PARP-1 and p53 Expression in Sertoli Cell during Rat Ontogenesis

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KEYWORDS: PARP, p53, Sertoli Cell

Abstract

Poly ADP-ribose polymerase-1 (PARP-1), a nuclear 113-kDa Zinc-finger protein, catalyzes poly ADP-ribosilation reactions and is involved in many physiological and pathological conditions such as cell differentiation and proliferation, transcriptional events, carcinogenesis and apoptosis. Sertoli cells are essential for reproduction, providing germ cells with nutrients and hormonal signals. Poly ADP-ribosylation has been well studied during sperm cell maturation but the role of such reactions at the Sertoli cell level remains unknown. In the present work we analyzed the expression of PARP-1 during the postnatal differentiation of Sertoli cells isolated from rat testis. We compared PARP-1 expression with that of p53, whose activity is modulated by poly ADP-ribosylation. Quantitative RT-PCR technique was employed. Our data demonstrate for the first time PARP-1 expression in rat Sertoli cell both in the neonatal and peripubertal period. p53 expression pattern was found opposite to that of PARP-1, suggesting that PARP-1 is possibly in charge for protecting the developing Sertoli cells when the activity of p53 lowers.

Introduction

The Sertoli cells play essential roles for testis functions both in fetal and adult life. During embryonic development they are of central importance for the early determination of male somatic sex. After birth, immature Sertoli cells continue to divide and differentiate, but their proliferation declines and finishes at the onset of puberty. Mature differentiated Sertoli cells are in close contact with germ cells in the seminiferous tubule and fill a crucial nursing function by providing germ cells with nutrients, paracrine and hormonal signals, thus regulating spermatogenesis during adult life [1]. The presence of poorly differentiated Sertoli cells in the adult testis is suggested to be involved in the genesis of testicular diseases such as sterility and cancer [2]. Thus, the study of the mechanisms underlying the postnatal differentiation of Sertoli cells is of high interest.

Nuclear poly ADP-ribosylation reactions are involved in many physiological and patophysiological processes such as cellular differentiation and proliferation, transcriptional control, genome integrity, carcinogenesis, cell survival and cell death [3]. Within the testis, poly ADP-ribosylation reactions have been studied during spermatogenesis [4-7], when dramatic changes in chromatin structure take place and poly ADP-ribose polymerase-1 (PARP-1) seems to act as a modulator of chromatin remodeling steps of sperm cell maturation [8]. On the contrary, the specific expression of enzymes of the PARP family in Sertoli cells has not been so far elucidated. In the present work we studied the expression of PARP-1 during the postnatal differentiation of Sertoli cells isolated from rat testis. We compared the pattern of PARP-1 expression with that of p53, the tumor suppressor protein whose expression, activity and stability is regulated by poly ADP-ribosylation, thus providing a target for PARP-1 activity [9].

Materials and methods

All chemicals, unless otherwise indicated, were of analytical grade and obtained from Sigma-Aldrich (Milano, Italy). Animals were obtained from Harlan Italy and housed under conditions of controlled temperature and light, according to national guidelines for animal care and use. Sertoli cell cultures were obtained from Wistar pre-and peri-pubertal rats (7, 14, 21 and 28 day-old), as previously described [10, 11]. Briefly, the testicular tissue was minced and sequentially digested with 0.25%(w/v) trypsin and 0.1% (w/v) collagenase/0.2% (w/v) DNase. Sertoli cell-enriched aggregates were cultured at 32° C in serum-free DMEM. After 24 h, cell monolayers were subjected to hypotonic treatment to remove germ cell contaminants [12]. Sertoli cell preparations resulted more than 90% pure. The contamination with peritubular cells was about 5%, as evaluated by cytochemical detection of alkaline phosphatase activity [13]. Cell viability, detected by Trypan blue exclusion, was greater than 90%. Total RNA was isolated by the acid phenol-chloroform procedure [14] using the Trizol reagent according to the manufacturer's instructions. The purity of RNA was checked via absorption spectroscopy by measuring the 260/280 OD ratio. Only high purity samples (OD260/280 > 1.8) were subjected to further manipulation. The quality of isolated RNA was assessed by electrophoresis on 1.5% formaldehyde-agarose

gel to verify the integrity of the 18S and 28S rRNA bands. First strand cDNA was synthesized from 1 μg of total RNA using 200 ng oligo(dT)18-primer (TibMolBiol, Genoa, Italy), 200 units RevertAid H-Minus M-MuLV reverse transcriptase (Fermentas, Hannover MD, USA), 40 units RNAsin and 1 mM dNTPs (Promega, Milan, Italy) in a final volume of 20 μl. The reaction was performed in a Mastercycler apparatus (Eppendorf, Milan, Italy) at 42°C for 1 h after an initial denaturation step at 70° C for 5 min. The expression levels of genes were quantified in 96-well optical reaction by using a Chromo 4™ System real-time PCR apparatus (Biorad, Milan, Italy). Real-time PCR reactions were performed in quadruplicate in a final volume of 20 μl containing 10 ng of cDNA, 10 μl of iTaq SYBR Green Supermix with ROX (Biorad), and 0.25 μM of each primer pair (TibMolBiol). The Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) mRNA fragment was used as housekeeping gene to normalize the expression data. The following primer pairs were used: PARP-1 (Fwd 5'-TGCAGTCACCCATGTTCGATGG-3' and Rev 5'-AGAGGAGGCTAAAGCCCTTG-3'), p53 (Fwd 5'-GGCTCCTCCCCAACATCTTATC-3' and Rev 5'-ACCACCACGCTGTGCCGAAAA-3'), GAPDH (Fwd 5'-GACCCCTTCATTGACCTCAAC-3' and Rev 5'-CGCTCCTGGAAGATGGTGATGGG-3') [15, 16]. The thermal protocol included an enzymatic activation step at 95° C (3 min) and 40 cycles of 95° C (15 s), 60° C (30 s) and 72° C (20 s). The melting curve of the PCR products (55–94° C) was also recorded to check the reaction specificity. The relative gene expression of target genes in comparison to the GAPDH reference gene was conducted following the comparative CT threshold method [17] using the Biorad software tool Genex-Gene Expression Macro™ [18]. The normalized expression refers to the amount of mRNA (fold induction) with respect to the control sample (28 days) taken as 1.
ANOVA and Bonferroni post hoc test were performed using the GraphPad InStat, version 3.05 package (GraphPad Software, San Diego, CA).

Results

The levels of PARP-1 transcripts as measured by Quantitative RT-PCR are depicted in fig. 1. Data are reported as fold induction in Sertoli cells isolated from rats of 7, 14 and 21 days, with respect to the value corresponding to 28 day-old rats, which was taken as 1. This choice was made because the age of 28-30 days is considered the onset of puberty in the rat, leading to adulthood thereafter [2]. As can be seen in the figure, during the pre-pubertal period the levels of PARP-1 mRNA increase starting from a low basal expression at 7 days and reaching a maximum at 21 days. The increase in PARP-1 expression observed at protein and mRNA levels in the developing rat Sertoli cells was consistent with the measurement of specific enzyme activity performed by radiometric assay [19] in intact cell monolayers (data not shown).
The relative expression of p53 gene in Sertoli cells during the postnatal development of rat testis is also shown in Fig.

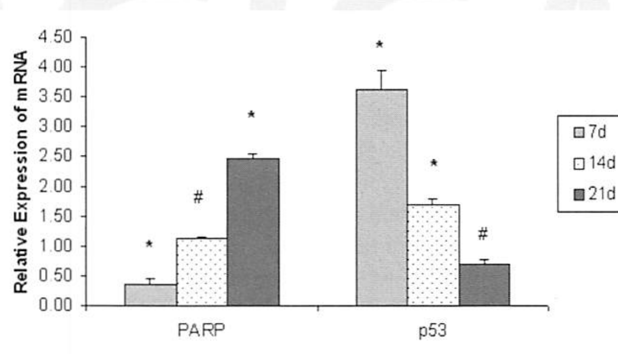

*Fig. 1 - Expression of PARP-1 and p53 in rat Sertoli cells at 7, 14, 21 days of development evaluated by quantitative RT-PCR. Data are calculated as mean±S.D. of 3 independent experiments and expressed as fold induction with respect to sample 28 days taken as 1, after normalization for GAPDH mRNA as standard. Statistical analysis was done using ANOVA test followed by Bonferroni ad hoc post test. #=p< 0.01; *=p<0.001.*

1. p53 transcript levels are high in the neonatal period and decrease thereafter.

Discussion

In Mammals, after birth the whole testis undergoes extensive morphological, physiological and biochemical changes that take place during sexual maturation. The somatic Sertoli cells play an essential role in the development of a functional testis [1]. Immature Sertoli cells continue to divide and differentiate, but, as puberty approaches, they lose the proliferative capacity, begin to produce seminiferous fluid and alter their pattern of protein expression, starting to produce, e. g., transferrin and IL-1α [20, 21]. Fully differentiated Sertoli cells regulate the flow of nutrients and growth factors to the germ cells, thus becoming essential for a correct spermatogenesis [22]. Therefore, the process leading to Sertoli cell differentiation is of crucial importance for the testis to perform one of its major tasks: the production of haploid germ cells. Several investigations have focused on hormonal and paracrine regulation of the functions of the mature Sertoli cell, but the mechanisms underlying the differentiation of these cells remain obscure. Cellular events such as proliferation, differentiation, transcriptional control, DNA repair, genomic stability and cell death are known to be regulated by poly ADP-ribosylation reactions. Poly ADP-ribosylation is a post-translational modification carried out by a family of poly ADP-ribose polymerases (PARPs) which transfer ADP-ribose from nicotinamide adenine dinucleotide (NAD) to certain aminoacid residues onto target proteins, thus modulating their activity. In humans, the PARP family members are encoded by 17 different genes, while PARP-1 is the best studied and founding member [23]. Since poly ADP-ribosylation reactions are implicated in the regulation of cellular proliferation and differentiation, the present work was aimed at investigating the pattern of PARP-1 expression in the developing Sertoli cells. We have isolated the cells from rat testis of 7, 14, 21 and 28 days of age. In the rat, the pubertal period ranges from 15 to 30 days of age, so that it

overlaps in part with the neonatal period [2]; in any case, by 28-30 days of life, proliferation ends and the adult number of Sertoli cells is already present. Therefore, in our experiments we referred our data to the 28-day-old values, taken as 1. By using quantitative RT-PCR, we demonstrate for the first time PARP-1 expression and activity in rat Sertoli cells, both in the neonatal and peripubertal period of life, when the maturation of this cell type occurs. Moreover, the expression levels of PARP-1 varies during the postnatal development, being low at 7 days of age and increasing thereafter. The maximum level of expression is reached at 21 days of life, while in 28-day old rats there is a stabilization of the expression at values possibly typical of the adult life. The mRNA levels are in total agreement with the results obtained through the biochemical assay, which measures the enzyme activity at the different ages (not shown). It would be of interest to discover which acceptor proteins are poly ADP-ribosylated in this contest. A classical target of PARP-1 is p53, the tumor suppressor protein whose activity and stability is regulated via poly ADP-ribosylation [9]. In our experiments, we measured the levels of p53 expression at mRNA level. Quantitative RT PCR analyses revealed that the pattern of p53 expression is opposite to that of PARP-1, being high at 7 days of life and decreasing thereafter. Both p53 and PARP-1 are considered as "guardians of the genome", being implicated in the maintenance of genome integrity and in the regulation of cell proliferation, survival and death. Therefore, it is conceivable that when p53 expression lowers, PARP-1 expression increases. Our data may indicate that PARP-1 is possibly in charge for protecting the developing Sertoli cells when the activity of p53 lowers. In conclusion, our results demonstrate for the first time the modulation of poly ADP ribosylation reactions during the postnatal development of the rat Sertoli cell. Moreover, they identify PARP-1 as a marker of differentiation of this cell type, because of its peculiar pattern of expression.

Acknowledgments

The authors are indebted to Dr. S. Candiani for designing PARP-1 primers.

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