Asian Journal of Andrology (2015) 17, 381–393

www.asiaandro.com; www.ajandrology.com

Open Access

INVITED REVIEW

Germ Cell Biology

Etiology and early pathogenesis of malignant testicular germ cell tumors: towards possibilities for preinvasive diagnosis

Jenny E Elzinga-Tinke[1], Gert R Dohle[1], Leendert HJ Looijenga[2]

Malignant testicular germ cell tumors (TGCT) are the most frequent cancers in Caucasian males (20–40 years) with an 70% increasing incidence the last 20 years, probably due to combined action of (epi)genetic and (micro)environmental factors. It is expected that TGCT have carcinoma *in situ* (CIS) as their common precursor, originating from an embryonic germ cell blocked in its maturation process. The overall cure rate of TGCT is more than 90%, however, men surviving TGCT can present long-term side effects of systemic cancer treatment. In contrast, men diagnosed and treated for CIS only continue to live without these long-term side effects. Therefore, early detection of CIS has great health benefits, which will require an informative screening method. This review described the etiology and early pathogenesis of TGCT, as well as the possibilities of early detection and future potential of screening men at risk for TGCT. For screening, a well-defined risk profile based on both genetic and environmental risk factors is needed. Since 2009, several genome wide association studies (GWAS) have been published, reporting on single-nucleotide polymorphisms (SNPs) with significant associations in or near the genes *KITLG*, *SPRY4*, *BAK1*, *DMRT1*, *TERT*, *ATF7IP*, *HPGDS*, *MAD1L1*, *RFWD3*, *TEX14*, and *PPM1E*, likely to be related to TGCT development. Prenatal, perinatal, and postnatal environmental factors also influence the onset of CIS. A noninvasive early detection method for CIS would be highly beneficial in a clinical setting, for which specific miRNA detection in semen seems to be very promising. Further research is needed to develop a well-defined TGCT risk profile, based on gene-environment interactions, combined with noninvasive detection method for CIS.

Asian Journal of Andrology (2015) **17**, 381–393; doi: 10.4103/1008-682X.148079; published online: 20 March 2015

Keywords: carcinoma *in situ*; etiology; environmental exposure; semen diagnostics; single nucleotide polymorphism; testicular germ cell tumor pathogenesis

INTRODUCTION TO GERM CELL TUMORS AND ITS CLASSIFICATION

Germ cell tumors (GCT) are a heterogeneous group of neoplasms that most frequently occur in the gonads, both testes and ovaries.[1] GCT may also rarely occur at specific extragonadal sites along the midline of the body, such as the pineal gland-hypothalamic region, mediastinum, retroperitoneum, and sacrum, probably as a consequences of the migration route of the primordial germ cells (PGCs), being the cells of origin of gametogenesis during early embryogenesis.[2–4]

Historically, GCT were based on histological classification only.[5–8] However, in 2005, Oosterhuis and Looijenga[9] proposed a classification system based on site of presentation, age of the patient at diagnosis, histological composition, pattern of genomic imprinting, and chromosomal constitution. This classification system includes five categories of GCT, which has been adopted by specialized pathologists and the World Health Organization (WHO).[10,11] The first group (type I) consists of benign teratomas and malignant yolk sac tumors (YSTs), predominantly diagnosed in neonates and infants, with an annual incidence of about 0.12 per 100 000. The per definition malignant seminomas and nonseminomatous GCT (type II) are the second group, which is the most common form of GCT, with an annual incidence of about 6.0 per 100 000. The third group is spermatocytic seminoma (type III), which usually affect men ≥ 40 years of age with an incidence of approximately 0.2 per 100 000 per year. The last two groups are type IV (dermoid cysts, mainly the ovary) and type V (the hydatidiform mole in fertile woman).

In this review, we will focus on type II GCT of the testis (i.e. testicular germ cell tumors [TGCT]), which have their origin in a blocked maturation of a PGC.[10,12] As indicated, the type II TGCT can be divided into seminoma (SE) and nonseminoma (NS). About 50% of all TGCT are seminomas, with a median age of patients at diagnosis of 35 years. Nonseminomas develop earlier in life, with a median age at diagnosis of 25 years. The latter can contain different histological tumor components, that is, the stem cell component embryonal carcinoma, teratoma (somatic differentiation), choriocarcinoma (extra-embryonic differentiation), and YST.[5,13] The nonseminomas account for 40% of cases. The remaining group consist of both seminoma and nonseminoma components and occur at an intermediate age.

[1]Department of Urology, Subsection of Andrology, Erasmus MC, University Medical Center Rotterdam, Rotterdam, The Netherlands; [2]Department of Pathology, Laboratory of Experimental Patho-Oncology, Erasmus MC Cancer Institute, University Medical Center Rotterdam, Rotterdam, The Netherlands.
Correspondence: Prof. LHJ Looijenga (l.looijenga@erasmusmc.nl)
Received: 16 September 2014; Revised: 25 November 2014; Accepted: 09 December 2014

TYPE II TESTICULAR GERM CELL TUMORS: INTRODUCTION

The malignant TGCT, also known as germ cell cancers, are the most frequent malignancy in Caucasian males between 20 and 40 years of age. Overall, TGCT account for approximately 1% of all solid cancers, but in young men, it is almost 60%.[6,9] In the last 20 years, there has been a 70% increase in the incidence of TGCT,[14–16] probably due to the action of (micro)environmental factors in relationship with (epi) genetic constitution,[15,17,18] to be discussed in detail later. Annually, about 12 000 new cases of TGCT are diagnosed in Europe.[19]

It is currently accepted that the majority, and possibly all, type II TGCT (i.e. the seminomas and all histological elements of the nonseminomas) have carcinoma *in situ* (CIS) as common precursor.[20] However, this has not been proven so far, which also accounts for the possible presence of CIS without progression to a full blown cancer. CIS is also referred to as intratubular germ cell neoplasia unclassified (IGCNU, WHO definition) and testicular intraepithelial neoplasia, but throughout this review, the term CIS will be used in accordance with most literature.[9] CIS originates from an embryonic germ cell, either a PGC or a gonocyte (i.e., a PGC located in the genital ridge/undifferentiated and bipotential gonad), blocked in its process of maturation.[21] After puberty, CIS has a high risk to progress into an invasive cancer, shown to be 70% in 7 years, and assumed to be up to 100% after 10 years.[22,23]

The overall cure rate of TGCT at 5 years, based on surgical interventional and depending on stage, combined with irradiation and/or chemotherapy,[24,25] is more than 90%, even in case of presence of metastatic disease. However, due to treatment resistance of the cancer in some patients, TGCT-related death is the second cause of death in men between 15 and 45 years. In addition, men surviving TGCT can present long-term side effects of systemic cancer treatment, such as chronic fatigue,[26,27] cardiovascular disease,[28] metabolic syndrome,[29,30] infertility,[31,32] and even second cancers.[33–36]

In contrast to the survival rates of patients with TGCT, the cure rate of CIS is 100%.[37] Local treatment is sufficient to effectively eradicate CIS, that is, a low doses of testicular irradiation[38] or orchidectomy. The consequence of this local therapy can be infertility and hypogonadism, especially in case of bilateral disease or monotestis.[39] For these patients, semen preservation must be performed in advance and hormonal support may be indicated after this local treatment. Contrary to patients with an advanced stage of TGCT, men diagnosed and treated for CIS without an invasive component continue to live without long-term side effects of systemic treatment.

Early detection of CIS is however difficult due to lack of symptoms and specific markers for screening.[37] A testis biopsy is currently the only reliable method to diagnose CIS of the testis. So far, it is proven to be difficult to develop a noninvasive test for CIS in men with an increased risk of TGCT, which can be used for screening purposes and case finding.[40] Defined risk factors for TGCT are cryptorchidism,[41,42] testis atrophy,[43] infertility,[44,45] a history of unilateral TGCT,[46,47] and familial predisposition.[48,49] However, many of the men having one or more of these risk factors will never develop a TGCT. Furthermore, many of the patients diagnosed with TGCT lack one or more of these risk factors. Therefore, these risk factors are not found to be highly informative on an individual level and it must be concluded that these risk factors alone are not specific enough to be used for screening of TGCT. In recent literature, various environmental factors may also result in a higher risk for TGCT,[50,51] but the role of these environmental factors is still unclear. They may play a role in the early development of CIS or in the transition of CIS into TGCT. In addition, a number of recent independent genome wide association studies (GWAS) have been conducted, indicating an association between a selected number of single-nucleotide polymorphisms (SNPs) and presence of a TGCT.[52–54] Interestingly, the genes likely linked to these SNPs are also known to be involved in early gonadal development and regulation of germ cell survival.

In this review, the early pathogenesis of CIS of the testis and TGCT will be discussed. A better understanding of these early pathogenetic steps, influenced by (micro) environmental and (epi)genetic factors, may help to develop an informative clinical approaches for early diagnosis of CIS, allowing local gonadal treatment and prevention of long-term side effects of systemic treatment.

PATHOGENESIS OF MALIGNANT TESTICULAR GERM CELL TUMORS (TYPE II)

Normal testicular development

During early embryogenesis, the early germ cells undergo subsequent maturation and differentiation influenced by the micro-environment of these cells.[55] Understanding these processes is essential to create insight into the mechanisms involved in the earliest events of the pathogenesis of CIS and of the derived invasive lesions, that is, TGCT.

The initial totipotent early germ cells, named PGC, initially start to migrate from their origin in the posterior wall of the yolk sac into the hindgut along the midline of the body in the 3rd week of embryogenesis.[2,56,57] Thereafter, they move into the mesoderm and travel to the genital ridge.[3] The KIT/KITLG (stem cell factor, SCF, c-KIT ligand) pathway is crucial for this migration.[58,59]

The PGC express a number of specific (embryonic) markers during and early after their migration to the genital ridge (**Figure 1**), which are involved in different biological mechanisms during this period of embryogenesis.[60,61] Some of these markers, like OCT3/4 (also known as POU5F1),[62,63] c-KIT,[58] placenta like alkaline phosphatase (PLAP),[64] NANOG, SOX2 (only for nonseminoma),[60] and SOX17,[65] have diagnostic value for TGCT as well as CIS.[66–68]

During the 5th week of development, still the pluripotent PGC enters the genital ridge and are now called gonocytes. In the beginning of week sixth of the embryogenesis in the male genomic constitution (XY) Sertoli cells are formed, due to the expression of the transcription factor Sex-determining Region on the Y-chromosome (SRY) and subsequently SOX9 (SRY related HMG box 9).[69–71] If no functional SRY and the subsequent downstream pathway is activated, no Sertoli cells will be generated, but the precursors will follow the female pathway and become granulosa cells.

The primitive seminiferous cords are formed in the 7th week of embryogenesis, in which the germ cells and Sertoli cells are not yet organized. Subsequently, the germ cells migrate toward the basal lamina of the seminiferous tubules and start to lose the expression of some of the forementioned embryonic markers (i.e. PLAP, OCT3/4, and c-KIT), happening during the 13th week of embryogenesis.[68,72,73] In fact, OCT3/4 and PLAP disappear completely, while c-KIT can still be detected at a relatively low level.[61,67] This is in contrast to the markers VASA[74] and SOX17:[65] they continue to be expressed after birth and remain even positive throughout life.

At the 13th week of development, the male gonocytes also begin to express Testis Specific Protein on the Y chromosome,[75] which regulates the normal proliferation of spermatogonia and remain positive when the spermatogonia enter meiotic division.[76] For a good regulation of the proliferation of the gonocytes and the protection of the cells against apoptosis, the contact between the Sertoli cells and now referred to prespermatogonia is necessary. The expression pattern of proteins on the gonocytes and prespermatogonia changes during embryogenesis

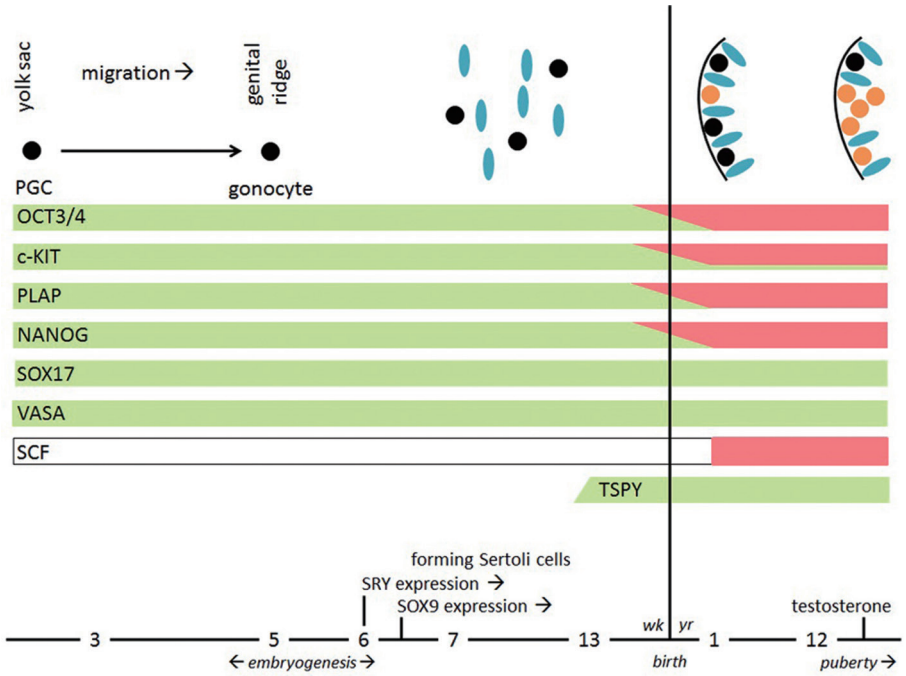

Figure 1: Marker expression in normal and impaired testicular development. Marker expression from early embryogenesis till puberty during the normal and impaired testicular development leading to carcinoma *in situ* (CIS). See text for further explanation. Black sphere: germ cell (not specified for different stages of maturation). Blue sphere: Sertoli cell. Orange sphere: CIS cell. Green bar: normal testicular development (physiological). Red bar: impaired testicular development (pathological).

substantially: the specific embryonic proteins decrease gradually in number and most of them eventually disappear.[64,65,67,68]

Impaired testicular development and the onset of carcinoma in situ

The origin of TGCT probably starts at early embryogenesis, and is hypothesized to be part of the Testicular Dysgenesis Syndrome (TDS).[77] This syndrome was first described by Skakkebaek *et al*.[77] and assumes that cryptorchidism, hypospadias, impaired spermatogenesis, and TGCT may all be manifestations of impaired testicular development during early embryogenesis.

This impaired testicular development means that some early PGC/gonocytes are blocked in their process of differentiation, and as such, these germ cells retain their early (embryonic) marker profile.[66,67,78] The reason for this development block is not yet entirely clear. It is probably a combination of (micro)environmental factors and (epi) genetic defects.[10] For example, it is believed that xeno-estrogens and anti-testosterones negatively affect the development of Sertoli cells and Leydig cells,[79] causing a suboptimal environment for germ cell differentiation and leading to development of CIS cells,[80] which are, as mentioned above, blocked embryonic germ cells. Furthermore, it is assumed that the KIT/KITLG pathway, which regulates the survival, migration and proliferation of PGC/gonocytes, is central to this development.[81–83]

As indicated, these CIS cells resemble PGC/gonocytes on different aspects.[78] Both have for example expression of embryonal markers (**Figure 1**: OCT3/4, PLAP, alkaline phosphatase (AP-2γ), c-KIT, *etc.*).[61,72] Normally, in the time window of about 6–12 months after birth all proteins related to the embryonal stage are disappeared, while VASA[84] and TPSY remain present.[75] However, there is on ongoing expression of these embryonal markers in CIS cells after birth,[68,85] wherein the expression of SCF (KITLG) is likely more selective for the niche of the CIS cells.

In the testis, these CIS cells remain, that is, resistant to apoptosis and maturation, and eventually progress after puberty, likely due to a rise in serum testosterone, into an invasive tumor.[22,23] The current hypothesis is that all patients with this abnormality will develop an invasive TGCT,[9] due to the fact that in the male Caucasian population, the incidence of CIS similar is to the lifetime risk of developing a TGCT. However, the exact cause for progression of CIS to TGCT is currently unknown. It has been suggested that loss of PTEN exposure to certain and environmental factors among others play a role.[86] For the onset of CIS and the development of CIS into an invasive tumor, there is likely an interplay between genetic, epigenetic, and (micro)environmental factors also referred as the genvironment.[87,88]

ETIOLOGY OF TESTICULAR GERM CELL TUMORS AND THE GENVIRONMENTAL HYPOTHESIS

Genetic risk factors

Ethnic differences in the incidence and geographical clustering of TGCT suggest a genetic component in the etiology of this disease in addition to possible environmental influences.[89] African and Asian men have a very low risk of developing TGCT.[90] Even if these men migrate to a western country they maintain their low risk, which is observed in African-Americans living in the USA for many generations.[91] This shows that the supposed absence of genetic risk factors in African and Asian men, have greater influence than exposure to environmental risk factors on the incidence of TGCT.[92] This is in contrast to selective Caucasian men migrating to another country with a higher incidence of TGCT. The second generation of these men has the same risk of TGCT as the local men.[89]

Another important argument for a genetic component in the etiology of TGCT is that family history is a strong known risk factor for these malignancies.[93–96] Several studies have shown that the risk for a brother of a TGCT patient is 8–10 fold higher as compared with

the general male population.[97–104] The risk for a son of a father with a history of TGCT is 4–6 fold higher.[97–99,101,103] Also twin studies, both mono- and dizygotic twins, have confirmed a strong genetic component to TGCT.[105–108] It is estimated that the genetic effects accounts for 25% of TGCT, which is a high rate in comparison to other cancers and even the third among all cancer types.[94] Nevertheless most men with TGCT have no family member who also has the disease.

However, familial linkage studies and candidate gene approaches have not been very successful so far in defining genes predisposing to TGCT. An association study of the gr/gr deletion on the Y chromosome,[109] which carries a number of testis - and germ cell-specific genes, demonstrated that this deletion provides an approximately two-fold risk of TGCT. It is however, not clear whether it is an effect specially related to this gene mutation or to the associated infertility, also a known risk factor for TGCT.

Since 2009 there are new genetic insights starting from two TGCT GWAS, one from the UK[54] and one from the US.[81] Subsequently, several additional TGCT GWAS have been done[83] and recently a meta-analysis is performed.[52] SNPs with significant associations were identified in or near the genes *KITLG* (KIT ligand), *SPRY4* (sprouty 4: sprout-related, EVH1 domain containing 2), *BAK1* (BCL2-antagonist/killer 1), *DMRT1* (doublesex and mab-3-related transcription factor 1), *TERT* (telomerase reverse transcriptase), *ATF7IP* (activating transcription factor 7 interacting protein), *HPGDS* (hematopoietic prostaglandin D synthase), *MAD1L1* (mitotic arrest deficient-like 1), *RFWD3* (ring finger WD domain 3), *TEX14* (testis expressed 14) and *PPM1E* (protein phosphatase, Mg^{2+}/Mn^{2+} dependent, 1E). **Table 1** highlights these genes, wherein also their chromosomal localization as well as relevant SNPs are listed.

Single-nucleotide polymorphisms at 12q22 likely related to *KITLG* have the strongest association with the development of TGCT, with a risk greater than 2.5-fold of TGCT per major allele.[54,81] *KITLG* has been shown to be required for multiple aspects of PGC/gonocyte development.[112] This gene is involved in the KITLG–KIT pathway, which system regulates the survival, proliferation, and migration of embryonic germ cells.[113] Delayed differentiation of PGC/gonocytes and subsequent development of TGCT can be the result of retained activation of this pathway.[114]

In addition, for TGCT somatic alterations in KIT have been described.[72,115,116] In 21% of seminomas and 9% of nonseminomas, an increased copy number of the *KIT* gene has been observed.[117,118] Probably, somatic KIT mutations can occur very early in embryogenesis, because there are known cases of bilateral disease with the same mutation in both testes.[82,119]

It is suggested that there is a relationship with these genes and infertility.[2,114,120] The link between cancer and infertility can, therefore, further be investigated in studies of KITLG variation in men with a history of infertility with and without TGCT.

SPRY4, on chromosome 5, is an inhibitor of the mitogen-activated protein kinase pathway, which is activated by the KITLG–KIT pathway.[121] An increased expression of SPRY4 can induce downregulation of the KIT–KITLG pathway.[122] A risk of 1.5-fold of TGCT per major allele on chromosome 5 loci is described.[54,81] BAK1, on chromosome 6, binds and then provide apoptosis by antagonizing the apoptosis suppressor activity of BCL2 and other anti-apoptotic proteins.[123] In testicular germ cells, expression of BAK1 is repressed by the KIT–KITLG pathway and interaction of BAK1 with anti-apoptotic proteins provides apoptosis in germ cell. Therefore, TGCT susceptibility might also come through BAK1 and its response to signaling through the KIT–KITLG pathway. A 1.5-fold increased risk was identified per major allele for the chromosome 5 and 6 loci.[54] It is suggested that a positive interaction between the loci on chromosomes 5, 6, and 12 gives a combined risk greater than the sum of the individual risks.

DMRT1 is a transcription factor with a highly conserved DNA-binding motif, which is similar to many vertebrate sex-determination pathways.[114,124] There is much evidence that this gene is related to testicular determination, differentiation and tumorigenesis.[114,110] Higher expression levels of DMRT1 are required for testicular differentiation and lower expression levels result in ovarian differentiation.[111,125,126] DMRT1 deficiency in mice is associated with a high rate of teratomas, so type I TGCT.[127] In humans seems a lower expression level of DMRT1 more strongly associated with nonseminomas type II TGCT, of which teratoma is one type, than with seminomas.[126]

Telomerase reverse transcriptase encodes the catalytic subunit of the telomerase ribonucleoprotein complex. Telomerase counterbalances loss of chromosome ends, which is normal in actively dividing cells, by extending the TTAGGG telomeric nucleotide repeats.[114] Telomere shortening is associated with increased genome instability and neoplasia.[128–131] TERT is endogenously expressed during embryogenesis, but testicular germ cells are the only normal adult tissue in which telomerase continues to be expressed.[132,133] Seminomas show high TERT expression, high telomerase activity and long telomeres, as seen in normal testicular germ cells.[110] Minimal TERT expression and no telomerase activity are seen at nonseminomas, such as teratomas.[132,133] This is also confirmed by the fact that SNP identified at this locus were more strongly associated with seminomas than nonseminomas.[110] ATF7IP activates the expression of TERT and its associated RNA component, TERC. Knock down of ATF7IP results in a significant decrease in TERT and TERC expression and also in telomerase activity.[134] In short, both TERT and ATF7IP maintain telomere length and are reactivated in a range of tumor types.

Hematopoietic prostaglandin D synthase is expressed in the early embryonic male gonad in mice and seems to control nuclear localization

Table 1: The TGCT risk-SNPs and their likely related genes

Gene	*Chromosome*	*SNP*	*References*
UCK2	1	rs4657482	Rapley *et al.* 2009[54]
HPGDS	4	rs17021463	Chung *et al.* 2013[52]
CENPE	4	rs4699052	Rapley *et al.* 2009[54]
TERT	5	rs2736100	Turnbull *et al.* 2010[110]
TERT/CLPTM1L	5	rs4635969	Turnbull *et al.* 2010[110]
SPRY4	5	rs4624820	Rapley *et al.* 2009[54]
		rs4324715	Kanetsky *et al.* 2009[81]
		rs6897876	Kanetsky *et al.* 2009[81]
BAK-1	6	rs210138	Rapley *et al.* 2009[54]
MAD1L1	7	rs12699477	Chung *et al.* 2013[52]
DMRT1	9	rs755383	Turnbull *et al.* 2010[110] Kanetsky *et al.* 2011[111]
		rs7040024	Kanetsky *et al.* 2011[111]
AFT7IP	12	rs2900333	Turnbull *et al.* 2010[110]
KITLG	12	rs995030	Rapley *et al.* 2009[54]
		rs1508595	Rapley *et al.* 2009[54]
		rs3782179	Kanetsky *et al.* 2009[81]
		rs4474514	Kanetsky *et al.* 2009[81]
RFWD3	16	rs4888262	Chung *et al.* 2013[52]
TEX14	17	rs9905704	Chung *et al.* 2013[52]
PPM1E	17	rs7221274	Chung *et al.* 2013[52]

The Single-Nucleotide Polymorphisms (SNPs) with their chromosomal localization that appear to be associated with the presence of TGCT, which is demonstrated in recent independent Genome Wide Association Studies (GWAS). The genes likely linked to these SNPs are also known to be involved in early gonadal development and regulation of germ cell survival. See text for further explanation

of the SOX9 protein.[52,135] Disruption of HPGDS leads to modification of the phenotype of $apc^{Min/+}$mice.[136] *MAD1L1* gene encodes MAD1, prevents aneuploidy and maintains genomic stability.[52] MAD1 is in fact a spindle assembly checkpoint protein that delays the onset of anaphase in the mitotic cell cycle until all sister chromatid s achieve proper alignment and microtubule attachment.[137] Remarkably, the risk allele (C) at the most significant SNP without study heterogeneity, rs12699477, is more prevalent in population of European (29%) than those of African ancestry (8%) in 1000 Genomes.[138] This might indeed be one of the possible explanations for the intrinsic incidence of TGCT among Caucasians compared to Africans, as well as Asians, as mentioned above. This also indicates that the genomic constitution of these SNPs is dominant over the exposure to environmental compounds. RFWD3 is an E3 ubiquitin ligase that controls p53 stability by forming a RFWD3-MDM2-p53 complex, thereby protecting p53 from degradation by MDM2 polyubiquitination.[139,140] The role of p53 in the pathogenesis is on interest in this context as well.

In conclusion, there are identified a number of SNPs for TGCT predisposition within fourteen genes that are biologically plausible candidates for disease susceptibility. This increased understanding of the genetic etiology of TGCT will lead to further improvement in the clinical management of this disease.

Environmental and other risk factors

In recent years, various potential risk factors for TGCT have been investigated, both prenatal, perinatal as well as postnatal. These include a variety of risk factors, such as related to environmental exposures, lifestyle factors, and prenatal characteristics. The knowledge on the impact of these particular risk factors is based on epidemiological research only,[141] because there are so far no informative animal models available for TGCT.[50] Some studies have focused on the etiology of TGCT in utero and early life, because CIS is supposed to develop in that period (as discussed above), and other studies have examined potential risk factors during childhood and adolescence. Overall, the results of these studies are inconsistent,[142] partly because of their small sample sizes, multiple testing, self-report data, and recall bias. Recent meta-analyses have provided insight into the influence of these factors on the development of TGCT.[50,51,143] In this review only the most important environmental factors in the context of development of TGCT will be highlighted.

The following prenatal risk factors for the development of TGCT have been investigated; maternal exposure to estrogens and nausea during pregnancy (being related to the first), diethylstilbestrol (synthetic estrogen DES), maternal hypertension, preeclampsia, maternal bleeding during pregnancy, maternal smoking, and maternal age.[17,18,51] No association is found for nausea,[143] and excess of endogenous hormones (likely estrogen) that can cause nausea, during pregnancy,[144] similarly as for maternal hypertension, preeclampsia, and maternal age. DES, a nonsteroidal estrogen, has been used from the late 1940s to the early 1970s to prevent abortions and pregnancy complications,[145] has been suggested that it can cause TGCT as well, but it remains uncertain so far.[145–147] Maternal bleeding during pregnancy is a risk factor for TGCT with an odds ratio (OR) of 1.33 (95% confidence interval (CI) 1.02–1.73) in a recent meta-analysis.[51] The association between maternal smoking and TGCT is inconsistent.[148–154]

Many perinatal factors have been investigated, including cryptorchidism, birth weight, gestational age, inguinal hernia, twinning, and hypospadias. Cryptorchidism is the most defined factor associated with TGCT, with a population attributable risk between 4.3% and 10%.[143,155–159] Low birth weight was associated with an increased risk TGCT (OR 1.19–1.34)[18,143] and gestational age was inversely related to the risk of TGCT.[143] A meta-analysis of two other perinatal factors, inguinal hernia and twinning, shows OR of, respectively, 1.63 (95%CI 1.37–1.94) and 1.22 (95% CI 1.03–1.44).[143] Due to the small number of patients who participated in only a few studies no meta-analysis of hypospadias as a risk factor for TGCT could be performed.[43,160,161] The variables birth length, breast feeding, and neonatal jaundice were not associated in a meta-analysis with TGCT.[143] Some perinatal factors of the mother were examined and birth order and sibship size were inversely associated with the risk of TGCT.[51] For breech delivery and cesarean, no association with TGCT was found.[51]

Various postnatal factors influence the development of TGCT, which are subdivided in direct case-related factors, lifestyle of men, and environmental influences. Most studied case-related factors are age at puberty, body mass index (BMI), height, infertility, history of TGCT, and socioeconomic status (SES). Late age at puberty is associated with a large reduction in the risk of TGCT.[162–165] The results of studies of BMI are often inconsistent[166–172] as well as height as TGCT risk factor.[164,167,169,170,172] Infertility is associated with a higher risk for TGCT.[44,164,173,174] A history of TGCT has a strong relationship with the risk of TGCT.[46,47,175] SES has been studied in various ways, as well as social class as education, however, the association with TGCT is uncertain.[163,176–179] The influence of some lifestyle factors on risk of TGCT has also been studied. There is no association with smoking and TGCT risk.[17] The effect of physical activity on the risk of TGCT is unclear.[160,179–181]

The environmental influences during life on the risk of TGCT developing have been studied extensively, but there are no clear associations between TGCT and environmental exposure so far. Various industrial exposures have been investigated, like working in the paper industry[182–193] plastic-related industries[190,192] and metal industries,[188,194–197] with different results and no clear association with TGCT. Studies on construction and related occupations,[187,188,192,193,196,198–200] like construction workers, electrical workers, painters, wood workers, and lumber-jacks, find some with inconsistent results and others with no association. Other jobs that haven been studied, like firemen,[201–205] policemen,[185,196,206,207] and military and related occupations[187,196,208–215] shows divergent results. In general, no significant results were found in all studies who investigated TGCT risk with pesticides exposures among agricultural workers, pesticides applicators or in occupations associated.[187,188,193,196,199,216–224]

Other environmental influences that have been studied are magnetic and electric field exposure, organochlorines exposure and living in rural areas. Magnetic and electric field exposure have been investigated in five studies[193,199,225–227] without a clear association with TGCT. Environmental exposure to organochlorines was studied by using blood samples[220,223,228–233] or questionnaires,[220,223] and overall no relation was found. Living in rural areas has been suggested as a surrogate for environmental exposure to pesticides,[192,193,223,234–236] but the several studies are inconsistent in their results. In conclusion, there are a number of well-defined prenatal and perinatal risk factors for the development of TGCT. The only prenatal factor that indicates a risk for TGCT is maternal bleeding during pregnancy. Perinatal risk factors of the son are cryptorchidism, a lower gestational age, inguinal hernia, and twinning. Birth order and sibship size are the two perinatal factors of the mother who are inversely associated with the risk of TGCT. There is a lack of association of most postnatal factors who could be influence the development of TGCT, which is in line with current hypotheses of the early embryogenic origin of TGCT. Only infertility and a late age at puberty are well-established postnatal factors.

Genvironmental hypothesis

Current evidence for the etiology of TGCT included genetic and environmental factors as mentioned above, but at this time, these factors separately are insufficient to make a risk assessment for TGCT on an individual level. Probably the combined action of (epi)genetic factors and (micro)environmental factors will lead to the development of TGCT,[229] named as the genvironmental hypothesis (**Figure 2**).[87,88]

EARLY DETECTION OF TESTICULAR GERM CELL TUMORS

Current methods

To date, an open testicular biopsy is still the gold standard for the diagnosis of CIS.[237] A single biopsy of at least 3 mm is usually sufficient to detect CIS,[238,239] since sensitivity for CIS detection is above 99% provided that at least 10% of tubules contain CIS cells. Contralateral biopsy is not routinely performed in most countries.[240] It may be considered in selected cases, such as in men with cryptorchidism, atrophic testes and ultrasonographic abnormalities.

Guidelines on the use of fixatives for testicular biopsies are contradictory on the use of Stieve's or Bouin's solution or formalin.[241,242] Formalin is commonly used for fixation, despite some shrinkage artefacts, along with at least one solid immunohistochemical marker such as OCT3/4,[63,243] PLAP,[244] AP-2γ[61,245] or c-KIT.[246] With the use of these robust CIS markers only in less than 0.5% false-negative biopsies occur,[247] as a consequence of a heterogeneous distribution of CIS within the testis[248–250] or surgical damage of the tissue.

An open testicular biopsy is, however, an invasive procedure with potential complications, although infrequent: overall, 2.8% complications were noted,[251] like superficial wound infections, intra-testicular hematoma and decline in semen quality secondary to the biopsy. A diagnostic testicular biopsy is nowadays only considered in men at high risk of TGCT. These include men with risk factors for TGCT, like infertility,[174,252] cryptorchidism,[43,253] atrophic testes[43] and a history of TGCT,[254] together with ultrasonographic abnormalities.[255,256] The most significant abnormalities found on the ultrasound are testicular microlithiasis (TM, **Figure 3a**), an inhomogeneous parenchyma (**Figure 3b**) and solid testicular lesions (**Figure 3c**).[257] TM is defined as all hyperechogenic foci smaller than 3 mm without shadowing irrespective of their number.[258] An inhomogeneous parenchyma of the testis is described as a heterogeneous parenchyma with hypo- and hyper-echoic areas and often very small cysts.[257] Testicular lesions are presented as focal solid hypo-echoic or hyper-echoic structures inside the parenchyma.[257]

Use of markers in semen of infertile males and testicular germ cell tumors patients

Efforts have been made to develop a noninvasive method for the detection of CIS cells in semen in the last decades, since these CIS cells are exfoliated from the seminiferous tubules into seminal fluid.[259] The first studies were based on cytological examination by plain microscopy[260–263] or after immunohistochemistry (IHC) using PLAP[264] and IHC with magnetic beads and the M2A antibody,[265,266] which all proved to be unsuccessful or too laborious. Another approach was to use the aneuploid DNA content in CIS cells as a marker.[267–270] However, normal semen contains cells with differences in numbers of chromosomal copies, and possible CIS cells could be proved only in a very small window.

After the discovery of novel stem cell-related markers of CIS, including AP-2γ,[271,272] NANOG,[272] and OCT3/4,[272,273] an immunocytochemical assay for the detection of CIS cells in semen was tested again (**Figure 4**). In contrast to previous studies, which used cytoplasmic markers of CIS, these proteins are localized in the nucleus, and thereby better protected from degradation in semen or during processing of the sample. In these studies, the identification of CIS cells in semen has a high specificity; nevertheless, the sensitivity remained relatively low. Even the double staining method with of immunocytological staining for AP-2γ or OCT3/4 and rapid cytochemical AP reaction has its limitations, like unspecific cross-reaction and still some false-positive results due to a weak staining of AP-2γ and OCT3/4 in the epithelial cells of the epididymis, prostate gland, and seminal vesicles.[274,275]

Most recently, the two cancer-testis antigens MAGE-A4[276] and NY-ESO-1, which are expressed by TGCT, were tested in semen by

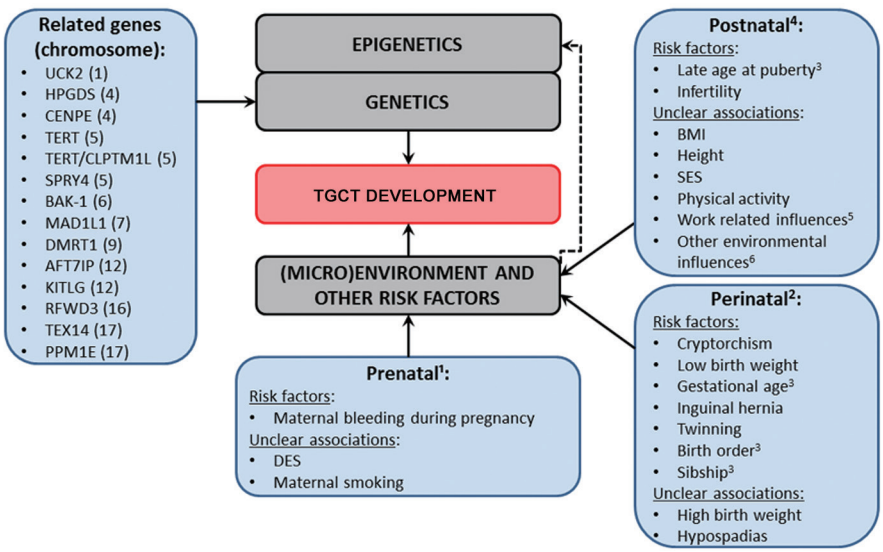

Figure 2: Genvironmental risk model. The genvironmental risk model shows the different etiological factors which have an influence on the development of testicular germ cell tumors. [1]No association is found for nausea during pregnancy, maternal hypertension, preeclampsia and maternal age. [2]No association is found for birth length, breastfeeding and neonatal jaundice. [3]Inversely related. [4]No association is found for smoking and organochlorines exposure. [5]Working on the paper industry, construction and related occupations, fireman, policemen and military and related occupations. [6]Pesticides exposures, magnetic and electric field exposure and living in rural areas.

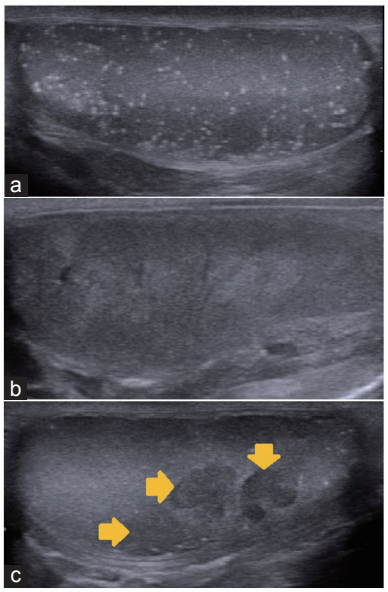

Figure 3: Ultrasonographic abnormalities that may be present in men at risk for testicular germ cell tumors. (**a**) Testicular microlithiasis in the parenchyma of the testis. (**b**) Inhomogeneous parenchyma of the testis. (**c**) Hypo-echoic lesions in the parenchyma of the testis.

immunological staining in TGCT patients and healthy semen donors.[277] Although this may seem promising results, there are too much false-positive cells. Moreover, there is only assumed that these markers also are expressed in CIS cells, but this has never been demonstrated in semen of men with CIS.

Despite some progress, none of the above-mentioned semen-based methods for CIS detection are currently used in standard clinical practice. This is due to different causes, like for example the low numbers of CIS cells in seminal samples and degradation of the CIS cells or impaired shedding from the seminiferous tubules. In addition to this, the relatively high number of false-positive results in the most promising immunocytochemical techniques is a problem too. False-positive cells were observed in some patients, probably due to a weak expression of some of the used markers, that is, OCT3/4, AP-2γ, NANOG, in the epithelial cells of epididymis, prostate and seminal vesicles.[278] The limits of these investigated markers are an important reason not to apply these techniques in standard clinical practice. Thus, this field still awaits a noninvasive method for CIS detection, ultra-sensitive and fail-safe, which could be applied to routine screening of population at risk for TGCT.

Detection of microRNA in blood and semen of testicular germ cell tumors patients and infertile males

microRNAs (miRNAs) are small noncoding RNA molecules, with approximately 22 nucleotides in length.[279,280] In the early 1990s, the first miRNAs were characterized in C. elegans,[281] but their role in biological processes is better understood since the beginning of this age. These relatively small sequences interact with messenger RNA (mRNA) in the mammalian system, and they are involved in the fine-tuning of the translation process from mRNA to protein.[282] It has been found that these miRNAs are crucial for normal development, of which some in stem cell formation.[283,284]

Over 500 miRNAs exist within the human genome[285] and different sets of expressed miRNAs are found in various cell types and tissues.[283] It is found that aberrations in regulation of expression of miRNAs can be involved in the development of cancers,[286–291] so they can act as both

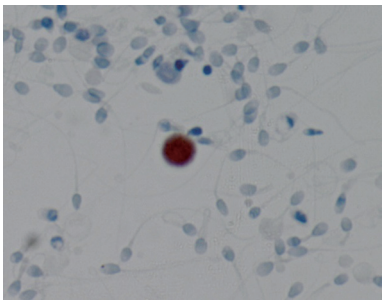

Figure 4: Carcinoma *in situ* (CIS)-cell in semen with OCT3/4 staining. A positive CIS-cell in semen, detected with the immunocytochemical marker OCT3/4.

oncogenes and tumor suppressor genes.[290,292,293] There is increasing interest in their potential use as biomarkers in various cancer types and other disorders due to their secretion into body liquids, in which they have proved to be very stable.[294–296]

Recently, it is demonstrated that miRNAs of the miR-371~373 (miR-371-372-373)[297,298] and miR-302/367 (miR302a, b,c,d/367) clusters[299] are overexpressed in all TGCT,[300] regardless of patient age, histologic subtype (except teratoma), or anatomic site of the tumor. All main members of the miR-371~373 and miR-302/367 clusters were elevated in the serum and returned to normal after treatment of the disease within only a few days or even less.[301–303] The hypothesis reported that these miRNAs are not only expressed in all TGCT, but also in CIS.[304] Remarkable is that these miRNAs are not up-regulated in other tumor types or disorders.

Clinical management of TGCT is greatly based on serum biomarker monitoring.[305] At the moment the markers alfa-fetoprotein, b-human chorionic gonadotropin, and lactate dehydrogenase are used, but only 60% of all TGCT patients have elevations of these markers.[306] For this reason, the above-mentioned miRNA are promising candidate biomarkers for disease monitoring and potentially also in the diagnosis of TGCT.[303,304,307] Possibly, these miRNAs may also be used as screening method of semen in men at risk for CIS/TGCT. To date, no studies have been performed of these TGCT miRNA in semen and further research is required.

However, specific miRNA for infertility in semen has been tested in several studies in infertile population.[308–313] Gene expression is active during spermatogenesis and miRNAs are differentially expressed during this differentiation period.[314,315] To understand the role of miRNAs in the different forms of spermatogenic failure, miRNA expression profiles have been studied in normal and infertile testicular tissues in mice.[316] With this knowledge, several clinical case–control studies were conducted in men and five of these studies have mapped the well-defined expression profiles of miRNAs in semen between normal and infertile men (**Table 2**).[308–311,317] Each study has made its own choice which miRNAs are tested in the semen and they all made a different case-selection. Therefore, the results of the studies are difficult to compare with each other. Yet, because of these studies, there is sufficient evidence that it is possible to detect in semen specific miRNAs related to infertility.

It can be concluded that specific seminal plasma miRNAs have been explored as potential biomarkers for the diagnosis and classification of male factor infertility. Therefore miRNAs, both for male factor infertility and CIS/TGCT, need to be further investigated in different patient population and follow-up studies.

CONCLUSIONS AND REMARKS

This review focused on the etiology and early pathogenesis of TGCT, its

Table 2: Case–control studies of detection specific miRNAs for infertility in semen

	Cases	*Controls*[a]	*A*[b]*/C*	*AO*[c]*/C*	*O*[d]*/C*	*OA*[e]*/C*	*IF*[f]*/C*	*NOA*[g]*/C*	*OA*[e]*/A*[2]
Wang *et al*. 2011[308]	*n*=181[h]	*n*=68	↓ miR-34c-5p, miR-122, miR-146b-5p, miR-181a, miR-374b, miR-509-5p, miR-513a-5p	↓ miR-34c-5p, miR-122, miR-146b-5p, miR-181a, miR-374b, miR-509-5p, miR-513a-5p	-	-	-	-	-
Liu *et al*. 2012[309]	*n*=86[i]	*n*=86	-	-	-	-	↑ miR-122, miR-185, miR-193b, miR-297, miR-373, miR-574-5p, miR-1275 ↓ miR-16, miR-19b, miR-23b, miR-26a, miR-100, miR-512-3p	-	-
Wu *et al*. 2012[310]	*n*=192[j]	*n*=96	-	-	Not sign	-	-	↑ miR-19b, let-7b	-
Wu *et al*. 2013[311]	*n*=100[k]	*n*=100	-	-	-	-	-	↑ miR-7-1-3p, miR-141, miR-429	-
Abu-Halima *et al*. 2013[317]	*n*=18[l]	*n*=9	↑ miR-141, miR-200a ↓ miR-34b, miR-122	-	-	↑ miR-141, miR-200a ↓ miR-16, miR-34b, miR-34c-5p, miR-122	-	-	Not sign

To date, in 5 case–control studies of infertile men miRNA in semen is investigated. The studies examined different miRNA in semen and also have made a different case-selection. [a]Controls (*n*): fertile controls; [b]A: azoospermia; [c]AO: asthenozoospermie; [d]O: oligozoospermia; [e]OA: obstructive azoospermia; [f]IF: infertile males; [g]NOA: nonobstructive azoospermia; [h]N: 181 – A: 73, AO: 79, O: 34; [i]N: 86 – IF: 86; [j]N: 192 – O: 96, NOA: 96; [k]N: 100 – NOA: 100; [l]N: 18 – A: 9, OA: 9. ↑: significant upregulated miRNA; ↓: significant downregulated miRNA. miRNA used in different studies: miR-34c-5p, miR-122, miR-16, miR-19b, let-7b, miR-141. Used miRNA per study – Wang *et al*. 2011.[308], *n*=7: miR-34c-5p, miR-122, miR-146b-5p, miR-181a, miR-374b, miR-509-5p, miR-513a-5p. Liu *et al*. 2012.[309], *n*=21: miR-574-5p, let-7b, miR-297, miR-122 (1, 2, 5), miR-1275, miR-1281, miR-373, miR-185, miR-193b, miR-145, miR-214, miR-31, miR-455-3p, miR-100, miR-517a, miR-512-3p, miR-16, miR-523, miR-19b, miR-23b, miR-26a. Wu *et al*. 2012.[310], *n*=2: miR-19b, let-7b. Wu *et al*. 2013.[311], *n*=3: miR-141, miR-429, miR-7-1-3p. Abu-Halima *et al*. 2013.[317], *n*=6: miR-141, miR-200a, miR-122, miR-34c-5p, miR-34b, miR-16. miRNAs: microRNAs

relation to testicular development and the current and future possibilities for noninvasive early detection methods of TGCT. Currently, the early detection methods for TGCT by the detection of CIS are not well-suited for clinical use, while there are considerable health benefits if CIS could be detected in a noninvasive way. All men diagnosed and treated for CIS only instead of a TGCT continue to live without long-term side effects of systemic treatment, such as chronic fatigue, cardiovascular disease, metabolic syndrome, infertility, and even second cancers. An open testicular biopsy is currently the only reliable method to diagnose CIS of the testis. Therefore, this biopsy is only considered for a specific group of patients, such as in men with cryptorchidism, atrophic testes, and ultrasonographic abnormalities. However, for screening purposes, it is required to develop a sensitive and specific noninvasive early detection method and to compose a well-defined TGCT risk profile.

Key aspects of the etiology of TGCT were discovered in the past few years. Since 2009, a several GWAS have found SNPs with significant associations in or near the genes *KITLG*, *SPRY4*, *BAK1*, *DMRT1*, *TERT*, *ATF7IP*, *HPGDS*, *MAD1L1*, *RFWD3*, *TEX14*, and *PPM1E*. Many of these genes are involved in the early gonadal development, which explains their involvement in the pathogenesis of CIS. Prenatal, perinatal, and postnatal risk factors also influence the onset of CIS. These genetic and environmental factors play an essential role in the pathogenesis of TGCT but are individually insufficient to identify men at high risk for TGCT. Additional national and international collaborative studies, to obtain enough power, into the combined effects of these factors are required to develop a well-defined TGCT risk profile for screening purposes.

Research has been done on a noninvasive method for the detection of CIS cells in semen for many years. Diagnostic methods like cytological examination and IHC with cytoplasmic and nucleus markers, like OCT3/4, AP-2c, NANOG, *etc*., have been assessed. None of these semen-based methods for CIS cell detection proved to be sufficiently valid, due to the high number of false positive results. A promising noninvasive method for CIS screening seems to be the detection of specific TGCT miRNAs in semen, because there was demonstrated that miRNAs of the miR-371~373 and miR-302/367 clusters are highly overexpressed in serum in all TGCT and specific miRNA associated to infertility has already been found in semen.

In summary, a screening method for population at increased risk for TGCT is needed to diagnose men at the CIS-stage, such that they do not have to undergo systemic treatment and suffer from the related long-term effects. Further research is needed to develop a well-defined TGCT risk profile, based on environmental interactions, and a noninvasive detection method, in which the miRNA detection in semen seems to be very promising.

REFERENCES

1 Friedman NB. Tumors of the testis. *Am J Pathol* 1946; 22: 635–7.
2 Runyan C, Schaible K, Molyneaux K, Wang Z, Levin L, *et al*. Steel factor controls midline cell death of primordial germ cells and is essential for their normal proliferation and migration. *Development* 2006; 133: 4861–9.
3 Donovan PJ. Growth factor regulation of mouse primordial germ cell development. *Curr Top Dev Biol* 1994; 29: 189–225.
4 Wylie C. Germ cells. *Cell* 1999; 96: 165–74.
5 Ulbright TM. Germ cell tumors of the gonads: a selective review emphasizing problems in differential diagnosis, newly appreciated, and controversial issues. *Mod Pathol* 2005; 18 Suppl 2: S61–79.
6 Looijenga LH. Human testicular (non) seminomatous germ cell tumours: the clinical implications of recent pathobiological insights. *J Pathol* 2009; 218: 146–62.
7 Mostofi FK, Sesterhenn IA, Davis CJ Jr. Immunopathology of germ cell tumors of the testis. *Semin Diagn Pathol* 1987; 4: 320–41.
8 Grigor KM. A new classification of germ cell tumours of the testis. *Eur Urol* 1993; 23: 93–100.
9 Oosterhuis JW, Looijenga LH. Testicular germ-cell tumours in a broader perspective. *Nat Rev Cancer* 2005; 5: 210–22.
10 Reuter VE. Origins and molecular biology of testicular germ cell tumors. *Mod Pathol* 2005; 18 Suppl 2: S51–60.
11 Ye H, Ulbright TM. Difficult differential diagnoses in testicular pathology. *Arch Pathol*

Lab Med 2012; 136: 435–46.
12 Skakkebaek NE, Berthelsen JG, Giwercman A, Müller J. Carcinoma-*in-situ* of the testis: possible origin from gonocytes and precursor of all types of germ cell tumours except spermatocytoma. *Int J Androl* 1987; 10: 19–28.
13 Honecker F, Stoop H, Mayer F, Bokemeyer C, Castrillon DH, *et al.* Germ cell lineage differentiation in non-seminomatous germ cell tumours. *J Pathol* 2006; 208: 395–400.
14 Huyghe E, Matsuda T, Thonneau P. Increasing incidence of testicular cancer worldwide: a review. *J Urol* 2003; 170: 5–11.
15 Bergström R, Adami HO, Möhner M, Zatonski W, Storm H, *et al.* Increase in testicular cancer incidence in six European countries: a birth cohort phenomenon. *J Natl Cancer Inst* 1996; 88: 727–33.
16 Shanmugalingam T, Soultati A, Chowdhury S, Rudman S, Van Hemelrijck M. Global incidence and outcome of testicular cancer. *Clin Epidemiol* 2013; 5: 417–27.
17 Garner MJ, Turner MC, Ghadirian P, Krewski D. Epidemiology of testicular cancer: an overview. *Int J Cancer* 2005; 116: 331–9.
18 Richiardi L, Pettersson A, Akre O. Genetic and environmental risk factors for testicular cancer. *Int J Androl* 2007; 30: 230–40.
19 Looijenga LH, Stoop H, Biermann K. Testicular cancer: biology and biomarkers. *Virchows Arch* 2014; 464: 301–13.
20 Skakkebaek NE. Possible carcinoma-*in-situ* of the testis. *Lancet* 1972; 2: 516–7.
21 Almstrup K, Ottesen AM, Sonne SB, Hoei-Hansen CE, Leffers H, *et al.* Genomic and gene expression signature of the pre-invasive testicular carcinoma *in situ*. *Cell Tissue Res* 2005; 322: 159–65.
22 Jørgensen N, Müller J, Giwercman A, Skakkebaek NE. Clinical and biological significance of carcinoma *in situ* of the testis. *Cancer Surv* 1990; 9: 287–302.
23 Giwercman A, Müller J, Skakkebaek NE. Prevalence of carcinoma *in situ* and other histopathological abnormalities in testes from 399 men who died suddenly and unexpectedly. *J Urol* 1991; 145: 77–80.
24 Schmoll HJ, Souchon R, Krege S, Albers P, Beyer J, *et al.* European consensus on diagnosis and treatment of germ cell cancer: a report of the European Germ Cell Cancer Consensus Group (EGCCCG). *Ann Oncol* 2004; 15: 1377–99.
25 Einhorn LH, Donohue J. Cis-diamminedichloroplatinum, vinblastine, and bleomycin combination chemotherapy in disseminated testicular cancer. *Ann Intern Med* 1977; 87: 293–8.
26 Orre IJ, Fosså SD, Murison R, Bremnes R, Dahl O, *et al.* Chronic cancer-related fatigue in long-term survivors of testicular cancer. *J Psychosom Res* 2008; 64: 363–71.
27 Skaali T, Fosså SD, Andersson S, Cvancarova M, Langberg CW, *et al.* Self-reported cognitive problems in testicular cancer patients: relation to neuropsychological performance, fatigue, and psychological distress. *J Psychosom Res* 2011; 70: 403–10.
28 Huddart RA, Norman A, Shahidi M, Horwich A, Coward D, *et al.* Cardiovascular disease as a long-term complication of treatment for testicular cancer. *J Clin Oncol* 2003; 21: 1513–23.
29 Altena R, Hummel YM, Nuver J, Smit AJ, Lefrandt JD, *et al.* Longitudinal changes in cardiac function after cisplatin-based chemotherapy for testicular cancer. *Ann Oncol* 2011; 22: 2286–93.
30 de Haas EC, Altena R, Boezen HM, Zwart N, Smit AJ, *et al.* Early development of the metabolic syndrome after chemotherapy for testicular cancer. *Ann Oncol* 2013; 24: 749–55.
31 Huddart RA, Norman A, Moynihan C, Horwich A, Parker C, *et al.* Fertility, gonadal and sexual function in survivors of testicular cancer. *Br J Cancer* 2005; 93: 200–7.
32 Brydøy M, Fosså SD, Klepp O, Bremnes RM, Wist EA, *et al.* Paternity following treatment for testicular cancer. *J Natl Cancer Inst* 2005; 97: 1580–8.
33 Alanee SR, Feldman DR, Russo P, Konety B. Long-term mortality in patients with germ cell tumors: effect of primary cancer site on cause of death. *Urol Oncol* 2014; 32: 26.e9–15.
34 Zagars GK, Ballo MT, Lee AK, Strom SS. Mortality after cure of testicular seminoma. *J Clin Oncol* 2004; 22: 640–7.
35 Travis LB, Curtis RE, Storm H, Hall P, Holowaty E, *et al.* Risk of second malignant neoplasms among long-term survivors of testicular cancer. *J Natl Cancer Inst* 1997; 89: 1429–39.
36 Haugnes HS, Bosl GJ, Boer H, Gietema JA, Brydøy M, *et al.* Long-term and late effects of germ cell testicular cancer treatment and implications for follow-up. *J Clin Oncol* 2012; 30: 3752–63.
37 Dieckmann KP, Skakkebaek NE. Carcinoma *in situ* of the testis: review of biological and clinical features. *Int J Cancer* 1999; 83: 815–22.
38 Dieckmann KP, Wilken S, Loy V, Matthies C, Kleinschmidt K, *et al.* Treatment of testicular intraepithelial neoplasia (intratubular germ cell neoplasia unspecified) with local radiotherapy or with platinum-based chemotherapy: a survey of the German Testicular Cancer Study Group. *Ann Oncol* 2013; 24: 1332–7.
39 Beyer J, Albers P, Altena R, Aparicio J, Bokemeyer C, *et al.* Maintaining success, reducing treatment burden, focusing on survivorship: highlights from the third European consensus conference on diagnosis and treatment of germ-cell cancer. *Ann Oncol* 2013; 24: 878–88.
40 Hoei-Hansen CE. Application of stem cell markers in search for neoplastic germ cells in dysgenetic gonads, extragonadal tumours, and in semen of infertile men. *Cancer Treat Rev* 2008; 34: 348–67.
41 Lip SZ, Murchison LE, Cullis PS, Govan L, Carachi R. A meta-analysis of the risk of boys with isolated cryptorchidism developing testicular cancer in later life. *Arch Dis Child* 2013; 98: 20–6.
42 Møller H, Skakkebaek NE. Testicular cancer and cryptorchidism in relation to prenatal factors: case-control studies in Denmark. *Cancer Causes Control* 1997; 8: 904–12.
43 Møller H, Prener A, Skakkebaek NE. Testicular cancer, cryptorchidism, inguinal hernia, testicular atrophy, and genital malformations: case-control studies in Denmark. *Cancer Causes Control* 1996; 7: 264–74.
44 Jacobsen R, Bostofte E, Engholm G, Hansen J, Olsen JH, *et al.* Risk of testicular cancer in men with abnormal semen characteristics: cohort study. *BMJ* 2000; 321: 789–92.
45 Jørgensen N, Vierula M, Jacobsen R, Pukkala E, Perheentupa A, *et al.* Recent adverse trends in semen quality and testis cancer incidence among Finnish men. *Int J Androl* 2011; 34: e37–48.
46 Colls BM, Harvey VJ, Skelton L, Thompson PI, Frampton CM. Bilateral germ cell testicular tumors in New Zealand: experience in Auckland and Christchurch 1978-1994. *J Clin Oncol* 1996; 14: 2061–5.
47 Osterlind A, Berthelsen JG, Abildgaard N, Hansen SO, Hjalgrim H, *et al.* Risk of bilateral testicular germ cell cancer in Denmark: 1960-1984. *J Natl Cancer Inst* 1991; 83: 1391–5.
48 Mai PL, Chen BE, Tucker K, Friedlander M, Phillips KA, *et al.* Younger age-at-diagnosis for familial malignant testicular germ cell tumor. *Fam Cancer* 2009; 8: 451–6.
49 Mai PL, Friedlander M, Tucker K, Phillips KA, Hogg D, *et al.* The International Testicular Cancer Linkage Consortium: a clinicopathologic descriptive analysis of 461 familial malignant testicular germ cell tumor kindred. *Urol Oncol* 2010; 28: 492–9.
50 Béranger R, Le Cornet C, Schüz J, Fervers B. Occupational and environmental exposures associated with testicular germ cell tumours: systematic review of prenatal and life-long exposures. *PLoS One* 2013; 8: e77130.
51 Cook MB, Akre O, Forman D, Madigan MP, Richiardi L, *et al.* A systematic review and meta-analysis of perinatal variables in relation to the risk of testicular cancer – experiences of the mother. *Int J Epidemiol* 2009; 38: 1532–42.
52 Chung CC, Kanetsky PA, Wang Z, Hildebrandt MA, Koster R, *et al.* Meta-analysis identifies four new loci associated with testicular germ cell tumor. *Nat Genet* 2013; 45: 680–5.
53 LeBron C, Pal P, Brait M, Dasgupta S, Guerrero-Preston R, *et al.* Genome-wide analysis of genetic alterations in testicular primary seminoma using high resolution single nucleotide polymorphism arrays. *Genomics* 2011; 97: 341–9.
54 Rapley EA, Turnbull C, Al Olama AA, Dermitzakis ET, Linger R, *et al.* A genome-wide association study of testicular germ cell tumor. *Nat Genet* 2009; 41: 807–10.
55 Kristensen DM, Sonne SB, Ottesen AM, Perrett RM, Nielsen JE, *et al.* Origin of pluripotent germ cell tumours: the role of microenvironment during embryonic development. *Mol Cell Endocrinol* 2008; 288: 111–8.
56 Molyneaux K, Wylie C. Primordial germ cell migration. *Int J Dev Biol* 2004; 48: 537–44.
57 Fujimoto T, Ukeshima A, Miyayama Y, Kuwana T, Yoshinaga K, *et al.* The primordial germ cells in amniotes: their migration *in vivo* and behaviors *in vitro*. *Prog Clin Biol Res* 1989; 296: 13–21.
58 Horie K, Fujita J, Takakura K, Kanzaki H, Suginami H, *et al.* The expression of c-kit protein in human adult and fetal tissues. *Hum Reprod* 1993; 8: 1955–62.
59 Rijlaarsdam MA, Looijenga LH. An oncofetal and developmental perspective on testicular germ cell cancer. *Semin Cancer Biol* 2014; 29C: 59–74.
60 Gillis AJ, Stoop H, Biermann K, van Gurp RJ, Swartzman E, *et al.* Expression and interdependencies of pluripotency factors LIN28, OCT3/4, NANOG and SOX2 in human testicular germ cells and tumours of the testis. *Int J Androl* 2011; 34: e160–74.
61 Hoei-Hansen CE, Nielsen JE, Almstrup K, Sonne SB, Graem N, *et al.* Transcription factor AP-2gamma is a developmentally regulated marker of testicular carcinoma *in situ* and germ cell tumors. *Clin Cancer Res* 2004; 10: 8521–30.
62 Yeom YI, Fuhrmann G, Ovitt CE, Brehm A, Ohbo K, *et al.* Germline regulatory element of Oct-4 specific for the totipotent cycle of embryonal cells. *Development* 1996; 122: 881–94.
63 Looijenga LH, Stoop H, de Leeuw HP, de Gouveia Brazao CA, Gillis AJ, *et al.* POU5F1 (OCT3/4) identifies cells with pluripotent potential in human germ cell tumors. *Cancer Res* 2003; 63: 2244–50.
64 Hustin J, Collette J, Franchimont P. Immunohistochemical demonstration of placental alkaline phosphatase in various states of testicular development and in germ cell tumours. *Int J Androl* 1987; 10: 29–35.
65 de Jong J, Stoop H, Gillis AJ, van Gurp RJ, van de Geijn GJ, *et al.* Differential expression of SOX17 and SOX2 in germ cells and stem cells has biological and clinical implications. *J Pathol* 2008; 215: 21–30.
66 Jørgensen N, Rajpert-De Meyts E, Graem N, Müller J, Giwercman A, *et al.* Expression of immunohistochemical markers for testicular carcinoma *in situ* by normal human fetal germ cells. *Lab Invest* 1995; 72: 223–31.
67 Honecker F, Stoop H, de Krijger RR, Chris Lau YF, Bokemeyer C, *et al.* Pathobiological implications of the expression of markers of testicular carcinoma *in situ* by fetal germ cells. *J Pathol* 2004; 203: 849–57.
68 Rajpert-De Meyts E, Hanstein R, Jørgensen N, Graem N, Vogt PH, *et al.* Developmental expression of POU5F1 (OCT-3/4) in normal and dysgenetic human gonads. *Hum Reprod* 2004; 19: 1338–44.

69 Koopman P. Sry and Sox 9: mammalian testis-determining genes. *Cell Mol Life Sci* 1999; 55: 839–56.
70 Capel B, Albrecht KH, Washburn LL, Eicher EM. Migration of mesonephric cells into the mammalian gonad depends on Sry. *Mech Dev* 1999; 84: 127–31.
71 Martineau J, Nordqvist K, Tilmann C, Lovell-Badge R, Capel B. Male-specific cell migration into the developing gonad. *Curr Biol* 1997; 7: 958–68.
72 Strohmeyer T, Reese D, Press M, Ackermann R, Hartmann M, *et al*. Expression of the c-kit proto-oncogene and its ligand stem cell factor (SCF) in normal and malignant human testicular tissue. *J Urol* 1995; 153: 511–5.
73 Mauduit C, Hamamah S, Benahmed M. Stem cell factor/c-kit system in spermatogenesis. *Hum Reprod Update* 1999; 5: 535–45.
74 Castrillon DH, Quade BJ, Wang TY, Quigley C, Crum CP. The human VASA gene is specifically expressed in the germ cell lineage. *Proc Natl Acad Sci U S A* 2000; 97: 9585–90.
75 Schnieders F, Dörk T, Arnemann J, Vogel T, Werner M, *et al*. Testis-specific protein, Y-encoded (TSPY) expression in testicular tissues. *Hum Mol Genet* 1996; 5: 1801–7.
76 Lau YF. Gonadoblastoma, testicular and prostate cancers, and the TSPY gene. *Am J Hum Genet* 1999; 64: 921–7.
77 Skakkebaek NE, Rajpert-De Meyts E, Main KM. Testicular dysgenesis syndrome: an increasingly common developmental disorder with environmental aspects. *Hum Reprod* 2001; 16: 972–8.
78 Rajpert-de Meyts E, Hoei-Hansen CE. From gonocytes to testicular cancer: the role of impaired gonadal development. *Ann N Y Acad Sci* 2007; 1120: 168–80.
79 Sharpe RM, Skakkebaek NE. Testicular dysgenesis syndrome: mechanistic insights and potential new downstream effects. *Fertil Steril* 2008; 89: e33–8.
80 Looijenga LH, Gillis AJ, Stoop H, Biermann K, Oosterhuis JW. Dissecting the molecular pathways of (testicular) germ cell tumour pathogenesis; from initiation to treatment-resistance. *Int J Androl* 2011; 34: e234–51.
81 Kanetsky PA, Mitra N, Vardhanabhuti S, Li M, Vaughn DJ, *et al*. Common variation in KITLG and at 5q31.3 predisposes to testicular germ cell cancer. *Nat Genet* 2009; 41: 811–5.
82 Looijenga LH, de Leeuw H, van Oorschot M, van Gurp RJ, Stoop H, *et al*. Stem cell factor receptor (c-KIT) codon 816 mutations predict development of bilateral testicular germ-cell tumors. *Cancer Res* 2003; 63: 7674–8.
83 Rapley EA, Nathanson KL. Predisposition alleles for testicular germ cell tumour. *Curr Opin Genet Dev* 2010; 20: 225–30.
84 Zeeman AM, Stoop H, Boter M, Gillis AJ, Castrillon DH, *et al*. VASA is a specific marker for both normal and malignant human germ cells. *Lab Invest* 2002; 82: 159–66.
85 Jørgensen N, Giwercman A, Müller J, Skakkebaek NE. Immunohistochemical markers of carcinoma *in situ* of the testis also expressed in normal infantile germ cells. *Histopathology* 1993; 22: 373–8.
86 Di Vizio D, Cito L, Boccia A, Chieffi P, Insabato L, *et al*. Loss of the tumor suppressor gene PTEN marks the transition from intratubular germ cell neoplasias (ITGCN) to invasive germ cell tumors. *Oncogene* 2005; 24: 1882–94.
87 Looijenga LH, Van Agthoven T, Biermann K. Development of malignant germ cells - The genvironmental hypothesis. *Int J Dev Biol* 2013; 57: 241–53.
88 van der Zwan YG, Biermann K, Wolffenbuttel KP, Cools M, Looijenga LH. Gonadal maldevelopment as risk factor for germ cell cancer: towards a clinical decision model. *Eur Urol* 2014 Sep 17. doi: 10.1016/j.eururo.2014.07.011. [Epub ahead of print].
89 Hemminki K, Li X. Cancer risks in Nordic immigrants and their offspring in Sweden. *Eur J Cancer* 2002; 38: 2428–34.
90 Gajendran VK, Nguyen M, Ellison LM. Testicular cancer patterns in African-American men. *Urology* 2005; 66: 602–5.
91 McGlynn KA, Devesa SS, Graubard BI, Castle PE. Increasing incidence of testicular germ cell tumors among black men in the United States. *J Clin Oncol* 2005; 23: 5757–61.
92 Kolonel LN, Altshuler D, Henderson BE. The multiethnic cohort study: exploring genes, lifestyle and cancer risk. *Nat Rev Cancer* 2004; 4: 519–27.
93 Chia VM, Li Y, Goldin LR, Graubard BI, Greene MH, *et al*. Risk of cancer in first- and second-degree relatives of testicular germ cell tumor cases and controls. *Int J Cancer* 2009; 124: 952–7.
94 Czene K, Lichtenstein P, Hemminki K. Environmental and heritable causes of cancer among 9.6 million individuals in the Swedish family-cancer database. *Int J Cancer* 2002; 99: 260–6.
95 Dong C, Hemminki K. Modification of cancer risks in offspring by sibling and parental cancers from 2,112,616 nuclear families. *Int J Cancer* 2001; 92: 144–50.
96 Krausz C, Looijenga LH. Genetic aspects of testicular germ cell tumors. *Cell Cycle* 2008; 7: 3519–24.
97 Bromen K, Stang A, Baumgardt-Elms C, Stegmaier C, Ahrens W, *et al*. Testicular, other genital, and breast cancers in first-degree relatives of testicular cancer patients and controls. *Cancer Epidemiol Biomarkers Prev* 2004; 13: 1316–24.
98 Sonneveld DJ, Sleijfer DT, Schrafford Koops H, Sijmons RH, van der Graaf WT, *et al*. Familial testicular cancer in a single-centre population. *Eur J Cancer* 1999; 35: 1368–73.
99 Heimdal K, Olsson H, Tretli S, Flodgren P, Børresen AL, *et al*. Familial testicular cancer in Norway and southern Sweden. *Br J Cancer* 1996; 73: 964–9.
100 Forman D, Oliver RT, Brett AR, Marsh SG, Moses JH, *et al*. Familial testicular cancer: a report of the UK family register, estimation of risk and an HLA class 1 sib-pair analysis. *Br J Cancer* 1992; 65: 255–62.
101 Westergaard T, Olsen JH, Frisch M, Kroman N, Nielsen JW, *et al*. Cancer risk in fathers and brothers of testicular cancer patients in Denmark. A population-based study. *Int J Cancer* 1996; 66: 627–31.
102 Gundy S, Babosa M, Baki M, Bodrogi I. Increased predisposition to cancer in brothers and offspring of testicular tumor patients. *Pathol Oncol Res* 2004; 10: 197–203.
103 Hemminki K, Chen B. Familial risks in testicular cancer as aetiological clues. *Int J Androl* 2006; 29: 205–10.
104 Spermon JR, Witjes JA, Nap M, Kiemeney LA. Cancer incidence in relatives of patients with testicular cancer in the eastern part of the Netherlands. *Urology* 2001; 57: 747–52.
105 Neale RE, Carrière P, Murphy MF, Baade PD. Testicular cancer in twins: a meta-analysis. *Br J Cancer* 2008; 98: 171–3.
106 Swerdlow AJ, De Stavola BL, Swanwick MA, Maconochie NE. Risks of breast and testicular cancers in young adult twins in England and Wales: evidence on prenatal and genetic aetiology. *Lancet* 1997; 350: 1723–8.
107 Braun MM, Ahlbom A, Floderus B, Brinton LA, Hoover RN. Effect of twinship on incidence of cancer of the testis, breast, and other sites (Sweden). *Cancer Causes Control* 1995; 6: 519–24.
108 Swerdlow AJ, De Stavola BL, Swanwick MA, Mangtani P, Maconochie NE. Risk factors for testicular cancer: a case-control study in twins. *Br J Cancer* 1999; 80: 1098–102.
109 Nathanson KL, Kanetsky PA, Hawes R, Vaughn DJ, Letrero R, *et al*. The Y deletion gr/gr and susceptibility to testicular germ cell tumor. *Am J Hum Genet* 2005; 77: 1034–43.
110 Turnbull C, Rapley EA, Seal S, Pernet D, Renwick A, *et al*. Variants near DMRT1, TERT and ATF7IP are associated with testicular germ cell cancer. *Nat Genet* 2010; 42: 604–7.
111 Kanetsky PA, Mitra N, Vardhanabhuti S, Vaughn DJ, Li M, *et al*. A second independent locus within DMRT1 is associated with testicular germ cell tumor susceptibility. *Hum Mol Genet* 2011; 20: 3109–17.
112 Roskoski R Jr. Signaling by Kit protein-tyrosine kinase – The stem cell factor receptor. *Biochem Biophys Res Commun* 2005; 337: 1–13.
113 Boldajipour B, Raz E. What is left behind – Quality control in germ cell migration. *Sci STKE* 2007; 2007: pe16.
114 Gilbert D, Rapley E, Shipley J. Testicular germ cell tumours: predisposition genes and the male germ cell niche. *Nat Rev Cancer* 2011; 11: 278–88.
115 Rapley EA, Hockley S, Warren W, Johnson L, Huddart R, *et al*. Somatic mutations of KIT in familial testicular germ cell tumours. *Br J Cancer* 2004; 90: 2397–401.
116 Bokemeyer C, Kuczyk MA, Dunn T, Serth J, Hartmann K, *et al*. Expression of stem-cell factor and its receptor c-kit protein in normal testicular tissue and malignant germ-cell tumours. *J Cancer Res Clin Oncol* 1996; 122: 301–6.
117 Izquierdo MA, Van der Valk P, Van Ark-Otte J, Rubio G, Germa-Lluch JR, *et al*. Differential expression of the c-kit proto-oncogene in germ cell tumours. *J Pathol* 1995; 177: 253–8.
118 McIntyre A, Summersgill B, Grygalewicz B, Gillis AJ, Stoop J, *et al*. Amplification and overexpression of the KIT gene is associated with progression in the seminoma subtype of testicular germ cell tumors of adolescents and adults. *Cancer Res* 2005; 65: 8085–9.
119 Kemmer K, Corless CL, Fletcher JA, McGreevey L, Haley A, *et al*. KIT mutations are common in testicular seminomas. *Am J Pathol* 2004; 164: 305–13.
120 Galan JJ, De Felici M, Buch B, Rivero MC, Segura A, *et al*. Association of genetic markers within the KIT and KITLG genes with human male infertility. *Hum Reprod* 2006; 21: 3185–92.
121 Sasaki A, Taketomi T, Kato R, Saeki K, Nonami A, *et al*. Mammalian Sprouty4 suppresses Ras-independent ERK activation by binding to Raf1. *Nat Cell Biol* 2003; 5: 427–32.
122 Tsumura Y, Toshima J, Leeksma OC, Ohashi K, Mizuno K. Sprouty-4 negatively regulates cell spreading by inhibiting the kinase activity of testicular protein kinase. *Biochem J* 2005; 387: 627–37.
123 Yan W, Samson M, Jégou B, Toppari J. Bcl-w forms complexes with Bax and Bak, and elevated ratios of Bax/Bcl-w and Bak/Bcl-w correspond to spermatogonial and spermatocyte apoptosis in the testis. *Mol Endocrinol* 2000; 14: 682–99.
124 Ferguson-Smith M. The evolution of sex chromosomes and sex determination in vertebrates and the key role of DMRT1. *Sex Dev* 2007; 1: 2–11.
125 Smith CA, McClive PJ, Western PS, Reed KJ, Sinclair AH. Conservation of a sex-determining gene. *Nature* 1999; 402: 601–2.
126 Raymond CS, Shamu CE, Shen MM, Seifert KJ, Hirsch B, *et al*. Evidence for evolutionary conservation of sex-determining genes. *Nature* 1998; 391: 691–5.
127 Krentz AD, Murphy MW, Kim S, Cook MS, Capel B, *et al*. The DM domain protein DMRT1 is a dose-sensitive regulator of fetal germ cell proliferation and pluripotency. *Proc Natl Acad Sci U S A* 2009; 106: 22323–8.
128 Feldser DM, Hackett JA, Greider CW. Telomere dysfunction and the initiation of genome instability. *Nat Rev Cancer* 2003; 3: 623–7.
129 Hackett JA, Feldser DM, Greider CW. Telomere dysfunction increases mutation rate and genomic instability. *Cell* 2001; 106: 275–86.
130 Wu X, Amos CI, Zhu Y, Zhao H, Grossman BH, *et al*. Telomere dysfunction: a potential

cancer predisposition factor. *J Natl Cancer Inst* 2003; 95: 1211–8.
131 Fernandez-Garcia I, Ortiz-de-Solorzano C, Montuenga LM. Telomeres and telomerase in lung cancer. *J Thorac Oncol* 2008; 3: 1085–8.
132 Schrader M, Burger AM, Müller M, Krause H, Straub B, *et al*. Quantification of human telomerase reverse transcriptase mRNA in testicular germ cell tumors by quantitative fluorescence real-time RT-PCR. *Oncol Rep* 2002; 9: 1097–105.
133 Schrader M, Burger AM, Müller M, Krause H, Straub B, *et al*. The differentiation status of primary gonadal germ cell tumors correlates inversely with telomerase activity and the expression level of the gene encoding the catalytic subunit of telomerase. *BMC Cancer* 2002; 2: 32.
134 Liu L, Ishihara K, Ichimura T, Fujita N, Hino S, *et al*. MCAF1/AM is involved in Sp1-mediated maintenance of cancer-associated telomerase activity. *J Biol Chem* 2009; 284: 5165–74.
135 Moniot B, Farhat A, Aritake K, Declosmenil F, Nef S, *et al*. Hematopoietic prostaglandin D synthase (H-Pgds) is expressed in the early embryonic gonad and participates to the initial nuclear translocation of the SOX9 protein. *Dev Dyn* 2011; 240: 2335–43.
136 Park JM, Kanaoka Y, Eguchi N, Aritake K, Grujic S, *et al*. Hematopoietic prostaglandin D synthase suppresses intestinal adenomas in ApcMin/+ mice. *Cancer Res* 2007; 67: 881–9.
137 Kim S, Sun H, Tomchick DR, Yu H, Luo X. Structure of human Mad1 C-terminal domain reveals its involvement in kinetochore targeting. *Proc Natl Acad Sci U S A* 2012; 109: 6549–54.
138 International HapMap Consortium, Altshuler DM, Gibbs RA, Peltonen L, Altshuler DM, *et al*. Integrating common and rare genetic variation in diverse human populations. *Nature* 2010; 467: 52–8.
139 Fu X, Yucer N, Liu S, Li M, Yi P, *et al*. RFWD3-Mdm2 ubiquitin ligase complex positively regulates p53 stability in response to DNA damage. *Proc Natl Acad Sci U S A* 2010; 107: 4579–84.
140 Liu S, Chu J, Yucer N, Leng M, Wang SY, *et al*. RING finger and WD repeat domain 3 (RFWD3) associates with replication protein A (RPA) and facilitates RPA-mediated DNA damage response. *J Biol Chem* 2011; 286: 22314–22.
141 McGlynn KA, Cook MB. Etiologic factors in testicular germ-cell tumors. *Future Oncol* 2009; 5: 1389–402.
142 Dieckmann KP, Pichlmeier U. Clinical epidemiology of testicular germ cell tumors. *World J Urol* 2004; 22: 2–14.
143 Cook MB, Akre O, Forman D, Madigan MP, Richiardi L, *et al*. A systematic review and meta-analysis of perinatal variables in relation to the risk of testicular cancer – Experiences of the son. *Int J Epidemiol* 2010; 39: 1605–18.
144 Henderson BE, Benton B, Jing J, Yu MC, Pike MC. Risk factors for cancer of the testis in young men. *Int J Cancer* 1979; 23: 598–602.
145 Jensen TK, Toppari J, Keiding N, Skakkebaek NE. Do environmental estrogens contribute to the decline in male reproductive health? *Clin Chem* 1995; 41: 1896–901.
146 Leary FJ, Resseguie LJ, Kurland LT, O'Brien PC, Emslander RF, *et al*. Males exposed in utero to diethylstilbestrol. *JAMA* 1984; 252: 2984–9.
147 Strohsnitter WC, Noller KL, Hoover RN, Robboy SJ, Palmer JR, *et al*. Cancer risk in men exposed in utero to diethylstilbestrol. *J Natl Cancer Inst* 2001; 93: 545–51.
148 Baron JA, La Vecchia C, Levi F. The antiestrogenic effect of cigarette smoking in women. *Am J Obstet Gynecol* 1990; 162: 502–14.
149 Brown LM, Pottern LM, Hoover RN. Prenatal and perinatal risk factors for testicular cancer. *Cancer Res* 1986; 46: 4812–6.
150 Coupland CA, Forman D, Chilvers CE, Davey G, Pike MC, *et al*. Maternal risk factors for testicular cancer: a population-based case-control study (UK). *Cancer Causes Control* 2004; 15: 277–83.
151 Pettersson A, Kaijser M, Richiardi L, Askling J, Ekbom A, *et al*. Women smoking and testicular cancer: one epidemic causing another? *Int J Cancer* 2004; 109: 941–4.
152 Weir HK, Marrett LD, Kreiger N, Darlington GA, Sugar L. Pre-natal and peri-natal exposures and risk of testicular germ-cell cancer. *Int J Cancer* 2000; 87: 438–43.
153 Clemmesen J. Is pregnancy smoking causal to testis cancer in sons? A hypothesis. *Acta Oncol* 1997; 36: 59–63.
154 McGlynn KA, Zhang Y, Sakoda LC, Rubertone MV, Erickson RL, *et al*. Maternal smoking and testicular germ cell tumors. *Cancer Epidemiol Biomarkers Prev* 2006; 15: 1820–4.
155 Boyle P, Zaridze DG. Risk factors for prostate and testicular cancer. *Eur J Cancer* 1993; 29A: 1048–55.
156 Swerdlow AJ, Higgins CD, Pike MC. Risk of testicular cancer in cohort of boys with cryptorchidism. *BMJ* 1997; 314: 1507–11.
157 Schottenfeld D, Warshauer ME, Sherlock S, Zauber AG, Leder M, *et al*. The epidemiology of testicular cancer in young adults. *Am J Epidemiol* 1980; 112: 232–46.
158 Giwercman A, Grindsted J, Hansen B, Jensen OM, Skakkebaek NE. Testicular cancer risk in boys with maldescended testis: a cohort study. *J Urol* 1987; 138: 1214–6.
159 Moss AR, Osmond D, Bacchetti P, Torti FM, Gurgin V. Hormonal risk factors in testicular cancer. A case-control study. *Am J Epidemiol* 1986; 124: 39–52.
160 Social, behavioural and medical factors in the aetiology of testicular cancer: results from the UK study. UK Testicular Cancer Study Group. *Br J Cancer* 1994; 70: 513–20.
161 Prener A, Engholm G, Jensen OM. Genital anomalies and risk for testicular cancer in Danish men. *Epidemiology* 1996; 7: 14–9.
162 Coupland CA, Chilvers CE, Davey G, Pike MC, Oliver RT, *et al*. Risk factors for testicular germ cell tumours by histological tumour type. United Kingdom Testicular Cancer Study Group. *Br J Cancer* 1999; 80: 1859–63.
163 Møller H, Skakkebaek NE. Risks of testicular cancer and cryptorchidism in relation to socio-economic status and related factors: case-control studies in Denmark. *Int J Cancer* 1996; 66: 287–93.
164 Swerdlow AJ, Huttly SR, Smith PG. Testis cancer: post-natal hormonal factors, sexual behaviour and fertility. *Int J Cancer* 1989; 43: 549–53.
165 Weir HK, Kreiger N, Marrett LD. Age at puberty and risk of testicular germ cell cancer (Ontario, Canada). *Cancer Causes Control* 1998; 9: 253–8.
166 Garner MJ, Birkett NJ, Johnson KC, Shatenstein B, Ghadirian P, *et al*. Dietary risk factors for testicular carcinoma. *Int J Cancer* 2003; 106: 934–41.
167 Akre O, Ekbom A, Sparén P, Tretli S. Body size and testicular cancer. *J Natl Cancer Inst* 2000; 92: 1093–6.
168 Petridou E, Roukas KI, Dessypris N, Aravantinos G, Bafaloukos D, *et al*. Baldness and other correlates of sex hormones in relation to testicular cancer. *Int J Cancer* 1997; 71: 982–5.
169 Davies TW, Prener A, Engholm G. Body size and cancer of the testis. *Acta Oncol* 1990; 29: 287–90.
170 Dieckmann KP, Pichlmeier U. Is risk of testicular cancer related to body size? *Eur Urol* 2002; 42: 564–9.
171 Rasmussen F, Gunnell D, Ekbom A, Hallqvist J, Tynelius P. Birth weight, adult height, and testicular cancer: cohort study of 337,249 Swedish young men. *Cancer Causes Control* 2003; 14: 595–8.
172 Richiardi L, Askling J, Granath F, Akre O. Body size at birth and adulthood and the risk for germ-cell testicular cancer. *Cancer Epidemiol Biomarkers Prev* 2003; 12: 669–73.
173 Haughey BP, Graham S, Brasure J, Zielezny M, Sufrin G, *et al*. The epidemiology of testicular cancer in upstate New York. *Am J Epidemiol* 1989; 130: 25–36.
174 Møller H, Skakkebaek NE. Risk of testicular cancer in subfertile men: case-control study. *BMJ* 1999; 318: 559–62.
175 Colls BM. Causes of testicular cancer. *Lancet* 1998; 351: 214.
176 Nethersell AB, Sikora K. Testicular cancer and social class in East Anglia. *Br J Cancer* 1984; 50: 537–40.
177 Swerdlow AJ, Skeet RG. Occupational associations of testicular cancer in south east England. *Br J Ind Med* 1988; 45: 225–30.
178 Prener A, Hsieh CC, Engholm G, Trichopoulos D, Jensen OM. Birth order and risk of testicular cancer. *Cancer Causes Control* 1992; 3: 265–72.
179 Srivastava A, Kreiger N. Relation of physical activity to risk of testicular cancer. *Am J Epidemiol* 2000; 151: 78–87.
180 Aetiology of testicular cancer: association with congenital abnormalities, age at puberty, infertility, and exercise. United Kingdom Testicular Cancer Study Group. *BMJ* 1994; 308: 1393–9.
181 Thune I, Lund E. Physical activity and the risk of prostate and testicular cancer: a cohort study of 53,000 Norwegian men. *Cancer Causes Control* 1994; 5: 549–56.
182 Andersson E, Nilsson R, Torén K. Testicular cancer among Swedish pulp and paper workers. *Am J Ind Med* 2003; 43: 642–6.
183 Andersson E, Westberg H, Bryngelsson IL, Magnuson A, Persson B. Cancer incidence among Swedish pulp and paper mill workers: a cohort study of sulphate and sulphite mills. *Int Arch Occup Environ Health* 2013; 86: 529–40.
184 Band PR, Le ND, Fang R, Astrakianakis G, Bert J, *et al*. Cohort cancer incidence among pulp and paper mill workers in British Columbia. *Scand J Work Environ Health* 2001; 27: 113–9.
185 Guo J, Kauppinen T, Kyyrönen P, Heikkilä P, Lindbohm ML, *et al*. Risk of esophageal, ovarian, testicular, kidney and bladder cancers and leukemia among finnish workers exposed to diesel or gasoline engine exhaust. *Int J Cancer* 2004; 111: 286–92.
186 Rix BA, Villadsen E, Engholm G, Lynge E. Hodgkin's disease, pharyngeal cancer, and soft tissue sarcomas in Danish paper mill workers. *J Occup Environ Med* 1998; 40: 55–62.
187 Swerdlow AJ, Douglas AJ, Huttly SR, Smith PG. Cancer of the testis, socioeconomic status, and occupation. *Br J Ind Med* 1991; 48: 670–4.
188 Van den Eeden SK, Weiss NS, Strader CH, Daling JR. Occupation and the occurrence of testicular cancer. *Am J Ind Med* 1991; 19: 327–37.
189 Hansen J. Risk for testicular cancer after occupational exposure to plastics. *Int J Cancer* 1999; 82: 911–2.
190 Hardell L, Malmqvist N, Ohlson CG, Westberg H, Eriksson M. Testicular cancer and occupational exposure to polyvinyl chloride plastics: a case-control study. *Int J Cancer* 2004; 109: 425–9.
191 Langård S, Rosenberg J, Andersen A, Heldaas SS. Incidence of cancer among workers exposed to vinyl chloride in polyvinyl chloride manufacture. *Occup Environ Med* 2000; 57: 65–8.
192 Ohlson CG, Hardell L. Testicular cancer and occupational exposures with a focus on xenoestrogens in polyvinyl chloride plastics. *Chemosphere* 2000; 40: 1277–82.
193 Walschaerts M, Muller A, Auger J, Bujan L, Guérin JF, *et al*. Environmental, occupational and familial risks for testicular cancer: a hospital-based case-control study. *Int J Androl* 2007; 30: 222–9.

194 Behrens T, Pohlabeln H, Mester B, Langner I, Schmeisser N, *et al*. Exposure to metal-working fluids in the automobile industry and the risk of male germ cell tumours. *Occup Environ Med* 2012; 69: 224–6.
195 Hobbesland A, Kjuus H, Thelle DS. Study of cancer incidence among 8530 male workers in eight Norwegian plants producing ferrosilicon and silicon metal. *Occup Environ Med* 1999; 56: 625–31.
196 Pollán M, Gustavsson P, Cano MI. Incidence of testicular cancer and occupation among Swedish men gainfully employed in 1970. *Ann Epidemiol* 2001; 11: 554–62.
197 Rhomberg W, Schmoll HJ, Schneider B. High frequency of metalworkers among patients with seminomatous tumors of the testis: a case-control study. *Am J Ind Med* 1995; 28: 79–87.
198 Dement J, Pompeii L, Lipkus IM, Samsa GP. Cancer incidence among union carpenters in New Jersey. *J Occup Environ Med* 2003; 45: 1059–67.
199 Hayes RB, Brown LM, Pottern LM, Gomez M, Kardaun JW, *et al*. Occupation and risk for testicular cancer: a case-control study. *Int J Epidemiol* 1990; 19: 825–31.
200 Tynes T, Andersen A, Langmark F. Incidence of cancer in Norwegian workers potentially exposed to electromagnetic fields. *Am J Epidemiol* 1992; 136: 81–8.
201 Bates MN. Registry-based case-control study of cancer in California firefighters. *Am J Ind Med* 2007; 50: 339–44.
202 Bates MN, Fawcett J, Garrett N, Arnold R, Pearce N, *et al*. Is testicular cancer an occupational disease of fire fighters? *Am J Ind Med* 2001; 40: 263–70.
203 Giles G, Staples M, Berry J. Cancer incidence in Melbourne metropolitan fire brigade members, 1980-1989. *Health Rep* 1993; 5: 33–8.
204 Ma F, Fleming LE, Lee DJ, Trapido E, Gerace TA. Cancer incidence in Florida professional firefighters, 1981 to 1999. *J Occup Environ Med* 2006; 48: 883–8.
205 Stang A, Jöckel KH, Baumgardt-Elms C, Ahrens W. Firefighting and risk of testicular cancer: results from a German population-based case-control study. *Am J Ind Med* 2003; 43: 291–4.
206 Davis RL, Mostofi FK. Cluster of testicular cancer in police officers exposed to hand-held radar. *Am J Ind Med* 1993; 24: 231–3.
207 Finkelstein MM. Cancer incidence among Ontario police officers. *Am J Ind Med* 1998; 34: 157–62.
208 Bullman TA, Watanabe KK, Kang HK. Risk of testicular cancer associated with surrogate measures of agent orange exposure among Vietnam veterans on the agent orange registry. *Ann Epidemiol* 1994; 4: 11–6.
209 Foley S, Middleton S, Stitson D, Mahoney M. The incidence of testicular cancer in Royal Air Force personnel. *Br J Urol* 1995; 76: 495–6.
210 Grayson JK, Lyons TJ. Cancer incidence in United States air force aircrew, 1975-89. *Aviat Space Environ Med* 1996; 67: 101–4.
211 Knoke JD, Gray GC, Garland FC. Testicular cancer and Persian Gulf War service. *Epidemiology* 1998; 9: 648–53.
212 Milanov L, Dimitrov D, Danon S. Cancer incidence in Republic of Bulgaria aircrew, 1964-1994. *Aviat Space Environ Med* 1999; 70: 681–5.
213 Ryder SJ, Crawford PI, Pethybridge RJ. Is testicular cancer an occupational disease? A case-control study of Royal Naval personnel. *J R Nav Med Serv* 1997; 83: 130–46.
214 Tarone RE, Hayes HM, Hoover RN, Rosenthal JF, Brown LM, *et al*. Service in Vietnam and risk of testicular cancer. *J Natl Cancer Inst* 1991; 83: 1497–9.
215 Yamane GK, Johnson R. Testicular carcinoma in U.S. Air Force aviators: a case-control study. *Aviat Space Environ Med* 2003; 74: 846–50.
216 Alavanja MC, Sandler DP, Lynch CF, Knott C, Lubin JH, *et al*. Cancer incidence in the agricultural health study. *Scand J Work Environ Health* 2005; 31 Suppl 1: 39–45.
217 Dich J, Wiklund K, Holm LE. Testicular cancer in pesticide applicators in Swedish agriculture. *Scand J Work Environ Health* 1996; 22: 66.
218 Fleming LE, Bean JA, Rudolph M, Hamilton K. Cancer incidence in a cohort of licensed pesticide applicators in Florida. *J Occup Environ Med* 1999; 41: 279–88.
219 Frost G, Brown T, Harding AH. Mortality and cancer incidence among British agricultural pesticide users. *Occup Med (Lond)* 2011; 61: 303–10.
220 Giannandrea F, Gandini L, Paoli D, Turci R, Figà-Talamanca I. Pesticide exposure and serum organochlorine residuals among testicular cancer patients and healthy controls. *J Environ Sci Health B* 2011; 46: 780–7.
221 Kelleher C, Newell J, MacDonagh-White C, MacHale E, Egan E, *et al*. Incidence and occupational pattern of leukaemias, lymphomas, and testicular tumours in western Ireland over an 11 year period. *J Epidemiol Community Health* 1998; 52: 651–6.
222 Knight JA, Marrett LD, Weir HK. Occupation and risk of germ cell testicular cancer by histologic type in Ontario. *J Occup Environ Med* 1996; 38: 884–90.
223 Nori F, Carbone P, Giordano F, Osborn J, Figà-Talamanca I. Endocrine-disrupting chemicals and testicular cancer: a case-control study. *Arch Environ Occup Health* 2006; 61: 87–95.
224 Zandjani F, Høgsaet B, Andersen A, Langård S. Incidence of cancer among nitrate fertilizer workers. *Int Arch Occup Environ Health* 1994; 66: 189–93.
225 Baumgardt-Elms C, Ahrens W, Bromen K, Boikat U, Stang A, *et al*. Testicular cancer and electromagnetic fields (EMF) in the workplace: results of a population-based case-control study in Germany. *Cancer Causes Control* 2002; 13: 895–902.
226 Floderus B, Stenlund C, Persson T. Occupational magnetic field exposure and site-specific cancer incidence: a Swedish cohort study. *Cancer Causes Control* 1999; 10: 323–32.
227 Stenlund C, Floderus B. Occupational exposure to magnetic fields in relation to male breast cancer and testicular cancer: a Swedish case-control study. *Cancer Causes Control* 1997; 8: 184–91.
228 Biggs ML, Davis MD, Eaton DL, Weiss NS, Barr DB, *et al*. Serum organochlorine pesticide residues and risk of testicular germ cell carcinoma: a population-based case-control study. *Cancer Epidemiol Biomarkers Prev* 2008; 17: 2012–8.
229 Chia VM, Li Y, Quraishi SM, Graubard BI, Figueroa JD, *et al*. Effect modification of endocrine disruptors and testicular germ cell tumour risk by hormone-metabolizing genes. *Int J Androl* 2010; 33: 588–96.
230 Hardell L, Bavel B, Lindström G, Eriksson M, Carlberg M. In utero exposure to persistent organic pollutants in relation to testicular cancer risk. *Int J Androl* 2006; 29: 228–34.
231 McGlynn KA, Quraishi SM, Graubard BI, Weber JP, Rubertone MV, *et al*. Persistent organochlorine pesticides and risk of testicular germ cell tumors. *J Natl Cancer Inst* 2008; 100: 663–71.
232 McGlynn KA, Quraishi SM, Graubard BI, Weber JP, Rubertone MV, *et al*. Polychlorinated biphenyls and risk of testicular germ cell tumors. *Cancer Res* 2009; 69: 1901–9.
233 Purdue MP, Engel LS, Langseth H, Needham LL, Andersen A, *et al*. Prediagnostic serum concentrations of organochlorine compounds and risk of testicular germ cell tumors. *Environ Health Perspect* 2009; 117: 1514–9.
234 Kristensen P, Andersen A, Irgens LM, Bye AS, Vagstad N. Testicular cancer and parental use of fertilizers in agriculture. *Cancer Epidemiol Biomarkers Prev* 1996; 5: 3–9.
235 Møller H. Work in agriculture, childhood residence, nitrate exposure, and testicular cancer risk: a case-control study in Denmark. *Cancer Epidemiol Biomarkers Prev* 1997; 6: 141–4.
236 Sonneveld DJ, Schaapveld M, Sleijfer DT, Meerman GJ, van der Graaf WT, *et al*. Geographic clustering of testicular cancer incidence in the northern part of the Netherlands. *Br J Cancer* 1999; 81: 1262–7.
237 Oosterhuis JW, Stoop H, Dohle G, Boellaard W, van Casteren N, *et al*. A pathologist's view on the testis biopsy. *Int J Androl* 2011; 34: e14–9.
238 Holstein AF, Lauke H. Histologic diagnostics of early testicular germ-cell tumor. *Int J Urol* 1996; 3: 165–72.
239 Rørth M, Rajpert-De Meyts E, Andersson L, Dieckmann KP, Fosså SD, *et al*. Carcinoma *in situ* in the testis. *Scand J Urol Nephrol Suppl* 2000; 205: 166–86.
240 Dieckmann KP, Kulejewski M, Heinemann V, Loy V. Testicular biopsy for early cancer detection – Objectives, technique and controversies. *Int J Androl* 2011; 34: e7–13.
241 Winstanley AM, Mikuz G, Debruyne F, Schulman CC, Parkinson MC, *et al*. Handling and reporting of biopsy and surgical specimens of testicular cancer. *Eur Urol* 2004; 45: 564–73.
242 Krege S, Beyer J, Souchon R, Albers P, Albrecht W, *et al*. European consensus conference on diagnosis and treatment of germ cell cancer: a report of the second meeting of the European Germ Cell Cancer Consensus group (EGCCCG): part I. *Eur Urol* 2008; 53: 478–96.
243 de Jong J, Stoop H, Dohle GR, Bangma CH, Kliffen M, *et al*. Diagnostic value of OCT3/4 for pre-invasive and invasive testicular germ cell tumours. *J Pathol* 2005; 206: 242–9.
244 Jacobsen GK, Nørgaard-Pedersen B. Placental alkaline phosphatase in testicular germ cell tumours and in carcinoma-*in-situ* of the testis. An immunohistochemical study. *Acta Pathol Microbiol Immunol Scand A* 1984; 92: 323–9.
245 Pauls K, Jäger R, Weber S, Wardelmann E, Koch A, *et al*. Transcription factor AP-2gamma, a novel marker of gonocytes and seminomatous germ cell tumors. *Int J Cancer* 2005; 115: 470–7.
246 van Casteren NJ, de Jong J, Stoop H, Steyerberg EW, de Bekker-Grob EW, *et al*. Evaluation of testicular biopsies for carcinoma *in situ*: immunohistochemistry is mandatory. *Int J Androl* 2009; 32: 666–74.
247 Dieckmann KP, Loy V. False-negative biopsies for the diagnosis of testicular intraepithelial neoplasia (TIN) – An update. *Eur Urol* 2003; 43: 516–21.
248 Loy V, Wigand I, Dieckmann KP. Incidence and distribution of carcinoma *in situ* in testes removed for germ cell tumour: possible inadequacy of random testicular biopsy in detecting the condition. *Histopathology* 1990; 16: 198–200.
249 Prym C, Lauke H. Carcinoma-*in situ* of the human testis: tumour cells are distributed focally in the seminiferous tubules. *Andrologia* 1994; 26: 231–4.
250 van Casteren NJ, Boellaard WP, Dohle GR, Weber RF, Kuizinga MC, *et al*. Heterogeneous distribution of ITGCNU in an adult testis: consequences for biopsy-based diagnosis. *Int J Surg Pathol* 2008; 16: 21–4.
251 Dieckmann KP, Heinemann V, Frey U, Pichlmeier U, German Testicular Cancer Study Group. How harmful is contralateral testicular biopsy? – An analysis of serial imaging studies and a prospective evaluation of surgical complications. *Eur Urol* 2005; 48: 662–72.
252 Olesen IA, Hoei-Hansen CE, Skakkebaek NE, Petersen JH, Rajpert-De Meyts E, *et al*. Testicular carcinoma *in situ* in subfertile Danish men. *Int J Androl* 2007; 30: 406–11.
253 Giwercman A, Bruun E, Frimodt-Møller C, Skakkebaek NE. Prevalence of carcinoma *in situ* and other histopathological abnormalities in testes of men with a history of cryptorchidism. *J Urol* 1989; 142: 998–1001.
254 Dieckmann KP, Loy V. Prevalence of contralateral testicular intraepithelial neoplasia in patients with testicular germ cell neoplasms. *J Clin Oncol* 1996; 14: 3126–32.
255 Tan IB, Ang KK, Ching BC, Mohan C, Toh CK, *et al*. Testicular microlithiasis predicts concurrent testicular germ cell tumors and intratubular germ cell neoplasia of unclassified

type in adults: a meta-analysis and systematic review. *Cancer* 2010; 116: 4520–32.
256 van Casteren NJ, Looijenga LH, Dohle GR. Testicular microlithiasis and carcinoma *in situ* overview and proposed clinical guideline. *Int J Androl* 2009; 32: 279–87.
257 Elzinga-Tinke JE, Sirre ME, Looijenga LH, van Casteren N, Wildhagen MF, *et al.* The predictive value of testicular ultrasound abnormalities for carcinoma *in situ* of the testis in men at risk for testicular cancer. *Int J Androl* 2010; 33: 597–603.
258 Doherty FJ, Mullins TL, Sant GR, Drinkwater MA, Ucci AA Jr. Testicular microlithiasis. A unique sonographic appearance. *J Ultrasound Med* 1987; 6: 389–92.
259 Giwercman A, Marks A, Skakkebaek NE. Carcinoma-*in-situ* germ-cells exfoliated from seminiferous epithelium into seminal fluid. *Lancet* 1988; 1: 530.
260 Czaplicki M, Rojewska J, Pykalo R, Szymanska K. Detection of testicular neoplasms by cytological examination of seminal fluid. *J Urol* 1987; 138: 787–8.
261 Rojewska J, Pykalo R, Czaplicki M. Urine and semen cytomorphology in patients with testicular tumors. *Diagn Cytopathol* 1989; 5: 9–13.
262 Howard GC, Hargreave TB, McIntyre MA. Carcinoma *in-situ* of the testis diagnosed on semen cytology. *Clin Radiol* 1989; 40: 323–4.
263 Yu DS, Wang J, Chang SY, Ma CP. Differential diagnosis of testicular mass by cytological examination of seminal fluid collected by ejaculation or prostatic massage. *Eur Urol* 1990; 18: 193–6.
264 Brackenbury ET, Hargreave TB, Howard GC, McIntyre MA. Seminal fluid analysis and fine-needle aspiration cytology in the diagnosis of carcinoma *in situ* of the testis. *Eur Urol* 1993; 23: 123–8.
265 Giwercman A, Marks A, Bailey D, Baumal R, Skakkebaek NE. A monoclonal antibody as a marker for carcinoma *in situ* germ cells of the human adult testis. *APMIS* 1988; 96: 667–70.
266 Meng FJ, Zhou Y, Skakkebaek NE, Marks A, Giwercman A. Detection and enrichment of carcinoma-*in-situ* cells in semen by an immunomagnetic method using monoclonal antibody M2A. *Int J Androl* 1996; 19: 365–70.
267 Giwercman A, Clausen OP, Skakkebaek NE. Carcinoma *in situ* of the testis: aneuploid cells in semen. *Br Med J (Clin Res Ed)* 1988; 296: 1762–4.
268 Giwercman A, Hopman AH, Ramaekers FC, Skakkebaek NE. Carcinoma *in situ* of the testis. Detection of malignant germ cells in seminal fluid by means of *in situ* hybridization. *Am J Pathol* 1990; 136: 497–502.
269 Meng FJ, Zhou Y, Giwercman A, Skakkebaek NE, Geurts van Kessel AD, *et al.* Fluorescence *in situ* hybridization analysis of chromosome 12 anomalies in semen cells from patients with carcinoma *in situ* of the testis. *J Pathol* 1998; 186: 235–9.
270 Salanova M, Gandini L, Lenzi A, Chiarenza C, Filippini A, *et al.* Is hyperdiploidy of immature ejaculated germ cells predictive of testis malignancy? A comparative study in healthy normozoospermic, infertile, and testis tumor suffering subjects. *Lab Invest* 1999; 79: 1127–35.
271 Hoei-Hansen CE, Rajpert-De Meyts E, Carlsen E, Almstrup K, Leffers H, *et al.* A subfertile patient diagnosed with testicular carcinoma *in situ* by immunocytological staining for AP-2gamma in semen samples: case report. *Hum Reprod* 2005; 20: 579–82.
272 Hoei-Hansen CE, Carlsen E, Jorgensen N, Leffers H, Skakkebaek NE, *et al.* Towards a non-invasive method for early detection of testicular neoplasia in semen samples by identification of fetal germ cell-specific markers. *Hum Reprod* 2007; 22: 167–73.
273 van Casteren NJ, Stoop H, Dohle GR, de Wit R, Oosterhuis JW, *et al.* Noninvasive detection of testicular carcinoma *in situ* in semen using OCT3/4. *Eur Urol* 2008; 54: 153–8.
274 Almstrup K, Lippert M, Mogensen HO, Nielsen JE, Hansen JD, *et al.* Screening of subfertile men for testicular carcinoma *in situ* by an automated image analysis-based cytological test of the ejaculate. *Int J Androl* 2011; 34: e21–30.
275 Nielsen JE, Kristensen DM, Almstrup K, Jørgensen A, Olesen IA, *et al.* A novel double staining strategy for improved detection of testicular carcinoma *in situ* cells in human semen samples. *Andrologia* 2012; 44: 78–85.
276 Aubry F, Satie AP, Rioux-Leclercq N, Rajpert-De Meyts E, Spagnoli GC, *et al.* MAGE-A4, a germ cell specific marker, is expressed differentially in testicular tumors. *Cancer* 2001; 92: 2778–85.
277 Satie AP, Auger J, Chevrier C, Le Bon C, Jouannet P, *et al.* Seminal expression of NY-ESO-1 and MAGE-A4 as markers for the testicular cancer. *Int J Androl* 2009; 32: 713–9.
278 Kristensen DM, Nielsen JE, Kalisz M, Dalgaard MD, Audouze K, *et al.* OCT4 and downstream factors are expressed in human somatic urogenital epithelia and in culture of epididymal spheres. *Mol Hum Reprod* 2010; 16: 835–45.
279 He L, Hannon GJ. microRNAs: small RNAs with a big role in gene regulation. *Nat Rev Genet* 2004; 5: 522–31.
280 Karp X, Ambros V. Developmental biology. Encountering microRNAs in cell fate signaling. *Science* 2005; 310: 1288–9.
281 Lee RC, Feinbaum RL, Ambros V. The C. elegans heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14. *Cell* 1993; 75: 843–54.
282 Miska EA. How microRNAs control cell division, differentiation and death. *Curr Opin Genet Dev* 2005; 15: 563–8.
283 Mineno J, Okamoto S, Ando T, Sato M, Chono H, *et al.* The expression profile of microRNAs in mouse embryos. *Nucleic Acids Res* 2006; 34: 1765–71.
284 Shcherbata HR, Hatfield S, Ward EJ, Reynolds S, Fischer KA, *et al.* The microRNA pathway plays a regulatory role in stem cell division. *Cell Cycle* 2006; 5: 172–5.
285 Cummins JM, He Y, Leary RJ, Pagliarini R, Diaz LA Jr, *et al.* The colorectal microRNAome. *Proc Natl Acad Sci U S A* 2006; 103: 3687–92.
286 Catto JW, Alcaraz A, Bjartell AS, De Vere White R, Evans CP, *et al.* microRNA in prostate, bladder, and kidney cancer: a systematic review. *Eur Urol* 2011; 59: 671–81.
287 Calin GA, Croce CM. microRNA signatures in human cancers. *Nat Rev Cancer* 2006; 6: 857–66.
288 Lu J, Getz G, Miska EA, Alvarez-Saavedra E, Lamb J, *et al.* microRNA expression profiles classify human cancers. *Nature* 2005; 435: 834–8.
289 Hwang HW, Mendell JT. microRNAs in cell proliferation, cell death, and tumorigenesis. *Br J Cancer* 2006; 94: 776–80.
290 Esquela-Kerscher A, Slack FJ. Oncomirs-microRNAs with a role in cancer. *Nat Rev Cancer* 2006; 6: 259–69.
291 Farazi TA, Spitzer JI, Morozov P, Tuschl T. miRNAs in human cancer. *J Pathol* 2011; 223: 102–15.
292 Cho WC. OncomiRs: the discovery and progress of microRNAs in cancers. *Mol Cancer* 2007; 6: 60.
293 Zhang B, Pan X, Cobb GP, Anderson TA. Micrornas as oncogenes and tumor suppressors. *Dev Biol* 2007; 302: 1–12.
294 Mitchell PS, Parkin RK, Kroh EM, Fritz BR, Wyman SK, *et al.* Circulating microRNAs as stable blood-based markers for cancer detection. *Proc Natl Acad Sci U S A* 2008; 105: 10513–8.
295 Chen X, Ba Y, Ma L, Cai X, Yin Y, *et al.* Characterization of microRNAs in serum: a novel class of biomarkers for diagnosis of cancer and other diseases. *Cell Res* 2008; 18: 997–1006.
296 Reis LO, Pereira TC, Lopes-Cendes I, Ferreira U. microRNAs: a new paradigm on molecular urological oncology. *Urology* 2010; 76: 521–7.
297 Gillis AJ, Stoop HJ, Hersmus R, Oosterhuis JW, Sun Y, *et al.* High-throughput microRNAome analysis in human germ cell tumours. *J Pathol* 2007; 213: 319–28.
298 Voorhoeve PM, le Sage C, Schrier M, Gillis AJ, Stoop H, *et al.* A genetic screen implicates miRNA-372 and miRNA-373 as oncogenes in testicular germ cell tumors. *Cell* 2006; 124: 1169–81.
299 Suh MR, Lee Y, Kim JY, Kim SK, Moon SH, *et al.* Human embryonic stem cells express a unique set of microRNAs. *Dev Biol* 2004; 270: 488–98.
300 Palmer RD, Murray MJ, Saini HK, van Dongen S, Abreu-Goodger C, *et al.* Malignant germ cell tumors display common microRNA profiles resulting in global changes in expression of messenger RNA targets. *Cancer Res* 2010; 70: 2911–23.
301 Murray MJ, Halsall DJ, Hook CE, Williams DM, Nicholson JC, *et al.* Identification of microRNAs From the miR-371 ~ 373 and miR-302 clusters as potential serum biomarkers of malignant germ cell tumors. *Am J Clin Pathol* 2011; 135: 119–25.
302 Belge G, Dieckmann KP, Spiekermann M, Balks T, Bullerdiek J. Serum levels of microRNAs miR-371-3: a novel class of serum biomarkers for testicular germ cell tumors? *Eur Urol* 2012; 61: 1068–9.
303 Murray MJ, Coleman N. Testicular cancer: a new generation of biomarkers for malignant germ cell tumours. *Nat Rev Urol* 2012; 9: 298–300.
304 Novotny GW, Belling KC, Bramsen JB, Nielsen JE, Bork-Jensen J, *et al.* microRNA expression profiling of carcinoma *in situ* cells of the testis. *Endocr Relat Cancer* 2012; 19: 365–79.
305 Albers P, Albrecht W, Algaba F, Bokemeyer C, Cohn-Cedermark G, *et al.* EAU guidelines on testicular cancer: 2011 update. *Eur Urol* 2011; 60: 304–19.
306 Barlow LJ, Badalato GM, McKiernan JM. Serum tumor markers in the evaluation of male germ cell tumors. *Nat Rev Urol* 2010; 7: 610–7.
307 Dieckmann KP, Spiekermann M, Balks T, Flor I, Löning T, *et al.* microRNAs miR-371-3 in serum as diagnostic tools in the management of testicular germ cell tumours. *Br J Cancer* 2012; 107: 1754–60.
308 Wang C, Yang C, Chen X, Yao B, Yang C, *et al.* Altered profile of seminal plasma microRNAs in the molecular diagnosis of male infertility. *Clin Chem* 2011; 57: 1722–31.
309 Liu T, Cheng W, Gao Y, Wang H, Liu Z. Microarray analysis of microRNA expression patterns in the semen of infertile men with semen abnormalities. *Mol Med Rep* 2012; 6: 535–42.
310 Wu W, Hu Z, Qin Y, Dong J, Dai J, *et al.* Seminal plasma microRNAs: potential biomarkers for spermatogenesis status. *Mol Hum Reprod* 2012; 18: 489–97.
311 Wu W, Qin Y, Li Z, Dong J, Dai J, *et al.* Genome-wide microRNA expression profiling in idiopathic non-obstructive azoospermia: significant up-regulation of miR-141, miR-429 and miR-7-1-3p. *Hum Reprod* 2013; 28: 1827–36.
312 Li Y, Li M, Liu Y, Song G, Liu N. A microarray for microRNA profiling in spermatozoa from adult men living in an environmentally polluted site. *Bull Environ Contam Toxicol* 2012; 89: 1111–4.
313 Marczylo EL, Amoako AA, Konje JC, Gant TW, Marczylo TH. Smoking induces differential miRNA expression in human spermatozoa: a potential transgenerational epigenetic concern? *Epigenetics* 2012; 7: 432–9.
314 Yang Q, Hua J, Wang L, Xu B, Zhang H, *et al.* microRNA and piRNA profiles in normal human testis detected by next generation sequencing. *PLoS One* 2013; 8: e66809.
315 McIver SC, Roman SD, Nixon B, McLaughlin EA. miRNA and mammalian male germ cells. *Hum Reprod Update* 2012; 18: 44–59.
316 Papaioannou MD, Nef S. microRNAs in the testis: building up male fertility. *J Androl* 2010; 31: 26–33.
317 Abu-Halima M, Hammadeh M, Schmitt J, Leidinger P, Keller A, *et al.* Altered microRNA expression profiles of human spermatozoa in patients with different spermatogenic impairments. *Fertil Steril* 2013; 99: 1249–55.e16.