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The role of RhoA kinase inhibition in human placenta-derived multipotent cells on neural phenotype and cell survival

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ABSTRACT

Current advances in stem cell biology have brought much hope for therapy of neuro-degenerative diseases. However, neural stem cells (NSCs) are rare adult stem cells, and the use of non-NSCs requires efficient and high-yielding lineage-specific differentiation prior to transplantation for efficacy. We report on the efficient differentiation of placental-derived multipotent cells (PDMCs) into a neural phenotype with use of Y-27632, a clinically compliant small molecular inhibitor of Rho kinase (ROCK) which is a major mediator of cytoskeleton dynamics. Y-27632 does not induce differentiation of PDMC toward the mesodermal lineages of adipogenesis and osteogenesis, but rather a neural-like morphology, with rapid development of cell extensions and processes within 24 h. Compared with conventional neurogenic differentiation agents, Y-27632 induces a higher percentage of neural-like cells in PDMCs without arresting proliferation or cell cycle dynamics. Y-27632-treated PDMCs express several neural lineage genes at the RNA and protein level, including nestin, MAP2, and GFAP. The effect of the ROCK inhibitor is cell-specific to PDMCs, and is mainly mediated through the ROCK2 isoform and its downstream target, myosin II. Our data suggest that ROCK inhibition and cytoskeletal rearrangement may allow for induction of a neural phenotype in PDMCs without compromising cell survival.

1. Introduction

Adult neural stem cells (NSCs) are a group of cells that have the capacity to self-renew and to differentiate into neuronal and glial lineages in the developed nervous system [1]. However, these stem cells are scarce, and are difficult to access or culture *ex-vivo*. In addition to NSCs, recent data has shown that a number of non-neural adult stem cells—which are more abundant and include bone marrow mesenchymal stem cells (BMMSCs)—can be differentiated into neural lineage cells [2–4]. The mechanisms involved in this process, however, remain relatively unclear.

While non-NSCs capable of neural differentiation broaden the sources available for neural regeneration, the fact remains that adult stem cells—including BMMSCs— require invasive procedures to obtain cells, and cell numbers decrease with increasing age of the donor [5]. In the pursuit of alternative sources of human stem cells, we have isolated multilineage progenitors from the term human placenta, a source without ethical concerns [6]. These placenta-derived multipotent cells (PDMCs) express a surface marker profile similar to BMMSCs and are capable of differentiation into all three germ layers including neural phenotypes [6,7]. Moreover, PDMCs harbor significant immunosuppressive effects towards T lymphocytes and natural killer lymphocytes [8,9]. These results suggest that PDMCs may be a potential source of easily accessible human MSCs for therapeutic use [10].

Currently, many protocols have been used to induce neural differentiation from various ASCs. One common drawback has been the toxic nature of many of the reagents used (for review, see Ref. [11]). Indeed, one of the most commonly used compound for neural differentiation, retinoic acid (RA), is cytotoxic at high concentrations in *in vitro* cultures, making therapeutic application difficult [12]. Thus, in this study, we explore the use of Y-27632—a

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clinically compliant inhibitor of Rho kinase (ROCK), which is an important mediator of cytoskeletal dynamics [13]—for neural differentiation of PDMCs. The specificity of the phenomenon and mechanisms involved is explored in this study.

2. Methods and materials

2.1. Isolation and culture of PDMCs

PDMCs were isolated from term human placenta tissue (38- to 40-week gestation) obtained from healthy donors with informed consent approved by the institutional review board. As reported previously, placental tissue was dissected enzymatically digested with 0.25% trypsin-EDTA (Gibco-Invitrogen Corp., Grand Island, NY, USA) and cultured in expansion medium consisting of Dulbecco's modified Eagle medium (DMEM; Gibco-Invitrogen) supplemented with 10% fetal bovine serum (FBS; Hyclone, Logan, UT, USA), 100 U/ml penicillin, and 100 μg/ml streptomycin (Gibco-Invitrogen) [6]. At 80–90% confluence, the cells were subcultured at the dilution of 1:2 to 1:3.

2.2. Differentiation studies

Osteogenic and adipogenic differentiation studies were performed as previous described [6]. For adipogenic differentiation, cells were cultured in complete medium with the addition of 0.5 μM isobutyl-methylxanthine, 1 μM dexamethasone, 10 μM insulin, and 60 μM indomethacin (all from Sigma–Aldrich, St Louis, MO, USA) for induction. For osteogenic differentiation, cells were cultured in complete medium along with 0.1 μM dexamethasone, 10 mM β-glycerol phosphate, and 50 μM

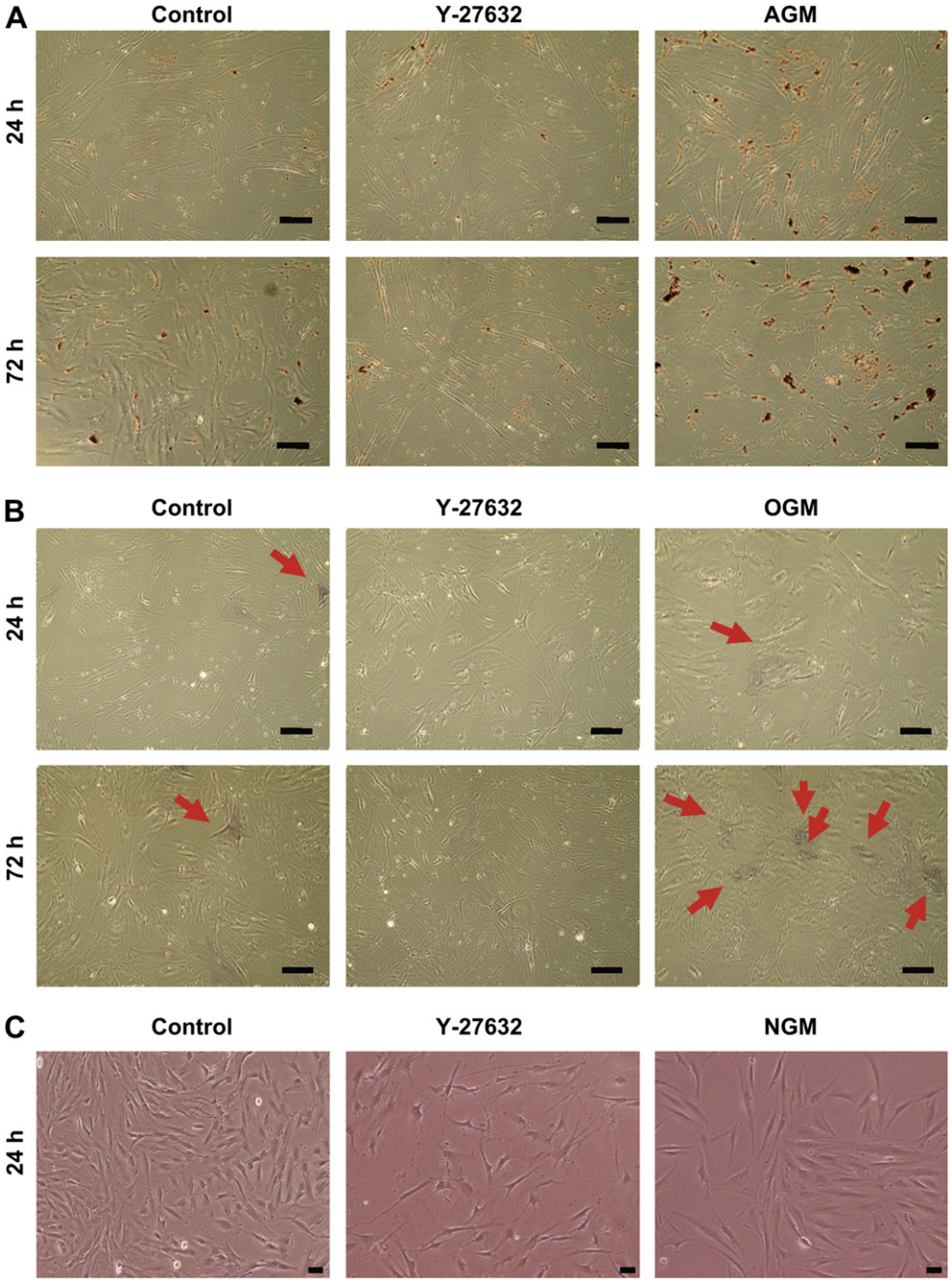

Fig. 1. Rho kinase (ROCK) inhibition in placenta-derived multipotent cells (PDMCs) does not induce adipogenic or osteogenic differentiation, but rather a neural-like morphology. Comparison of PDMCs cultured in complete medium (CM) to (A) adipogenic medium (AGM), (B) osteogenic medium (OGM), or (C) neurogenic (NGM) with or without the ROCK inhibitor Y-27632 (10 μM in CM), for 24 and 72 h. Adipogensis was assessed by Oil Red O staining for the presence of oil droplets, and osteogenesis was assessed by staining for alkaline phosphatase (red arrow). Bar: 100 μm. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)