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MINIREVIEW

Pharmacological Modulation of Poly(ADP-ribose) Polymerase-Mediated Cell Death: Exploitation in Cancer Chemotherapy

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Poly(ADP-ribose) polymerases (PARPs) are defined as a family of cell-signaling enzymes present in eukaryotes, which are involved in poly(ADP-ribosylation) of DNA-binding proteins. PARP enzymes are activated in response to DNA damage induced by ionizing radiation, oxidative stress, and DNA-binding antitumor drugs (Lindahl et al., 1995; D'Amours et al., 1999). Poly(ADP-ribose) polymerase-1 (PARP-1; EC 2.4.2.30), also known as poly(ADP-ribose) synthetase and poly(ADP-ribose) transferase, is the main member of the PARP enzyme family. PARP-1 is an abundant and highly conserved chromatin-bound enzyme that binds to nicked DNA as a homodimer [molecular weight = 2 × 113 kDa] and mediates protection against DNA damage. Upon binding to DNA breaks, activated PARP-1 cleaves NAD^+ into nicotinamide and ADP-ribose moieties and polymerizes the latter onto nuclear acceptor proteins and PARP-1 itself. When DNA is moderately damaged, PARP-1 participates in the DNA repair process and the cell survives. However, in the case of extensive DNA damage, PARP-1 overactivation induces a decrease of NAD^+ and ATP levels, leading to cell dysfunction or even to necrotic cell death (Martin et al., 2000). PARP-1 and other poly(ADP-ribosyl) transferases are localized not only in the nucleus but also in the mitochondria. In fact, it has been recently reported that intramitochondrial poly(ADP-ribosylation) contributes to NAD^+ depletion and cell death induced by oxidative stress in neurons (Du et al., 2003). Overactivation of PARP-1 have been implicated in the pathogenesis of several diseases, including stroke, myocardial infarction, diabetes, shock, neurodegenerative disorder, allergy, and several other inflammatory processes (Tentori et al., 2002). Therefore, PARP-1 may be considered a potential target for pharmacological intervention against various pathophysiological states. In addition, because of PARP-1 involvement in cell death, pharmacological modulation of PARP activity may constitute a suitable target to enhance the activity of antitumor drugs. In fact, several adjuvant strategies directed to modulate PARP activity, such as the use of PARP-1 inhibitors (Southan and Szabó, 2003) or ATP-depleting agents (Martin et al., 2000), have been recently reported. The present review gives an update of the pharmacological modulation of PARP activity in cancer therapy and tries to shed further light on this important subject.

Structure and Activity of PARP-1

Structural Data. PARP-1 consists of three main domains (Fig. 1A): The N-terminal DNA-binding domain (DBD; MW, 46 kDa), which contains two zinc fingers; the automodification domain (MW, 22,000 kDa); and the C-terminal region (MW, 54 kDa) accommodating the catalytic center (de Murcia and Menissier de Murcia, 1994; de Murcia et al., 1994; Virág and Szabó, 2002). In the DBD, two zinc fingers are responsible for DNA binding and some protein-protein interactions. This domain (DBD), a DNA nick sensor, also contains a nuclear localization signal within which the caspase-cleavage site (DEVD) can be found. The automodification domain, a central regulating segment, contains a breast cancer susceptibility protein C terminus motif, which is common in many

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ABBREVIATIONS: 6-AN, 6-aminonicotinamide; BEC, bioenergetics cellular; Da, dalton; β-F1-ATPase, β subunit of the mitochondrial H^+-ATP synthase; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; Hsp, heat shock protein; IP_3, inositol 1,4,5-triphosphate; MAP regime, 6-methylmercaptopurine riboside + 6-aminonicotinamide + *N*-(phosphonacetyl)-L-aspartic acid; MW, molecular weight; PALA, *N*-(phosphonacetyl)-L-aspartic acid; PARG, poly(ADP-ribose) glycohydrolase; PARP, poly(ADP-ribose) polymerase; PARP-CF, poly(ADP-ribose) polymerase catalytic fragment.

DNA repair and cell cycle proteins. Through this motif (i.e., the breast cancer susceptibility protein C terminus), PARP-1 participates in various protein-protein interactions. There is high homology in the primary structure of the PARP-1 enzyme, with the catalytic domain showing the highest degree of amino acid sequence homology between different species. This catalytic domain, found in the C-terminal part of the protein, contains the active site, which is highly conserved in eukaryotes. The active site, also known as "the PARP signature", is formed by a sequence of 50 amino acids that shows 100% homology among vertebrates (Virág and Szabó, 2002). The C-terminal region (54 kDa) can be cut down to a 40-kDa C-terminal polypeptide without losing the basal catalytic activity (Simonin et al., 1993). The PARP catalytic fragment (PARP-CF) is included within the catalytic domain and comprises residues 655 to 1014 (human numbering). PARP-CF is composed of two parts: a purely α-helical N-terminal domain of residues 662 to 784 and a C-terminal domain of residues 785 to 1010 bearing the putative NAD^+ binding site (Ruf et al., 1996). The N-terminal domain is formed by an up-up-down-up-down-down motif of helices in which the connections are 9 to 14 residues long. The core of C-terminal region consists of a five-stranded antiparallel β-sheet and four-stranded mixed β-sheet. These two sheets are connected via a single pair of hydrogen bonds between two strands that run at an angle of 90°. The sheets are consecutive. The central β-sheets are surrounded by five α-helices, three 3_{10}-helices, and by a three- and a two-stranded β-sheet in a 37-residue excursion between two central β-strands (Ruf et al., 1996). The block of 50 amino acids (residues 859–908) that is identical for all PARP-1 sequences of vertebrates is contained in a segment formed by a β-sheet, an α-helix, a 3_{10}- helix, a β-sheet, and an α-helix, consecutively. Both His-862 on a β-strand and Glu-988 on another β-strand are involved in NAD^+-binding or catalysis (Marsischky et al., 1995; Masson et al., 1995). Some PARP enzymes (PARP-1 and -2; Vault PARP (vPARP); Tankyrases —TANK1 and 2—) show differences in the domain structures (Virág and Szabó, 2002).

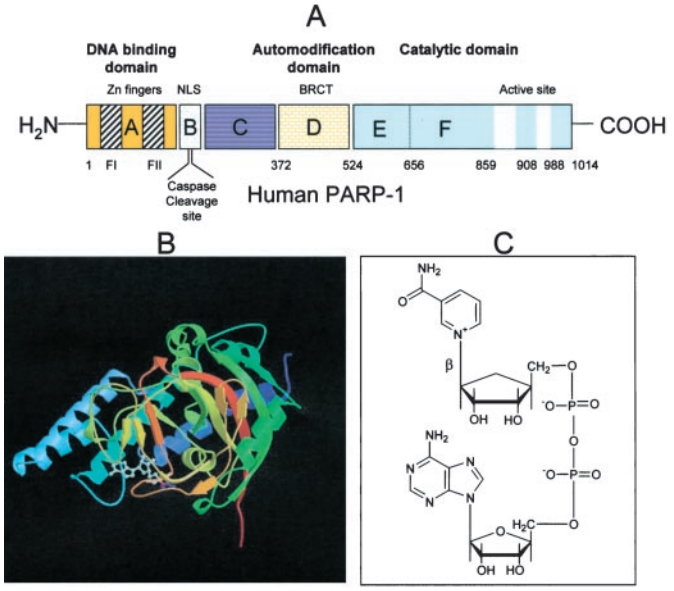

Fig. 1. Structure of poly(ADP-ribose) polymerase-1 (PARP-1). A, schematic representation of the modular organization of human PARP-1 (hPARP-1), the location of some modules is indicated by amino acid numbering. B, ribbon representation of chicken's PARP-1 catalytic fragment (C-terminal end, amino acids 662 to 1014), which was cocrystallized with the NAD analog carba-NAD. The ribbon diagram shows the interaction of carba-NAD (inhibitor substrate analog) with the NAD^+-binding site of PARP-CF. The observed bound ADP moiety of carba-NAD is shown; it marks the acceptor site. (Adapted from Ruf A, Rolli V, de Murcia G, and Schulz GE (1998) The mechanism of the elongation and branching reaction of poly(ADP-ribose) polymerase as derived from crystal structures and mutagenesis. *J Mol Biol* **278:**57–65. Copyright © 1998 Academic Press. Used with permission.) C, structure of carba-NAD: the ring oxygen of the nicotinamide ribose is replaced by a methylene group, which prevents ADP-ribosyl transfer and hydrolysis of the nicotinamide moiety by cleavage of the β-glycosidic bond.

PARP-1 inhibitors are very useful tools for studying the biological functions of the PARP enzyme. Moreover, PARP inhibitors may have additional applications as anticancer agents and therapeutic agents against many other diseases (Southan and Szabó, 2003). Most PARP inhibitors act as competitive inhibitors of the enzyme, blocking NAD^+ binding to the catalytic domain of PARP. In addition, a common structural feature of PARP inhibitors is the presence of either a carboxamide or an imide group built in a polyaromatic heterocyclic skeleton or a carbamoyl group attached to an aromatic ring (Virág and Szabó, 2002). The oxygen atom from the carbonyl group seems to function as a hydrogen acceptor, and the hydrogen atom from the amide or imide groups acts as a proton donor in the hydrogen-bond interaction with PARP (Virág and Szabó, 2002). Nicotinamide, the smaller cleavage product of NAD^+, and 3-aminobenzamide were the first reported PARP inhibitors. Both compounds inhibit PARP with a low potency, have limited intracellular accumulation, and exert a variety of nonspecific actions, including antioxidants effects (Farber et al., 1990). The inhibitory effect of nicotinamide on PARP-1 allows negative feedback regulation of the enzyme. In recent years, several potent and selective PARP inhibitors have been synthesized. Figure 1B shows the binding of the NAD^+ analog carba-NAD to the NAD^+-binding site of the C-terminal of the PARP-1 catalytic fragment. As shown in Fig. 1C, carba-NAD has a methylene group that replaces the ring oxygen of the nicotinamide riboside of NAD^+ so that ADP-ribosyl transfer and hydrolysis of the nicotinamide moiety cannot take place, thereby inhibiting PARP activity (Ruf et al., 1998).

Mechanisms of PARP-1 Activation. PARP-1 catalyzes the cleavage of NAD^+ into nicotinamide and ADP-ribose and then uses the latter to synthesize branched nucleic acid-like polymers of poly(ADP-ribose) covalently attached to nuclear acceptor proteins (de Murcia et al., 1994; de Murcia and Menissier de Murcia, 1994; Lindahl et al., 1995; Bürkle, 2001a). As shown in Fig. 2, in response to DNA breaks produced by genotoxic stimuli, PARP catalyzes the covalent attachment of ADP-ribose units from NAD^+ to itself (automodification) and to a limited number of nuclear DNA-binding proteins (heteromodification) such as histones, adaptor factors, and DNA repair effectors (Lautier et al., 1993; Ruf et al., 1996). The reaction is $NAD^+ + X \rightarrow X - 1'$-ribose-$5'$-ADP + nicotinamide, where X is a side chain of a protein to be modified (generally a glutamate, chain initiation, the $2'$- or $3'$-OH group of the ADP moiety of monomeric or polymeric ADP-ribose (chain elongation or branching), or water (hydrolysis). When PARP-1 is activated through binding to DNA breaks, the enzyme acts as a transferase, the so-called poly(ADP-ribose) transferase, adding ADP-ribose units to carboxyl groups of aspartic and glutamic residues. This reaction continues ahead by a short-lived ($t_{1/2}$ = 1 min) polymeriza-

tion of ADP-riboses [i.e., poly(ADP)-ribosylation (Kupper et al., 1990; Satoh et al., 1994)]. If DNA damage is repaired, then the cell survives. However, in the event of irreparable DNA damage, PARP-1 is overactivated, producing depletion of both NAD^+ and ATP stores, and then the cell may die by necrosis. As also shown in Fig. 2, poly(ADP-ribosylation) is a dynamic process in which poly(ADP-ribose) polymers are degraded by poly(ADP-ribose) glycohydrolase (PARG). PARP activation leads to automodification through poly(ADP-ribosylation), resulting in PARP inhibition. So, removal of inhibitory poly(ADP-ribose) units by PARG enzyme from the automodification domain of PARP is required to reactivate PARP and to allow for continuous NAD^+ turnover (Davidovic et al., 2001).

PARP may be also activated through a signal transduction pathway linked to phospholipase C–inositol 1,4,5-triphospate (IP_3)–calcium route. In fact, some evidence for a fast activation of nuclear PARP-1 by electrical activity generated from high $[K^+]$-induced depolarization on the cell membrane of brain cortical neurons has been reported (Homburg et al., 2000). This novel mode of signaling to the cell nucleus may be an alternative mechanism of PARP-1 activation that would not involve the formation of DNA breaks. PARP-1 would be activated by intracellular Ca^{2+} mobilization into the nucleoplasm from IP_3-gated stores. Hence, IP_3-induced Ca^{2+} release into the nucleoplasm would be a possible mechanism for the depolarization-induced activation of PARP-1. In this sense, it is interesting to point out that an increase in intracellular $[Ca^{2+}]$ together with an enhanced IP_3 production has been detected in neurons during membrane depolarization (Al-Mohanna et al., 1994). Therefore, it has been hypothesized that PARP-1 might be a downstream target of phospholipase C that would modulate, through poly(ADP-ribosylation), the activity of transcription factors in response to signals promoting phosphoinositides turnover and phosphatidyl-inositol 4,5-bisphosphate hydrolysis (Fruman et al., 1998; Toker, 1998). Moreover, the role of PARP-1 in DNA repair and transcription might underlie the effect of depolarization in protecting growth factor-deprived neurons from apoptotic cell death (D'Mello et al., 1993; Galli et al., 1995). However, the physiological relevance of the phospholipase C–IP_3–calcium pathway in PARP-1 activation remains unclear.

PARP-1 Involvement in the Mechanisms of Cell Death

In the 1990s, cell biologists used the expression "death substrate" (Tewari et al., 1995) to name PARP-1, one of the first identified substrates of caspases, the main executioners of apoptosis (Kaufmann et al., 1993). Therefore, a role for PARP-1 in the regulation of apoptosis has been suggested. Some data in the literature point toward a possible role of PARP-1 in apoptosis; however, more convincing evidence suggests the involvement of PARP-1 in necrosis. In this regard, it is important to know the role that PARP-1 plays in these two main pathways of cell death.

Role of PARP-1 in Apoptotic Pathways. Apoptosis, also called "programmed cell death" or "cell suicide", is considered a controlled biochemical pathway of cell demise. The apoptotic process is generally divided in three main phases (initiation, effector, and execution) and requires ATP (González et al., 2001). The last phase of the apoptotic process is a common irreversible execution stage in which cysteine proteases called caspases digest cellular proteins. Proteins that are cleaved by caspases during apoptosis include, among others, pro-caspase-3 and pro-caspase-7, Bcl-2 family proteins (Bid, BcL-X_L, and Bcl2), structural proteins (actin, cytokeratin, and focal adhesion kinase), neural cell adhesion molecules, signal-transduction proteins (e.g., Ca^{2+} /calmodulin-dependent protein kinase), and DNA repair and cell cycle regulatory proteins (PARP, DNA polymerase-ϵ, cyclin D, and p53) (Orrenius et al., 2003). At the end of the execution phase, the cell is dismantled through the formation of apoptotic bodies, and nuclear DNA is cleaved into oligonucleosomal fragments by an endonuclease. During apoptosis,

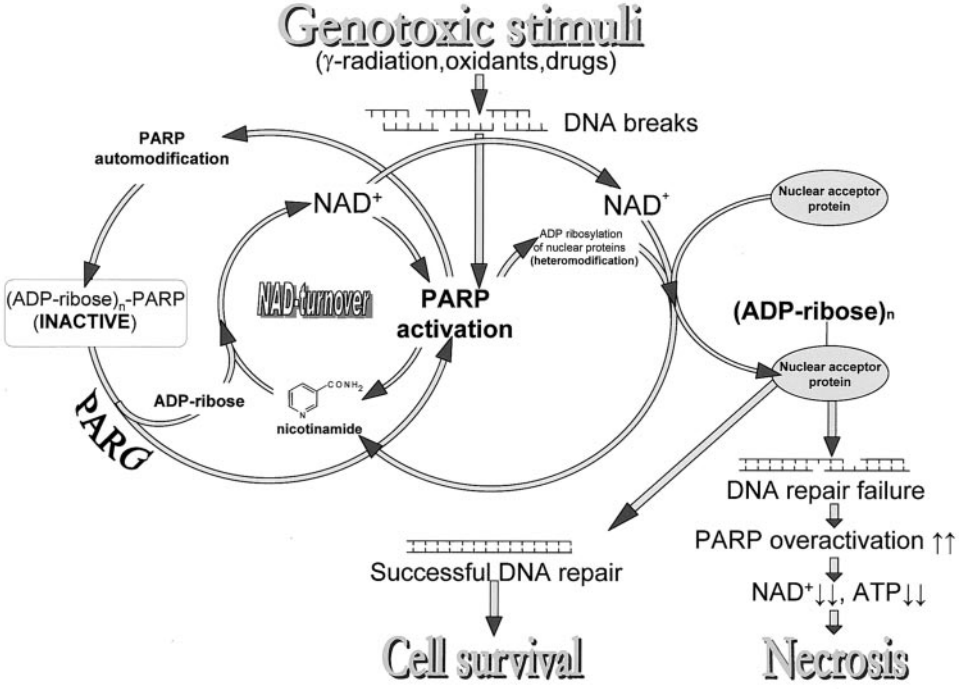

Fig. 2. Activation of PARP-1 through the formation of DNA strand breaks after genotoxic stimuli. DNA damage causes PARP activation and subsequent cleavage of NAD^+ into nicotinamide and ADP-ribose. ADP-ribose units are polymerized onto nuclear acceptor proteins (heteromodification). Poly(ADP-ribosylation) allows DNA repair and cell survival. PARP activation also leads to automodification of PARP by ADP-ribosylation, which results in PARP inhibition. By removing poly(ADP-ribose) from PARP, PARG reactivates PARP and allows NAD^+ turnover. Severe DNA damage leads to overactivation of PARP, leading to NAD^+ and ATP depletion and subsequent necrotic cell death.

PARP-1 is cleaved into two fragments, p89 and p24, by the caspase-3,-6,-7 complex (Germain et al., 1999), which recognizes the DEVD motif in the nuclear localization signal of PARP-1 (Lazebnik et al., 1994). Caspase cleavage at this site separates the DBD from the catalytic domain, resulting in the inactivation of PARP. Cleavage fragments contribute to the suppression of PARP activity because p89 and p24 inhibit homoassociation and DNA binding of intact PARP-1, respectively (Kim et al., 2000; D'Amours et al., 2001). This is a positive feedback loop in caspase-mediated PARP-1 inactivation, which suggests that blocking PARP-1 activation is vital for the proper function of the apoptotic machinery. So, PARP cleavage aims at preventing the activation of PARP by the ensuing DNA fragmentation and thereby aims at preserving cellular energy for certain ATP-sensitive steps of apoptosis (Virág and Szabó, 2002). In conclusion, PARP cleavage seems to be vital for the appropriate function of apoptosis.

Role of PARP-1 in Necrotic Cell Death. Considering that cell death takes place in a tissue or organ, necrosis may not be simply regarded as an accidental type of cell demise but rather as a form of cell death more severe than apoptosis. (Leist et al., 1997). There are several biochemical and morphological differences between apoptosis and necrosis; however, the most distinctive characteristic of necrosis is the disintegration of the plasma membrane, as opposed to the compaction of apoptotic cells (Eguchi et al., 1997). During apoptosis, cells are rapidly cleared from the tissues by macrophages, whereas in necrosis, the leakage of cell content from necrotic cells into the surrounding tissue may contribute to organ injury (Golstein et al., 1991). It has been proposed that apoptosis and necrosis are at two ends of a continuum of possible modes of cell death in which apoptosis and necrosis are triggered by mild and by severe genotoxic stimuli, respectively (Bonfoco et al., 1995; Nicotera et al., 1999). Moreover, it has also been suggested that ATP is an important determinant of the mode of cell death, especially in oxidatively injured cells or in cells treated with DNA-binding antineoplastic drugs such as cisplatin, doxorubicin, and etoposide (Nicotera et al., 1998; Leist et al., 1999; Ran et al., 1999; Crowley et al., 2000; Fuertes et al., 2003b). The reason is that severe ATP depletion, which causes necrosis, is brought about by the fall in mitochondrial permeability transition induced by cytotoxic stimuli. Fall in mitochondrial permeability transition produces inhibition of mitochondrial oxidative phosphorylation that generates ATP so that ATP depletion blocks caspase cleavage of PARP, leading to continued PARP activity. PARP overactivation results in NAD^+ depletion and further ATP depletion (Green and Reed, 1998). Therefore, it is likely that PARP, as a NAD^+-catabolizing enzyme, may serve as a molecular switch between apoptosis and necrosis. Several groups have reported that inhibition of PARP activity induces protection against necrotic cell death. However, PARP inhibition does not protect cells from apoptosis (Palomba et al., 1999; Szabó et al., 2001; Tentori et al., 2001). Thus, a role of PARP activation in necrosis is consistent with the fact that the inhibition or absence of PARP provides the most remarkable protection in disease models such as stroke, myocardial infarction, or mesenteric ischemia-reperfusion injury, which are characterized predominantly by necrotic-type cell death (Miesel et al., 1995). PARP moderately activated may decrease cellular NAD^+ content without being fatal to the cells. In these conditions, cellular energetics, moderately compromised, may cause cell dysfunction. It seems that pharmacological inhibition of PARP, by improving cellular energetics, may rescue dysfunctional cells and thereby can restore cell function.

Drug-Induced DNA Damage and PARP-Mediated Cellular Responses. As shown in Fig. 3, it is currently accepted that cells exposed to DNA-damaging drugs and other genotoxic stimuli may undergo three pathways depending on the intensity of the DNA injury (Virág and Szabó, 2002). Thus, a mild DNA damage activates PARP-1, which

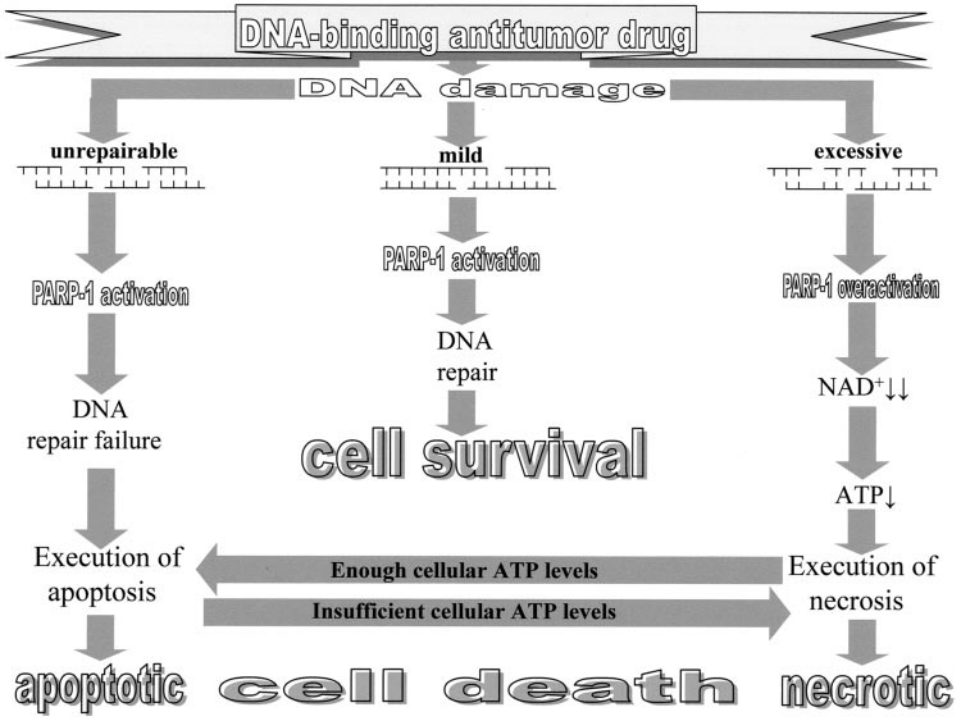

Fig. 3. The fate of a tumor cell after exposure to a DNA-binding drug depends on the degree of DNA damage as well as on the availability of energy and the metabolic condition of the cell. Mild DNA damage induces PARP activation and efficient DNA repair (cell survival pathway). Intense DNA damage may activate p53-dependent (or independent) apoptosis after DNA repair failure (apoptotic pathway). Very intense DNA damage may induce PARP overactivation and subsequent depletion of NAD^+ and ATP stores, leading to necrotic cell death. Necrotic and apoptotic pathways are interconnected so that there is a continuum of possible modes of cell death between apoptosis and necrosis, depending on the energetic status of the cell (i.e., ATP levels). Therefore, a cell that initiates the apoptotic program may finally die by necrosis if during the course of apoptosis ATP levels descend to cell death-inducing levels. Conversely, if PARP overactivation does not decrease ATP levels to cell death-inducing levels, then the cell may die by apoptosis.

subsequently interacts via its zinc-finger domains with several proteins involved in DNA repair (i.e., XXRCC1, polymerase β, and DNA ligase III) and modifies these repair proteins through poly(ADP-ribosylation). If DNA repair proceeds successfully, then the cell survives. Alternatively, if DNA damage is too severe to be repairable, p53-dependent (or even independent) apoptosis takes place, so that the caspase-3,-6,-7 complex cleaves PARP-1. Hence, by inactivating PARP, the caspase-3,-6,-7 complex relieves necrosis-mediated cell death by virtue of preventing the depletion in NAD^+ and ATP (Green and Reed, 1998; Herceg and Wang, 1999). A third pathway may be induced by extensive DNA breakage in which overactivation of PARP cleaves NAD^+ into nicotinamide and ADP-ribose moieties and polymerizes the latter onto nuclear acceptor proteins. Decrease of NAD^+ levels inhibits production of ATP through oxidative phosphorylation, leading to ATP depletion and necrotic cell death (Martin et al., 2000).

It is known that certain types of cancer cells, when exposed to antineoplastic drugs, show features such as internucleosomal DNA degradation, blebbing of the cell surface, and cell shrinkage, which are consistent with apoptosis as a form of cell death (Henkels and Turchi, 1997). In contrast, other cell lines, particularly those with resistance to chemotherapy, show characteristic features of necrosis (Guchelaar et al., 1998; Pérez et al., 1999). Besides, it has been reported that in the same population of cancer cells treated with antitumor drugs (i.e., *cis*- and *trans*-platinum compounds), necrotic and apoptotic cell death may take place together (Montero et al., 2002). Apoptosis and necrosis have been usually considered morphologically and mechanistically separate pathways of cell death (Wyllie, 1987). In the 1980s, necrosis was considered the mode of cell death induced by DNA-damaging anticancer drugs because of the activity of PARP (Tanizawa et al., 1989). However, by the 1990s, apoptosis was thought to be the usual form of cell death induced by most clinically effective anticancer agents that bind to DNA (Eastman, 1999). The end of the 1990s saw these two hypotheses unified by the discovery that intracellular ATP levels dictate whether antitumor drugs induce cell death by necrosis or apoptosis and that both processes of cell death are interconnected. Thus, ATP depletion is the cause of necrosis, whereas ATP is necessary for the execution of the apoptotic program. As shown in Fig. 3, if DNA damage is unrepaired and ATP levels are sufficient to maintain caspase activity, PARP-1 is cleaved and antitumor drug-induced cell death may proceed through apoptosis. Conversely, if ATP levels are too low to maintain apoptosis, then the cell dies by necrosis (Eguchi et al., 1997).

Pharmacological Modulation of PARP in Cancer Chemotherapy

As mentioned above, it is currently believed that within a population of tumor cells, necrosis and apoptosis may take place together (but in different cells) in response to cytotoxic drugs. One reason is that different drug concentrations reach different cancer cells; low concentrations induce apoptosis, and higher concentrations produce necrotic cell death. However, the particular mode of cell death induced after drug treatment is dependent not only on the drug and its concentration but also on the particular cell line and the metabolic status (mainly ATP levels) of the cell (González et al., 2001). On the other hand, occurrence of resistance is a common drawback of cancer chemotherapy. Drug resistance in tumor cells is often multifactorial, including decreased drug accumulation, intracellular drug detoxification, enhanced DNA repair/tolerance, and failure of apoptotic pathways (Fuertes et al., 2003a). In certain cases of drug resistance, apoptotic cell death pathways may be inhibited. Highly resistant tumor cells may bear genetic alterations of caspases or they may contain endogenous caspase inhibitors (Schimmer et al., 2003). Moreover, highly resistant tumor cells may prevent apoptosis because of a drastic reduction of the bioenergetic cellular (BEC) index (Cuezva et al., 2002). The BEC index is a nondimensional ratio that expresses the mitochondrial activity (oxidative phosphorylation), as indirectly evaluated by the β-F1ATPase:70-kDa heat shock protein ratio, relative to the cellular glycolytic potential as assessed from the amount of GAPDH (Cuezva et al., 2002). This index is an indirect measure of the energetic status of the cell and therefore of the cellular ATP levels. Cancer cells with low BEC indices (i.e., low cellular ATP levels) may be highly resistant to programmed cell death because apoptosis is an energy-dependent biochemical pathway. Therefore, it is very likely that antitumor drugs provoke cell death by necrosis in most cancer cell lines with low BEC indices. Taking into account all the above-mentioned considerations about the degree of sensitivity of the tumor to cancer chemotherapy, several approaches may be taken to enhance the cytotoxic effect of antitumor drugs through pharmacological modulation of PARP activity.

PARP Inhibitors as Adjuvant Drugs in Cancer Chemotherapy. It has been reported that PARP-1 activity may show an inverse correlation with the degree of cell differentiation, meaning that most tumors would have accelerated poly(ADP-ribose) metabolism. For instance, increased PARP activity has been found in hepatocellular tumors in comparison with healthy liver cells (Shiobara et al., 2001). Some studies have reported that PARP inhibitors such as 6(5H)-phenanthridinone or 4-iodo-3-nitrobenzamide may have a direct cytotoxic effect on tumor cells (Weltin et al., 1994; Mendeleyev et al., 1995). However, most studies have focused on the synergistic cytotoxic effect of PARP inhibitors in combination with γ-radiation or DNA-binding drugs, including alkylating agents and topoisomerase inhibitors (Bowman et al., 1998; Tentori et al., 2002). DNA-binding antitumor drugs directly damage DNA, inducing DNA breaks and subsequent PARP activation. As may be deduced from Fig. 3, inhibition of PARP in cells exposed to DNA-damaging drugs would decrease DNA repair and would induce apoptotic cell death, decreasing necrotic cell death and preventing the pathological side effects of necrosis. It is interesting to note that PARP inhibitors might be more effective against tumor cells than against normal cells. For example, in low-grade malignant non-Hodgkin lymphoma cells and hepatocellular carcinomas, increased PARP-1 activity has been reported compared with healthy lymphocytes or hepatocytes (Shiobara et al., 2001). Therefore, PARP inhibition may render tumor cells more sensitive to cytotoxicity induced by DNA-damaging antitumor drugs. For instance, it has been recently reported that PARP inhibitors 3-aminobenzamide or NU1025 increase apoptosis and reduce necrosis induced by the DNA minor groove binder $MeOSO_2(CH_2)_2$-lexitropsin (Me-Lex) (Tentori et al., 2001). Then, the use of PARP inhibitors in combination

with DNA-binding drugs may result in the enhancement of the apoptotic activity of this type of antitumor drugs.

ATP-Depleting Agents as Adjuvant Therapy in Antitumor Drug-Induced PARP Overactivation. Tumors usually contain neoplastic cells with high resistance to the anticancer drugs. In addition, highly resistant tumors are usually treated with high doses of chemotherapy (Mueller et al., 2003). Intensive chemotherapy induces severe DNA damage, which leads to PARP overactivation, resulting in the depletion of NAD^+ and ATP and consequently in necrotic cell death. During necrosis, cellular contents are released into the tissue, exposing neighboring cells to potential damage by proteases and other released factors (Golstein et al., 1991). Although necrotic cell death induces local toxic effects in the body, annihilation of tumor cells resistant to chemotherapy may prevent not only a tumor recurrence but also the formation of metastases from the primary tumor site. On the other hand, it is known that after exposure of a resistant tumor to high doses of antitumor drugs, the PARP overactivation-induced reduction in levels of both NAD^+ and ATP may drop overall but will still be elevated in a few subpopulations of highly-resistant cells. Therefore, intensive chemotherapy may not be enough to kill these highly resistant tumor cells (Martin et al., 2000). Moreover, other subpopulations of highly resistant tumor cells may be able to survive under severe conditions of ATP depletion, meaning that they may be injured sublethally and may recover after drug treatment. In both cases, pharmacological manipulation of tumor cell energy to further depress NAD^+ and ATP to levels sufficiently low to cause necrotic death may provoke the killing of highly resistant tumor cells (Nord et al., 1997). In fact, it has been recently reported that a concomitant ATP-depleting strategy, called MAP regime, enhances antitumor drug-induced cell killing in sublethally injured cancer cells through activation of the PARP-associated biochemical mechanism of necrotic cell death. This therapeutic strategy was based on data showing that extensive drug-induced DNA damage leads to overactivation of the PARP enzyme (Martin et al., 2000). The MAP regime is a combination of 6-methylmercaptopurine riboside plus 6-aminonicotinamide (6-AN) plus *N*-(phosphonacetyl)-L-aspartic acid (PALA). Whereas 6-AN, a NAD^+ antagonist, inhibits glycolytic production of ATP (Street et al., 1996), 6-methylmercaptopurine riboside is an inhibitor of de novo purine biosynthesis that limits adenine supplies for ATP production (Shantz et al., 1973). PALA inhibits aspartate transcarbamylase and selectively lowers pyrimidine nucleotide levels in tumors (Martin et al., 1983; Fuertes et al., 2003b). The MAP regime not only depletes ATP levels but also affects the pyridine nucleotide pool (NAD^+/NADH plus $NADP^+$/NADPH). ATP depletion to lethal levels by MAP regime prevents caspase activity from completing anticancer drug-induced apoptosis because the caspase-3,-6,-7 complex cannot cleave PARP (Martin et al., 2000). Then, ATP depletion is further continued via PARP-induced NAD^+ depletion so that the cell is forced to die by necrosis because there is not enough energy to support apoptosis (Boulares et al., 1999). Interestingly, the MAP regime has shown to improve the antitumor activity of several antitumor agents (i.e., doxorubicin, etoposide, paclitaxel, 5-fluorouracil, and cisplatin) in mice bearing human tumor xenografts (Martin et al., 2001). In addition, it should be mentioned that the combination of PALA with some drugs does not provoke severe toxicity in animals. Moreover, 6-AN, as a single agent, has been safely administered to patients with disseminated cancer in phase I clinical trials (Martin et al., 2000).

Summary and Outlook

Preclinical studies have shown that pharmacological modulation of PARP-1 activity may represent a useful tool to treat cancer cells with different degrees of sensitivity to DNA-binding antitumor drugs. In sensitive tumor cells, the use of PARP inhibitors may avoid efficient DNA repair, allowing low doses of anticancer drug to induce the elimination of tumor cells by apoptosis. Moreover, it is interesting to note that PARP inhibitors might be more effective against some type of tumor cells than against normal cells. For instance, in low-grade malignant non-Hodgkin lymphoma cells and hepatocellular carcinomas, increased PARP-1 activity has been reported compared with healthy lymphocytes or hepatocytes (Wielckens et al., 1980; Shiobara et al., 2001). In addition, it has also been found that the sensitivity of certain tumor cells to chemotherapeutic regimens is higher than that of normal cells because of an inappropriate overexpression of oncogenes. In fact, overexpression of E1A and c-*myc* oncogenes increases tumor cell susceptibility to apoptosis induced by several DNA-binding anticancer agents, including etoposide, doxorubicin, 5-fluorouracil, and cisplatin (Lowe and Lin, 2000). Neoplastic cell populations usually contain tumor cells of variable sensitivity to antineoplastic drugs. In addition, when a tumor relapses, it usually becomes highly resistant to chemotherapy (acquired resistance). Moreover, some tumors are highly resistant to cancer chemotherapy (intrinsic resistance). Therefore, in drug-resistant tumors, the use of ATP-depleting agents as potentiators of the effect of PARP overactivation may induce the killing of sublethally injured cells through necrosis, increasing the chances of complete tumor regression (Martin et al., 2000, 2001). From a biochemical point of view, specific differences in the BEC index may be the key determinants of sensitivity to antitumor drug-induced genotoxic stimuli (Fuertes et al., 2003b). Because the BEC index is directly correlated with the cellular ATP levels, tumor cells with low BEC index (low ATP levels), such as liver, kidney, and colon carcinomas, are usually highly resistant to the execution of programmed cell death in response to antitumor drugs (Cuezva et al., 2002). Thus, in highly resistant tumors with a low BEC index, the use of anticancer drugs in combination with ATP-depleting agents might further depress ATP levels to cell death-inducing levels without jeopardizing the viability of normal cells (Martin et al., 2000). We think that the use of PARP inhibitors (apoptotic tumor cell death) and of ATP-depleting regimes (necrotic tumor cell death) as complementary anticancer therapies merits future clinical exploration. Figure 4 summarizes these two therapeutic strategies of modulation of PARP activity, taking into account the degree of tumor cell resistance. However, in terms of the overall host immune response to dying tumor cells, apoptotic destruction of tumor cells might have immunological advantages for cancer patients relative to necrotic tumor cell death. It is known that the host tries to fight against an established tumor through presentation of antigens from peripheral tumor cells to CD8 cytotoxic T cells, a process known as "cross-presentation (den Haan et al., 2000). Interestingly enough, it has been recently reported that in-

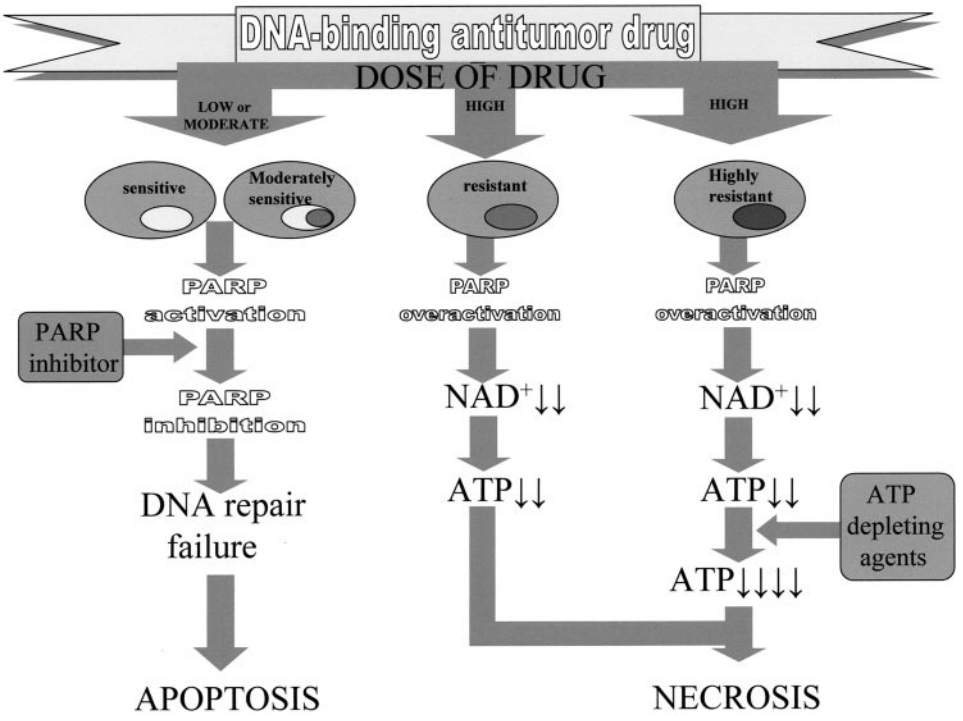

Fig. 4. Pharmacological modulation of PARP activity in cancer chemotherapy. In tumor cells sensitive or moderately sensitive to chemotherapy, a low or moderate dose of drug in combination with PARP inhibition may result in efficient block of DNA repair and subsequent apoptotic cell death. In contrast, in resistant or highly resistant tumor cells, PARP overactivation through intensive chemotherapy in combination with ATP-depleting agents may increase tumor cell killing. Resistant cells would die as a result of PARP overactivation, ATP depletion, and necrosis. However, highly resistant cells might not receive enough damage to reduce ATP levels to cell death-inducing levels. To prevent survival of highly-resistant tumor cells, the use of high doses of chemotherapy together with ATP-depleting agents might further depress ATP to cell death-inducing levels, provoking necrotic cell death of these extremely resistant cells.

duction of tumor cell apoptosis in mice may increase the efficiency of cross-presentation and the response of tumor specific cytotoxic T cells (Nowak et al., 2003).

Recently, several additional enzymes catalyzing poly(ADP-ribosylation) have been identified. Taking into account their intracellular localization and dependence or independence for activation on DNA damage, these new members of the PARP-family may have specific biological functions (Bürkle, 2001b). Therefore, future research must be also focused on how PARP inhibitors may affect the function of these other PARP isoforms and its potential pharmacological exploitation in cancer and other diseases.

It has been hypothesized that cancer cells and trypanosomes may share some basic molecular mechanism of proliferation and/or cell survival. Thus, indirect evidence suggests the presence of a PARP activity in *Trypanosoma cruzi* (Isola et al., 1987). On the other hand, the isolation and partial purification of a PARP-like enzyme from the trypanosomatid protozoan *Crithidia fasciculata* has been recently reported (Villamil et al., 2001). Of interest was the observation that this PARP-like enzyme was inhibited by the lipophilic drug *o*-naphthoquinone β-lapachone, which induces PARP cleavage in cancer cells and PARP inhibition in Chinese hamster ovary cells (Vanni et al., 1998; Pink et al., 2000). Hence, inhibition of PARP activity might be also exploited in the future to increase the antiparasitic effect of DNA-binding drugs such as pentamidine and related analogs. In summary, PARP enzymes are pharmacological targets that offer several opportunities of intervention to increase the cytotoxic activity of chemotherapy against cancer and other proliferative malignancies.

References

Al-Mohanna FA, Caddy KW, and Bolsover SR (1994) The nucleus is insulated from large cytosolic calcium ion changes. *Nature (Lond)* **367:**745–750.

Bonfoco E, Krainc D, Ankarcrona M, Nicotera P, and Lipton SA (1995) Apoptosis and necrosis: two distinct events induced, respectively, by mild and intense insults with N-methyl-D-aspartate or nitric oxide/superoxide in cortical cell cultures. *Proc Natl Acad Sci USA* **92:**7162–7166.

Boulares AH, Yokovlev AG, Ivanova V, Stoica BA, Wang G, Iyer S, and Smulson M (1999) Role of poly(ADP-ribose) polymerase (PARP) cleavage in apoptosis. Caspase 3-resistant PARP mutant increases rates of apoptosis in transfected cells. *J Biol Chem* **274:**22932–22940.

Bowman KJ, White A, Golding BT, Griffin RJ, and Curtin NJ (1998) Potentiation of anti-cancer agent cytotoxicity by the potent poly(ADP-ribose) polymerase inhibitors NU1025 and NU1064. *Br J Cancer* **78:**1269–1277.

Bürkle A (2001a) Physiology and pathophysiology of poly(ADP-ribosyl)ation. *Bioessays* **23:**795–806.

Bürkle A (2001b) PARP-1: a regulator of genomic stability linked with mammalian longevity. *ChemBioChem* **2:**725–728.

Crowley CL, Payne CM, Bernstein H, Bernstein C, and Roe D (2000) The NAD^+ precursors, nicotinic acid and nicotinamide protect cells against apoptosis induced by a multiple stress inducer, deoxycholate. *Cell Death Differ* **7:**314–326.

Cuezva JM, Krajewska M, López de Heredia M, Krajewski S, Santamaría G, Kim H, Zapata JM, Marusawa H, Chamorro M, and Reed JC (2002) The bioenergetic signature of cancer: a marker of tumor progression. *Cancer Res* **62:**6674–6681.

D'Amours D, Desnoyers S, D'Silva I, and Poirier GG (1999) Poly(ADPribosyl)ation reactions in the regulation of nuclear functions. *Biochem J* **342:**249–268.

D'Amours D, Sallmann FR, Dixit VM, and Poirier GG (2001) Gain-of-function of poly(ADP-ribose) polymerase-1 upon cleavage by apoptotic proteases: implications for apoptosis. *J Cell Sci* **114:**3771–3778.

D'Mello SR, Galli C, Ciotti T, and Calissano P (1993) Induction of apoptosis in cerebellar granule neurons by low potassium: inhibition of death by insulin-like growth factor I and cAMP. *Proc Natl Acad Sci USA* **90:**10989–10993.

Davidovic L, Vodenicharov M, Affar EB, and Poirier GG (2001) Importance of poly(ADP-ribose) glycohydrolase in the control of poly(ADP-ribose) metabolism. *Exp Cell Res* **268:**7–13.

de Murcia G and Menissier de Murcia J (1994) Poly(ADP-ribose) polymerase: a molecular nick-sensor. *Trends Biochem Sci* **19:**172–176.

de Murcia G, Schreiber V, Molinete M, Saulier B, Poch O, Masson M, Niedergang C, and Menissier de Murcia J (1994) Structure and function of Poly(ADP-ribose) polymerase. *Mol Cell Biochem* **138:**15–24.

den Haan JM, Lehar SM, and Bevan MJ (2000) $CD8^+$ but not $CD8^-$ dendritic cells cross-prime cytotoxic T cells in vivo. *J Exp Med* **192:**1685–1696.

Du L, Zhang X, Han YY, Burke NA, Kochanek PM, Watkins SC, Graham SH, Carcillo JA, Szabó C, and Clark RSB (2003) Intra-mitochondrial Poly(ADP-ribosylation) contributes to NAD^+ depletion and cell death induced by oxidative stress. *J Biol Chem* **278:**18426–18433.

Eastman A (1999) The mechanism of action of cisplatin: from adducts to apoptosis, in *Cisplatin, Chemistry and Biochemistry of a Leading Anticancer Drug* (Bernhard Lippert ed) p 111, Wiley-VCH, Basel, Switzerland.

Eguchi Y, Shimizu S, and Tsujimoto Y (1997) Intracellular ATP levels determine cell death fate by apoptosis or necrosis. *Cancer Res* **57:**1835–1840.

Farber JL, Kyle ME, and Coleman JB (1990) Mechanisms of cell injury by activated oxygen species. *Lab Invest* **62:**670–679.

Fruman DA, Meyers RE, and Cantley LC (1998) Phosphoinositide kinases. *Annu Rev Biochem* **67:**481–507.

Fuertes MA, Alonso C, and Pérez JM (2003a) Biochemical modulation of cisplatin mechanism of action: from cytotoxicity to induction of cell death through interconnections between apoptotic and necrotic pathways. *Curr Med Chem* **10:**257–266.

Fuertes MA, Castilla J, Alonso C, and Pérez JM (2003b) Cisplatin biochemical

mechanisms of action: enhancement of antitumor activity and circumvention of drug resistance. *Chem Rev* **103:**645–663.
Galli C, Meucci O, Scorziello A, Werge TM, Calissano P and Schettini G (1995) Apoptosis in cerebellar granule cells is blocked by high KCl, forskolin and IGF-I through distinct mechanisms of action: the involvement of intracellular calcium and RNA synthesis. *J Neurosci* **15:**1172–1179.
Germain M, Affar EB, D'Amours D, Dixit VM, Salvesen GS, and Poirier GG (1999) Cleavage of automodified poly(ADP-ribose) polymerase during apoptosis. Evidence for involvement of caspase-7. *J Biol Chem* **274:**28379–28384.
Golstein P, Ojcius DMA, and Young JDE (1991) Cell death mechanisms and the immune system. *Immunol Rev* **121:**29–65.
González VM, Fuertes MA, Alonso C, and Pérez JM (2001) Is cisplatin-induced cell death always produced by apoptosis? *Mol Pharmacol* **59:**657–663.
Green DR and Reed JC (1998) Mitochondria and apoptosis. *Science (Wash DC)* **281:**1309–1312.
Guchelaar HJ, Vermes I, Koopmans RP, Reutelingsperger CPM, and Haanen C (1998) Apoptosis- and necrosis-inducing potential of cladribine, cytarabine, cisplatin and 5-fluorouracil in vitro: a quantitative pharmacodynamic model. *Cancer Chemother Pharmacol* **42:**77–83.
Henkels KM and Turchi JJ (1997) Induction of apoptosis in cisplatin-sensitive and -resistant human ovarian cancer cell lines. *Cancer Res* **57:**4488–4492.
Herceg Z and Wang ZQ (1999) Failure of poly(ADP-ribose) polymerase cleavage by caspases leads to induction of necrosis and enhanced apoptosis. *Mol Cell Biol* **19:**5124–5133.
Homburg S, Visochek L, Moran N, Dantzer F, Priel E, Asculai E, Schwartz D, Rotter V, Dekel N, and Cohen-Armon M (2000) A fast signal-induced activation of poly-(ADP-ribose) polymerase: a novel downstream target of phospholipase C. *J Cell Biol* **150:**293–307.
Isola ELD, Lammel EM, and González Cappa SM (1987) *Trypanosoma cruzi*: differentiation to metacyclic trypomastigotes in the presence of ADP-ribosyltransferase inhibitors. *Exp Parasitol* **64:**424–429.
Kaufmann SH, Desnoyers S, Ottaviano Y, Davidson NE, and Poirier GG (1993) Specific proteolytic cleavage of poly(ADP-ribose) polymerase: an early marker of chemotherapy-induced apoptosis. *Cancer Res* **53:**3976–3985.
Kim JW, Kim K, Kang K, and Joe CO (2000) Inhibition of homodimerization of poly(ADP-ribose) polymerase by its C-terminal cleavage products produced during apoptosis. *J Biol Chem* **275:**8121–8125.
Kupper JH, de Murcia G, and Burkle A (1990) Inhibition of poly(ADP-ribosyl)ation by overexpressing the poly(ADP-ribose)polymerase DNA-binding domain in mammalian cells. *J Biol Chem* **265:**18721–18724.
Lautier D, Lagueux J, Thibodeau J, Ménard L, and Poirier GG (1993) Molecular and biochemical features of poly (ADP-ribose) metabolism. *Mol Cell Biochem* **122:**171–193.
Lazebnik YA, Kaufmann SH, Desnoyers S, Poirier GG, and Earnshaw WC (1994) Cleavage of poly(ADP-ribose) polymerase by a proteinase with properties like ICE. *Nature (Lond)* **371:**346–347.
Leist M, Single B, Castoldi AF, Kühnle S, and Nicotera P (1997) Intracellular adenosine triphosphate (ATP) concentration: a switch in the decision between apoptosis and necrosis. *J Exp Med* **185:**1481–1486.
Leist M, Single B, Naumann H, Fava E, Simon B, Kuhnle S, and Nicotera P (1999) Inhibition of mitochondrial ATP generation by nitric oxide switches apoptosis to necrosis. *Exp Cell Res* **249:**396–403.
Lindahl T, Satoh MS, Poirier GG, and Klungland A (1995) Post-translational modification of poly(ADP-ribose)polymerase induced by DNA strand breaks. *Trends Biochem Sci* **20:**405–412.
Lowe SW and Lin AW (2000) Apoptosis in cancer. *Carcinogenesis* **21:**485–495.
Marsischky GT, Wilson BA, and Collier RJ (1995) Role of glutamic acid 988 of human poly(ADP-ribose) polymerase in polymer formation. Evidence for active site similarities to the ADP-ribosylating toxins. *J Biol Chem* **270:**3247–3255.
Martin DS, Stolfi RL, Sawyer RC, Spiegelman S, Casper ES, and Young CW (1983) Therapeutic utility of utilizing low doses of N-(phosphonacetyl)-L-aspartic acid in combination with 5-fluorouracil: a murine study with clinical relevance. *Cancer Res* **43:**2317–2321.
Martin DS, Bertino JR, and Koutcher JA (2000) ATP depletion + pyrimidine depletion can markedly enhance cancer chemotherapy: Fresh insight for a new approach. *Cancer Res* **60:**6776–6783.
Martin DS, Spriggs D, and Koutcher JA (2001) A concomitant ATP-depleting strategy markedly enhances anticancer activity. *Apoptosis* **6:**125–131.
Masson M, Rolli V, Dantzer F, Trucco C, Schreiber V, Fribourg S, Molinete M, Ruf A, Alves Miranda E, Niedergang C, et al. (1995) Poly(ADP-ribose) polymerase: structure-function relationship. *Biochimie* **77:**456–461.
Mendeleyev J, Kirsten E, Kaham A, Buki KG, and Kun E (1995) Potential chemotherapeutic of 4-iodo-3-nitrobenzamida. Metabolic reduction to the 3-nitroso derivatives and induction of cell death in tumor cells in culture. *Biochem Pharmacol* **50:**705–714.
Miesel R, Kurpisz M, and Kroger H (1995) Modulation of inflammatory arthritis by inhibition of poly(ADP ribose) polymerase. *Inflammation* **19:**379–387.
Montero EI, Pérez JM, Schwartz A, Fuertes MA, Malinge J-M, Alonso C, Leng M, and Navarro-Ranninger C (2002) Apoptosis induction and DNA interstrand cross-link formation by cytotoxic *trans*-[$PtCl_2(NH(CH_3)_2)(NHCH(CH_3)_2)$]: cross-linking between d(G) and complementary d(C) within oligonucleotide duplexes. *ChemBioChem* **3:**61–67.
Mueller T, Voigt W, Simon H, Fruehauf A, Bulankin A, Grothey A, and Schmoll HJ (2003) Failure of activation of caspase-9 induces a higher threshold for apoptosis and cisplatin resistance in testicular cancer. *Cancer Res* **63:**513–521.
Nicotera P, Leist M, and Ferrando-May E (1998) Intracellular ATP, a switch in the decision between apoptosis and necrosis. *Toxicol Lett* **102–103:**139–142.
Nicotera P, Leist M, and Ferrando-May E (1999) Apoptosis and necrosis: different execution of the same death. *Biochem Soc Symp* **66:**69–73.
Nord LD, Stolfi RL, Alfieri AA, Netto G, Reuter V, Sternberg SS, Colofiore JR, Koutcher JA and Martin DS (1997) Apoptosis induced in advanced CD8F1-murine mammary tumors by the combination of PALA, MMPR and 6AN precedes tumor regression and is preceded by ATP depletion. *Cancer Chemother Pharmacol* **40:**376–384.
Nowak AK, Lake RA, Marzo AL, Scott B, Heath WR, Collins EJ, Frelinger JA, and Robinson BWS (2003) Induction of tumor cell apoptosis in vivo increases tumor antigen cross-presentation, cross-priming rather than cross-tolerizing host tumor-specific CD8 T cells. *J Immunol* **170:**4905–4913.
Orrenius S, Zhivotovsky B, and Nicotera P (2003) Regulation of cell death: the calcium-apoptosis link. *Nat Rev Mol Cell Bio* **4:**552–565.
Palomba L, Sestili P, Columbaro M, Falcieri E, and Cantoni O (1999) Apoptosis and necrosis following exposure of U937 cells to increasing concentrations of hydrogen peroxide: the effect of the poly(ADP-ribose)polymerase inhibitor 3-aminobenzamide. *Biochem Pharmacol* **58:**1743–1750.
Pérez JM, Montero EI, Gónzalez AM, Alvarez-Valdés A, Alonso C, and Navarro-Ranninger C (1999) Apoptosis induction and inhibition of H-ras overexpression by novel *trans*-[$PtCl_2$(isopropylamine)(amine′)] complexes. *J Inorg Biochem* **77:**37–42.
Pink JJ, Planchon Sm, TAgliarino C, Varnes Me, Siegel D and Boothman DA (2000) NADP(H):quinone oxidoreductase activity is the principal determinant of β-lapachone cytotoxicity. *J Biol Chem* **275:**5416–5424.
Ran Z, Rayet B, Rommelaere J, and Faisst S (1999) Parvovirus H-1-induced cell death: influence of intracellular NAD consumption on the regulation of necrosis and apoptosis. *Virus Res* **65:**161–174.
Ruf A, Ménissier de Murcia J, de Murcia G, and Schulz GE (1996) Structure of the catalytic fragment of poly(ADP-ribose) polymerase from chicken. *Proc Natl Acad Sci USA* **93:**7481–7485.
Ruf A, Rolli V, de Murcia G, and Schulz GE (1998) The mechanism of the elongation and branching reaction of poly(ADP-ribose) polymerase as derived from crystal structures and mutagenesis. *J Mol Biol* **278:**57–65.
Satoh MS, Poirier GG, and Lindahl T (1994) Dual effect for poly(ADP-ribose) synthesis in response to DNA strand breakage. *Biochemistry* **33:**7099–7106.
Schimmer AD, Pedersen IM, Kitada S, Eksioglu-Demiralp E, Minden MD, Pinto R, Mah K, Andreeff M, Kim Y, Suh WS, et al. (2003) Functional blocks in caspase activation pathways are common in leukemia and predict patient response to induction chemotherapy. *Cancer Res* **63:**1242–1248.
Shantz GD, Smith CM, Fontenella LJ, Lau HKF, and Henderson JF (1973) Inhibition of purine nucleotide metabolism by 6-methylthiopurine ribonucleoside and structurally related compounds. *Cancer Res* **33:**2867–2871.
Shiobara M, Miyazaki M, Ito H, Togawa A, Nakajima N, Nomura F, Morinaga N, and Noda M (2001) Enhanced polyadenosine diphosphate-ribosylation in cirrhotic liver and carcinoma tissues in patients with hepatocellular carcinoma. *J Gastroenterol Hepatol* **16:**338–344.
Simonin F, Höfferer L, Panzeter P, L Muller S, de Murcia G and Althaus FR (1993) The carboxyl-terminal domain of human poly(ADP-ribose) polymerase. Overproduction in *Escherichia coli*, large scale purification and characterization. *J Biol Chem* **268:**13454–13461.
Southan GJ and Szabó C (2003) Poly(ADP-ribose) polymerase inhibitors. *Curr Med Chem* **10:**321–340.
Street JC, Mahmoud V, Ballon D, Alfieri AA, and Koutcher JA (1996) 13C and 31P NMR investigation of effect of 6-aminonicotinamide on metabolism of RIF-1 tumor cells in vitro. *J Biol Chem* **271:**4113–4119.
Szabó E, Virag L, Bakondi E, Gyure L, Hasko G, Bai P, Hunyadi J, Gergely P, and Szabó C (2001) Peroxynitrite production, DNA breakage and poly(ADP-ribose) polymerase activation in a mouse model of oxazolone-induced contact hypersensitivity. *J Invest Dermatol* **117:**74–80.
Tanizawa A, Kubota M, Hashimoto H, Shimizu T, Takimoto T, Kitoh T, Akiyama Y, and Mikama H (1989) VP-16-induced nucleotide pool changes and poly(ADP-ribose) synthesis: the role of VP-16 in interphase death. *Exp Cell Res* **185:**237–246.
Tentori L, Balduzzi A, Portarena I, Levati L, Vernole P, Gold B, Bonmassar E and Graziani G (2001) Poly(ADP-ribose) polymerase inhibitor increases apoptosis and reduces necrosis induced by a DNA minor groove binding methyl sulfonate ester. *Cell Death Differ* **8:**817–828.
Tentori L, Portarena I, and Graziani G (2002) Potential clinical applications of poly(ADP-ribose) polymerase (PARP) inhibitors. *Pharmacol Res* **45:**73–85.
Tewari M, Quan LT, O'Rourke K, Desnoyers S, Zeng Z, Beidler DR, Poirier GG, Salvesen GS, and Dixit VM (1995) Yama/CPP32 beta, a mammalian homolog of CED-3, is a CrmA-inhibitable protease that cleaves the death substrate poly(ADP-ribose) polymerase. *Cell* **81:**801–809.
Toker A (1998) The synthesis and cellular roles of phosphatidylinositol 4, 5-bisphosphate. *Curr Opin Cell Biol* **10:**254–261.
Vanni A, Fiore M, De Salvia R, Cundari E, Ricordy R, Ceccarelli R, and Degrassi F (1998) DNA damage and cytotoxicity induced by β-lapachone. relation to poly-(ADP-ribose) polymerase inhibition. *Mutat Res* **401:**55–63.
Villamil SF, Podestá D, Molina M, and Stoppani A (2001) Characterization of poly(ADP-ribose) polymerase from *Crithidia fasciculata*: enzyme inhibition by β-lapachone. *Mol Biochem Parasitol* **115:**249–256.
Virág L and Szabó C (2002) The therapeutic potential of poly (ADP-ribose) polymerase inhibitors. *Pharmacol Rev* **54:**375–429.
Weltin D, Marchal J, Dufour P, Potworowski E, Oth D, and Bischoff P (1994) Effect of 6(5H)-phenanthridinone, an inhibitor of poly(ADP-ribose) polymerase, on cultured tumor cells. *Oncol Res* **6:**399–403.
Wielckens K, Garbrecht M, Kittler M, and Hilz H (1980) ADP-ribosylation of nuclear proteins in normal lymphocytes and in low-grade malignant non-Hodgkin lymphoma cells. *Eur J Biochem* **104:**279–287.
Wyllie AH (1987) Apoptosis: cell death in tissue regulation. *J Pathol* **153:**313–316.

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