

MOLECULAR MECHANISMS OF INITIATION OF CARCINOGENESIS IN THE TESTIS

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In this review, literature data on the study of precancerous changes in testicular tissue and molecular changes, as well as the influence of environmental factors that can initiate carcinogenesis, were analyzed and summarized for the future determination of early diagnosis of germ cell tumors of the testis and the development of preventive measures. The review also discusses the significant new changes presented in the Fourth Edition of the World Health Organization Classification of Urogenital Tumors, published in 2016, and modern concepts of the etiology and pathogenesis of these diseases. Among the environmental factors that can initiate carcinogenesis, the most noteworthy are the biological effects of low doses of ionizing radiation, such as the effect of radiation-induced genome instability, which increases the risk of carcinogenesis, the “bystander effect”, and chronic oxidative stress. Disruption of ubiquitin-proteasomal proteolysis, impaired molecular-level components of the blood-testis barrier, and impaired regulatory action of TGF- β on the cell cycle can play a crucial role in the pathogenesis of male infertility and the initiation of carcinogenesis in the testis. The effect of low doses of ionizing radiation as an additional etiological factor leads to changes in the structural, as well as molecular, components of the testis, including epigenetic changes, which can be characterized as environmental pathomorphosis, which leads to impaired spermatogenesis and increased risk of malignancy. Summarizing the literature review data, we can state that patients with blocked spermatogenesis, in which atypical germ cell neoplasia *in situ* cells are detected in testicular tissue, constitute a group at increased risk of testicular carcinogenesis. The presence of additional etiological factors, such as chronic low doses of ionizing radiation, can initiate the progression of carcinogenesis in the testicle.

Key Words: germ cell tumors, germ cell neoplasia *in situ*, male infertility, ionizing radiation, ubiquitin-proteasomal proteolysis, blood-testis barrier, TGF- β .

In recent years, there has been a trend in Ukraine of an increase in the incidence of germ cell tumors (GCT), which occur in 3–4 cases per 100,000 of the male population per year, ranking fourth among the causes of cancer mortality among young men [1]. The incidence of testicular tumors in the US is 3.1, in England and Canada 2.5, and in Japan — 0.97 per 100,000 of the male population per year [2]. Peak morbidity for GCT is attributed to men of reproductive age from 20 to 40 years [3]. Among the etiologic factors in testicular carcinogenesis, particular attention is paid to the association of GCT with male infertility, which has been confirmed in many studies [4, 5].

According to the European Society for Reproduction and Human Embryology, more than 1 million married couples suffer from infertility in Ukraine, accounting for 15–17% of this population [6]. In recent decades, men’s reproductive health rates have been declining in many countries, but in Ukraine they have had a pronounced negative trend [7].

The situation with population morbidity in Ukraine is complicated by anthropogenic contamination with radioactive waste due to the Chernobyl accident. The medical effects of the accident have been the

subject of much research in recent years. There is no longer any doubt about the significant carcinogenic effects of low doses of ionizing radiation on the human body [8, 9]. It has become clear that under the influence of ionizing radiation, chronic inflammation develops, causing changes at a molecular level and increasing the risk of carcinogenesis [10]. It is also known that ionizing radiation has a negative effect on spermatogenesis [11, 12].

Identification of molecular markers is a new promising research area, especially in view of the high diagnostic value of molecular signatures allowing targeted therapies for treatment of immune, genetic and oncological diseases [13, 14]. Immunohistochemical methods are being used to detect proteins associated with such signature markers that can be further linked to the pathogenetic mechanisms.

In particular, molecular studies have established that the ubiquitin-proteasome system of intracellular proteolysis is involved in the most important processes of cell development and differentiation, proliferation, cell responses to stress and injury as well as in the process of neoplasia [15]. Structural and functional damage to the components of the ubiquitin-proteasomal system can play a significant role in the disruption of spermatogenesis in male infertility and in the carcinogenesis of testicular tissue.

The transmembrane proteins, claudin 11 and occludin, are key elements of tight junctions that form tissue barriers, including the blood-testis barrier (BTB). These tight junction (TJ) proteins, as well as cytokines, in particular TGF- β , play an important role in regula-

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Abbreviations used: BTB — blood-testis barrier; CIS — carcinoma *in situ*; FSH — follicle-stimulating hormone; GCNIS — germ cell neoplasia *in situ*; GCT — germ cell tumors; RIGI — radiation-induced genome instability; ROS — reactive oxygen species; SUMO — small ubiquitin-related modifier; TJ — tight junction; UBL — ubiquitin-like; WHO — World Health Organization; YST — yolk sac tumor.

tion of the BTB, spermatogenesis and are involved in carcinogenesis of testicular tumors [16].

Many questions remain to be answered, including: What are the morphological changes in testicular tissue that are precancerous with a high risk of neoplasia?; What are the pathogenetic molecular mechanisms of male infertility and testicular GCT?; What conditions promote malignancy?; and What environmental factors can initiate testicular carcinogenesis? These topics are covered in this review. We focus on analysis of the literature on the study of precancerous changes in testicular tissue and the molecular changes, as well as the influence of environmental factors that can initiate carcinogenesis for the future determination of early diagnosis of GCT of the testis and the development of preventive measures.

RELATIONSHIP OF DISORDERS OF SPERMATOGENESIS WITH THE DEVELOPMENT OF GCT OF THE TESTIS

According to the literature, the majority (90–95%) of testicular neoplasms are GCT [17]. The pre-invasive lesion associated with post-pubertal malignant GCT of the testis was first recognized in the early 1970s and confirmed by a number of observational and follow-up studies [18, 19]. Until today, this scientific theory has been confused by disagreement on its name. Initially termed “carcinoma *in situ*” (CIS), it has also been known as “intratubular germ cell neoplasia, unclassified” and “testicular intraepithelial neoplasia”. One paper [20] reviews the history and controversy concerning these names and introduces the reasoning for uniting behind a new name, endorsed unanimously at the World Health Organization (WHO) consensus classification 2016: germ cell neoplasia *in situ* (GCNIS).

The fourth edition of the classification of urogenital tumors of the WHO, published in 2016, contains significant changes that were made on the basis of modern data obtained by molecular, genetic and immunohistochemical studies. GCNIS is the WHO-recommended term for precursor lesions of invasive GCT. Testicular GCT are now separated into two fundamentally different groups: those derived from GCNIS and those unrelated to GCNIS. Spermatocytic seminoma has been designated as a spermatocytic tumor and placed within the group of non-GCNIS-related tumors [21, 22].

GCNIS-derived tumors include seminoma, embryonal carcinoma, yolk sac tumor (YST) (sarcomatoid YST/sarcoma NOS), trophoblastic (choriocarcinoma, other trophoblastic tumors), and teratoma, post-pubertal type (somatic malignancy).

Not GCNIS-derived include spermatocytic tumor (spermatocytic tumor with sarcoma), YST, prepubertal type, and teratoma, prepubertal type [22].

The causes of testicular tumors have not yet been fully established, but there are known factors that play an important role in their development. It is known that the development of GCT is accompanied by changes in the spermogram in the form of oligozoospermia and

azoospermia, indicating impaired spermatogenesis in such patients. Many studies indicate the possible association of disorders of spermatogenesis with the development of GCT [4, 5, 23, 24]. In particular, a survey of 3847 infertile men and analysis of a similar group of men in the general population revealed that the number of cases of diagnosed testicular cancer in infertile men is 20 times greater than in the general male population [24].

The study of testicular biopsies performed for diagnosis of male infertility allowed Skakkebek *et al.* [4, 25] to link such changes to an increased risk of developing a testicular tumor in such patients. However, some researchers have found no risk for GCT in spermatogenesis disorders [26].

The testis is a hormone-active and hormone-dependent organ, and the literature indicates the impact of endocrine disorders in the etiology of testicular tumors. Thus, it is a known fact that age peaks of tumors coincide with the rise of gonadotropins in the blood in the antenatal period, during puberty and age involution [27, 28].

Similar hormonal changes occur in patients with male infertility, in particular those with secretory infertility. There are studies reporting the development of GCT in infertile male patients treated with chorionic gonadotropin or synthetic analogues of testosterone (clomiphene, mesterolone) for oligozoospermia [29]. Many researchers have linked cryptorchidism to an increased risk of testicular cancer [30, 31].

Specific genetic alterations characteristic of this group of tumors have been identified in all histological types of GCT. However, it has been confirmed that an isochromosome of 12p, i (12p), is the only consistent structural chromosomal abnormality in GCT [32].

Testicular intratubular neoplasia (considered the precursor for the majority of GCT) showed similar chromosomal changes as well as mutations of the p53 gene in 66% of cases. GCNIS is a non-invasive cancer because anaplastic cells are located within the family tubule. GCNIS is not found in spermatocytic seminomas seen in older men, yolk sac tumors and mature teratoma in infants [33]. GCNIS can be observed in the testicular tissue surrounding the tumor, according to some researchers in 90% of cases [34], as well as in the testicle tissue in men at risk of developing testicular neoplasms due to a history of cryptorchidism, Klinefelter syndrome, family relationship with a testicular cancer patient (father or brother), contralateral testicular tumor, gonadal dysgenesis, or male infertility [4, 5, 30, 31, 35, 36].

Many additional risk factors for the development of GCT have been described, including testicular atrophy, microlithiasis, low birth weight, excess estrogen, and adverse environmental factors [37, 38]. It is possible that the trigger mechanism for GCNIS and GCT is dysregulation of the development program of polypotent germ cells.

Various modern ideas about the etiology and pathogenesis of germ cells *in situ* neoplasia have been presented [39]. Comparative studies of cell surface proteins (e.g. PLAP and KIT), some of the germ cell-

specific markers (e.g. MAGEA4, VASA, TSPY and NY-ESO-1), supported by studies of regulatory elements of the cell cycle (e.g. p53, CHK2 and p19-INK4d) demonstrated a close similarity of CIS to primordial germ cells and gonocytes, consistent with the pre-meiotic origin of CIS. Recent gene expression profiling studies showed that CIS cells closely resemble embryonic stem cells. The abundance of factors associated with pluripotency (NANOG and OCT-3/4) and undifferentiated state (AP-2g) may explain the remarkable pluripotency of germ cell neoplasms, which are capable of differentiating to various somatic tissue components of teratomas.

Modern GCNIS markers include M2A C-KIT and OCT4/ NANOG, PLAP [40, 41]. The prevailing theory of GCT histogenesis is that GCNIS cells are pluripotent and any type of testicular tumor can develop from them [39, 42, 43]. In particular, immunohistochemically high levels of OCT4 and PLAP are detected in classical seminomas and GCNIS compared with non-seminal tumors, in our opinion, demonstrating a close relationship between GCNIS and typical seminomas, thereby confirming the hypothesis that examines GCNIS and GCT [44].

Epidemiological studies [45, 46] indicate that environmental factors, including ionizing radiation, may play a role in testicular carcinogenesis.

BIOLOGICAL EFFECTS OF IONIZING RADIATION AND IMPACT OF IONIZING RADIATION ON SPERMATOGENESIS

The biological effects of ionizing radiation after the Chernobyl accident have been extensively studied with particular attention focused on the problems of radiation-induced genome instability (RIGI), the role of the “bystander effect” in the formation of RIGI, its connection with individual sensitivity and proof that RIGI is an etiological factor of radiation-induced carcinogenesis [47, 48].

A characteristic feature of RIGI is the increased level of genomic changes in the offspring of irradiated cells. RIGI is the structural and functional variability of genetic material and the disturbance of the genomic balance under the influence of radiation that occurs in the offspring of multiple cells. RIGI can manifest as DNA breaks, chromatin compaction, chromosome aberrations, sister chromatid exchanges, aneuploidy and polyploidy, spontaneous gene expression, gene and chromosomal mutations, accompanied by impaired cellular function, and inducible apoptosis. These lesions lead to gene mutations or chromosomal aberrations and remain in the form of potential long-term injury [48–50]. There is crucial importance of the repair processes of radiation-induced DNA breaks for the survival of the cell and its progeny [47, 51, 52].

One important RIGI mechanism is the “bystander effect”. In particular, researchers [53, 54] have shown that the radiation-induced “bystander effect” can cause cell death, cell cycle arrest, apoptosis, changes in gene expression, increased frequency of mutations

and chromosomal instability in non-irradiated cells. Known mechanisms of the “bystander effect” include signaling to non-irradiated cells through direct intercellular contacts, the production of cytokines or growth factors, and the generation of free radicals and the release of various signaling factors [55, 56].

A key role in the formation of the “bystander effect” and the maintenance of RIGI is played by chronic oxidative stress, enhanced production of reactive oxygen species (ROS) as a manifestation of altered redox metabolism inherited from parental cells that have passed into this state after irradiation [47]. ROS formation is associated with p53 status of irradiated cells and the role of mitochondrial signaling cascades in the initiation and development of mediated “bystander effect” [57, 58]. One hypothesis attributes the mechanism of genome instability induced to a steady increase in the formation of ROS, which leads to oxidative damage to DNA and, consequently, to an increase in cell death and the frequency of chromosome aberrations [47, 59, 60].

When studying the effects of small doses of radiation, researchers have shown that the effects of ionizing radiation manifest themselves even after several cell generations. These effects are induced at doses less than 50 mSv of rare ionizing radiation and 10 mSv of tightly ionizing radiation, and can be detected after 50 or more cellular divisions [61]. Another way of inducing chromosomal instability in irradiated cells is through circulating “bystander” factors that can cause damage in intact cells [62]. It has been shown that in the blood of Chernobyl accident victims, even 20 or more years after the exposure, such “bystander” factors continue to circulate [63]. The problem of carcinogenic effects of low doses of radiation is significant [64]. Studies of dose-response dependence indicate an effect of accumulation upon irradiation in low doses. According to some authors, the effect of accumulation is already manifested in irradiation at doses of 0.1–0.2 Gy, in particular, in the form of gene mutations [65].

Chromosomal instability is believed to be one of the major manifestations of RIGI and neoplastic transformation. It has been noted that chromatid-type aberrations characteristic of a malignant phenotype are more likely to persist in a number of irradiated cell generations, which may also serve as a criterion for low-dose carcinogenic risk [66].

It has been shown that radiation exposure revealed a specific polymorphism of genes of repair, XRCC1, XRCC3, XRCC6, hRAD51, XPD1, and others, as well as genes of metabolism of xenobiotics, contributing to the increase of the frequency of double DNA breaks, and the formation of aberrations by development of breast, stomach, rectal and other cancers [67, 68].

Scientific papers published during the last 15 years by the State Institution “Institute of Urology of NAMS of Ukraine”, together with scientists in Japan, Sweden and Spain, confirmed that chronic long-term ionizing radiation in small doses induces cancer development in urinary bladder and prostate gland in humans

residing in radionuclide-contaminated ^{137}Cs regions of Ukraine, with molecular features (primarily early stages) of the carcinogenesis of such tumors [69–71].

Morphological studies showed that exposure to small doses of radiation (0.25 and 1 Gy) causes profound changes in the spermatogenic epithelium of mice in the form of a decrease in the number of spermatogenic cells and an increase in multinucleated spermatocytes and spermatids. The appearance of multinucleated cells indicates the late maturation and differentiation of cells at some stages of spermatogenesis [72].

Other studies have investigated changes in testicular tissue in Chernobyl disaster eliminators 10–15 years after they received high doses of ionizing radiation. According to the authors [73], morphological changes of the testicular tissue were absent in men who underwent general body irradiation at a dose of 1 to 10 Gy. Some changes in the tubules appeared after exposure at a dose of 10 to 20 Gy. Lymphoid infiltration of the seminiferous tubules occurred 5 years after the accident. Interstitial tissue featured lymphoid infiltrate 10–15 years after exposure to radiation at a dose of 20–30 Gy. Autoimmune orchitis affected spermatogenesis shortly after irradiation exposure. Lymphoid infiltration of the seminiferous tubules and interstitial tissue was observed 5 years after 30–50 Gy irradiation. Sclerosis of 50% of the seminiferous tubules, Leydig cell hyperplasia with lymphoid infiltration, and fibrosis of the interstitial tissue were seen 10–15 years after irradiation.

Subsequently, ultrastructural, immunohistochemical and molecular studies have shown that ionizing radiation can subsequently lead to malformations of the male reproductive system and infertility [74, 75].

In particular [76], x-ray irradiation of the whole body at a dose of 5 Gy resulted in expression of apoptosis and proliferation genes associated with Bcl-2 and p53 in kidneys and testes of neonatal rats (4–5 days) after 2, 4, 6, 8 and 24 h exposure. Apoptosis peaked after 4 h in the testes and after 6 h in the kidney; mitosis after exposure in both tissues was almost non-existent.

Various parameters of Sertoli cells (cell count, androgen-binding protein, transferrin, inhibin, follicle-stimulating hormone (FSH)) have been investigated after exposure to 19-day low-dose gamma-irradiation in rats. Differentiated germ cells are the primary target of gamma rays, leading to the diminution of maturation, consistently and irreversibly affecting all types of germ cells [77].

The study of the influence of electromagnetic pulses on the state of permeability of the BTB in an experiment in mice showed that TGF- β_3 is a key molecule involved in the regulation of BTB permeability. The results showed that an increase in the rate of apoptosis of testicular cells and an increase in TGF- β_3 expression may be mechanisms induced by electromagnetic radiation to diminish tightness of the BTB [78].

In recent years, studies on the diagnosis and correction of male fertility disorders, depending on the status of free radical processes, have emerged [79,

80]. In particular, the work of Trifonova [79] showed the effect of environmental pollution (small-dose irradiation due to living in radioactively contaminated territories) on sperm counts 15–20 years after the Chernobyl accident. The dependence of spermograms on the distance to the Chernobyl and the activity of free radical processes have been established. The dependence of spermogram indicators on sperm chemiluminescence in infertile patients and patients with chronic prostatitis have been shown. It has been shown that sperm phospholipids are an important substrate for the action of free radicals in male infertility, accompanied by oligozoospermia.

These data correlate with the results of experimental studies that investigated the dynamics of change in the morphologic and functional characteristics of sperm and composition of rabbit sperm after total ionizing radiation at doses of 1.0–7.0 Gy. A dose-dependent increase in sperm morphological abnormalities (damage to acrosomes, heads and tails), loss of sperm motility and decrease in their linear velocity were detected [80].

Among the biological effects of low doses of ionizing radiation, RIGI increases the carcinogenic risk, the “bystander effect”, and chronic oxidative stress. The effect of ionizing radiation on spermatogenesis investigated in relatively few studies showed the extreme sensitivity of all stages of spermatogenesis.

MOLECULAR MECHANISMS OF DISORDERS OF SPERMATOGENESIS IN MALE INFERTILITY AND CARCINOGENESIS IN THE TESTIS

Among the various molecular markers used to detect spermatogenesis in male infertility [81, 82], it is important to identify those that play a role in the pathogenesis of male infertility and oncogenesis in testicular tissue, and those that could be used for early diagnosis of testicular cancer and for therapeutic interventions.

Ubiquitin

In 2004, Drs. Chekhanover and Avram Gershko were awarded the Nobel Prize in Chemistry for the discovery of a mechanism known as ubiquitin-mediated intracellular protein cleavage. It is known that the exchange of proteins in the cell, like other substances, is normally in a dynamic equilibrium between the processes of synthesis and decay (proteolysis). Intracellular proteins perform certain functions assigned to them, and then the cell needs to remove them. The real breakthrough in this area was the discovery of the “ubiquitin signaling pathway”, which distinguishes two basic phases: 1) covalent attachment to the protein to be degraded, the polyubiquitin chain and 2) protein degradation in the proteasome.

Proteins subject to ubiquitin-dependent proteolysis include the following essential substrates: a) cell cycle regulators: CLN1, CLN2, CLN3, Sic1, Cyclin D1, P27, Cyclin A, Cyclin B, Cdc6, Cyclin E; b) components of different signaling pathways; c) transcription factors: NF- κ B-p105 (p50 precursor), I κ B (NF- κ B inhibitor) MyoD, Rpn4; d) tumor oncoproteins and protein sup-

pressors: c-Jun, c-Fos, c-Mos, p53; e) enzymes: RNA polymerase II (large subunit), ornithine decarboxylase, fructose-1,6-diphosphatase; f) mutated proteins; and g) proteins damaged post-translationally [83]. Proteasomal degradation of proteins is actively involved in various cellular processes, including regulation of the cell cycle, apoptosis, and regulation of the rate of transcription, all of which play important roles in oncogenesis [84].

Along with the described proteolytic function of the proteasome, there are additional proteins responsible for protein processing and refolding [84, 85]. The essence of protein processing is that by partial hydrolysis the structure of the protein changes and its active centers are opened. An example of processing in the proteasome is the conversion of Spt23 protein (its active site is released in the proteasome after ubiquitination from the inactive form) and the activation of p50, one of the components of nuclear transcription factor kappa B (NF- κ B). Protein refolding in the proteasome has been studied *in vitro*. It has been found that the proteasome can bind damaged “unwound” proteins and “twist” them again.

Post-translational modification of intracellular proteins is accomplished not only by ubiquitination of protein molecules, but also by 11 ubiquitin-like (UBL) proteins. Their structure is similar to that of ubiquitin. The interaction of ubiquitin proteins with intracellular proteins does not cause their degradation, but leads to modification or modulation of their activity.

One of the most studied UBL proteins is SUMO (small ubiquitin-related modifier). Mammals have four SUMO homologues. Unlike the ubiquitin-dependent system, which has many E2-conjugating enzymes, SUMO proteins interact with only the Ubc9 enzyme. In this case, SUMO E-3 ligases play the role of adapters that interact with Ubc9 and facilitate the transfer of SUMO to substrate proteins. The process of sumoylation is involved in many cellular processes, including nuclear cytosolic transport, regulation of transcription, apoptosis, stabilization of proteins, reaction to stress, and regulation of the cell cycle [86, 87].

Proteins that regulate the rate of transcription can be either oncoproteins, or, conversely, suppressors of tumor growth. An example of such transformations is the p53 protein, whose activity in a healthy cell maintains the relationship between proliferation and apoptosis. When infected with papillomavirus, the E6 viral protein finds the p53 protein and signals to an E3-ligase family enzyme the need to attach the ubiquitin chain to p53. Thus, p53 protein becomes a substrate for proteasomal degradation. As a result of the rapid destruction of p53, the regulation of the cell cycle is disrupted and the cell embarks on the path of malignant rebirth.

Changes in ubiquitin-dependent proteolysis are also associated with impaired apoptosis. It has been found that by reducing the activity of NF- κ B, inhibitors can trigger apoptosis of transformed cells, thereby preventing activation of angiogenesis in the form of intense growth of capillaries in cancer tissue and metastasis. Thus, the decrease in the catalytic activity of the proteasome leads to the accumulation of in-

hibitors of cell growth and proapoptotic proteins. Non-destroyed proteasome-disrupted short-lived proteins, such as p53 and p27kip1, are also capable of initiating a cascade of biochemical and morphological processes that lead to apoptosis, which can be used to develop a new direction in the development of advanced drugs in oncology.

To study the function of the proteasome, inhibitors of its activity have been used. One of these proteasomal inhibitors is bortezomib, a potent inhibitor of chymotrypsin-like and trypsin-like proteasome activity. Bortezomib has been approved by the FDA for the treatment of multiple myeloma [88]. Studies have found that bortezomib produces positive results in the treatment of multiple myeloma, lymphoma and other hematologic diseases, but is generally not effective as a monotherapy in the treatment of solid tumors [89–91]. This drug has been used to treat central nervous system tumors in studies on human tissue cell culture and in experimental animal models [92].

Of note, bortezomib treatment causes long-term gonadal dysfunction in male mice. Within 2 days after the end of an 11-day bortezomib cycle, 80% of tubules showed hypo-spermatogenesis with arrest at the level of spermatogonia, spermatocytes and round spermatids. Even six months after bortezomib treatment, testicular weight, sperm concentration, and length of the tubules remained at a reduced level, indicating that spermatogenesis could not fully recover. In combination with the ever-increasing levels of FSH in the serum of these mice, these results indicate that the use of bortezomib may have long-term effects on testicular function [93].

In summary, ubiquitin-proteasomal proteolysis provides degradation of a large number of cell nuclear proteins, cytoplasmic regulatory proteins, endoplasmic reticulum membranes, cell membranes, and the presentation of antigens on the cell surface.

Studies on the role of the ubiquitin-proteasomal system in the development of male infertility are few, but the authors note the important role of components of the ubiquitin system in various stages of gametogenesis, including controlling meiosis and reorganization of the chromatin structure. Recent studies have shown that inactivation of ubiquitin protein ligase, localized in male germ cells, results in the blocking of spermatogenesis and the development of infertility [94, 95].

In studies performed at the State Institution “Institute of Urology of NAMS of Ukraine” [96, 97], the authors found an increase in the processes of ubiquitination in dysregulation of spermatogenesis. There was a significant increase in cytoplasmic expression of ubiquitin in Sertoli cells (from 2.70 ± 0.09 with preserved spermatogenesis, 8.00 ± 0.17 for spermatogenesis block and 7.40 ± 0.60 for “only Sertoli cell” syndrome). Substantial intensification of ubiquitination processes has been determined in residents of ^{137}Cs radionuclide contaminated regions of Ukraine. Significant increase in cytoplasmic expression of ubiquitin in Sertoli cells, spermatogenic epithelium and Leydig cells has been observed compared to similar testicu-

lar cells in residents from non-contaminated areas. Expression of the ubiquitin SUMO protein correlated with abnormalities of ubiquitin protein. In blocked spermatogenesis, a significant increase in cytoplasmic and nuclear expression was found in Sertoli cells (1.7–1.8-fold increase, $p \leq 0.001$), indicating increased processes of sumoylation in Sertoli cells and spermatogenic epithelium. In a group of patients from regions of Ukraine contaminated with ^{137}Cs with blocked spermatogenesis, a significant increase in the process of sumoylation was observed: a 3-fold increase in the index of immunohistochemical coefficient of ubiquitin SUMO in Sertoli cells and a 2-fold increase in the cytoplasm of Leydig cells compared to groups from “clean” regions of Ukraine.

Epigenetic phenomena interacting with the genome due to external factors, without disrupting the nucleotide DNA sequence, are being actively researched [98–100]. Epigenetic regulation is a complex process that involves DNA methylation, micro-RNA effects, and posttranscriptional modifications of histones through their phosphorylation, acetylation, ubiquitination, and sumoylation [100, 101]. According to recent data, the dysfunction of any components of the process of epigenetic regulation can cause impaired spermatogenesis, infertility, and cancer [50, 101, 102].

Therefore, the increased processes of ubiquitination and sumoylation seen after exposure to small doses of radionuclides are a sign of impaired epigenetic mechanisms of regulation of spermatogenesis.

Transforming growth factor beta (TGF- β)

TGF- β is a multifunctional regulatory polypeptide, a member of a large family of cytokines that regulates the most important cellular processes (growth, differentiation, proliferation, migration, adhesion, regeneration, apoptosis) and also plays an important role in the immune response and immunogenesis. The TGF- β family includes more than 40 members, which are grouped into several subfamilies, including activins, inhibitors, and other cytokines. The prototypes of such subfamilies are TGF- β_1 , TGF- β_2 , TGF- β_3 , which are expressed in mammalian cells and play an important regulatory role in the body, starting with fertilization [103].

The most studied and important member of this family is TGF- β_1 , which regulates the immune system being usually secreted by suppressor cells and inhibiting the activation of the immune system. At high concentrations, TGF- β_1 can cause the death of normal human cells (mainly by apoptosis, especially of the cells of the immune system) [104]. TGF- β_1 is a major regulator of carcinogenesis. TGF- β_1 has been shown to inhibit the growth and division of normal human cells, but enhance the growth and migration of highly transformed cancer cells [105].

TGF- β_1 binds to type I and type II receptors on the surface of the target cell. Due to this spatial approximation, the type I receptor phosphorylates receptor II, thereby activating it. The type II recep-

tor phosphorylates Smad2 and Smad3 proteins that bind to the Smad4 protein, forming a hetero-hexameric complex composed of two Smad2, two Smad3, and two Smad4 proteins. This complex is transported to the nucleus, where it binds to the promoters of the target genes, regulating their activity. Smad7 protein is a suppressor protein that inhibits the phosphorylation of Smad2 and Smad3 proteins, decreases their transport to the nucleus and enhances their degradation. Smad2 and Smad3 proteins are often called R-Smad-s (from the R-receptor); Smad-7 is called I-Smad-s (from the I-inhibitor) [106].

Studies have shown that TGF- β cytokine inhibits proliferation, induces apoptosis in most normal epithelial cells, and enhances mesenchymal cell proliferation. In addition, under the influence of TGF- β , epithelial cells lose adhesion and polarity and acquire a mesenchymal phenotype. In the early stages of carcinogenesis, the inhibitory effect of TGF- β on tumor cells was detected by blocking the cell cycle and inducing apoptosis [107].

The TGF- β family is known to regulate such testicular functions as spermatogenesis, steroid synthesis in Leydig cells, and testicular development. Recent studies indicate that TGF- β_3 regulates Sertoli cell TJ dynamics *in vitro* via a mitogen-p38-MAP protein kinase-activated signaling pathway. TGF- β is thought to play an important role in regulating the opening/closing of TJ BTB. This, in turn, regulates the passage of preleptotene and leptotene spermatocytes through the BTB in stages VIII–XI of the spermatogenesis cycle. The regulatory role of TGF- β has been shown in TJ dynamics in the testis [108, 109]. Several cytokines have been shown to selectively regulate the restructuring (“opening” and “closing”) of TJ/adherent junctions during spermatogenesis [110].

Hormones and cytokines are known to regulate the cellular functions of all organs, including the testes. These two groups of molecules exhibit a wide range of effects on different aspects of spermatogenesis. One of the regulatory effects of hormones is the restructuring of intercellular contacts between Sertoli cells and between Sertoli cells and germ cells; the role of hormones (FSH and testosterone) and the cytokines, tumor necrosis factor alpha (TNF- α) and TGF- β , have been demonstrated [111].

TJ proteins of the BTB

It is known that in the male reproductive system there is a hematotesticular barrier (BTB) formed by TJ between Sertoli cells (the main component) and structures that separate mature sperm from peripheral blood to prevent them from interacting [112, 113]. The structure of TJ is formed by three groups of integral transmembrane proteins, including the large claudin family (predominantly claudin-11) and occludin (a key element of TJ), and the junctional adhesion molecule proteins. In addition, cytoplasmic proteins, zonula occludens (ZO-1, ZO-2, ZO-3), are found to be associated with TJ contacts. New cytoplasmic PDZ-containing signaling proteins have also been described, which

play a key role in the attachment of receptor proteins in the membrane to cytoskeleton components and are involved in synaptic signal transduction and regulation, and tumor suppressor proteins that attach to the surface of TJ, as discussed below [114, 115].

TJ are heteropolymers of integral membrane proteins, occludin and claudins, which are embedded in the plasma membrane in epithelial and endothelial cells, forming a boundary between the apical and basolateral domains. Accordingly, TJ act as a fence, delimiting the lateral diffusion of lipids and proteins by the apical and basolateral domains of the membrane. Because the extracellular space is completely obliterated by TJ, integral proteins with large extracellular structures cannot pass through the dense TJ contacts. The disassembly of TJ leads to the mixing of membrane proteins from the apical and basolateral domains.

The basis of TJ between Sertoli cells that generate the BTB is the transmembrane protein, claudin-11. The biological regulation of claudin-11 in the testis has been demonstrated [116, 117]. In mice, it was shown that the absence of claudin-11 protein at the BTB leads to azoospermia. Deficiency of claudin-11 causes the cells of the spermatogenic epithelium to slip into the adjacent branch of the tortuous seminiferous tubules. Understanding this pathophysiology together with immunohistochemical features can improve histological diagnosis [118].

Expression of claudin-11 in normal spermatogenesis and in GCNIS in humans has been investigated [119]. In normal spermatogenesis, clear expression of claudin-11 is found in the basal branch of the seminiferous tubules. GCNIS is accompanied by more scattered and cytoplasmic expression of claudin-11. It has been concluded that claudin-11 is a major protein of TJ, and the disruption of the BTB in GCNIS is related to claudin-11 dysfunction, not its complete loss.

Opposite results were obtained by North Korean researchers who found an increase in the expression of claudin-11 in TJ in hypo-spermatogenesis and in the block of spermatogenesis at the level of spermatocytes in humans. In Sertoli cell only syndrome, increased expression of claudin-11 was observed in the cytoplasm of Sertoli cells [120].

Structural analysis showed that the main components of the BTB, claudin-3, claudin-11, occludin, and ZO-1, are decreased in the area of the basal and adluminal compartment during the stages of movement (VIII–IX) preleptotene/leptotene spermatocytes [121].

It was shown that the loss of claudin-11 in TJ leads to separation from the basement membrane of the Sertoli cell, which becomes fibroblast-like and is excreted through lumens together with apoptotic spermatogenic cells found in the epididymitis [122].

Testosterone has been shown to enhance the expression of claudin-11 in Sertoli cell culture (maximum effect — 0.06 μ M after 72 h of treatment). Administration of flutamide at a dose of 2 mg/kg reduces the expression of claudin-11 in prepubescent eggs and

testes of adult rats. However, there is no change in the expression of claudin-11 in the testes of adult rats with the introduction of 10 mg/kg flutamide [123].

In our studies an increase in the degradation of the proteins of occludin and claudin-11 in observations with blocked spermatogenesis, as well as the negative effect of ionizing radiation on the BTB was shown [124, 125].

It is known that the BTB performs a trophic function and also provides the specific hormonal environment required for spermatogenesis in the tubules. Therefore, BTB injury is an important factor in the emergence of disorders of spermatogenesis (oligo-, terato- and azoospermia). In addition, it has become known that TJ attach to their cytoplasmic domains proteins of the tumor suppressor gene, cell-polarity-related gene products (PAR-3, PAR-6, cdc42) and vesicular-transport-related products (Rab3b, Rab13, Sec6/Sec8), which may cause the antitumor function of TJ [126, 127].

In a comprehensive study [128], causal relationships between changes in spermatogenesis, environmental factors and testicular carcinogenesis have been demonstrated. The author identified the molecular epigenetic mechanisms underlying impaired spermatogenesis, activation of processes of ubiquitination and sumoylation in testicular cells and enhanced degradation of damaged hematotesticular barrier proteins occludin and claudin-11. It is established that the influence of small doses of ionizing radiation leads to changes in the structural components of the testicle, as well as to molecular changes, including epigenetic ones, characterized as an ecological pathomorphosis of male infertility, causing malignancy.

The participation of the ubiquitin-proteolysis system in the initiation of carcinogenesis in the testicle has been determined. In the peritumoral testicular tissue, more intensive expression of ubiquitin is observed compared to testicular tissue with blocked spermatogenesis: 3.5–4 times higher than in observations with blocked spermatogenesis from “clean” regions and 2.5–3 times higher than in observations with blocked spermatogenesis from regions contaminated with radionuclides ^{137}Cs . GCNIS cells differ from spermatogenesis (spermatogonia) cells due to the altered significantly lower expression of ubiquitin [129, 130].

We propose a scheme of pathogenetic mechanisms of disorders of spermatogenesis and the initiation of carcinogenesis in the testis (Figure) [128] with changes according to [22].

CONCLUSIONS

Among the environmental factors that can initiate carcinogenesis, the most noteworthy are the biological effects of low doses of ionizing radiation, such as the effect of RIGI, which increases the risk of carcinogenesis, the “bystander effect”, and chronic oxidative stress.

Disruption of ubiquitin-proteasomal proteolysis, impaired molecular-level components of the BTB, and

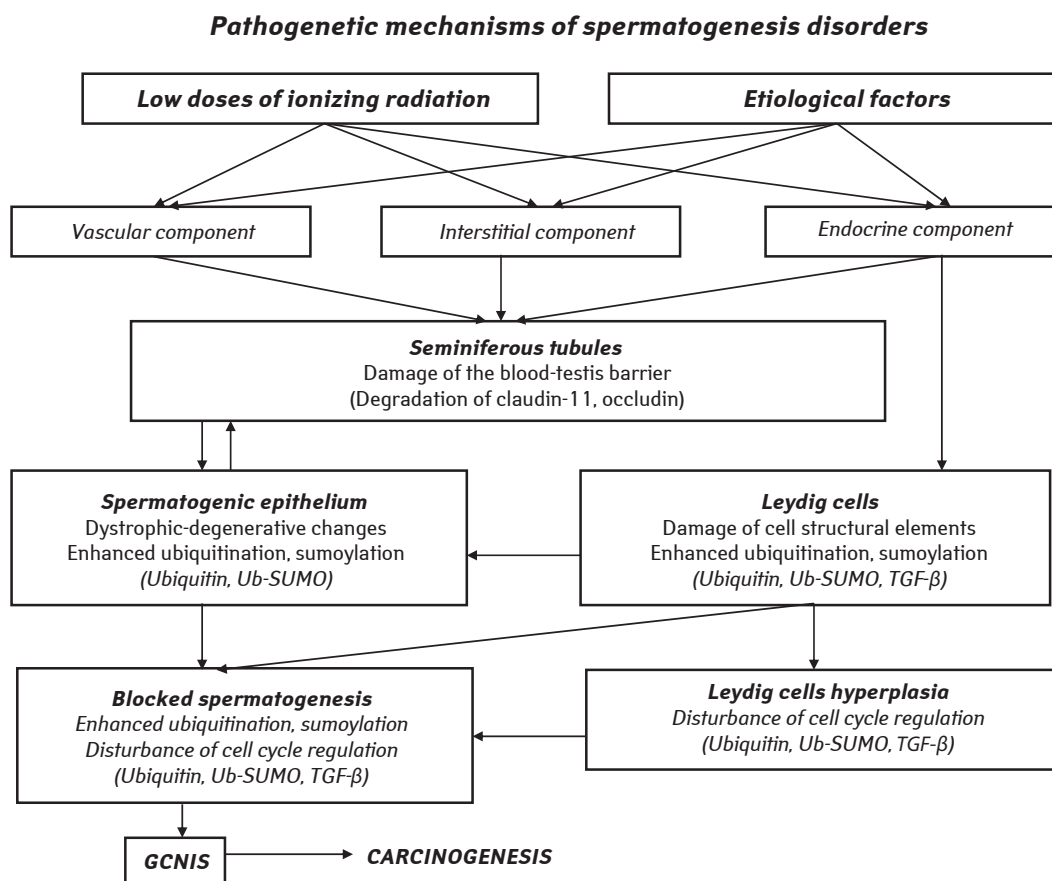


Figure. Pathogenetic mechanisms of disorders of spermatogenesis and initiation of carcinogenesis in the testis [128] with changes according to [22]

impaired regulatory action of TGF- β on the cell cycle can play a crucial role in the pathogenesis of male infertility and the initiation of carcinogenesis in the testis.

The effect of doses of ionizing radiation as an additional etiological factor leads to changes in the structural, as well as molecular, components of the testis, including epigenetic changes, which can be characterized as environmental pathomorphosis, which leads to impaired spermatogenesis and increases the risk of malignancy.

Summarizing the literature, we can state that patients with blocked spermatogenesis, in which atypical cells GCNIS (according to the WHO classification of urogenital tumors, published in 2016) are found in testicular tissue, constitute a group at increased risk of testicular carcinogenesis. The presence of additional etiological factors, such as chronic low doses of ionizing radiation, can initiate the progression of carcinogenesis in the testicle.

REFERENCES

1. Fedorenko ZP, Mikhailovich YuY, Gulak LO, *et al.* Cancer in Ukraine, 2016–2017. Morbidity, mortality, indicators of oncology service activity. Bulletin of the National Cancer Registry of Ukraine. Kyiv, 2018; vol. 19. Available from: http://www.ncru.inf.ua/publications/BULL_19/index_e.htm
2. Feldman PS, Howards SS, Harris C, *et al.* A geographic cluster of testicular seminomas. J Urol 1983; **129**: 839–40.
3. Chissov VI, Davydova MI. Oncology: national guide. Moscow: GEOTAR-Media, 2008. 1072 p. (in Russian).
4. Moller H, Skakkebaek NE. Risk of testicular cancer in subfertile men: case-control study. Br Med J 1999; **318**: 559–62.
5. Pasqualotto FF, Pasqualotto EB, Agarwal A, *et al.* Detection of testicular cancer in men presenting with infertility. Rev Hosp Clin Fac Med Sao Paulo 2003; **58**: 173–8.
6. Dankovich NA. Treatment of infertility in Ukraine: from a doctor of women's consultation to a reproductive specialist. Zdorov'ya Ukrainy. Pediatriya. Akusherstvo. Ginekologiya 2010; **4**: 3 (in Ukrainian).
7. Gorpichenko II, Nikitin OD. Infertile marriage in Ukraine. New realities. Zdorov'ye muzhchiny 2010; **3**: 184–90 (in Russian).
8. Bazyka DA. The evolution of radiobiology and radiation medicine after the Chernobyl disaster. In: Vozianov AF, Bebesko VG, Bazyka DA, eds. Medical Consequences of the Accident at the Chernobyl Nuclear Power Plant. Kyiv: DIA, 2007: 7–31 (in Ukrainian).
9. Health effects of the Chernobyl accident — a quarter of century aftermath. Serdiuk A, Bebesko V, Bazyka D, Yamashita Sh, eds. Kyiv: DIA, 2011. 648 p.
10. Dickey JS, Redon CE, Nakamura AS, *et al.* H2AX: functional roles and potential applications. Chromosoma 2009; **118**: 683–92.
11. Klepko AV, Gorban LV, Motryna OA, *et al.* Study of the dynamics of changes in physiological parameters of rabbit sperm after irradiation of animals with X-rays. Probl Rad Med Radiobiol 2013; **18**: 338–48.
12. Klepko AV, Motryna OA, Bulavitskaya VM, *et al.* Analysis of the activity of the antioxidant sperm system under the conditions of total X-ray irradiation of animals. Probl Rad Med Radiobiol 2014; **19**: 407–18.

13. Koenders MI, Van den Berg WB. Novel therapeutic targets in rheumatoid arthritis. *Trends Pharm Sci* 2015; 36: 189–95.
14. Pal A, Di Magliano MP, Peterson L, *et al.* Usp9x as a novel therapeutic target in human pancreatic cancer. *Cancer Res* 2015; 75: Abstr 1748.
15. Ciechanover A. The ubiquitin proteolytic system and pathogenesis of human diseases: a novel platform for mechanism-based drug targeting. *Biochem Soc Trans* 2003; 31: 474–85.
16. Lui WY, Lee WM, Cheng CY. TGF- β s: their role in testicular function and Sertoli cell tight junction dynamics. *Int J Androl* 2003; 26: 147–60.
17. Albers P, Albrecht W, Algaba F, *et al.* Testicular tumors [homepage in the Internet]. European Association of Urology; Russian Society of Oncurologists [cited 2019 August 22]. Available from: http://uroweb.org/wp-content/uploads/5_Testicular_Cancer.pdf
18. Persidskiy YuV. Morphological and histogenetic features of germinal testicular tumors: Abstract of thesis... cand med sci. Kiev: AA Bogomolets Medical Institute, 1984. 23 p. (in Russian).
19. Berney DM, Looijenga LH, Idrees M, *et al.* Germ cell neoplasia in situ (GCNIS): evolution of the current nomenclature for testicular pre-invasive germ cell malignancy. *Histopathology* 2016; 69: 7–10.
20. Moch H, Cubilla AL, Humphrey PA, *et al.* The 2016 WHO classification of tumours of the urinary system and male genital organs — Part A: renal, penile, and testicular tumours. *Eur Urol* 2016; 70: 93–105.
21. World Health Organization Classification of Tumours: Pathology and Genetics of Tumours of the Urinary System and Male Genital Organs. Eble JN, Sauter G, Epstein JI, Sesterhenn IA, eds. Lyon: IARC Press, 2004. 359 p.
22. World Health Organization Classification of Tumours of the Urinary System and Male Genital Organs. Moch H, Humphrey PA, Ulbright TM, Reuter VE, eds. 4th ed. Lyon: IARC Press, 2016.
23. Tal R, Holland R, Belenky A, *et al.* Incidental testicular tumors in infertile men. *Fertil Steril* 2004; 82: 469–71.
24. Schottenfeld D, Warshauer ME, Sherlock S, *et al.* The epidemiology of testicular cancer in young adults. *Am J Epidemiol* 1980; 112: 232–46.
25. Skakkebaek NE, Berthelsen JG, Müller J. Carcinoma-in-situ of the undescended testis. *Urol Clin North Am* 1982; 9: 377–85.
26. Giwercman A, Thomsen JR, Hertz J, *et al.* Prevalence of carcinoma *in situ* of the testis in 207 oligozoospermic men from infertile couples: prospective study of testicular biopsies. *Br Med J* 1997; 315: 989–91.
27. Ishida H, Isurugi K, Nijima T, *et al.* Carcinoma *in situ* of germ cells and subsequent development of an invasive seminoma in a hyperprolactinaemic man. *Int J Androl* 1983; 6: 229–34.
28. Albers DD, Males JL. Seminoma in hypogonadotropic hypogonadism associated with anosmia (Kallmann's syndrome). *J Urol* 1981; 126: 57–8.
29. Neoptolemos JP, Locke TJ, Fossard DP. Testicular tumour associated with hormonal treatment for oligospermia. *Lancet* 1981; 2: 754.
30. Hutson JM, Balic A, Nation T, *et al.* Cryptorchidism. *Semin Pediatr Surg* 2010; 19: 215–24.
31. Robin G, Boitrelle F, Marcelli F, *et al.* Cryptorchidism: from physiopathology to infertility. *Gynecol Obstet Fertil* 2010; 38: 588–99.
32. Van Echten J, Oosterhuis JW, Looijenga LH, *et al.* No recurrent structural abnormalities in germ cell tumors of the adult testis apart from i(12p). *Genes Chromosomes Cancer* 1995; 14: 133–44.
33. Dieckmann KP, Skakkebaek NE. Carcinoma *in situ* of the testis: review of biological and clinical features. *Int J Cancer* 1999; 83: 815–22.
34. Huyghe E, Soulie M, Escourrou G, *et al.* Conservative management of small testicular tumors relative to carcinoma *in situ* prevalence. *J Urol* 2005; 173: 820.
35. Dieckmann KV, Loy P, Büttner P. Prevalence of bilateral testicular germ cell tumours and early detection based on contralateral testicular intra-epithelial neoplasia. *Br J Urol* 1993; 71: 340–45.
36. Olbert P, Wille S, Von Vietsch H, *et al.* Contralateral spermatogenesis and endocrine profile in patients with testicular germ cell tumors (TGCT). *Eur Urology* 2000; 37: 88.
37. Pedersen MR, Rafaelsen SR, Møller H, *et al.* Testicular microlithiasis and testicular cancer: review of the literature. *Int Urol Nephrol* 2016; 48: 1079–86.
38. Müller J, Skakkebaek NE. Testicular carcinoma *in situ* in children with the androgen insensitivity (testicular feminisation) syndrome. *Br Med J (Clin Res Ed)* 1984; 288: 1419–20.
39. Rajpert-De Meyts E. Developmental model for the pathogenesis of testicular carcinoma *in situ*: genetic and environmental aspects. *Hum Reprod Update* 2006; 12: 303–23.
40. Jonnes TD, MacLennan GT, Varsegi MF, *et al.* Screening for intratubular neoplasia of the testis using OCT4 immunohistochemistry. *Am J Surg Pathol* 2006; 30: 1427–31.
41. Hoei-Hansen CE. Application of stem cell markers in search for neoplastic germ cells in dysgenetic gonads, extragonadal tumours, and in semen of infertile men. *Cancer Treat Rev* 2008; 34: 348–67.
42. Romanenko AM, Persidskiy YuV. Role of atypical sex cells in the histogenesis of germinative testicular tumors. *Arkh Pathol* 1982; 44: 22–8.
43. Hoei-Hansen CE, Rajpert-De Meyts E, Daugaard G, *et al.* Carcinoma *in situ* testis, the progenitor of testicular germ cell tumours: a clinical review. *Ann Oncol* 2005; 16: 863–8.
44. Sakalo AV, Romanenko AM, Bondarenko YuM, *et al.* OST-3/4 and PLAP in the diagnosis of testicular intraepithelial neoplasia. *Zdorov'ye muzhchiny* 2013; (47): 153–4 (in Russian).
45. Joffe M. What has happened to human fertility? *Hum Reprod* 2010; 25: 295–307.
46. Povorozniuk MV. Prevalence and main causes of infertility in men. *Medycni aspekty zdorov'ya cholovika* 2012; (3): 62–73 (in Ukrainian).
47. Ryabchenko NN, Demina EA. Radiation-induced instability of the human genome. *Probl Rad Med Radiobiol* 2014; 19: 48–58.
48. Morgan W. Radiation-induced genomic instability. *Health Phys* 2011; 100: 281–8.
49. Smirnova SG, Orlova NV, Zamulaeva IA, *et al.* Monitoring the frequency of lymphocytes mutant for genes of the t-cell receptor in the liquidators of the consequences of the Chernobyl accident in the remote post-radiation period. *Radiation and Risk* 2012; 21: 20–9 (in Russian).
50. Demina EA. Genetic and epigenetic determinants of radiation carcinogenesis. *Visnyk Ukrainy's'koho tovarystva henetykiv i selektsioneriv* 2014; 12: 103–12 (in Ukrainian).
51. Jackson S, Bartek J. The DNA-damage response in human biology and disease. *Nature* 2009; 461: 1071–8.
52. Mothersill C, Seymour CB. Changing paradigm in radiobiology. *Mutat Res* 2012; 750: 85–95.
53. Mothersill C, Seymour CB. Radiation induced bystander effects and the DNA paradigm: an “out of field” perspective. *Mutat Res* 2006; 597: 5–10.

54. Shemetun EV, Talan OA, Pilinskaya MA, *et al.* Cytogenetic features of induction and persistence of the radiation-induced effect of a witness in human blood lymphocytes. *Tsytol Genet* 2014; **48**: 51–8 (in Russian).
55. Azzam EI, de Toledo SM, Little JB. Oxidative metabolism, gap junctions and the ionizing radiation induced bystander effect. *Oncogene* 2003; **22**: 7050–7.
56. Sokolov MV, Dickey JS, Bonner WM, *et al.* Gamma-H2AX in bystander cells: not just a radiation-triggered event, a cellular response to stress mediated by intercellular communication. *Cell Cycle* 2006; **6**: 2210–2.
57. Chen S, Zhao Y, Han W, *et al.* Mitochondria-dependent signaling pathways are involved in the early process of radiation-induced bystander effects. *Br J Cancer* 2008; **98**: 1839–44.
58. He M, Zhao M, Shen B, *et al.* Radiation-induced intercellular signaling mediated by cytochrom c via p53 depended pathway hepatoma cells. *Oncogene* 2011; **30**: 1947–55.
59. Rugo RE, Schiestl RH. Increases in oxidative stress in the progeny of X-irradiated cells. *Radiat Res* 2004; **162**: 416–25.
60. Limoli CL, Giedzinski E. Induction of chromosomal instability by chronic oxidative stress. *Neoplasia* 2003; **5**: 339–46.
61. Barber C, Hickenbotham L, Hatch T, *et al.* Radiation-induced transgenerational alterations in genome stability and DNA damage. *Oncogene* 2006; **25**: 7336–42.
62. Morgan WF. Non-targeted and delayed effects of exposure to ionizing radiation: II. Radiation-induced genomic instability and bystander effects *in vivo*, clastogenic factors and transgenerational effects. *Radiat Res* 2003; **159**: 581–96.
63. Morozik PM, Mosse IB, Melnov SB, *et al.* A study of the nature of “bystander” factors in vitro and in vivo. Materials of VI Congress on Radiation Research. Vol. 2. M: RUDN, 2010: 72 (in Russian).
64. Mills KD, Ferguson DO, Alt FW. The role of DNA breaks in genomic instability and tumorigenesis. *Immunol Rev* 2003; **194**: 77–95.
65. Gorgojo L, Little JB. Expression of lethal mutations in progeny of irradiated mammalian cells. *Int J Radiat Biol* 1989; **55**: 619–30.
66. Bonassi S, El-Zein R, Bolognesi C, *et al.* Micronuclei frequency in peripheral blood lymphocytes and cancer risk: evidence from human studies. *Mutagenesis* 2011; **26**: 93–100.
67. Bau DT, Tsai CW, Wu CN. Role of the XRCC5/XRCC6 dimer in carcinogenesis and pharmacogenomics. *Pharmacogenomics* 2011; **12**: 515–34.
68. Ryabchenko NM, Glavin OA, Shtefura VV, *et al.* Chromosomal radiosensitivity in Ukrainian breast cancer patients and healthy individuals. *Exp Oncol* 2012; **3**: 1–4.
69. Romanenko AM, Morell-Quadreny L, Lopez-Guerrero JA, *et al.* P16INK4a and p15INK4b gene alteration associated with oxidative stress in renal cell carcinomas after the Chernobyl accident. *Diagn Molec Pathol* 2002; **11**: 163–9.
70. Romanenko A, Kakehashi A, Morimura K, *et al.* Urinary bladder carcinogenesis induced by chronic exposure to persistent low-dose ionizing radiation after Chernobyl accident. *Carcinogenesis* 2009; **30**: 1821–31.
71. Romanenko A, Chekalova A, Harkonen P, *et al.* Latent prostate cancer in association with benign prostatic hyperplasia after the Chernobyl accident in Ukraine. *Virchows Arch* 2010; **457**: 253.
72. Mamina VP, Sheiko LD. Influence of ionizing radiation and xenobiotics on spermatogenesis of the epithelium of laboratory animals. *Gigiyena Sanitariya* 2001; **6**: 24–7 (in Russian).
73. Cheburakov BYu, Cheburakov SYu, Belozarov NYu. Morphological changes in testicular tissue in liquidators after the Chernobyl accident. *Arkh Pathol* 2004; **6**: 19–21 (in Russian).
74. Garcia CR, Sammel MD, Coutifaris Ch, *et al.* Occupational exposures and male infertility. *Am J Epidemiol* 2005; **162**: 729–33.
75. Muratori M, Luconi M, Marchiani S, *et al.* Molecular markers of human sperm functions. *Int J Androl* 2009; **32**: 25–45.
76. Gobé GC, Harmon B, Leighton J, *et al.* Radiation-induced apoptosis and gene expression in neonatal kidney and testis with and without protein synthesis inhibition. *Int J Radiat Biol* 1999; **75**: 973–83.
77. Guitton N, Touzalin AM, Sharpe RM, *et al.* Regulatory influence of germ cells on sertoli cell function in the pre-pubertal rat after acute irradiation of the testis. *Int J Androl* 2000; **23**: 332–9.
78. Lu Y, Wang X, Chen Y, *et al.* Effects of electromagnetic radiation on morphology and TGF- β_3 expression in mouse testicular tissue. *Toxicology* 2013; **310**: 8–14.
79. Trifonova YP. Diagnosis and correction of male fertility disorders depending on the state of free radical processes: dis. ... cand. medical sciences: 14.01.06. Institute of Urology, Academy of Medical Sciences of Ukraine. Kyiv, 2005. 128 p.
80. Klepko AV, Gorban LV, Motryn OA, *et al.* The study of the dynamics of changes in the physiological parameters of rabbit sperm after irradiation of animals with x-rays. *Probl Rad Med Radiobiol* 2013; **18**: 338–48.
81. El-Domyati MM, Al-Din AB, Barakat MT, *et al.* The expression and distribution of deoxyribonucleic acid repair and apoptosis markers in testicular germ cells of infertile varicocele patients resembles that of old fertile men. *Fertil Steril* 2010; **93**: 795–801.
82. Hegazy R, Hegazy A, Ammar M, *et al.* Immunohistochemical measurement and expression of Mcl-1 in infertile testes. *Front Med* 2015; **9**: 361–7.
83. Abramova EB, Karpov VL. Proteasoma: destruction in the name of creation. *Nature* 2003; **7**: 36–45.
84. Sorokin AV, Kim ER, Ovchinnikov LP. Proteasomal system of protein degradation and processing. *Uspekhi Biologicheskoi Khimii* 2009; **49**: 3–76.
85. Martino GN, Slaughter CA. The proteasome, a novel protease regulated by multiple mechanisms. *J Biol Chem* 1999; **274**: 22123–6.
86. Ciechanover A, Martino GN, Slaughter CA. The ubiquitin-proteasome pathway: on protein death and cell life. *EMBO J* 1998; **17**: 7151–60.
87. Glickman MH, Ciechanover A. The ubiquitin-proteasome proteolytic pathway: destruction for the sake of construction. *Physiol Rev* 2002; **82**: 373–428.
88. Kane RC, Bross PF, Farrell AT, *et al.* Velcade: US FDA approval for the treatment of multiple myeloma progressing on prior therapy. *Oncologist* 2003; **8**: 508–13.
89. Kane RC, Dagher R, Farrell A, *et al.* Bortezomib for the treatment of mantle cell lymphoma. *Clin Cancer Res* 2007; **13**: 5291–4.
90. Somlo G, Lashkari A, Bellamy W, *et al.* Phase II randomized trial of bevacizumab versus bevacizumab and thalidomide for relapsed/refractory multiple myeloma: A California Cancer Consortium trial. *Br J Haematol* 2011; **154**: 533–5.
91. Messinger YH, Gaynon PS, Sposto R, *et al.* Bortezomib with chemotherapy is highly active in advanced B-precursor acute lymphoblastic leukemia: Therapeutic Advances in Child-

hood Leukemia & Lymphoma (TACL) Study. *Blood* 2012; **120**: 285–90.

92. **Michaelis M, Fichtner I, Behrens D, et al.** Anti-cancer effects of bortezomib against chemoresistant neuroblastoma cell lines *in vitro* and *in vivo*. *Int J Oncol* 2006; **28**: 439–46.

93. **Hou M, Eriksson E, Svechnikov K, et al.** Bortezomib treatment causes long-term testicular dysfunction in young male mice. *Mol Cancer* 2014; **13**: 155.

94. **Lui WW, Lee M.** cAMP perturbs inter-Sertoli tight junction permeability barrier *in vitro* via its effect on proteasome-sensitive ubiquitination of occluding. *J Cell Physiol* 2005; **203**: 564–72.

95. **Wang YL, Liu W, Sun YJ, et al.** Overexpression of ubiquitin carboxyl-terminal hydrolase L1 arrests spermatogenesis in transgenic mice. *Mol Reprod Dev* 2006; **73**: 40–9.

96. **Bazalytska SV, Romanenko AM.** Ubiquitin-proteolysis system in the pathogenesis of male infertility after the Chernobyl accident. *Urolohiya* 2011; **15**: 37–45 (in Ukrainian).

97. **Bazalytska SV, Romanenko AM.** Processes of ubiquitination and sumoylation in the pathogenesis of male infertility. *Patolohiya* 2011; **8**: 35–9 (in Ukrainian).

98. **Vaiserman A.** Early-Life epigenetic programming of human disease and aging. In: Trygve O, eds. *Epigenetics in human disease*. Tollefsbol—Amsterdam, Boston: Elsevier, 2012: 545–67.

99. **Vayserman AM, Voitenko VP, Mekhova LV.** Epigenetic epidemiology of age-related diseases. *Ontogenesis* 2011; **42**: 1–21.

100. **Labonte B, Turecky G.** Epigenetics: a link between environment and genome. *Sante mentale au Quebec* 2012; **37**: 31–44.

101. **Tammen SA, Friso S, Choi SW.** Epigenetics — the link between nature and nurture. *Mol Aspects Med* 2013; **34**: 753–64.

102. **Kurilo LF, Stout MI.** Genetic and epigenetic mechanisms of regulation, chronology and dynamics of spermatogenesis in mammals. *Andrology Genital Surgery* 2015; **1**: 31–40 (in Russian).

103. **Clark DA, Coker R.** Transforming growth factor-beta (TGF-beta). *Int J Biochem Cell Biol* 1998; **30**: 293–8.

104. **Tiemessen MM, Kunzmann S, Schmidt-Weber CB, et al.** Transforming growth factor β inhibits human antigen specific CD4⁺ T cell proliferation without modulating the cytokine response. *Int Immunol* 2003; **15**: 1495–1504.

105. **Ikushima H, Miyazono K.** TGF-beta signalling: a complex web in cancer progression. *Nat Rev Cancer* 2010; **10**: 415–24.

106. **Dahl M, Maturi V, Lönn P, et al.** Fine-tuning of Smad protein function by poly(ADP-ribose) polymerases and poly(ADP-ribose) glycohydrolase during transforming growth factor β signaling. *PLoS One* 2014; **9**: e103651.

107. **Xu J, Lamouille S, Derynck R.** TGF-beta-induced epithelial to mesenchymal transition. *Cell Res* 2009; **19**: 156–72.

108. **Li MW, Mruk D, Lee WM, et al.** Cytokines and junction restructuring events during spermatogenesis in the testis: an emerging concept of regulation. *Cytokine Growth Factor Rev* 2009; **20**: 329–38.

109. **Lui WY, Lee WM.** Molecular mechanisms by which hormones and cytokines regulate cell junction dynamics in the testis. *J Mol Endocrinol* 2009; **43**: 43–51.

110. **Xia W, Mruk DD, Lee WM, et al.** Cytokines and junction restructuring during spermatogenesis — a lesson to learn from the testis. *Cytokine Growth Factor Rev* 2005; **16**: 469–93.

111. **Xia W, Wong EW, Mruk DD, et al.** TGF-beta3 and TNF alpha perturb blood-testis barrier (BTB) dynamics

by accelerating the clathrin-mediated endocytosis of integral membrane proteins: a new concept of BTB regulation during spermatogenesis. *Dev Biol* 2009; **327**: 48–61.

112. **Mruk DD, Cheng CY.** Tight junctions in the testis: new perspectives. *Philos Trans R Soc Lond B Biol Sci* 2010; **365**: 1621–35.

113. **Jiang XH, Bukhari I, Zheng W, et al.** Blood-testis barrier and spermatogenesis: lessons from genetically-modified mice. *Asian J Androl* 2014; **16**: 572–80.

114. **Van Itallie CM, Anderson JM.** Architecture of tight junctions and principles of molecular composition. *Semin Cell Dev Biol* 2014; **36**: 157–65.

115. **Tao YX, Johns RA.** Neuronal PDZ domains: a promising new molecular target for inhaled anesthetics? *Mol Interv* 2004; **4**: 215–21.

116. **Furuse M, Tsukita S.** Claudins in occluding junctions of humans and flies. *Trends Cell Biol* 2006; **16**: 181–8.

117. **Wu X, Peppi M, Vengalil MJ, et al.** Transgene-mediated rescue of spermatogenesis in Cldn11-null mice. *Biol Reprod* 2012; **86**: 1–11.

118. **Mazaud-Guittot S, Gow A, Le Magueresse-Battistoni B.** Phenotyping the claudin 11 deficiency in testis: from histology to immunohistochemistry. *Methods Mol Biol* 2011; **763**: 223–36.

119. **Fink C, Weigel R, Fink L, et al.** Claudin-11 is overexpressed and dislocated from the blood-testis barrier in Sertoli cells associated with testicular intraepithelial neoplasia in men. *Histochem Cell Biol* 2009; **131**: 755–64.

120. **Nah WH, Lee JE, Park HJ, et al.** Claudin-11 expression increased in spermatogenic defect in human testes. *Fertil Steril* 2011; **95**: 385–8.

121. **Chihara M, Otsuka S, Ichii O, et al.** Molecular dynamics of the blood-testis barrier components during murine spermatogenesis. *Mol Reprod Dev* 2010; **77**: 630–9.

122. **Mazaud-Guittot S, Meugnier E, Pesenti S, et al.** Claudin 11 deficiency in mice results in loss of the Sertoli cell epithelial phenotype in the testis. *Biol Reprod* 2010; **82**: 202–13.

123. **Florin A, Maire M, Bozec A, et al.** Androgens and postmeiotic germ cells regulate claudin-11 expression in rat Sertoli cells. *Endocrinology* 2005; **146**: 1532–40.

124. **Bazalytska SV, Romanenko AM, Persidskiy YuV.** Peculiarities of expression of claudin protein (Claudin 11) in various forms of male infertility. *Patolohiya* 2012; **26**: 11–4 (in Ukrainian).

125. **Bazalytska SV.** Features of Claudin 11 protein expression and blood-testis barrier status in various forms of male infertility. *Svit Medytsyny Biolohiyi* 2015; **49**: 80–3 (in Ukrainian).

126. **Aranda V, Haire T, Nolan ME, et al.** Par6-aPKC uncouples ErbB2 induced disruption of polarized epithelial organization from proliferation control. *Nat Cell Biol* 2006; **8**: 1235–45.

127. **Canman JC, Lewellyn L, Laband K, et al.** Inhibition of Rac by the GAP activity of centralspindlin is essential for cytokinesis. *Science* 2008; **322**: 1543–6.

128. **Bazalytska SV.** Male infertility in Ukraine: features of patho- and morphogenesis. Kyiv: The Fourth Wave, 2016. 262 p (in Ukrainian).

129. **Bazalytska SV, Romanenko AM, Sakalo VS, et al.** Processes of ubiquitination in the peritumoral tissue of patients with germinogenic tumors of the testicle. *Urolohiya* 2013; **17**: 81–4 (in Ukrainian).

130. **Bazalytska SV, Romanenko AM.** Expression of Ubiquitin protein in peritumoral tissue in germinogenic testicular tumors. *Patolohiya* 2015; **33**: 26–30 (in Ukrainian).