Invited review

Potential roles of poly (ADP-ribose) polymerase in male reproduction

Reda Z. Mahfouz[1], Tamer M. Said[2], Devna Mangrola, Ashok Agarwal[1]

[1]Center for Reproductive Medicine, Glickman Urological and Kidney Institute and OB-GYN and Women's Health Institute, Cleveland Clinic, Cleveland, OH, USA
[2]The Toronto Institute for Reproductive Medicine, Toronto, ON, Canada

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Corresponding author:
Prof. Ashok Agarwal
Center for Reproductive Medicine
Glickman Urological and Kidney Institute and OB-GYN and Women's Health Institute
Cleveland Clinic
9500 Euclid Avenue
Desk A19.1, Cleveland
Ohio 44195, USA
Phone: 216-444-9485
Fax: 216-445-6049
E-mail: Agarwaa@ccf.org

Abstract

Poly (ADP-ribose) polymerase (PARP) is an abundant nuclear enzyme involved in DNA repair and transcriptional regulation. It is now recognized as a key regulator of cell survival and cell death in response to noxious stimuli. Poly (ADP-ribose) polymerase becomes activated in response to oxidative DNA damage and depletes cellular energy pools, leading to cellular dysfunction in various tissues. Poly (ADP-ribose) polymerase over-activation depletes its substrate nicontinamide adenine dinucleotide, slowing the rate of glycolysis, electron transport and adenosine triphosphate formation. Eventually this leads to functional impairment or cell death, as well as upregulation of various pro-inflammatory pathways. Therefore, novel antioxidants and PARP inhibitors have entered clinical development as experimental therapies for various diseases and possibly defective spermatogenesis. This review focuses on the data available on the pathophysiological relevance of the PARP in various stages of spermatogenesis ranging from testicular to ejaculated spermatozoa.

Key words: apoptosis, DNA fragmentation, oxidative stress, sperm.

Introduction

Poly (ADP-ribose) polymerase (PARP) is a nuclear enzyme important for the detection of DNA strand breaks caused by genotoxic agents such as reactive oxygen species (ROS), ionizing radiation and alkylating agents or those caused by enzymatic incision of DNA-base lesions [1]. In testicular germ cells, PARP has a particularly well-researched role in base excision repair (BER), which is one of the primary repair mechanisms to resolve DNA lesions caused by endogenous and exogenous processes [2-6]. However, a similar role for PARP in human ejaculated spermatozoa is still being investigated and remains to be controversial [7-10].

Male germ cells are exposed to a wide variety of endogenous and exogenous genotoxic agents [11, 12]. Endogenous agents include reactive oxygen and nitrogen species generated during the metabolic activities of cells [13, 14]. Exogenous agents include various environmental factors that can inflict damage to genomic DNA. These genotoxic agents can introduce DNA lesions in the form of DNA single-strand breaks, double-strand breaks, base damage, inter- and intra-stand cross links and DNA-protein cross-links.

To counteract this wide spectrum of DNA lesions, eukaryotic cells possess efficient DNA repair pathways. In addition to BER, these include nucleotide excision repair (NER), mismatch repair (MMR), non-homologous end-joining (NHEJ), and homologous recombination (HR). These five major pathways operate alone or in combination to remove the wide array of DNA lesions and restore genomic stability. Some proteins like PARP participate in multiple DNA repair pathways [15-17].

Not only does PARP have a well-defined role in DNA repair, it can also serve as biochemical marker of caspase-dependent apoptosis [15, 21]. During apoptosis, numerous DNA strand breaks lead to PARP activation. This may be an attempt by the dying cell to repair the DNA damage caused by nuclease activation [18]. However, this attempt to repair damage proves futile as PARP is cleaved by caspase-3 into a catalytic fragment of 89 kDa and DNA binding unit of 24 kDa [19, 20].

Recently, there has been growing interest in the role of PARP in the management malignancy including the use of PARP inhibition as an adjuvant therapy with chemotherapeutic drugs [22]. As our focus lies primarily in male infertility, we highlight in this review the possibilities of PARP involvement in male infertility as well as the possible role of PARP modulation to control DNA damage in male germ cells. This may reveal a new therapeutic option for the correction and repair of sperm DNA damage.

Structural and functional classifications of poly (ADP-ribose) polymerase

The PARP family contains 18 homologues with a conserved catalytic domain made up of 50 amino acids that serve as the "PARP signature" [23]. This is the site where PARP chains are initiated and elongated and where branching of the chains can occur [24]. Besides this catalytic domain, other PARP family members may have other domains, including DNA binding domains, macrodomains, BRCT (BRCA1 C terminus) domain found in proteins responding to DNA damage, ankyrin repeats, and WWE domains found in proteins associated with ubiquitination. All of these special types of domains contribute to the unique functions of each family member [23, 25].

Poly (ADP-ribose) polymerase family members can be divided into several subcategories based on each protein's established functional domains and precise functions. The first category consists of the DNA-dependent PARP (PARP-1 and PARP-2), which are activated by DNA strand breaks. The second group is the tankyrases (tankyrase-1 and tankyrase-2), which serve diverse functions such as telomere regulation and mitotic segregation. The third group is the CCCH-type PARP (PARP-12, PARP-13, and TCDD-inducible PARP), which contains special CCCH-type zinc fingers. Lastly, the fourth group (PARP-9, PARP-14, and PARP-15) consists of macro-PARP that have one to three macro-domains connected to a PARP domain. PARP-8, PARP-11, PARP-16, and PARP-6 do not have sufficient known domains functions to be assigned a role [26].

A recent classification system by Hassa and Hottiger compared the catalytic domain sequences of these enzymes. They divided the PARP family into three separate groups: group 1 consists of PARP-1, PARP-1b (short PARP-1, PARP-2, and PARP-3). Group 2 consists of only PARP-4, and group 3 consists of tankyrase-1, tankyrase-2a, and its isoform tankyrase-2b (also known as PARP-5 and PARP-6a/b) [24]. The various PARP enzymes also can have different subcellular localization patterns. PARP-1 and PARP-2 are considered nuclear enzymes and are found in the nucleus of cells. In contrast, the tankyrases and PARP-3 are found in both the nucleus and cytoplasm [27].

Perhaps the best studied member of the PARP family is PARP-1, a 113 kD enzyme encoded by the ADPRT gene in humans located on chromosome 1 [28, 29]. The protein structure of PARP-1 is very well characterized. It is made up of four functional domains, including a DNA binding domain consisting of structures known as zinc fingers that can bind to DNA breaks. A second domain contains the nuclear localization signal (NLS), which ensures PARP-1 is found in the nucleus, and it is also a site of cleavage by caspase-3. PARP-1 also includes an automodification domain and a domain that holds the enzyme's catalytic activity [23].

Modulation of poly (ADP-ribose) polymerase activity

Modulation of PARP activity is important for exploring this enzyme's therapeutic options. Several types of molecules have been identified as activators of PARP activity, including histones, the common target of PARP activity. Although histones are modified by PARP-1, histones H1 and H3 actually can activate PARP-1. An important enzyme involved in regulating histone structure, SIRT-1 (a histone deacetylase) enzyme also is involved in regulating PARP-1 activity. In the absence of SIRT-1, PARP is not regulated, and cell death regulated by apoptosis-inducing factor (AIF) occurs [49]. Magnesium ions, calcium ions, and polyamines are allosteric activators of the auto-(poly-ADP-ribosyl)ation activity of these enzymes. It should be noted that calcium ions also play an important role in oxidative stress pathophysiology [50].

Poly (ADP-ribose) polymerase has an extensive list of inhibitors that are used extensively to study PARP activity. Purines such as hypoxanthine and inosine are endogenous inhibitors [51]. Interestingly,

caffeine derivatives can inhibit PARP-1 activity as well [52-55], in addition tetracycline derivative also have the ability to inhibit PARP-1 activity [56]. Phosphorylation of PARP is a more complicated type of modulation. It was found that phosphorylation of PARP-1 by ERK 1 and 2 (extracellular signal-regulated kinases) was important for regulating neuronal cell death [57]. Poly (ADP-ribose) polymerase also can be phosphorylated by DNA-PK (DNA-dependent protein kinase) a major protein that takes part in double-stranded break repair. DNA-PK phosphorylates PARP and suppresses PARP activity [58].

Poly (ADP-ribose) polymerase in the context of male reproduction

Poly (ADP-ribose) polymerase plays a crucial role in maintaining the genomic integrity in a variety of cell types. Perhaps nowhere is this genomic integrity more important than in germ cells. Cases of male infertility are associated with abnormal sperm chromatin and DNA structure. The problems that arise in genomic integrity of sperm come from a variety of sources, including spermatogenesis defects, abortive apoptosis, problems with spermatid maturation, and oxidative stress [76]. Problems in spermatogenesis could include double-stranded breaks that are not resolved after crossing over during meiosis I [76].

The role of PARP in male fertility is not as well defined as its role in cellular processes. However, there is enough evidence to suggest that such a role exists due to the documented presence of PARP in the testis, during spermatogenesis, and just recently in ejaculated spermatozoa [72]. Furthermore, PARP as an important DNA repair enzyme could maintain the sperm genomic integrity. Similarly, the role of PARP in cell death pathways may have important implications for the elimination of abnormal spermatozoa, especially during the processes of spermatogenesis.

Germ cell apoptosis

Apoptosis is a normal component of mammalian spermatogenesis. It is orchestrated spontaneously during the entirety of spermatogenesis to produce mature spermatozoa and to eliminate any abnormal spermatozoa. In fact, a very large number of spermatozoa die during spermatogenesis. This may be due to the ability of the Sertoli cells to maintain only a limited number of germ cells so excess cells must be eliminated. Apoptosis also may function to destroy cells that do not make it past certain cellular checkpoints [59]. In a recent study by Codelia et al., the cell death pathway involved in pubertal rat spermatogenesis was identified as the extrinsic pathway of cell death involving the Fas-FasL system. The study also showed significantly less cleaved PARP and a reduction in the number of apoptotic germ cells when using a caspase-8 inhibitor and a pan-caspase inhibitor [61]. Thus, PARP cleavage may play a key role in the cellular death pathways of spermatogenesis.

The significant presence of PARP in human spermatocytes during maturation arrest was suggested to represent the greater amount of DNA strand breaks occurring during spermatogenesis impairment [70]. Poly (ADP-ribose) polymerase-2 also has been implicated in abnormal spermatogenesis. In a recent study by Dantzer et al., an increased incidence of apoptosis was found in the testis of PARP-2 null mice, specifically in the spermatocyte and spermatid layers. However, the layers containing spermatogonia and preleptotene spermatocytes did not show any markers for apoptosis. Chromosome segregation was abnormal during metaphase I, and spindle assembly was also abnormal in these PARP-2-deficient mice. Thus, the decrease in fertility seen in these mice could be related to defective meiosis I and spermiogenesis [71].

Poly (ADP-ribose) polymerase in ejaculated spermatozoa

The quest to detect PARP in ejaculated spermatozoa has demonstrated success only recently. Initially, the presence of PARP-1 in human ejaculated sperm samples when analyzing semen for apoptotic markers was not detected (Taylor, Weng et al. 2004). However, in a recent study by Jha et al., several PARP isoforms were detected in ejaculated spermatozoa, including PARP-1, PARP-2, and PARP-9. Immunolocalization patterns showed that PARP was found near the acrosomal regions in sperm heads. Furthermore, a direct correlation was seen between sperm maturity and the presence of PARP. An increased presence of PARP-1, PARP-2, and PARP-9 was seen in mature sperm samples when compared with immature sperm samples of fertile and infertile men. In addition, a possible relationship between PARP and male infertility was also demonstrated. Poly (ADP-ribose) polymerase activity was then modulated to determine its role in responding to oxidative and chemical damage in sperm. In the presence of the PARP inhibitor 3-aminobenzamide, chemical- and oxidative stress-induced apoptosis increased by nearly twofold. This recent finding suggested that PARP could play an important protective role for spermatozoa responding to oxidative and chemical damage [72].

The research presented by our group was the first in showing the presence of cleaved PARP in ejaculated spermatozoa. The presence of cleaved PARP was similar in mature and immature sperm samples following exposure to oxidative stress and chemical damage. Modulating PARP activity was

also shown to alter the incidence of cell death. When the same sperm samples were exposed to PARP inhibitors after chemical and oxidative stress, apoptosis decreased [75].

Poly (ADP-ribose) polymerase and oxidative stress

Oxidative stress can cause DNA damage, inflammation, and modification of proteins. Poly (ADP-ribose) polymerase responds to all three of these changes that can occur in the cell as a result of oxidative damage. However, there is a great deal of variability in PARP activation as a result of this type of stress; the cell's metabolic stage or its microenvironment could affect the activity of PARP [38]. In response to DNA damage caused by ROS, PARP-1 recruits the DNA repair protein XRCC1 to damage sites [40]. DNA is not the only structure modified by ROS; histones could be damaged as well. Ullrich et al. showed that PARP can activate the 20S proteosome involved in breaking down oxidatively damaged histones. Also, in response to oxidative stress caused by exposure of histones to hydrogen peroxide, PARP, PAR, and the nuclear proteosome all bind together [41]. This could be important for protecting DNA against oxidative damage because it has been found that a condensed chromatin structure may prevent DNA strand breaks induced by hydroxyl radicals [42].

Poly (ADP-ribose) polymerase and aging

Two main aspects of PARP activity may influence aging: PARP's regulation of immune responses through its interaction with NF-κB and PARP's central role in maintaining genomic stability through DNA repair, telomere maintenance, spindle stability, and cell death [43]. Both PARP-1 and PARP-2 are involved in telomere functioning. PARP-1 and PARP-2 bind to TRFII (telomere repeat binding factor II) and modify it, affecting its ability to bind to telomere regions [44, 45]. Poly (ADP-ribose) polymerase-1 also is found at telomere regions of DNA damaged by genotoxic agents, and it may play a role in preventing damage to genomic instability [45]. Tankyrases also are involved in telomere regulation. Tankyrase can bind to TRF1, a telomeric factor that shortens telomere length, and TRF-1 is in turn modified by ADP-ribosylation, thus making it more difficult for TRF-1 to bind to telomeric regions of DNA *in vitro* [46]. It has even been shown that mice lacking PARP exhibited telomere shortening, a symptom of ageing [47].

Chromosomal instability increases with an organism's age. Poly (ADP-ribose) polymerase is essential for chromosomal stability through its role in DNA repair. Poly (ADP-ribose) polymerase-1-deficient mice created by Simbulan-Rosenthal et al. showed a higher incidence of chromosomal aberrations and polyploidy. Reintroduction of PARP in the form of cDNA allowed for restoration of chromosomal integrity [48].

Potential therapeutic options for poly (ADP-ribose) polymerase

The key role of PARP in cell death has made it an attractive candidate for modulation in cancer therapies. Poly (ADP-ribose) polymerase inhibition is currently being explored as an adjunct to chemotherapy. It is based on the hypothesis that inactivating PARP will render malignant cells exposed to chemotherapy unable to repair DNA damage resulting from therapy, leading to their death. Non-malignant cells will not be susceptible to cell death at these low doses of chemotherapy. Thus PARP-inhibitors are way of sensitizing cancerous cells to chemotherapy [82]. Inactivating PARP through cleavage is also being explored. In a recent study by Zhang et al., PARP cleavage through activation of caspases induced cytotoxicity in human leukemia cells [83].

Whether PARP can function in therapy for male infertility remains to be seen. Our group has previously reported that PARP inhibition may protect against chemically induced injury of ejaculated spermatozoa *in vitro*, but it could not protect against damage induced by oxidative stress [75]. PARP inhibition may have a potential role in testicular cancer as well as cancer that may have spread to the testes. Inflammatory processes as result of infections could also be another area to explore in terms of PARP and male fertility.

Future directions

Poly (ADP-ribose) polymerase homologues seem to have diverse roles in spermatogenesis and even in ejaculated sperm. Their expression has showed a correlation with spermatozoa maturity and fertility potential. The presence of cleaved PARP in ejaculated spermatozoa confirms this hypothesis. The available preliminary data show that PARP inhibition may protect against chemically induced damage in sperm. This will pave the way for future studies to confirm these findings and to examine the role of PARP in other types of induced sperm damage.

Poly (ADP-ribose) polymerase modulation may also create new therapeutic options for infertile patients, especially those suffering from sperm DNA damage, oxidative stress-induced sperm DNA damage, or perhaps even idiopathic male infertility. Poly (ADP-ribose) polymerase inhibition may be used as an *in vitro* treatment for inducing death in spermatozoa that carry damaged DNA. Cleaved PARP could serve as an apoptotic marker that may prove useful for identifying healthy spermatozoa. Not only this, but the therapeutic potential of PARP

as an anti-tumor agent could help point the way to new ways of preserving fertility in cancer patients even after the genotoxic stress of chemotherapy and radiation. The current widespread use of assisted reproductive technologies and the limited knowledge available about the consequences of sperm DNA damage both demand further exploration of DNA repair mechanisms in spermatozoa. Poly (ADP-ribose) polymerase may hold the key to a better understanding of these repair mechanisms inherent in spermatozoa and the importance of such mechanisms in producing healthy pregnancies.

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