleotide exchange factors catalyze the activation by exchanging GDP to GTP [4,5], while GTPase activating proteins promote the hydrolysis of GTP to GDP. A subgroup of Rho GTPases, the "atypical" Rho GTPases, however, is unable to hydrolyze GTP and is considered to be constitutively active [6]. They are regulated by expression and protein turnover. Based on their sequence similarity, classical and atypical Rho GTPases can be divided into subgroups: Among the classical Rho GTPases one distinguishes the Rho group (RhoA, RhoB, RhoC), the Rac group (Rac1, Rac2, Rac3, RhoG), the Cdc42 group (Cdc42, TC10/RhoQ, TCF/RhoJ), and the RhoD, Rif/RhoF group. The atypical Rho GTPases are divided into the Rnd group (Rnd1, Rnd2, Rnd3/RhoE), and smaller groups (CHP/RhoV, Wrch1/RhoU; RhoBTB1, RhoBTB2; RhoH).

Best characterized are the ubiquitously expressed RhoA, Rac1 and Cdc42 that were initially described as master regulators of the actin cytoskeleton. Research of the last 20 years however has revealed many additional functions of Rho GTPases in nearly all cellular processes. As a consequence, even partial overviews of Rho GTPase dependent functions are confusingly complex due to the large number of effector molecules, crosstalk of Rho GTPases with each other, and crosstalk of the Rho GTPase dependent signaling pathways. This complexity raises two important questions. Firstly, to which extent are cell culture models relevant to understand Rho GTPase function in cells of a living organism? The use of immortalized, mutated cell lines, artificial growth conditions and cellular environment might result in overestimation of some and underestimation of other functions. And secondly, how cell type specific are the functions of Rho GTPases and what determines it? Is Rho

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GTPase function mainly dependent on the respective effector molecules expressed in a given cell or does the activation pathway channel already predispose Rho GTPases to rather specific functions due to the formation of supramolecular complexes? *Drosophila*, *Caenorhabditis elegans* and *Dictyostelium* have been important model systems for understanding Rho GTPase function in vivo, but due to space limitations we will focus in this review on mice and mammalian development.

Mice with targeted mutations in specific Rho GTPase genes are excellent tools to address these questions. They allow studying the function of specific Rho GTPases in specific cell types in vivo without the problems of partial inhibition or off-target effects which inhibitors, dominant negative mutants or siRNA have. Moreover, applying established disease models to these mice, the cell type specific function of Rho GTPases in different illnesses can be investigated with the potential to identify Rho GTPase dependent pathways as useful drug targets.

Few years ago, the in vivo functions of Rho GTPases have been superbly reviewed by Heasman and Ridley [7]. Therefore we will focus in this short overview article only on the most recent findings in this area.

Rho

The ubiquitously expressed RhoA controls via the kinase ROCK the phsophorylation of myosin light chain and thus cell contractility. Via the formin mDia, RhoA is promoting actin polymerization. ROCK is also affecting the cleavage of F-actin by inhibiting cofilin activity. The related GTPases RhoB and Rho C share many effectors with RhoA, suggesting partially overlapping functions. Their localization, however, is different. While RhoA and C are at the cell membrane, RhoB is found at the membrane of intracellular vesicles. Inhibition of RhoA by toxins or dominant negative inhibitors in vitro suggested essential functions of this subfamily in cytokinesis, stress fiber formation and maintenance of cell–cell contacts, and cell migration. Mice lacking RhoB or RhoC did not show an obvious developmental phenotype. RhoA-null mice are dying during embryogenesis (Brakebusch et al., unpublished observations). Mice with a tissue-restricted deletion of RhoA have revealed interesting cell type specific differences in RhoA function.

In skin, keratinocyte-specific deletion of the RhoA gene by keratin-5 cre did not interfere with normal development and maintenance of skin and hair follicles (K5 cre; [8]). In contrast to the expectations, no change was observed in cell–cell contacts or wound healing in vivo. This mild phenotype might be related to the posttranscriptional upregulation of RhoB. Yet, phosphorylation of MLC and cofilin was strongly decreased in epidermal lysates, suggesting decreased ROCK activation and indicating that the loss of RhoA is not completely compensated by RhoB or other pathways. Culturing primary keratinocytes in vitro revealed additional defects: Slight increase in number of binucleated cells, mild defect in the formation of cell–cell contacts and defective migration, independent of ROCK but corresponding to decreased activation of Cdc42 and Rac1, which were not altered in vivo. Environmental conditions, therefore, have a huge impact on the phenotype of RhoA-null keratinocytes. In general, the skin phenotype is much milder than expected form in vitro studies in keratinocytes and other cell types, which suggested severe defects in adherens junctions and cytokinesis.

While reduced RhoA/ROCK dependent contractility has apparently little consequences for skin development, it is crucial for epithelial invagination morphogenesis during lens pit formation in early eye development. Ablation of the RhoA gene in lens epithelium (Le cre) resulted in a more open shape of the lens pit due to reduced contraction at the apical side [9]. In the absence of RhoA, Rac1 activity was increased causing elongation of the epithelial cells. Deletion of the Rac1 gene had the opposite effect with increased RhoA activity, increased contraction at the apical side, shorter cells, and more closed shape of the lens pit, indicating that RhoA and Rac1 cross-regulate each other. Interestingly, simultaneous loss of RhoA and Rac1 resulted in loss of F-actin and defective cell–cell junctions. These data illustrate the importance of crosstalk between Rho GTPases for the in vivo function of Rho GTPases. Due to their ubiquitous expression and crucial functions, it seems advisable to check activation of all three Rho GTPases, even if one manipulates only one of them.

A role for RhoA in regulating cell junctions has also been described in several mouse strains with cell type specific deletions of the RhoA gene in the central nervous system. Loss of RhoA in the spinal cord neuroepithelium (Brn4 cre), midbrain (Wnt1 cre), and forebrain (Foxg1 cre) all lead to a loss of adherens junctions and apical–basal polarity [10,11]. Loss of adherens junctions is not only of structural importance, but also affects cadherin dependent signaling inside the cells. Similar to adhesion defective αE catenin-knockouts, midbrain restricted RhoA-null mice (Wnt1 cre) showed accelerated proliferation of neuronal progenitors correlating with increased expression of hedgehog target genes, which might contribute to the phenotype [11]. Brn4 cre mutant mice died at late embryonic stages and displayed severe defects in the organization of the ventricular region [10]. Similar defects were observed by mDia1 inhibition in vivo through in utero electroporation of dominant negative mDia1, suggesting that this F-actin promoting formin is the major RhoA effector in these cells [10]. In these mice proliferation of neural progenitors was decreased, different to the Wnt1 cre mutants, suggesting that loss of adherens junction can have different effects. The reason for the different effects of RhoA knockout on adherens junctions in the central nervous system and in the epidermis is not clear. Probably, redundant pathways to maintain adherens junction integrity exist in skin, but not in brain.

Deletion of the RhoA gene in the cerebral cortex by Emx-cre revealed no gross behavioral abnormalities [12]. The mouse brains, however, showed subcortical band heterotopia, characterized by a heterotopic cortex underlying a normotopic cortex, as well as cobblestone lisencephaly, where neurons are protruding at the pial surface. Surprisingly, this phenotype was not caused by a primary migration defect of RhoA-null neurons, which showed rather normal migration in vitro and in a wild type brain in vivo. Instead, Cappello et al. observed that the radial glia scaffold, which guides the migrating neurons, is impaired. This was due to defects in the actin cytoskeleton of the glia cells, which abolished apical anchoring and decreased microtubule formation. In the RhoA null neurons the cytoskeleton defects were not as pronounced as in the glia cells, and the dendrite axon polarity was normal. Cytokinesis was not affected, but a transiently increased proliferation was observed. The reason for the