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DNA vector-based RNAi approach for stable depletion of poly(ADP-ribose) polymerase-1 ☆

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Abstract

RNA-mediated interference (RNAi) is a powerful technique that is now being used in mammalian cells to specifically silence a gene. Some recent studies have used this technique to achieve variable extent of depletion of a nuclear enzyme poly(ADP-ribose) polymerase-1 (PARP-1). These studies reported either transient silencing of PARP-1 using double-stranded RNA or stable silencing of PARP-1 with a DNA vector which was introduced by a viral delivery system. In contrast, here we report that a simple RNAi approach which utilizes a pBS-U6-based DNA vector containing strategically selected PARP-1 targeting sequence, introduced in the cells by conventional $CaPO_4$ protocol, can be used to achieve stable and specific silencing of PARP-1 in different types of cells. We also provide a detailed strategy for selection and cloning of PARP-1-targeting sequences for the DNA vector, and demonstrate that this technique does not affect expression of its closest functional homolog PARP-2.

Keywords: RNA interference; Small interfering RNA; Small hairpin RNA; Poly(ADP-ribose) polymerase-1; PARP-2

Mammalian and other higher eukaryotic cells respond to DNA damage with rapid catalytic activation of a nuclear enzyme poly(ADP-ribose) polymerase-1 (PARP-1). Activated PARP-1 utilizes the substrate NAD to form polymers of ADP-ribose (pADPr) on itself and several chromatin structure- and DNA metabolism-related proteins. PARP-1 and its metabolism have been shown to participate in different responses to DNA damage, such as maintenance of genomic integrity, DNA repair or cell death [1–4]. More recently, other roles of PARP-1 in the absence of DNA damage are being discovered, such as its effect on transcription [5,6], post-mitotic cell cycle checkpoints [7], and in memory [8]. In view of these expanding roles, there is a need to develop new tools that can achieve targeted and specific depletion of PARP-1 in diverse types of cells from different species in which these processes can be best analyzed. This is more important because there are 18 known PARP-homologs [1], and some of the earlier approaches, such as use of pharmacological inhibitors or depletion of substrate NAD, cannot specifically target PARP-1, whereas gene-knockout approach, although specific for PARP-1, is restricted to the mouse model.

RNA-mediated interference (RNAi) is a reverse-genetics technique that is extensively used in plants, *Caenorhabditis elegans* and *Drosophila* to achieve inhibition of expression of specific gene by introduction of a large double-stranded RNA (dsRNA) [9–12]. This dsRNA is trimmed by the nuclease Dicer to create a short 22–25 nucleotide small interfering RNA (siRNA), which is used by a multi-protein RNA-induced silencing

☆ *Abbreviations:* dsRNA, double-stranded RNA; GFP, green fluorescent protein; MEF, mouse embryonic fibroblasts; MNNG, *N-methyl N'-nitro-N-nitrosoguanidine*; pADPr, poly(ADP-ribose); PARP or PARP-1, poly(ADP-ribose) polymerase-1; RNAi, RNA-mediated interference; siRNA, small interfering RNA.

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complex (RISC) to search and destroy cellular mRNA containing the sequence complementary to the siRNA. The application of this technique to mammalian cells became feasible with the discovery that short synthetic dsRNA can mimic the dicer product siRNA and induce gene-specific silencing in mammalian cells [9–12]. Since exogenously supplied dsRNA method can provide only transient gene-silencing in mammalian cells, DNA vector-based RNAi methods were developed to permit stable gene-silencing. In this approach, a DNA vector directs cellular RNA polymerases to either make two single strands of RNA which can combine and form siRNA or make a short hairpin double-stranded RNA (shRNA) which can be processed by the Dicer to form siRNA in the cell [10,13,14].

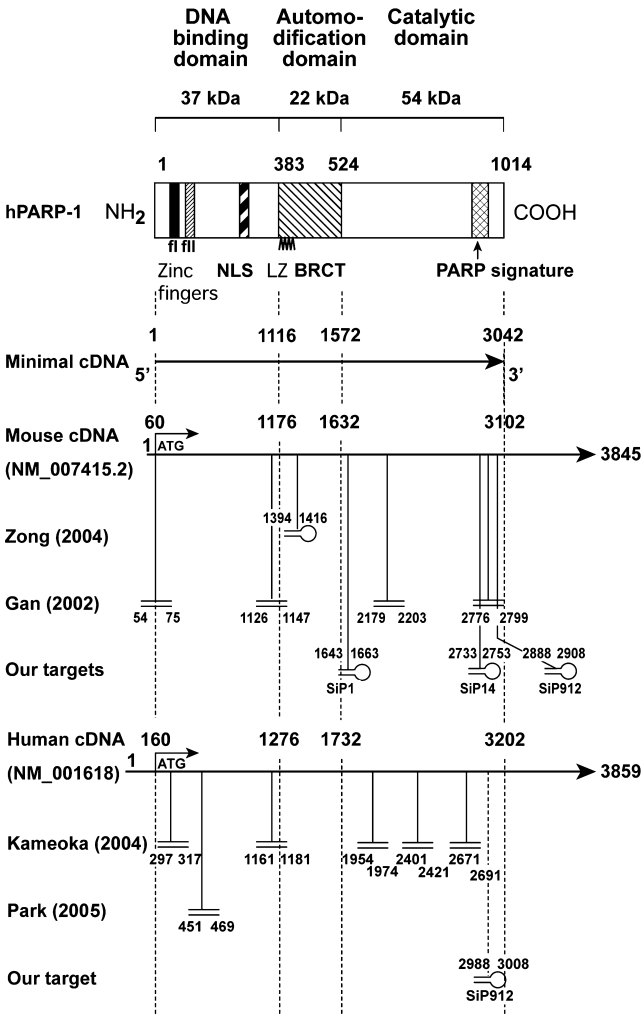

Fig. 1. Comparison of different RNAi approaches against PARP-1. PARP-1 was targeted by RNAi approach in four earlier studies, three of which used short dsRNA (represented by two parallel lines) and one used DNA vector-based RNAi approach, i.e., shRNA (represented by a short hairpin). The location of each of these targeting sequences is shown in relation to PARP-1 protein, and mouse or human PARP-1 cDNA. We targeted PARP-1 using DNA vector-based RNAi approach using three constructs SiP1, SiP14, and SiP912. The target sequence of SiP1 is present in mouse and rat PARP-1, that of SiP14 only in mouse PARP-1, and SiP912 target sequence is present in mouse, rat, human, hamster, and bovine PARP-1.

Some recent studies have reported using RNAi approach against PARP-1 [15–18], three of which used exogenous dsRNA approach, which can only transiently knock down PARP-1 [15–17]. One previous study used DNA vector approach [18], but employed viral delivery system, a technique that is not readily available in many laboratories. In these studies, no clear rationale was provided for selection of a particular PARP-1 targeting sequence, and since extent of PARP-1 depletion was variable with different targeting sequences (Fig. 1), no consensus emerged as to the best strategy for silencing PARP-1 by RNAi approach. Here, we report that a simple DNA vector-based RNAi approach, which employs pBluescript-based DNA vector with U6 promoter [14] and a routine $CaPO_4$ transfection, can be used to create stable PARP-1-depleted mouse, hamster, and human cell lines. We also provide a strategy for selection of PARP-1 targeting sequence, which can be applied to silence PARP-1 in any species, and also demonstrate that PARP-2, the closest functional homolog of PARP-1, is not affected by the RNAi silencing of PARP-1.

Materials and methods

Cells and transfection. *PARP-1*$^{+/+}$ and *PARP-1*$^{-/-}$ mouse embryonic fibroblasts (MEF), obtained from de Murcia [19], were grown in DMEM high glucose medium (Gibco). The hamster cell lines CHO (sub-line WT-5) and AP-1, obtained from Grinstein [20], were grown in αMEM (Gibco). The human diploid SV-40 transformed fibroblast cells GM00637 (Coriell Cell Repository, Camden, NJ) were cultured in MEM (Gibco). All the cells were grown at 37 °C in a humidified incubator with 5% CO_2 in the appropriate medium supplemented with 10% foetal bovine serum, 100 U/ml penicillin, and 100 μg/ml streptomycin. Cells were transfected by $CaPO_4$ protocol [21] with DNA vector and either pGFP-C1 plasmid (9:1) for transient studies or PTK-hygromycin plasmid (4:1) for developing stable clones. The stable hygromycin-resistant clones were isolated and maintained in the presence of 200 μg/ml hygromycin.

PARP-1 target sequences and creation of pBS-U6 DNA vector for PARP-1 knockdown. The pBS-U6, a pBluescript plasmid (Stratagene) containing RNA polymerase III-specific U6 promoter inserted in the polylinker at *Kpn*I site, was obtained from Shi [14]. In the pBS-U6 vector, one of the following three PARP-1-targeting sequences 5′-GGGAGGAGCAGCCGTTGATCC-3′, 5′-GGGAAAGGGATCTACTTTGCC-3′, and 5′-GGGCAAGCACAGTGTCAAAGG-3′ was introduced under the control of U6 promoter to create three new DNA vectors SiP1, SiP14, and SiP912, respectively. For each target sequence, four oligos were synthesized (Invitrogen), two of which expressed this sequence in forward or reverse direction. As an example, the four oligos required for cloning SiP14 in PBS-U6 were:

Oligo 1 (forward strand): 5′-GGAAAGGGATCTACTTTGCCA-3′
Oligo-2 (complementary to oligo1): 3′-CCTTTCCCTAGATGAAACGGTTCGA-5′.
Oligo-3 (reverse strand): 5′-AGCTTGGCAAAGTAGATCCCTTTCCCTTTTTG-3′

Oligo-4 (complementary to oligo3): 3′-ACCGTTTCATCTAGG GAAAGGGAAAAACTTAA-5′

In each of these oligos, target sequences are underlined and additional nucleotides represent either the restriction sites required for cloning or the termination signal of 5Ts. The forward double-stranded oligo pair (1 and 2) was cloned in pBS-U6 at *Apa*I (blunt)–*Hin*dIII sites, followed by cloning of the reverse oligo pair (3 and 4) at *Hin*dIII–*Eco*RI sites. The nucleotide sequence in the final DNA construct was verified by DNA sequencing from both directions using T4 and T7 promoters present in the pBS plasmid. The final SiP14 vector had the following sequence just after the U6 promoter: 5′-GGGAAAGG GATCTACTTTGCCAAGCTTGGCAAAGTAGATCCCTTTCCCT TTTTG-3′.

This DNA would direct cellular RNA polymerase III to create an RNA that would form a short hairpin (shRNA) with the two underlined complementary sequences forming double-stranded stem and intervening AAGCTT sequence (*Hin*dIII site) forming the loop of the hairpin.

Indirect immunofluorescence for PARP-1. After transient transfection with SiP DNA vectors for silencing PARP-1 and pGFP-C1, the cells were fixed at 48 or 72 h and reacted with monoclonal anti-PARP-1 C-2-10 (Aparptosis, 1:500), followed by anti-mouse second antibody tagged with Alexa 594-red (Molecular Probes, 1:200) [22]. Cells were examined at 40× in Nikon Eclipse E1000 microscope for green fluorescence of GFP and red fluorescence of PARP-1. Two-color fluorescence merged images were processed with MetaMorph software (Universal Imaging) [22].

Western blotting and activity-Western blotting. Western blotting was carried out as described earlier [23,24]. In brief, aliquots representing 200,000 cells were loaded on 8% SDS–PAGE, blotted on nitrocellulose, and probed with monoclonal anti-PARP-1 C-2-10 (Aparptosis, 1:10,000), polyclonal anti-PARP-2 [25] (1:5000) or polyclonal anti-pADPr LP96-10 (Aparptosis, 1:10,000). Activity-Western blotting was carried out exactly as described [26]. In brief, proteins transferred on the membrane were incubated in the presence of nicked DNA and 1 mM non-isotopic NAD to activate PARP-1; the pADPr-modified PARP-1 was identified with monoclonal anti-pADPr 10H (1:100) [27].

Results and discussion

Strategy for selection of PARP-1 targeting sequences and cloning in pBS-U6 DNA vector

To silence PARP-1, we used a pBS-U6-based DNA vector method, in which a 21 nucleotide gene-targeting sequence is cloned in tandem in forward and reverse directions between a RNA pol III promoter U6 and a stretch of 5Ts [14]. This DNA vector would be transcribed intracellularly to form a short hairpin RNA (shRNA), which upon processing by Dicer would form siRNA with a 3′-overhang, a structure that is required for processing and selection of the antisense strand of siRNA as the target strand by RISC complex to silence the gene [11,12]. The most critical aspect of a successful RNAi approach is selection of the right gene-targeting sequence. Earlier RNAi studies for PARP-1 have reported variable extent of PARP-1 depletion using target sequences derived from different regions of PARP-1 cDNA (Fig. 1). However, neither the rationale for selection of target sequences was provided nor a consensus about ideal targeting sequence could be reached from these studies, because some found better results with targeting sequence from N-terminal domain rather than C-terminal domain of PARP-1 [15,17], while others observed the inverse [16] and yet another group that used DNA vector-based approach used a target sequence from the automodification domain [18].

To identify a suitable PARP-1 targeting sequence, we used the following principles, some of which were general rules for all siRNA studies, some specific for the DNA vector approach [12] and the rest specific for targeting PARP-1. (a) Selection of target sequence was initially restricted to sequence from cDNA of PARP-1 (i.e., exons), although it could be extended to non-coding sequences, which have been shown to silence other genes [12]. (b) We searched for 21 nucleotide sequences starting with a GGG, because three Gs in proximity to the promoter U6 allow precise initiation of transcription by RNA polymerase III [12]. (c) Only those sequences with GC content between 45% and 65% were selected [12], so as to provide a stable double-stranded hairpin structure. (d) There were about 20 target sequences in cDNA of mouse and human PARP-1 that met these general criteria, and 2–4 of these sequences from mouse or human PARP-1 were excluded because they were present within 100 nucleotides of the translation start codon ATG [12]. (e) We then focused on selecting a sequence within or as close as possible to the PARP-1 signature sequence in the C-terminal catalytic domain of PARP-1 (Fig. 1), because this area of the protein is highly conserved among different PARP-1 [1]; hence, one DNA vector could potentially knock down PARP-1 from different species. (f) Finally, we ensured that the selected sequence was having at least two or more nucleotide mismatch with PARP-2 or other members of the PARP family.

Using above criteria for selection, two candidate sequences were identified in mouse PARP-1 cDNA between nucleotide 2733–2753 (SiP14) and 2888–2908 (SiP912) (Fig. 1). Although both target sequences represent amino acid sequences that are fully conserved in PARP-1 from many different species, the nucleotide sequence for SiP14 was found only in mouse PARP-1, and it had 2–6 nucleotide mismatch with PARP-1 from many other species due to degeneracy of the genetic code. In contrast, amino acid and nucleotide sequences for SiP912 were 100% conserved in PARP-1 from many different species, including human (nucleotide 2988–3008), mouse (nucleotide 2888–2908), hamster (nucleotide 2826–2846, NCBI No. AF168781), rat (nucleotide 2859–2879, NCBI No. NM_013063.1), and bovine (nucleotide 2835–2855, NCBI No. NM_174751.1) PARP-1. For comparison with the earlier published

work that used a DNA vector which targeted PARP-1 in the automodification domain [18], we also selected a third PARP-1 targeting sequence (SiP1) from the middle of the mouse PARP-1 cDNA at nucleotide 1643–1663 (Fig. 1). The SiP1 sequence was also fully conserved in rat PARP-1, but had 4 or more mismatches with PARP-1 from many other species. We cloned these three sequences in the pBS-U6 vector after RNA pol III promoter U6 [14] to create three DNA vectors, namely SiP1, SiP14, and SiP912, and examined their capacity to knock down PARP-1 in mouse, hamster or human cells.

Depletion of PARP-1 in mouse fibroblasts

PARP-1 is an abundant protein in the cell; hence, we first examined in a transient transfection assay, the rapidity and extent to which some of our DNA vectors could deplete PARP-1 in *PARP-1*$^{+/+}$ mouse fibroblasts (Fig. 2A). The SiP1 and SiP14 DNA vectors were co-transfected with a green fluorescent protein (GFP)-expressing plasmid DNA to distinguish transfected cells from untransfected cells. In the cells expressing GFP protein (green), red nuclear signal of PARP-1, identified by indirect immunofluorescence with monoclonal anti-PARP antibody, was reduced by 48 h (yellow nuclei) and abolished by 72 h (only green nuclei), whereas red signal of PARP-1 remained unchanged in neighboring untransfected cells. Thus, both SiP1 and SiP14 vectors could efficiently deplete PARP-1 in mouse fibroblasts within 72 h.

We then established stable mouse cell lines by co-transfection of SiP14 vector with selection plasmid pTK-Hyg, which confers resistance to hygromycin. As controls, *PARP-1*$^{+/+}$ cells were transfected with empty pBS-U6 vector along with pTK-Hyg, and several stable U6 clones were isolated. Several SiP14 clones were observed to have significant depletion of PARP-1, as shown here in PARP-1 immunoblot for two of these clones #3 and 15 (Fig. 2B, top panel). Activity-Western blot technique is a functional in vitro test for the enzymatic activity present in the PARP-1 protein which has been immobilized on the membrane for a Western blot [26]. Using this test, we confirmed that PARP-1 protein was indeed decreased in the SiP14 clones, because signal for in vitro activated and pADPr-modified PARP-1 in these clones was very weak as compared to control U6 clones (Fig. 2B, middle panel). Next, we examined whether PARP-1-depleted SiP14 clones would be able to form pADPr, when exposed to DNA damaging alkylating agent *N-methyl N′-nitro-N-nitrosoguanidine* (MNNG) (Fig. 2C). In the polymer-immunoblot of cells exposed for 15 min to MNNG, signal for pADPr-modified proteins was very strong in the control pBS-U6 clone, but it was highly suppressed in both the SiP14 clones. Thus, SiP14 could significantly deplete PARP-1 protein and significantly suppress the capacity of mouse cells to make pADPr in response to DNA damage.

Depletion of PARP-1 in hamster cells

The hamster PARP-1 has three nucleotide mismatch with the target sequence of SiP14 vector. To determine whether PARP-1 in hamster cells would be silenced by

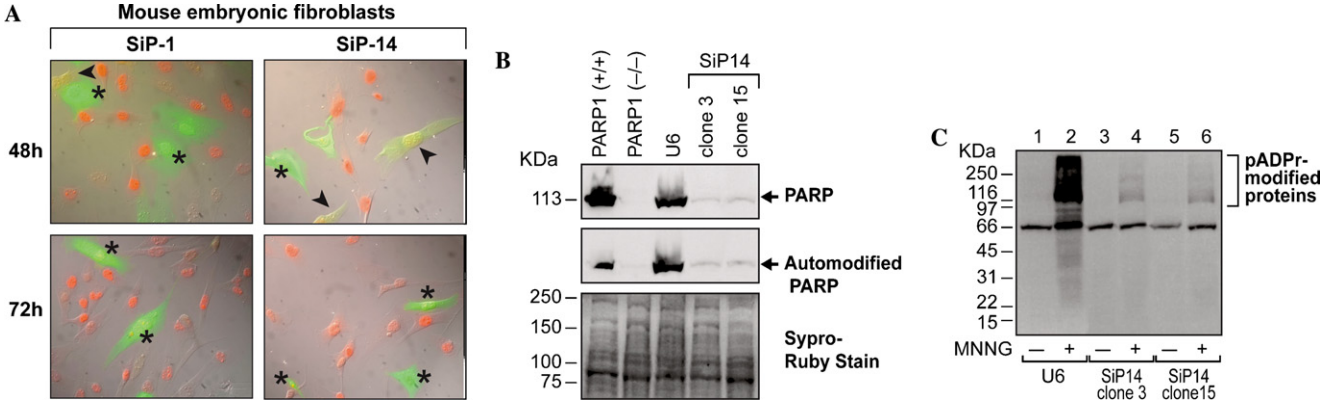

Fig. 2. Silencing of PARP-1 in mouse cells. (A) Transient depletion of PARP-1 with SiP1 and SiP14 DNA vectors. *PARP-1*$^{+/+}$ MEF were transiently co-transfected with GFP-expressing plasmid and either SiP1 or SiP14, and cells were harvested at 48 or 72 h for indirect immunofluorescence analysis of PARP-1 using monoclonal C-2-10, followed by staining with Alexa 594 (red) second anti-mouse antibody. The merged images at 40× magnification showed untransfected cells with red nuclei due to PARP-1, whereas nuclei of transfected green cells were either yellow (arrowheads) or green (stars), which indicated that PARP-1 was either decreased or significantly depleted. This experiment was repeated at least four times and representative panels are shown here. (B) Stable depletion of PARP-1 in mouse cell lines. Stable hygromycin-resistant clones were isolated after transfection of *PARP-1*$^{+/+}$ MEF with either SiP14 DNA or control vector pBS-U6. PARP-1 depletion in two of the SiP14 clones and a pBS-U6 clone was compared with parental *PARP-1*$^{+/+}$ or with *PARP-1*$^{-/-}$ MEF by Western blotting (top panel) or by activity-Western blotting, which revealed pADPr-modified PARP-1 (middle panel). Membrane was stained with Sypro-Ruby as a loading control. The results shown here with two SiP14 clones and pBS-U6 clone are similar to results with at least 4 more clones in each category. (C) Significantly reduced capacity of PARP-1-depleted cells to form pADPr in response to DNA damage. Control U6 and SiP14 clones 3 and 15 were treated with 300 μM MNNG or DMSO (control) for 15 min, harvested and analyzed for formation of pADPr-modified proteins by polymer-immunoblotting. The panel represents identical results observed in four independent experiments.

SiP14 vector despite three mismatches, we created three different stable clones of CHO cells after transfection with SiP14. There was no reduction in PARP-1 levels in any of the three SiP14-CHO clones, as compared to the U6-transfected control CHO cells (Fig. 3A). Thus, SiP14-induced PARP-1 knockdown in mouse cells, observed earlier (Fig. 2), was due to a sequence specific targeting of mouse PARP-1 and not a non-specific response to introduction of a DNA vector. In contrast to SiP14, the target sequence in SiP912 vector has 100% nucleotide sequence match with hamster PARP-1. Therefore, we prepared stable SiP912 clones in two different hamster cell lines, CHO and its Na/H^+ exchanger-deficient mutant cell line AP-1. The PARP-1 immunoblotting revealed that three different SiP912-CHO clones had significant depletion of PARP-1 as compared to U6 control, whereas there was a variable extent of PARP-1-depletion in three different AP-1 clones (Fig. 3B, top panel). The activity-Western blot analyses of the enzymatic function of PARP-1 in these blots (Fig. 3B, middle panel) closely reflected the extent of depletion of PARP-1 protein that was earlier observed in the PARP-1-immunoblot. Finally, ability of the SiP912 clones to activate PARP-1 and form pADPr in response to DNA damage by MNNG was examined (Fig. 3C). The formation of pADPr-modified proteins was significantly suppressed in all three CHO-derived SiP912 clones (lanes 4, 6, and 8), as compared to U6 controls (lanes 2 and 10). In contrast, all three AP-1-derived SiP912 clones had reduced but easily detectable amounts of pADPr-modified proteins. Thus, targeting hamster PARP-1 with SiP912 was more successful in CHO cells than in AP-1 cells, indicating that endogenous factors within each cell line may also contribute towards the success of the targeting strategy. These results strongly suggest that it is best to carefully verify PARP-1 knockdown phenotype in a given cell line by a functional assay rather than relying solely on identification of depletion of PARP-1 protein by a Western blot.

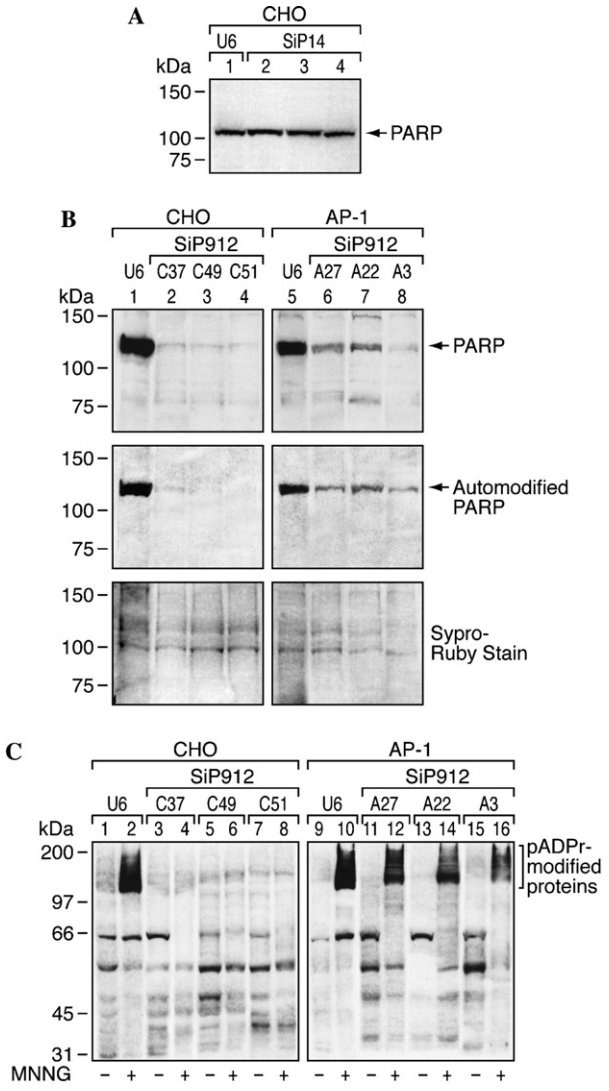

Fig. 3. Silencing of PARP-1 in hamster cells. (A) Inability of a mismatched SiP14 DNA vector to deplete PARP-1 in CHO cells. CHO cells were transfected with SiP14 or pBS-U6 vector to create hygromycin-resistant stable cell lines as described in Fig. 2B. Levels of PARP-1 in these clones were examined by Western blotting for PARP-1. (B) Stable depletion of PARP-1 by SiP912 DNA vector in two hamster cells CHO and AP-1. Stable hygromycin-resistant clones were derived from CHO and AP-1 cells after transfection with pBS-U6 control or SiP912 vector. One U6 control clone and three SiP912 clones from each cell line were examined for the extent of depletion of PARP-1 by Western blotting (top panel) or activity-Western blotting (middle panel). Membrane was stained with Sypro-ruby as a loading control. The results with clones shown here represent identical results obtained with at least 4–6 more clones in each category. (C) Variable extent of suppression in capacity of PARP-1 depleted clones to form pADPr after DNA damage. The CHO and AP-1-derived U6 or SiP912 clones were treated with 300 μM MNNG, harvested after 15 min, and analyzed for formation of pADPr-modified proteins by polymer-immunoblotting. The panel represents identical results observed in three independent experiments.

Depletion of PARP-1 in human skin fibroblasts

The target sequence in SiP912 is also 100% conserved in human PARP-1, therefore, we examined its capacity to silence PARP-1 in human skin fibroblasts (Fig. 4). During transient co-transfection with GFP plasmid, it was evident by 72 h that cells expressing GFP protein (green) were depleted of PARP-1 protein (red) (Fig. 4A). After stable transfection with SiP912, several clones were isolated and found to have significantly depleted levels of PARP-1, as shown here for one of the SiP912 clones (Fig. 4B, lane 3). When exposed to DNA damaging agents H_2O_2 or MNNG, the SiP912 clone was unable to activate PARP-1 and form pADPr-modified proteins, whereas U6 clone had strong activation of PARP-1 (Fig. 4C). Thus, SiP912 could significantly deplete PARP-1 in human cells.

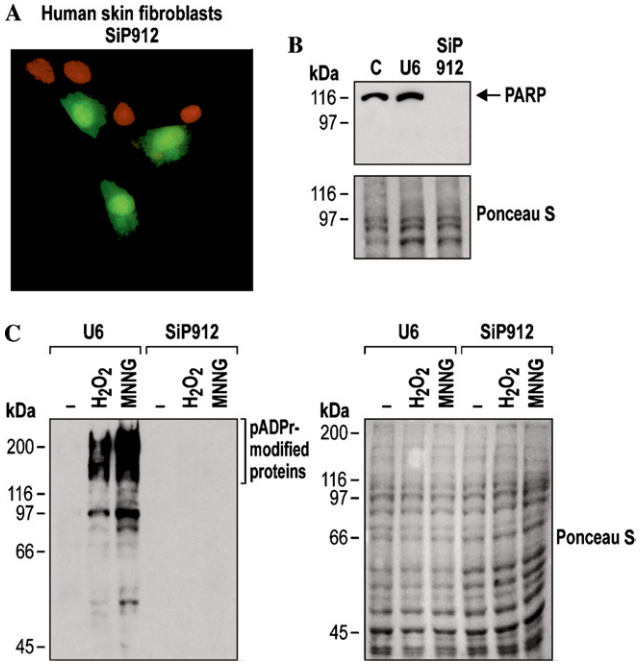

Fig. 4. Silencing of PARP-1 in human cells. (A) Rapid PARP-1 knockdown in human fibroblasts. Human skin fibroblasts were transiently co-transfected with GFP-expressing plasmid and SiP912 (1:9), and analyzed by immunofluorescence for depletion of PARP-1, as described for Fig. 2A. The transfected cells identified by GFP expression were devoid of red nuclear signal of PARP-1, whereas untransfected neighboring cells showed red nuclear signal due to PARP-1. (B) Stable depletion of PARP-1 by SiP912 in human cell lines. Stable hygromycin-resistant clones of human skin fibroblasts were isolated after transfection with either SiP912 or pBS-U6 vector. The PARP-1 levels in one of the U6 and SiP912 clones were compared with those in untransfected control cells by immunoblotting for PARP-1 (top panel). Membrane was stained with Ponceau S as a loading control. The clones shown here represent identical results obtained with at least three more clones. (C) Significant reduction in the capacity of PARP-1-depleted cells to form pADPr in response to DNA damage. Control U6 and SiP912 clones were treated with 300 μM MNNG for 15 min or 100 μM H_2O_2 for 10 min and analyzed for the formation of pADPr-modified proteins by polymer-immunoblotting. Membrane was stained with Ponceau S as a loading control. The results shown here are identical to results obtained with at least two more clones in each category.

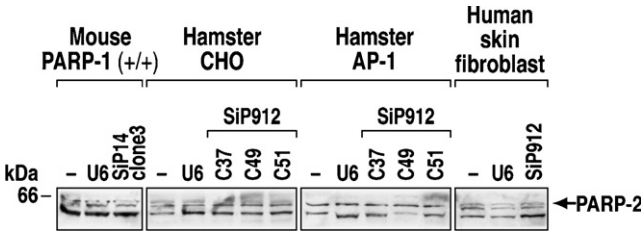

Fig. 5. PARP-2 is not affected by silencing of PARP-1 by SiP14 or SiP912. The mouse, hamster, and human cell lines, whether U6 control or PARP-1 depleted, were probed by immunoblotting with polyclonal PARP-2 antibody. No changes in PARP-2 levels occurred in PARP-1-depleted clones.

PARP-2 is not affected in PARP-1-depleted cells

A major side-effect of RNAi approach in mammalian cells is non-specific silencing of other genes, which may occur despite few sequence mismatches [10,11]. It is interesting, therefore, to examine whether efforts to silence PARP-1 may cause silencing of its most closely related homolog PARP-2, which has quite significant amino acid and nucleotide sequence similarity with PARP-1 in the C-terminal domain [1]. Moreover, PARP-2 is known to interact with PARP-1, it is present in many sub-nuclear locations along with PARP-1, and it is also activated, although weakly, in response to DNA damage [1,28]. The target sequence of SiP14 has 3 nucleotide mismatch with mouse PARP-2, and that of SiP912 has greater than 5 nucleotide mismatch with human or hamster PARP-2. We observed that at this level of mismatch, both SiP14 and SiP912 vectors could not influence expression levels of PARP-2 in all of the PARP-1-depleted mouse, hamster or human cell lines described above (Fig. 5). Since, other PARP-homologs have greater mismatch than PARP-2 with the targeting sequences in SiP14 and SiP912 vectors, they are not likely to be affected by this approach, although it has not been examined.

Our results along with four earlier reports [15–18] show that siRNA-based reverse-genetics can be applied to PARP-1 in mammalian cells. We have described a detailed strategy for selection of PARP-1 targeting sequences, which can be suitably modified to knock down PARP-1 from cells of any species. Moreover, the simple pBS-U6-based DNA vector and $CaPO_4$ transfection procedure used in our method can be easily manipulated in any laboratory. This DNA vector-based RNAi approach may be a simpler alternative to technically challenging, expensive, and time-consuming *PARP-1*$^{-/-}$ model. It will allow rapid exploration of the role of PARP-1 under the conditions where PARP-1 knockout is not be feasible or has not been tried, such as cells derived from species other than mouse. Moreover, in cases where PARP-1 knockout has created lethality in conjunction with knock out of another gene, such as PARP-2 [29], ATM [30] or Ku80 [31,32], the RNAi approach offers a simpler way to transiently introduce PARP-1 depletion and examine its impact. An inducible version of this DNA vector [33] can also permit creation of cells or mice with conditional knock down of PARP-1.

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