Institute of Experimental Morphology, Pathology and Anthropology with Museum
Bulgarian Anatomical Society

Acta morphologica et anthropologica, 21
Sofia ● 2015

Apoptotic Changes in Aging Testis

D. Barbutska, Y. Koeva

Department of Anatomy and Histology, Medical University, Plovdiv, Bulgaria

Cell apoptosis is an active process which occurs in the course of aging. The direct result of the aging of the testis is reduction in levels of testosterone (T) and results of this are disrupting of physiological processes and dramatically worsened the quality of life for millions of older men. According to the theory of oxidative stress, with the leading factors free radicals (ROS- Reactive oxygen species), mitochondria are the biological clock of aging and the main place of oxidative injury in the course of this process. ROS attack primarily mitochondria and their DNA (mt DNA), increased permeability of the mitochondrial membrane, reduced membrane potential of mitochondria and these are the early signs of beginning apoptotic changes in the cells. Mitochondrial damage caused by ROS disrupts steroidogenesis in the testis and at the same time the process of steroidogenesis generates sub-products, which could also be responsible for functional insufficiency and apoptotic changes in aging LC. Apoptosis is a complex process which is regulated from multiple levels pro-and anti-apoptotic proteins and long-term damage to the balance between the two groups causes apoptotic changes in cells. One of this proteins is tumor suppressive protein p53 – promotes the expression of a number apoptotic genes. At this stage, the role of p53 in the control of apoptosis in human steroidogenic LC in the aging testis is not well investigated which determines the aim of the present study. We used material from human testis of patients between 56, 68 and 80 years old, embedded in paraffin and prepared for immunohistochemistry. Our results demonstrated the increased intensity of the immunoexpression of p53 in the course of the aging and these findings categorically point out the role of this protein in the regulation of testis apoptosis.

Key words: apoptosis, Leydig cells, aging, immunoexpression, p53.

Introduction

A lot of external factors and intracellular mechanisms occurring in the course of aging lead to a flow of active processes in the cells as apoptosis that affects the cells of the germinative epithelium and endocrine Leydig cells (LC). The direct result of the aging of the testis is reduction in levels of testosterone (T), such as changes in hormonal values, disrupting a number of physiological processes and dramatically worsened quality of life for millions of older men.

Well known is the theory of oxidative stress with the leading factors free radicals (ROS- Reactive oxygen species), influencing a wide range of physiological and pathological processes, including aging. According to this theory, mitochondria are the biological clock of aging and the main place of oxidative injury in the course of this

process [10]. ROS attack primarily mitochondria and their DNA (mt DNA) as oxidative lesions accumulate with age, losses of mt DNA are often more critical, than those of the nuclear DNA. The increased permeability of the mitochondrial membrane and the reduced membrane potential of mitochondria are the early signs of beginning apoptotic changes in the cells [10]. Mitochondrial damage caused by ROS, together with the deterioration of the synthesis of ATP, as well as participation in the steroidogenic process enzyme P-450 are key factors not only in the process of aging, but in general in the process of apoptosis [7]. The mitochondria of endocrine cells like LC release free radicals which exceed the antioxidant cellular protection and this fact is the basis of mitochondrial theory of aging [10]. The process of steroidogenesis as a cascade of several enzymatic activities generates so-called sub-products, which could also be responsible for functional insufficiency of aging LC and for apoptotic changes in it [7].

Apoptosis is a complex process involving two main paths – indoor and outdoor, each of which is regulated at multiple levels by a wide spectrum-pro-and anti-apoptotic proteins [14]. Long-term damage to the balance between the two groups causes apoptotic changes in cells [5]. Bcl-2 family is the main regulator of the internal apoptotic way in the testis, essential for the normal balance between survival and death of germ cells [1, 2, 12, 13]. The internal mechanism of apoptosis is regulated by the tumor suppressive protein p5 – which promotes the expression of a number apoptotic genes (transcription factor), also directly modulates the activity of theBcl-2 family members [3, 6, 8, 9]. Reported on the important role of p53 in apoptosis in normal spermatogenesis and in response to DNA damage [2, 4, 11], facts demonstrating the importance of p53 gene in the modulation of apoptosis and spermatogenesis.

At this stage, the role of p53 in the control of apoptosis in human steroidogenic LC in the aging testis is not well investigated that determines the aim of the present study.

Materials and Methods

In the present study, material was used from human testis of patients aged between 56 and 80 years, obtained after an orchidectomy in connection with the treatment of prostate carcinoma (Urology Clinic at the Medical University, Plovdiv). Testicular fragments (4-5 mm thick) were fixed by immersion in Bouin's fluid for 24 hours, embedded in paraffin and prepared for routine histological analysis and immunohistochemistry. Expression of p53 in aging testis was visualized by ABC method with kit Immuno-Cruz™ rabbit ABC Staining System (Santa Cruz Biotechnology, Inc., USA), with DAB chromogen and primary polyclonal anti-p53 antibody (Santa Cruz Biotechnology, Inc., USA; 1: 100). As negative controls were used cuts in which the primary antibody was replaced with phosphate buffered saline (PBS).

Results

By using light microscopic observation and immunohistochemical analysis we determine significant immunoexpression of p53 in the curved seminal tubules and greatly reduced immunostaining in the testis interstitium, that indicates that the control of cell death of LC, does not include only the p53 and probably involved and other gene products.

We observed atrophy of LCs with aging (56, 68 and 80 years of age) rather than reduction in their numbers and as consequence the interstitium of aging testis appeared enlarged (**Fig. 1A-C**).

Our results demonstrated the increased intensity of the immunoexpression of p53 in the course of aging (**Fig. 1A-C**) fact which categorically points out the role of this protein in the regulation of testis apoptosis.

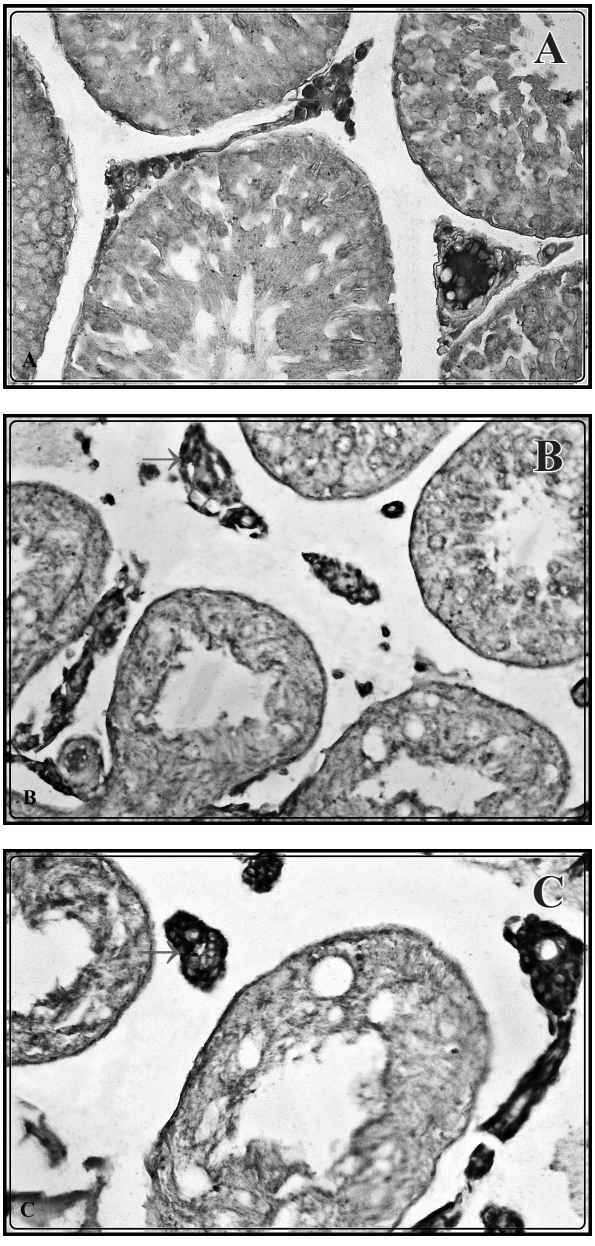

Fig. 1. A, B, C – Immunohistochemistry for p-53. Expression of p-53 increased in testis 80 years (C) compared to 56 and 68 years (A, B)

Discussion

External factors and intracellular mechanisms occurring in the course of aging lead to active processes in the cells as apoptosis, and this affects not only the cells of the germ epithelium and endocrine Leydig cells (LC). The theory of oxidative stress with the leading factors free radicals (ROS- Reactive oxygen species), is known and ROS influencing a wide range of physiological and pathological processes, including the process of aging [10]. ROS attack mitochondria and mt DNA. Increased permeability of the mitochondrial membrane and the reduced membrane potential of mitochondria are the early signs of beginning apoptotic changes in the cells [10]. Pro-and anti-apoptotic proteins and long-term damage to the balance between the two groups caused apoptotic changes in cells [5, 14]. Tumor suppressive protein p53 promotes the expression of numerous apoptotic genes in the processes of cellular differentiation and cellular life cycle, as well as in response to different stress signals [3, 6, 8, 9]. P53 affects programmed cell death through direct interaction with cellular proteins or directly with DNA and this makes it a unique marker for development of apoptosis in cells [2, 4, 11]. Our study revealed the role of this protein in the regulation of testis apoptosis.

References

1. **Adams, J. M., S. Cory.** The Bcl-2 protein family: arbiters of cell survival. – Science, **28**, 1998, 1322-1326.
2. **Baum, J. S., J. P. St George, K. McCall.** Programmed cell death in the germline. – Semin. Cell. Dev. Biol., **16**, 2005, 245-259.
3. **Bennett, M., K. Macdonald, S. W. Chan, J. P. Luzio, R. Simari, P. Weissberg.** Cell surface trafficking of Fas: a rapid mechanism of p53-mediated apoptosis. – Science, **282**, 1998, 290-293.
4. **Beumer, T. L., H. L. Roepers-Gajadien, I. S. Gademan, P. P. van Buul, G. Gil-Gomez, D. H. Rutgers, D. G. de Rooij.** The role of the tumor suppressor p53 in spermatogenesis. – Cell. Death. Differ., **5**, 1998, 669-677.
5. **Bozec, A., F. Chuzel, S. Chater, C. Paulin, R. Bars, M. Benahmed M, C. Mauduit.** The mitochondrial-dependent pathway is chronically affected in testicular germ cell death in adult rats exposed in utero to anti-androgens. – Journal of Endocrinology, **183**, 2004, 79-90.
6. **Chipuk, J. E., T. Kuwana, L. Bouchier-Hayes, N. M. Droin, D. D. Newmeyer, M. Schuler, D. R. Green.** Direct activation of Bax by p53 mediates mitochondrial membrane permeabilization and apoptosis. – Science, **303**, 2004, 1010-1014.
7. **Lacombe, A., V. Lelievre, C. E. Roselli, W. Salameh, Y-H. Lue, G. Lawson, J-M. Muller, J. A. Waschek, E. Vilain.** Delayed testicular aging in pituitary adenylate cyclase-activating peptide (PACAP) null mice. – PNAS, **103**(10), 2006, 3793-3798.
8. **Miyashita, T., J. C. Reed.** Tumor suppressor p53 is a direct transcriptional activator of the human bax gene. – Cell., **80**, 1995, 293-299.
9. **Oda, E., R. Ohki, H. Murasawa, J. Nemoto, T. Shibue, T. Yamashita, T. Tokino, T. Taniguchi, N. Tanaka.** Noxa, a BH3-only member of the Bcl-2 familyand candidate mediator of p53-induced apoptosis. – Science, **288**, 2000, 1053-1058.
10. **Sastre, J., F. V. Pallardo, G. J. De la Asuncion.** Mitochondria, oxidative stress and aging. – Free Radic. Res., **32**(3), 2000, 189-198.
11. **Schwartz, D., N. Goldfinger, Z. Kam, V. Rotter.** p53 Controls low DNA damage-dependent premeiotic checkpoint and facilitates DNA repair during spermatogenesis. – Cell. Growth. Differ., **10**, 1999, 665-675.
12. **Taylor, M. F., I. Woolveridge, A. D. Metcalfe, C. H. Streuli, J. A. Hickman, I. D. Morris.** Leydig cell apoptosis in the rat testes after administration of the cytotoxin ethane dimethanesulphonate: role of the Bcl-2 family members. – Journal of Endocrinology, **157**, 1998, 317-326.

13. **Tsujimoto, Y.** Cell death regulation by the Bcl-2 protein family in the mitochondria. – J. Cell. Physiol., **195**, 2003, 158-167.
14. **Xu, G., K. S. Vogel, C. A. McMahan, D. C. Herbert, C. A. Walter.** BAX and Tumor Suppressor TRP53 are important in regulating mutagenesis in spermatogenic cells in mice. – Biology of Reproduction, **83**, 2010, 979-987.

Corresponding author:
Assist. Prof. Darina Barbutska, DM
Medical University, Plovdiv
Department of Anatomy,
Histology and Embryology
15A Vassil Aprilov Str., Plovdiv
tel: 032602304,
e-mail: darinas5@abv.bg