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PARP activity and inhibition in fetal and adult oligodendrocyte precursor cells: Effect on cell survival and differentiation

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ABSTRACT

Poly (ADP-ribose) polymerase (PARP) family members are ubiquitously expressed and play a key role in cellular processes, including DNA repair and cell death/survival balance. Accordingly, PARP inhibition is an emerging pharmacological strategy for cancer and neurodegenerative diseases. Consistent evidences support the critical involvement of PARP family members in cell differentiation and phenotype maturation. In this study we used an oligodendrocyte precursor cells (OPCs) enriched system derived from fetal and adult brain to investigate the role of PARP in OPCs proliferation, survival, and differentiation. The PARP inhibitors PJ34, TIQ-A and Olaparib were used as pharmacological tools. The main results of the study are: (i) PARP mRNA expression and PARP activity are much higher in fetal than in adult-derived OPCs; (ii) the culture treatment with PARP inhibitors is cytotoxic for OPCs derived from fetal, but not from adult, brain; (iii) PARP inhibition reduces cell number, according to the inhibitory potency of the compounds; (iv) PARP inhibition effect on fetal OPCs is a slow process; (v) PARP inhibition impairs OPCs maturation into myelinating OL in fetal, but not in adult cultures, according to the inhibitory potency of the compounds. These results have implications for PARP-inhibition therapies for diseases and lesions of the central nervous system, in particular for neonatal hypoxic/ischemic encephalopathy.

1. Introduction

Oligodendrocytes, the cells wrapping the axons of the central nervous system (CNS) with the myelin sheath, derive from the oligodendrocyte precursor cells (OPCs) generated during development from multipotent neuroectodermal derivatives in the cortex and spinal cord (Bergles and Richardson, 2015). These cells proliferate and migrate to populate the entire adult CNS, where they account for approximately 5–8% of the entire cell population (Dawson et al., 2003). New OPCs can be also generated in the adult CNS from neural stem cells (NSCs; Agathou et al., 2013) and by mitosis, as OPCs are the major proliferating population of the CNS under appropriate stimuli (Fernandez-Castaneda and Gaultier, 2016). These cells are responsible for myelin formation during development (Bergles and Richardson, 2015), and for myelin turnover and repair during adulthood (Young et al., 2013).

The process of myelin formation is highly orchestrated in time and space, involves different cell types, and a key step of this process is the OPC maturation toward myelinating oligodendrocyte (Zuchero and Barres, 2013). Internal and external cues targeting genetic, epigenetic and cytoplasmic mechanisms provide the appropriate microenvironment that regulates the molecular machinery triggering OPCs out of the cell cycle to terminal differentiation (Zuchero and Barres, 2013; Liu et al., 2016; Fernández et al., 2016).

It has been suggested that remyelination after lesions in the adult CNS recapitulates developmental myelination (Franklin and Hinks, 1999). However, substantial differences could well be expected between a normal turnover process and a repair process (Fancy et al., 2011) triggered by pathological events like inflammation and hypoxic/ischemic damage. These events actually mobilize a large number of inhibitory factors leading to a differentiation block of OPC (Gaesser and Fyffe-Maricich, 2016). Thus, there is an urgent need to more clearly elucidate the molecular bases of the relationship between OPCs proliferation and differentiation, carefully dissecting developmental myelination vs. myelin turnover in adulthood, and physiological vs. pathological conditions.

In the present study, we investigated the role of poly(ADP-ribose) polymerases (PARPs) in OPCs survival, proliferation and differentiation. PARPs are members of nuclear enzyme family that catalyse the formation of $(ADP\text{-}ribose)_n$ chains from NAD^+ on acceptor proteins after DNA double strands breaks (Amé et al., 2004). Because of the PARPs well described role in DNA repair and apoptosis induction (Heeres and Hergenrother, 2007), PARPs inhibition is currently considered a

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therapeutic option for cancer, including glioblastoma (Curtin and Szabo, 2013). Moreover, PARPs inhibition was proposed as a neuroprotective strategy for neonatal asphyxia and hypoxia/ischemia encephalopathy (Neira-Peña et al., 2015), a CNS injury that occurs during a critical period of developmental myelination (Dimou and Götz, 2014). In order to study the effect of PARP inhibition in all stages of differentiation, from NSCs to mature oligodendrocytes, we used OPCs-enriched cultures obtained from the fetal forebrain- and the adult sub-ventricular zone of the mouse to study the different role of PARPs in adult and fetal OPCs differentiation and maturation. We used PARP inhibitors having a different inhibitory concentration as pharmacological toll, including the clinically approved Olaparib (LYNPARZA™).

2. Materials and methods

2.1. Cell cultures

All animal protocols described herein were carried out according to the European Community Council Directives (86/609/EEC), and comply with the guidelines published in the *NIH Guide for the Care and Use of Laboratory Animals*.

Fetal and adult NSCs were isolated from E.13–14 fetal mouse forebrain or 2.5 month old mice sub-ventricular zone (SVZ), following the Ahlenius and Kokaia protocol (Ahlenius and Kokaia, 2010). Oligodendrocyte differentiation was performed following the Chen protocol (Chen et al., 2007) with some modifications. Tissues were enzymatically dissociated using trypsin (SIGMA), hyaluronidase (SIGMA) and DNase (SIGMA), than mechanically dissociated by piepetting. The solution was filtered, centrifuged and the resulting pellet was washed twice in HBSS. After 7 min centrifugation at 400 ×*g*, the cellular pellet was resuspended in serum-free medium (DMEM/F12 GlutaMAX 1×; 8 mmol/L HEPES; 100 U/100 μg Penicillin/Streptomycin; 0.1 × B27; 1 × N-2; 20 ng/ml bFGF; 20 ng/ml EGF) and, after cell count, cells were plated in suspension at a density of 10 cells/μl in a final volume of 3 ml in low-attachment 6-well plates (NUNC). Half medium was changed every three days, centrifuging the cell suspension at 300 ×*g* for 5 min and gently resuspending the cellular pellet in fresh medium. Neurospheres were allowed to proliferate until they attained a diameter of about 100 μm.

To obtain oligospheres, primary neurospheres were centrifuged at 300 ×*g* for 5 min. The pellet was mechanically dissociated by pipetting and cells were counted and plated again at a density of 10 cells/μl in a final volume of 3 ml of OPCs medium (DMEM/F12 GlutaMAX 1×; 8 mmol/L HEPES; 100 U/100 μg Penicillin/Streptomycin; 0.1 × B27; 1 × N-2; 20 ng/ml bFGF; 20 ng/ml PDGF) in low-attachment 6-well plates (NUNC). Oligospheres were centrifuged and the pellet was mechanically dissociated to obtain a single cell suspension. After cell count, cells were plated at a density of 3000 cells/cm^2 on poly-D,L-ornithine (50 μg/ml)/laminin (5 μg/ml) coating, in OPC medium.

In order to induce oligodendrocyte differentiation and maturation, after 3 DIVs OPC medium was replaced with the oligodendrocyte differentiation medium (DMEM/F12 GlutaMAX 1×; 8 mmol/L HEPES; 100 U/100 μg Penicillin/Streptomycin; 0.1 × B27; 1 × N-2; 50 nM T3; 10 ng/ml CNTF; 1× *N*-acetyl-L-cysteine-NAC-).

2.2. PARP inhibitor treatments

The following PARPi (PARP inhibitors) were used in this study: TIQ-A (IC50 140–450 nM), PJ34 (IC50 20 nM) and Olaparib (IC50 5 nM; Ferraris, 2010; Hans et al., 2011). OPCs were treated 24 h before the T3-mediated differentiation induction (−1 DIV), and cell viability was analysed at DIV 12 of differentiation (Fig. 1A). In order to study effects of PARPi on fetal and adult cultures, different treatment were performed: i) in order to study PARP1–2 expression and PARP activity, cells were analysed 24 h after treatment (DIV 0; Fig. 1A); ii) a dose-response curve of PARPi were performed, treating cultures with a series of 7 concentrations (0–10 μM). PARPi treatments were maintained after the oligodendrocyte differentiation medium change, until DIV 12 (Fig. 2A); iii) fetal cultures were also treated with PJ34 10 μM and analysed for cell number and cell death at DIV 0, 6 and 12 (Fig. 3A); iv) both fetal and adult cultures were treated with lower dose of PARPi (1 μM and 10 μM) and analysed for mature oligodendrocytes at DIV 12 (Fig. 4A).

2.3. Immunocytochemistry

Indirect immunofluorescence was used to identify OPCs (NG2-positive cells), mature (CNPase-positive cells) and myelinating (MBP-positive cells) OLs, neurons (β-III-tubulin-positive cells) and astrocytes (GFAP-positive cells). The following primary antisera were used: mouse anti-β-III-tubulin (R&D system, Trento, Italy) 1:3000; rabbit anti-GFAP (Glial Fibrillary Acidic Protein, Dako) 1:1000; rabbit anti-NG2 (chondroitin sulphate proteoglycan, neural/glial antigen 2, Millipore, Merck S.p.a., Milan, Italy) 1:350; mouse anti-CNPase (2′, 3′-cyclic nucleotide 3′-phosphodiesterase, Millipore) 1:500; rabbit anti-MBP (Myelin Basic Protein, Dako) 1:500. Donkey Alexa 488-conjugated anti mouse and donkey Alexa 568-conjugated anti-rabbit (Invitrogen) were used as secondary antisera. After immunofluorescence staining, cells were incubated with the nuclear dye Hoechst 33,258 (1 μg/ml in PBS, 0.3% Triton-X 100) for 20 min at RT. Cells were finally washed in PBS and mounted in glycerol and PBS (3:1, v/v) containing 0.1% paraphenylendiamine.

2.4. High content screening

For HCS analysis cells were grown in 96 flat-bottom well HCS plates (NUNC). Analysis of condensed nuclei, cell number and lineage/differentiation markers were performed with Cell Insight™ CX5 High Content Screening (HCS; Thermo Scientific), using the *Compartmental analysis* BioApplication. Based on nuclear staining, the software is able to recognise nuclei and calculate the percentage of high intensity/small sized condensed nuclei. Moreover, based on the nuclei identification, the software is able to detect the presence of the marker-specific stain in the cell body, calculating the percentage of the immunoreactive cells. Lineage/differentiation markers analysis was performed only on cells showing intact nuclei, excluding condensed nuclei from the percentage calculation.

2.5. RNA isolation and reverse transcription

Total RNA isolation was performed by using the RNeasy Mini kit (Qiagen, Milan, Italy) following manufacturer's instructions. Total RNA was eluted in RNase Free Water and concentration estimated through absorbance values at 260, 280 and 320 (Nanodrop 2000 spectrophotometer, Thermo Scientific). First strand cDNAs were obtained using the iScript™ cDNA Synthesis Kit (BioRad), incubating samples at 42 °C for 30 min. An RNA sample with no reverse transcriptase enzyme in the reaction mix was processed as a no-reverse transcription control sample.

2.6. Semi-quantitative real-time PCR

Semi-quantitative real-time PCR was performed using the CFX96 real-time PCR system (BioRad, CA, USA). The reactions were performed in a final volume of 20 μl consisting of 1× SYBR Green qPCR master mix (BioRad) and 0.4 μM forward and reverse primers. In order to avoid possible contamination of genomic DNA in isolated RNA, the no-reverse transcriptase sample was processed in parallel with the others and tested by real-time PCR for every pair of primers used. All primers used were designed using Primer Blast software (NCBI, MD, USA) and synthesised by IDT (Coralville, IA, USA). The following primer

sequences were used: PARP-1 (FW: 5′-GCCACACATCTCAGGGAGAC-3′; REV: 5′-CCCAAACCTTTGACACTGTGC-3′), PARP-2 (FW: 5′-GAAGGACGCAGACAGGACAA-3′; REV: 5′-ACATGAGCCTTTCCCAGCTT-3′). GAPDH (FW: 5′-GGCAAGTTCAATGGCACAGTCAAG-3′; REV: 5′-ACATACTCAGCACCAGCATCACC-3′) was used as housekeeping gene to normalize the amount of reverse-transcribed RNA used for PCR. Thermal profile of PCR reactions consisted first of a denaturation step (95 °C, 2 min) and 40 cycles of amplification (95 °C for 15 s and 60 °C for 60 s). At the end of the amplification cycles the melting curve of amplified products was performed according to the following temperature/time scheme: heating from 55 °C to 95 °C with a temperature increase of 0.5 °C/s.

Primers efficiency values for all primers were 95–102%. The $2^{(-\Delta\Delta CT)}$ method was used for the calculation of gene expression.

2.7. PARP activity assay

Fetal and adult OPCs at DIV 0, after 24 h of PARPi treatment, were lysed and proteins were quantified using the Lowry method (BioRad) following the manufacturer's instruction. PARP enzymatic activity was measured using HT Colorimetric PARP Apoptosis Assay Kit (Trevingen), following manufacturer's instructions.

2.8. Statistical analysis

Data are reported as mean ± SD. Prism software (GraphPad) was used for statistical analyses and graph generation. Student's *t*-test or one-way ANOVA and Dunnett's multiple comparison post-hoc were used to analyse data. Results were considered significant when the probability of their occurrence as a result of chance alone was <5% ($P < 0.05$).

3. Results

3.1. PARP expression and activity in fetal and adult OPCs

The cell systems used in this study are presented in Fig. 1. After cell expansion as neurospheres and oligospheres, cells were seeded for 3 DIVs in OPC medium, and then differentiated for 12 days in the presence of T3 (Fig. 1A). Lineage progression from NG2-positive OPC (DIV0) through CNPase-mature OL as far as myelinating MBP-positive OL is illustrated in Fig. 1C–E (representative images). The culture composition changes during the 12 DIV differentiation period, starting from DIV0, where the majority of cells are NG2 positive (NG2-positive cells 80%; CNPase-positive cells 5%), passing thorough DIV 6, where CNPase cells are growing in number (NG2-positive cells 60%; CNPase-positive cells

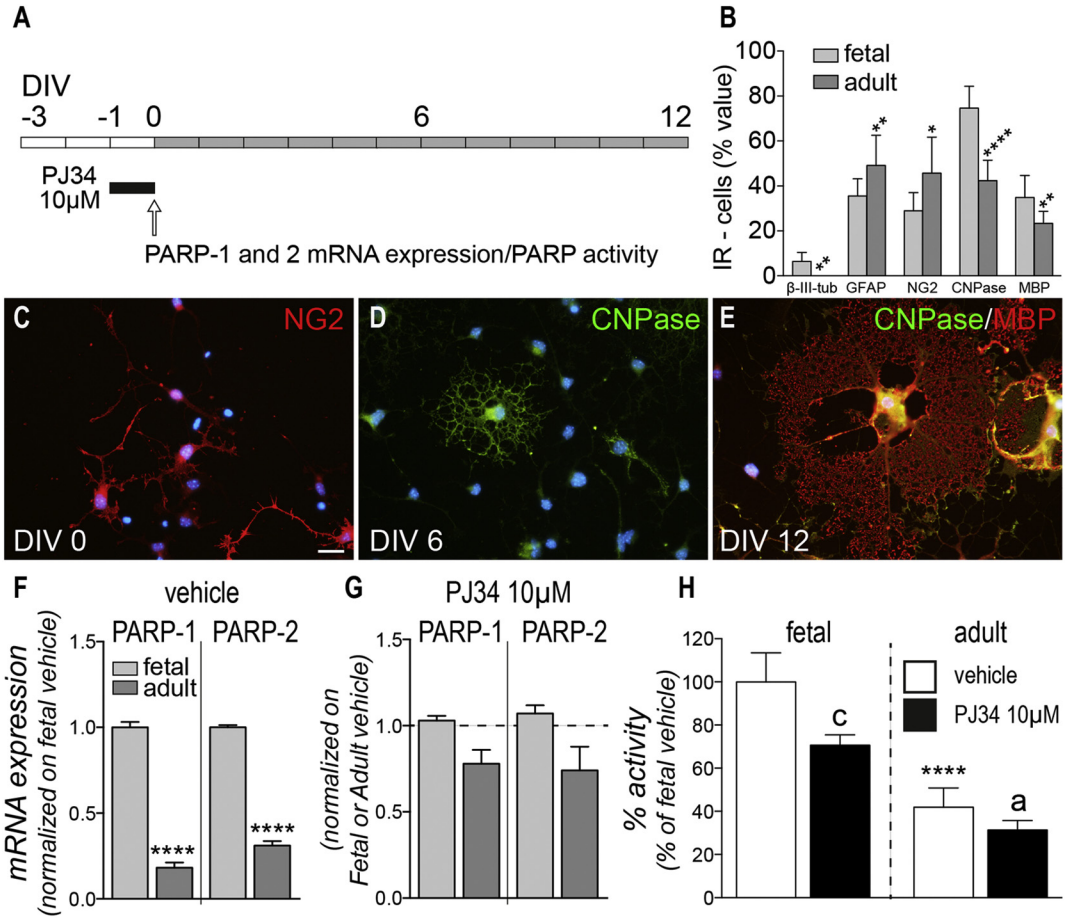

Fig. 1. PARP activity and expression in OPCs-enriched cultures. (A) Experimental design: in order to study PARP mRNA expression and activity, cells were treated for 24 h, form DIV −1 to DIV 0, with PJ34 10 μM. Arrow shows the time point of the represented analysis. Vehicle treated cells were also cultured for 12 DIV after T3-differentiation induction. Gray rectangles represent T3 exposure phase. (B) Fetal and adult OPC culture composition at 12 DIV. Specific markers were used in order to visualize neurons (beta-III-tubulin), astrocytes (GFAP; Glial Fibrillary Acidic Protein), OPCs (NG2; chondroitin sulphate proteoglycan, neural/glial antigen 2), Mature oligodendrocytes (CNPase; 2′,3′-cyclic nucleotide 3′-phosphodiesterase) and myelinating oligodendrocytes (MBP; myelin basic protein). (C–E) Representative images of oligodendrocyte maturation at 0, 6 and 12 DIV. Bar: 40 μm. (F–G) PARP1 and 2 mRNA level quantification in vehicle-treated cells (F) and in cells exposed for 24 h to PJ34 10 μM (G; dashed line represents the level of expression in vehicle-treated cells). (H) PARP activity assay, performed on fetal and adult OPCs at DIV 0, treated or untreated with PJ34 for 24 h. Data are represented as mean ± SD. Statistical analysis: Student's *t*-test. Asterisks indicate differences between adult and fetal (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; ****$P < 0.0001$), letters represent differences between treated and non-treated cultures (a = $P < 0.05$; c = $P < 0.001$).

30%), reaching the end of the differentiation with CNPase/MBP positive cells representing the majority of cells. Culture composition at 12 DIV is presented in Fig. 1B. Adult cultures after 12 DIVs of differentiation show a less mature cell composition, with a high percentage of NG2-positive cells ($P = 0.016$) and less CNPase ($P < 0.0001$) and MBP ($P = 0.008$) positive cells. Fetal cultures show less astrocytes (GFAP-positive cells; $P = 0.0023$) and around 6% of neurons (β-III-tubulin positive cells) that are not detected in adult cultures ($P = 0.002$).

In order to characterize the effect of PARP inhibition in fetal and adult OPCs on oligodendrocyte differentiation and maturation, cells were exposed to PARPi 24 h before differentiation induction (Fig. 1A). We first investigated the gene expression of the two major and more extensively studied PARPs, PARP-1 and 2 (Fig. 1F–G), and PARP activity (Fig. 1H), before the induction of differentiation, 24 h after PARPi treatment (DIV 0). Both genes are expressed in fetal ($\Delta Cq = 3.28 \pm 0.08$) and adult ($\Delta Cq = 5.79 \pm 0.4$) OPCs; gene expression is significantly higher in fetal compared to adult OPCs (PARP-1, $P < 0.0001$; PARP-2, $P = 0.0002$; Fig. 1F), and PJ34 treatment did not influence the mRNA expression levels (Fig. 1G). PARP activity analysis was based on the capability of PARP to parrylate histons in an ELISA-based assay. PARP activity was significantly higher in fetal OPCs compared to adult ($P < 0.0001$), and PJ34 treatment was able to decrease the activity in both cultures (fetal, $P = 0.0005$; adult $P = 0.0284$), thus confirming that the enzyme is active in both cell systems (Fig. 1H).

3.2. Effect of PARP inhibition on fetal and adult OPCs viability

In order to establish the cytostatic/toxic profile of PARPs inhibition on OPCs, we first constructed a dose-response curve on the cell culture which displayed the higher activity and expression of PARPs, i.e. fetal OPCs, using two standard PARPi, i.e. PJ34 and TIQ-A. The cytostatic effect is evaluated by the cell number (Fig. 2B), the cytotoxic effect by the

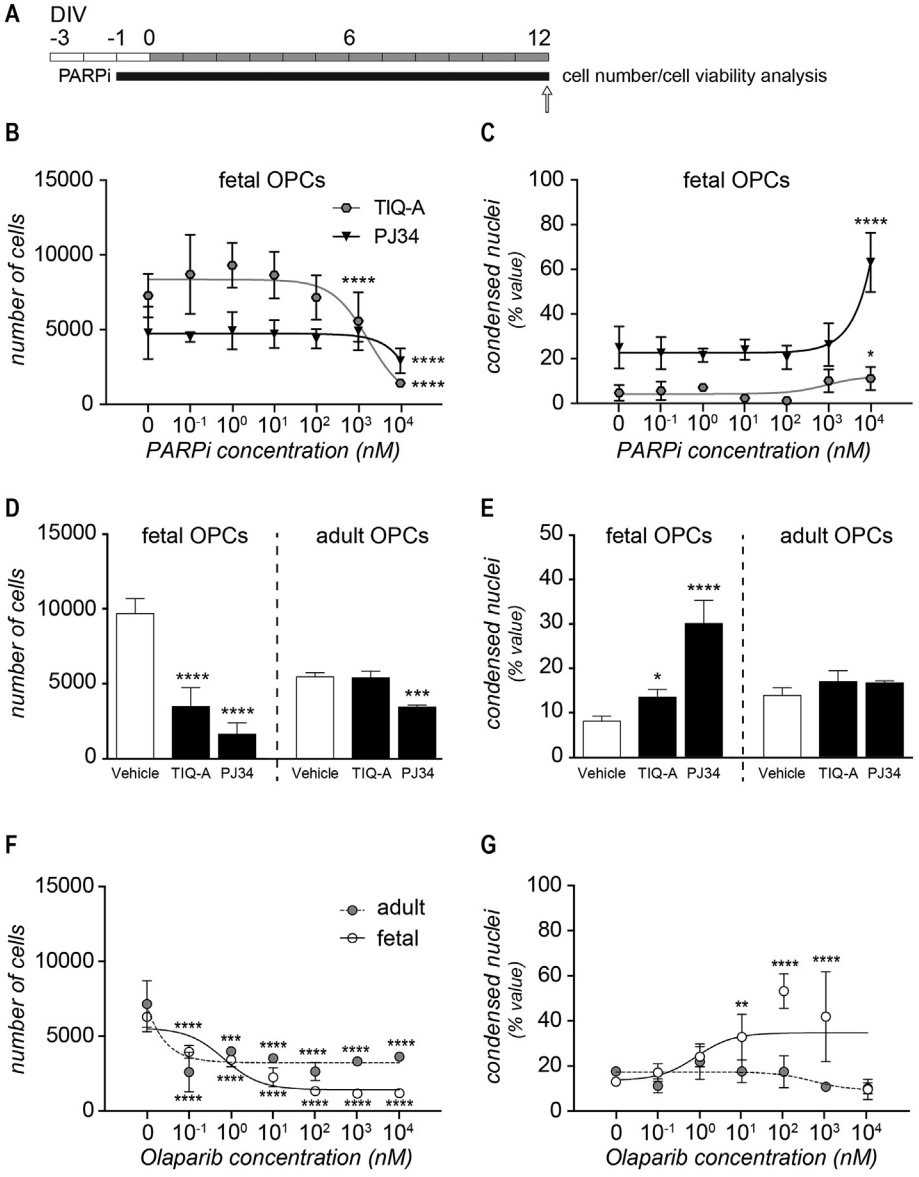

Fig. 2. Effect of PARP inhibition on fetal and adult OPC viability. (A) Experimental design: in order to study PARP inhibition effect on cell number and cell viability, cells were treated with PARPi form DIV −1 to DIV 12. Gray rectangles represent T3 exposure phase; arrow shows the time point of the represented analysis. (B—C) Dose-response curves of PJ34 and TIQ-A treatment of fetal OPCs cultures. Results are shown as number of cells (B; total number of nuclei detected by the HCS software) and percentage of condensed nuclei (C) obtained at 12 DIV. (D–E) Comparison between percentage of number of cells (D) and condensed nuclei (E) at 12 DIV (day in vitro) in fetal and adult cultures treated with PJ34 and TIQ-A at 10 μM. (F–G) Dose-response curves of Olaparib treated fetal and adult OPCs. Results are shown as number of cells (F) and percentage of condensed nuclei (G) at 12 DIV. Data are represented as mean ± SD. Statistical analysis: One-way ANOVA followed by Dunnet's post hoc. Asterisks represent difference between treated and untreated groups (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; ****$P < 0.0001$).

percentage of condensed nuclei (Fig. 2C), as established by HCS in three independent experiments. The cytostatic effect was observed at 1 μM and 10 μM using TIQ-A ($P < 0.0001$); at 10 μM using PJ34 ($P < 0.0001$). Both molecules induced an increase in the percentage of condensed nuclei at the highest concentration (10 μM) according to the respective potency (TIQ-A, $P < 0.0001$; PJ34, $P = 0.0362$).

We then investigated the effect of PJ34 and TIQ-A on adult OPCs using the toxic concentration for fetal OPCs (10 μM) finding that PJ34, but not TIQ-A, reduced the cell number in adult cultures (Fig. 2D; $P = 0.0004$) without affecting the percentage of condensed nuclei (Fig. 2E).

We also tested the Olaparib, a clinically approved PARPi, showing the highest PARP inhibitory activity. Olaparib treatment caused a reduction in cell number in both fetal and adult cultures at low concentrations (Fig. 2F; 10^{-1} nM; $P < 0.0001$) but is cytotoxic only for fetal OPCs (Fig. 2G; 10^{1} nM; $P < 0.0001$).

In order to better elucidate the timing of the PARP inhibition effect, we performed a time course analysis of cell number and cell death, by using PJ34 at a 10 μM toxic dose, (Fig. 3A). We found that after 24 h from the treatment there were no effects on cell number (Fig. 3B) and cell death (Fig. 3C). In vehicle treated cells, cell number increases over time, while in PARPi-treated cells remain at the same level for the whole differentiation period (Two Ways ANOVA, time $F(2, 90) = 20.37$, $P < 0.0001$; treatment $F(1, 90) = 63.13$, $P < 0.0001$; interaction $F(2, 90) = 15.28$, $P < 0.0001$), leading to a strong difference between the two groups at DIV 6 ($P < 0.0001$) and DIV 12 ($P < 0.0001$; Fig. 3B). In the opposite way, the percentage of condensed nuclei remain constant in the vehicle-treated, while increasing over time in the PARPi-treated group (Two Ways ANOVA, time $F(2, 86) = 12.49$, $P < 0.0001$; treatment $F(1, 86) = 46.65$, $P < 0.0001$; interaction $F(2, 86) = 15.60$, $P < 0.0001$), starting from DIV6 ($P = 0.0003$) and reaching >30% at DIV 12 ($P < 0.0001$; Fig. 3C).

3.3. Effect of PARP inhibition on fetal and adult OLs maturation

We finally investigated the effect of PARP inhibition on the differentiation of remaining OPCs using the two highest concentrations, being either toxic or non-toxic. Fetal OPCs were treated with TIQ-A, PJ34 and Olaparib at 1 μM (non-toxic concentration of TIQ-A and PJ34, toxic concentration of Olaparib) and 10 μM (toxic concentration of all PARPi). Adult OPCs were treated with all 10 μM and 1 μM (both non-toxic concentration of all PARPi). Representative HCS images of CNPase/MBP-positive cells in the different treatments of both fetal and adult OPCs are presented in Fig. 4B; results of the quantitative HCS analysis in Fig. 4C (fetal) and Fig. 4D (adult). The number of cells included in this analysis ranged from 1199 to 7152/well. Three wells were included in each experiment. PARP inhibition impairs OPCs maturation in fetal, but not in adult, cultures. In cell preparations derived from fetal brain, TIQ-A, the less potent PARPi, does not affect OPC maturation, as indicated by the percentage of MBP-IR cells. On the contrary, PJ34 treatment, already at non-toxic concentrations (1 μM) resulted in a reduction of mature OPCs ($P = 0.0013$), as when used in a toxic dose (10 μM) ($P < 0.0001$). Olaparib treatment at both concentrations resulted in a strong reduction of the percentage of mature and myelinating OPCs ($P < 0.0001$). Notably, all PARPi had no effect on adult OPC differentiation (1 μM treatment, data not shown).

4. Discussion

Several in vivo and in vitro studies indicate that OPCs have substantially different properties according to their chronological age. For example, human fetal OPCs from different gestational stages exhibit differences in the myelination profile (Cui et al., 2012), such as human OPCs derived from fetal brain during the period of maximum oligoneogenesis or from adult subcortical white matter (Windrem et al., 2004). However, the mechanisms behind the different age-related OPC biological properties are not known. The aim of this study was to investigate if PARP is involved in determining the age-related OPCs properties. Thus, we compared OPCs derived from NSCs obtained from fetal and adult brains. This cell system was preferred to primary and purified OPCs to better mimic developmental biology of OPC, including lineage specification, proliferation/survival and differentiation. PARP is a family of enzymes comprising 17 homologues involved in multiple cell functions (Jubin et al., 2016a). Apart from the role in DNA repair, PARP-1 and 2 participate in cell homeostasis maintenance (Bai, 2015), intracellular transports (Abd Elmageed et al., 2012), cell cycle (Madison et al., 2011) inflammation and immunity (Bai and Virág, 2012) and gene expression regulation (Bock et al., 2015) through >100 substrates (Hottiger, 2015). Moreover, PARPs are critically involved in inflammation and in the tissue damage caused by ischemia/reperfusion conditions (Li et al., 2015).

We first showed that PARP mRNA expression and PARP activity are much higher in fetal- than in adult-derived OPCs. Due to the role of

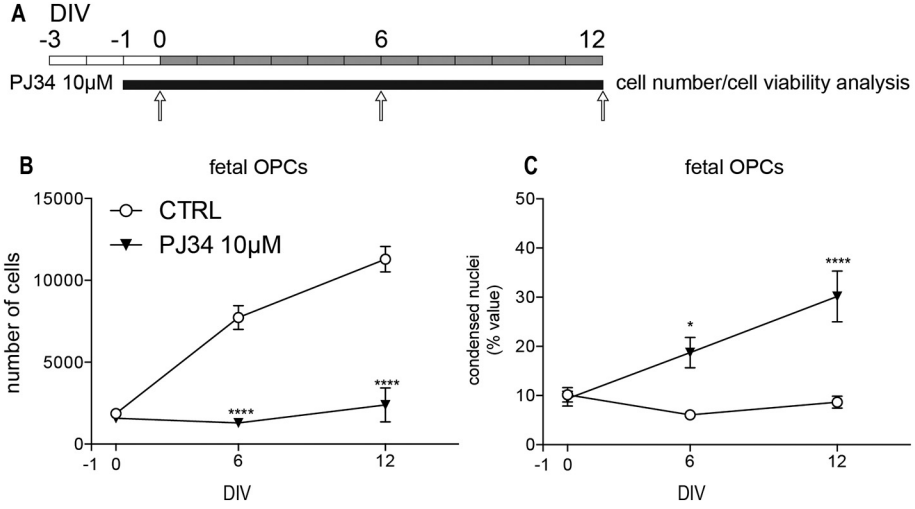

Fig. 3. Time course of PARP inhibition effect on fetal OPCs. (A) Experimental design: in order to study PARP inhibition effect on cell number and cell viability, cells were treated with PARPi form DIV −1 to DIV 12. Gray rectangles represent T3 exposure phase; arrows show the time points of the represented analysis. (B—C) Time course analysis of number of cells (B) and percentage of condensed nuclei (C) in fetal OPCs treated with vehicle or PJ34 10 μM. Data are represented as mean ± SD. Statistical analysis: Two-way ANOVA followed by Tukey's post hoc. Asterisks represent difference between treated and untreated groups at the same time point (*$P < 0.05$; ****$P < 0.0001$).

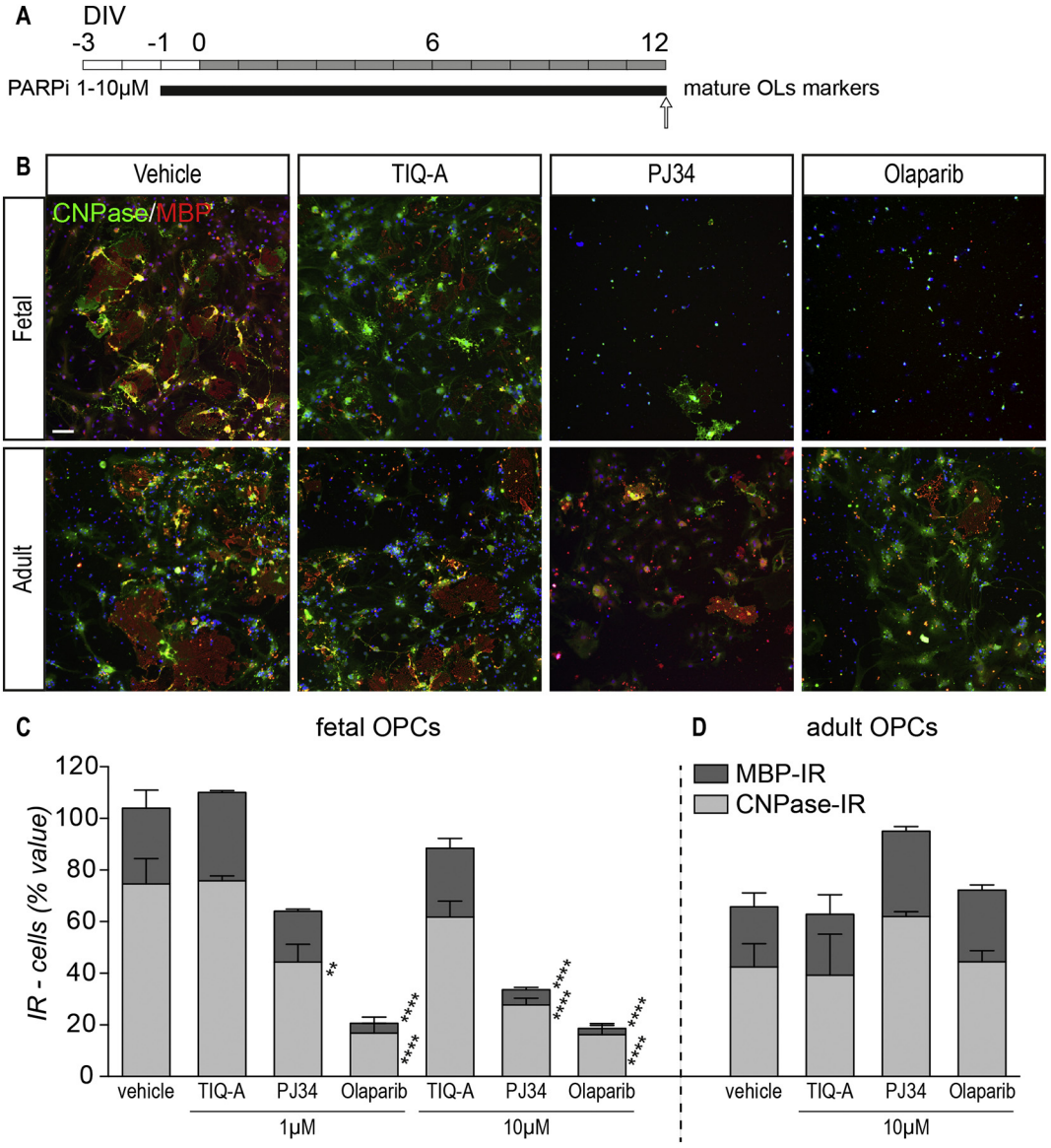

Fig. 4. Effect of PARP inhibition on oligodendrocyte maturation. (A) Experimental design: in order to study PARP inhibition effect on OLs maturation, cells were treated with PARPi form DIV −1 to DIV 12. Gray rectangles represent T3 exposure phase; arrow shows the time point of the represented analysis. (B) Representative HCS images of fetal and adult cultures at 12 DIV. Specific markers were used in order to visualize mature (CNPase; 2′,3′-cyclic nucleotide 3′-phosphodiesterase) and myelinating (MBP; myelin basic protein) oligodendrocytes. Bar: 100 μm. (C–D) Percentage of CNPase- and MBP-positive cells at 12 DIV in fetal (C) or adult (D) cultures. Data are represented as mean ± SD. Statistical analysis: One-way ANOVA followed by Dunnet's post hoc. Asterisks represent difference between treated and untreated groups (**$P < 0.01$; ***$P < 0.001$; ****$P < 0.0001$).

PARPs in DNA repair and apoptosis induction (Heeres and Hergenrother, 2007), the reduction in PARP mRNA expression level and activity observed in adult compared to fetal-derived OPC might reflect the age-related decrease of DNA repair capability (Bürkle et al., 2015). In fact, PARP activity also varies according to the age in different brain regions (Strosznajder et al., 2000), and PARylation is developmentally regulated (Jubin et al., 2016b).

We then showed that PARP inhibition produces substantially different effects in OPCs derived from fetal and adult brain. In particular: (i) the culture treatment with PARP inhibitors is cytotoxic for OPCs derived from fetal, but not from adult, brain; (ii) PARP inhibition reduces cell number in proportion to the inhibitory potency of the compounds; (iii) the PARP inhibition effect in fetal OPCs is a slow process (iv) PARP inhibition impairs OPC maturation into myelinating OL in fetal, but not in adult OPCs, according to the inhibitory potency of the compounds. A primary role of PARP in the differentiation process of different cell types was recently recognized, possibly though chromatin plasticity and epigenetic regulation (Li et al., 2015). In embryonic stem cells, PARP interacts with the Wnt pathway, Sonic Hedgehog and Pax6 signalling (Hemberger et al., 2003; Yoo et al., 2011). PARP-1 has also a role in neuroectoderm differentiation (Yoo et al., 2011) and favours the transit of NSCs toward a glial fate (Plane et al., 2012). Here we confirmed and further extend this latter observation, showing that PARP inhibition reduced proliferation in fetal-derived neurospheres and oligospheres (see supplemental material), and reduces OPCs differentiation into mature OLs. Notably, key transcription factors involved in OPC maturation, i.e. retinoic acid receptors, thyroid hormone receptors and their hetero-dimerization, such as PDGF signalling, a critical pathway for OPC biology, are targets of PARP regulation (Pavri et al., 2005; Allen, 2008). Overall, these results suggest that a different PARP signalling in fetal and adult OPCs might be part in the biological properties of OPCs at different chronological ages.

These results have also therapeutic implications. In fact, PARP inhibition has even been proposed as a pharmacological strategy in a number of acquired inflammation/demyelinating disorders in which OPCs play a key pathogenic role, occurring both in perinatal/neonatal (e.g. neonatal

hypoxia/ischemia encephalopathy) and adult age (e.g. spinal cord injury and multiple sclerosis; Komjáti et al., 2005; Moroni, 2008; Cavone and Chiarugi, 2012). Contradictory results have been obtained in mice models of inflammatory/demyelinating diseases (Selvaraj et al., 2009; Veto et al., 2010; Casaccia, 2011; Kamboj et al., 2013). PARP-2 deletion in conventional transgenic mice results in a protection from experimental allergic encephalomyelitis (EAE), the most widely used animal model for multiple sclerosis (Kamboj et al., 2013). Conversely, knockout of PARP-1 gene leads to an exacerbated EAE and an increase in the mortality rate (Selvaraj et al., 2009). Pharmacological PARP inhibition in EAE mice has a protective effect, preventing OL death and attenuating inflammation (Veto et al., 2010), although this effect is attributed to the reduction of CNS inflammation and immunomodulation (Scott et al., 2004).

Our data, showing that PARP inhibition is cytotoxic in OPCs derived from the fetal brain, suggest that the OPCs chronological age should be taken into account in considering PARP inhibition as a neuroprotective strategy. In particular, PARP inhibition in ischemic damage should distinguish perinatal/neonatal from adult conditions, considering that the time-window for the treatment of neonatal hypoxia/ischemia encephalopathy is the key period for OPCs proliferation, maturation and developmental myelination.

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Appendix A. Supplementary data

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