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

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

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 pre-spermatogonia 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-
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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 isochromosome 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.
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
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## Table 2. Common genetic and epigenetic alterations in TGCT

<table>
<thead>
<tr>
<th>TGCT subtype</th>
<th>Alteration (Effect)</th>
<th>Role in TGCT pathogenesis</th>
</tr>
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<tbody>
<tr>
<td><strong>Chromosomal</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12p</td>
<td>Invasive TGCT; Intratubular seminoma; Intratubular embryonal carcinoma</td>
<td>Gain of 12p, mostly isochromosome 12p (amplification of a cluster of genes, promoting tumor invasion)</td>
</tr>
<tr>
<td><strong>Single-gene mutations</strong></td>
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<tr>
<td>KIT</td>
<td>IGCNU; Seminoma &gt; NSGCT</td>
<td>Activating mutation in the receptor for KITLG (suppressing apoptosis)</td>
</tr>
<tr>
<td>TP53</td>
<td>Seminoma</td>
<td>Inactivating mutation in cell cycle regulator p53 (leading to impaired DNA repair and cell cycle dysregulation)</td>
</tr>
<tr>
<td>KRAS</td>
<td>Seminoma &gt; NSGCT</td>
<td>Activating mutation in a small GTPase (promoting cell proliferation, differentiation, and migration)</td>
</tr>
<tr>
<td>NRAS</td>
<td>Seminoma &gt; NSGCT</td>
<td>Same as KRAS</td>
</tr>
<tr>
<td>BRAF</td>
<td>Seminoma &lt; NSGCT</td>
<td>Activating mutations in a serine/threonine kinase (promoting cell proliferation and differentiation)</td>
</tr>
<tr>
<td><strong>Differentially expressed genes</strong></td>
<td></td>
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</tr>
<tr>
<td>Tumor vs normal</td>
<td></td>
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</tr>
<tr>
<td>IGCNU</td>
<td>Up-regulated: KITLG, PDLPN, ANXA3, C12orf35, DOCK11, IL22RA1, KIT, L1TD1, LIN28A, MYC1, NAG1, NSMAF, OSBP1, POU5F1 (OCT3/4), SLC25A16, SOX17, TCL6, TFCP21L, UPP1</td>
<td></td>
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<tr>
<td>Seminoma</td>
<td>Upregulated: JUP, CCND2, LITTL1, PIM2, BAX, CACNB3, CCNF, CDC25B, CDH3, ET1, IL6R, KCM8, MMP12, MYCN, OSBP1, PIM1, SLC43A1, SOX4, TCL1B, TUBB</td>
<td>Downregulated: CLU</td>
</tr>
<tr>
<td><strong>Subtype-specific signatures</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Seminoma</td>
<td>LITTL1</td>
<td></td>
</tr>
<tr>
<td>Embryonal carcinoma</td>
<td><strong>DNMT3B</strong>, DPPA4, GAL, GPC4, POU5F1, TERF1</td>
<td></td>
</tr>
<tr>
<td>Yolk sac tumor</td>
<td>AFB, APOA2, B4GALT4, BMP2, C5, CYP26A1, DSCAM, EOMES, FAM89A, FERMT2, FLRT3, FOXA2, LEPREL1, LRRN1, NEK2, NRXN3, OTX2, RAGE, SEBOX, VTN</td>
<td></td>
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<tr>
<td>Choriocarcinoma</td>
<td>CGA</td>
<td></td>
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<tr>
<td>Teratoma</td>
<td><strong>EMP1</strong>, CDH17, MFAP4, NFKBIZ, TSPAN8</td>
<td></td>
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<tr>
<td><strong>Epigenetic changes</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Global DNA methylation</td>
<td>IGCNU; Seminoma</td>
<td>Low level (Promoter hypomethylation allows gene transcription)</td>
</tr>
<tr>
<td>Embryonal carcinoma</td>
<td>Intermediate level</td>
<td>1. Associated with transformed undifferentiated tumor 2. Hypermethylation → chemoresistance</td>
</tr>
<tr>
<td>Yolk sac tumor; Choriocarcinoma; Teratoma</td>
<td>High level: similar to other solid tumors (Promoter hypermethylation suppresses gene transcription)</td>
<td>1. Associated with more differentiated tumors 2. Hypermethylation → chemoresistance</td>
</tr>
</tbody>
</table>

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, PTK1, 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 IGCCU, most seminomas, and some NSGCT do [61-63]. Interestingly, KITLG showed a similar expression pattern in IGCCU, 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 tumorogenesis [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 IGCCU. 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-
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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 of 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 PDYN (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 IG-CNU 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, choriocarcinomas, 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, Loojenga 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 cisplatin-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 TGCT. 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 TGCT [76]. Although a high ratio of proapoptotic Bax to anti-apoptotic Bcl-2 was found in invasive TGCT, the Bax:Bcl2 ratio seems not to be associated with TGCT 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 generic 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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