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Role of poly (ADP-ribose) polymerase-1 (PARP-1) in the inflammatory response

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PAPEL DE LA ENZIMA POLI (ADP-RIBOSA) POLIMERASA-1 EN LA RESPUESTA INFLAMATORIA

RESUMEN

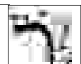
La respuesta inflamatoria se caracteriza por una expresión exacerbada de genes pro-inflamatorios en respuesta a diferentes estímulos. El estudio de los mecanismos moleculares involucrados en las rutas de señalización inducidas por estos estímulos, es muy importante para tratar de controlar los procesos inflamatorios. Recientemente, se ha sugerido que la enzima muclear poli-(ADP-ribosa) polimerasa (PARP-1), puede jugar un papel muy importante en la regulación de la respuesta inflamatoria. PARP-1 es una proteína de unión al ADN que detecta de forma específica roturas en el ADN y, utilizando NAD^+ como sustrato, sintetiza y transfiere ADP-ribosa a otras proteínas nucleares. Numerosos estudios, utilizando ratones deficientes en PARP-1 o animales tratados con inhibidores de PARP, han demostrado que la inactivación de PARP-1 mejora la respuesta del animal a una variedad de procesos fisiopatológicos asociados con inflamación tisular o sistémica. Se han propuesto diferentes mecanismos para explicar el papel jugado por PARP-1 en la respuesta inflamatoria. Tras un proceso inflamatorio, diferentes tipos celulares activan la síntesis de óxido nítrico, el cual da lugar a peroxinitrito, un derivado genotóxico. El daño en el ADN inducido por el peroxinitrito da lugar a una sobre-activación de PARP-1, consumo de NAD^+ y el consiguiente agotamiento energético que conduce a la alteración celular y necrosis. PARP-1 se ha implicado también en la regulación de la actividad transcripcional de genes eucarióticos. PARP-1 puede modular la expresión génica mediante diferentes mecanismos: (i) interacción física con otras proteínas, especialmente factores de transcripción; (ii) unión directa a secuencias reguladoras del gen; y (iii) modificación de proteínas nucleares mediante poli-ADP-ribosilación.

PALABRAS CLAVE: Poli-(ADP-ribosa) polimerasa/ Inflamación/ Transcripción.

ABSTRACT

Excessive expression of pro-inflammatory genes in response to different stimuli is a hallmark of the inflammatory response. The study of the molecular events involved in the cell signaling pathways induced by these stimuli could be of extreme importance for controling inflammation. Recently, it has been suggested that the nuclear enzyme poly(ADP-ribose) polymerase-1 (PARP-1) might play a significant role in the regulation of the inflammatory response. PARP-1 is a DNA-binding protein that specifically detects DNA-strand breaks or nicks and, using NAD^+ as a substrate, synthesises and transfers ADP-ribose onto several nuclear proteins. 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 pathophysiological 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. After an inflammatory stress, different cell types activate a massive synthesis of nitric oxide, which is in turn converted into a genotoxic derivative, peroxynitrite. Rapid DNA single-stranded breaks are induced by peroxynitrite, leading to overactivation of PARP-1, NAD^+ consumption and consequently depletion of cellular energy resulting in cell dysfunction or necrosis. PARP-1 has also been implicated in the transcriptional activity regulation of several eukaryotic genes. PARP-1 might modulate gene expression through different mechanisms: (i) physical interactions with other proteins, specially transcription factors; (ii) direct binding to the gene-regulating sequences; and (iii) transient post-translational modifications of nuclear proteins by poly (ADP-ribosyl) ation.

KEY WORDS: *Poly(ADP-ribose) polymerase-1/ Inflammation/ Transcription.*



INTRODUCTION

The inflammatory immune response is characterised by an excessive pro-inflammatory gene expression in response to different stimuli such as bacterial lipopolysaccharides (LPS), superantigens or potent oxidants (1). These stimuli induce signaling pathways to the effector cell nucleus, mediated by NF- B and other stress-responsive transcription factors which play a critical role in reprogramming gene expression. However, the molecular mechanisms involved in the onset and/or regulation of the inflammatory immune response are incompletely understood, and its study could allow the identification of new therapeutic targets. Recently, it has been suggested that the nuclear enzyme poly (ADP-ribose) polimerase-1 (PARP-1) might play a significant role in the innate immune response. This enzyme has been implicated in the pathways (in monocytes and macrophages) leading to the expression of the inducible genes coding for nitric oxide synthase (iNOS), IL-6 and TNF alpha (2,3). PARP-1 has also been directly involved in the regulation of gene expression during the inflammatory response. An important tool for the analysis of these issues has been the development of PARP-1 knockout mice (PARP-1$^{-/-}$) (4-6). Studies using these mice showed that they are protected against a variety of experimentally induced disorders with a clear inflammatory component. In this review we summarise the most recent results regarding the role of PARP-1 in inflammation and explore different mechanisms by which PARP-1 could be involved in the inflammatory response.

PARP-1: STRUCTURE AND FUNCTION

PARP-1 (EC 2.4.2.30) is an abundant and highly conserved nuclear zinc-finger DNA-binding protein of 113 kDa in molecular weight that specifically detects DNA-strand breaks or nicks generated by different genotoxic agents (7). PARP-1 belongs to a large family of enzymes (Table I) (8) that, using NAD$^+$ as a substrate, synthesise and transfer ADP-ribose onto aspartic and glutamic acid residues of proteins (Fig. 1). These include PARP-1 itself (automodification) as well as several nuclear proteins that interact with DNA (6). Poly-ADP-ribosylation is terminated by the release of extensively poly-ADP-ribosylated (negatively charged) PARP molecules from DNA (9). ADP-ribose polymers are then subjected to degradation by poly-ADP-ribose-glycohydrolase (9,10). Poly (ADP-ribosyl)ation is therefore an immediate, covalent, but transient post-translational modification of nuclear proteins, induced by DNA lesions. Recently, a fast signal-induced activation of PARP in brain cortical neurones, mediated by inositol 1,4,5,-trisphosphate (IP3)-induced Ca^{2+} mobilisation has also been demonstrated, which does not involve DNA damage (11).

PARP-1 structure has been divided into three functional domains (Fig. 2). The N-terminal 42 kDa DNA-binding domain (DBD) consists of two zinc fingers and contains a nuclear localisation signal and a caspase-3 cleavage site. The C-terminal 55 kDa catalytic domain includes the NAD$^+$ binding site. The central 17 kDa domain has been described as the auto-modification domain (7,12-14).

The physiological function of PARP-1 is still under debate but it seems to participate in processes involving nicking and resealing DNA strands, such as recovery from DNA damage and cell proliferation (5), maintenance of genomic stability (15,16), differentiation and genetic recombination (17), apoptosis (18-20), control of telomere length (21), as well as regulation of transcription (22).

PARP-1 IN INFLAMMATORY DISEASE

A considerable number of studies on either PARP-1 deficient-mice or normal animals treated

Table I
Members of the poly (ADP-ribose) polymerase (PARP) family

Protein	*MW (kDa)*	*Deficient mice*	*Sensitivity to PARP-1 Inhibitors*	*Intracellular localisation*	*Reference*
PARP-1	113	Yes	Yes	Nuclear	(7)
PARP-2	62	Yes	Yes	Nuclear	(75,76)
PARP-3	60	No	ND*	Nuclear	(76)
TANKYRASE	142	No	Yes	Cytoplasm and nuclear	(77,78)
TANKYRASE-2	130	No	ND*	Cytoplasm	(79,80)
VPARP	193	No	Yes	Cytoplasm and nuclear	(81)

*ND: not done

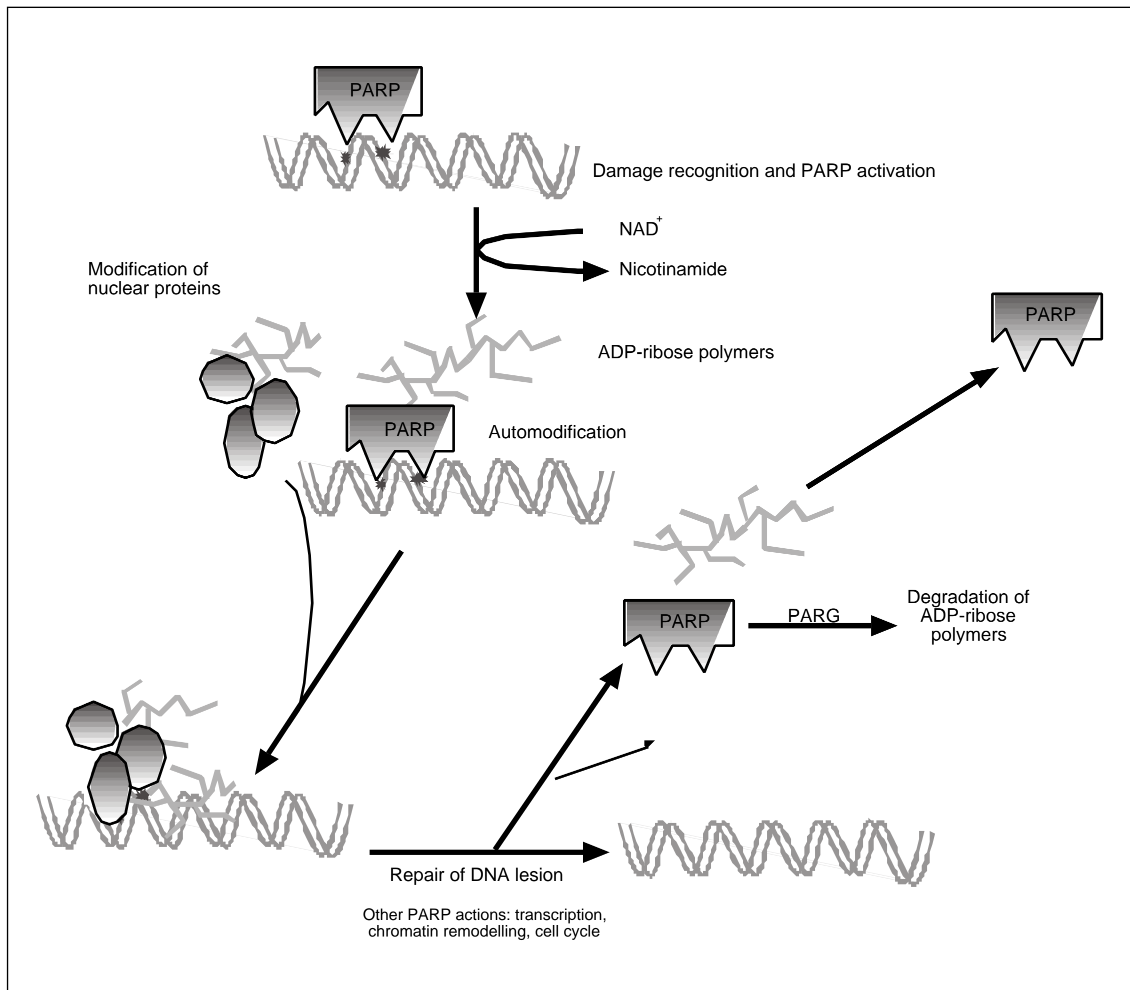


Figure 1. *Schematic representation of poly(ADP-ribose) metabolism during DNA damage. Synthesis and transfer of poly(ADP-ribose) to acceptor proteins is catalysed by PARP. Degradation of the poly(ADP-ribose) chains are performed by the enzyme poly(ADP-ribose) glycohydrolase (PARG).*

with PARP inhibitors have revealed a crucial role of PARP-1 in cell death after ischemia-reperfusion injury and in various inflammation processes. Part of the more relevant will be discussed in the subsequent paragraphs.

Ischemia-reperfusion damage

Ischemia/reperfusion injury is a common process that initiates a pathophysiological cascade including an inflammatory response with liberation of cytokines and oxygen-derived free radicals. Recently, it has been shown that PARP-1 is involved in the pathogenesis of various forms of ischemia/reperfusion injury. The beneficial effects of PARP-1 deficiency was first demonstrated by Eliasson et al., in a model of cerebral ischemia in PARP-1$^{-/-}$ mice (23). These authors demonstrated that genetic disruption of PARP-1 provides profound protection against glutamate-NO-mediated ischemic insults *in vitro* and a major decrease in infarct volume after reversible middle cerebral artery occlusion, providing evidence for a primary involvement of PARP-1 activation in neuronal damage following focal ischemia. Also in 1997, Thiemermann et al. (24) and Zingarelli et al. (25) demonstrated, independently, that pharmacological inhibition of PARP reduces myocardial necrosis and improves cardiac function after coronary ischemia-reperfusion injury. Later on, extensive number of studies have demonstrated that pharmacological inhibition of PARP reduces reperfusion injury in the brain (26), kidney (27), bowel (28), retina (29), liver (30), skeletal muscle (24), lung (31) as well as during heart transplantation (32) and chronic heart failure (33).

Haemorrhagic shock

Haemorrhagic shock and resuscitation leads to widespread production of oxidant species. Recently, it has been shown that PARP-1$^{-/-}$ mice are

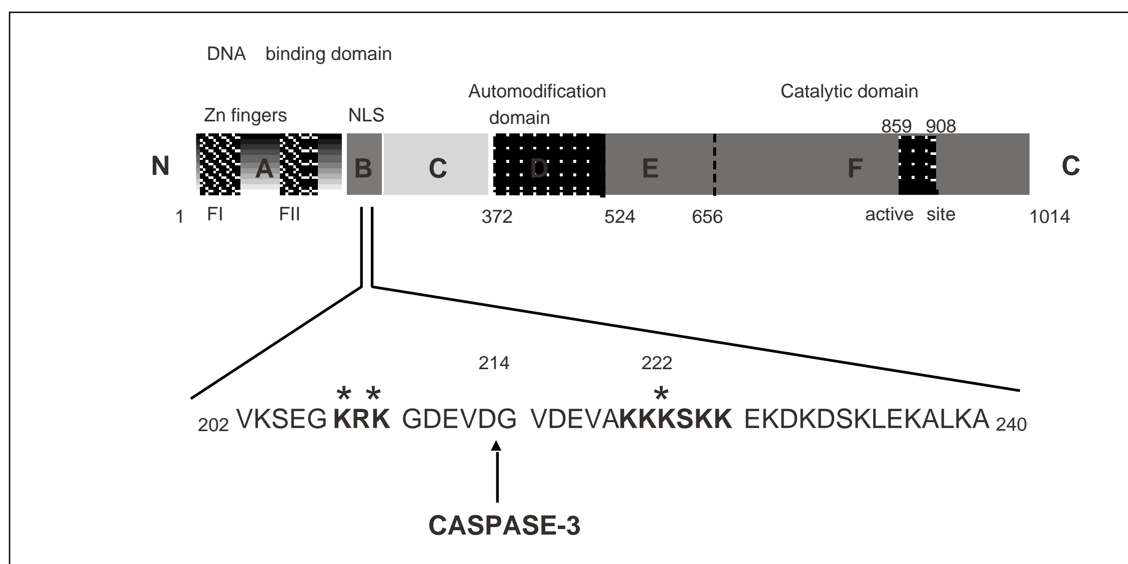


Figure 2. *Schematic representation of the domain structure of PARP. Sequence of the nuclear location signal and the caspase-3 cleavage site are represented. NLS, nuclear localisation signal.*

protected from the rapid decrease in blood pressure after resuscitation and showed and increase of survival time, as well as reduced lung neutrophil sequestration (34). Moreover, pharmacological inhibition of PARP in a model of haemorrahage and resuscitation in rats reduces organ dysfunction and metabolic acidosis (35).

Septic shock

Septic shock is the most common cause of death in intensive care units and it is usually the result of a systemic Gram-negative bacterial infection resulting in hypotension and failure of a number of organ systems, in particular the liver, kidney and heart (36). The bacterial membrane component LPS, when injected into animals, causes a shock-like state leading to death. The mechanism by which LPS induces endotoxic shock is related to its ability to activate the NF- B/Rel family of transcription factors, enabling the expression of several critical genes involved in the pathogenesis of septic shock: TNF- , interleukins, adhesion molecules, cyclooxygenase-2 and inducible nitric oxide synthase (iNOS) (37). The response to LPS-induced septic shock has been tested in PARP-1-deficient mice and the results showed that these animals were extremely resistant to LPS-induced endotoxic shock (38,39). The resistance of PARP-1$^{-/-}$ mice to the septic shock was similar to that found for interleukin-1 converting enzyme (ICE)-deficient mice (40) but more dramatic than that reported for TNF- knockout mice (41).

Diabetes mellitus

Type-1 diabetes results from the selective destruction of insulin-producing pancreatic -cells as consequence of an inflammatory reaction induced by autoantibodies. Several groups have shown that PARP-1$^{-/-}$ mice are completely resistant to diabetes induction by the -cell toxin streptozotocin (42-44). Likewise, it has been demonstrated that the administration of PARP inhibitors to 90% depancreatized rats induces islet regeneration (45). However, unexpectedly, nonobese diabetic mice (NOD) with a disrupted poly (ADP-ribose) polymerase-1 gene are highly sensitive to the diabetes induced by a single high dose of streptozotocin, suggesting that NOD mice are characterised not only by their immune dysfunction but also by a peculiarity of their islets leading to a PARP-1-independent mechanism of streptozotocin-induced -cell death (46).

PARP-1 also seems to play a crucial role in the pathogenesis of endothelial dysfunction in diabetes (47). Diabetic vascular dysfunction is a major clinical problem that predisposes patients to a variety of cardiovascular diseases. Vascular rings from PARP-1$^{-/-}$ mice were fully resistant to endothelial dysfunction induced by high glucose level. Likewise, inhibition of PARP by PJ34, a new inhibitor of PARP, also protected against the development of high glucose – induced endothelial dysfunction (47). Moreover, pharmacological inhibition of PARP not only prevents the development of endothelial cell dysfunction but it is also able to reverse it (48).

Chronic inflammatory disorders

PARP-1 has also been involved in different forms of chronic inflammatory conditions such as arthritis and Crohn´s disease. Inhibition of PARP has shown to prevent inflammation in animal models of collagen-induced arthritis, supporting the view that PARP-1 is involved in the progression of the inflammatory process (49). Crohn's disease is a chronic inflammatory bowel disease characterised by oxidant-induced tissue injury and increased intestinal permeability. Pharmacological inhibition of PARP activity in a mouse model of Crohn's disease results in a marked improvement of colonic inflammatory disease and a normalisation of cellular metabolic function and intestinal permeability (50).

MECHANISMS TO EXPLAIN THE ROLE OF PARP-1 IN THE INFLAMMATORY RESPONSE

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 *in vivo* outcome of a variety of pathophysiological conditions associated with an exacerbated tissue or systemic inflammation (Fig. 3).

The "suicide hypothesis"

The initial observation that the activation of PARP-1 due to DNA damage can lead to massive NAD^+ consumption led to propose that this NAD^+ depletion can affect cellular energetic balance and function (51). In the 1980s, many investigators observed rapid depletion of NAD^+ due to PARP activation, leading to cellular ATP depletion and functional alterations of the cell, with eventual necrotic-type cell death. 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. Rapid single-stranded DNA breaks are induced by peroxynitrite, leading to over-activation of PARP-1 and depletion of cellular energy resulting in mitochondrial free radical generation and cell necrosis (52,53). This hypothesis is consistent with the enzymology of poly (ADP-ribosyl) ation and also explains *in vitro* and *in vivo* effects of PARP-1 inactivation in experimental models in which DNA damage is a common denominator (54). 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 (55). The suicide hypothesis, therefore, might be valid only in conditions of massive DNA rupture and intense PARP-1 activation.

Very recently a key observation on the mechanism by which PARP-1 activation and NAD^+ consumption could lead, under overwhelming DNA damage, to cell death has been reported. This pathway implies the mitochondrial release of an apoptosis-inducing factor (AIF), directly linked to the massive synthesis of poly (ADP-ribose), and the activation of a caspase-independent cell death pathway (20).

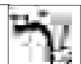
Regulation of gene expression

A large body of evidence has implicated PARP-1 in the regulation of the transcriptional activity of several eukaryotic genes. PARP-1 might modulate gene expression in both a positive and a negative fashion, with the final effects depending on the cell type, the gene and the transcription factor involved (56). This transcriptional regulatory function may be acting through different mechanisms, which makes PARP-1 a unique regulator of transcription, capable of integrating chromatin remodelling, cis- and trans-activation (Fig. 3).

1. *Modification of transcription factors and other nuclear proteins by poly (ADP-ribosyl) ation.* PARP-1-dependent gene regulation involves poly (ADP-ribosyl) ation of transcription factors, which, in turn, affect their binding to specific promoter sequences (22). The basal transcription factors TFIIF and TEF-1, TATA box-binding protein, YY1, SP-1, cAMP-response element-binding protein, p53, and NF- B are all highly specific substrates for poly (ADP-ribosyl) ation (22,57-59). ADP-ribose chains are also transferred to histones, proteins of the high mobility group, topoisomerases, as well as PARP-1 itself. By so doing, poly (ADP-ribosyl) ation participates together with acetylation, methylation and phosphorylation in the architectural remodelling of chromatin and, therefore, transcription.

2. *Direct protein-protein interaction with different regulator proteins.* A physical interaction between PARP-1 and different transcription factors such as AP-2 (60) B-MYB (61), Oct-1 (62), YY1 (63), TEF-1 (58) and NF- B (64) has been demonstrated.

3. *Direct binding of PARP-1 to gene-regulating sequences.* Recently, a key role of PARP-1 in the regulation of transcriptional activity through its binding to cis-acting elements has been demonstrated. PARP-1 regulates the expression of the Reg gene (a gene for insulin-producing -cell regeneration) in -cells in response to IL-6 and dexamethasone by binding to its promoter region (45). The DNA/protein complex formation was inhibited depending on the autopoly (ADP-ribosyl)ation of



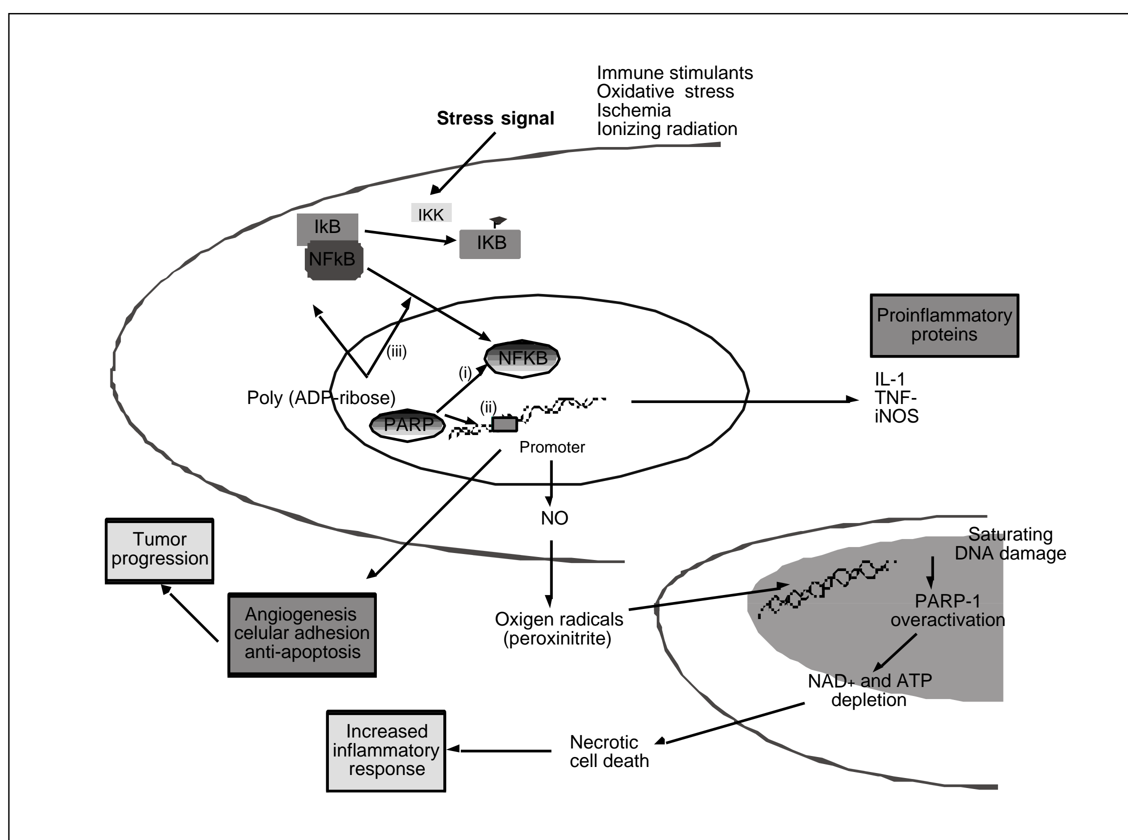


Figure 3. *PARP-1 is involved in the inflammatory response at different levels. (a) Regulation of transcription upon stimulation of the cells through different mechanisms: (i) physical interactions with transcription factors, specially NF-kB; (ii) binding to the gene-regulating sequences; and/or (iii) transient post-translational modifications of proteins by poly(ADP-ribosyl)ation. (b) PARP-1 acts as a sensor of DNA damage in cells with increased production of oxygen radicals. Subsequent PARP-1 overactivation leads to energy depletion and necrosis.*

PARP-1 in the complex. Thus, PARP inhibitors enhance the DNA/protein complex formation for Reg gene transcription and stabilise the complex by inhibiting the autopoly (ADP-ribosyl) ation of PARP-1. Similarly, PARP-1 is involved in the transcriptional regulation of CXCL1, a chemokine involved in inflammation and progression of melanocytes into malignant melanoma, by binding to the CXCL1 promoter. 3-aminobenzamide, an inhibitor of PARP-1, inhibits CXCL1 promoter activity (65). However, the current paradigm linking PARP-1 function to the presence of DNA strand-breaks does not provide a comprehensive mechanism by which it may be recruited to gene-regulating domains in the absence of DNA damage. Recently, Soldatenkov et al. (66) have shown that PARP-1 can bind to the DNA secondary structures in heteroduplex DNA in a DNA end-independent fashion. Auto modification of PARP-1 in the presence of NAD^+ inhibited its hairpin binding activity.

PARP-1 as an essential transcriptional coactivator for NF- B

Special mention requires the role played by PARP-1 in the regulation of NF- B mediated-gene transcription. NF- B encompasses a family of inducible transcription factors with key roles in the regulation of genes involved in the immune and inflammatory responses (67). In most unstimulated cells, NF- B is sequestered in the cytoplasm as an inactive transcription factor in a complex with I B proteins. Many agents, including TNF- , LPS, mitogens, phorbol esters, ionising radiation, as well as oxidative stress lead to the rapid phosphorylation of I B that results in ubiquitination of I B and subsequent degradation. Dissociation of NF- B unmasks nuclear localisation sequences of p65 and p50, which leads to NF- B nuclear translocation, subsequent binding to specific B consensus sequences in the chromatin and activation of specific subsets of genes (67). In

immune responses, NF- B regulates the expression of TNF- , iNOS, interleukins IL-1 , IL-2, IL-6 and IL-8 as well as the adhesion molecules ICAM-1 and E-selectin. Recently, evidence has been provided that PARP-1 might be required *in vivo* for specific NF- B-dependent gene expression (38). Using PARP-1$^{-/-}$ mice and cells derived from these animals, it has been demonstrated that PARP-1 is necessary for the induction of NF- B expression after exposure of the cells or mice to LPS, TNF- or hydrogen peroxide (38). However, the translocation of NF- B to the nucleus occurred in PARP-1$^{-/-}$ cells as it did in the wild-type (38), suggesting a new level of control of NF- B, which occurs after its translocation to the nucleus, where PARP-1 seems to play a crucial role. Likewise, a physical interaction between NF- B and PARP-1 has been reported which does not seem to require DNA (64). Furthermore, direct protein-protein interaction of PARP-1 with both subunits of NF- B is required for its coactivator function (68), thus expanding the role of PARP-1 as an essential and novel classical transcriptional coactivator for B-dependent gene expression *in vivo*.

INHIBITORS OF PARP-1 AS TOOLS FOR THERAPY

PARP activity can be inhibited by a variety of agents which, in view of the above-mentioned role of PARP-1 overactivation in a wide range of pathophysiological processes, will probably result in clinical applications for therapy and /or prevention (69). The first-generation of PARP inhibitors (3-aminobenzamide, nicotinamide, benzopyrone derivatives and isoquinolones) (70) have significant drawbacks, such as poor solubility, lack of potency and limited specificity of the compounds. In recent years, more sophisticated PARP-inhibitory compounds have been synthesised, with Ki values in the low nanomolar range (71-73). Some of them have been tested in animal models and the results are promising. However, a deeper and exhaustive information regarding the pharmacokinetics of these PARP inhibitors, their uptake into various organs, and the length of time they remain inside the cell are necessary. Finally, development of new potent inhibitors aimed at specific inhibition of particular enzymes of the PARPs family (Table I) could be useful in elucidating the functions of individual PARPs as well as for clinical strategies.

CONCLUDING REMARKS AND FUTURE PROSPECTS

A large body of evidence supports a role of PARP-1 as a signaling molecule, which controls the expression of multiple genes involved in the inflammatory response. An important tool for this finding has been the development of PARP-1 deficient mice. The results obtained with these animals in different experimental models point out to PARP-1 as a new target molecule for therapy in various forms of inflammation and ischemia-reperfusion injury. Differential gene expression profile studies between PARP-1$^{-/-}$ and their correspondent PARP-1$^{+/+}$ cells under different activation conditions will allow us to complete the picture of gene expression in which PARP-1 plays, directly or indirectly, an important role (74). Moreover, the identification of new nuclear factors that interact with PARP-1 could help to a better understanding of the mechanisms involved in the regulation of gene expression by PARP-1 as well as to identify new potential targets for therapy.

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