Modulation of Transcription by PARP-1: Consequences in Carcinogenesis and Inflammation

R. Aguilar-Quesada1, J.A. Muñoz-Gámez2, D. Martín-Oliva3, A. Peralta-Leal1, R. Quiles-Pérez4, J.M. Rodríguez –Vargas3, M. Ruiz de Almodóvar2, C. Conde5, A. Ruiz-Extremera4 and F.J Oliver1

1Instituto de Parasitología y Biomedicina López Neyra, CSIC, Granada
2IBIMER, Universidad de Granada
3Dpto. de Biología Celular, Universidad de Granada
4Hospital Universitario San Cecilio, Granada
5Hospital Xeral de Galicia, Santiago de Compostela

Abstract: Post-translational modification of proteins by poly(ADP-ribosyl)ation is involved in the regulation of a number of biological functions. While an 18 member superfamily of poly(ADP-ribose) polymerase (PARP) has been described PARP-1 accounts for more than 90% of the poly(ADP-ribosyl)ating capacity of the cells. PARP-1 has been studied as a DNA nick sensor and is activated by DNA breaks to cleave NAD+ into nicotinamide and ADP-ribose to synthesize long branching poly(ADP-ribose) polymers (PAR) covalently attached to nuclear acceptor proteins. Whereas activation of PARP-1 by mild genotoxic stimuli facilitates DNA repair and cell survival, severe DNA damage triggers different pathways of cell death including PARP-mediated cell death through the translocation of apoptosis inducing factor (AIF) from the mitochondria to the nucleus. PAR and PARP-1 have also been described as having a function in transcriptional regulation through their ability to modify chromatin-associated proteins and as a cofactor of different transcription factors, notably NF-κB and AP-1. Pharmacological inhibition or genetic ablation of PARP-1 not only provides remarkable protection from tissue injury in various oxidative stress-related disease models but it results in a clear benefit in the treatment of cancer by different mechanisms including selective killing of homologous recombination-deficient tumor cells, down regulation of tumor-related gene expression and decrease in the apoptotic threshold in the co-treatment with chemotherapeutic drugs and radiotherapy. We will summarize in this review the current findings and concepts for the role of PARP-1 and poly(ADP-ribosyl)ation in transcription, oxidative stress and carcinogenesis.

INTRODUCTION

PARP-1 is the founding member of the PARP family that contains as many as 18 distinct proteins in humans [1]. PARP-1 is an abundant nuclear protein found in most eukaryotes apart from yeast. It binds to DNA strand breaks and concomitantly synthesizes oligo- or poly(ADP-ribose) chains using NAD+ as substrate and covalently couples to various acceptor proteins or to itself resulting in the attachment of linear or branched polymer of poly(ADP-ribose). PARP-1’s full activation is strictly dependent on the presence of strand breaks in DNA and is modulated by the level of automodification [1, 2] (Fig. (1)).

PARP family members share a conserved catalytic domain that contains the "PARP signature" motif, a highly conserved sequence (100% conserved in PARP-1 among vertebrates) that forms the active site [1]. Some PARP family members identified solely on homology, however, have not yet been shown to possess intrinsic PARP enzymatic activity [3]. In addition to a catalytic domain, PARP family members typically contain one or more additional motifs or domains, including zinc fingers, "BRCA1 C-terminus-like" (BRCT) motifs, ankyrin repeats, macro domains, and WEE domains (a protein–protein interaction motif), conferring specific properties to the different PARP members [4].

PARP-1 has a highly conserved structural and functional organization including an N-terminal double zinc finger DNA-binding domain (DBD) [1], a nuclear localization signal [2], a central automodification domain [3], and a C-terminal catalytic domain [4] (Fig. (2)). PARP-1’s basal enzymatic activity is very low, but is stimulated dramatically in the presence of a variety of allosteric activators, including damaged DNA, some undamaged DNA structures, nucleosomes, and a variety of protein-binding partners [3, 5]. The targets of PARP-1’s enzymatic activity include PARP-1 itself, which is the primary target in vivo, core histones, the linker histone H1, and a variety of transcription-related factors that interact with PARP-1 [3, 5-7] (Fig. (1)).

The DNA binding domain contains a repeated sequence (residues 2-97 and 106-207) in which 35 amino acids are duplicated. Interestingly these residues are strictly conserved during evolution and are crucial for DNA interaction. These crucial residues for DNA-binding consist in two zinc finger residues (FI and FII) [8] (Fig. (2)). The automodification domain of PARP-1 is rich in glutamic acid residues, consistent with the fact that poly(ADP-ribosyl)lation occurs on such residues. This domain also comprises a BRCT motif that is present in many DNA damage repair and cell-cycle checkpoint proteins [5, 8-9]. Globally, the structure and activities of PARP-1 suggest important roles for this in a variety of cell functions. The activities and functions of the other PARP family members have not been studied to the same extent as PARP-1, although a clearer picture for some of the PARP family members has been emerging, as noted below and reviewed in more detail elsewhere [1, 4].

PARP in Genome Integrity

Although the most recent findings challenge the concept that the obligatory trigger of PARP-1 activation are the nicks and breaks in the DNA strand [4], this stimulus remains the most studied and well known. DNA damage can be induced by a variety of environmental stimuli including free radical oxidation, alkylatation’s, and ionizing radiation. The binding of PARP-1 to damaged DNA, including single-strand breaks (SSBs) and double-strand breaks (DSBs), through its double zinc finger DNA-binding domain potently activates PARP-1 enzyme activity (as much as 500-fold) [10, 11]. As such,
Fig. (1). DNA damage induces PARP-1 activation. A number of genotoxic agents that produces DNA strand breaks activates PARP-1 leading to poly(ADP-ribose) polymer formation from NAD+ and consequent covalent modification by poly(ADP-ribosyl)ation of different nuclear acceptors proteins.

PARP-1 can function as a DNA damage sensor (Fig. (1)). With low levels of DNA damage, PARP-1 acts as a survival factor involved in

DNA damage detection and repair. In contrast, with high levels of DNA damage, PARP-1 promotes cell death [12]. PARP-1 has been implicated in multiple DNA repair pathways, including the SSB, DSB, and base excision repair (BER) pathways [13]. PARP-1 interacts physically and functionally with various proteins involved in these DNA repair pathways, and may recruit the repair proteins to sites of DNA damage [14]. PAR itself, as a covalent attachment of automodified PARP-1, may also act to recruit repair proteins to sites of DNA damage.

PARP-2, the only other PARP enzyme whose catalytic activity is known to be stimulated by damaged DNA, has also been implicated in BER through interactions with XRCC-1 and PARP-1 [15, 16].

The DNA damage response is currently viewed as a signal-transduction pathway involving sensors that activates signal transducers upon detection of damaged DNA. These transducers in turn modulate the activity of effectors that redirect cellular functions while the damage is being repaired. Cellular responses to genomic insults include activation of DNA repair pathways, cell cycle arrest, and initiation of cell death processes [17]. A function of PARP-1 as a nick sensor has been proposed [18]. Its rapid activation in response to DNA strand breaks may result in the poly(ADP-ribosyl)ation of key enzymes such as transducers of the DNA damage. Alternatively, PARP-1 auto-poly(ADP-ribosyl)ation could result in the recruitment of transducers to the damaged site (Fig. (1)). How PARP and poly(ADP-ribosyl)ation participate in the initial cell’s response to DNA damage and their interaction with key players of this pathway (such as p53 and ATM) has been extensively reviewed previously [13].

Fig. (2). Structural domains of human PARP-1. DNA binding domain (DBD) contains two zinc finger structures (FI and FII), the nuclear localization signal (NLS) and caspases -3 and -7 cleavage site; Automodification domain contains BRCT (BRCA1 like C-terminal) motif and Leucine Z ipper motif (LZ); and C-terminal Catalytic domain. Also, the interactions of PARP domains with others proteins are showed.
In the present review we will focus on different aspects of PARP’s role in the regulation of transcription and the consequences in carcinogenesis and inflammation.

PARP-1 AND TRANSCRIPTION

A very important aspect of PARP-1 is its involvement in the modulation of chromatin structure and transcription [17]. PARP-1’s enzymatic activity is stimulated dramatically by the binding of PARP-1 to damaged DNA and hence, most studies of PARP-1 have focused on its role in DNA repair and cell death pathways [4]. Considerably less is known about the chromatin-dependent gene regulatory activities of PARP-1 under physiological conditions where the integrity of the genome is maintained. As mentioned above, the PARP-1’s literature is replete with the view that

PARP-1 enzymatic activity is strictly dependent on damaged DNA as an allosteric activator [5]. However, studies identifying other allosteric activators, including certain undamaged DNA structures [18] and PARP-1 binding proteins [19], have challenged this view. Nucleosomes, for example, are potent activators of PARP-1 auto(ADP-ribosyl)ation, more than damaged DNA [20].

Two modes of PARP-1 regulatory activity of transcription have been proposed: (1) a histone-modifying enzymatic activity that can regulate chromatin structure and (2) an enhancer/promoter binding cofactor activity that can act in conjunction with other transcription-related factors [17] (Fig. (3)). In summary, PARP-1 can function both as a structural component of chromatin and as a modulator of chromatin structure through an intrinsic enzymatic activity.

PARP-1-Dependent Histone-Modifying Activity and Transcription

PARP-1 exerts its effects in modulating chromatin by directly (ADP-ribosyl)ating core histones and chromatin associated proteins, thereby promoting the dissociation of nucleosomes and the decondensation of chromatin [5, 17, 21, 23]. Although it cannot be excluded that trans-modification is not necessary for PARP-1-dependent regulation of chromatin structure.

PARP-1 is an abundant nuclear protein supporting the idea that either itself or PARP-1-related proteins (with functional redundancy) can function as a structural component of chromatin in vivo [22]. In this model, PARP-1, when incorporates into compact transcriptionally repressed chromatin structures, is poised for NAD+-dependent activation, automodification, and subsequent release from chromatin, facilitating chromatin decondensation and transcription by Pol II [22]. DNA binding transcriptional activators could provide the trigger for PARP-1 by recruiting NAD+-synthesizing enzymes. Autophosphorylation of PARP-1 is acutely sensitive to small changes in ATP concentration. Thus, the numerous transcription-related factors that PARP-1 and transcriptional activators supporting the idea that either itself or PARP-1-related proteins (with functional redundancy) can function as a structural component of chromatin in vivo. In this model, PARP-1, when incorporated into compact transcriptionally repressed chromatin structures, is poised for NAD+-dependent activation, automodification, and subsequent release from chromatin, facilitating chromatin decondensation and transcription by Pol II. DNA binding transcriptional activators could provide the trigger for PARP-1 by recruiting NAD+-synthesizing enzymes. Autophosphorylation of PARP-1 is acutely sensitive to small changes in ATP concentration. Thus, the numerous transcription-related factors that consume ATP have the potential to reduce local ATP concentrations and increase PARP-1 enzymatic activity. On the other hand, the incorporation of PARP-1 protein into chromatin promotes the formation of higher-order chromatin structures that localize to discrete chromatin domains in vivo and this incorporation has a repressive effect on Pol II transcription in vitro [20].

A specific example of histone-modifying PARP-1 activity is puff formation in Drosophila polytene chromosomes, which presents PARP-1-dependent accumulation of PAR at decondensed, transcriptionally active loci [22]. PARP protein is widely distributed in Drosophila polytene chromosomes but is normally inactive. However, upon exposure to a heat shock stimulus, PARP accumulates rapidly at heat shock gene loci,

Fig. (3). Models of regulation of transcription activity by PARP-1. Two modes of PARP-1-dependent transcription regulation have been proposed. First, a histone-modifying by PARP-1 that can regulate chromatin structure; second, a transcriptional cofactor PARP-1 activity can enhancer the transcription with other transcription-related factors (adapted of Krauss WL and Lis JT, 2003).
where it develops intense poly(ADPribosyl)ation activity. Similarly, PARP accumulates at sites of edcsyone-induced puffs in polytene chromosomes of fruit fly larvae shortly before pupation. Inactive PARP is recruited, presumably by certain transcription factors, to target genes where it becomes activated. PARP then adds long ADP-ribose tails to the histone proteins of nucleosomes around which the DNA is wrapped. Nucleosomes containing poly-ADP-ribosylated histones are unable to remain tightly packed, resulting in “loosening” or decondensation of the chromatin. In vitro experiments suggest that transcription is initially facilitated by PARP, but as soon as transcription factors dissociate from the DNA, they too become inactivated through poly-ADP-ribosylation, thus preventing repeated cycles of transcription [23]. In this way, PARP ensures a strong but transient transcriptional response to a heat shock or edcsyone stimulus. Ultimately, PARP poly-ADP-ribosylates itself and dissociates from the DNA. The mechanism of PARP action seems adapted to facilitate sudden bursts of transcriptional activity in response to transient environmental signals [24].

Further support for the importance of PARP-1-mediated poly(ADP-ribosyl)ation of chromatin modulators in the regulation of DNA-dependent processes [25] is the functional link between PARP-1 and FACT. FACT (facilitates chromatin transcription) is a heterodimer composed of hSpt16 and SSRP1 [27] that allows RNA polymerase II to proceed along the chromatin template. Biological functions of FACT are regulated by poly(ADP-ribosylation) [26] and in this way, both, PARP-1 and FACT, are involved in the global regulation of chromatin architecture. hSpt16 but not SSRP1 is poly(ADP-ribosylated) in vivo especially following genotoxic stress, and additionally, there is a direct interaction between hSpt16 and PARP-1 [26]. Nucleosome-binding activity of hSpt16 (and FACT) is decreased after poly(ADP-ribosylation). Whether poly(ADP-ribosylated) FACT dissociate from nucleosome through the disruption of its interaction with the histones remains to be determined. These results suggest that pol II dependent transcription may be regulated through the modulation of chromatin-binding property of FACT by PARP-1 [28].

A recent report of Ju et al. [29] show that estrogen-dependent transcription of pS2 requires a promoter intermediate containing a double-strand break (DSB) that is generated by a protein complex containing topoisomerase II (TopoIIβ) and PARP-1. TopoIIβ and PARP-1 collaborate in an interesting way that alters the molecular composition and structure of the pS2 promoter during an estradiol (E2)-dependent transcriptional response. E2 not only rapidly induces an increase in binding of estrogen receptor α (ERα) to the promoter but also causes a concomitant rapid exchange of the co-repressors for what appears to be an activation complex containing PARP-1 and TopoIIβ. Both PARP-1 and TopoIIβ enzymatic activities are critical for the activation of pS2 transcription, although the definitive target of this PARP-1 enzymatic activity has yet to be determined [30]. Prior to treatment with E2, PARP-1 is associated with three adjacent nucleosomes in the promoter region (NucE and a nucleosome on each side, NucU and NucT), possibly acting as part of a repression complex [29] or as a direct nucleosome binding factor [20]. Upon estrogen treatment, PARP-1 departs from NucU and NucT, perhaps as a consequence of auto poly(ADP-ribosyl)ation, resulting in a loss of nucleosome binding activity [20]. In addition, a PARP-1/TopoIIβ activation complex containing the nuclear receptor coactivator ASC2 becomes concentrated on NucE. The factor dynamics on the three nucleosomes are accompanied by changes in chromatin structure. H1 is lost from NucE and is replaced with HMGB1/2, a non histone structural protein. H1 could be a target for poly(ADP-ribosyl)ation by PARP-1, causing its release. Alternatively, PARP-1’s ability to compete with H1 for binding to nucleosomes could function in the dissociation of H1 from NucE [20]. In this system, a transient DSB occurs in promoter DNA adjacent to a nucleosome containing the DNA binding sequence for Estα (NucE) and the formation of this DSB requires TopoIIβ enzymatic activity and participates in the subsequent exchange of factors at the promoter. A possible role for the TopoIIβ-dependent DSB in stimulating PARP-1 enzymatic activity has yet to be addressed. In addition to pS2, Ju et al. [29] observed recruitment of TopoIIβ and PARP-1 and other components of the complex to the PSA, RARβ, Diol1, and MMP12 promoters upon gene activation, as well as promoter cleavage.

PARP-1 as an Enhancer/Promoter Binding Cofactor

With respect to the second mode of PARP-1 regulatory activity, the role of PARP-1 in transcription is well established with several independent studies revealing its potent effect on activators like AP-2 (activator protein 2), p53, NF-κB, B-Myb, TEF-1/Max, SP-1, YY-1 and STATs [31-40]. PARP-1 modulates the activity of key transcription factors involved in tumor promotion such as AP-1 (whose defective activation by either PARP inhibition or genetic deletion of PARP-1 results in an effective blockage of gene expression, [41]) and HIF-α [70]. In addition, PARP-1 was identified among the constituents of positive cofactor-1 complex [42], essential for the activity of transcription factors such as NF-κB, Sp1 and Oct-1. Nevertheless, the exact mechanism by which PARP-1 affects transcription lacks clarity and the ambiguity is evident in some cases.

PARP-1 has been shown to play different roles on transcription factors, depending on the presence of specific binding partners, the proliferative status of the cell, the concentration of NAD+ and the presence of DNA strand breaks. In the presence of NAD+, PARP-1 dependent silencing of transcription involves poly(ADP-ribosylation) of specific transcription factors like p53 and fos [43, 44], which prevents both their binding to the respective DNA consensus sequences and the formation of active transcription complexes [23, 45-48], reporting a negative role for PARP-1 in transcription regulation. Direct interaction of PARP-1 protein with its own gene promoter resulted in suppression of transcription [48]. However, in response to DNA damage, PARP-1 catalytic activity was stimulated and automodification of PARP-1 subsequently prevented its interaction with the promoter. This relieved the PARP-mediated block on the promoter and allowed for transcription of PARP-1 and other genes suppressed by PARP-1. In the absence of NAD, PARP-1 promotes activator-dependent transcription by interacting with RNA polymerase II-associated factors [46, 49], transcription enhancer factor 1 (TEF1) and an increasing number of transcription factors, including AP-2, B-Myb, YY-1, Oct 1, NF-κB, and p53 [32, 33, 36, 46, 50-53]. Other example is HSF-1, which requires nuclear presence of PARP-1, but not its catalytic activity, for the DNA binding. On the other hand, PARP-1, either alone or in a heterodimeric complex with Ku protein, has been shown to specifically bind internal sequences of matrix attachment regions (MARs) [54] that are required for extending chromatin domains and enhancer-distal positions accessible to transcription factors.

PARP-1 may have a dual regulatory role with opposing effects and it is possible that PARP-1 studies on some transcription factors had characterized and interpreted only one of the two effects. The case of AP-2α-dependent transcription [55] is an example of dual regulation, where the C-terminal enzymatic domain of PARP-1 strongly interacts with AP-2α to poly(ADP-ribosyl)ate it affecting negatively its DNA binding and thereby its transcriptional activation. However, the low-affinity interaction of the middle region has an enzymatic
activity-independent positive effect on AP-2α transcription and it is possible that PARP-1 connect AP-2α to the general transcriptional machinery. Griesenbeck et al. [56] revealed that automodification of PARP-1 plays a crucial role in choosing partners to interact with. It is possible that the state of automodification determines the time of interaction with AP-2α and histones. The structural overlap of the automodification region with the co-activator domain may represent an important built-in regulatory mechanism. This may also explain the existence of a weaker interaction of AP-2α with this region. In view of this, PARP-1 has an important biological function beyond its enzymatic activity and warrants a new look at this molecule as a multifaceted protein rather than as one with a single catalytic function with multiple effects.

On the contrary, PARP-1 does not exert a dual effect on E2F-1 transcriptional activation because E2F-1 is not a substrate for modification by PARP-1 [57]. PARP-1 binds E2F-1 through the automodification domain of PARP-1 and together, as a complex, augments binding to the E2F-1 promoter region and expression of E2F-1-responsive genes (including E2F-1 itself) [58] thus verifying that PARP-1 acts as a positive co-activator of E2F-1-mediated transcription. That PARP-1 neither binds internal sequences of the E2F-1 promoter nor modifies E2F-1 by poly(ADP-riboseylation) is consistent with the fact that PARP-1-E2F-1 binding does not require the DNA-binding domain or the catalytic active site of PARP-1. PARP-1 also enhances the transactivation of B-Myb independently of PARP-1 enzymatic activity [33]. Given that binding sites for E2F-1 are also present in b-myb promoters [59], it is possible that, in addition to direct binding of PARP-1 to B-Myb, PARP-1 can induce b-myb transcription by its coactivation of E2F-1. In this case, PARP-1 does not play a direct role in the transcription of E2F-1-responsive genes by binding to internal E2F-1 promoter sequences and acting as a transcription factor itself, unlike its sequence-specific interaction with other DNA elements such as MCAT1 elements, the Reg gene promoter, the IUR element in the CXCL1 gene, and HTLV-1 TxREs [46, 60-62]. Within the CXCL1 promoter, the IUR element binds PARP-1 [61]. In normal melanocytes, PARP-1 activity is silent, leading to binding of PARP-1 to the promoter of CXCL1 and preventing NF-kB from binding to the promoter. However, in cancer cells exhibiting bioenergetic malfunction, this balance is shifted, resulting in more auto-poly(ADP-riboseylation) of PARP-1, dissociating PARP-1 from the promoter and allowing for an increased binding of NF-kB to the promoter and activated transcription. Here, aberrant activation of PARP-1 in melanoma cells regulates the transcriptional activity of NF-kB. Thus, it appears that the physical interaction of PARP-1 with the CXCL1 promoter asserts a negative effect in transcription, whereas the activity of PARP-1 is important in promotion of CXCL1 transcription. PARP-1 regulates CXCL1 gene expression both negatively and positively, once more having a dual role as a transcriptional modulator [63], where a fine balance exists between the inactive and active state of PARP-1. Moreover, the cell/tissue- and pathway-specific roles of PARP-1 in transcription have been clearly demonstrated [40].

PARP-1 has been suggested to act as a promoter-specific coactivator [65]. PARP-1 has been identified as an interaction partner not only of NF-kB but also of several sequence specific transcription factors and cofactors including Oct-1, and PC3/topoisomerase-I [65] and has been shown to increase the transcriptional activity of these transcription factors [65].

**PARP-1 in the NF-kB, HIF and TCF-4/β-Catenin Pathways: Implications in Carcinogenesis**

PARP-1 can act both as an inhibitor and activator of NF-kB-dependent transcription. In the context of NF-kB target gene transcriptional regulation, Chang and Alvarez-Gonzalez. [64] reported that direct PARP-1 interaction with NF-kB inhibits the binding of NF-kB to its element and this inhibition is relieved by the auto-poly(ADP-riboseylation) of PARP-1. Several reports demonstrated that coactivator activity of PARP-1 for NF-kB-dependent gene expression seems to be dependent on the stimuli and cell type [65]. PARP-1 directly interacted with both subunits of NF-kB (p65 and p50) *in vitro* and *in vivo* [66]. Remarkably, neither the DNA binding nor the enzymatic activity of PARP-1 was required for full activation of NF-kB in response to various stimuli *in vivo* [66]. Note that PARP-1 is an important regulator of skin carcinogenesis and this is due, at least in part, to its ability to modulate the response through NF-kB [41]. In addition, PARP-1 directly interacted with p300/CREB and synergistically coactivated NF-kB-dependent transcription [51]. NF-kB-dependent trans-activation of PARP-1-dependent promoters not only requires the enzymatic activity of the coactivator of NF-kB p300/CREBB but also that PARP-1 itself is acetylated *in vivo* in response to inflammatory stimuli. However, the exact molecular mechanism by which acetylation of PARP-1 regulates the co-activator activity of PARP-1 in the context of chromatin remains to be investigated. Acetylation of PARP-1 is required for the interaction with p50 and the transcriptional activation of NF-kB in response to inflammatory stimuli. Acetylation of PARP-1 might be mainly required for the NF-kB-dependent promoter activity. However, it remains to be further investigated whether acetylation of PARP-1 could also strongly influence other sequence-specific transcription factors or cofactors under certain conditions. The synergistic coactivation of PARP-1, p300/CREB, and also the Mediator complex was dependent on acetylation of PARP-1. Based on the multistep interaction model of transcriptional activation proposed by Malik et al. [67], PCI/PARP-1 might facilitate together with other structural/architectural positive cofactors the co-operative interactions between sequence-specific activators and different co-activator complexes such as p300/CREB and Mediator, thereby providing an architectural function in stabilizing the pre-initiation complex [65].

PARP-1 interacts *in vivo* with the Mediator complex under physiological conditions and directly interacted *in vitro* with the Mediator subunits MED14 and CDK8 as well as the TFIIF subunit RAP74 but not with TFIIB, TATAbox-binding protein (TBP) and the tested TBP-associated factors. PARP-1 might only function during assembly of the pre-initiation complex [49]. Whether acetylation of PARP-1 might regulate the PARP-1 activity at this level in the context of chromatin remains to be investigated. However, it seems unlikely since the Mediator subunits DRIP150 and CDK8 did not bind to the acetylated domain in PARP-1. Acetylation of PARP-1 might be mechanistically required for the stabilization of preformed PARP-1 containing transcriptional coactivator-cofactor complexes and this modification is important for its role as transcriptional coactivator and the different physiological functions of PARP-1 might be in general regulated by post-translational modifications in a stimulus-dependent manner [68].

Expression and stabilization of Hifoxia Inducible Factor-alpha (HIF-α) differs drastically between wild type and *parp-1*-deficient cells and also in the presence of the PARP inhibitor DPQ [41]. In some types of cancer models it has been reported that PARP-1 deletion contribute to a defective activation of transcription factors that play a key role in tumor development such as NF-kB, AP-1 and HIF [41, 70]. In the case of skin carcinogenesis the decreased susceptibility of *parp-1/-* mice could also be attributed to the reduced inflammatory/oxidative stress component in *parp-1/-* mice. In the absence of PARP-1, the oxidative cell damage produced during the inflammatory response in the initial steps of skin neoplasia is prevented
through the inactivation of key transcription factors involved in carcinogenesis [70] (Fig. (4)). Thus, inhibition or genetic elimination of PARP-1 interferes with the promotion of tumors of epithelial origin, in which inflammatory processes play a critical role [71]. Finally, another example of PARP-1 as transcriptional cofactor is TCF-4/ß-catenin-evoked gene transactivation. PARP-1 physically interacts with the transcription factor TCF-4 and augments its transcriptional activity evoked by oncogenic ß-catenin, participating in the transcriptional regulation of target genes. In addition, PARP-1 may be indirectly regulated by the TCF-4/ß-catenin complex establishing a positive feedback loop that enhances PARP-1 expression [69].

These findings together with the fact that monotherapy with PARP inhibitors is effective in BRCA-1 -/- and BRCA-2 -/- cancer cells and tumors (due to the inability of these cells to repair by homologous recombination the stalled replication fork damages induced by PARP inhibitors) [72, 73] and the radio and chemopotentiation with the use of PARP inhibitors (Fig. (4)), place the PARP’s field in the cutting edge in the pre-clinical advances in cancer treatment.

PARP-1 IN INFLAMMATION, OXIDATIVE STRESS AND CELL DEATH

It has been demonstrated in the last years that PARP-1 might play a significant role in the regulation of the inflammatory response. A considerable number of studies on either PARP-1 deficient mice or PARP inhibitors have revealed that the inactivation of PARP-1 improves the outcome of a variety of patho-physiological conditions associated with an exacerbated tissue or systemic inflammation. Different mechanisms have been proposed to explain the role of PARP-1 in the inflammatory response.

PARP-1 in Inflammatory Diseases

Inflammation is the first response of the immune system to infection, irritation or other injury, which occurs as a defensive response. Inflammation is characterized by the immediate infiltration at the site of injury or infection with immune system components and is manifest by increased blood supply and vascular permeability which allows chemotactic peptides, neutrophils, and mononuclear cells to leave the intravascular compartment. Peroxynitrite is a labile, toxic oxidant species produced from the reaction of superoxide and nitric oxide (NO) [74]. Peroxynitrite, as well as hydroxyl radical, are the key pathophysiologically relevant triggers of direct DNA single strand breakage [75]. Moreover, several studies have demonstrated that peroxynitrite produces mitochondrial injury as well as an increase in mitochondria-derived reactive oxygen species generation [76, 77]. Endogenous production of peroxynitrite and other oxidants by immunostimulated macrophages and neutrophils induce prolonged DNA damage in neighboring cell [78-80]. Likewise, in brain slices (upon activation of NMDA receptors that trigger for enhanced NO, superoxide and peroxynitrite production) and smooth muscle cells, led to oxidant species-mediated DNA single strand

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Fig. (4). Proposed model of action of PARP inhibitor in tumors. PARP inhibitors might interfere with tumor growth at different levels counteracting with the transcriptional activation of tumor-related transcription factors, angiogenesis process and in promotion of apoptosis in combination with chemotherapy, and by induction of cell death in homologous recombination deficient tumor cells (BRCA-1 and 2 deficient cells).
breakage and PARP related cell injury [81, 82]. A considerable number of studies have revealed a crucial role of PARP-1 in cell death after various inflammation processes like ischemia-reperfusion damage, haemorrhagic shock, septic shock, lung inflammation, diabetes mellitus and chronic inflammatory disorders such as arthritis and inflammatory bowel diseases (ulcerative colitis and Crohn’s disease), diseases of the central nervous system, such as allergic encephalomyelitis and multiple sclerosis, uveitis, periodontal inflammation, meningitis, asthma and possibly in various forms of dermal inflammation [83]. Experimental evidence supports that Reactive Oxygen Species (peroxynitrite, hydrogen peroxide, nitric oxide, etc.) generated during inflammation response, induce DNA strand breakage and PARP activation [83, 84] (Fig. (4)). This activation significantly contributes to the pathophysiology of various forms of inflammation and its inactivation reduced the onset and progression of these illnesses. As will be outline below, different mechanisms have been proposed to explain that the inactivation of PARP-1 (either pharmacologically or using genetically engineered mice lacking PARP-1), improve the outcome of a variety of pathophysiological conditions associated with an exacerbated tissue or systemic inflammation [83].

Mechanisms to explain the role of PARP-1 in the inflammatory response. The first mechanism described is “The suicide hypothesis” in which Berger proposed that the excessive DNA damage induces massive PARP-1 activation that leads to a rapid depletion of NAD+ and ATP, to an irreversible cellular energy failure, to a drastic reduction of energy dependent processes and to necrotic-type cell death consequent to disruption of oxidative metabolism [84]. This suicide model gained new support in the mid-1990s because after an inflammatory stress (LPS, ischemia-reperfusion injury, etc.), different cell types, including macrophages and endothelial cells, activate a massive synthesis of nitric oxide (NO), which is in turn converted into a genotoxic derivative, peroxynitrite that generates single-strand ed DNA breaks and hyperactivation of PARP-1 and depletion of cellular NAD+ and ATP. Moreover, the peroxynitrite also induces mitochondrial free radical generation that produces more DNA damage and PARP-1 activation and finally causes cell necrosis [85, 86]. It has also been described the PARP-1 activation rapidly modulates the mitochondrial functioning and triggers mitochondrial dysfunction [87-89]. The level of PARP-1 activation has been considered as a molecular switch between necrosis versus apoptosis [90] and the NAD+ as a metabolic link between DNA damage and cell death [91]. The treatment with NADH or pyruvate blocks PARP-1-mediated cell death [92, 93]. The pathophysiological significance of PARP-1 hyperactivation is well exemplified by the remarkable therapeutic efficacy of PARP-1 inhibitors in experimental models of disorders characterized by DNA damage such as ischemia, diabetes, shock, inflammation and cancer [36, 83] (Fig. (4)).

Recently a key observation on the mechanism by which PARP-1 activation and NAD+ consumption could lead, under overwhelming DNA damage, to cell death is the pathway that involves the mitochondrial release of apoptosis inducing factor (AIF) and cytochrome c, directly link to the massive synthesis of poly (ADP-ribose), and the activation of a caspase-independent cell death pathway [87, 94-97].

However, several lines of evidence suggest that under specific conditions the beneficial effects of PARP-1 inhibition are independent from the prevention of energy failure [98]. The suicide hypothesis, therefore, might be valid only in conditions of massive DNA rupture and intense PARP-1 activation.

PARP-1 may also influence the stress/inflammation response through regulation of transcription factors and associated gene transcription. PARP-1 has been reported either to activate or repress transcription activity [5]. Nuclear factor-xB (NF-xB)/Rel transcription factors play a central role in the regulation of genes involved in the immune and inflammatory response. NF-xB regulates the expression of TNF-α, iNOS, interleukins IL-1β, IL-2, IL-6 and IL-8 as well as the adhesion molecules ICAM-1 and E-selectin. As has been treated above, reports by different groups, including ours, have shown that PARP-1 inhibitor have minimal or no effect on NF-xB activation, while cells and mice lacking PARP-1 display a dramatic deficiency in this transcription factor activation [34, 36, 40, 41, 51] (Fig. (4)).

These interesting findings have implicated PARP-1 in upstream events of inflammatory signalling (Fig. (4)). PARP inhibitors may affect the signalling pathways and they might play a key role in an inflammatory model as well as a significant role in cell survival. Recent data showed that PARP inhibition-induced Akt activation is dominantly responsible for the cytoprotection in pathophysiological conditions associated with oxidative stress and inflammation [99, 100]. Furthermore, PARP inhibitors down-regulated two elements of the MAP kinase system, ERK 1/2 and p38 mitogen-activated protein (MAP) kinase but not JNK in a tissue-specific manner [40, 101]. However, the exact nature of the regulation of phosphatidylinositol 3-kinase-Akt/protein kinase B and MAP kinase by PARP-1 remains to be elucidated.

These beneficial effects of PARP inhibitors probably result from improvement of cellular energetic status leading to cell survival and from inhibition of signal transduction leading to suppressed expression of inflammatory mediators. The contribution of these two mechanisms to the effect of PARP inhibitors in various disease models may likely differ to a great extent.

The marked beneficial effect of PARP inhibitor in many animal models of various diseases suggests that they can be exploited to treat human inflammatory diseases. However, crucial safety experiments must be done, due to the fact that PARP has been involved in DNA repair and maintenance of genomic integrity and its log-term inactivation could increase the possible risk of mutation rate and cancer formation (Fig. (4)).

CONCLUSIONS

Here we have summarised several independent lines of evidence that are all supporting an involvement of members of the PARP-1 in transcription, carcinogenesis and inflammation (Fig. (4)). As genotoxic stress – mainly induced by ROS – is believed to be the major driving force for tissue damage and inflammation-related carcinogenesis, mechanisms that counteract it or reverse its consequences should be crucial for maintaining genetic integrity. The role of PARP and PAR in cellular physiology has greatly diverged in the last decade. For many years PARP (and more exactly, PARP-1) has been envisaged solely (and importantly) as a protein involved in detection and signalling of DNA damage. The number of PARP/PAR-associated cellular functions currently goes from DNA damage detection and repair to cell death pathways, telomeric function, transcription, chromatin structure, etc., with important consequences in the physiology and pathophysiology of processes such as the control of genome integrity, carcinogenesis, the inflammatory response and neuronal function. The next challenges for this exciting field has to address how these ubiquitous factors can have so many different functions, the insights of the PARPs activation to synthesize the polymer in the absence of DNA damage, the role of the polymer as a signalling molecule in the nucleus and cytosol, and a better understanding of cellular poly(ADP-
Modulation of Transcription by PARP-1


