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Rho-kinase signaling controls nucleocytoplasmic shuttling of class IIa Histone Deacetylase (HDAC7) and transcriptional activation of orphan nuclear receptor NR4A1

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ABSTRACT

Rho-kinase (ROCK) has been well documented to play a key role in RhoA-induced actin remodeling. ROCK activation results in myosin light chain (MLC) phosphorylation either by direct action on MLC kinase (MLCK) or by inhibition of MLC phosphatase (MLCP), modulating actin–myosin contraction. We found that inhibition of the ROCK pathway in induced pluripotent stem cells, leads to nuclear export of HDAC7 and transcriptional activation of the orphan nuclear receptor NR4A1 while in cells with constitutive ROCK hyperactivity due to loss of function of the RhoGTPase activating protein Oligophrenin-1 (*OPHN1*), the orphan nuclear receptor *NR4A1* is downregulated. Our study identify a new target of ROCK signaling via myosin phosphatase subunit (MYPT1) and Histone Deacetylase (HDAC7) at the nuclear level and provide new insights in the cellular functions of ROCK.

1. Introduction

Rho GTPase proteins (Rho, Rac, Cdc42) have relevant functions in regulating various aspects of cell development such as differentiation, migration and synaptogenesis. Rho GTPase activity is modulated through positive (GTPases activating proteins GAPs) and negative regulators (guanine nucleotide exchange factors, GEFs or guanine nucleotide dissociation inhibitors, GDIs) [1]. Loss of function of the RhoGTPase activating protein *OPHN1*, the first Rho-linked gene associated with X-linked intellectual disability and cerebellar hypoplasia [2,3], leads to hyperactivation of the downstream RhoA/Rho-kinase (ROCK) pathway and dendritic spine immaturity [4,5]. ROCK acts through phosphorylation of the Myosin Phosphatase targeting subunit 1 (MYPT1) leading to inhibition of myosin light chain phosphatase (MLCP) which triggers actin–myosin contractility [6]. Interestingly, MYPT1 and the catalytic subunit of MLCP (PP1β) were found to interact specifically with Histone Deacetylase 7 (HDAC7) dephosphorylating it. HDAC7 belongs to class IIa HDACs (including also HDAC4/5/9), and is part of a transcriptional repressor complex involved in regulation of gene expression. Nucleocytoplasmic shuttling depending on HDACs phosphorylation status, has been demonstrated as a mechanism controlling their function [7]. Nuclear import of HDAC7 leads to repression of *NR4A1* transcription in developing thymocytes [8]. *NR4A1* (also known as *NUR77*, *TR3* or nerve growth factor induced *NGFI-B*) belongs to the family of nuclear orphan receptors which act as immediate early response genes, and is important for neuronal differentiation, T cell tolerance induction and apoptosis [9–11]. In this study we demonstrate that ROCK signaling controls nucleocytoplasmic shuttling of HDAC7 and modulates the expression of its target gene *NR4A1*.

Abbreviations: ROCK, Rho kinase; OPHN1, Oligophrenin-1; HDAC7, Histone Deacetylase 7; MYPT1, myosin phosphatase subunit 1; MLCP, myosin light chain phosphatase; MLCK, myosin light chain kinase; NR4A1, nuclear receptor subfamily 4, group A, member 1; iPSCs, induced pluripotent stem cells; PKA, protein kinase A; CREB, cAMP responsive element binding; MAP2, microtubule-associated protein 2; STAT3, signal transducer and activator of transcription 3.

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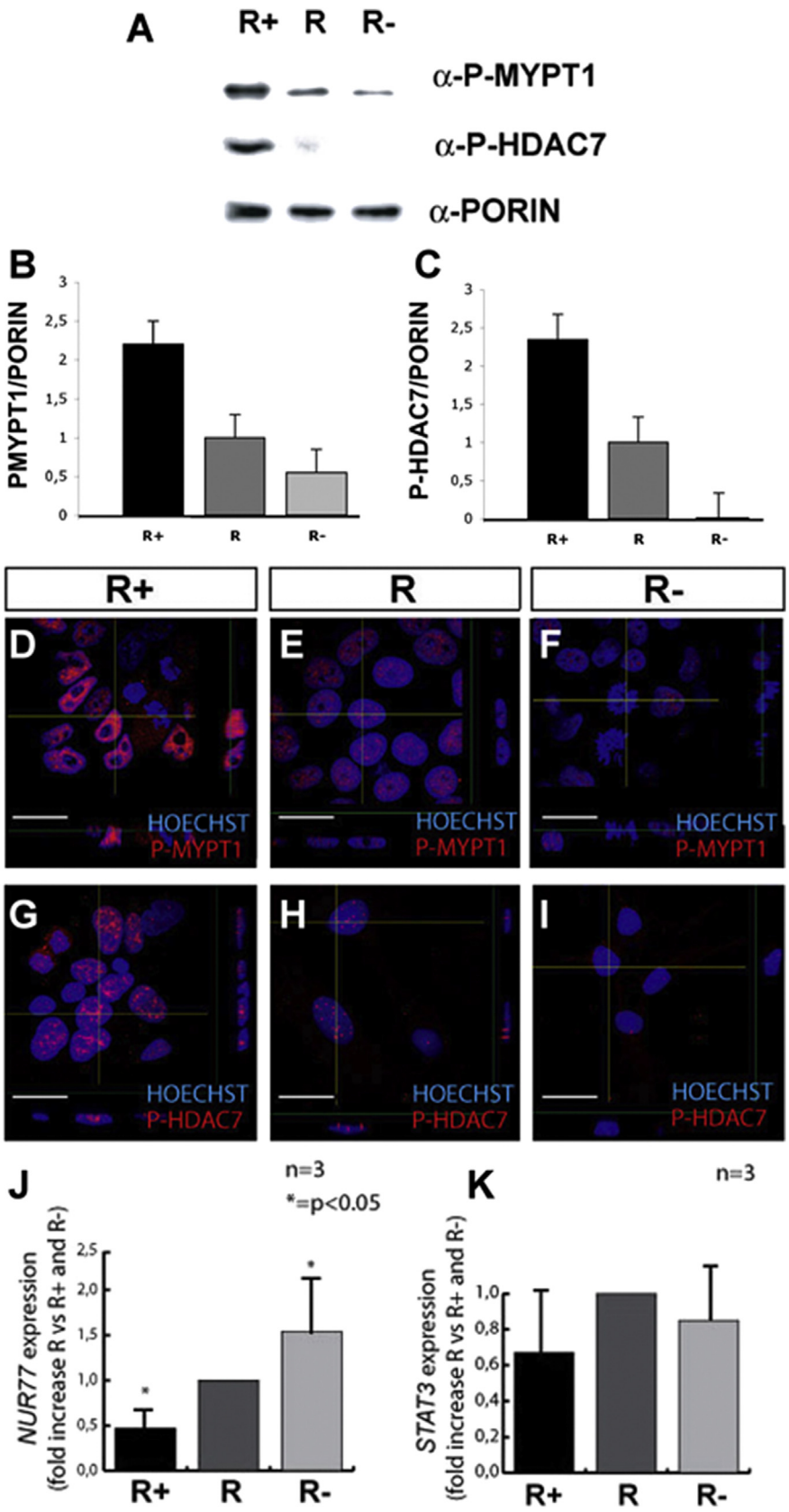

Fig. 1. Analysis of MYPT1 and HDAC7 phosphorylation status (upper panel), and *NR4A1* expression (lower panel) in iPSCs with different levels of ROCK activity. (A) Western blots of protein extracts obtained from iPS cells with *OPHN1* loss of function (R+), control iPS cells (R) and iPS cells treated with the ROCK inhibitor Y27632 (10 μM), (R−) showing phosphorylation of Thr853 in MYPT1 (P-MYPT1), phosphorylation of S318 in HDAC7 (P-HDAC7), and PORIN (used as internal standard). The data show different phosphorylation status of MYPT1 and HDAC7 at different levels of ROCK activity. (B–C) Densitometric analyses of Western blot where P-MYPT1 and P-HDAC7 values were normalized with respect to PORIN values showing decreased P-MYPT1 and P-HDAC7, respectively, in parallel with decreasing levels of ROCK activity. (D–I) Immunofluorescence analysis of iPSC colonies