Am J Cancer Res 2012;2(2):153-167
www.ajcr.us /ISSN:2156-6976/ajcr0000104

Review Article

Molecular genetics of testicular germ cell tumors

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Received January 16, 2012; accepted February 4, 2012; Epub February 15, 2012; Published February 28, 2012

Abstract: Testicular germ cell tumors (TGCT) are the most common malignancy in young men. While most TGCT are potentially curable, approximately 5% of patients with TGCT may develop chemoresistance and die from the disease. This review article summarizes current knowledge in genetics underlying the development, progression and chemoresistance of TGCT. Most post-pubertal TGCT originate from intratubular germ cell neoplasia unclassified (IGCNU), which are transformed fetal gonocytes. Development of IGCNU may involve aberrantly activated KITLG/KIT pathway and overexpression of embryonic transcription factors such as *NANOG* and *POU5F1*, which leads to suppression of apoptosis, increased proliferation, and accumulation of mutations in gonocytes. Invasive TGCT consistently show gain of chromosome 12p, typically isochromosome 12p. Single gene mutations are uncommon in TGCT. *KIT*, *TP53*, *KRAS/NRAS,* and *BRAF* are genes most commonly mutated in TGCT and implicated in their pathogenesis. Different histologic subtypes of TGCT possess different gene expression profiles that reflect different directions of differentiation. Their distinct gene expression profiles are likely caused by epigenetic regulation, in particular DNA methylation, but not by gene copy number alterations. Resistance of TGCT to chemotherapy has been linked to karyotypic aberrations, single-gene mutations, and epigenetic regulation of gene expression in small-scale studies. The study of TGCT genetics could ultimately translate into development of new molecular diagnostic and therapeutic modalities for these tumors and improve the care of patients with these malignancies.

Keywords: Pathology, genetics, testis, germ cell tumor

INTRODUCTION

Testicular cancers account for approximately 1% of all human malignancies and their incidence has been increasing worldwide in the last fifty years [1, 2]. In young men, they are the most common malignancy and the second leading cause of death [1, 2]. Testicular germ cell tumors (TGCT) comprise more than 95% of all testicular malignancies [2]. Patients with TGCT have a high cure rate, as these tumors are highly sensitive to either radiation or chemotherapy, but approximately 5% of patients develop treatment resistance [3]. TGCT have been studied as a model system to understand mechanisms of tumor chemosensitivity and resistance. However, molecular mechanisms underlying development and progression of TGCT are still not clear. Many studies have been recently published describing genetic profiles of TGCT, which have generated new insights in understanding of tumorigenesis of these neoplasms [4-7]. In this article, we will review current knowledge in the field of TGCT genetics, focusing on genetic susceptibility, somatic genetic and epigenetic events, and mechanisms of chemotherapy resistance.

I. NORMAL DEVELOPMENT OF TESTICULAR GERM CELLS

Development of TGCT is determined by a series of genetic and environmental events that occur mainly during fetal testicular development but partly also after birth [8]. Primordial germ cells (PGCs) are progenitors of the germ cell lineage that are selected from the embryonic stem cells. PGCs can be identified in human embryos at 5-6 weeks of gestational age [9, 10]. Orchestrated by the KIT ligand (KITLG, also known as the stem cell factor, SCF) and its receptor KIT as well as the chemokine SDF1 (CXCL12) and its

Table 1. Classification of TGCT based on molecular genetics and histomorphology

Tumors with IGCNU as precursor and gain of 12p in invasive tumor (> 90%) (Type II)
Seminoma
Non-seminomatous germ cell tumor (NSGCT)
NSGCT of one histologic type
Embryonal carcinoma
Yolk sac tumor (Endodermal sinus tumor)
Trophoblastic tumors: choriocarcinoma and others
Teratoma
NSGCT of more than one histologic type
Non-seminomatous mixed germ cell tumor
Tumor not associated with IGCNU and 12p abnormalities (< 10%)
Spermatocytic seminoma (Type III); Dermoid cyst; Epidermoid cyst; Pediatric TGCT (Type I)

receptor CXCR4, PGCs migrate from the proximal epiblast (yolk sac) through the hindgut and mesentery to the genital ridge and become gonocytes [11-14]. PGCs and gonocytes can be identified by stem cell markers including PLAP, NANOG, KIT, SOX2, POU5F1 (also known as OCT3/4), and SALL4 [15-21]. PGCs and gonocytes are both undifferentiated embryonic germ cells, with their original genomic imprinting pattern completely erased by DNA demethylation which allows development of gender-specific germ cell lineages [22, 23]. In the presence of a Y chromosome, the gonadal stromal cells express transcription factor SRY and its target gene *SOX9* and give rise to Sertoli cells [24]. The Sertoli cells create a microenvironment that allows differentiation of gonocytes into prespermatogonia and spermatogonia. During the differentiation process, the germ cells gradually lose expression of *NANOG*, *PLAP*, and *POU5F1*, partially lose expression of *KIT* and *SALL4*, and acquire expression of other genes including *MAGE4A*, *VASA*, and *TSPY* [19, 20, 25]. The testis continues to develop after birth, resulting in production of mature spermatozoa with a male imprinting pattern after puberty.

II. HISTOGENESIS OF TGCT

The precursor lesion of almost all postpubertal TGCT is intratubular germ cell neoplasia unclassified (IGCNU), defined as malignant germ cells confined to the seminiferous tubules (*in situ* carcinoma). The widely accepted theory of TGCT tumorigenesis is that development of IGCNU starts in utero, with important predisposing factors being elevated maternal estrogen levels or other environmental toxins with estrogenic activities [26, 27]. The disturbance of germ cell development by estrogen results in arrest of fetal germ cells at the gonocyte stage. Fetal gonocytes have a completely erased genomic imprinting pattern by DNA demethylation, and therefore they are susceptible to mutational events which are accumulated during cell replication. With a combination of oncogene activating mutation and silencing mutations of tumor suppressor genes, gonocytes are transformed to IGCNU [18]. Proliferation of IGCNU cells is believed to occur during puberty and early adulthood, in the setting of hormonal stimulation. IGCNU subsequently progresses to invasive TGCT with differentiation into the various histologic subtypes either before or after invasion.

III. CLASSIFICATION OF TGCT

TGCT are a heterogeneous group of tumors. **Table 1** is a modified molecular and histomorphologic classification of TGCT based on the World Health Organization (WHO) 2004 Classification [28]. This review article focuses on "Type II" TGCT, which account for >90% of TGCT. These tumors share the same precursor lesion IGCNU, and the same chromosomal abnormality associated with tumor invasion, which is gain of chromosome 12p. "Type II" TGCT is subclassified into seminomas (~ 55%) and non-seminomatous TGCT (NSGCT) (~ 45%). Seminomas morphologically and immunophenotypically resemble PGC/gonocytes, and are sensitive to both radiation and platinum-based chemotherapy. NSGCT consists of embryonal carcinoma, yolk sac tumor, choriocarcinoma, and teratoma. Different histologic subtypes reflect different directions of differentiation, either undifferentiated (embryonal carcinoma, composed of transformed pluripotent embryonic stem cells resembling the inner cell mass of the blastocyst) or differentiated (teratoma with somatic differen-

tiation, yolk sac tumor and choriocarcinoma with extraembryonic differentiation). NSGCT are sensitive to chemotherapy but not to radiation. There are a few exceptions in the TGCT family that do not share the common genetic trait and are mostly indolent in behavior, including spermatocytic seminoma, dermoid cyst, and epidermoid cyst in postpubertal individuals, as well as pediatric TGCT (yolk sac tumor and teratoma). The genetics of these rare tumors are not discussed in this review.

IV. ETIOLOGY

The etiology of TGCT is still unclear. A number of risk factors have been recognized, including prior TGCT in the contralateral testicle, cryptorchidism, impaired fertility, disorders of sex development, family history, and prenatal and perinatal risk factors including birth weight, gestational age, maternal age, and maternal smoking [29, 30]. Although there is some evidence for a difference in risk factors among the different histologic subtypes, the majority of risk factor analyses support a shared etiology of TGCT subtypes [29, 31].

V. TUMOR GENETICS

V-1. Genetic Susceptibility

In familial TGCT cases, genome-wide linkage analysis studies have failed to identify any consistent genetic linkages [32]. In sporadic TGCT cases, a 1.6-Mb gr/gr deletion on Y chromosome has been found to be the most common genetic alteration in infertility patients which results into a two-fold increase in risk for TGCT [33]. Interesting findings were recently discovered by two groups through genome-wide single nucleotide polymorphism (SNP) association studies [34, 35] and were reviewed by Turnbull et al [6]. Eight TGCT predisposition SNPs have been found in six chromosomal loci at 5p15, 5q31, 6p21, 9p24, 12p13, and 12q21. Of these, SNPs in loci 5q31, 9p24 and 12q21 were confirmed by independent studies [6]. The 12q21 locus has a much lower incidence in the African American population compared to the Caucasians, which may explain a much lower incidence of TGCT in African Americans [36, 37]. More importantly, five loci contain biologically plausible candidate genes for TGCT susceptibility. First, loci 12q21, 5q31, and 6p21 contain genes directly or indirectly associated with the KITLG/KIT signaling pathway, including *KITLG* on chromosome 12q21, *SPRY4* on 5q31, and *BAK1* (*BCL2*-antagonist/killer 1) on 6p21 [38]. SPRY4 is an inhibitor of the mitogen-activated protein kinase (MAPK) pathway, which is a downstream pathway activated by the KITLG/KIT interaction. BAK1 is an apoptosis-promoter, whose gene expression is suppressed by KITLG/KIT pathway [39]. It was hypothesized that these genomic variants lead to KITLG/KIT signaling pathways that are highly susceptible to oncogenic stimulants [6]. Additional plausible susceptibility genes include *TERT* (human telomerase reverse transcriptase) and *CLPTM1L* (cisplatin resistance related protein CRR9p) on chromosome 5p15, and *DMRT1* (doublesex and mab-3 related transcription factor 1) on 9P24. DMRT1 is a key protein in sex-determining pathways, whose high expression leads to testicular differentiation and low expression to ovarian differentiation [40]. Although these SNPs may be biologically significant, these six loci together with gr/gr deletion account only for approximately 15% of the excess familial risk of TGCT [6]. The remaining 85% of genetic predisposition is yet unexplained and requires further investigation.

V-2. Somatic Genetic Events

V-2.1. Chromosomal Aberrations/DNA Copy Number Alterations

Chromosome 12p alterations are the hallmark of TGCT, identified in nearly all invasive TGCT, as well as intratubular embryonic carcinoma and intratubular seminoma [41, 42] (**Figure 1** and **Table 2**). Specifically, isochromosome 12p is the most common alteration (~ 80%), with duplication of 12p and amplification of shorter stretches of 12p being much less common [43]. Interestingly, IGCNU without adjacent invasive TGCT does not contain isochromsome 12p in most studies [44], which suggests that isochromosome 12p is not required for the development of IGCNU. It has been hypothesized that amplified region on 12p may harbor genes associated with Sertoli-cell-independent or invasive growth of TGCT cells. Several candidate genes have been suggested, including *KITLG*, *NANOG* (and its pseudogenes), *KRAS2*, *BCAT1*, and *CCND2* [45-48]. However, the exact genes still have not been identified. Further studies are required before we fully understand the role of chromosome 12p in TGCT carcinogenesis.

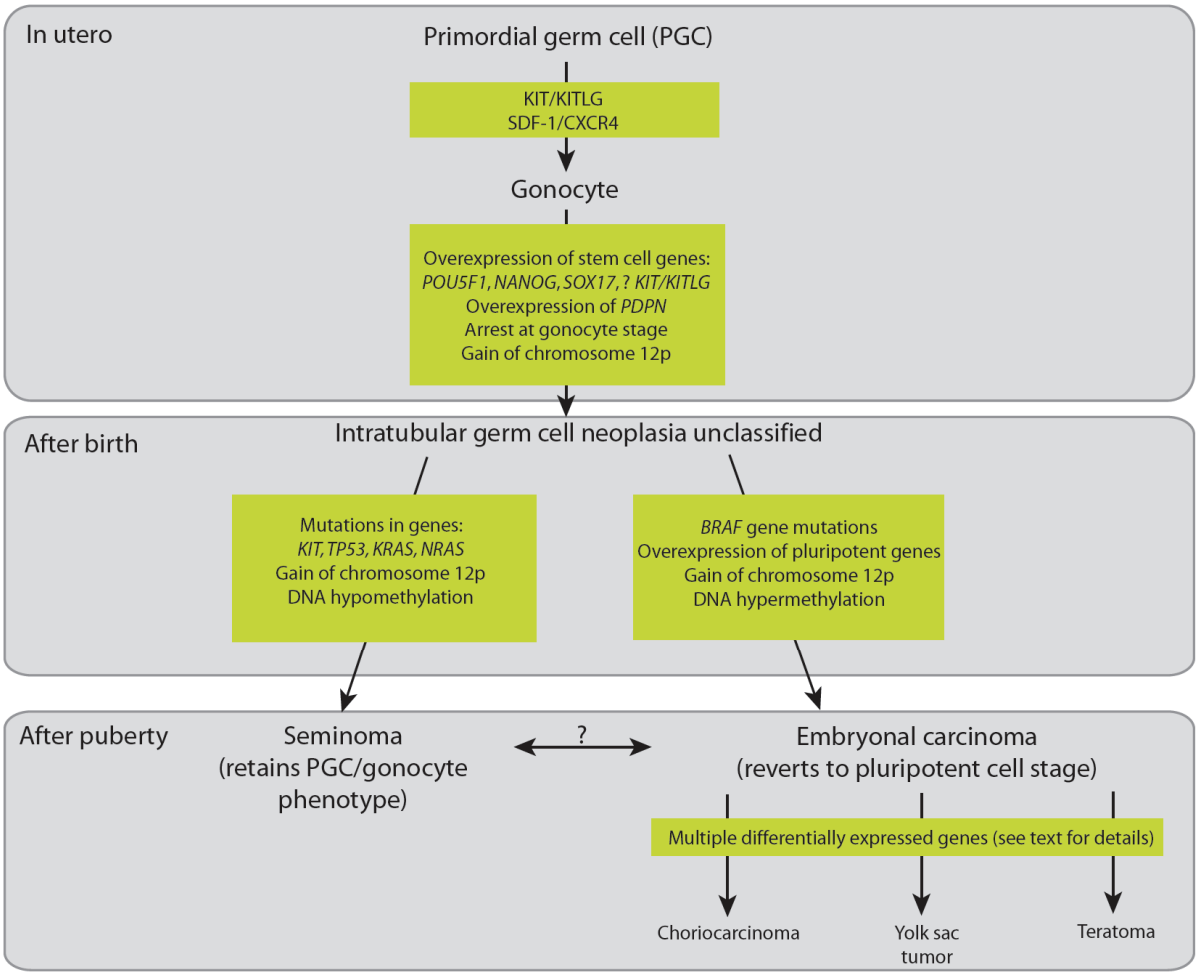

Figure 1. Selected genetic events involved in TGCT pathogenesis**.** Multiple genetic factors are involved at each stage of TGCT development. In utero, hormonal and environmental factors and KIT activating mutation induce arrest of embryonic germ cells at the gonocyte stage. Gonocytes have almost completely demethylated genomic DNA, which facilitates accumulation of mutations during cell replication and development of IGCNU. IGCNU may present as early as before birth. Hormonal stimulation during puberty facilitates proliferation of IGCNU. Gain of chromosome 12p is associated with the invasion of IGCNU through the basement membranes of seminiferous tubules. Individual gene mutations such as *TP53*, *KRAS*, *NRAS*, and *BRAF*, and selected gene expression may determine the development of seminomas and nonseminomas. Within nonseminomas, selected gene expression may determine different directions of differentiation. Yolk sac tumor, choriocarcinoma, and teratoma may develop either directly from IGCNU or indirectly through embryonal carcinoma as an intermediate phase.

Less frequent cytogenetic aberrations found in TGCT include over-presentation of chromosomes 7, 8, 12, 17 and X, and under-presentation of chromosomes 4, 11, 13, 18 and Y [49].

NSGCT are typically hypotriploid and seminomas are usually hypertriploid [50]. A few cytogenetic aberrations have been reported to be tumor subtype-specific. For example, one study showed that seminomas were associated with gains of 15q and 22q, while NSGCT were associated with high-level amplification of 12p, gain of 17q and loss of 10q [51]. Another study showed that gain of 17p and loss of 2p were associated with embryonal carcinoma, while gain of 8p and loss of 5p, 11q, and 13q were specific for seminoma [52]. The significance of these findings awaits to be confirmed by further studies.

Using genomic approaches (classic CGH, array CGH, and SNP arrays), studies using human tissue or tumor cell lines have detected certain

Table 2. Common genetic and epigenetic alterations in TGCT

	TGCT subtype	Alteration (Effect)	Role in TGCT pathogenesis
Chromosomal			
12p	Invasive TGCT; Intratubular seminoma; Intratubular embryonal carcinoma	Gain of 12p, mostly isochromosome 12p (amplification of a cluster of genes, promoting tumor invasion)	1. Most common single genetic event 2. Genetic hallmark of TGCT
Single-gene mutations			
KIT	IGCNU; Seminoma > NSGCT	Activating mutation in the receptor for KITLG (suppressing apoptosis)	1. Most common single gene mutation 2. May be associated with TGCT initiation 3. Mutant tumors responsive to imatinib mesylate
TP53	Seminoma	Inactivating mutation in cell cycle regulator *p53* (leading to impaired DNA repair and cell cycle dysregulation)	Role in TGCT pathogenesis unclear
KRAS	Seminoma > NSGCT	Activating mutation in a small GTPase (promoting cell proliferation, differentiation, and migration)	KRAS wild type → chemoresistance
NRAS	Seminoma > NSGCT	Same as *KRAS*	Mutation frequency in NSGCT is controversial
BRAF	Seminoma < NSGCT	Activating mutations in a serine/threonine kinase (promoting cell proliferation and differentiation)	Mutation → chemoresistance
Differentially expressed genes*			
Tumor vs normal	IGCNU	Up-regulated: ***KITLG, PDPN,*** *ANXA3, C12orf35, DOCK11, IL22RA1, KIT, L1TD1, LIN28A, MYCL1, NANOG, NSMAF, OSBPL3, PLBD1, POU5F1 (OCT3/4), SLC25A16, SOX17, TCL6, TFCP2L1, UPP1*	
	Seminoma	Upregulated: ***JUP****, CCND2, LZTS1, PIM2, BAX, CACNB3, CCNF, CDC25B, CDH3, ETV1, IL6R, KCNJ8, MMP12, MYCN, OSBPL3, PIM1, SLC43A1, SOX4, TCL1B, TUBB* Downregulated: *CLU*	
Subtype-specific signatures	Seminoma	*LZTS1*	
	Embryonal carcinoma	***DNMT3B****, DPPA4, GAL, GPC4, POU5F1, TERF1*	
	Yolk sac tumor	*AFP, APOA2,B4GALT4, BMP2, C5, CYP26A1, DSCAM, EOMES, FAM89A, FERMT2, FLRT3, FOXA2, LEPREL1, LRRN1, NEK2, NRXN3, OTX2, RAGE, SEBOX, VTN*	
	Choriocarcinoma	*CGA*	
	Teratoma	***EMP1****, CDH17, MFAP4, NFKBIZ, TSPAN8*	
Epigenetic changes			
Global DNA methylation	IGCNU; Seminoma	Low level (Promoter hypomethylation allows gene transcription)	1. Associated with undifferentiated tumors 2. Hypermethylation → chemoresistance
	Embryonal carcinoma	Intermediate level	1. Associated with transformed undifferentiated tumor 2. Hypermethylation → chemoresistance
	Yolk sac tumor; Choriocarcinoma; Teratoma	High level: similar to other solid tumors (Promoter hypermethylation suppresses gene transcription)	1. Associated with more differentiated tumors 2. Hypermethylation → chemoresistance

IGCNU = intratubular germ cell neoplasia unclassified, NSGCT = non-seminomatous germ cell tumor *: Genes in bold fonts are those identified by 3-4 independent studies. Genes in regular fonts are those identified by 2 independent studies.

chromosomal aberrations (mainly amplifications) associated with cisplatin resistance of TGCT [53-55]. However, these studies did not find consistent aberrations in the majority of resistant tumors.

V-2.2. Single Gene Mutations

Mutations in single genes are uncommon in TGCT. A search in Wellcome Trust Sanger Institute's CatalOgue of Somatic Mutations In Cancer (COSMIC, http://www.sanger.ac.uk/cosmic, search performed on January 23, 2012) [56] showed the top five genes mutated in TGCT to be *KIT*, *TP53*, *KRAS /NRAS,* and *BRAF* (**Table 2**). Among these genes, *KIT*, *TP53*, and *KRAS / NRAS* were found to be more frequently mutated in seminomas (6-19%) compared to NSGCT (0-2%). Other mutated genes associated with TGCT were *FGFR3*, *HRAS*, *PTEN*, *SIK1*, *SMAD4*, *STK10*, and *STK11*, but they were detected in very few cases each and will not be reviewed here.

KIT

One of the most commonly mutated genes in TGCT is *KIT*, a proto-oncogene located on chromosome 4q11-q12. KIT is also known as stem cell growth factor receptor (SCFR), or CD117. KIT is a tyrosine kinase receptor that dimerizes and becomes phosphorylated when bound to its ligand KITLG [57-59]. As mentioned above, KIT is crucial for survival, proliferation, and migration of germ cells [60]. Normal spermatogonia rarely express *KIT*, while almost all IGCNU, most seminomas, and some NSGCT do [61-63]. Interestingly, KITLG showed a similar expression pattern in IGCNU, seminomas, and NSGCT, with focal or no expression in normal spermatogonia [64]. It has been proposed that production of both KIT and KITLG by malignant germ cells forms a temporary autocrine or paracrine system to stimulate tumor cell growth. Gain-of-function mutations in *KIT* most commonly occur in juxtamembrane or cytoplasmic kinase domains and lead to its constitutive activation which drives tumorigenesis [65]. A recent search of COSMIC database shows that *KIT* is mutated in 19% of seminomas (45/233) and 2% of NSGCT (2/120). Two studies [43, 66] reported a significantly higher rate of *KIT* mutation in patients with bilateral TGCT compared to those with unilateral TGCT (93% vs 1.3% and 63.6% vs 6.4%), while other studies found no increase in *KIT* mutation frequency in patients with bilateral disease [29, 67]. *KIT* mutation was detected in some but not all TGCT-associated IGCNU. Some but not all bilateral TGCT shared the same *KIT* mutation [43, 66]. Therefore, it is controversial whether *KIT* mutation is an initiation event in TGCT development and whether it has any predictive value for bilateral disease. Detection of specific *KIT* mutations may have therapeutic implications for cisplatin-resistant TGCT. Two of the seminoma-associated mutations detected by Kemmer et al [62] made KIT susceptible to imatinib mesylate *in vitro*. Two case reports of complete regression have been reported in patients with *KIT*-mutated TGCT after treatment with KIT inhibitor imatinib mesylate [68, 69]. The prospect of using this information for clinical decision-making should instigate further studies documenting the frequency of specific *KIT* mutations in TGCT and investigation of their effect on therapy with tyrosine kinase inhibitors.

TP53

TP53 encodes p53, a cell cycle regulating protein. *TP53* is located on chromosome 17p13. Its mutations leading to non-functional p53 result in lack of cell cycle regulation and repair of DNA damage and, not surprisingly, are associated with a high number of cancers [70]. Earlier reports showed that *TP53* mutations seem to be infrequent in sporadic TGCT [71, 72]. A recent search of COSMIC [56] showed that *TP53* was mutated in 7% seminomas (10/135) and 0% of NSGCT (0/9), which is higher than previously reported. Although TGCT constantly express p53, it is thought to be intrinsic due to their germ cell nature but not due to mutations. The potential role of detecting *TP53* mutations for prediction of TGCT disease outcome and chemoresistance is controversial and needs to be further clarified [73-76].

KRAS/NRAS

KRAS and NRAS are small receptor tyrosine kinase-coupled GTPases. They interact with effector proteins that in turn activate the Raf/MEK/ERK pathway, the PI3K/PKB/Akt pathway, and other downstream pathways [77, 78]. Activating *KRAS* and *NRAS* mutations (codons 12, 13, and 61) lead to their constitutive activation which promotes carcinogenesis [77]. Presence of these mutations in colorectal and lung cancer patients determines poor response to anti-EGFR -directed therapies and tyrosine kinase inhibi-

tors [78]. A recent search of COSMIC [56] showed that *KRAS* (13/214) and *NRAS* (8/145) mutations are present in approximately 6% seminomas each, while none of the NSGCT had *KRAS* (0/138) and *NRAS* mutations (0/71). *RAS* mutations in NSGCT were reported by a few studies not deposited in COSMIC. For example, Sommerer et al [79] detected *KRAS* mutations in 7% of seminomas (2/30) and 9% of NSGCT (3/32); Ganguly et al [80] detected *NRAS* gene mutations in 59% (13/22) seminomas and 78% (7/9) NSGCT. A recent study by Honecker et al [81] assessed 100 control (50 seminomas and 50 NSGCT) and 35 cisplatin-resistant cases of TGCT (3 seminomas and 32 NSGCT) and found that only two tumors in the control group (one seminoma and one NSGCT, 2%) harbored a *KRAS* mutation. The functional link between *KRAS* mutation status and TGCT chemoresponsiveness needs to be explored in the future.

BRAF

BRAF is located on chromosome 7q34 and encodes protein BRAF. BRAF is a member of the RAF family of serine/threonine kinases, playing a role in regulating the MAP kinase/ERK signaling pathway, which affects cell proliferation and differentiation. A recent search of COSMIC [56] showed that *BRAF* is mutated in 1% of seminomas (1/112) and 2% of NSGCT (2/100). Sommerer et al [79] analyzed 62 TGCT and found a *BRAF* V599E mutation in 9% of NSGCT (3/32, all in embryonal carcinoma component), while none of the seminomas harbored this mutation. No correlation was detected between *BRAF* mutation status and prognostic parameters. Interestingly, *BRAF* V599E mutation was recently linked to chemoresistance of TGCT. In a study by Honecker et al [81], 26% of cisplatin-resistant TGCT (9/35) harbored the *BRAF* V599E mutation, compared to 1% (one NSGCT) in the group of 100 chemosensitive TGCT. Patients with TGCT harboring the *BRAF* mutation frequently presented with a mediastinal primary tumor or suffered from late relapse of disease; both groups known to be associated with a poor outcome [82, 83]. Further studies investigating BRAF association with chemoresistance and outcomes are warranted.

V-2.3. Transcriptomic Signatures

Results from transcriptomic studies suggest that alterations in gene expression may play an essential role in TGCT differentiation into various histologic subtypes (**Table 2**). A recent meta-analysis summarized gene expression signatures of IGCNU and TGCT with their various subtypes [5]. The authors reviewed 22 transcriptomic studies published from 2002 to 2009 and extracted dysregulated genes that were listed by at least 2 independent studies.

IGCNU, Seminoma, and TGCT vs Normal Testis

IGCNU cells were found to closely resemble normal gonocytes in their transcriptional profile. *PDPN* was found to be consistently overexpressed in IGCNU cells in 3 of 3 studies in comparison to normal testis. *PDPN* encodes podoplanin (recognized by antibody D2-40), which is a membrane glycoprotein involved in intercellular adhesion. *PDPN* was found to be overexpressed in fetal but not adult germ cells [84]. Additional genes found to be upregulated in IGCNU in 2 of 3 studies include *KIT, NANOG, POU5F1,* and *SOX17* (**Table 2**). Among 5 studies examining pure seminoma in comparison to normal testis, overexpression of *JUP* was detected by 4 studies, overexpression of *CCND2, BAX, MYCN,* and *SOX4* in 3 studies, and downregulation of *CLU* in 2 studies (**Table 2**). Analysis of 7 studies comparing invasive TGCT as a group to normal testis showed overexpression of *KRAS*, *CCND2* and *TPD52* in 3 studies, upregulation of *CCT6A, IGFB3, JUP, LYN, MYCN, RAB25*, and *SALL2* in 2 studies, and downregulation of *C11orf70* and *CADM1* in 2 studies.

Seminoma vs NSGCT

Although seminomas and NSGCT do not seem to be etiologically different [29, 31], they were found to exhibit distinct gene expression signatures. A study by Port et al [85] found that almost 90% of genes were discordantly regulated between seminomas and NSGCT. Overall seminomas exhibited up-regulated genes (mostly oncogenes, genes encoding intracellular transducers, genes related to DNA synthesis, proliferation and repair) while NSGCT had mostly down-regulated genes.

Signatures of Individual Histologic Subtypes

Seminoma-specific signature found by 2 of 4 individual studies was *LZTS1*, encoding a tumor suppressor involved in cell cycle control. Embryonal carcinoma was found to have a six-gene signature which was detected in 3 of 5 studies

and included *DNMT3B, DPPA4, GAL, GPC4, POU5F1,* and *TERF1*. All six genes play important roles in embryonic development and pluripotency, which is the evidence that embryonic carcinoma resembles inner cell mass. Yolk sac tumor was found to have a 20-gene signature, which included *AFP* (**Table 2**). Choriocarcinoma repetitively overexpressed *CGA*, a gene encoding alpha polypeptide of four glycoprotein hormones including human chorionic gonadotropin (**Table 2**). Teratoma had a 5-gene signature, which included *EMP1*, and which was detected by 3 out of 5 studies (Table 2). Analysis of 4 studies investigating differences in gene expression between embryonal carcinoma and seminoma showed that embryonal carcinoma exhibited upregulation of genes including stem cell genes *BCAT1, DNMT3B, GAL, GDF3, GPC4,* and *SOX2*, and downregulation of genes including *KIT, SOX17,* and *PDPN* [5].

Mechanisms of Differential Gene Expression in Different TGCT Subtypes

Although different histologic subtypes of TGCT share surprisingly similar chromosomal aberrations [49], they display remarkable difference on the gene expression level. Studies have found a low level of correlation between histology-specific gene expression profiles and reported histology-specific genomic gains and losses [5]. Based on these observations, it is now recognized that gene copy number alterations are unlikely to be the main driving force in the differentiation process of TGCT into different subtypes, although it has been shown to be critical for the initiation and development of TGCT. Other mechanisms such as epigenetic regulation, in particular DNA methylation of gene promoter regions, may play an important role in differentiation of TGCT. Studies have shown that seminomas exhibit hypomethylation of cancer-related genes (such as *p16*, *APC* and others), while NSGCT are characterized by hypermethylation of those same genes [86]. One of the potential DNA methylation regulators is *DNMT3B,* which was found to be differentially expressed in different TGCT subtypes (see below). These findings support a role of epigenetic regulation in TGCT differentiation.

Pitfalls of Current Transcriptomic Studies

Studies investigating gene expression in TGCT need to be interpreted with caution. TGCT are relatively rare tumors, therefore many individual studies had small case numbers. It is difficult to perform meta-analysis combining studies using different gene array chips, as their coverage varied from 1,000 to 20,000 genes. A common technical limitation in these studies is the pureness of tumor and control tissue. TGCT tumor samples may contain non-neoplastic tubules and stroma. Tumors from mixed NSGCT may contain an admixture of different subtypes. Many studies used normal testis as control tissue, while some used non-neoplastic testicular tissue from the same patient. However, normal testicular tissue contains an admixture of cell types, and therefore is not the best control for TGCT. An ideal design is to use microdissected pure tumor as study tissue material and microdissected normal fetal gonocytes as normal control. However, this was not done in the majority of studies performed to date. Furthermore, different gene array studies used different statistical analyses. These limitations have made it difficult to accrue sufficient number of individual studies to conduct adequately powered meta-analysis. Finally, only a few studies have validated array findings using RT-PCR and immunohistochemistry. Therefore, most the above gene signatures need to be verified in the future.

Clinical Applications of Transcriptomic Findings

One immediate clinical application of results from transcriptomic studies is to develop immunomarkers to assist pathologists to make accurate pathologic diagnoses. To date, a few dysregulated genes such as *PDPN* (D2-40), *c-KIT, POU5F1* (OCT3/4), *GPC3* (Glypican 3), *SALL4*, *SOX2,* and *SOX17* have been successfully translated into new diagnostic tools for TGCT. A recent review by Emerson and Ulbright [87] provides an excellent overview of old and new immunohistochemical markers in diagnosing IGCNU and TGCT with their different histologic subtypes. Secondly, one may find gene signatures to predict chemoresistance of TGCT and therefore alter clinical management. For example, one study identified *CCND1* (Cyclin D1) overexpression in chemoresistant TGCT cell lines [88]. Using RT-PCR, the authors confirmed overexpression of *CCND1* in 8 of 12 clinical samples of resistant TGCT. Whether *CCND1* is a potential predictor of TGCT chemoresistance needs to be confirmed by further investigations.

V-2.4. Epigenetic Alterations

Epigenetic mechanisms include methylation of cytosine bases, posttranslational modification of histones, positioning of nucleosomes along the DNA molecule, and regulation by noncoding RNAs (especially microRNAs) [89]. DNA methylation is the most studied mechanism of epigenetic regulation. It primarily occurs in CpG islands that is often found near or in the gene promoter regions, and is maintained by DNA methyltransferases. Methylation suppresses gene transcription or silences the genes [89]. Epigenetic changes are necessary for normal development and maturation of germ cells [22]. Fetal germ cell DNA is generally hypomethylated, but after birth it becomes hypermethylated. IGCNU and seminomas exhibit low levels of DNA methylation and permissive chromatin structure associated with high transcriptional and proliferative activity [90]. More differentiated TGCTs (yolk sac tumors, choriocarcionomas, and teratomas) show a higher degree of methylation that is close to that seen in other solid tumors. Embryonal carcinomas show an intermediate pattern [91, 92] (**Table 2**). The different extent of global methylation in TGCT subtypes supports the model that IGCNU develops into seminoma and embryonal carcinoma, and the latter subsequently differentiates into other NSGCT (**Figure 1**). The shift of methylation status may be associated with the embryonal carcinoma-specific gene *DNMT3B*, which encodes DNA methyltransferase 3 beta. *DNMT3B* is normally expressed in pluripotent embryonic cells and induces *de novo* methylation at this stage of development. *DNMT3B* was found to be upregulated in embryonal carcinoma compared to seminoma (**Table 2**). It may regulate differentiation from embryonal carcinoma into different subtypes via regulating DNA methylation. Regarding treatment and prognosis of TGCTs, methylation has also been found to be associated with tumor chemoresistance. In general, undifferentiated tumors, which are often hypomethylated, are much more susceptible to chemotherapy than well differentiated tumors. Most seminomas are hypomethylated and sensitive to chemotherapy, while chemoresistant seminomas often show high degree of methylation (**Table 2**) [92, 93]. *In vitro*, demethylation of a seminoma cell line led to an increased chemosensitivity as well as an increased expression of stem cell markers *NANOG* and *POU5F1* [92]. In addition, it has been shown that methylation may regulate chemosensitivity in a gene-specific manner [92, 93]. A recent study examining differentially methylated regions between TGCT and normal testicular tissue showed that of more than 35,000 differentially methylated regions including noncoding DNA, only a small number mapped to gene promoters [94]. The biologic significance of these findings is unclear and requires further investigation.

V-3. Summary of Genetic Events in Tumorigenesis

Figure 1 summarize key genetic events in each phase of TGCT development, from PGC to gonocytes, transformation from arrested gonocytes to IGCNU, progression to invasive TGCT, and differentiation into different histologic subtypes. Recently, Looijenga et al [4] proposed a model describing that IGCNU development is associated with KIT activating mutation and KITLG expression, while the progression to invasive tumors is associated with gain of copies of chromosome 12p resulting in amplification of *KRAS2* and *NANOG* (and its pseudogenes). Their model needs to be tested in the future, as the roles of *KIT* and specific genes in 12p are still unclear (see above).

V-4. Genetics Underlying Chemoresistance

TGCTs are highly sensitive to cisplastin-based chemotherapy, with the exception of teratoma. Patients with TGCT have a cure rate of more than 95% if chemotherapy is given at early stages. Approximately 5% of all patients with TGCT and 10–20% of patients diagnosed with metastatic TGCT will develop *de novo* or therapy -induced resistance [3] [95]. They either will have incomplete response or develop recurrence after initial treatment. Many genetic and epigenetic alterations have been found to be associated with TGCT chemoresistance, as described individually above. Here we will discuss the molecular mechanisms behind those observations.

Cisplatin acts through covalent binding to DNA and formation of DNA adducts which alter DNA structure. Altered DNA is recognized by DNA damage repair proteins, leading to cell cycle arrest and apoptosis [95]. Resistance to cisplatin may be mediated via two mechanisms. 1) *Insufficient DNA binding*: Platinum enters TGCT tumor cells through transmembrane transport-

ers, mainly CTR1 [96]. Platinum is exported out of the cells by export pumps ATP7A and ATP7B [97], or multidrug resistance-related protein MRP after platinum molecules are conjugated with glutathione by glutathione S-transferases (GSTs) [98, 99]. Studies have shown that most TGCTs lack export pumps with affinity for platinum and exhibit low level of GST activity, which makes export-mediated resistance an unlikely mechanism in these tumors [7]. 2) *Resistance post DNA binding:* Among 4 DNA repair pathways in human cells including nucleotide-excision repair (NER), base-excision repair (BER), mismatch repair (MMR), and double-strand-break repair, MMR is found to be an important platinum-resistant mechanism in TCGT. Although NER is the major pathway to remove platinum-induced adducts and is known to contribute to high platinum-sensitivity of TGCT, NER does not seem to be a common resistance mechanism [100, 101]. MMR plays a role in damage recognition and initiation of apoptosis [102]. Loss or defects of MMR proteins can lead to failure to initiate apoptosis and resistance to platinum. Studies have shown that resistant TGCTs had a higher incidence of microsatellite instability (MSI), resulting from impaired MMR [81, 103]. Similar to MSI in colorectal cancer, MSI in TGCT showed a strong correlation with *BRAF* mutations (described above). Both MSI and mutated BRAF were associated with diminished MMR protein hMLH1 expression, which was correlated with promoter hypermethylation [81]. Based on these findings, platinum resistance in TGCT may be induced by DNA hypermethylation of MMR proteins and a subsequently impaired MMR pathway. Further, it suggests that chemoresistant TGCT patients may one day benefit from genetic tests and targeted therapies in a manner similar to that currently applied to patients with colorectal cancer. Downstream of MMR proteins, resistance could result from defects in initiation and execution of apoptosis. However, no apoptotic regulators have been proven to be determinants for chemoresistance in TGCT. *TP53*, an apoptosis regulator commonly mutated in other solid tumors, is rarely mutated in TGCT including resistant TCGT [76]. Although a high ratio of pro-apoptotic Bax to anti-apoptotic Bcl-2 was found in invasive TGCT, the Bax:Bcl2 ratio seems not to be associated with TCGT chemoresistance [104-106]. Resistance mechanisms downstream of DNA repairing machineries need to be elucidated in the future.

SUMMARY

TGCT are a heterogeneous group of tumors. While they show a great similarity in etiology, precursor lesions, and chromosome 12p alterations, different TGCT subtypes display distinct tumor genetics that may explain their different histology and biological behavior. **Table 2** summarizes common genetic alterations identified in TGCT initiation, differentiation, and progression to chemoresistance. Genomic/epigenomic signatures may be useful for molecular subclassification and prognostication of these tumors. An understanding of how TGCT develop resistance to cisplatin and how new therapeutic targets can be discovered in chemoresistant TGCT will be essential to further improve clinical care of patients with these malignancies.

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References

[1] Jemal A, Tiwari RC, Murray T, Ghafoor A, Samuels A, Ward E, Feuer EJ and Thun MJ. Cancer statistics, 2004. CA Cancer J Clin 2004; 54: 8-29.

[2] Cancer facts & figures 2010. American Cancer Society 2010; www.cancer.org/acs/groups/content/@nho/documents/document/acspc-024113.pdf: last accessed on Jan 18, 2012.

[3] Raghavan D. Testicular cancer: maintaining the high cure rate. Oncology (Williston Park) 2003; 17: 218-228; discussion 228-219, 234-215, passim.

[4] Looijenga LH, Gillis AJ, Stoop H, Biermann K and Oosterhuis JW. Dissecting the molecular pathways of (testicular) germ cell tumour pathogenesis; from initiation to treatment-resistance. Int J Androl 2011; 34(4 Pt 2): e234-251.

[5] Alagaratnam S, Lind GE, Kraggerud SM, Lothe RA and Skotheim RI. The testicular germ cell tumour transcriptome. Int J Androl 2011; 34(4 Pt 2):e133-50.

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

[7] Piulats JM, Jimenez L, Garcia del Muro X, Villanueva A, Vinals F and Germa-Lluch JR. Molecular mechanisms behind the resistance of cisplatin in germ cell tumours. Clin Transl Oncol 2009; 11: 780-786.

[8] Looijenga LH, Hersmus R, de Leeuw BH, Stoop H, Cools M, Oosterhuis JW, Drop SL and Wolffenbuttel KP. Gonadal tumours and DSD. Best Pract Res Clin Endocrinol Metab 2010; 24: 291-310.
[9] Donovan PJ. The germ cell--the mother of all stem cells. Int J Dev Biol 1998; 42: 1043-1050.
[10] McLaren A. Primordial germ cells in the mouse. Dev Biol 2003; 262: 1-15.
[11] Donovan PJ. Growth factor regulation of mouse primordial germ cell development. Curr Top Dev Biol 1994; 29: 189-225.
[12] Godin I, Deed R, Cooke J, Zsebo K, Dexter M and Wylie CC. Effects of the steel gene product on mouse primordial germ cells in culture. Nature 1991; 352: 807-809.
[13] Wylie CC. The biology of primordial germ cells. Eur Urol 1993; 23: 62-66; discussion 67.
[14] Runyan C, Schaible K, Molyneaux K, Wang Z, Levin L and Wylie C. Steel factor controls midline cell death of primordial germ cells and is essential for their normal proliferation and migration. Development 2006; 133: 4861-4869.
[15] de Jong J, Stoop H, Gillis AJ, van Gurp RJ, van de Geijn GJ, Boer M, Hersmus R, Saunders PT, Anderson RA, Oosterhuis JW and Looijenga LH. Differential expression of SOX17 and SOX2 in germ cells and stem cells has biological and clinical implications. J Pathol 2008; 215: 21-30.
[16] Kerr CL, Hill CM, Blumenthal PD and Gearhart JD. Expression of pluripotent stem cell markers in the human fetal testis. Stem Cells 2008; 26: 412-421.
[17] Wu Q, Chen X, Zhang J, Loh YH, Low TY, Zhang W, Sze SK, Lim B and Ng HH. Sall4 interacts with Nanog and co-occupies Nanog genomic sites in embryonic stem cells. J Biol Chem 2006; 281: 24090-24094.
[18] Honecker F, Stoop H, de Krijger RR, Chris Lau YF, Bokemeyer C and Looijenga LH. Pathobiological implications of the expression of markers of testicular carcinoma in situ by fetal germ cells. J Pathol 2004; 203: 849-857.
[19] Gaskell TL, Esnal A, Robinson LL, Anderson RA and Saunders PT. Immunohistochemical profiling of germ cells within the human fetal testis: identification of three subpopulations. Biol Reprod 2004; 71: 2012-2021.
[20] Gashaw I, Dushaj O, Behr R, Biermann K, Brehm R, Rubben H, Grobholz R, Schmid KW, Bergmann M and Winterhager E. Novel germ cell markers characterize testicular seminoma and fetal testis. Mol Hum Reprod 2007; 13: 721-727.
[21] Wong CC, Gaspar-Maia A, Ramalho-Santos M and Reijo Pera RA. High-efficiency stem cell fusion-mediated assay reveals Sall4 as an enhancer of reprogramming. PLoS One 2008; 3: e1955.
[22] Biermann K and Steger K. Epigenetics in male germ cells. J Androl 2007; 28: 466-480.
[23] Lind GE, Skotheim RI and Lothe RA. The epigenome of testicular germ cell tumors. APMIS 2007; 115: 1147-1160.
[24] Polanco JC and Koopman P. Sry and the hesitant beginnings of male development. Dev Biol 2007; 302: 13-24.
[25] Cao D, Li J, Guo CC, Allan RW and Humphrey PA. SALL4 is a novel diagnostic marker for testicular germ cell tumors. Am J Surg Pathol 2009; 33: 1065-1077.
[26] Depue RH, Pike MC and Henderson BE. Estrogen exposure during gestation and risk of testicular cancer. J Natl Cancer Inst 1983; 71: 1151-1155.
[27] Swerdlow AJ, Huttly SR and Smith PG. Prenatal and familial associations of testicular cancer. Br J Cancer 1987; 55: 571-577.
[28] Eble JN, World Health Organization and International Academy of Pathology. Pathology and genetics of tumours of the urinary system and male genital organs. Lyon: IARC Press, 2004.
[29] McGlynn KA and Cook MB. Etiologic factors in testicular germ-cell tumors. Future Oncol 2009; 5: 1389-1402.
[30] Kratz CP, Mai PL and Greene MH. Familial testicular germ cell tumours. Best Pract Res Clin Endocrinol Metab 2010; 24: 503-513.
[31] Stang A and Kuss O. Etiologic differences between seminoma and nonseminoma of the testis: a systematic review of epidemiologic studies. Hematol Oncol Clin North Am 2011; 25: 473-486, vii.
[32] Crockford GP, Linger R, Hockley S, Dudakia D, Johnson L, Huddart R, Tucker K, Friedlander M, Phillips KA, Hogg D, Jewett MA, Lohynska R, Daugaard G, Richard S, Chompret A, Bonaiti-Pellie C, Heidenreich A, Albers P, Olah E, Geczi L, Bodrogi I, Ormiston WJ, Daly PA, Guilford P, Fossa SD, Heimdal K, Tjulandin SA, Liubchenko L, Stoll H, Weber W, Forman D, Oliver T, Einhorn L, McMaster M, Kramer J, Greene MH, Weber BL, Nathanson KL, Cortessis V, Easton DF, Bishop DT, Stratton MR and Rapley EA. Genome-wide linkage screen for testicular germ cell tumour susceptibility loci. Hum Mol Genet 2006; 15: 443-451.
[33] Nathanson KL, Kanetsky PA, Hawes R, Vaughn DJ, Letrero R, Tucker K, Friedlander M, Phillips KA, Hogg D, Jewett MA, Lohynska R, Daugaard G, Richard S, Chompret A, Bonaiti-Pellie C, Heidenreich A, Olah E, Geczi L, Bodrogi I, Ormiston WJ, Daly PA, Oosterhuis JW, Gillis AJ, Looijenga LH, Guilford P, Fossa SD, Heimdal K, Tjulandin SA, Liubchenko L, Stoll H, Weber W, Rudd M, Huddart R, Crockford GP, Forman D, Oliver DT, Einhorn L, Weber BL, Kramer J, McMaster M, Greene MH, Pike M, Cortessis V, Chen C, Schwartz SM, Bishop DT, Easton DF, Stratton MR and Rapley EA. The Y deletion gr/gr and susceptibility to testicular germ cell

tumor. Am J Hum Genet 2005; 77: 1034-1043.
[34] Kanetsky PA, Mitra N, Vardhanabhuti S, Li M, Vaughn DJ, Letrero R, Ciosek SL, Doody DR, Smith LM, Weaver J, Albano A, Chen C, Starr JR, Rader DJ, Godwin AK, Reilly MP, Hakonarson H, Schwartz SM and Nathanson KL. Common variation in KITLG and at 5q31.3 predisposes to testicular germ cell cancer. Nat Genet 2009; 41: 811-815.
[35] Rapley EA, Turnbull C, Al Olama AA, Dermitzakis ET, Linger R, Huddart RA, Renwick A, Hughes D, Hines S, Seal S, Morrison J, Nsengimana J, Deloukas P, Rahman N, Bishop DT, Easton DF and Stratton MR. A genome-wide association study of testicular germ cell tumor. Nat Genet 2009; 41: 807-810.
[36] Gajendran VK, Nguyen M and Ellison LM. Testicular cancer patterns in African-American men. Urology 2005; 66: 602-605.
[37] McGlynn KA, Devesa SS, Graubard BI and Castle PE. Increasing incidence of testicular germ cell tumors among black men in the United States. J Clin Oncol 2005; 23: 5757-5761.
[38] Boldajipour B and Raz E. What is left behind--quality control in germ cell migration. Sci STKE 2007; 2007: pe16.
[39] Yan W, Samson M, Jegou B and 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: 682-699.
[40] Smith CA, McClive PJ, Western PS, Reed KJ and Sinclair AH. Conservation of a sex-determining gene. Nature 1999; 402: 601-602.
[41] Chaganti RS and Houldsworth J. Genetics and biology of adult human male germ cell tumors. Cancer Res 2000; 60: 1475-1482.
[42] Reuter VE. Origins and molecular biology of testicular germ cell tumors. Mod Pathol 2005; 18 Suppl 2: S51-60.
[43] Looijenga LH, de Leeuw H, van Oorschot M, van Gurp RJ, Stoop H, Gillis AJ, de Gouveia Brazao CA, Weber RF, Kirkels WJ, van Dijk T, von Lindern M, Valk P, Lajos G, Olah E, Nesland JM, Fossa SD and Oosterhuis JW. Stem cell factor receptor (c-KIT) codon 816 mutations predict development of bilateral testicular germ-cell tumors. Cancer Res 2003; 63: 7674-7678.
[44] Ottesen AM, Skakkebaek NE, Lundsteen C, Leffers H, Larsen J and Rajpert-De Meyts E. High-resolution comparative genomic hybridization detects extra chromosome arm 12p material in most cases of carcinoma in situ adjacent to overt germ cell tumors, but not before the invasive tumor development. Genes Chromosomes Cancer 2003; 38: 117-125.
[45] Almstrup K, Hoei-Hansen CE, Nielsen JE, Wirkner U, Ansorge W, Skakkebaek NE, Rajpert-De Meyts E and Leffers H. Genome-wide gene expression profiling of testicular carcinoma in situ progression into overt tumours. British journal of cancer 2005; 92: 1934-1941.
[46] Sperger JM, Chen X, Draper JS, Antosiewicz JE, Chon CH, Jones SB, Brooks JD, Andrews PW, Brown PO and Thomson JA. Gene expression patterns in human embryonic stem cells and human pluripotent germ cell tumors. Proc Natl Acad Sci U S A 2003; 100: 13350-13355.
[47] Korkola JE, Houldsworth J, Chadalavada RS, Olshen AB, Dobrzynski D, Reuter VE, Bosl GJ and Chaganti RS. Down-regulation of stem cell genes, including those in a 200-kb gene cluster at 12p13.31, is associated with in vivo differentiation of human male germ cell tumors. Cancer Res 2006; 66: 820-827.
[48] Giuliano CJ, Kerley-Hamilton JS, Bee T, Freemantle SJ, Manickaratnam R, Dmitrovsky E and Spinella MJ. Retinoic acid represses a cassette of candidate pluripotency chromosome 12p genes during induced loss of human embryonal carcinoma tumorigenicity. Biochim Biophys Acta 2005; 1731: 48-56.
[49] Skotheim RI and Lothe RA. The testicular germ cell tumour genome. APMIS 2003; 111: 136-150; discussion 150-131.
[50] Gilbert D, Rapley E and Shipley J. Testicular germ cell tumours: predisposition genes and the male germ cell niche. Nat Rev Cancer 2011; 11: 278-288.
[51] Kraggerud SM, Skotheim RI, Szymanska J, Eknaes M, Fossa SD, Stenwig AE, Peltomaki P and Lothe RA. Genome profiles of familial/bilateral and sporadic testicular germ cell tumors. Genes Chromosomes Cancer 2002; 34: 168-174.
[52] Korkola JE, Heck S, Olshen AB, Reuter VE, Bosl GJ, Houldsworth J and Chaganti RS. In vivo differentiation and genomic evolution in adult male germ cell tumors. Genes Chromosomes Cancer 2008; 47: 43-55.
[53] Rao PH, Houldsworth J, Palanisamy N, Murty VV, Reuter VE, Motzer RJ, Bosl GJ and Chaganti RS. Chromosomal amplification is associated with cisplatin resistance of human male germ cell tumors. Cancer Res 1998; 58: 4260-4263.
[54] Wilson C, Yang J, Strefford JC, Summersgill B, Young BD, Shipley J, Oliver T and Lu YJ. Overexpression of genes on 16q associated with cisplatin resistance of testicular germ cell tumor cell lines. Genes Chromosomes Cancer 2005; 43: 211-216.
[55] Noel EE, Perry J, Chaplin T, Mao X, Cazier JB, Joel SP, Oliver RT, Young BD and Lu YJ. Identification of genomic changes associated with cisplatin resistance in testicular germ cell tumor cell lines. Genes Chromosomes Cancer 2008; 47: 604-613.

[56] Forbes S, Clements J, Dawson E, Bamford S, Webb T, Dogan A, Flanagan A, Teague J, Wooster R, Futreal PA and Stratton MR. Cosmic 2005. Br J Cancer 2006; 94: 318-322.
[57] Besmer P, Murphy JE, George PC, Qiu FH, Bergold PJ, Lederman L, Snyder HW, Jr., Brodeur D, Zuckerman EE and Hardy WD. A new acute transforming feline retrovirus and relationship of its oncogene v-kit with the protein kinase gene family. Nature 1986; 320: 415-421.
[58] Yarden Y, Kuang WJ, Yang-Feng T, Coussens L, Munemitsu S, Dull TJ, Chen E, Schlessinger J, Francke U and Ullrich A. Human proto-oncogene c-kit: a new cell surface receptor tyrosine kinase for an unidentified ligand. Embo J 1987; 6: 3341-3351.
[59] Flanagan JG, Chan DC and Leder P. Transmembrane form of the kit ligand growth factor is determined by alternative splicing and is missing in the Sld mutant. Cell 1991; 64: 1025-1035.
[60] Vliagoftis H, Worobec AS and Metcalfe DD. The protooncogene c-kit and c-kit ligand in human disease. J Allergy Clin Immunol 1997; 100: 435-440.
[61] Rajpert-De Meyts E and Skakkebaek NE. Expression of the c-kit protein product in carcinoma-in-situ and invasive testicular germ cell tumours. Int J Androl 1994; 17: 85-92.
[62] Kemmer K, Corless CL, Fletcher JA, McGreevey L, Haley A, Griffith D, Cummings OW, Wait C, Town A and Heinrich MC. KIT mutations are common in testicular seminomas. Am J Pathol 2004; 164: 305-313.
[63] Madani A, Kemmer K, Sweeney C, Corless C, Ulbright T, Heinrich M and Einhorn L. Expression of KIT and epidermal growth factor receptor in chemotherapy refractory non-seminomatous germ-cell tumors. Ann Oncol 2003; 14: 873-880.
[64] Stoop H, Honecker F, van de Geijn GJ, Gillis AJ, Cools MC, de Boer M, Bokemeyer C, Wolffenbuttel KP, Drop SL, de Krijger RR, Dennis N, Summersgill B, McIntyre A, Shipley J, Oosterhuis JW and Looijenga LH. Stem cell factor as a novel diagnostic marker for early malignant germ cells. J Pathol 2008; 216: 43-54.
[65] Masson K and Ronnstrand L. Oncogenic signaling from the hematopoietic growth factor receptors c-Kit and Flt3. Cell Signal 2009; 21: 1717-1726.
[66] Biermann K, Goke F, Nettersheim D, Eckert D, Zhou H, Kahl P, Gashaw I, Schorle H and Buttner R. c-KIT is frequently mutated in bilateral germ cell tumours and down-regulated during progression from intratubular germ cell neoplasia to seminoma. J Pathol 2007; 213: 311-318.
[67] Coffey J, Linger R, Pugh J, Dudakia D, Sokal M, Easton DF, Timothy Bishop D, Stratton M, Huddart R and Rapley EA. Somatic KIT mutations occur predominantly in seminoma germ cell tumors and are not predictive of bilateral disease: report of 220 tumors and review of literature. Genes Chromosomes Cancer 2008; 47: 34-42.
[68] Pedersini R, Vattemi E, Mazzoleni G and Graiff C. Complete response after treatment with imatinib in pretreated disseminated testicular seminoma with overexpression of c-KIT. Lancet Oncol 2007; 8: 1039-1040.
[69] Pectasides D, Nikolaou M, Pectasides E, Koumarianou A, Valavanis C and Economopoulos T. Complete response after imatinib mesylate administration in a patient with chemoresistant stage IV seminoma. Anticancer Res 2008; 28: 2317-2320.
[70] Petitjean A, Mathe E, Kato S, Ishioka C, Tavtigian SV, Hainaut P and Olivier M. Impact of mutant p53 functional properties on TP53 mutation patterns and tumor phenotype: lessons from recent developments in the IARC TP53 database. Hum Mutat 2007; 28: 622-629.
[71] Heimdal K, Lothe RA, Lystad S, Holm R, Fossa SD and Borresen AL. No germline TP53 mutations detected in familial and bilateral testicular cancer. Genes Chromosomes Cancer 1993; 6: 92-97.
[72] Peng HQ, Hogg D, Malkin D, Bailey D, Gallie BL, Bulbul M, Jewett M, Buchanan J and Goss PE. Mutations of the p53 gene do not occur in testis cancer. Cancer Res 1993; 53: 3574-3578.
[73] Pectasides D, Papaxoinis G, Nikolaou M, Valavanis C, Aravantinos G, Fountzilas G, Tamvakis N, Pectasides E, Lekka I, Arapantoni-Dadioti P, Zizi A, Ghiconti I and Economopoulos T. Analysis of 7 immunohistochemical markers in male germ cell tumors demonstrates the prognostic significance of p53 and MIB-1. Anticancer Res 2009; 29: 737-744.
[74] Houldsworth J, Xiao H, Murty VV, Chen W, Ray B, Reuter VE, Bosl GJ and Chaganti RS. Human male germ cell tumor resistance to cisplatin is linked to TP53 gene mutation. Oncogene 1998; 16: 2345-2349.
[75] Kersemaekers AM, Mayer F, Molier M, van Weeren PC, Oosterhuis JW, Bokemeyer C and Looijenga LH. Role of P53 and MDM2 in treatment response of human germ cell tumors. J Clin Oncol 2002; 20: 1551-1561.
[76] Mayer F, Honecker F, Looijenga LH and Bokemeyer C. Towards an understanding of the biological basis of response to cisplatin-based chemotherapy in germ-cell tumors. Ann Oncol 2003; 14: 825-832.
[77] Colicelli J. Human RAS superfamily proteins and related GTPases. Sci STKE 2004; 2004: RE13.
[78] Lin JS, Webber EM, Senger CA, Holmes RS and Whitlock EP. Systematic review of pharmacogenetic testing for predicting clinical benefit to anti-EGFR therapy in metastatic

colorectal cancer. Am J Cancer Res 2011; 1: 650-662.
[79] Sommerer F, Hengge UR, Markwarth A, Vomschloss S, Stolzenburg JU, Wittekind C and Tannapfel A. Mutations of BRAF and RAS are rare events in germ cell tumours. Int J Cancer 2005; 113: 329-335.
[80] Ganguly S, Murty VV, Samaniego F, Reuter VE, Bosl GJ and Chaganti RS. Detection of preferential NRAS mutations in human male germ cell tumors by the polymerase chain reaction. Genes Chromosomes Cancer 1990; 1: 228-232.
[81] Honecker F, Wermann H, Mayer F, Gillis AJ, Stoop H, van Gurp RJ, Oechsle K, Steyerberg E, Hartmann JT, Dinjens WN, Oosterhuis JW, Bokemeyer C and Looijenga LH. Microsatellite instability, mismatch repair deficiency, and BRAF mutation in treatment-resistant germ cell tumors. J Clin Oncol 2009; 27: 2129-2136.
[82] Oldenburg J, Martin JM and Fossa SD. Late relapses of germ cell malignancies: incidence, management, and prognosis. J Clin Oncol 2006; 24: 5503-5511.
[83] Bokemeyer C, Nichols CR, Droz JP, Schmoll HJ, Horwich A, Gerl A, Fossa SD, Beyer J, Pont J, Kanz L, Einhorn L and Hartmann JT. Extragonadal germ cell tumors of the mediastinum and retroperitoneum: results from an international analysis. J Clin Oncol 2002; 20: 1864-1873.
[84] Sonne SB, Herlihy AS, Hoei-Hansen CE, Nielsen JE, Almstrup K, Skakkebaek NE, Marks A, Leffers H and Rajpert-De Meyts E. Identity of M2A (D2-40) antigen and gp36 (Aggrus, T1A-2, podoplanin) in human developing testis, testicular carcinoma in situ and germ-cell tumours. Virchows Arch 2006; 449: 200-206.
[85] Port M, Schmelz HU, Stockinger M, Sparwasser C, Albers P, Pottek T and Abend M. Gene expression profiling in seminoma and nonseminoma. J Clin Oncol 2005; 23: 58-69.
[86] Honorio S, Agathanggelou A, Wernert N, Rothe M, Maher ER and Latif F. Frequent epigenetic inactivation of the RASSF1A tumour suppressor gene in testicular tumours and distinct methylation profiles of seminoma and non-seminoma testicular germ cell tumours. Oncogene 2003; 22: 461-466.
[87] Emerson RE and Ulbright TM. Intratubular germ cell neoplasia of the testis and its associated cancers: the use of novel biomarkers. Pathology 2010; 42: 344-355.
[88] Noel EE, Yeste-Velasco M, Mao X, Perry J, Kudahetti SC, Li NF, Sharp S, Chaplin T, Xue L, McIntyre A, Shan L, Powles T, Oliver RT, Young BD, Shipley J, Berney DM, Joel SP and Lu YJ. The association of CCND1 overexpression and cisplatin resistance in testicular germ cell tumors and other cancers. Am J Pathol 2010; 176: 2607-2615.
[89] Sharma S, Kelly TK and Jones PA. Epigenetics in cancer. Carcinogenesis 2010; 31: 27-36.
[90] Almstrup K, Nielsen JE, Mlynarska O, Jansen MT, Jorgensen A, Skakkebaek NE and Rajpert-De Meyts E. Carcinoma in situ testis displays permissive chromatin modifications similar to immature foetal germ cells. Br J Cancer 2010; 103: 1269-1276.
[91] Smiraglia DJ, Szymanska J, Kraggerud SM, Lothe RA, Peltomaki P and Plass C. Distinct epigenetic phenotypes in seminomatous and nonseminomatous testicular germ cell tumors. Oncogene 2002; 21: 3909-3916.
[92] Wermann H, Stoop H, Gillis AJ, Honecker F, van Gurp RJ, Ammerpohl O, Richter J, Oosterhuis JW, Bokemeyer C and Looijenga LH. Global DNA methylation in fetal human germ cells and germ cell tumours: association with differentiation and cisplatin resistance. J Pathol 2010; 221: 433-442.
[93] Koul S, McKiernan JM, Narayan G, Houldsworth J, Bacik J, Dobrzynski DL, Assaad AM, Mansukhani M, Reuter VE, Bosl GJ, Chaganti RS and Murty VV. Role of promoter hypermethylation in Cisplatin treatment response of male germ cell tumors. Mol Cancer 2004; 3: 16.
[94] Cheung HH, Lee TL, Davis AJ, Taft DH, Rennert OM and Chan WY. Genome-wide DNA methylation profiling reveals novel epigenetically regulated genes and non-coding RNAs in human testicular cancer. Br J Cancer 2010; 102: 419-427.
[95] Kelland L. The resurgence of platinum-based cancer chemotherapy. Nat Rev Cancer 2007; 7: 573-584.
[96] Ishida S, Lee J, Thiele DJ and Herskowitz I. Uptake of the anticancer drug cisplatin mediated by the copper transporter Ctr1 in yeast and mammals. Proc Natl Acad Sci U S A 2002; 99: 14298-14302.
[97] Safaei R, Holzer AK, Katano K, Samimi G and Howell SB. The role of copper transporters in the development of resistance to Pt drugs. J Inorg Biochem 2004; 98: 1607-1613.
[98] Ishikawa T. The ATP-dependent glutathione S-conjugate export pump. Trends Biochem Sci 1992; 17: 463-468.
[99] Masters JR, Thomas R, Hall AG, Hogarth L, Matheson EC, Cattan AR and Lohrer H. Sensitivity of testis tumour cells to chemotherapeutic drugs: role of detoxifying pathways. Eur J Cancer 1996; 32A: 1248-1253.
[100] Reed E. Platinum-DNA adduct, nucleotide excision repair and platinum based anticancer chemotherapy. Cancer Treat Rev 1998; 24: 331-344.
[101] Honecker F, Mayer F, Stoop H, Oosterhuis JW, Koch S, Bokemeyer C and Looijenga LH. Xeroderma pigmentosum group a protein and chemotherapy resistance in human germ cell tumors. Lab Invest 2003; 83: 1489-1495.

[102] Zhou BB and Elledge SJ. The DNA damage response: putting checkpoints in perspective. Nature 2000; 408: 433-439.
[103] Mayer F, Gillis AJ, Dinjens W, Oosterhuis JW, Bokemeyer C and Looijenga LH. Microsatellite instability of germ cell tumors is associated with resistance to systemic treatment. Cancer Res 2002; 62: 2758-2760.
[104] Mayer F, Stoop H, Scheffer GL, Scheper R, Oosterhuis JW, Looijenga LH and Bokemeyer C. Molecular determinants of treatment response in human germ cell tumors. Clin Cancer Res 2003; 9: 767-773.
[105] Burger H, Nooter K, Boersma AW, Kortland CJ, van den Berg AP and Stoter G. Expression of p53, p21/WAF/CIP, Bcl-2, Bax, Bcl-x, and Bak in radiation-induced apoptosis in testicular germ cell tumor lines. Int J Radiat Oncol Biol Phys 1998; 41: 415-424.
[106] Baltaci S, Orhan D, Turkolmez K, Yesilli C, Beduk Y and Tulunay O. P53, bcl-2 and bax immunoreactivity as predictors of response and outcome after chemotherapy for metastatic germ cell testicular tumours. BJU Int 2001; 87: 661-666.