Journal of Controlled Release 235 (2016) 291–305

Contents lists available at ScienceDirect

Journal of Controlled Release

journal homepage: www.elsevier.com/locate/jconrel

MicroRNA-124 loaded nanoparticles enhance brain repair in Parkinson's disease

C. Saraiva [a], J. Paiva [b,c], T. Santos [a], L. Ferreira [b,c,d], L. Bernardino [a,*]

[a] Health Sciences Research Centre, Faculty of Health Sciences, University of Beira Interior, 6201-506 Covilhã, Portugal
[b] CNC-Center for Neuroscience and Cell Biology, 3004-504 Coimbra, Portugal
[c] Biocant - Center of Innovation in Biotechnology, 3060-197 Cantanhede, Portugal
[d] Institute for Interdisciplinary Research, University of Coimbra (IIIUC), 3030-789 Coimbra, Portugal

ARTICLE INFO

Article history:
Received 9 March 2016
Received in revised form 29 May 2016
Accepted 2 June 2016
Available online 3 June 2016

Keywords:
Neural stem cells
MiR-124
Nanoparticles
Neurogenesis
Parkinson's disease

ABSTRACT

Modulation of the subventricular zone (SVZ) neurogenic niche can enhance brain repair in several disorders including Parkinson's disease (PD). Herein, we used biocompatible and traceable polymeric nanoparticles (NPs) containing perfluoro-1,5-crown ether (PFCE) and coated with protamine sulfate to complex microRNA-124 (miR-124), a neuronal fate determinant. The ability of NPs to efficiently deliver miR-124 and prompt SVZ neurogenesis and brain repair in PD was evaluated. *In vitro*, miR-124 NPs were efficiently internalized by neural stem/progenitors cells and neuroblasts and promoted their neuronal commitment and maturation. The expression of Sox9 and Jagged1, two miR-124 targets and stemness-related genes, were also decreased upon miR-124 NP treatment. *In vivo*, the intracerebral administration of miR-124 NPs increased the number of migrating neuroblasts that reached the granule cell layer of the olfactory bulb, both in healthy and in a 6-hydroxydopamine (6-OHDA) mouse model for PD. MiR-124 NPs were also able to induce migration of neurons into the lesioned striatum of 6-OHDA-treated mice. Most importantly, miR-124 NPs proved to ameliorate motor symptoms of 6-OHDA mice, monitored by the apomorphine-induced rotation test. Altogether, we provide clear evidences to support the use of miR-124 NPs as a new therapeutic approach to boost endogenous brain repair mechanisms in a setting of neurodegeneration.

1. Introduction

Neurogenesis occurs constitutively in the subventricular zone (SVZ) of the adult mammalian brain, including in humans [1]. Within this region, neural stem cells (NSCs) can self-renew, proliferate and give rise to new neurons, astrocytes and oligodendrocytes. In rodents, newborn neurons generated in the SVZ migrate through the rostral migratory stream (RMS) towards the olfactory bulb (OB) where they fully differentiate as mature interneurons [2]. Adult neurogenesis homeostasis is altered in several brain disorders including Parkinson's disease (PD) [3]. PD is characterized by the loss of dopaminergic (DA) neurons present in the *substantia nigra* (SN) and degeneration of DA terminals in the striatum, leading to movement coordination impairments and cognitive deficits. Several factors have already been reported to improve functional recovery in PD animals models by increasing endogenous neurogenesis and migration of newly born neurons into the lesioned striatum [4,5]. However, an efficient therapy aiming at full regeneration has not yet been found. Therefore, it is imperative to find new platforms to efficiently deliver pro-neurogenic factors to NSCs and to boost the endogenous regenerative capacity of adult brain.

A tightly controlled network of intrinsic and extrinsic signals, including small non-coding RNAs (*e.g.* microRNAs) [6–9] regulate the neurogenic niche. MicroRNAs (miR) are able to regulate hundreds of genes [10] at the post-transcriptional level by inhibiting mRNA translation or inducing mRNA degradation [11]. MiR-124 is one of the most abundant miR in the adult brain [12]. The expression of miR-124 is initiated during the transition from NSC to progenitor cell and it is enhanced with neuronal maturation [13,14]. Several studies have shown that the overexpression of miR-124 induces neuronal differentiation of both progenitor cells [15,16] and HeLa cells [10]. More recently, lentiviral overexpression of the miR-124 precursor (among other factors) was capable of inducing the differentiation of human neonatal foreskin fibroblasts into functional mature neurons [17,18]. *In vivo*, miR-124 overexpression in the SVZ niche increased the number of newborn neurons without affecting their migratory capability [13,14]. Additionally, miR-124 is intimately associated with brain pathologies and neurodegenerative disorders, such as PD. Indeed, within the miR-124 validated targets, one-fourth are de-regulated in PD (49 genes out of 202, MIRECORDS database) [19]. Significant decrease of miR-124 was described in the SN of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine

* Corresponding author.
E-mail address: libernardino@fcsaude.ubi.pt (L. Bernardino).

http://dx.doi.org/10.1016/j.jconrel.2016.06.005
0168-3659/

(MPTP)-intoxicated mice (PD mouse model) as well as in MN9D dopaminergic neurons treated with methyl phenyl pyridinium (MPP) iodide, while its overexpression improved cell survival [20,21]. Therefore, increasing miR-124 intracellular levels likely stands for a novel therapeutic strategy to improve functional outcome in PD (reviewed by Sun et al. [3]).

Due to the short half-life and poor stability of miR, their efficient delivery into cells in a safe and controlled way remains a challenge [22]. Viral vectors have a high capacity to deliver miR, however safety issues such as immunogenicity and the risk of triggering oncogenic transformation limits its translation potential [23]. Non-viral vectors such as polymeric nanoparticles (NPs), which avoid these safety issues, have been used by us and others to efficiently deliver proneurogenic molecules, including miR, into cells [7,23–25]. As so, we hypothesized that the use of polymeric NPs to deliver miR-124 could promote neurogenesis of SVZ NSCs both *in vitro* and *in vivo* and ultimately promote brain repair. To confirm this hypothesis we used a NP formulation formed by poly(lactic acid-*co*-glycolic acid) (PLGA) and perfluoro-1,5-crown ether (PFCE), a fluorine compound that can be tracked non-invasively by Fluorine (^{19}F)-magnetic resonance imaging (MRI), and coated with protamine sulfate to complex miR-124 [26]. The neurogenic potential of this NP formulation was assessed both in physiologic conditions and in a 6-hydroxydopamine (6-OHDA) mouse model for PD. We have found that miR-124-loaded NPs (miR-124 NPs) enhanced neuronal differentiation and axonogenesis *in vitro*. Accordingly, mRNA and protein levels of both Sox9 and Jagged1, two key non-neuronal genes, were also decreased upon miR-124 NP treatment. *In vivo*, a single administration of miR-124 NPs into the lateral ventricles of both healthy and 6-OHDA-lesioned mice was able to significantly increase the number of migrating neuroblasts that reached the granule cell layer of the OB. Importantly, miR-124 NPs also potentiated the migration of SVZ-derived new neurons towards the 6-OHDA lesioned striatum and decreased the motor impairments found in 6-OHDA treated mice. Altogether, our results support the therapeutic potential of miR-124 NPs as an enhancer of endogenous brain repair mechanisms.

2. Materials and methods

2.1. Preparation of PLGA NPs

PLGA NPs were prepared as described by Gomes and colleagues [26]. Briefly, PLGA (Resomers 502 H; 50:50 lactic acid/glycolic acid) (Boehringer Ingelheim Lda, Ingelheim, Germany) was covalently conjugated to fluoresceinamine (Sigma-Aldrich Co. LLC). NPs were prepared by dissolving PLGA (100 mg) in a solution of dichloromethane/trifluoro-ethanol (1:8) containing PFCE (100 mg) (Fluorochem, Derbyshire, UK). This solution was then added dropwise to a poly(vinyl alcohol) (PVA) solution (5% w/v in water) and stirred. NPs were centrifuged and washed with distilled water before freeze-drying. NPs were coated with protamine sulfate (PS) in 1:1 ratio by agitation at room temperature (RT). After this incubation period, NPs were dialyzed (MWCO of 50 kDa) against distilled water, frozen and lyophilized to obtain a dry powder that was stored in a desiccator at RT.

2.2. Complexation of NPs with miR and cell transfection

NPs were weighed and sterilized under ultraviolet light before being resuspended in SVZ cell culture medium devoid of growth factors and sonicated (Transsonic T460/HH, Elma Schmidbauer GmbH, Singen, Germany). To this suspension (1 to 20 μg/mL final concentration, specified in the text) a total of 200 nM of miR (miR-124 or scramble-miR, both from GE Healthcare Dharmacon Inc., Chicago, USA) were added and allowed to complex for 45 min at 37 °C with intermittent agitation (Fig. 1A). Void NPs were prepared using the same procedure but without adding miR. Cells were then incubated with void or miR-loaded NPs for 4 h at 37 °C in an incubator with 5% CO_2 and 95% atmospheric air. All miR are from GE Healthcare Dharmacon Inc. and were provided annealed, desalted and in the 2′-hydroxyl form and were resuspended in sterile RNA free water. MiR-124 mature sequence is 5′ UAAGGCACGCGGUGAAUGCC3′.

2.3. Characterization of NPs

Particle size and zeta potential of NP suspensions were determined using light scattering *via* a Zeta PALS zeta potential analyzer and ZetaPlus Particle Sizing Software (Brookhaven Instruments Corporation, NY, USA). Size measurements were performed at 25 °C, and data were recorded at a 90° angle, with an equilibration time of 2.5 min and individual run times of 60 s. The average diameters described are number-weighted average diameters. The zeta potential of NPs was determined in aqueous solutions, at 25 °C.

2.4. SVZ cell cultures and experimental treatments

SVZ cell cultures were prepared from 1 to 3 day-old C57BL/6 mice as described by us [27]. Briefly, brains were removed and placed into Hank's balanced salt solution (HBSS) solution supplemented with 100 U/mL penicillin and 100 μg/mL streptomycin (all from Life Technologies, Carlsbad, CA, USA). SVZ fragments were dissected from 450 μm-thick coronal brain sections and digested in 0.025% trypsin, 0.265 mM EDTA (all from Life Technologies), followed by mechanical dissociation. The single cell suspension was diluted in serum-free medium (SFM) composed of Dulbecco's modified Eagle medium [(DMEM)/F12 + GlutaMAX™-I)] supplemented with 100 U/mL penicillin, 100 μg/mL streptomycin, 1% B27 supplement, 10 ng/mL epidermal growth factor (EGF), and 5 ng/mL basic fibroblast growth factor-2 (FGF)-2 (all from Life Technologies) and plated onto uncoated petri dishes (Corning Life Science, NY, USA) and allowed to develop in an incubator with 5% CO_2 and 95% atmospheric air at 37 °C. In these conditions, SVZ cells grow in suspension and generate neurospheres that are rich in neural and progenitor stem cells at distinct stages of differentiation and with proliferative and self-renewing abilities, as previously shown by us [7,24,27–30]. Five to six-day-old neurospheres were seeded onto 0.1 mg/mL poly-D-lysine- (PDL; Sigma-Aldrich Co. LLC, St. Louis, MO, U.S.A.) coated glass coverslips, or in PDL-coated 6-well plates for quantitative (q)PCR analysis and in 24-well plates for all the remaining experiments, in SFM medium devoid of growth factors. Two days after, medium was renewed and the cell monolayer was allowed to develop for different time frames in the presence of void, scramble-miR or miR-124 NPs.

2.5. Internalization studies

SVZ cells were transfected with 10 μg/mL of fluorescein-labeled NPs (FITC-NPs) complexed with 400 nM of miR-Dy547 (GE Healthcare Dharmacon Inc.) for 4 h at 37 °C, rinsed and maintained in SFM medium devoid of growth factors for 24 h. Thereafter cells were fixed with 4% paraformaldehyde (PFA), permeabilized and blocked with 6% bovine serum albumin (BSA; Amresco LLC, Solon, USA) and 0.5% Triton X-100 (Fisher Scientific, Pittsburgh, PA, USA) in 0.1 M of phosphate buffered-saline (PBS) for 1 h at RT. SVZ cells were then incubated overnight (ON) with the following primary antibodies: mouse monoclonal anti-nestin (1:100, Abcam Plc., Cambridge, UK), rabbit polyclonal anti-GFAP (1:200, Abcam Plc.), goat polyclonal anti-FITC (1:200, Abcam Plc.), goat polyclonal anti-DCX (1:200, Santa Cruz Biotechnology, Inc., Dallas, TX, U.S.A.) and mouse monoclonal anti-FITC (1:100, Sigma-Aldrich Co. LLC), all prepared in 0.3% BSA and 0.1% Triton X-100 solution. The secondary antibodies used were the following: Alexa Fluor 647 donkey anti-mouse, Alexa Fluor 350 goat anti-rabbit, Alexa Fluor 488 donkey anti-goat or mouse and Alexa Fluor 647 donkey anti-goat (all 1:200, Life Technologies). Whenever appropriated, nuclei were stained with Hoechst-33342 (4 μg/mL in PBS, Life Technologies) for 5 min at RT. After, cells were mounted in Fluoroshield mounting medium (Abcam Plc.) and photomicrographs