[Frontiers In Bioscience, Landmark, 22, 1073-1098, March 1, 2017]

Epigenetic and risk factors of testicular germ cell tumors: a brief review

Daniel Adrian Landero-Huerta[1,2,3], Rosa Maria Vigueras-Villasenor[1], Emiy Yokoyama-Rebollar[4], Elena Arechaga-Ocampo[5], Julio Cesar Rojas-Castaneda[1], Francisco Jimenez-Trejo[1], Margarita Chavez-Saldana[1]

[1]Laboratory of Reproductive Biology, National Institute of Pediatrics, Mexico City, Mexico, [2]Postgraduate of Natural Sciences and Engineering, Autonomous Metropolitan University Cuajimalpa Campus, Mexico City, Mexico, [3]Coordination of Medical School, Autonomous University of the State of Mexico Branch Chimalhuacan, State of Mexico, Mexico, [4]Department of Human Genetics, National Institute of Pediatrics, Mexico City, Mexico,[5]Department of Natural Sciences and Engineering, Autonomous Metropolitan University Cuajimalpa Campus, Mexico City, Mexico

TABLE OF CONTENTS

1. ABSTRACT

Testicular germ cell cancer (TGCT) is the most common malignancy among young adult males, which has become important due to its increased incidence and mortality in the population worldwide. The etiology is multifactorial. Recent studies have shown some associations between the development of isolated TGCT and certain risk factors, such as exposure to endocrine disruptors, cryptorchidism, and family history of cancer, in order to identify the key pieces in carcinogenesis. Some of the most important findings in recent years is the association of different genes, such as *c-KIT*/*KITLG*, expression of the miR-371-373 cluster and protein expression as c-KIT and *POU5F1* in the development of this neoplasia, and the identification of new molecular markers as *TGFBR3* gene, identifying aberrant methylation patterns in promoter regions of several genes, expression of miR-1297 which regulates *PTEN* and protein expression as DMTR1. In the future, a multidisciplinary research strategy could provide valuable new insights into the etiology of TGCTs, which support clinical diagnosis of TGCT in the next years to increase survival in this kind of patients.

2. INTRODUCTION

Testicular germ cell tumor (TGCT; OMIM 273300) is the most common solid tumor in men and young adults between 15 and 45 years old and represents a major cause of death in this group of individuals (1). Its incidence has been increasing worldwide in recent decades, probably due to increased exposure to etiological factors (2), although this Figure

varies in different populations: a high incidence is reported in Scandinavia, Switzerland and Germany, intermediate in the United States, Britain and Mexico, while in Africa and Asia a low incidence is reported (3-4). In 2012, a worldwide incidence was estimated with 55,226 new cases and 10,351 deaths. In Mexico, in the same year, 1,602 new cases and a total of 347 deaths from this disease were reported (5). When comparing TGCT incidence rates in different populations, it was observed that rates were similar among Hispanic/Latino populations, specifically in Mexico, compared to the United States and Great Britain (6). However, in Mexico the mortality rate is higher than in those countries, probably due to late diagnosis. Overall, the risk for the development of TGCT is higher in Caucasian populations than in African and Asian populations; however, the reasons for these differences in the incidence of TGCT among different ethnic groups are unknown (7). Clinical and epidemiological studies suggest that both environmental and genetic factors play an important role in the origin and development of TGCT. In humans, early stages of development (embryonic, fetal and infant) are vulnerable to environmental effects. It has been proposed that intrauterine exposure to endocrine disruptors (EDs) could have different effects on fetal development and induces genital malformations in boys (8-10). Although epidemiological studies have shown some controversy, it is proposed that the damage caused by EDs may depend on dosage, exposure time, developmental stage of each individual, maternal lifestyle, genetic factors and the genetic variability of susceptibility to the exposure to EDs (8,11). It has been observed that certain genes and miRNAs that have not been reported can be involved in the pathogenesis of TGCT. Moreover, it is documented that cryptorchidism or no testicular descent (CO; OMIM # 219050) is a risk factor for the development of TGCT. Patients with CO are 7 to 10 times more likely to develop TGCT than the general population, although this association is established, the mechanisms that lead to this disease are not yet known, but it is proposed that the TGCT is originated from a histological precursor called intratubular germ cell neoplasia unclassified (IGCNU), is now known as germ cell neoplasia *in situ* (GCNIS) (12-15). It has been documented in several studies the association of CO and the risk of developing malignancy in adulthood, proposing CO as a symptom of a future alteration with higher impact to cancer and reproductive level (16-20). Undoubtedly, advances in knowledge of TGCT have led to improved strategies to optimize a timely diagnosis, so this review aims to describe some of the risk factors contributing to the pathophysiology of this cancer, such as environmental, genetics and epigenetics mechanisms.

3. TESTICULAR GERM CELL TUMOR

The TGCT is the most common solid testicular tumor. About 95 percent of them are TGCT, and the remaining 5 percent correspond to non-germ cell line tumors such as Sertoli cell tumors, Leydig cell tumors, or lymphomas. The TGCT occurs early in men, affecting one or both testicles. It is considered a heterogeneous genitourinary malignancy clinically asymptomatic in its early stages, which is commonly treated successfully, although a significant rate of morbidity and mortality has been reported (21). The World Health Organization (WHO) has histologically classified TGCT into two groups: 1) the testicular germ cell tumors of seminoma type (sTGCT) representing about 55 percent of cases being reported, in most cases sensitive to treatment with radiation therapy and cisplatin-based chemotherapy; 2) the testicular germ cell tumor type nonseminoma (nsTGCT) representing about 45 percent of cases, in this group mixed tumors (tumors of the type nonseminoma with seminoma component) are included, such tumor usually does not respond to radiation therapy, but is sensitive to chemotherapy. The sTGCT represents a set of undifferentiated cells that resemble primordial germ cells (PGC) and can be classified into groups: 1) the classical or typical sTGCT representing about 95 percent of cases, originating from gonocytes or PGC with a predominance in young adult patients, and 2) spermatocytic sTGCT with a source in spermatocytes, occurs in adults 50 and older. The nsTGCT usually occurs in younger patients and brings together 2 cell lines: 1) tumors of embryonic lineage as embryonal carcinoma and teratoma, classified as immature teratoma, mature teratoma and teratoma with malignant transformation, and 2) tumors with extra embryonic tissue components, such as yolk sac tumor and choriocarcinoma. It is because of this classification that efforts have been made to understand the etiology of TGCT in order to influence the diagnosis and treatment of this neoplasia (22, 23).

4. GONOCYTE THEORY IN THE DEVELOPMENT OF THE GERM CELL NEOPLASIA *IN SITU* (GCNIS)

It is suggested that the origin of the GCNIS is during embryonic development and gonocytes (also called pre-spermatogonia) are responsible for this neoplasm. Gonocytes are cells that arise from the differentiation of PGCs that have migrated to the gonadal ridge and the allantois. Once established in this structure, they no longer migrate and differentiate into gonocytes (24). Gonocytes show morphological characteristics similar with atypical cells of GCNIS, also sharing the same profile protein expression of pluripotency and lifespan, as OCT3/4, now known as POU5F1, TFAP2C, c-KIT, PLAP and NANOG (14, 25-28). It is important to note that some of these proteins are used as biomarkers for diagnosing the GCNIS. An animal model that develops GCNIS has been proposed, exposing rabbits to 17-beta estradiol, considered DEs, which induces bilateral CO and GCNIS long term.

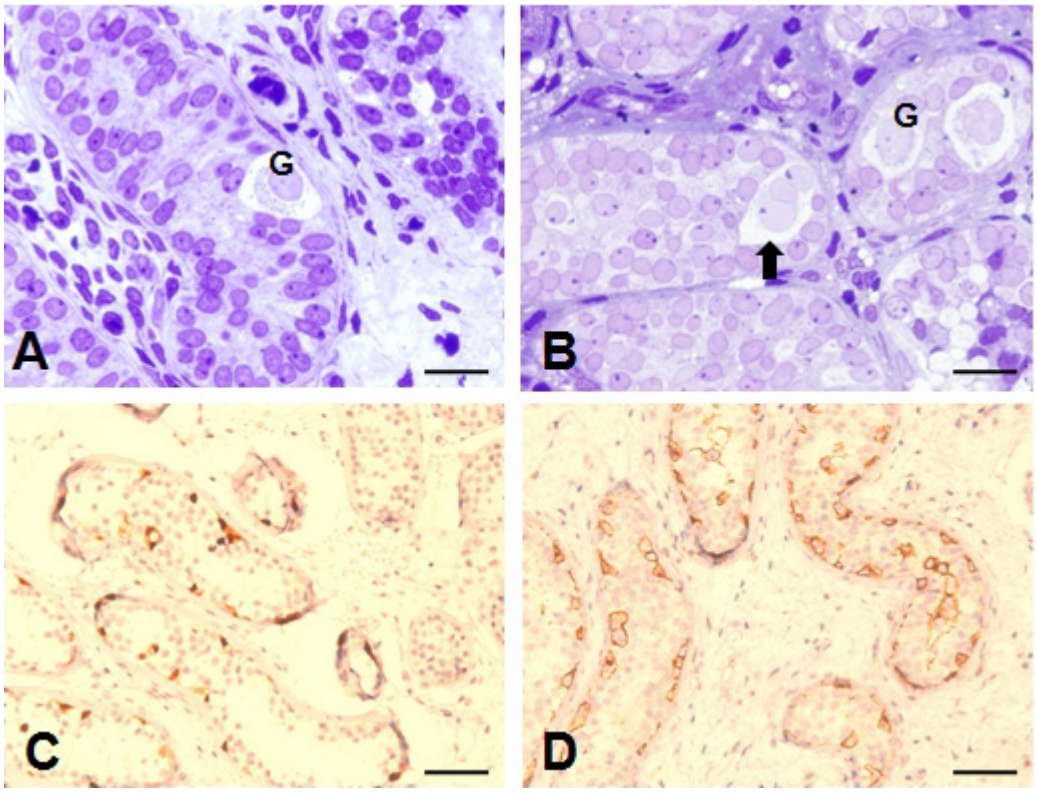

Figure 1. Histological images of seminiferous tubules from patients with cryptorchidism: A) a 2 years-old: the gonocytes shows mitochondrial with a half-moon shape, around cell nucleus (G); B) a 7 years-old: the gonocytes shows disruption in central region with the presence of degenerating gonocytes (arrow); C) and D) 7 years old: Immunohistochemistry of POU5F1 and C-KIT in testicular tissues, respectively. Scale bars: 20 micrometers A and B, 50 micrometers C and D.

During the development of neoplasia, it has been found that gonocytes persisted expressing pluripotency proteins like POU5F1, c-KIT and PLAP beyond its normal downregulation period (80 days old in the rabbit). This supports the theory of the origin of GCNIS from germ cells as they remain undifferentiated and pluripotent (29). In healthy humans, gonocytes begin their differentiation into spermatogonia during the second to third trimester of pregnancy and concludes in the first months after birth (30, 31). During this period, pluripotency proteins are regulated downward until their total disappearance, at about four months of age (28, 31, 32). The lack of differentiated gonocytes in patients with CO, suggests that these cells are kept alive, undifferentiated and capable of pluripotency, which makes them susceptible to the development of GCNIS (33, 34). Recently one study showed, by immunohistochemistry and RT-PCR, the presence of proteins POU5F1, c-KIT, AP2-gamma, Sall4 and PLAP in 5.4. percent (2/37) of patients with CO older than 12 months old, far beyond their normal differentiation period (16, 35, 36), which proposed that GCNIS originates from fetal germ cells or gonocytes that reside latent in the testis, until they initiate proliferation after puberty by activation of hypothalamic-pituitary-testicular axis (37). This causes an increased slow in testicular volume after puberty, leading to a delayed diagnosis around 25 to 35 years (38, 39). There are reports where it is shown that in diseases such as gonadal dysgenesis, undifferentiated testicular tissue was positive to POU5F1 protein, c-KIT and PLAP during childhood gonadal tumor that develop in later stages (36) (Figure 1). In this sense, there have been studies in animal models documenting differentiation gonocytes, which is due to the contribution of factors produced by functional Sertoli cells, such as retinoic acid, stem cells factors, molecular cell adhesion, among others (40-43).

5. RISK FACTORS FOR THE DEVELOPMENT OF TGCT

Molecular, clinical and epidemiological studies have established evidence linking environmental and genetic factors in the development of TGCT (44, 45). It is believed that development of TGCT comes from the GCNIS (12-15). These factors can influence the differentiation process avoiding PGC. Among the environmental factors, they have included EDs such as some pesticides and chemicals that can interfere in various stages of development male since gestational stage (46). Furthermore, previous

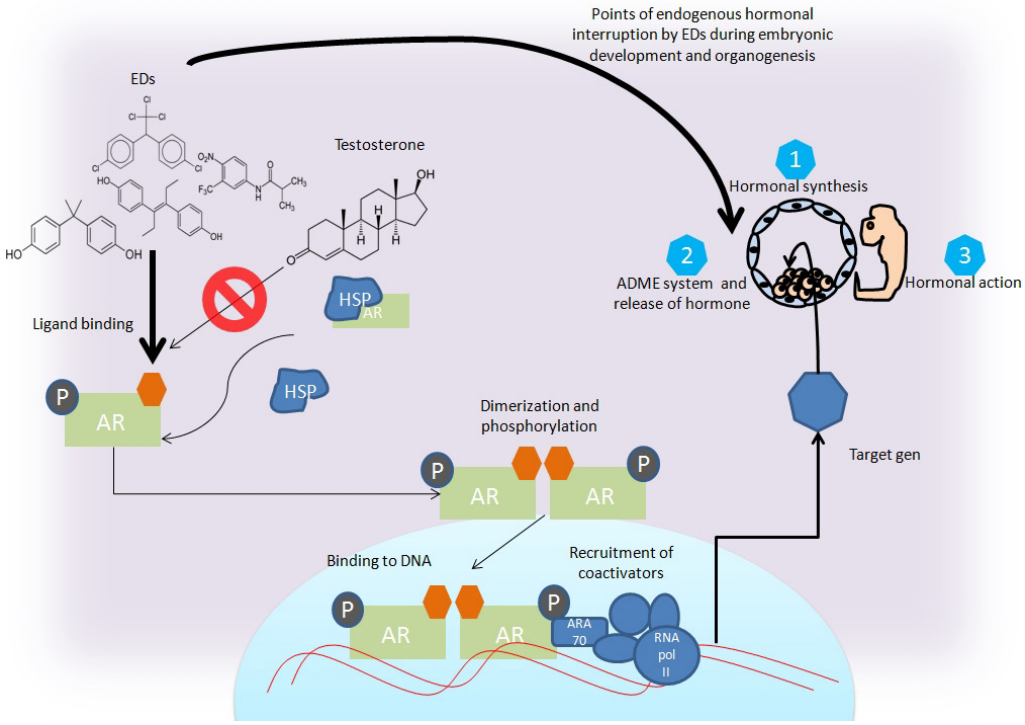

Figure 2. Action of endocrine disruptors on the endogenous hormonal production, which showing the competition of endocrine disruptors for the action site of action in the androgen receptor, that may change the synthesis, release, transport, metabolism, binding, action or elimination of endogenous hormones during embryonic development, as well as before, during or after organogenesis.

reports have identified genetic and epigenetic factors involved in the development of TGCT at different stages of development: 1) *in uterus*, in PGC over-expression of factors such as *c-KIT*/*KITLG*, *SDF-1*/*CXCR4*, and in gonocytes over-expression of genes that would have to be adjusted downward such as *POU5F1*, *NANOG*, *SOX17*, *c-KIT*/*KITLG*, *PDPN* in addition to the gain of chromosome 12p; 2) during childhood, mutations in *c-KIT*, *TP53*, *KRAS*, *NRAS* and *BRAF*, gain in chromosome 12p, over-expression of genes of pluripotency, and hypomethylation and hypermethylation of DNA; and 3) after puberty, mutations, allelic variants and differential expression of genes such as *AR*, *DMRT1*, *SPRY4* and *BAK1* reported in both nsTGCT and sTGCT (23).

5.1. Environmental factors

From the 1950s, promising to improve quality of life standards, introduction of various chemicals structurally related to hormonal compounds was given, such as the EDs, including pesticide and some industrial waste products, were documented as compounds that affect the normal development in humans, after several studies. Epidemiological studies and animal models have demonstrated that exposure to EDs during organogenesis correlates with the increased incidence of malformations of the male genital tract, a decrease in sperm quality and testicular cancer development (47). EDs have also shown that they alter the reproductive process in both men and women, modifying the neuroendocrine profile, aggressive behavior, metabolism, developing prostate and thyroid cancer (48). The term of EDs arises in the Wingspread Conference in 1991, through an epidemiological study in European and North American population, and for the first time a relationship between exposure to these chemicals and decreased semen quality is proposed, in addition to finding a increased incidence of congenital malformations, such as cryptorchidism, hypospadias and an increased incidence of testicular and breast cancer. The action mechanism of EDs is proposed by the interruption in the synthesis, release, transport, metabolism, binding, action or elimination of endogenous hormones during embryonic development before, during or after organogenesis (Figure 2). Experiments have shown that hormone level variations can cause morphological and functional alterations in organisms. Fetal exposure to EDs occurs through the mother during pregnancy, to the infant through breastfeeding and during childhood by consuming formula wherein the containers have these compounds (47). One of the first documented cases between the 1940s and 1970s was the use of diethylstilbestrol, which is a synthetic form of estrogen, indicated to prevent spontaneous abortions, premature births and other pregnancy-related complications (48). Years later it was found that this compound was not effective in preventing these problems, discovering in those prenatally exposed, the

development of a type of cervical and vagina cancer (clear cell adenocarcinoma) (49-50). Epidemiological data support the hypothesis that small increments in estrogen levels during fetal development increase the risk of developing hormone-dependent cancers such as the TGCT. Another ED studied is Bisphenol A (BPA), which is currently used in many products, such as baby bottles, water bottles, food containers, and for the production of epoxy resins among others. A study in USA, detected BPA in urine in 92 percent of the population tested. The use of BPA has already been legislated in some countries such as Canada, the European Union and United States of America, as well as some Latin American countries like Argentina, Colombia and Peru, but in Mexico is not still clear (51). Animal models have reported that prenatal BPA exposure is associated with increased development of pre-cancerous lesions as GCNIS, which appear in adolescence. It has also been demonstrated in animal models exposed to BPA during breastfeed an increased risk of developing TGCT, compared to unexposed animals (52). Several studies have found a risk association between exposure to EDs in agricultural areas and genital abnormalities in males. In fact, blood concentrations of organochlorines have been detected in mothers of patients with TGCT after several decades of diagnosis (53). Moreover, some studies have found a correlation between high levels of phthalates in urine of pregnant women and their undervirilized offspring (54). This interaction of disease and the environment, has been linked to epigenetic processes, like DNA and histone modifications, mainly in fetal adaptation to an environment in which the dose, the period of development and duration of this exposition are of great importance. In men, the exposure to EDs has been implicated in the development of testicular dysgenesis syndrome (TDS), which includes TGCT, CO, hypospadias and infertility, which suggests that fetal exposure to these compounds alter the functioning of the Leydig and Sertoli cells, modifying the development of germ cells (20, 55).

5.2. Cryptorchidism as risk factor in the development of TGCT

The CO is the most common genitourinary birth defect in man and is important due to the complications in adulthood; including infertility and the TGCT risk (9, 56). The CO has a variable prevalence from one to another country; Denmark has one of the highest incidences (9.0. percent) compared to Finland, which has one of the lowest incidences (2.4. percent) (9). In Mexico, until now there are no epidemiological studies of this malformation. The CO can occur in one testicle (unilateral) or both (bilateral). The classification can be based on the location of the testicle: 1) abdominal, above the inguinal canal; 2) inguinal; and 3) ectopic, outside of the normal testicular descent path (10). In embryonic stage, the testicle is located at the abdomen and then moves into the scrotum. In humans, the testicle in the abdominal position is suspended by two ligaments, one that connects the kidney to the gonad, called cranial suspensory ligament (LSC), and the other connecting the testis and epididymis to the floor of the scrotum called gubernaculum. Testicular descent in humans is presented in two phases called trans-abdominal and inguinoescrotal between week 8 to 17 and during the week 26 to 32 of gestation, respectively (57, 58) (Figure 3). This descent process occurs in order to provide testes a temperature of 1.5. to 4.0. degrees centigrade lower than the body temperature, allowing adequate spermatogenesis and optimal epididymal function (56, 58, 59). Despite widespread knowledge of the testicular physiology, etiology of CO remains largely unknown. However, it is considered a multifactorial disease in which various mechanisms that regulate testicular descent have been proposed (59, 60), possibly affected by endocrine, anatomical and genetic factors. It is suggested that environmental and genetic factors involved can intervene in early stages of embryonic development (61). Based on its etiology, CO may occur as an isolated pathology; but it may also occur as part of any of the more than 250 genetic syndromes associated with this malformation (10, 60). It has been documented that this malformation is the second risk factor for the development of testicular neoplasia. Patients with CO are 7 to 10 times more likely to develop TGCT (62-64). Although this association is established, the mechanisms that lead to this disease are still unknown (12-15, 61). It has been documented that among individuals with unilateral CO, one of every four cases develops TGCT in the contralateral normally descended testicle (16, 17). Also, it has been demostrated that the testicle with intraabdominal CO has an increased risk of developing malignancy, comparing with the others that stay at inguinal level. Indeed, patients with bilateral CO compared to unilateral cases have also increased risk (9, 18-20). Based on these observations, it can be inferred that there are shared underlying genetic factors in the presence of CO and TGCT. Thus, CO can be the first symptom of a future alteration where TGCT and infertility can be manifestations of the same defect (20). Different genes involved in the process of testicular descent, such as androgen receptor *(AR), INSL3*, *GREAT* and *HOXA10* among others, as well as the prevalence of CO in different populations, is probably the result of ethnic differences (18, 19). It is important to note, that certain genes interactions, specifically *INSL3* and *AR*, have also been documented in both CO and TGCT. Several studies have shown that the production of INSL3 is sensitive to estrogenic or anti-androgenic compounds exposure, as EDs (Figure 3), which clearly suggests that exposure to these compounds during pregnancy can result in cryptorchidism, a factor that predisposes to TGCT (65). The impact of EDs in steroidogenesis could result in inhibition of the cholesterol supply and gene

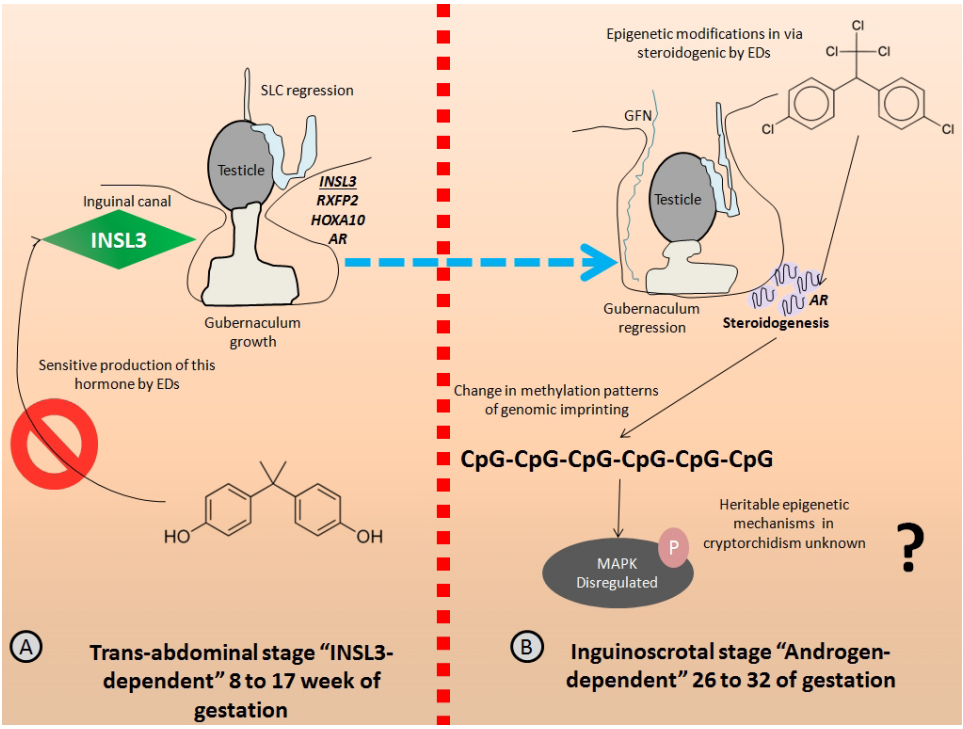

Figure 3. Action of endocrine disruptors (EDs) at different stages of testicular descent: A) During the transabdominal stage, the EDs can limit the production of INSL3 hormone; B) During the inguinoscrotal stage, the presence of can produce heritable changes in DNA methylation, which promotes MAPK deregulation, as a possible epigenetic mechanism of cryptorchidism, which is considered a risk factor of TGCT.

expression in the related pathway (66). Another study with great importance in the etiology of CO reviewed the role of dioxin or Di (2-ethylhexyl) phthalate, (DEHP) which is also an ED, and demonstrated that it promotes transgenerational epigenetic inheritance of CO. This component was administered in the gonadal development period in pregnant female rats, which is a critical period, inducing the occurrence of CO in the offspring during pregnancy. The incidence of CO in the first generation (F1) was 30 percent, the F2 was 12.5. percent, while for the F3 and F4 generations was no development of CO. Results revealed many DNA sequences differentially methylated between F1 and F4. DEHP damages male reproductive function in rats affecting the expression of the DNA methyltransferase enzyme, which in turn leads to changes in methylation patterns of genomic imprinting, especially within CpG and promoter island regions, representing an important damage mechanism to the male reproductive system (Figure 3). However, the authors attributed this phenomenon to a phosphorylation and negative regulation of MAPK cascade that could play an important role in epigenetic inheritance of the male reproductive system. This study represents one of the most important analyses about the influence between environment and development of CO, and the possible risk of developing TGCT by epigenetic mechanisms (67). However, the pathogenesis of CO remains uncertain, and it is unclear whether CO is a risk indicator for the development of TGCT, or is itself a precursor for malignancy. Therefore, it is difficult to speculate whether the prevalence of CO would be elevated among individuals with a predisposition to develop TGCT.

5.3. Family history in TGCT

Family history increases the risk of TGCT, however, this affects approximately 1 to 3 percent of patients who have one or more first-degree relatives affected by this neoplasia (62). The relative risk is 7 to 10 times higher among siblings of patients with TGCT, while the relative risk of parents or children of affected individuals is 4 to 6 times, which is higher compared to other malignancies, emphasizing that not only the shared environmental factors among kindred, could be important in this kind of cancer, but also genetic susceptibility is an important factor. However, it is difficult to realize such studies because this cancer directly affects male reproduction (62-64), therefore it is complicated to study genetic predisposition, between one generation and the next, and identify precursor lesions *in utero*. However, histological differentiation produced at random, coupled with the influence from environmental exposure of EDs could explain the lack of a distinctive phenotype between cases of familial testicular germ cell tumor (FTGCT) and sporadic cases (1). The phenomenon of genetic anticipation is one in which the hereditary disorders become more severe in future generations, as manifested by decreasing the

age at onset of disease and increased clinical severity of the disease. This pattern has been described in affected families with parents and children with TGCT, noting a significant decrease in the age of diagnosis of TGCT in the younger generation, although it is possible that the observed difference is a secondary device of several statistical bias (68-70). Another possible explanation of what resembles genetic anticipation is that younger men with more aggressive disease may have decreased fertility after treatment, while the grandparents and parents have the disease in older age and a less aggressive histological type, so they may be more likely to reproduce before being diagnosed with cancer, and more likely to remain fertile after treatment. Most young parents have presented nsTGCT at a time when the chances of healing were more complicated by monetary conditions compared to older patients with sTGCT who had better financial means, plus the fact that the treatment was often gonadotoxic and bound to fertility (i.e., "the bias fertility"). With the introduction of cisplatin and more effective treatment regimens, younger patients with TGCT are more likely to survive and remain fertile after treatment. The increased disease severity is an important aspect of anticipation; unfortunately, it does not have enough epidemiological information so far (1). In 2000, the International Testicular Cancer Linkage Consortium (ITCLC) reported the association between a *locus* on Xq27 chromosome and 134 families with confirmed diagnosis of TGCT from the NIH Clinical Center and other participating countries (HLOD equal to 2, 01 and 4.7. in familial cases) (71). Eighty-eight percent of the families in this study had only two affected males, while 53 families (12 percent) had 3 or more cases, with 5 being the highest number of affected family members. It is important to highlight that of the families linked to this *locus,* 73 percent had a history with cryptorchidism, while 26 percent did not (statistical significance equal to 0.0.3), providing evidence of the possible association of *locus* Xq27 and TGCT and also with CO, which is a major risk factor for the development of this neoplasm, so is important to find risk variants for both diseases (71, 72). Later work decided to replicate the 2000 study of 134 families by the same consortium in order to expand and corroborate the association of the Xq27 locus in patients with FTGCT, however, the new linkage analysis in 237 families could not corroborate previous findings, additionally this study failed to identify additional candidate genes for new susceptibility loci with high penetrance (73). Due to the complexity of the etiology of FTGCT and the results obtained, a new hypothesis of genetic susceptibility for this tumor has changed the approach in the investigation of this neoplasm, including the combined effect of multiple low penetrance risk alleles are part of the genetic load of this disease. The collaboration of ITCLC found a high impact prevalence of the microdeletion gr/gr in the genomic region AZFc analyzed because it is the most common genetic cause of infertility and sterility, and a known risk factor for development the TGCT. This deletion was present in 13/431 (3.0. percent) of the FTGCT cases, 28/1376 (2.0. percent) with sporadic TGCT, and 33/2599 (1.3. percent) men without TGCT, obtaining an odds ratio of 3.2. and 2.1., respectively (74). The authors concluded that this Y chromosome microdeletion is a low penetrance variant in susceptibility. It should be noted that there are other genes reported with inconclusive results (*DND1*, *ERS1*, *ERS2*, *RLN*, *LHCCGR*, *DICER1*, *AKT1*, *PTEN*, *PDE11A*). However, it is noteworthy that the three genes (*ERS1/ERS2, RLN*, *LHCCGR*) involved for the first time by Genome-wide association studies (GWAS) were part of a central signaling pathway to the biology of normal germ cells (72). Currently, there are another 30 gene variants published in 18 genomic regions significantly associated, detected by GWAS (75-80). Besides, c-KIT/KITLG is present in other important biological pathways, including fertility, spermatogenesis, testicular differentiation, double-stranded DNA repair, chromosome segregation, chromatin remodeling, and maintenance of telomeres, apoptosis, c-AMP signaling pathway and sexual determination. Supporting the hypothesis of genetic susceptibility, all these risk alleles represent approximately 15 to 22 percent risk in the FTGCT (79, 81). Attempts of association between risk SNPs and other risk factors such as FTGCT, cryptorchidism, inguinal hernia, age at diagnosis, bilateral TGCT, and histology of the tumor had not any success (73). Some of the GWAS studies have agreed on various genes such as *KITLG*, *BAK1*, *DMRT1*, and *TERT* (62), in both FTGCT and sporadic TGCT without distinction, corroborating the genomic similarities (72). Both TGCT and prostate cancer (CaP) could be plausibly seen as hormonal related tumors and as such, several common low risk susceptibility alleles may resemble the pattern of the associations observed in other endocrine disorders, such as diabetes mellitus type 2, which has more than 70 modifying risk variants described (82) (Table 1).

6. MOLECULAR ASPECTS ASSOCIATED WITH TESTICULAR GERM CELL TUMORS

6.1. Single nucleotide polymorphisms (SNPs) and mutations related to the development of TGCT

In recent years, genetic studies have been performed based on the search for biomarkers for TGCT, with the purpose of revolutionizing the field of diagnosis and treatment, documenting in various populations genetic factors involved with TGCT. It is important to note that so far no strong differences in the patterns of alterations or set of single nucleotide polymorphisms (SNPs) and expression patterns between sporadic and familial cases of TGCT have been found (83). They have identified some *loci* of susceptibility in several

Table 1. Some of the genes involved in the TGCT

Gene	*Locus*	Function
	Loci Xq27	
Micro deletion gr/gr of *AZFc*	*Loci* Y cromosome	Implicated on infertility and sterility
DND1	5q31.3.	Inhibiting microRNA-mediated repression
ERS1	6q25.1.	Estrogen receptor
ERS2	6q25.1.	Estrogen receptor
RLN	9p24.1.	Enhancing sperm motility
LHCCGR	2p16.3.	Receptor for luteinizing hormone and choriogonadotropin
DICER1	14q32-13	Ribonuclease
AKT1	14q32.3.3	Serine-threonine protein kinase
PTEN	10q23.3.1	Tumor supressor
PDE11A	2q31.2.	Regulation of cyclic nucleotides concentrations
cKIT/KITLG	4q11-q12/12q21.3.2	Proliferation and differentiation
BAK1	6p21.3.1	Act as a promoter of apoptosis
DMRT1	9p24.3.	Mitotic regulator, germ cell proliferation
TERT	5p15.3.3	Transcription factor
ATF7IP	12p13.1.	Nuclear protein that associates with heterochromatin
SPRY4	5q31.3.	Inhibitor of protein kinase pathway
BCL2	18q21.3.	Apoptosis
CLPTM1	19q13.3.2	Transcription factor
AR	Xq12	Androgen metabolisms
POU5F1	6p21.3.3	Stem cell pluripotency
NANOG	12p13.3.1	Pluripotency, proliferation and renewal
TGFBR3	1p22.1.	Inhibits the secretion of FSH

GWAS studies, such as chromosome 12 associated to *KITLG* and *ATF7IP* genes, and specifically the ligand-receptor c-KIT/KITLG pathway has been implicated in the development of PGC. c- KIT is a proto-oncogene which plays an important role in the physiological regulation of cell proliferation and differentiation, so that changes in conformation or expression may promote transformation to malignancy and tumor progression; c-KIT is the cellular counterpart of the oncogene v-kit Hardy-Zuckerman 4 feline viral sarcoma. In humans, the *c-KIT* gene is located on chromosome region 4q11-q12 with a length of 82.7.87 bp, encoding a tyrosine kinase activity receptor, involved in hematopoiesis, melanogenesis and spermatogenesis (75, 84). This protein functions as a transduction signal system essential for the survival, migration and differentiation of early germ cells. It is strongly expressed in gonocytes in fetal and pediatric stages. Many alterations in this gene, result in the activation of c-KIT in an uncontrolled manner (84). To date, there have been several points likely to have gene mutations, in exons 9, 11, 13 and 17; in the latter, it has been characterized the D816H and D816V alterations associated with development of neoplasia, including performing studies of genotype-phenotype correlation determining that these variants are frequently present in patients with bilateral TGCT and not in patients with the unilateral phenotype. *KITLG*, also known as stem cell factor (SCF), is the other component of this pathway, as described as the ligand of c-KIT. This gene is located on chromosome region 12q21.3.2, which is indispensable to carry out the dimerization and auto phosphorylation of c-KIT, activating the signaling pathway c-KIT-KITLG. Kanetsky *et al*., document variations in the sequence of *KITLG* (rs995030, rs4474514 and rs3782179) giving predisposition to develop TGCT, suggesting that *KITLG* plays a decisive role in pigmentation, also postulating that these variations could give an explanation, in part, why the incidence of TGCT is higher in whites than in African-Americans, besides being associated with infertility in males (83). Two of the genes associated via c-KIT/KITLG are *SPRY4* and *BAK1* genes. *SPRY4* is located on chromosome 5, it has been described as an inhibitor of protein kinase pathway linked to TGCT. On the other side *BAK1* located on chromosome 6, has been associated with neoplasia because the same signaling pathway suppresses it, additionally acting as a promoter of apoptosis in the germ cells by binding the repressor *BCL2*. *TERT-CLPTM1* located on

chromosome 5 is a transcription factor that regulates *ATF7IP*, usually overexpressed in tumors, not being the exception TGCT (72). Another important factor of TGCT development is sex steroids, which play an important role in the risk and progression of malignancy. The classical genomic androgen action is mediated by the androgen receptor (AR); its deficiency leads to an increased number of gonocytes during the fetal period. GCNIS cells express AR unlike normal stem cells in the adult male that not express it. The protein functions as a signal transduction system on nuclear level, regulating gene transcription, which modifies cellular functions. This dimeric receptor is activated by androgens and interacts with the aminoterminal structure or transcriptional activation domain and after activation zinc fingers joins are set in the DNA binding domain, and subsequently the androgen binds by a hinge region to the ligand binding domain, and initiates transcription of genes that regulate androgen response. The *AR* gene is located in the Xq11q12 region where SNPs are associated with the development of TGCT (P390S, A279T, rs12014709) in addition to the repeated glutamine and glycine tracts (85-87). One of the most widely SNPs characterized in the *AR* gene in humans are these tracts located in the trans-activation domain, which is encoded by a polymorphic CAG and GGC repeats respectively, which is inversely correlated to the trans-activation *in vitro*, such that less trans-activation is expected for men with higher number of repeats. Thus, individuals that have been detected with a mutated copy of *AR*, which has long repeated low transactivation and high levels of circulating androgens. It is postulated that the presence of these polymorphic sequences may be involved in increasing the risk of TGCT, since these variants alter receptor function that leads to insensitivity of androgens, causing high concentrations of testosterone and estrogen in circulation. Dao *et al*. conducted a study and found that short repeats are strongly associated with TGCT patients, specifically seminoma (88). Furthermore, Västermark *et al*. showed that rs12014709 variant is associated with an increased risk of neoplasia in spite of being located in the noncoding region of this gene (87). *DMRT1* is a transcription factor, a member of the DNA binding gene family, one of the most important genes of the DMRT group, distinguished to have a DM domain, which is a highly conserved zinc finger across species, with strong implications in testicular development in vertebrates. This is expressed as a pluripotency gene in TGCT, like other genes as *POU5F1* and *NANOG*, whereas in normal adult germ cells these genes are not expressed, so that *DMRT1* expressed in the gonad during maturation of Sertoli cells, participates in the regulation of differentiation there of, in gametogenesis and sexual determination in certain vertebrates (89, 90). Recently, studies with animal models have shown that *DMRT1* participates in modulating, signaling and tumor pluripotency. *DMRT1* is located on chromosome region 9q24.3. and is also one of the genes involved in tumor development, with variants (rs7040024 and rs755383) in this gene having a strong relationship with susceptibility to TGCT (89), besides being associated with gonadoblastoma when this gene is deleted. It is proposed that the epistasis between risk variants and the development of TGCT is characteristic in this kind of tumor, and so far has not been able to detect rare mutations with high penetrance. One of the studies conducted in recent years analyzed gonadal dysgenesia syndrome which consists of TGCT, CO, hypospadias and infertility, where identifying new genes involved in the development of this syndrome (*TGFBR3* and *BMP7*) belong to the TGFB signaling pathway using SNPs microarray. This also corroborates what has been described for c-KIT/KITLG pathway, highlighting the fact gonadal dysgenesis is a real syndrome, even when questioning the relationship between the same pathologies in isolation for the development of TGCT, which is predominantly dependent on the environment, proposing that the pathogenesis of this tumor has a relatively weak genetic component due to the current lifestyle of man. This study also highlights the close relationship found between TGCT and CO by which *TGFBR3* gene has not been associated to date. This encoding TGFBR3 protein is expressed in most endocrine tissues, including the testis; this in turn has been identified in Sertoli and Leydig, both normal cells and in the GCNIS (91).

6.2. Epigenetic mechanisms in the development of TGCT

One of the most relevant fields today, is the study of heritable changes in gene function that occur without a change in DNA sequence, known as epigenetic changes, which are of great importance in the research of the development of several tumors. Different epigenetic mechanisms modulate gene expression, activating or repressing, and can work together or in an isolated manner, such as DNA methylation, histone modification and silencing RNA genes associated by the action of miRNAs(92) (Figure 4).

6.2.1. DNA methylation

Is the most studied epigenetic process and it has been recognized as an important mechanism for TGCT progression (93). In addition, patterns of DNA methylation appear to correlate with histological features of different types of TGCT, identifying hypomethylation in seminoma, GCNIS and gonadoblastoma, while hypermetilation in teratoma, yolk sac tumor, and choriocarcinoma; finally, embryonal carcinoma cases showed an intermediate methylation pattern. Furthermore, the expression pattern of DNMT3b has been widely studied and demonstrated that it could be used as a predictive marker for relapse

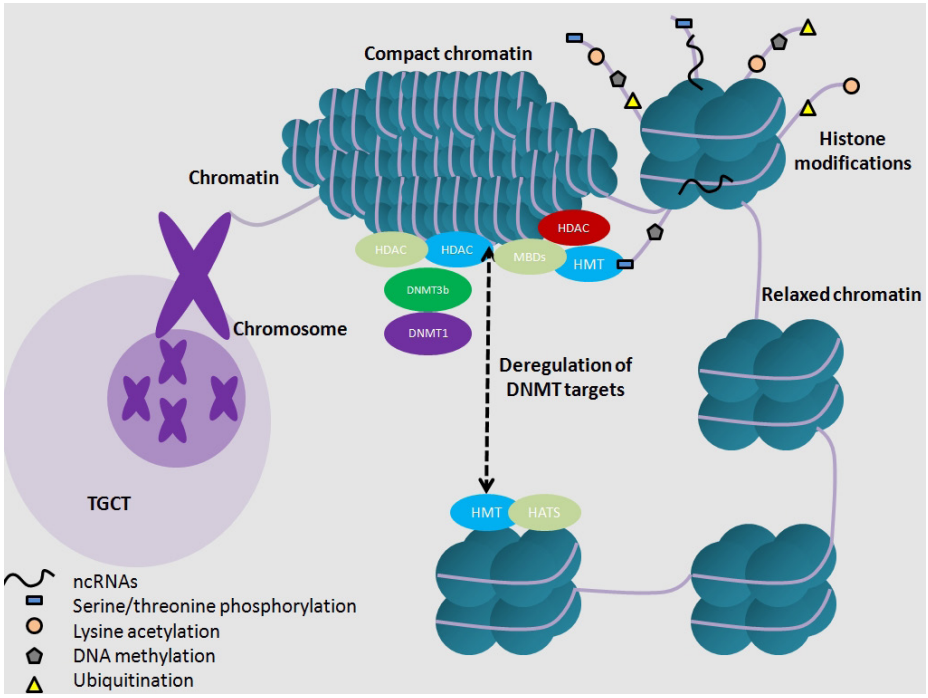

Figure 4. Regulation of gene expression by epigenetic modifications in the development of TGCT, including DNA methylation, histone modification by methylation and acetylation, and finally gene silencing by miRNAs.

of sTGCT in stage I, while overexpressed DNMT3l is found in nsTGCT (94). Two of the most important findings are identifying methylation patterns in *NANOG* and *POU5F1* genes that aim to regulate pluripotency in fetal germ cell tumors and in undifferentiated germ cells, such as the hypomethylation of DNA in the promoter of *NANOG* gene in spermatogonia and hypermethylation in the sperm, that could reflect that cells need this selective mechanism to delete their pluripotent character in order to prevent malignant transformation, in addition identifying methylation CpG islands in the promoter of *NANOG* gene in germ cell tumors. Additionally, the study showed DNA hypomethylation in *POU5F1* gene, in both sTGCT and embryonal carcinoma (95) (Table 2).

Normally in the male germline, there are various epigenetic modifications, until spermatogenesis is fully functional. In fact, it has been shown that through the different stages of spermatogenesis, from the gonocytes to sperm, germ cells experience dynamic epigenetic modifications, highlighting changes in the expression patterns of various enzymes such as DNA methyltransferases (DNMTs), which is mainly expressed in spermatogonia, while the HMTs is expressed mainly in spermatocytes (96). As described throughout this review, environmental factors represent a fundamental factor in the development of TGCT. There are several studies showing that the presence of EDs may impact the epigenome, interestingly, has been shown in the last decade that these effects could also have an impact on subsequent generations by modifying DNA methylation in the germline (97). Such effect was demonstrated for the first time with exposure to vinclozolin, a compound with anti-androgenic activity, identifying differential expression of genes such as DNMT, which is responsible for histone modifications (98). These changes in epigenetic programming through the EDs are of particular interest because it is suspected that they are responsible for increasing the frequency of pathologies related to the TDS. Indeed, it appears that epigenetic tumor cells show a similar pattern to undifferentiated spermatogonial stem cells, GCNIS have a fetal-like chromatin structure (99), and also the precursor of TGCT has already been identified in testis of embryos. Another key mechanism associated with the development of TGCT is the modification on an epigenetic level in aberrant DNA methylation. Several studies have focused on trying to elucidate the epigenetic hallmark of this neoplasia. It has been identified in testicular tissues that methylation on promoters inactivate tumor suppressor genes whereas hypomethylation cases have been reported on a somatic level (100). Since TGCT have not been able to identify rare disorders with high penetrance for development, a trend has emerged that suggests that this neoplasia may be due to aberrant DNA methylation, this being an alternative pathway that could explain the etiology of this disease. In 2010 the global methylation of *LINE-1* was assessed by pyrosequencing in 446 families of 101 multple-case testicular cancer familiar and found a strong positive association between mother-daughters (r= 0.4.8; p= <0.0.01) and father-daughter pairs (r= 0.3.1; p= 0.0.2)

Table 2. Some of epigenetic changes in the TGCT

Type of modification	Gene	Type of cancer
Methylation of DNA	*LINE-1*	FTGCT
Hypo methylation of DNA	*KITLG*	FTGCT
Hyper methylation of DNA	*SPRY4*	FTGCT
Hyper methylation of DNA	*BAK1*	FTGCT
Hyper methylation of DNA	*PDE11A*	FTGCT
Hyper methylation of DNA	*DND1*	FTGCT
Hyper methylation of DNA	*APOLD1*	Sporadic TGCT
Hyper methylation of DNA	*RGAG1*	Sporadic TGCT
Hyper methylation of DNA	*PCDH10*	Sporadic TGCT
Hypo methylation of DNA	*NANOG*	Sporadic TGCT
Hypo methylation of DNA	*POU5F1*	Sporadic TGCT
Methylation of H3-K4 and K9	*LSD1/KDM1*	Sporadic TGCT

suggesting gender-specific inheritance of methylation. When they analyzed cancer status, they observed a strong correlation in LINE-1 methylation leves only among affected father-affected song pairs (r= 0.4.9; p= 0.0.3). These data suggest that the heritability of the methylation pattern of *LINE-1* could be gender specific, i.e. the epigenetic inheritance may be associated with risk of developing TGCT (101). Another follow-up study of the same families investigated the promoter methylation of some candidate genes (*KITLG*, *SPRY4*, *BAK1*, *PDE11A*, *DND1*) that might be associated with a risk in the FTGCT. Comparing the affected family members with those unaffected ones, increasing promoter methylation in *PDE11A*, *SPRY4*, and *BAK1* and decreasing promoter methylation in *KITLG*. were observed. This data suggests that susceptibility in patients with FTGCT may be associated with promoter methylation in genes risk modifiers (102), findings that deserve further follow-up in larger study populations. Other studies focused on sporadic TGCT have searched for methylation profiles in the complete genome, identifying deregulating *APOLD1*, *RGAG1* and *PCDH10* genes by hypermethylation of promoter regions. (103) These studies show that epigenetic reprogramming could be important in the pathogenesis of TGCT, providing a possible interesting relationship between environmental risk factors, particularly with respect to hormonal mechanisms, susceptibility to disease, and development risk of TGCT (104). On the other hand, epigenetic studies of sporadic cases of TGCT have identified that DNA methylation is critical for the development of germ cells, and these enzymatic modifications depend on DNMTs. Specifically during germ cell development in the prenatal stage, establishing *de novo* methylation by expression profile consists initially of DNMT3a and DNMT3l, while DNMT1 and DNMT3b occur after birth in the male. Therefore, these isoforms that increase DNMTs. must be involved in the maintenance of methylation patterns in spermatogonial proliferation (105). The primary role of these epigenetic alterations has been demonstrated in models of carcinogenesis; in fact, it has been shown that DNA methylation is associated with repressed expression of tumor suppressor genes.

6.2.2. Histone methylation

These modifications are carried out in spermatogenesis by several members of the histone methyltranferases family (HMTs), which may mediate the dimethylation or trimethylation in histone 3 (H3) of lysine 9. The G9a, is a HMTase candidate for methylation of H3-K9 in a non-heterochromatic loci. Mice lacking G9a are sterile, which produces apoptosis in germ cells during the pachytene stage (109) (Table 2). In addition, it was shown that G9a was a target of retinoid signaling via a key regulator of germ cell differentiation. It has been suggested that dimethylation of arginine 3, histones H2A and H4 may be a mechanism by which sTGCT and GCNIS maintain their undifferentiated state; while the loss of these histone modifications could be involved in somatic differentiation observed in nsTGCT (107). Added to this, methylation of H3-K4 and K9 histones could be associated with abnormal expression of LSD1/KDM1 in nsTGCT, which suggest that is a histone demethylase that suppress gene expression by converting to monomethylated to dimethylated H3K4 Interestingly, the level of protein LSD1 is very high in cancer cells and tissues, expressing sTGCT *POU5F1* (108).

6.2.3. Acetylation of histone

Acetylation of histones also leads to gene repression and is performed by specific enzymes called histone deacetylases (HDCA). The hyperacetilation of histone H4 during spermatogenesis is one of the

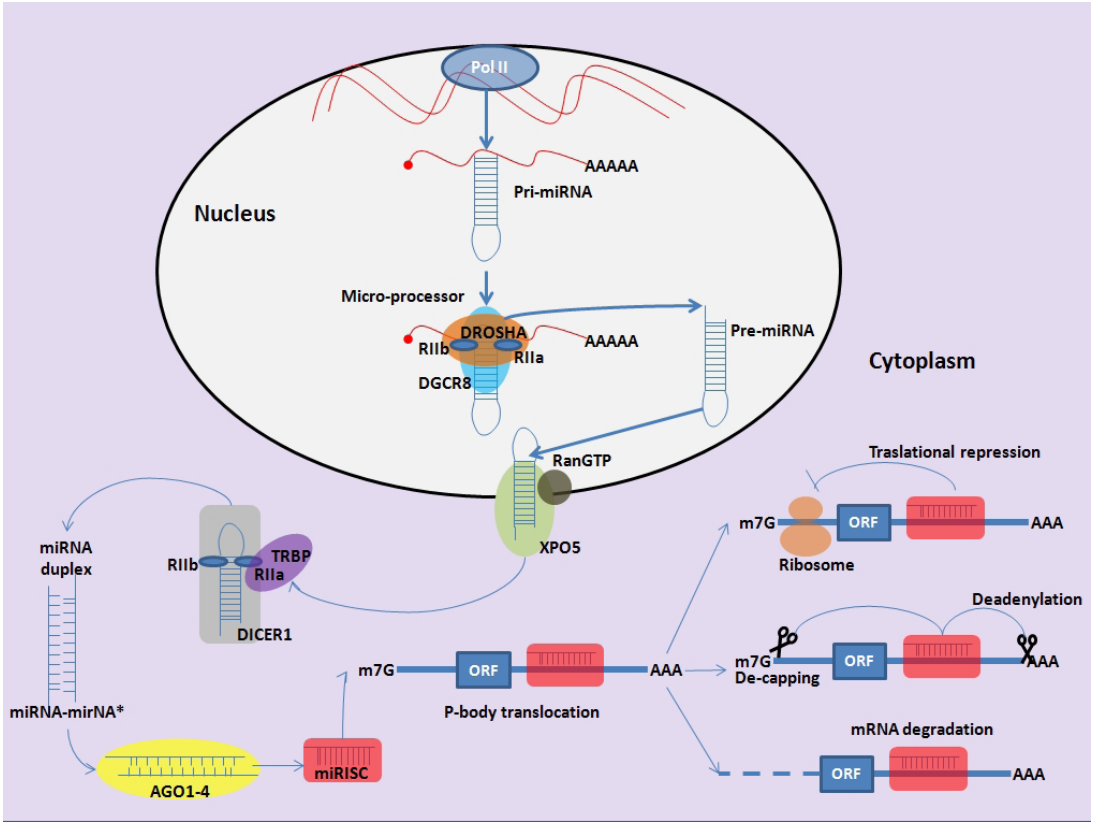

Figure 5. Biogenesis of miRNAs in ncRNA processing, seen from nucleus to cytoplasms, showing three mechanisms of regulation of gene expression; the first by translational repression, the second by mRNA degradation, and the last eliminating adenylation and capping signals.

most important processes in the sperm, as it appears crucial for replacement of histones by protamines way, allowing condensation of the nucleus and compaction of the genetic material, and thus the formation of sperm. Interestingly, over-expression of the class I HDAC 1, 2 and 3 were present in TGCT of choriocarcinoma type, but not in other histological type of testicular neoplasia (111, 112).

6.2.4. Participation of microRNAs (miRNAs) in the development of TGCT

Currently one of the mechanisms studied extensively in the origin of TGCT and timely diagnosis is leaded by microRNAs (miRNAs/miRs), which are a class of small non-coding single RNA's strand. The miRNAs are synthesized and processed in the nucleus, then are exported to the cytoplasm where they bind to complementary mRNA sequences, altering their expression through a silencer complex or inducer RNA (RISC, RNA-induced silencing complex). The process of miRNAs biogenesis starts with primary transcripts or pri-miRNA containing a cap 5' and a tail in 3' of poly-adenine (poly-A) that are processed in the cell nucleus in short fragments of 70 nucleotides of length in a stem-loop form, which are known as pre-miRNA. This processing is performed by a protein complex called microprocessor that comprises the nuclease Drosha and the RNA double helix binding protein, Pasha. Subsequently these pre-miRNAs mature in miRNA, having a length of 19 to 23 nucleotides in the cytoplasm by interaction of Dicer endonuclease, initiating the RISC complex formation (RNA-induced silencing complex) then obtaining double strand miRNAs, which finally will join a specific or non-specific mRNA. It should be noted that non-classical form may have the alternative biogenesis of miRNA stem-loops derived from intronic sequences; in this case are processed by Dicer but not Drosha. Both, the sense strand and the antisense strand of DNA can function as a template to produce miRNAs. The miRNAs negatively control the expression of genes at post-transcriptional level by binding to the 3'UTR region of their target mRNA, and induce their degradation or inhibit translation. By function, miRNAs have been described as fundamental and specific regulators of several cellular biological processes (113-114) (Figure 5).

It is estimated that more than 50% of all cellular transcripts are targets of miRNAs, and each miRNA can potentially regulate hundreds of targets. Some miRNAs show a restricted distribution in tissues and can also be located through various biological fluids (115, 116). It has been shown that there are changes in

the expression profile of miRNAs that may correlate with various pathophysiological conditions, including certain types of malignant tumors. Thus, it has recently been discovered that deregulation of expression of various miRNAs is strongly associated with carcinogenesis as it directly acts over the expression of oncogenes and tumor suppressor genes, thus miRNAs are key in regulating the hallmarks of cancer such as cell growth and survival, apoptosis, angiogenesis and metastasis (117). In the TGCT one of the main lines of research related to miRNAs focuses on determining its function as diagnostic markers, prognostic risk and response to treatment, and the classification type of TGCT. In this sense it has been reported that miR-302-367 and mir-371-373 clusters are expressed in embryonic stem cells as well as in patients with seminoma and not others histotypes. Furthermore, these clusters are able to differentiate tumor tissue from non-tumor tissue (118). Other reports show that miR-17-5p and miR-154 are suppressed in teratomas, while the miR-301 is over-expressed in teratomas, seminomas spermatocytic and yolk sac tumors. Recently, Dieckmann *et al*. have shown increased levels of miR-371, miR-373, miR-376 and miR-302 in patients with TGCT (119, 120). Interestingly, the expression of these miRNAs were higher in patients who developed metastases but surprisingly the levels of miRNAs decreased after orchiectomy, reaching expression levels similar to healthy controls. The authors also reported a significant decrease in expression of this group of miRNAs in patients with metastases treated with chemotherapy. These analyzes had a sensitivity of 98 percent from the 60 percent shown by alpha-fetoprotein (AFP), human chorionic gonadotrophin (hCG) and lactate dehydrogenase (LDH) (119, 120). Other studies have shown that miR-302a increases the sensitivity to cisplatin in cell lines derived from TGCT by increasing levels of apoptosis *in vitro* (121). Serum levels of miR-367-3p, 371a-3p, 372-3p and 373-3p increase significantly in patients with TGCT in locally advanced and metastatic stage compared with healthy individuals and patients with non-malignant testicular diseases. In particular miR-371a-3p allowed the identification of patients with TGCT with a sensitivity of 84.7. percent and a specificity of 99 percent surpassing the AFP and hCG (122). In the carcinogenic process of TGCT, miR-372 and miR-373 have been described as oncogenic regulators to modulate the proliferation and apoptosis. The oncogenic activity of miR-372 and miR-373 is based on cooperation with mutated *RAS*, at least in part by direct regulation *of LATS2* (tumor suppressor kinase 2). This inhibition interferes with cell arrest p53-p21 initiated by increasing CDK-cell proliferation in the testis. Interestingly, it was observed that the miR-302 to 367 is transcriptionally activated by *POU5F1*, *SOX2* and *NANOG*, which are regulators of pluripotency in embryonic pluripotent cells (123). In addition, an inverse association between resistance to treatment with cisplatin and expression of miR-106b and POU5F1 was demonstrated. This seems to be modulated by over-expression of p21 at a cytoplasmic level, suggesting the way of modulation POU5F1/miR-106b/p21 as a new alternative in the treatment of chemoresistant TGCT (124). On the other hand miRNA-21, miRNA-221 and miRNA-222 are overexpressed in seminoma compared to non-seminoma testicular tissue. The miR-21 negatively regulates *ETV5*, a transcription factor that plays an important role in stem cell renewal at the spermatogonia level (125). The miR-221 and miR-222 regulate the expression of *PTEN*, which regulates *AKT* and this event is related to radiosensitivity in sTGCT (126); miR-1297 decreases the expression of *PTEN* to promote proliferation of cells derived from TGCTs (127). Recently it was reported that Meg3, a long non-coding RNA, counteracts the action of miR-1297 on *PTEN*, allowing the tumor suppressor function of *PTEN* and its regulatory action on the PI3K/AKT in TGCT (128, 129). All of this data indicate that miRNAs can be key drivers of the carcinogenic process in TCGT, emphasizing that molecules are expressed differentially in tissues of various subtypes of TGCTs, and serum of patients with TGCTs representing a potential tool diagnostic and therapeutic that should be explored in clinical practice (Table 3; Figure 6).

6.3. Proteomics of TGCT

Testicular environment is considered a particular microenvironment, where spermatogenesis is carried out and is important to note that there are similarities between spermatogenesis and tumorigenesis at testicular level in humans, so it is of great importance to study these two biological processes. Currently one of the problems in identifying protein markers is the low number of associated proteins to TGCT, due to conventional technological limitations necessary fot the characterization of these markers. However, in 2013 Liu *et al*. conducted a massive search for tumor markers by proteomic techniques such as 2D HPLC-MS/MS, which were validated by immunohistochemistry with previous data from an online Human Testis Proteome Database (HTPD) and additionally this information was supported by the use of a GWAS study, using associated SNPs where some differential expression of these protein markers were found. This study characterized six new proteins (DMTR1, PIWIL1, TMPRSS12, TPPP2, PRSS55 and HEMGN) and SNPs in the respective genes, involved in TGCT; the last four proteins still have unknown functions in testis, although they are proposed to play important roles in spermatogenesis and tumorigenesis (130). The Mab-3 doublesex- and Related Transcription Factor 1 (DMTR1) together with the allelic variant rs408200 in this gene have been linked to cases of prostate cancer; this protein is a regulator of mitotic proliferation of germ cells (130, 131), which contains a zinc finger structural motif

Table 3. Some of miRNAs involved in the TGCT

miRNA	Target gene	Expression	Histologycal type of TGCT	Type of cancer	Clinical application
302-367	*PTEN*, *RAB23*, Activated by *POU5F1*, *SOX2* and *NANOG*	Upregulated	TGCT	Both of them	Patients with metastasis (upregulated) Patients with orchiectomy or chemotherapy (downregulated) *(miR-302a cisplatin sensibity)
371-373	*LATS2*	Upregulated	TGCT	Both of them	Patients with metastasis (upregulated) Patients with orchiectomy or chemotherapy (downregulated)
17-5p	*PTEN*, *NOR-1*	Downregulated	Teratoma	Both of them	Unknown
154	*ZEB2*, *CCND2*, *HMGA2*	Downregulated	Teratoma	Both of them	Unknown
301	*TIMP2, PTEN, SOCS6*	Upregulated	Teratoma Spermatocytic seminoma Yolk sac tumor	Both of them	Unknown
106b	*POU5F1/p21*	Upregulated	TGCT	Both of them	Chemoresistant of cisplatin
21	*ETV5*	Upregulated	Seminoma	Both of them	
221-222	*PTEN*	Upregulated	Seminoma	Both of them	Radiosensivity in seminoma
1297	*PTEN*	Downregulated	Seminoma	Both of them	Radiosensivity in seminoma

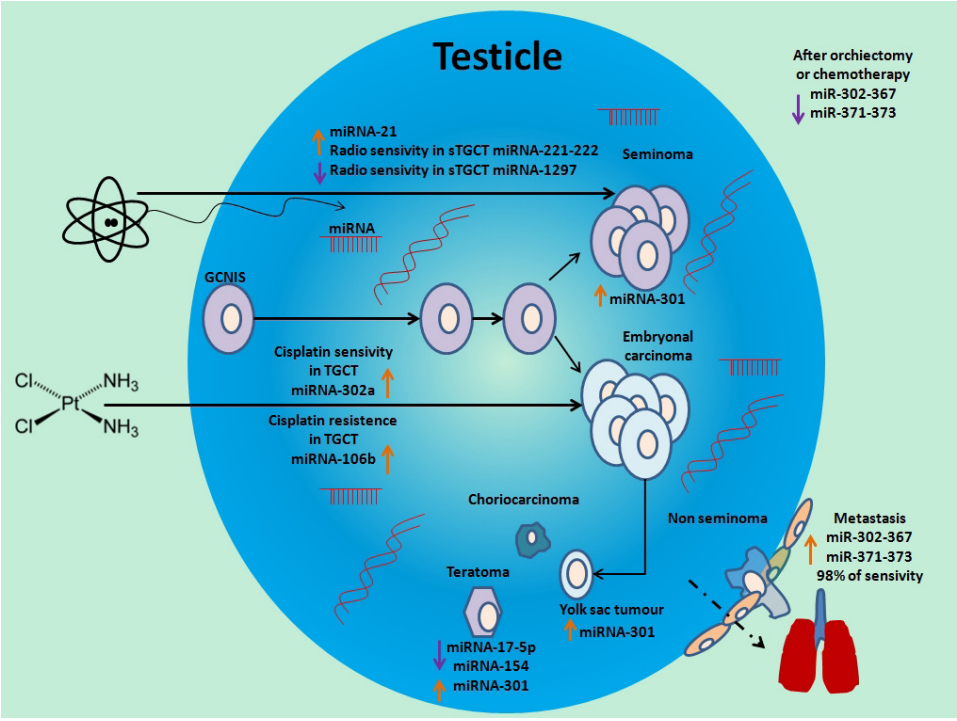

Figure 6. Examples of differential expression of miRNAs, shown in different histological kinds of TGCT; orange arrows represent miRNAs that are over-expressed; purple arrows represent down-expressed miRNAs. Some miRNAs showed are indicative of presence of metastasis or treatment resistance.

that could contribute to cell proliferation of cancer (130, 132). The second protein is Piwi-Like RNA-Mediated Gene silencing 1 (PIWIL1) which together with the allelic variant rs10773777 was also found in prostate cancer cases; this protein is a member of the evolutionarily conserved PIWI subfamily, and could play a role in RNA silencing during translational regulation. Previously, PIWIL1 was also detected in human sarcomas and pancreatic cancers, but was not seen as a particular protein in TGCT. It has been established that PIWIL1 has a role in tumorigenesis, increasing overall DNA methylation (130, 133-134). The third of these proteins is Transmembrane (C-Terminal) Protease, Serine 12 (TMPRSS12) that

Table 4. Proteins related proteomic analysis in TGCT

Protein	Gene *locus*	SNPs associated	Function	Conditions where the protein is expressed
DMRT1	9p24.3.	rs408200	Mitotic regulator germ cell proliferation	TGCT CaP
PIWIL1	12q24.3.3	rs10773777	RNA silencing in translational regulation in addition to has a role in tumorigenesis, increasing overall DNA methylation	TGCT CaP Sarcoma Pancreatic cancer
TMPRSS12	12q13.1.2	rs11169552	Serine-type endopeptidase activity	TGCT Colorectal cancer Spermatids Spermatocytes
TPPP2	14q11.2.	rs1952524	Joints involved in tubulin	TGCT Liver cancer
PRSS55	8p23.1.	rs4404875	Regulates fertility in males	TGCT CaP Ovarian cancer Leydig Sertoli
HEMGN	9q22.3.3	rs10984103 rs1443343 rs7024345 rs907580 rs925487	Regulates proliferation and differentiation	TGCT Thyroid cancer Hematopoyetic cells

had only been associated to colorectal cancer with the variant rs11169552; this protein is expressed in spermatids and spermatocytes (130, 135). The Tubulin Polymerization-Promoting Protein Family Member 2 (TPPP2) and its variant rs1952524 (present in tubulin unions), was associated with liver cancer (130, 136). Another protein is the Protease Serine 55 (PRSS55) along with the variant rs4404875, is associated with cases of prostate and ovarian cancer, and is mainly expressed in Leydig and Sertoli cells in testis and proposes that its primary role is to regulate fertility in males (130, 137). Finally Hemogein (HEMGN) and its 5 variants rs10984103, rs1443343, rs7024345, rs907580 and rs925487 have been associated with thyroid cancer; this protein regulates proliferation and differentiation in hematopoietic cells. Its overexpression blocks megakaryocyte differentiation induced by TPA K562 cell line as well as preventing apoptosis through the activation of nuclear factor-kappa B (NF-kB) (130, 138). In this study it was found that the total of 300 genes encoding proteins expressed in human testis, play an important role in spermatogenesis and fertilization. Only 22.7. percent (68 of 300) were genes encoding related TGCT proteins, of which only 66 proteins were evaluated. It is important to note that only INSL3 was not detected in germ cells, while the remaining 65 were detected in germ cells. Interestingly only 20 of the 65 identified were associated with TGCT, suggesting the existence of other candidate proteins, which have not been identified due to less sensitive methodologies in the area of proteomics. These six proteins mentioned, are important drug targets for personalized therapy in this neoplasm in the future candidates (130) (Table 4).

7. CONCLUSIONS

Comprehension of the pathophysiology of TGCT has to be approached in a multidisciplinary way, although we understand more of this neoplasm, much remains to be done regarding the research, focused on the carcinogenic process, both in the search for diagnostic markers in risk populations, and identification of prognostic markers, and response to treatment. One of the key pieces that will further help to understand the pathophysiology of this cancer is the study of the interactions between the different gene variants and the impact they have on the development of TGCT, without leaving aside the important role the environment plays in this condition, as well as the long-term effects that may trigger exposure to compounds such as EDs and their potential impact at the cellular level and epigenetics. Another line of research of great importance is to find the relationship between the development of TGCT and risk factors, such as CO, so the approach to this problem necessarily have to be done by massive analysis technologies to give ways to predict the development of neoplasia; making it clear that there is much to do about it, given the complexity of this disease.

8. ACKNOWLEDGEMENTS

The authors thank the CONACyT (Project 87101/2008) and the Ministry of Health, Federal Funds (INP-01/2016 project), who made the research possible. Daniel Adrian Landero Huerta was supported by a scholarship (Doctor´s degree) from CONACyT (537316).

9. REFERENCES

1. P. L. Mai, M. Friedlander, K. Tucker, K. A. Phillips, D. Hogg, M. A.Jewett, R. Lohynska, G. Daugaard, S. Richard, C. Bonaïti-Pellié, A. Heidenreich, P. Albers, I. Bodrogi, L. Geczi, E. Olah, P. A. Daly, P. Guilford, S. D. Fossa, K. Heimdal, L. Liubchenko, S. A. Tjulandin, H. Stoll, W. Weber, D. F.Easton, D. Dudakia, R. Huddart, M. R. Stratton, L. Einhorn, L. Korde, K. L. Nathanson, D. T. Bishop, E. A. Rapley, M. H.Greene: The International Testicular Cancer Linkage Consortium: a clinicopathologic descriptive analysis of 461 familial malignant testicular germ cell tumor kindred. Urol Oncol. 28, 492-499 (2010)
DOI: 10.1016/j.urolonc.2008.10.004
PMid:19162511 PMCid:PMC2891341

2. R. H. Jones, P. A. Vasey:Part I: testicular cancer--management of early disease. Lancet Oncol. 4, 730-7 (2003)
DOI: 10.1016/j.urolonc.2008.10.004
DOI: 10.1016/S1470-2045(03)01278-6

3. A. Jemal; F. Bray; M. M. Center.; J. Farlay; E. Ward; D. Forman:Global cancer statistics. CA Cancer JClin.61, 69-90 (2011)
DOI: 10.3322/caac.20107
PMid:21296855

4. J. Ferley, H.R. Shin, D. Forman, C. Mathers, D.M Parkin: Cancer incidence and mortality worldwide. IARC Press Lyon (2008)
PMid:18496893

5. International Agency for Research on Cancer The GLOBOCAN project. Octubre, 18, 2012, http://globocan.iarc.fr/

6. E. Huyghe, T. Matsuda, P. Thonneau: Increasing incidence of testicular cáncer worldwide: a review. J Urol. 170, 5-11 (2003)
PMid:12796635

7. F. Bray, J. Ferlay, S. S. Devesa, K. A. McGlynn, H. Moller: Interpreting the international trends in testicular seminoma and non seminoma incidence. Nat ClinPract Urol.3, 532-543 (2006)
DOI: 10.1038/ncpuro0606
PMid:17031378

8. S. F. Gilbert: Mechanisms for the environmental regulation of gene expression: ecological aspects of animal development. J Biosci. 30, 65-74 (200)

9. L. Ferguson, A. I. Agoulnik: Testicular cancer and Cryptorchidism. Front Endocrinol.4, 1-9 (2013)
DOI: 10.3389/fendo.2013.00032
PMid:23519268 PMCid:PMC3602796

10. H. E. Virtanen, R. Bejerknes, D. Cortes, N Jorgensen, E. Rajpert-De Meyts, A. V. Thorsson, J. Thorup K. M. Main: Cryptorchidism: classification, prevalence and long-term consequences. ActaPaediatr96, 611-616 (2007)

11. A. Kortenkamp: Breast cancer, oestrogens and environmental pollutans: a re-evaluation from a mixture perspective. INt J Androl. 29, 193-198 (2006)
DOI: 10.1111/j.1365-2605.2005.00613.x
PMid:16466540

12. K. M. DeGirolamo, D. Dix, M. Langer, J. Masterson: Intratubular Germ Cell Neoplasia in the Pediatric Population: A Case Report. UBCMJ. 3, 27-32(2012)

13. S. C. Mclver, S. Roman, B. Nixon, K. L. Loveland: The rise of testicular germ cell tumours: the search for causes, risk factors and novel therapeutic targets. F1000 Research. 2, 55 (2013)

14. E. Rajpert-De Meyts: Developmental model for the pathogenesis of testicular carcinoma *in situ*: genetic and environmental aspects. Hum Reprod Update. 12, 303–323 (2006)
DOI: 10.1093/humupd/dmk006
PMid:16540528

15. M. Patterson, D. N. Chan, I. Ha, D. Case, Y. Cui, B. V. Handel, H. K. A. Mikkola, W. E. Lowry: Defining the nature of human pluripotent stem cell progeny. Cell Research. 22,178-193 (2012)
DOI: 10.1038/cr.2011.133
PMid:21844894 PMCid:PMC3351932

16. L. H. Looijenga: Human testicular (non) seminomatous germ cell tumors: the clinical implications of recent pathobiological insights. J Pathol. 218, 146-162 (2009)
DOI: 10.1002/path.2522
PMid:19253916

17. D. Cortes, J. M. Thorup, J. Visfeldt: Cryptorchidism: aspects of fertility and neoplasias. A study including data of 1,335 consecutive boys who underwent testicular biopsy simultaneously with surgery for

cryptorchidism. Horm Res. 55, 21-27 (2001)
DOI: 10.1159/000049959
PMid:11423738

18. A. Ferlin; M. Simonato; L. Bartoloni; G. Rizzo; A. Bettella; T. Dottorini; B. Dallapiccola; C. Foresta: The INSL3-LGR8/GREAT ligand-receptor pair in human cryptorchidism. J Clin Endocrinol Metabol. 88, 4273-4279 (2003)
DOI: 10.1210/jc.2003-030359
PMid:12970298

19. K. Bay, A. M. Andersson: Human testicular insulin-like factor 3: in relation to development, reproductive hormones and andrological disorders. Int J Androl. 34, 97-109 (2011)
DOI: 10.1111/j.1365-2605.2010.01074.x
PMid:20550598

20. N. E. Skakkebaek, E. Rajpert-De Meyts, K. M. Main: Testicular dysgenesis syndrome: an increasingly common developmental disorder with environmental aspects. Hum Reprod.16, 972–978 (2001)
DOI: 10.1093/humrep/16.5.972
PMid:11331648

21. D. M. Berney, L- H. Looijenga, M. Idrees, J. W. Oosterhuis, E. Rajpert-De Meyts, T. M. Ulbright, N. E. Skakkebaek:Germ cell neoplasia *in situ* (GCNIS): evolution of the current nomenclature for testicular pre-invasive germ cell malignancy.Histopathology. 69, 7-10 (2016)
DOI: 10.1111/his.12958
PMid:26918959

22. C. Winter, P. Albers: Testicular germ cell tumors: pathogenesis, diagnosis and treatment: Nat ReviewsEndocrinol. 7, 43-53 (2011)
DOI: 10.1038/nrendo.2010.196
PMid:21116298

23. 23.Y. Sheikine; E. Genega; J. Melamed; P. Lee; V. Reuter; H. Ye: Molecular genetics of testicular germ cell tumours. Am J Cancer Res.2, 153-167 (2012)
PMid:22432056 PMCid:PMC3304567

24. 7. M. De Felici. Origin, Migration, and Proliferation of Human Primordial Germ Cells. In: Oogenesis Chapter 2. Eds Springer-Verlag. London (2013)

25. G. Q. Zhao, D. L. Garbers: Male germ cell specification and differentiation. Dev Cell. 2, 537-547 (2002)
DOI: 10.1016/S1534-5807(02)00173-9

26. C. Chazaud, M. Oulad-Abdelghani, P. Bouillet, D. Decimo, P. Chambon, P. Dolle: AP-2.2., a novel gene related to AP-2, is expressed in the forebrain, limbs and face during mouse embryogenesis. Mech Dev. 54, 83-94 (1996)
DOI: 10.1016/0925-4773(95)00463-7

27. M. A. Helal, H. Mehmet, N. S. B. Thomas, P. M. Cox, D. J. Ralph R. Bajoria, R. Chatterjee: Ontogeny of human fetal testicular apoptosis during first, second, and third trimesters of pregnancy. J Clin Endocrinol Metab. 87, 1189-1193 (2002)
DOI: 10.1210/jcem.87.3.7836
PMid:11889186

28. H. Wartenberg: Differentiation and development of the testes. In The testicle. Ed. New York: Raven Press pp 39-81. (1981)

29. R. M. Vigueras-Villaseñor, P. Montelongo-Solís, M. Chávez-Saldaña, O. Gutiérrez-Pérez, L. Cortés-Trujillo, J. C. Rojas-Castañeda: Development of germ cell neoplasia *in situ* in chinchilla rabbits. Histol Histopathol. 31, 573-84 (2016)

30. R. Paniagua, M. Nistal: Morphological and histometric study of human spermatogonia from birth to the onset of puberty. J. Anat. 139, 535–552 (1984)
PMid:6490534 PMCid:PMC1165067

31. M. Culty: Gonocytes, the forgotten cells of the germ cell lineage. Birth Defects Res C Embryo Today. 87, 1–26 (2009)
DOI: 10.1002/bdrc.20142
PMid:19306346

32. N. Jorgensen, E. Rajpert-De Meyts, N. Graem, A. Giwercman, N. E. Skakkebaek: Expression of immunohistochemical markers for testicular carcinoma *in situ* by normal human fetal germ cells. Lab Invest.72, 223–231 (1995)
PMid:7531795

33. T. L. Gaskell, A. Esnal, L. L. L. Robinson, R. A. Anderson, P. T. K. Saunders:Immunohistochemical profiling of germ cells within the human fetal testicle: identification of three subpopulations. Biol Reprod.71, 2012–2021 (2004)
DOI: 10.1095/biolreprod.104.028381
PMid:15317684

34. F. Honecker, H. Stoop, R. R. de Krijger, Y. F. Chris Lau, C. Bokemeyer, L. H.

Looijenga: Pathobiological implications of the expression of markers of testicular carcinoma *in situ* by fetal germ cells. J Pathol. 203, 849–857 (2004)
DOI: 10.1002/path.1587
PMid:15221945

35. R. M. Vigueras-Villaseñor, L. Cortés-Trujillo, M. Chávez-Saldaña, F. G. Vázquez, D. Carrasco-Daza , O. Cuevas-Alpuche, J. C. Rojas-Castañeda: Analysis of POU5F1, c-Kit, PLAP, AP2γ and SALL4 in gonocytes of patients with cryptorchidism. Acta Histochem. 117, 752-761 (2015)
DOI: 10.1016/j.acthis.2015.08.004
PMid:26315991

36. H. Stoop, F. Honecker, G. J. van de Geijn, A. J. Gillis, M. C. Cool, M. de Boer, K. P. Wolffenbuttel, R. R. de Krijger, B. Summersgill, A. McIntyre, J. Shipley, J. W. Oosterhuis, L. H. Looijenga: Stem cell factor as a novel diagnostic marker for early malignant germ cells. J Patho 16, 43–54 (2008)
DOI: 10.1002/path.2378
PMid:18566970

37. J. W. Oosterhuis, L. H. Looijenga: Testicular ger-cell tumors in broader perspective. Nat. Rev. Cancer. 5, 210-222 (2005)
DOI: 10.1038/nrc1568
PMid:15738984

38. N. E. Skakkebaek: Carcinoma in-situ of the undescended testicle. UrolClin North Am. 9, 377-85 (1982)
PMid:6128821

39. S. Liu, S. W. Wen, Y. Mao, L. Mery, J. Rouleau: Birth cohort effects underlying the increasing testicular cancer incidence in Canada. Can J Public Health. 9, 176–180 (1999)

40. J. Wu, F. William, Jr. Jester, j. M. Orth: Short-type PB-cadherin promotes survival of gonocytes and activates JAK-STAT signalling. Dev Biol. 284, 437-450 (2005)
DOI: 10.1016/j.ydbio.2005.05.042
PMid:16038895

41. Y. Wang, M. Culty: Identification and distribution of a novel platelet-derived growth factor receptor beta variant: effect of retinoic acid and involvement in cell differentiation. Endocrinol.148, 2233-22350 (2007)
DOI: 10.1210/en.2006-1206
PMid:17303670

42. S. Basciani: Platelet-derived growth factor receptor beta-subtype regulates proliferation and migration of gonocytes. Endocrinol. 149, 6226-6235 (2008)
DOI: 10.1210/en.2008-0349
PMid:18687785

43. Q. Zhou, Y. Li, R. Nie, P. Friel, D. Mitchell, R. M. Evanoff, D. Pouchnik, B. Banasik, J. R. McCarrey, C. Small, M. D. Griswold: Expression of stimulated by retinoic acid gene 8 (Stra8) and maturation of murine gonocytes and spermatogonia induced by retinoic acid *in vitro*. Biol Reprod. 78, 537-545 (2008)
DOI: 10.1095/biolreprod.107.064337
PMid:18032419 PMCid:PMC3208258

44. E. Rajpert-De Meyts, N. E. Skakkeebaek: Pathogenesis of testicular carcinoma *in situ* and germ cell cáncer: Still more cuestióntanasnwers. Int. J. Androl. 34, e2-e6 (2011)
DOI: 10.1111/j.1365-2605.2011.01213.x
PMid:21790651

45. P. Chieffi, S. Chieffi: Molecular biomarkers as potential targets for therapeutic strategies in human testicular germ cell tumors: An overview. J Cell Physiol 228, 1641-1646 (2013)
DOI: 10.1002/jcp.24328
PMid:23359388

46. A. M. Soto, C. Sonnenschein: Environmental causes of cáncer: endocrine disruptor as carcinogens. Nat Rev Endocrinol. 6, 363-370 (2010)
DOI: 10.1038/nrendo.2010.87
PMid:20498677 PMCid:PMC3933258

47. C. M. Markey, B. S. Rubin,A. M. Soto, C.Sonnenschein: Endocrine disruptors: from Wingspread to environmental developmental biology. J Steroid BiochemMol Biol. 83, 235-244 (2002)
DOI: 10.1016/S0960-0760(02)00272-8

48. E. Diamanti-Kandarakis, J. P. Bourguignon, L. C. Giudice, R. Hauser, G. S. Prins, A. M. Soto, R. T. Zoeller, A. C. Gore: Endocrine-disrupting chemicals: an endocrine society scientific statement. Endocr Rev.30, 293-342 (2009)
DOI: 10.1210/er.2009-0002
PMid:19502515 PMCid:PMC2726844

49. R. Mittendorf: Teratogen update: carcinogenesis and teratogenesis associated

with exposure to diethylstilbestrol (DES) in utero. Teratology.51, 435-445 (1995)
DOI: 10.1002/tera.1420510609
PMid:7502243

50. T. Kurita, A. A. Mills, G. R. Cunha: Role of p63 in the diethylstilbestrol-induced cervicovaginaladenosis. Development.131, 1639-1649 (2004)
DOI: 10.1242/dev.01038
PMid:14998922

51. N. E. SkakkebaekA Brief Review of the Link between Environment and Male Reproductive Health: Lessons from Studies of Testicular Germ Cell Cancer. Horm Res Pediatr. (2016)

52. M. Durando, L. Kass, J. Piva, C. Sonnenschein, A. M. Soto, E. H. Luque, M. Muñoz-de-Toro: Prenatal bisphenol a exposure induces preneoplasic lesion in the mammary gland in Winstar rats. Environ Health Perspect. 115, 80-86 (2007)
DOI: 10.1289/ehp.9282
PMid:17366824 PMCid:PMC1797838

53. A. M. Calafat, X. Ye, L. Y. Wong, J. A. Reidy, L. L. Needham: Exposure of the U. S. population to bisphenol A and 4-tertiary-octylphenol: 2003-2004. Environ Health Perspect.116, 39-44 (2008)
DOI: 10.1289/ehp.10753
PMid:18197297 PMCid:PMC2199288

54. Y. Suzuki, J. Yoshinaga, Y. Mizumoto, S. Serizawa, H. Shiraishi: Foetal exposure to phthalate esters and anogenital distance in male newborns.*Int. J. Androl.* 35, 236-244 (2012).
DOI: 10.1111/j.1365-2605.2011.01190.x
PMid:21696396

55. L. H. Looijenga., T. V. Agthoven, K. Biermann: Development of malignant germ cells – the genvironmentalhyphotesis. Int J Dev Biol.57, 241-253 (2013)
DOI: 10.1387/ijdb.130026ll
PMid:23784835

56. C. Foresta, D. Zuccarello, A. Garolla, A. Ferlin: Role of Hormones, Genes, and Environment in Human. Endocr Rev.29, 560–580 (2008)
DOI: 10.1210/er.2007-0042
PMid:18436703

57. J.S. Elder: The undescended testicle: hormonal and surgical management. SurgClin North Am. 68, 983-1005 (1988)
DOI: 10.1016/S0039-6109(16)44633-5

58. J. M. Hutson, M. Terada, B. Zhou, M. P. Williams: Normal testicular descent and the aetiology of cryptorchidism. AdvAnatEmbryol Cell Biol. 132, 1-56 (1995)
DOI: 10.1007/978-3-642-61026-4_1

59. R. Ivell, S. Hartung: The molecular basis of cryptorchidism. Mol. Hum. Reprod. 9, 175-81 (2003)
DOI: 10.1093/molehr/gag025
PMid:12651898

60. F. Massart, G. Saggese: Morphogenetic Targets and Genetics of Undescended Testicle. Sex Develop.4, 326-335 (2010)
DOI: 10.1159/000321006
PMid:20980787

61. R. Ashley; J. S. Barthold; T. F.Kolon: Cryptorchidism Pathogenesis, Diagnosis, Treatment and Prognosis. UrolClin N Am. 37, 183-193 (2010)
DOI: 10.1016/j.ucl.2010.03.002
PMid:20569797

62. C. Kratz; P. Mai, M. H. Greene: Familial testicular germ cell tumours. Best Practice and Research Clinical Endocrinol and Metab.24, 503-513 (2010)
DOI: 10.1016/j.beem.2010.01.005
PMid:20833340 PMCid:PMC2939736

63. M. Greene,C. Kratz,P. Mai,C. Mueller,J. Peters,G. Bratslavsky,A. Ling,P. Choyke,A. Premkumar,J. Bracci; R. Watkins; M. McMaster; L. Korde: Familial testicular germ cell tumors in adults: summary of genetic risk factors and clinical phenotype. EndocrRelat Cancer.17, R109-R121 (2010)
DOI: 10.1677/ERC-09-0254
PMid:20228134 PMCid:PMC3101798

64. M. Gerner, M. Turner,P. Ghadirian,D. Krewski: Epidemiology of testicular cancer: an overview. Int JCancer.116, 331-339 (2005)
DOI: 10.1002/ijc.21032
PMid:15818625

65. M. Adham, A. I. Agoulnik: Insulin-like 3 signalling in testicular descent. Int. J. Androl. 27257–27265 (2004)
DOI: 10.1111/j.1365-2605.2004.00481.x

66. C. Vanparys, T. L. M. Hectors, R. Blust, W. De Coen: Mechanistic profiling of the

cAMP-dependent steroidogenic pathway in the H295R endocrine disrupter screening system: new endpoints for toxicity testing. Toxicol. Lett. 208, 174–184 (2012)
DOI: 10.1016/j.toxlet.2011.10.014

67. Chen,S. Wu,S. Wen,L. Shen,J. Peng,C. Yan,X. Cao,Y. Zhou,C. Long,T. Lin,D. He,Y. Hua,G. Wei: The Mechanism of Environmental Endocrine Disruptors (DEHP) Induces Epigenetic Transgenerational Inheritance of Cryptorchidism. Plos One. 2, 10 (2015)

68. D. B. Fuller, H. P. Plenk: Malignant testicular germ cell tumors in a father and two sons. Case report and literature review. Cancer. 58, 955–958 (1986)
DOI: 10.1002/1097-0142(19860815)58:4<955::AID-CNCR2820580425>3.0.CO;2-D

69. P. K. Mills, G. R. Newell, D. E. Johnson: Familial patterns of testicular cancer. Urology. 24, 1–7 (1984)
DOI: 10.1016/0090-4295(84)90375-3

70. S. Han, R. E. Peschel: Father-son testicular tumors. Evidence for genetic anticipation? : A case report and review of the literature. Cancer. 88, 2319–2325(2000)
DOI: 10.1002/(SICI)1097-0142(20000515)88:10<2319::AID-CNCR16>3.3.CO;2-F
DOI: 10.1002/(SICI)1097-0142(20000515)88:10<2319::AID-CNCR16>3.0.CO;2-O

71. E. A. Rapley, G. P. Crockford, D. Teare, P. Biggs, S. Seal, R. Barfoot, S. Edwards, R. Hamoudi, K. Heimdal, S.D. Fossa, K. Tucker, J. Donald, F. Collins, M. Friedlander, D. Hogg, P. Goss, A. Heidenreich, W. Ormiston, P. A. Daly, D. Forman, T. D. Oliver, M. Leahy, R. Huddart, C.S. Cooper, J. G. Bodmer, D. F. Easton, M. R. Stratton,D. T. Bishop: Localization to Xq27 of a susceptibility gene for testicular germ-cell tumours. Nat Genet. 24, 197–200 (2000)
DOI: 10.1038/72877
PMid:10655070

72. M. H. Greene,P. L. Mia, J. T. Loud, A. Pathak, A. Peters, L. Mirabello, M. L. McMaster, P. Rosenberg,D. R. Stewart: Familial testicular germ cell tumors (FTGCT) – overview of a multidisciplinary etiologic study. Andrology. 3, 47-58 (2015)
DOI: 10.1111/andr.294
PMid:25303766

73. G. P. Crockford, R. Linger, S. Hockley, D. Dudakia, I. Johnson, R. Huddart, K. Tucker, M. Friedlander, K. A. Phillips, D. Hogg, M. A. Jewett, R. Lohynska, G. Daugaard, S. Richard, A. Chompret, C. Bonaiti-Pellie, A. Heidenreich, P. Albers, E. Olah, L. Geczi, I. Bodrogi, W.J. Ormiston, P.A. Daly, P. Guilford, S.D. Fossa, K. Heimdal, S.A. Tjulandin, L. Liubchenko, H. Stoll, W. Weber, D. Forman, T. Oliver, L. Einhorn, M. McMaster, J. Kramer, M. H. Greene, B. L. Weber, K. L. Nathanson, V. Cortessis, D. F. Easton, D. T. Bishop, M. R. Stratton, E. A. Rapley: Genome-wide linkage screen for testicular germ cell tumour susceptibility loci. Hum Mol Genet. 15, 443–451 (2006)
DOI: 10.1093/hmg/ddi459
PMid:16407372

74. K. L. Nathanson, P. A. Kanetsky, R. Hawes, D. J. Vaughn, R. Letrero, K. Tucker, M. Friedlander,K. A. Phillips, D. Hogg, M. A. Jewett, R. Lohynska, G. Daugaard, S. Richard, A. Chompret, C. Bonaiti-Pellie, A. Heidenreich, E. Olah, L. Geczi, I. Bodrogi, W.J. Ormiston, P.A. Daly, J. W. Oosterhuis, A. J. Gillis, L. H. Looijenga, P. Guilford, S. D. Fossa, K. Heimdal, S. A. Tjulandin, L. Liubchenko, H. Stoll, W. Weber, M. Rudd, R. Huddart, G. P. Crockford, D. Forman, D. T. Oliver, L. Einhorn, B. L. Weber, J. Kramer, M. McMaster, M. H. Greene, M. Pike, V. Cortessis, C. Chen, S. M. Schwartz, D. T. Bishop, D. F. Easton, M. R. Stratton, E. A. Rapley: The Y deletion gr/gr and susceptibility to testicular germ cell tumor. Am J Hum Genet. 77, 1034–1043 (2005)
DOI: 10.1086/498455
PMid:16380914 PMCid:PMC1285161

75. P. A. Kanetsky, N. Mitra, S. Vardhanabhuti, M. Li, D. J. Vaughn, R. Letrero, S. L. Ciosek, D. R. Doody, L. M. Smith, J. Weaver, A. Albano, C. Chen, J. R. Starr, D. J. Rader, A. K. Godwin, M. P. Reilly, H. Hakonarson, S. M. Schwartz, K. L.Nathanson: Common variation in KITLG and at 5q31.3. predisposes to testicular germ cell cancer. Nat Genet. 41, 811–815(2009)
DOI: 10.1038/ng.393
PMid:19483682 PMCid:PMC2865677

76. P. A. Kanetsky, N. Mitra, S. Vardhanabhuti, D. J. Vaughn, M. Li, S. L. Ciosek, R. Letrero, K. D'Andrea, M. Vaddi, D. R. Doody, J. Weaver, C. Chen, J. R. Starr, H. Hakonarson, D. J. Rader, A. K. Godwin, M. P. Reilly, S. M. Schwartz, K. L.Nathanson:

A second independent locus within DMRT1 is associated with testicular germ cell tumor susceptibility. Hum Mol Genet. 20, 3109–3117 (2011)
DOI: 10.1093/hmg/ddr207
PMid:21551455 PMCid:PMC3131044

77. C. Turnbull, E. A. Rapley, S. Seal, D. Pernet, A. Renwick, D. Hughes, M. Ricketts, R. Linger, J. Nsengimana, P. Deloukas, R. A. Huddart, D. T. Bishop, D. F. Easton, M. R. Stratton, N. Rahman, UK Testicular Cancer Collaboration: Variants near DMRT1, TERT and ATF7IP are associated with testicular germ cell cancer. Nat Genet. 42, 604–607 (2010)
DOI: 10.1038/ng.607
PMid:20543847 PMCid:PMC3773909

78. C. C. Chung, P. A. Kanetsky, Z. Wang, M. A. Hildebrandt, R. Koster, R. I. Skotheim, C. P. Kratz, C. Turnbull, V. K. Cortessis, A. C. Bakken, D. T. Bishop, M. B. Cook, R. L. Erickson, S. D. Fossa, K. B. Jacobs, L. A. Korde, A. Ragnhild, R. A. Lothe, J. T. Loud, N .Rahman, M. V. Rubertone, E.C. Skinner, D. C. Thomas, X. Wu, M. Yeager, F. R. Schumacher, M. H. Greene, S. M. Schwartz, K. A. McGlynn, S. J. Chanock, K. L.Nathanson: Meta-analysis identifies four new loci for testicular germ cell tumor. Nat Genet. 45, 680–685 (2013)
DOI: 10.1038/ng.2634
PMid:23666239 PMCid:PMC3723930

79. E. Ruark, S. Seal, H. McDonald, F. Zhang, A. Elliot, K. Lau, E. Perdeaux, E. Rapley, R. Eeles, J. Peto, Z. Kote-Jarai, K. Muir, J. Nsengimana, J. Shipley, UK Testicular Cancer Collaboration (UKTCC), D. T. Bishop, M. R. Stratton, D. F. Easton, R. A. Huddart, N. Rahman,C.Turnbull: Identification of nine new susceptibility loci for testicular cancer, including variants near DAZL and PRDM14. Nat Genet. 45, 686–689 (2013)
DOI: 10.1038/ng.2635
PMid:23666240 PMCid:PMC3680037

80. F. R. Schumacher, Z. Wang, R.I. Skotheim, R. Koster, C. C. Chung, M. A. Hildebrandt, C. P. Kratz, A. C. Bakken, D. T. Bishop, M. B. Cook, R. L. Erickson, S. D. Fossa, M. H. Greene, K. B. Jacobs, P. A. Kanetsky, L. N. Kolonel, J. T. Loud, L. A. Korde, L. Le Marchand, J. P. Lewinger, R. A. Lothe, M. C. Pike, N. Rahman, M. V. Rubertone, S. M. Schwartz, K. D. Siegmund, E. C. Skinner, C. Turnbull, D. J. Van Den Berg, X. Wu, M. Yeager, K. L. Nathanson, S. J. Chanock, V. K. Cortessis, K. A. McGlynn: Testicular germ cell tumor susceptibility associated with the UCK2 locus on chromosome 1q23. Hum Mol Genet. 22, 2748–2753 (2013)
DOI: 10.1093/hmg/ddt109
PMid:23462292 PMCid:PMC3674801

81. R. Koster, N. Mitra, K. D'Andrea, S. Vardhanabhuti, C. Chen, Z. Wang, D. R. Doody, R. L. Erickson, S. M. Schwartz, D. J. Vaughan, N. Rahman, M. H. Greene, K. A McGlynn, C. Turnbull, S. J. Chanock, K. L. Nathanson,P. A. Kanetsky: Pathway-based analysis of GWAS data identifies association of sex determination genes with susceptibility to testicular germ cell tumors. Hum Mol Genet. 23, 6061-6068(2014)
DOI: 10.1093/hmg/ddu305
PMid:24943593 PMCid:PMC4204765

82. H. J. Ng, A. L.Gloyn: Bridging the gap between genetic associations and molecular mechanisms for type 2 diabetes. CurrDiab Rep. 13, 778–785 (2013)
DOI: 10.1007/s11892-013-0429-1

83. M. Cook: A systematic review and metaanalysis of perinatal variables in relation to the risk of testicular cancer-experiences of son. Int J Epidemiol. 39, 1605-1608 (2010)
DOI: 10.1093/ije/dyq120
PMid:20660640 PMCid:PMC2992627

84. E. Rapley,C. Turnbull,A. Al Olama,E. Dermitzakis,R. Linger,R. Huddart,A. Renwick,D. Hughes,S. Hines,S. Seal,J. Morrison,J. Nsengimana, P.Deloukas,N. Rahman,D. Bishop,D. Easton,M. Stratton: A genome-wide association study of testicular germ cell tumor. Nat Genet.41, 807-810(2009)
DOI: 10.1038/ng.394
PMid:19483681 PMCid:PMC2871592

85. C. Turnbull, N. Rahman: Genome-wide association studies provide new insights into the genetic basis of testicular germ-cell tumour. Int J Androl. 34(4 Pt 2), e86-96-e96-7 (2011)

86. E. P. Gelmann: Molecular biology of the androgen receptor. J Clin Onc. 20, 3001-3015(2002)
DOI: 10.1200/JCO.2002.10.018

87. A. Västermark, Y. Giwercman, O. Hagströmer, E. De-Meyts, J. Eberhard, O. Stahl, G. Cedermark, H. Rastkhani, G. Daugaard, S. Arver, A.Giwercman: Polymorphic variation in the androgen receptor gene: Assosiation

with risk of testicular germ cell cáncer and metastatic disease. Europ J Cancer. 47, 413-419 (2011)
DOI: 10.1016/j.ejca.2010.08.017
PMid:20880698

88. C. Davis,K. Siegmund,D. Vandenverg,E. Skinner,G. Coetzee,D. Thomas,M. Pike,V. Cortessis:Heterogenous effect of androgen receptor CAG tract length on testicular germ cell tumor risk: shorter repeats associated with seminoma but not other histologic types. Carcinogenesis.32, 1238-1243 (2011)
DOI: 10.1093/carcin/bgr104
PMid:21642359 PMCid:PMC3202310

89. M. Pesce, X. Wang, D. J. Wolgemuth, H. Schöler: Differential expression of the Oct-4 transcription factor during mouse germ cell differentiation. Mech Dev.71, 89-98 (1998)
DOI: 10.1016/S0925-4773(98)00002-1

90. D. Zarkower: DMRT Genes in Vertebrate Gametogenesis. Current Topics in Develop Biol.102. 327-356 (2013)
DOI: 10.1016/B978-0-12-416024-8.00012-X
PMid:23287039

91. M. D. Dalgaard, N. Weinhold, D. Edsgard, J. D. Silver, T. H. Pers, J. E. Neilsen, N. Jorgensen, A. Juul, T. A. Gerd, A. Giwercman, Y. L. Giercman, G. Cohn-Cedermark, H. E. Virtanen, J. Toppari, G. Daugaard, T. S. Jensen, S. Brunak, E. Rajpert-De Meyts, N. E. Skakketbaek, H. Leffers.R. A. Gupta: A genome-wide association study of men with symptoms of testicular dysgenesis syndrome and its network biology interpretation. J Med Genet. 49, 58-65 (2012).
DOI: 10.1136/jmedgenet-2011-100174
PMid:22140272 PMCid:PMC3284313

92. M. Meaney, A. Ferguson: Epigenetic regulation of the neural transcriptome: the meaning of the marks. Nat. Neurosci.13 (11), 1313-1318 (2010).
DOI: 10.1038/nn1110-1313
PMid:20975754

93. J. Ellinger, P. Albers, F. G. Perabo, S. C. Müller, A. von Ruecker, P. J. Bastian: CpG island hypermethylation of cell-free circulating serum DNA in patients with testicular cancer.*J. Urol.*182, 324–329(2009)
DOI: 10.1016/j.juro.2009.02.106
PMid:19447423

94. A. Vega,M. Baptissart, F. Caira, F. Brugnon, J. M. A. Lobaccaro, D. H. Volle: Epigenetic: a molecular link between testicular cancer and environmental exposures. Front Endocrinol. 3, (2012)
DOI: 10.3389/fendo.2012.00150

95. D. Nettersheim, K. Biermann, A. J. M. Gillis, K. Steger, L. H. J. Looijenga, H. Schorle: NANOG promoter methylation and expression correlation during normal and malignant human germ cell development. *Epigenetics.*6, 114-122 (2011)
DOI: 10.4161/epi.6.1.13433
PMid:20930529 PMCid:PMC3052918

96. M. Godmann, R. Lambrot, S. Kimmins: The dynamic epigenetic program in male germ cells: its role in spermatogenesis, testicle cancer, and its response to the environment. *Microsc. Res. Tech.*72, 603–619 (2009)
DOI: 10.1002/jemt.20715
PMid:19319879

97. E. J. Schoevers, R. R. Santos, B. Colenbrander, J. Fink-Gremmels, B. A. J. Roelen: Transgenerational toxicity of Zearalenone in pigs.*Reprod. Toxicol.*34, 110-119 (2012)
DOI: 10.1016/j.reprotox.2012.03.004
PMid:22484360

98. M. D. Anway, A. S. Cupp, M. Uzumcu, M. K. Skinner: Epigenetic transgenerational actions of endocrine disruptors and male fertility.*Science.*308, 1466-1469 (2005)
DOI: 10.1126/science.1108190
PMid:15933200

99. K. Almstrup, J. E. Nielsen, O. Mlynarska, M. T. Jansen, A. Jørgensen, N. E. Skakkebæk: Carcinoma *in situ* testicle displays permissive chromatin modifications similar to immature foetal germ cells.*Br. J. Cancer.*103, 1269-1276 (2010)
DOI: 10.1038/sj.bjc.6605880
PMid:20823885 PMCid:PMC2967056

100. G. Lind, R. Skotheim, R. Lothe: The epigenome of testicular germ cell tumors. APMIS.115, 1147-1160 (2007)
DOI: 10.1111/j.1600-0463.2007.apm_660.xml.x
PMid:18042148

101. L. Mirabello, S. A.Savage, L. Korde, S. M. Gadalla, M. H. Greene: LINE-1 methylation is inherited in familial testicular cáncer kindred. BMC Med Genet. 11, 77 (2010)
DOI: 10.1186/1471-2350-11-77

102. L. Mirabello, S. A. Savage, M. H. Greene: Promoter methylation of candidate genes

associated with familial testicular cancer. Int J MolEpidemiol Genet. 3, 213-227 (2012)

103. C. C. Chung, P. A. Kanetsky, Z. Wang, M. A. T. Hildebrandt, R. Koster, R. I. Skotheim, C. P. Kratz, C. Turnbull, V. K. Cortessis, A. C. Bakken, D. T. Bishop, M. B. Cook, R. L. Erickson, S. D. Fossa, K. B. Jacobs, L. A. Korde, S. M. Kraggerud, R. A. Lothe, J. T. Loud, N. Rahman, E. C. Skinner, D. C. Thomas, X. Wu, M. Yeager, F. R. Schumacher, M. H. Greene, S. M. Schwartz, K. A. McGlynn, S. J. Chanock, K. L. Nathanson: Meta-analysis identofies four new loci for testicular germ cell tumor. Nat Genet. 45, 680-685 (2013)
DOI: 10.1038/ng.2634

104. C. L. Walker: Epigenomic re-programing of the developing reproductive tract and disease susceptibility in adulthood. Birth Defects A Clin Mol Teratol.91, 666-671 (2011)

105. M. Godmann, R. Lambrot, S. Kimmins: The dynamic epigenetic program in male germ cells: its role in spermatogenesis, testicle cancer, and its response to the environment. *Microsc. Res. Tech.*72, 603–619 (2009)
DOI: 10.1002/jemt.20715

106. J. Ellinger, P. Albers, F. G. Perabo, S. C. Müller, A. von Ruecker, P. J. Bastian: CpG island hypermethylation of cell-free circulating serum DNA in patients with testicular cancer.*J. Urol.*182, 324–329(2009)
DOI: 10.1016/j.juro.2009.02.106

107. A. Vega,M. Baptissart, F. Caira, F. Brugnon, J. M. A. Lobaccaro, D. H. Volle: Epigenetic: a molecular link between testicular cancer and environmental exposures. Front Endocrinol. 3, (2012)
DOI: 10.3389/fendo.2012.00150

108. D. Nettersheim, K. Biermann, A. J. M. Gillis, K. Steger, L. H. J. Looijenga, H. Schorle: NANOG promoter methylation and expression correlation during normal and malignant human germ cell development. *Epigenetics.*6, 114-122 (2011)
DOI: 10.4161/epi.6.1.13433

109. M. Tachibana, M. Nozaki, N. Takeda, Y. Shinkai: Functional dynamics of H3K9 methylation during meiotic prophase progression.*EMBO J.*26, 3346–3359 (2007)
DOI: 10.1038/sj.emboj.7601767

110. J. Wang, F. Lu, Q. Ren, H. Sun, Z. Xu, R. Lan: Novel histone demethylase LSD1 inhibitors selectively target cancer cells with pluripotent stem cell properties.*Cancer Res.*71, 7238-7249 (2011)
DOI: 10.1158/0008-5472.CAN-11-0896

111. B. E. Gryder, Q. H. Sodji, A. K. Oyelere: Targeted cancer therapy: giving histone deacetylase inhibitors all they need to succeed.*Future Med. Chem.*4, 505-524 (2012)
DOI: 10.4155/fmc.12.3

112. F. R. Fritzsche, A. Hasler, P. K. Bode, H. Adams, H. H. Seifert, T. Sulser:Expression of histone deacetylases 1, 2 and 3 in histological subtypes of testicular germ cell tumours.*Histol. Histopathol.*26, 1555–1561 (2011)

113. X. Ding, J. Weiler, H. Grosshans: Regulatin the regulator: mechanisms controlling the maturation of microRNAs. Trends in Biotechnol. 27, 27-32 (2008)
DOI: 10.1016/j.tibtech.2008.09.006

114. J. Yang, E. Lai: Alternative miRNA biogenesis pathways and the interpretation of core miRNA pathway mutants. Mol cell.43, 892-903 (2011)
DOI: 10.1016/j.molcel.2011.07.024

115. X. Chen, H. Liang, J. Zhang, K. Zen, C. Zhang: Secreted microRNAs: a new form of intracellular communication. Trends in Cell Biol.1-8 (2011)

116. J. Weber,D. Baxter, S. Zhang, D. Huang,K. Huang,M. Lee,D. Galas,K. Wang: The MicroRNA spectrum un 12 body fluids. Clin Chem.56, 1733-1741 (2010)
DOI: 10.1373/clinchem.2010.147405

117. Y. Peng, C. M. Croce: The role of MicroRNAs in human cancer. Signal Transduction and Targeted Therapy. (2016)

118. A. J. Gillis, H. J. Stoop, R. Hermus: High-throughput microRNAome analysis in human germ cell tumours. J Phatol. 213, 319-128 (2007)
DOI: 10.1002/path.2230

119. K. P. Dieckmann, M. Spiekermann, T. Balks, I. Flor, T. Löning, J. Bullerdiek, G. Belge: MicroRNAs miR-371-3 in serum as diagnostic tools in the management of

testicular germ cell tumours. Br J Cancer. 107, 1754-1760 (2012)
DOI: 10.1038/bjc.2012.469

120. M. Spiekermann, G. Belge, N. Winter, R. Ikogho, T. Balks, J. Bullerdiek, K. P. Dieckmann: MicroRNA miR-371a-3p in serum of patients with germ cell tumours: evaluations for establishing a serum biomarker. Andrology. 3, 78-84 (2015)
DOI: 10.1111/j.2047-2927.2014.00269.x

121. L. Liu, J. Lian, H. Zhang, H. Tian, M. Liang, M. Yin, F. Sun: MicroRNA-302a sensitizes testicular embryonal carcinoma cells to cisplatin-induced cell death. J Cell Physiol. 228, 2294-304 (2013)
DOI: 10.1002/jcp.24394

122. I. Syring, J. Bartels, S. Holdenrieder, G. Kristiansen, S. C. Müller, J. Ellinger: Circulating serum miRNA (miR-367-3p, miR-371a-3p, miR-372-3p and miR-373-3p) as biomarkers in patients with testicular germ cell cancer. J Urol. 193, 331-337 (2015)
DOI: 10.1016/j.juro.2014.07.010

123. A. Barroso de Jesus, G. Lucena-Aguilar, P. Mendez: The miR-302-367 cluster as a potential stemess regulator in ESCs. Cell Cycle. 8, 394-398 (2009)
DOI: 10.4161/cc.8.3.7554

124. Koster, A. di Pietro, H. Timmer-Bosscha, J. H. Gibcus, A. van den Berg, A. J. Suurmeijer: Cytoplasmic p21 expression levels determine cisplatin resistance in human testicular cancer.*J. Clin. Invest.* 120, 3594–3605 (2010).
DOI: 10.1172/JCI41939

125. Z. Niu, S. M. Goodyear, S. Rao, X. Wu, J. W. Tobias, M. R. Avarbock, R. L. Brinster: MicroRNA-21 regulates the self-renewal of mouse spermatogonial stem cells. Proc Natl AcadSci U S A. 108, 12740-12745 (2011)
DOI: 10.1073/pnas.1109987108

126. Z. Bing, S. R. Master, J. W. Tobias, D. A. Baldwin, X. W. Xu, J. E.Tomaszewski: MicroRNA expression profiles of seminoma from paraffin-embedded formalin-fixed tissue. Virchows Archiv. 461, 663-668 (2012)
DOI: 10.1007/s00428-012-1325-9

127. Z. Bing, R. Master, D. Tobias,D. Baldwin,X. Xu,D. Tomaszewsky: MicroRNA expression profiles of seminoma from paraffin-embedded formalin-fixed tissue. Virchows Arch.461, 663-668 (2012)
DOI: 10.1007/s00428-012-1325-9

128. N. Q. Yang, J Zhang, Q. Y. Tang, J. M. Guo, G. M. Wang: miRNA-1297 induces cell proliferation by targeting phosphatase and tensin homolog in testicular germ cell tumor cells. Asian Pac J Cancer Prev. 15, 6243-6. (2014)
DOI: 10.7314/APJCP.2014.15.15.6243

129. N. Q. Yang, X. J. Luo, J. Zhang, G. M. Wang, J. M. Guo: Crosstalk between Meg3 and miR-1297 regulates growth of testicular germ cell tumor through PTEN/PI3K/AKT pathway. Am JTransl Res. 8, 1091-1099 (2016)

130. M. Liu, Z. Hu, L. Qi, J. Wang, T. Zhou, Y. Gou, Y. Zeng, B. Zheng, Y. Wu, P. Zhang, X. Chen, W. Tu, T. Zhang, Q. Zhou, M. Jiang, X. Gou, Z. Zhou, J. Sha: Scanning of novel cáncer/testicle proteins by human testicle proteomic analysis. Proteomics.13, 1200-1210 (2013)
DOI: 10.1002/pmic.201200489

131. A. D. Krentz, M. W. Murphy, S. Kim, M. S. Cook, B .Capel, R. Zhu, A. Matin, A. L. Sarver, K. L. Parker, M. D. Griswold, L. H. Looijenga, V. J. Bardwell, D. Zarkower: TheDMdomainproteinDMRT1isadose-sensitiveregulator of fetal germ cell proliferation and pluripotency. Proc. Natl. Acad. Sci. USA. 106, 22323–22328 (2009)
DOI: 10.1073/pnas.0905431106

132. U. Fahrioglu, M. W. Murphy, D. Zarkower, V. J. Bardwell: mRNA expression analysis and the molecular basis of neonataltesticledefe ctsinDmrt1mutantmice. Sex Dev.1, 42–58 (2007)
DOI: 10.1159/000096238

133. S. Siddiqi, I. Matushansky: Piwisandpiwi-interactingRNAs in the epigenetics of cancer. J. Cell. Biochem. 113, 373–380 (2012)
DOI: 10.1002/jcb.23363

134. H. Taubert, T. Greither, D. Kaushal, P. Wurl: Expression of the stem cell self-renewal gene Hiwi and risk of tumourrelateddeathi npatientswithsoft-tissuesarcoma.Oncogene. 26, 1098–1100 (2007)
DOI: 10.1038/sj.onc.1209880

135. J. Vieira, S. Yoon, S.Domingues, S. Guimarães, A. V. Goltsev, E. Figueiredo, F. Mendes, O. Beirão, M. Fardilha:Amyloid

precursor protein interaction network in human testicle: sentinel proteins for male reproduction. BMC Bioinformatics.16, 12 (2015)
DOI: 10.1186/s12859-014-0432-9

136. O. Vincze, N. Tokési, J. Oláh, E. Hlavanda, A. Zotter, I. Horváth, A. Lehotzky, L. Tirián, K. F. Medzihradszky, J. Kovács, F. Orosz, J. Ovádi: Tubulin polymerization promoting proteins (TPPPs): members of a new family with distinct structures and functions. Biochem.46, 13818-13826 (2006)
DOI: 10.1021/bi061305e

137. P. Neth, B. Profanter, C. Geissler, D. K. Nägler, A. Nerlich, C. P. Sommerhoff, M. Jochum: T-SP1: a novel serine protease-like protein predominantly expressed in testicle. Biol Chem.12, 1495-1504 (2008)
DOI: 10.1515/bc.2008.170

138. C. Y. Li, Y. Q. Zhan, C. W. Xu, W. X. Xu, S. Y. Wang, J. Lv, Y. Zhou, P. B. Yue, B. Chen, X. M. Yang: EDAG regulates the proliferation and differentiation of hematopoietic cells and resists cell apoptosis through the activation of nuclear factor-kappa-B. Cell Death Diff.11, 1299-1308 (2004)
DOI: 10.1038/sj.cdd.4401490

Abbreviations: AFP: alpha-fetoprotein, AKT: Protein kinase B, AKT1: AKT serine/threonine kinase 1, APOLD1: apolipoprotein L domain containing 1, AR: androgen receptor, ATF7IP: activating transcription factor 7 interacting protein, AZFc: azoospermia factor c, BAK1: BCL2 antagonist/killer 1, BCL2: BCL2: apoptosis regulator, BMP7: bone morphogenetic protein 7, BPA: Bisphenol A, BRAF: B-Raf proto-oncogene: serine/threonine kinase, c-AMP: Cyclic adenosine monophosphate, CaP: prostate cancer, CDK: Cyclin-dependent kinases, c-KIT: Proto-Oncogene Receptor Tyrosine Kinase Kit, CLPTM1: CLPTM1: transmembrane protein, CO: cryptorchidism or no testicular descent, CXCR4: C-X-C chemokine receptor type 4, DEHP: Di (2-ethylhexyl) phthalate, DICER1: dicer 1: ribonuclease III, DMRT: DMRT like family A1, DMRT1: doublesex and mab-3 related transcription factor 1, DNA: Deoxyribonucleic acid, DND1: DND microRNA-mediated repression inhibitor 1, DNMT: DNA methyltransferase, DNMT3b: DNA methyltransferase 3 beta, DNMT3l: DNA methyltransferase 3 like, DNMTs: DNA methyltransferases, EDs: endocrine disruptors, ERS1: ethylene response sensor 1, ERS2: ethylene response sensor 2, ETV5: ETS variant 5, FTGCT: familial testicular germ cell tumor, G9a: euchromatic histone lysine methyltransferase 2, GCNIS: germ cell neoplasia in situ, GREAT: relaxin/insulin like family peptide receptor 2, GWAS: Genome-wide association study, H3: histone 3, hCG: human chorionic gonadotrophin, HDAC: histone deacetylases, HEMGN: hemogen, HLOD: Heterogeneity Lod Scores, HMTase: histone methyltransferase, HMTs: Histone Methyltransferases, HOXA10: homeobox A10, HPLC-MS: Liquid chromatography-mass spectrometry, HTPD: Human Testis Proteome Database, INSL3: insulin like 3, ITCLC: International Testicular Cancer Linkage Consortium, K4: keratin 4, K562: N-acetyltransferase 14 (putative), K9: keratin 9, KDM1: lysine demethylase 1A, KITLG: KIT ligand, KRAS: KRAS proto-oncogene: GTPase, LATS2: large tumor suppressor kinase 2, LDH: lactate dehydrogenase, LHCCGR: Luteinizing Hormone/Choriogonadotropin Receptor, LSC: cranial suspensory ligament, LSD1: lysine demethylase 1A, MAPK: Mitogen-activated protein kinase, Meg3: maternally expressed 3 (non-protein coding), miRNAs: microRNAs, miRs: microRNAs, mRNA: Messenger RNA, MS: mass spectrometry, NANOG: Nanog homeobox, NF-kB: nuclear factor-kappa B, IGCNU intratubular germ cell neoplasia unclassified, NIH: National Institutes of Health, NRAS: neuroblastoma RAS viral oncogene homolog, nsTGCT: testicular germ cell tumor type nonseminoma, OCT3/4: octamer-binding transcription factor 4, PCDH10: protocadherin 10, PDE11A: phosphodiesterase 11A, PDPN: podoplanin, PGC: primordial germ cells, PI3K: Phosphatidylinositol 3-kinases, PIWIL1: Piwi like RNA-mediated gene silencing 1, PLAP: placental alkaline phosphatase, poly-A: poly-adenine, POU5F1: POU domain: class 5: transcription factor 1, pri-miRNA: primary transcripts, PRSS55: protease: serine 55, PTEN: phosphatase and tensin homolog, RGAG1: retrotransposon gag domain containing 1, RISC: RNA-induced silencing complex, RNA: Ribonucleic acid, SCF: stem cell factor, SDF-1: stromal cell-derived factor 1, SNPs: single nucleotide polymorphisms, SOX17: SRY (sex determining region Y)-box 17, SOX2: SRY-box 2, SPRY4: sprouty RTK signaling antagonist 4, sTGCT: testicular germ cell tumors of seminoma type, TDS: testicular dysgenesis syndrome, TERT: telomerase reverse transcriptase, TFAP2C: Transcription Factor AP-2 Gamma, TGCT: Testicular germ cell tumor, TGFBR3: transforming growth factor beta receptor 3, TMPRSS12: transmembrane protease serine 12, TP53: tumor protein p53, TPA: transmembrane protein 165, TPPP2: tubulin polymerization promoting protein family member 2, WHO: The World Health Organization

Key Words: Epigenetic, Testicular germ cell tumor, Cryptorchidism; Endocrine Disruptors, Risk Factors, Genetic Factors.

Send mail to: Margarita Chavez Saldana, Laboratorio de Biologia de la Reproduccion, Instituto Nacional de Pediatria, Av. Insurgentes Sur 3700-C, Col, Insurgentes-CuicuilcoC.P. 04530 Ciudad de Mexico, Mexico, Tel: 52-55-1084-0900 Ext. 1453, Fax: 52-55-1084-5533, E-mail: mdoloreschs@gmail.com