Pharmacological inhibition of poly(ADP-ribose) polymerase activity down-regulates the expression of syndecan-4 and Id-1 in endothelial cells

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Abstract. Poly(ADP-ribose) polymerase (PARP) is a family of nuclear proteins which regulate a number of cell functions, such as DNA repair, transcription, remodelling of chromatin structure, cell division and cell death. We and others have recently demonstrated that down-regulation of cellular PARP activity, using pharmacological inhibitors, impairs a number of endothelial functions and angiogenesis. In the present study, we investigated the potential mechanisms underlying the anti-angiogenic effect exerted by the potent PARP inhibitor GPI 15427, analyzing gene expression in human endothelial cells shortly after treatment with this compound. Analysis of gene and protein expression indicated that a 2-h exposure of human endothelial cells to GPI 15427 induced a rapid decrease of syndecan-4 (SDC-4), a transmembrane protein involved in modulation of cell signalling during angiogenesis that plays a role in endothelial cell migration and adhesion. Moreover, treatment with the PARP inhibitor induced a reduction of a helix-loop-helix transcription factor, the inhibitor of DNA binding-1 (Id-1), also implicated in the control of endothelial functions. We suggest that the inhibitory effect exerted by GPI 15427 on the angiogenic process is likely due to the reduced activity of specific transcription factors, such as Oct-1 and CREB that contribute to the regulation of SDC-4 and Id-1 expression, respectively. In conclusion, these results strongly suggest that PARP activity is capable of modulating molecules required for endothelial cell migration, adhesion, proliferation or differentiation during the angiogenic process.

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Introduction

Poly(ADP-ribose) polymerase (PARP) is a family of eukaryotic nuclear proteins which plays a key role in regulating DNA repair, transcription, chromatin function, cell division and cell death (1). PARPs synthesize polymers of ADP-ribose using NAD^+ as a substrate and catalyse the attachment of poly(ADP-ribose) to acceptor proteins modifying their function. This post-translational modification is transient due to the prompt degradation of the polymers by poly(ADP-ribose) glycohydrolase. Among the PARP family members, PARP-1 is responsible for most of the cellular poly(ADP-ribosyl)ating activity and its primary targets include PARP-1 itself, histones and a variety of transcription factors. So far, only PARP-1 and PARP-2 are known to be activated by DNA damage, acting as sensor of genotoxic damage and co-ordinating repair (1). However, PARP-2 is less active than PARP-1 in the response to DNA damage and unique PARP-2 functions in various differentiation processes have been recently identified (2).

PARP-1 and -2 are involved in the base excision repair (3), which corrects DNA base lesions including N-methylpurines induced by methylating agents and oxidized bases or single strand breaks generated by ionizing radiations (4). Moreover, PARP-1 modulates the repair of double-strand breaks through its interaction with components of the non-homologous end joining (5). In its poly(ADP-ribos)ylated form PARP-1 counteracts the action of topoisomerase I poisons facilitating resealing of DNA strand (6). Recently, it has been demonstrated that PARP-1 may play a role also in the nucleotide excision repair pathway (7). Thus, PARP inhibitors have been exploited in cancer treatment to increase the efficacy of chemotherapeutic agents such as temozolomide, dacarbazine, carboplatin, irinotecan, topotecan or of radiotherapy, and are currently under evaluation in phase I and II clinical trials for the treatment of refractory solid and hematological tumors (4, www.clinicaltrials.gov). PARP inhibitors are also investigated as single agents for the therapy of ovarian or breast cancer carrying mutations of breast cancer associated gene 1 and 2 (BRCA-1 and BRCA-2) which are involved in the homologous

recombination repair machinery (8,9, www.clinicaltrials.gov).

In addition to DNA repair mechanisms, targeting PARP-1 has been shown to suppress angiogenesis, which is an essential requirement for the growth of tumours. We recently demonstrated a direct involvement of PARP-1 function in angiogenesis as indicated by the reduction of blood vessel neo-formation in response to angiogenic stimuli observed in PARP-1 KO mice (10). Similar results were obtained in animals treated with the potent PARP inhibitor GPI 15427 (10). The observed anti-angiogenic effect of GPI 15427 is associated with a decrease of endothelial cell migration in response to angiogenic factors, such as the vascular endothelial growth factor (VEGF) or the placenta growth factor, and cannot be directly related to increased DNA damage, since it is observed at drug concentrations that do not affect the viability and the proliferative potential of endothelial cells. Similar anti-angiogenic effects were obtained also with other PARP inhibitors (11-13). Notably, abrogation of PARP-1 expression by stable gene silencing reduced the aggressiveness of melanoma and this effect was associated with a decreased vasculature formation within the tumour (14). Altogether, these data strongly suggest that PARP inhibitors may also exert antitumor activity through a reduction of tumour-associated neo-angiogenesis, independently on their effect on DNA repair.

In the present study, we demonstrate that a short exposure of human endothelial cells to the PARP inhibitor GPI 15427 induces a rapid decrease of the expression of syndecan-4 (SDC-4), a transmembrane protein involved in modulating cell signalling during angiogenesis, and a later reduction of the expression of inhibitor of DNA binding-1 (Id-1), a helix-loop-helix transcription factor which regulates endothelial functions.

Materials and methods

Cell culture and treatment with PARP inhibitor. The immortalized human endothelial cell line HUV-ST was generated as previously described (15). Cells were maintained in culture in endothelial growth factor medium (EGM-2; Clonetics, BioWhittaker Inc., Walkersville, MD, USA) supplemented with 0.4 mg/ml geneticin and 5 μg/ml puromycin.

For PARP inhibition in endothelial cells, the recently developed compound GPI 15427 [10-(4-methyl-piperazin-1-ylmethyl)-2H-7-oxa-1,2-diaza-benzo[de]anthracen-3-one, Eisai, Baltimore, MD, USA] was used (16). The PARP inhibitor stock solution (1 mM) was prepared by dissolving GPI 15427 in 70 mM PBS without potassium.

PARP activity assay. To measure total cellular PARP activity, HUV-ST cells ($5x10^6$) were lysed in 0.5 ml of a buffer containing 0.1% Triton X, 50 mM Tris-HCl pH 8.0, 0.6 mM EDTA, 14 mM ß-mercaptoethanol, 10 mM $MgCl_2$ and protease inhibitors. Proteins (25 μg) were incubated with 2 μCi ^{32}P-NAD$^+$ (GE Healthcare, Milan, Italy), 10 μM NAD$^+$, 50 mM Tris-HCl, 10 mM $MgCl_2$, 14 mM ß-mercaptoethanol, 10 μg nuclease-treated salmon testes DNA. PARP activity was expressed as fmol of ^{32}P-NAD$^+$/μg of protein (16). For the analysis of the influence of GPI 15427 on cellular PARP, intact HUV-ST cells ($5x10^5$) were treated with the inhibitor and permeabilized with digitonin (0.1 mg/ml) in the presence of 0.25 μCi ^{3}H-NAD$^+$ (PerkinElmer, Milan, Italy) (17).

Microarray analysis of gene expression

Gene expression profiling. Total cellular RNA was isolated from endothelial cells using RNeasy kits (Qiagen, Germantown, MD, USA). Preparation of labeled cRNA and hybridization (GeneChip Human Genome U133A array, Affymetrix Inc., High Wycombe, UK) was performed according to the Affymetrix GeneChip expression analysis manual.

Data analysis. Affymetrix GeneChip scanning was analyzed by a customized R language-based script (see www.r-project.org) which utilizes the Bioconductor packages (see www.bioconductor.org) for quality control analysis, data normalization, hierarchical cluster and identification of differentially expressed transcripts. Specifically, the 'gcrma package' was used for chip normalization and background correction; the 'vsn package' provided calibration and transformation of the probe intensities. The genefilter package was used to separate genes with high variance according to the interquartile range method.

Prediction analysis. Prediction analysis of microarrays (PAM) is a statistical technique for class prediction utilizing gene expression data using shrunken centroids. The method of nearest shrunken centroids identifies subsets of genes that best characterize each class (18). In order to test the internal consistency and to explore the relationship among untreated or GPI 15427-treated cell samples and underlying features of gene expression the unsupervised cluster analysis was followed by PAM R supervised class prediction package.

Differentially expressed genes. To identify genes differentially expressed in GPI 15427-treated and untreated cell samples, we used a significant analysis of microarrays (SAM) test, which uses permutation to identify significant differences between different groups. Analysis was performed using the R package 'samr' which was applied in order to adjust P-values using a false discovery rate to identify genes that were significantly different between groups (19).

The lists of genes modulated by treatment with GPI 15427 that are shown in the Results section correspond to the genes that resulted to be up- or down-modulated in both PAM and SAM analysis.

Gene function analysis. Regulated biological processes and molecular functions were identified by the GEPAS web-based application according to the Gene Ontology (http://www.geneontology.org) Consortium classification (20).

Western blot analysis. Time-course experiments of specific protein expression were performed in semi-confluent cultures of HUV-ST cells, growing in 6-well plates. Cells were treated with 2 μM GPI 15427 for different periods of time; wells were, then, washed with PBS and cell lysates were prepared using a lysis buffer (150 mM NaCl, 1 mM EDTA, 0.5% NP-40, 50 mM Tris-HCl pH 8.0) containing phosphatase (PhoSTOP, Roche Diagnostics, Mannheim, Germany) and protease (Complete Mini, EDTA-free, Roche) inhibitors. Samples were run in a 10% SDS-polyacrylamide gel and proteins were then transferred to supported nitrocellulose membranes (Hybond-C; GE Healthcare). Membranes were blocked in blocking solution

Table I. Up-modulated genes after GPI 15427 treatment of endothelial cells.

Probe	Symbol	Description[a]	Score[b]	Fold change[b]
203035_s_at	PIAS3	Protein inhibitor of activated STAT, 3	3.21	1.85
220748_s_at	ZNF580	Zinc finger protein 580	3.03	1.81
209140_x_at	HLA-B	Major histocompatibility complex, class I, B	2.41	1.60
214459_x_at	HLA-C	Major histocompatibility complex, class I, C	2.40	1.76
200696_s_at	GSN	Gelsolin (amyloidosis, Finnish type)	2.23	1.51
201626_at	INSIG1	Insulin-induced gene 1	2.22	1.83
204067_at	SUOX	Sulfite oxidase	2.21	1.62
202245_at	LSS	Lanosterol synthase (2,3-oxidosqualene-lanosterol cyclase)	2.21	1.85
37462_i_at	SF3A2	Splicing factor 3a, subunit 2, 66 kDa	2.21	1.63
214246_x_at	MINK1	Misshapen-like kinase 1 (zebrafish)	2.17	1.59

[a]The list corresponds to the genes that resulted to be modulated in both PAM and SAM analysis. [b]Score and fold change parameters were obtained by SAM analysis.

(4% BSA, 0.1% Tween-20, 0.9% NaCl, 20 mM Tris-HCl, pH 7.4) and incubated in the same solution overnight at 4˚C with primary antibodies.

The rabbit polyclonal antibodies anti-Id-1 (C20, diluted 1:200), anti-ATF-3 (C-19, diluted 1:200), anti-Oct-1 (C21, diluted 1:200) and anti-ß-tubulin (H-235, diluted 1:1000), as well as the monoclonal antibodies anti-SDC-4 (5G9, diluted 1:200), anti-ATF-1/CREB (25C10G, diluted 1:200) and anti-phosphotyrosine (PY20, diluted 1:200) were all from Santa Cruz Biotechnology (Santa Cruz, CA, USA); the monoclonal antibody anti-phospho-CREB/ATF-1 (1B6, diluted 1:1000) was purchased from Cell Signaling (Beverly, MA, USA). After washing with TBST buffer (0.1% Tween-20, 0.9% NaCl, 20 mM Tris-HCl, pH 7.4), membranes were incubated for 1 h at room temperature with the appropriate horseradish-peroxidase conjugated secondary antibody (anti-rabbit or anti-mouse Ig from GE Healthcare, diluted 1:5000 in blocking solution). Membranes were then washed with TBST buffer and detection was carried out using the ECL Western blot detection reagents and Hyperfilm, both from GE Healthcare. Densitometric analysis was performed using a GS710 densitometer from Bio-Rad Laboratories (Hercules, CA, USA).

NF-κB and Oct-1 activation assays. Time-course experiments of transcription factors activity were performed in semi-confluent cultures of HUV-ST cells, growing in 6-well plates. Cells were treated with 2 μM GPI 15427 for different periods of time and then lysed to prepare nuclear extracts. Nuclear proteins were prepared using the Active motif nuclear extract kit (Active Motif Europe, Rixensart, Belgium) according to the manufacturer's instructions. Activation of the NF-κB p65 subunit in 10 μg of HUV-ST nuclear extracts was determined using an NF-κB p65 Enzyme-linked immunosorbent assay (ELISA)-based transcription factor assay kit (TransAM assay) (Active Motif Europe), according to the manufacturer's protocol. This kit contains a 96-well plate with immobilized oligonucleotides encoding an NF-κB consensus site (5'-GGGACTTTCC-3'). The active form of this transcription factor specifically binds to this oligonucleotide. The NF-κB detecting antibody recognizes an epitope on p65 that is accessible only when this polypeptide is activated and bound to its target DNA. A horseradish peroxidase-conjugated secondary antibody provides a sensitive colorimetric readout that is quantified by a Bio-Rad microplate reader 680 at 450 nm with a reference wavelength of 655 nm. The positive control Jurkat nuclear extract, provided within the kit, was used to assess assay specificity.

Oct-1 activity was assayed using a modified Oct-4 TransAM kit from Active Motif Europe. In brief, the 96-well plate with immobilized oligonucleotides encoding the octamer binding transcription factor family consensus sequence (5'-ATGCAAAT-3') was utilized to capture the activated Oct-1 protein present in the HUV-ST nuclear extracts (10 μg). Then, the assay was performed according to the manufacturer's instructions using an anti-Oct-1 rabbit polyclonal antibody from Santa Cruz (C-21) diluted 1:1000. A nuclear extract from P19 cells, provided with the kit, was used as positive control.

Results

Analysis of differential gene expression in untreated or GPI 15427-treated HUV-ST cells. HUV-ST cells were initially analyzed for PARP activity, measured in cell extracts in the presence of nuclease-treated salmon testes DNA and ^{32}P-NAD$^+$. The results indicated that total PARP activity of HUV-ST cells was 853±178 fmol/μg of protein. We then tested the ability of GPI 15427 to inhibit PARP activity of intact endothelial cells by exposure of HUV-ST cells for 1 h to graded concentrations of GPI 15427 (0.1-2 μM), followed by permeabilization with digitonin in the presence of ^{3}H-NAD$^+$. The results indicated that GPI 15427 easily penetrated into the cells and inhibited PARP activity with an IC_{50} of 237±27 nM.

To investigate the early molecular changes after exposure of endothelial cells with GPI 15427, HUV-ST cells were treated for 2 h with 2 μM GPI 15427, a concentration that completely inhibits PARP activity and angiogenesis and that does not affect the growth and clonogenic survival of endothelial cells (10). Total RNA was extracted from untreated (n=2) or drug-treated cultures (n=2) and gene expression was evaluated by microarray analysis. The identification of the differentially expressed genes showed that most of these genes were down-regulated by drug treatment (Tables I and II). The genes demonstrating early modulated expression mainly

Table II. Down-modulated genes after GPI 15427 treatment of endothelial cells.

Probe	Symbol	Description[a]	Score[b]	Fold change[b]
202071_at	SDC-4	Syndecan 4 (amphiglycan, ryudocan)	-4.56	0.41
210762_s_at	DLC1	Deleted in liver cancer 1	-4.44	0.31
208937_s_at	Id-1	Inhibitor of DNA binding 1, dominant negative helix-loop-helix protein	-4.34	0.22
218237_s_at	SLC38A1	Solute carrier family 38, member 1	-4.14	0.38
205321_at	EIF2S3	Eukaryotic translation initiation factor 2, subunit 3 γ, 52 kDa	-3.77	0.32
212582_at	OSBPL8	Oxysterol binding protein-like 8	-3.64	0.33
201123_s_at	EIF5A	Eukaryotic translation initiation factor 5A	-3.62	0.28
211136_s_at	CLPTM1	Cleft lip and palate associated transmembrane protein 1	-3.58	0.43
221806_s_at	SETD5	SET domain containing 5	-3.57	0.34
200852_x_at	GNB2	Guanine nucleotide binding protein (G protein), ß polypeptide 2	-3.47	0.41
205924_at	RAB3B	RAB3B, member RAS oncogene family	-3.45	0.50
213757_at	EIF5A	Eukaryotic translation initiation factor 5A	-3.29	0.33
209832_s_at	CDT1	Chromatin licensing and DNA replication factor 1	-3.28	0.42
208777_s_at	PSMD11	Proteasome (prosome, macropain) 26S subunit, non-ATPase, 11	-3.24	0.46
206445_s_at	PRMT1	Protein arginine methyltransferase 1	-3.20	0.49
202416_at	DNAJC7	DnaJ (Hsp40) homolog, subfamily C, member 7	-3.19	0.54
217756_x_at	SERF2	Small EDRK-rich factor 2	-3.17	0.40
222244_s_at	TUG1	RNA taurine upregulated gene 1	-3.16	0.33
204004_at	PAWR	PRKC, apoptosis, WT1, regulator	-3.03	0.29
203109_at	UBE2M	Ubiquitin-conjugating enzyme E2M (UBC12 homolog, yeast)	-3.01	0.29
210092_at	MAGOH	Mago-nashi homolog, proliferation-associated *(Drosophila)*	-3.00	0.43
212015_x_at	PTBP1	Polypyrimidine tract binding protein 1	-3.00	0.54
202976_s_at	RHOBTB3	Rho-related BTB domain containing 3	-2.98	0.50
208750_s_at	ARF1	ADP-ribosylation factor 1	-2.93	0.42
213980_s_at	CTBP1	C-terminal binding protein 1	-2.91	0.56
201801_s_at	SLC29A1	Solute carrier family 29 (nucleoside transporters), member 1	-2.89	0.42
211671_s_at	NR3C1	Nuclear receptor subfamily 3, group C, member 1 (glucocorticoid receptor)	-2.88	0.54
202407_s_at	PRPF31	PRP31 pre-mRNA processing factor 31 homolog *(S. cerevisiae)*	-2.84	0.55
203917_at	CXADR	Coxsackie virus and adenovirus receptor	-2.81	0.35
203093_s_at	TIMM44	Translocase of inner mitochondrial membrane 44 homolog (yeast)	-2.80	0.46
222029_x_at	PFDN6	Prefoldin subunit 6	-2.71	0.57
209042_s_at	UBE2G2	Ubiquitin-conjugating enzyme E2G 2 (UBC7 homolog, yeast)	-2.70	0.46
209208_at	MPDU1	Mannose-P-dolichol utilization defect 1	-2.69	0.57
209264_s_at	TSPAN4	Tetraspanin 4	-2.68	0.47
201164_s_at	PUM1	Pumilio homolog 1 *(Drosophila)*	-2.62	0.49
214383_x_at	KLHDC3	Kelch domain containing 3	-2.62	0.53
202656_s_at	SERTAD2	SERTA domain containing 2	-2.61	0.46
202839_s_at	NDUFB7	NADH dehydrogenase (ubiquinone) 1 beta subcomplex, 7, 18 kDa	-2.61	0.48
213887_s_at	POLR2E	Polymerase (RNA) II (DNA directed) polypeptide E, 25 kDa	-2.59	0.43
205748_s_at	RNF126	Ring finger protein 126	-2.57	0.57
212961_x_at	CXorf40B	Chromosome X open reading frame 40B	-2.57	0.47
207088_s_at	SLC25A11	Solute carrier family 25 (mitochondrial carrier; oxoglutarate carrier), member 11	-2.56	0.55
214911_s_at	BRD2	Bromodomain containing 2	-2.55	0.56
208645_s_at	RPS14	Ribosomal protein S14	-2.54	0.57
212125_at	RANGAP1	Ran GTPase activating protein 1	-2.53	0.45
200968_s_at	PPIB	Peptidylprolyl isomerase B (cyclophilin B)	-2.50	0.46
204212_at	ACOT8	Acyl-CoA thioesterase 8	-2.50	0.41
212040_at	TGOLN2	Trans-golgi network protein 2	-2.50	0.44
212251_at	MTDH	Metadherin	-2.49	0.42
218206_x_at	SCAND1	SCAN domain containing 1	-2.47	0.56
201865_x_at	NR3C1	Nuclear receptor subfamily 3, group C, member 1 (glucocorticoid receptor)	-2.46	0.53
204031_s_at	PCBP2	Poly(rC) binding protein 2	-2.46	0.46
218368_s_at	TNFRSF12A	Tumor necrosis factor receptor superfamily, member 12A	-2.45	0.43
217716_s_at	SEC61A1	Sec61 alpha 1 subunit *(S. cerevisiae)*	-2.45	0.55
204640_s_at	SPOP	Speckle-type POZ protein	-2.44	0.39
208625_s_at	EIF4G1	Eukaryotic translation initiation factor 4 γ, 1	-2.41	0.43
201506_at	TGFBI	Transforming growth factor, ß-induced, 68 kDa	-2.38	0.59
221516_s_at	SMCR7L	Smith-Magenis syndrome chromosome region, candidate 7-like	-2.38	0.60

Table II. Continued.

Probe	Symbol	Description[a]	Score[b]	Fold change[b]
212863_x_at	CTBP1	C-terminal binding protein 1	-2.37	0.50
200967_at	PPIB	Peptidylprolyl isomerase B (cyclophilin B)	-2.37	0.53
212596_s_at	HMG2L1	High-mobility group protein 2-like 1	-2.36	0.46
201050_at	PLD3	Phospholipase D family, member 3	-2.34	0.48
201075_s_at	SMARCC1	SWI/SNF related, matrix associated, actin dependent regulator of chromatin, Subfamily c, member 1	-2.34	0.58
218028_at	ELOVL1	Elongation of very long chain fatty acids (FEN1/Elo2, SUR4/Elo3, yeast)-like 1	-2.31	0.42
207826_s_at	ID3	Inhibitor of DNA binding 3, dominant negative helix-loop-helix protein	-2.31	0.45
210183_x_at	PNN	Pinin, desmosome associated protein	-2.30	0.44
212277_at	MTMR4	Myotubularin related protein 4	-2.30	0.58
201040_at	GNAI2	Guanine nucleotide binding protein (G protein), α inhibiting Activity polypeptide 2	-2.28	0.56
213236_at	SASH1	SAM and SH3 domain containing 1	-2.28	0.51
208887_at	EIF3S4	Eukaryotic translation initiation factor 3, subunit 4 δ, 44 kDa	-2.28	0.59
201095_at	DAP	Death-associated protein	-2.26	0.58
213746_s_at	FLNA	Filamin A, α (actin binding protein 280)	-2.23	0.56
204405_x_at	DIMT1L	DIM1 dimethyladenosine transferase 1-like *(S. cerevisiae)*	-2.23	0.61
201862_s_at	LRRFIP1	Leucine rich repeat (in FLII) interacting protein 1	-2.22	0.50
202646_s_at	CSDE1	Cold shock domain containing E1, RNA-binding	-2.20	0.62
218302_at	PSENEN	Presenilin enhancer 2 homolog *(C. elegans)*	-2.19	0.55
209953_s_at	CDC37	CDC37 cell division cycle 37 homolog *(S. cerevisiae)*	-2.19	0.50
202223_at	STT3A	STT3, subunit of the oligosaccharyltransferase complex, homolog A *(S. cerevisiae)*	-2.18	0.55
208685_x_at	BRD2	Bromodomain containing 2	-2.18	0.62
209008_x_at	KRT8	Keratin 8	-2.18	0.51
217802_s_at	NUCKS1	Nuclear casein kinase and cyclin-dependent kinase substrate 1	-2.17	0.57
220272_at	BNC2	Basonuclin 2	-2.16	0.47
214656_x_at	MYO1C	Myosin IC	-2.15	0.57
202024_at	ASNA1	ArsA arsenite transporter, ATP-binding, homolog 1 (bacterial)	-2.15	0.52
212515_s_at	DDX3X	DEAD (Asp-Glu-Ala-Asp) box polypeptide 3, X-linked	-2.12	0.57
202224_at	CRK	V-crk sarcoma virus CT10 oncogene homolog (avian)	-2.12	0.60
218473_s_at	GLT25D1	Glycosyltransferase 25 domain containing 1	-2.11	0.54
208447_s_at	PRPS1	Phosphoribosyl pyrophosphate synthetase 1	-2.11	0.56
221744_at	WDR68	WD repeat domain 68	-2.10	0.51
211177_s_at	TXNRD2	Thioredoxin reductase 2	-2.10	0.57
212783_at	RBBP6	Retinoblastoma binding protein 6	-2.10	0.59
200612_s_at	AP2B1	Adaptor-related protein complex 2, ß1 subunit	-2.09	0.54
208978_at	CRIP2	Cysteine-rich protein 2	-2.07	0.65
204980_at	CLOCK	Clock homolog (mouse)	-2.07	0.66
200641_s_at	YWHAZ	Tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, ς polypeptide	-2.06	0.54
201609_x_at	ICMT	Isoprenylcysteine carboxyl methyltransferase	-2.05	0.59
209897_s_at	SLIT2	Slit homolog 2 *(Drosophila)*	-2.05	0.42
215464_s_at	TAX1BP3	Tax1 (human T-cell leukemia virus type I) binding protein 3	-2.04	0.56
200922_at	KDELR1	KDEL (Lys-Asp-Glu-Leu) endoplasmic reticulum protein retention receptor 1	-2.04	0.58
204094_s_at	TSC22D2	TSC22 domain family, member 2	-2.04	0.61
209440_at	PRPS1	Phosphoribosyl pyrophosphate synthetase 1	-2.04	0.61
201729_s_at	KIAA0100	KIAA0100	-2.03	0.47
204117_at	PREP	Prolyl endopeptidase	-2.03	0.60
200885_at	PPM1J	Protein phosphatase 1J (PP2C domain containing)	-2.03	0.64
200869_at	RPL18A	Ribosomal protein L18a	-2.02	0.63
218384_at	CARHSP1	Calcium regulated heat stable protein 1, 24 kDa	-2.01	0.60
221039_s_at	DDEF1	Development and differentiation enhancing factor 1	-2.01	0.58
218618_s_at	FNDC3B	Fibronectin type III domain containing 3B	-2.01	0.33
200752_s_at	CAPN1	Calpain 1, (mu/I) large subunit	-1.99	0.57
212669_at	CAMK2G	Calcium/calmodulin-dependent protein kinase (CaM kinase) II γ	-1.97	0.62
200008_s_at	GDI2	GDP dissociation inhibitor 2	-1.97	0.64
203931_s_at	MRPL12	Mitochondrial ribosomal protein L12	-1.97	0.64

Table II. Continued.

Probe	Symbol	Description[a]	Score[b]	Fold change[b]
221699_s_at	DDX50	DEAD (Asp-Glu-Ala-Asp) box polypeptide 50	-1.97	0.67
222162_s_at	ADAMTS1	ADAM metallopeptidase with thrombospondin type 1 motif, 1	-1.97	0.59
212363_x_at	ACTG1	Actin, γ1	-1.96	0.70
204275_at	SOLH	Small optic lobes homolog *(Drosophila)*	-1.96	0.62
55705_at	FMN2	Formin 2	-1.95	0.68
32811_at	MYO1C	Myosin IC	-1.95	0.70
217234_s_at	VIL2	Villin 2 (ezrin)	-1.95	0.60
218496_at	RNASEH1	Ribonuclease H1	-1.94	0.58
210658_s_at	GGA2	Golgi associated, γ adaptin ear containing, ARF binding protein 2	-1.949	0.60
210555_s_at	NFATC3	Nuclear factor of activated T-cells, cytoplasmic, calcineurin-dependent 3	-1.93	0.61
215446_s_at	LOX	Lysyl oxidase	-1.91	0.68
201143_s_at	EIF2S1	Eukaryotic translation initiation factor 2, subunit 1α, 35 kDa	-1.90	0.61
210125_s_at	BANF1	Barrier to autointegration factor 1	-1.89	0.61
204875_s_at	GMDS	GDP-mannose 4,6-dehydratase	-1.89	0.64
209391_at	DPM2	Dolichyl-phosphate mannosyltransferase polypeptide 2, regulatory subunit	-1.89	0.62
216602_s_at	FARSLA	Phenylalanine-tRNA synthetase-like, α subunit	-1.88	0.60
40562_at	GNA11	Guanine nucleotide binding protein (G protein), α11 (Gq class)	-1.88	0.64
213509_x_at	CES2	Carboxylesterase 2 (intestine, liver)	-1.88	0.68
201516_at	SRM	Spermidine synthase	-1.88	0.68
218088_s_at	RRAGC	Ras-related GTP binding C	-1.87	0.62
211271_x_at	PTBP1	Polypyrimidine tract binding protein 1	-1.87	0.66
209100_at	IFRD2	Interferon-related developmental regulator 2	-1.85	0.67
208336_s_at	GPSN2	Glycoprotein, synaptic 2	-1.84	0.59
221693_s_at	MRPS18A	Mitochondrial ribosomal protein S18A	-1.83	0.66
208623_s_at	VIL2	Villin 2 (ezrin)	-1.80	0.64
208996_s_at	POLR2C	Polymerase (RNA) II (DNA directed) polypeptide C, 33 kDa	-1.79	0.68
212586_at	CAST	Calpastatin	-1.78	0.73
208991_at	STAT3	Signal transducer and activator of transcription 3 (acute-phase response factor)	-1.78	0.67
201979_s_at	PPP5C	Protein phosphatase 5, catalytic subunit	-1.75	0.66
210978_s_at	TAGLN2	Transgelin 2	-1.72	0.69
201667_at	GJA1	Gap junction protein, alpha 1, 43 kDa (connexin 43)	-1.71	0.72
219068_x_at	ATAD3A	ATPase family, AAA domain containing 3A	-1.71	0.69
208677_s_at	BSG	Basigin (Ok blood group)	-1.69	0.69
218001_at	MRPS2	Mitochondrial ribosomal protein S2	-1.67	0.72
200991_s_at	SNX17	Sorting nexin 17	-1.63	0.73
200638_s_at	YWHAZ	Tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, ζ polypeptide	-1.63	0.73
205178_s_at	RBBP6	Retinoblastoma binding protein 6	-1.62	0.71
211026_s_at	MGLL	Monoglyceride lipase	-1.61	0.74
212131_at	LSM14A	LSM14 homolog A (SCD6, *S. cerevisiae*)	-1.59	0.73
203342_at	TIMM17B	Translocase of inner mitochondrial membrane 17 homolog B (yeast)	-1.47	0.76

[a]The list corresponds to the genes that resulted to be modulated in both PAM and SAM analysis. [b]Score and fold change parameters were obtained by SAM analysis.

regulate transcription, protein modification/biosynthesis and RNA processing/metabolism (Table III). Only five of the down-modulated genes resulted to be involved in the angiogenic process: SDC-4, Id-1 and Id-3, tumor necrosis factor receptor superfamily member 12A (TNFRSF12A) and nuclear factor of activated T-cells C3 (NFATC3) (Tables III and IV). The protein inhibitor of activated STAT3 (PIAS3) was the only up-regulated gene involved in angiogenesis (Table III and IV).

Effect of GPI 15427 on SDC-4 and Id-1 expression. The influence of treatment with GPI 15427 on SDC-4 and Id-1 expression in endothelial cells was further studied at the protein level by Western blot analysis (Fig. 1). The results indicated that SDC-4 protein was strongly reduced (61 to 67% inhibition) in treated cells at short incubation times after drug exposure (2 and 6 h). At later time points, SDC-4 expression was up-regulated during the culture both in untreated or GPI 15427-treated endothelial cells. On the other

Table III. Functional analysis of genes modulated after GPI 15427 treatment of endothelial cells.

Biological process[a]	Genes	Percentage[b]
Regulation of transcription	STAT3 Id-3 SMARCC1 SERTAD2 PNN NFATC3 NR3C1 BNC2 CRK CSDE1 ID1 CLOCK CARHSP1 RPS14 PAWR SCAND1 HMG2L1 TSC22D2 LRRFIP1 ZNF580 PIAS3	19.8
Protein modification	LOX PPP5C DPM2 ICMT PPM1J CAMK2G PSENEN SPOP UBE2M RBBP6 GNA11 MTMR4 STT3A UBE2G2 CTBP1 PRMT1 RNF126 PIAS3	17.0
Protein biosynthesis	MRPL12 DPM2 MRPS18A EIF3S4 EIF2S3 RPL18A PUM1 EIF2S1 RPS14 PAWR STT3A FARSLA EIF4G1 EIF5A MRPS2	14.1
RNA processing and mRNA metabolism	RRAGC PTBP1 PNN DIMT1L SPOP RPS14 PRPF31 MAGOH PCBP2 SF3A2	9.4
Protein targeting	YWHAZ ICMT SEC61A1 TIMM17B CDC37 TIMM44	5.7
Proteolysis	SOLH ADAMTS1 PREP CAPN1 PSENEN UBE2G2	5.7
Angiogenesis	Id-1 Id-3 NFATC3 SDC-4 TNFRSF12A PIAS3	5.7
Actin filament-based process	ACTG1 CRK FLNA FMN2 VIL2 GSN	5.7
Protein folding	PPIB DNAJC7 CDC37 PFDN6	3.8
Glycoprotein metabolism	DPM2 PSENEN PAWR STT3A	3.8

[a]Regulated biological processes were identified by the GEPAS web-based application according to the Gene Ontology Consortium classification. [b]The values indicate the percentage of genes that have been assigned to each biological process among the total number of genes modulated by treatment of endothelial cells with GPI 15427.

Table IV. Genes known to be involved in the angiogenic process that are modulated by treatment of endothelial cells with GPI 15427.

Probe	Symbol	Description	Score[a]	Fold change[a]
203035_s_at	PIAS3	Protein inhibitor of activated STAT, 3	3.21	1.85
202071_at	SDC-4	Syndecan 4 (amphiglycan, ryudocan)	-4.56	0.41
208937_s_at	Id-1	Inhibitor of DNA binding 1, dominant negative helix-loop-helix protein	-4.34	0.22
218368_s_at	TNFRSF12A	Tumor necrosis factor receptor superfamily, member 12A	-2.45	0.43
207826_s_at	Id-3	Inhibitor of DNA binding 3, dominant negative helix-loop-helix protein	-2.31	0.45
210555_s_at	NFATC3	Nuclear factor of activated T-cells, cytoplasmic, calcineurin-dependent 3	-1.93	0.61

[a]Score and fold change parameters were those obtained by SAM analysis.

hand, Id-1 protein expression started to be down-modulated only 6 to 24 h after treatment, with a 55% reduction in treated cells with respect to the untreated control at 30 h. The decrease of Id-1 protein expression occurred later with respect to that of the corresponding mRNA transcription which, instead, was detected 2 h after treatment with the PARP inhibitor. Experiments performed to analyze basal expression of Id-1 in HUV-ST cells indicated that this protein was absent in the cell extracts prepared from cultures grown in medium lacking growth factor supplements (data not shown).

Effect of GPI 15427 on transcription factor activity. Gene functional analysis showed that the majority of modulated genes, including most of those involved in angiogenesis, are under the control of the transcription factor NF-κB, which is known to be regulated by PARP-1 (Table V). Other transcription factors known to be regulated by PARP-1 are indicated in Table V. Among them, Oct-1 regulates 13% of the genes down-modulated by GPI 15427 treatment (including SDC-4), while AP-1 modulates 6.2% of the genes (but none of them related to angiogenesis). Only one gene, affected by

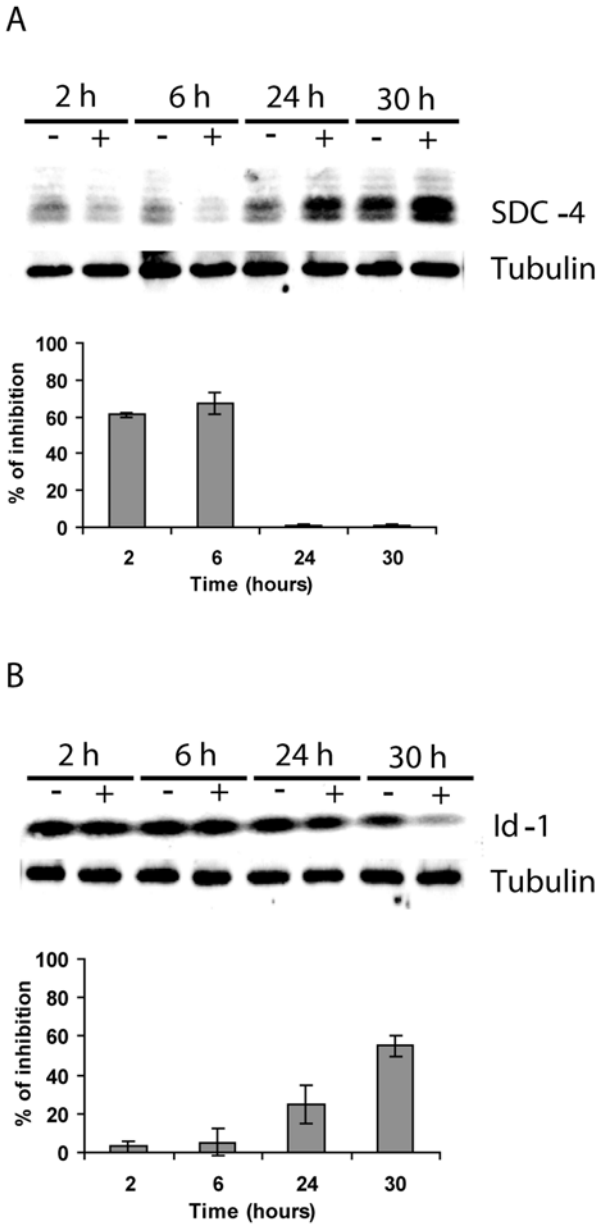

Figure 1. Effect of GPI 15427 on SDC-4 and Id-1 expression. HUV-ST cells, untreated or incubated with 2 μM GPI 15427 for the indicated periods of time, were analyzed for the expression of SDC-4 (A) and Id-1 (B) polypeptides by Western blotting. Blots are representative of 1 out of 3 independent experiments with similar results. SDC-4 and Id-1 proteins were quantified by densitometric analysis followed by normalization against the ß-tubulin content of the samples. Histograms represent the mean (± SD) percentage inhibition of SDC-4 or Id-1 expression in GPI 15427-treated cells with respect to untreated control using the data from 3 independent experiments.

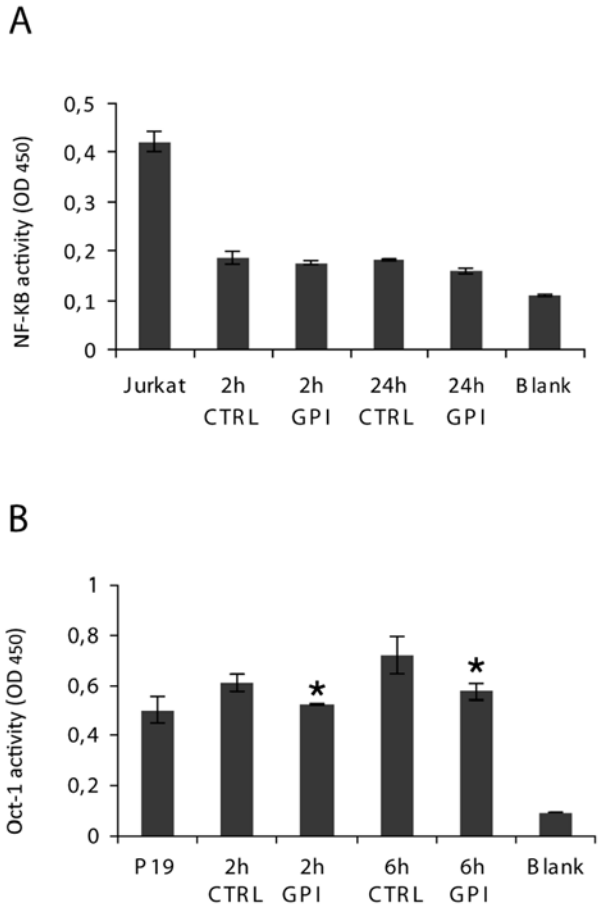

Figure 2. Effect of GPI 15427 on NF-κB and Oct-1 transcription factors. Nuclear extracts prepared from HUV-ST cells, untreated or incubated with 2 μM GPI 15427 for the indicated periods of time, were analyzed for the activity of NF-κB (A) or Oct-1 (B) as described in Materials and methods using a TransAm kit. Data are representative of 1 out of 3 independent experiments with similar results. Bars indicate the SD of the mean. A) Statistical analysis of NF-κB activity in control versus GPI 15427- treated groups by Student's t test: not significant. B) Statistical analysis of Oct-1 activity in control versus GPI 15427-treated groups by Student's t test, $^{*}P<0.05$.

treatment with the PARP inhibitor, but not related to angiogenesis, is under the control of HIF-1. Interestingly, the members of the activating transcription factor family c-AMP response element binding protein (CREB)/ATF-1 and ATF-3 regulate the transcription of Id-1 (Table V).

The activity and/or expression of transcription factors that might be involved in the effect of GPI 15427 on the angiogenic capabilities of human endothelial cells were tested. The results indicated that in the endothelial cells used for the present study the NF-κB activity was very low and was not modulated by the PARP inhibitor (Fig. 2A). In the conditions tested, Oct-1 activity resulted to be only slightly, but significantly, down-modulated (up to 16%) by treatment with the inhibitor for 2 or 6 h (Fig. 2B). Oct-1 protein is abundantly produced in HUV-ST cells, but its expression was not substantially modulated by GPI 15427 treatment (data not shown), suggesting that PARP-1 could directly modulate Oct-1 activity.

Analysis of CREB/ATF-1 levels and phosphorylation showed that both proteins were modulated during the period of culture analyzed, being barely detectable few hours after the beginning of culture and up-regulated at later time points. In the case of ATF-1, protein and phosphorylation levels were almost unaffected by the drug treatment (Fig. 3). On the other hand, up-regulation of CREB expression was blocked by the PARP inhibitor and this effect was paralleled by a reduced phosphorylation of the protein. In regard to ATF-3, no changes in the level of protein expression were observed and a modest decrease of phosphorylation of this transcription factor was detected in the first 2 h (data not shown).

Table V. Genes modulated after GPI 15427 treatment of endothelial cells that are under the control of specific transcription factors regulated by PARP-1.

Transcription factors[a]	Genes	Percentage[b]
NF-κB	FNDC3B MYO1C GLT25D1 GNAI2 Id-3 PLD3 PPP5C WDR68 GPSN2 NUCKS1 NFATC3 NR3C1 BNC2 SEC61A1 DIMT1L GDI2 SDC-4 EIF2S3 CAMK2G PRPS1 CAPN1 PSENEN FMN2 UBE2M ARF1 DDX50 CDC37 MTMR4 FARSLA CXADR EIF4G1 PRMT1 DAP TSPAN4 HMG2L1 NDUFB7 TXNRD2 SERF2 HLA-C GSN PIAS3 HLA-B	28.8
Oct-1	STAT3 SETD5 RHOBTB3 SMARCC1 MRPS18A CRK SDC-4 EIF2S3 PRPS1 CLOCK SRM BANF1 SCAND1 PRMT1 HMG2L1 NDUFB7 SLC29A1 TSC22D2 GSN	13.0
AP-1	GMDS BNC2 IFRD2 CAMK2G PRPS1 KIAA0100 SPOP TIMM44 RAB3B	6.2
CREB/ATF-1	GLT25D1 STAT3 Id-1 AP2B1 RBBP6 CDC37 DAP TSC22D2	5.5
ATF3	STAT3 IFRD2 Id-1 AP2B1 RBBP6 DAP TSC22D2	4.8
ATF4	SOLH STAT3 Id-3 AP2B1 TIMM44 NDUFB7 TSC22D2	4.8
ATF6	STAT3 RBBP6 CTBP1	2.0
HIF-1	CDT1	0.7

[a]Transcription factors were identified by the GEPAS web-based application. [b]The values indicate the percentage of genes, whose expression is regulated by each transcription factor with respect to the total number of genes modulated by GPI 15427 treatment of endothelial cells.

Discussion

Following our recent publication on the role of PARP-1 in angiogenesis and on the impairment of endothelial functions mediated by PARP inhibition with GPI 15427 (10), we investigated the early molecular mechanisms involved in this effect analyzing the modulation of gene expression in endothelial cells treated with non-toxic concentrations of the PARP inhibitor. In the present study we demonstrate, for the first time, that SDC-4 and Id-1 proteins, which are both involved in angiogenesis, are down-regulated by GPI 15427 treatment.

Treatment of endothelial cells with the PARP inhibitor for 2 h provoked a prompt decrease of SDC-4 both at the transcript and protein level. SDC-4 is the most ubiquitously expressed member of a transmembrane heparin sulfate proteoglycans family that binds to and can be activated by insoluble ligands, such as extracellular matrix molecules, and soluble ligands, including growth factors (21). Moreover, SDC-4 is activated by angiogenic factors and is involved in endothelial cell adhesion and migration (22,23). The effect of the treatment with the PARP inhibitor on SDC-4 expression in endothelial cells was transient, since 24 h after treatment the decrease of this protein was no longer detectable. Therefore, it is possible to hypothesize that SDC-4 protein might be required in the initial steps of the angiogenesis process, such as the triggering of endothelial cell migration from the pre-existing vessel, that is compatible with the known role of this protein in endothelial cell adhesion and migration (22,23).

The Id-1 protein was down-regulated by GPI 15427 at a later time point with respect to SDC-4. Id-1 is a member of the helix-loop-helix family of proteins that does not contain DNA binding sequences but prevents transcription factors from binding to DNA by direct physical interaction (24). Id-1 plays a key role in regulating angiogenesis, including that associated with tumor development (25). The expression of this protein can be induced in endothelial cells by VEGF and TGF, and its overexpression enhances I-CAM-1 and E-selectin expression, and provokes transmigration, matrix metalloproteinase production and tube formation (26). Knockdown of Id-1 in endothelial cells abolished proliferation and angiogenic processes induced by VEGF (26). Induction of the Id-1 protein by VEGF supports the hypothesis that the previously observed effects of GPI 15427 on VEGF-induced angiogenic processes (10) might indeed involve this protein.

A short exposure of endothelial cells to the PARP inhibitor resulted in transcription down-regulation of a limited number of genes known to play a role in angiogenesis. While SDC-4 and Id-1 were found to be the mostly affected genes, the other genes found to be inhibited were TNTRSF12A, a member of the TNF receptor family which interacts with TNF-like weak inducer of apoptosis (TWEAK) (27), Id-3, another member of the helix-loop-helix family of proteins (25,26) and NFATC3, a transcription factor belonging to a protein family controlled by calcineurin which regulates vascular development during angiogenesis (28). Among the up-regulated genes, PIAS3 was the most significantly up-regulated. PIAS3 is a negative

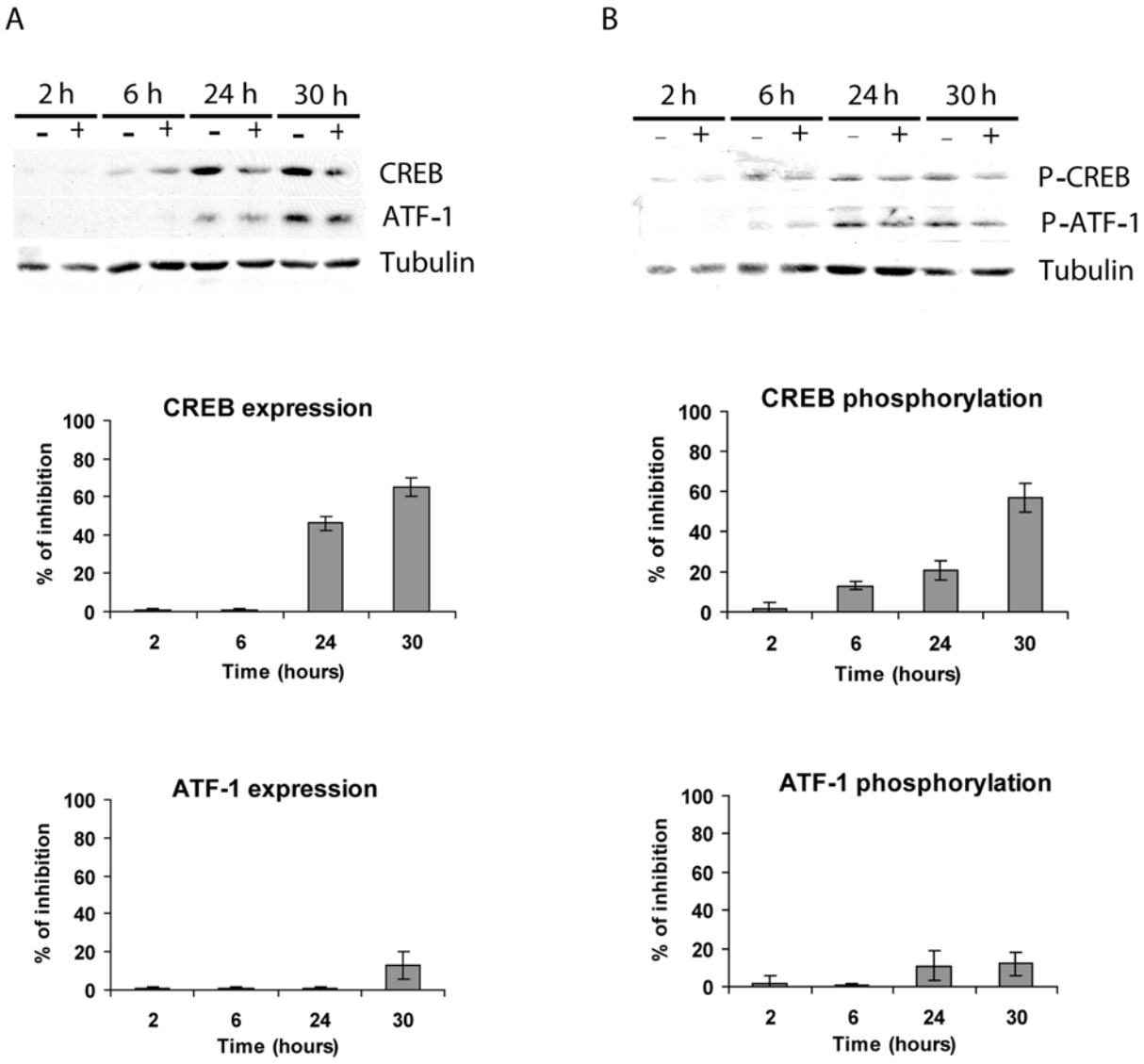

Figure 3. Effect of GPI 15427 on CREB and ATF-1 activation. Cell extracts were prepared at the indicated time points and analyzed by Western blotting for the presence of total (A) or phosphorylated (B) CREB and ATF-1 proteins. Results were quantified by densitometric analysis as indicated in the legend of Fig. 1. Blots are representative of 1 out of 3 independent experiments with similar results and histograms represent the mean (± SD) percentage inhibition of the protein or phosphoprotein analyzed in GPI 15427-treated cells with respect to untreated control.

regulator of STAT3, a transcription factor involved in VEGF expression (29,30). However, in HUV-ST cells or in HUVEC the levels of VEGF released by the cells was extremely low and was not modulated by GPI 15427 treatment (10).

Microarray analysis revealed a prevalent down-modulation of gene expression, the highest percentage of which is involved in regulation of gene transcription. It is well known that PARP-1 regulates transcription by at least two different mechanisms: modulating chromatin structure and acting as part of gene-specific enhancer/promoter-binding complexes (31). This activity is accomplished by PARP-1 through a direct protein-protein interaction or through covalent poly(ADP-ribosyl)ation of the target molecule (32). For instance, poly(ADP-ribosyl)ation of histones loosens chromatin and renders genes accessible to the transcriptional machinery. Moreover, non-covalent interaction of histones with poly (ADP-ribose), as free polymer or attached to proteins, may also allow release of histones from nucleosome rendering DNA accessible for transcription (32-34). Even though in cells not subjected to DNA damage the average length of ADP-ribose polymers is shorter than in cells exposed to genotoxic injury, ADP-ribose polymers may still participate to the regulation of several functions also in intact cells. In addition, PARP-1 interacts with a number of transcription factors such as NF-κB, p53, Oct-1, B-MYB, AP-1, HIF-1, acting as transcriptional co-regulator (32-37). It is likely that the reduction of gene transcription induced by GPI 15427 might be the result of a decrease of cellular ADP-ribose polymers content rather than of compromised protein interactions with PARP-1. In fact, similarly to the other PARP inhibitors presently available, GPI 15427 targets the NAD^+ binding domain of the enzyme hampering the use of NAD^+ as substrate for the synthesis of ADP-ribose polymers (16).

Gene functional analysis, performed according to the Gene Ontology Consortium classification, showed that about 28% of the genes (including Id-3, NFATC3, SDC-4 and PIAS3 which are involved in angiogenesis) modulated by GPI 15427 treatment of endothelial cells were under the control

of NF-κB. Even though modulation of NF-κB by PARP-1 presumably requires direct protein-protein interaction, it has been shown that NF-κB is capable of binding poly(ADP-ribose), indicating that its activity may be regulated also by non-covalent interaction with the polymer (38). Nevertheless, our results showed that in the endothelial cell model used NF-κB activity was barely detectable and was not modulated by treatment with the PARP inhibitor GPI 15427. Also HIF-1α was not found to be modulated by GPI 15427 in this model of endothelial cells (10). Gene functional analysis showed that the transcription of 13% of down-regulated genes (including SDC-4) is controlled by the octamer trans-acting factor Oct-1, a transcription regulator capable of interacting with PARP-1, which stabilizes its binding to the DNA octamer motif (39). This transcription factor was expressed by HUV-ST cells and its activity was slightly down-modulated by GPI 15427 in the assay used. Nevertheless, it cannot be excluded that the drug-induced reduction of Oct-1 activity might be underestimated. In fact, it is likely that the physical interaction between Oct-1 and PARP-1 might not be fully maintained during the preparation of the nuclear extracts or during the *in vitro* activity assay.

PARP-1 has also been involved in the modulation of the ATF family of transcription factors, favoring phosphorylation of these proteins (40). Functional analysis of genes modulated by GPI 15427 in endothelial cells indicated that members of the ATF protein family (ATF-1, CREB and ATF-3) regulate Ids gene expression. In particular, it has been shown in normal human keratinocytes that Ids transcription can be modulated through *cis*-acting elements in the distal portion of the promoter, including a CREB-binding site (41). Therefore, it is possible that the strong down-modulation of activated CREB protein after 6 h of treatment would contribute to the reduction in Id-1 protein expression. The delayed decrease of Id-1 protein with respect to the prompt reduction of mRNA expression might depend on the half-life of the previously synthesized protein. As described by Ling and colleagues, the half-life of Id-1 protein is quite variable depending on cell type (42). We observed that the increased stability of the protein appears to be a characteristic of endothelial cells maintained in medium with a mix of growth factors (bFGF, VEGF, EGF and IGF-1) (data not shown), some of which are known to sustain Id-1 expression (26). Finally, the modest decrease of ATF-3 phosphorylation detected 2 h after treatment is unlikely to account for the reduction of Id-1 transcript since ATF-3 has been found to be a negative regulator of this protein (43). Therefore, the initial inhibition of Id-1 transcript during the first 2 h of treatment with the PARP inhibitor might be due to the effect of the drug on other still undefined molecular mechanisms.

Previous studies have analyzed the effects of PARP-1 deficiency on gene expression at the genome wide-level, using murine PARP-$1^{-/-}$ embryonic stem cell lines and cells of different tissue origin (37,44,45) or cells derived from PARP-$1^{+/+}$ mice treated with a PARP inhibitor (37). The lack of PARP-1 expression was associated with both down-regulation and up-regulation of genes involved in a variety of processes such as metabolism, signal transduction, cell cycle control and transcription, which varied depending on the cell system investigated (44). In skin-derived cells obtained from P-$1^{-/-}$ mice or from PARP-$1^{+/+}$ mice treated with the PARP inhibitor 3,4-dihydro-5-[4-(1-piperidinyl)butoxyl]-1(2H)-isoquinolinone (DPQ), differences were found in genes involved in carcinogenesis and inflammation with respect to controls (37). In regard to endothelial cells, gene expression profiles in response to TNF-α treatment were investigated in cells derived from the heart vasculature of PARP-$1^{-/-}$ mice and the absence of PARP-1 was found to hamper the expression of genes up-regulated during the inflammatory process (45). However, since no studies have been performed to date on human endothelial cells, it is difficult to make comparisons between our results and the previously published studies, due to the differences in the species, tissue origin and stimuli used.

In conclusion, treatment of human endothelial cells with the potent PARP inhibitor GPI 15427 induced a strong down-modulation of at least two gene products that play an important role in endothelial functions: SDC-4 and Id-1. It can be hypothesized that the mechanism by which GPI 15427 exerts the inhibitory effect on angiogenesis (10), might be ascribed to a reduced PARP-mediated regulation of the activity of certain transcription factors that control endothelial functions. In this context, inhibition of Oct-1 and CREB/ATF family of transcription factors might be crucial in the down-modulation of molecules involved in endothelial cell migration, adhesion, proliferation or differentiation during the angiogenic process.

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