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Testicular Germ Cell Tumor Genomics

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Abstract

Purpose of review: Testicular Germ cell tumors (TGCT) are a model for curable cancer due to exquisite chemosensitivity and incorporation of multimodal therapy. Nevertheless, our ability to predict metastases in early-stage disease and responders to chemotherapy in advanced disease is limited. Treatment options for cisplatin-resistant disease are sparse. A further understanding of TGCT biology may allow for more precise patient counseling and identify novel therapies in patients with cisplatin-resistant disease.

Recent findings: Adult TGCTs are characterized by frequent chromosomal anomalies and low rates of somatic mutations. Large scale integrated molecular analysis of early-stage TGCT patients is actively underway. In addition to ubiquitous gain of isochromosome 12p, current molecular studies have confirmed mutations of previously described genes (i.e. *KIT* and *KRAS*) and described novel mutations. Analysis of cisplatin-resistant cases has identified high rates of alterations within the *TP53-MDM2* axis and a high proportion of patients with potentially actionable targets, including *TP53-MDM2*, PI3 Kinase, and MAPK signaling pathway alterations. The role of epigenetics in TGCT development and prognosis is also being further characterized.

Summary: Further molecular characterization of TGCT may allow for avoidance of unnecessary treatment in patients with early-stage disease and also provide new treatment options in patients with cisplatin-resistant disease.

Keywords

testicular cancer; genomics; germ cell tumor

INTRODUCTION

Testicular germ cell tumors (TGCTs) represent the most common solid malignancy in young men.¹ TGCTs are divided broadly into seminoma and nonseminoma (NSGCT) as these distinctions inform management decisions, treatment options, and prognosis. Even when presenting in an advanced setting, testicular cancer represents a rare oncologic success story: the 5-year overall survival rate is over 80% even among patients with metastatic disease with fewer than 400 deaths from TGCT in the United States estimated in 2016.^{1,2}

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The optimal treatment for many patients with TGCT is unknown given the high cancer-specific survival rates and relative young age at presentation for most men diagnosed with testicular cancer,³ which highlights the need to minimize treatment morbidity while maximizing cancer control. For example, stage I TGCTs can be effectively managed with surveillance, chemotherapy, and radiation (for seminoma) or RPLND (for NSGCT). With surveillance there is a subsequent need for intervention in about 20% of cases, and conversely the disadvantage of immediate intervention is potential overtreatment in 80% of cases along with attendant risks including surgical risks, infertility, organ damage, and future malignancies.⁴ In the advanced setting, up to 30% of advanced germ cell tumors exhibit cisplatin-resistance and require salvage treatment, which is effective less than 50% of the time.⁵ Significant uncertainty remains regarding when to perform post-chemotherapy lymph node dissection and decisions about optimal salvage chemotherapy regimens in the refractory clinical state.

Improving upon patient outcomes requires better understanding of the molecular and genetic basis of these tumors. TGCTs often represent a mix of histologies, which make genetic correlates with a particular histology difficult, necessitating careful microdissection.⁶ Furthermore, the degree of intratumoral heterogeneity is not yet well understood in TGCT, which complicates the implementation of precision medicine for this disease.⁷

TESTICULAR GERM CELL TUMOR DEVELOPMENT

Identified risk factors for TGCT include cryptorchidism, family history, previous germ cell tumor, subfertility, and disorders of sexual differentiation, yet the majority of TGCTs occur spontaneously without known genetic loci of susceptibility.^{8,9} Recent genomic studies suggest heritability of TGCT at 48.9%, with known single nucleotide polymorphisms (SNPs) representing only 9.1% of total heritability.¹⁰ Several gene loci were identified using genome-wide association studies (GWAS) that appear related to TGCT predisposition, including *KITLG*, *SPRY4*, *BAK1*, *DMRT1*, *TERT*, and *ATF7IP*, proposed gene *TGCT1* on Xq27, and gr/gr deletion in the AZFc region on Y-chromosome.^{11–18} Many of the loci identified on GWAS are directly involved in the KIT-KITLG signaling cascade, confirming the central role of this pathway in TGCT tumorigenesis.

TGCT development is a polygenic, multistep process; initial events occur in the embryo stage of development, followed by dormancy until puberty where hormonal stimulation involved in spermatogenesis results in further genetic events that result in the clinical manifestation of TGCT.¹⁹ TGCT are histologically divided into seminomas, which resemble primordial germ cells, and NSGCT, which are more undifferentiated (embryonal carcinomas) or differentiated towards embryonic (teratoma) or extra-embryonic (yolk sac or choriocarcinoma) elements.^{15,20} There is support that both histologic variants of TGCT arise from a common pathogenesis, suggested by the finding that about 15% of TGCTs contain both seminomatous and non-seminomatous histologies.^{21–23} Despite their heterogeneity, all TGCTs are believed to arise from intratubular germ cell neoplasia (ITGCN), a precursor lesions often visualized adjacent to invasive TGCT.²⁴ The genetic landscape of ITGCN is not well established, but *KIT* mutations are described in some cases.²⁵

TGCT GENETIC AND EPIGENETIC PROFILES

Chromosomal Abnormalities

The genetics of TGCT tend to reflect their embryonic origin, with a generally low incidence of mutations, loss of parental pattern of genomic imprinting, distinct DNA methylation profiles, and frequent uniparental disomies.²⁶ Seminoma and ITGCN cells are typically hypertriploid, while NSGCT cells are typically hypotriploid.²⁷ Compared to adult solid tumors such as lung, kidney, and bladder that have mutation rates between 8–11 mutations/megabase, the mutation frequency of TCGTs is much lower at approximately 0.5 mutations/megabase.²⁸

Gain of 12p – The only consistent chromosomal abnormality of TGCT—The presence of an isochromosome of chromosome 12 [i(12p)] on karyotype analysis in many TGCT specimens was the first reported genetic marker of TGCT.²⁹ Fluorescence in situ hybridization (FISH) confirmed extra copies of 12p in virtually all TGCT specimens, becoming a hallmark pathognomonic for TCGT. The exact mechanism through which gain of 12p results in TGCT is unclear, but a number of genes are implicated including the proto-oncogenes Cyclin D2 (*CCND2*)³⁰ and *KRAS*,³¹ the growth factor receptor *TNFRSF1A*, *GLUT3* (glucose transporter), *REA* (estrogen receptor), *FLJ22028*,³² *NANOG*, *STELLAR*/*DPPA3*, and *GDF3* (stem-cell associated genes)³³ that may result in transformation and maintenance of pluripotency.⁶

Other Chromosomal Abnormalities—A number of karyotypic analyses have uncovered other chromosomal anomalies associated with TGCTs.^{34,35} Increase in copy numbers of chromosomes 7, 8, 17, and X and decreases in copy numbers of chromosomes 4, 11, 13, and 18 are reported.³⁴ Cytogenetic analysis on 124 microdissected specimens identified nonrandom rearrangements with an association to certain histologies (1p32–36 and 7q11.2 rearrangements associated with teratoma; 1p22 rearrangements associated with yolk-sac tumor) and other clinical characteristics.³⁶ Genomic studies investigating the clinical significance of i12p and other chromosomal abnormalities have generally been plagued by inconsistent results.^{37–41}

Single Gene Mutations

KIT—The *KIT* gene, which encodes a proto-oncogene receptor tyrosine kinase protein and plays crucial roles in cell survival and proliferation, is well characterized in TGCT.⁴² A query of the Catalogue of Somatic Mutations in Cancer (COSMIC) database identified *KIT* mutations in 19% (52/261) of seminomas and just 2% (2/98) of NSGCT, confirming that while rare in nonseminomas, a significant proportion of seminomas harbor *KIT* mutations.^{9,33,43,44} *KIT* mutations in TCGT pose attractive treatment possibilities in light of highly effective response rates of gastrointestinal stromal tumor (GIST) that harbor *KIT* mutations to imatinib (Gleevec).⁴⁵ A small phase II study of imatinib in heavily pretreated GCTs demonstrating *KIT*-positivity on immunohistochemical staining failed to demonstrate any response,⁴⁶ though imatinib-sensitive *KIT* mutations are reported in TGCTs.^{47,48} Recent next generation sequencing (NGS) efforts identified that most patients with TGCT harbor

exon 17 mutations, which are generally imatinib-resistant, emphasizing the need for precise genotyping and potentially explaining the disappointing results of the phase II study.⁴⁹

KRAS/NRAS—KRAS and NRAS are proto-oncogene receptor tyrosine kinase proteins that activate multiple downstream pathways including the Raf/MEK/ERK and PI3Kinase pathways.⁵⁰ A query of the COSMIC database indicates *KRAS* and *NRAS* mutations in just 5–7% of seminomas and 0% of nonseminomas.³³ *RAS* mutations and *FGFR3* mutations were identified at a higher frequency in chemoresistant versus sensitive tumors⁵¹ and given the potential clinical relevance of RAS pathway mutations and potential therapeutic approaches,^{49,52} further research is needed to clarify the roles of such mutations in TGCTs.

BRAF—The *BRAF* gene encodes a serine/threonine kinase that regulates the MAP kinase/ERK pathway and plays an important role in cell proliferation and differentiation.⁹ COSMIC query indicates that *BRAF* mutations are rare in TGCT (1% of seminoma, 2% of nonseminomas).^{33,44} Honecker et al. compared unselected TGCTs to cisplatin-resistant TGCTs and found a significantly higher incidence of *BRAF* (*V600E*) mutations in the resistant vs. sensitive tumors (26% vs. 1%).⁵³ These findings were not reproduced in a subsequent study in patients with cisplatin-resistant TGCT, where *BRAF* mutations were not identified though hotspot variants in *PIK3CA*, *AKT1*, and *FGFR3* were noted.⁵¹ The disparate reported incidence of *BRAF* mutations highlights the need for further research with contemporary sequencing platforms.

TP53—The *TP53* gene encodes p53 – a cell-cycle regulatory protein that plays an important role in stress response through induction of apoptosis and cell-cycle arrest for DNA repair. Loss of p53 function results in lack of cell-cycle regulation and is associated with more aggressive tumors and chemotherapy-resistance.^{54–56} While *TP53* mutations are common in somatic tumors,⁵⁵ they are rare in TGCTs, with the COSMIC database finding *TP53* mutations in 7% of seminomas (10/148) and 0% of nonseminomas (0/9).^{44,54} Paradoxically, TGCTs often over-express *wild-type* p53^{57–59} though this p53 over-expression may occur in conjunction with the over-expression of various p53 inhibitors, including micro RNAs⁶⁰ and the oncoprotein murine double minute 2 (*mdm2*), which inhibits *wild-type* p53 through nuclear export and ubiquitination.⁶¹ Researchers have found that interference of *mdm2*-p53 binding with the small molecule inhibitors RITA and Nutlin-3 rescued the p53 pathway, resulting in cell-cycle arrest and apoptosis in tumor cells, which has exciting treatment implications.⁶² The incidence of *TP53* pathway mutations, including *MDM2* amplifications, was recently reported to be approximately 25% among patients with cisplatin-resistant disease.⁶³

The Future—The search for genetic alterations with clinical relevance to TGCT is being accelerated by the advent of NGS. Importantly, The Cancer Genome Atlas (TCGA) is conducting an integrative analysis of 150 TGCTs, which will provide comprehensive data on copy number changes, somatic mutation profile, RNA expression, methylation status, and other molecular characteristics of TGCT. To date, whole exome sequencing of 42 TGCT cases demonstrated that in addition to 12p amplification and *KIT* mutations, recurrent mutations were noted in the tumor suppressor gene *CDC27* (11.9%) and copy number

analysis demonstrated amplification of the spermatocyte development gene *FSIP2* (15.3%) in a 0.4 Mb region at Xq28 (15.3%).²⁸ These authors also reported missense mutations in the DNA repair gene *XRCC2* in two treatment-resistant tumors, implying that impaired DNA repair may lead to cisplatin resistance.²⁸ Another study which performed whole exome sequencing on 8 patients with seminoma found a total of 98 novel and distinct gene mutations, in addition to well-known *KIT* and *KRAS* mutations.⁶⁴

NGS may provide key information for clinical decision making. In a study of patients with cisplatin-resistant GCT treated with sunitinib, NGS identified a rare *RET* amplification known to be sensitizing to sunitinib in the lone responder.⁶⁵ Recent data suggests that actionable targets may be present in up to 50% of patients with cisplatin-resistant GCT, providing hope that other targeted solutions exist to these difficult clinical challenges.⁴⁹

Active research is ongoing to identify patients who may be at risk for treatment-related toxicities, including infertility, hypogonadism, secondary malignancies, metabolic syndrome, cardiovascular disease, and neurologic toxicity.⁵ Certain polymorphisms, including *GSTP1*, are associated with the development of neurotoxicity though genetic predisposition factors for the development of long-term sequelae of chemotherapy have not been identified for most treatment-related toxicities.⁶⁶

Gene Expression Profiling

Differential gene expression patterns plays a key role in determining the histologic phenotype and biology of the tumor. There are potential pitfalls in interpreting expression data since TGCTs represent a rare tumor; as such, most studies represent a small sample size using different gene array chip technologies and different statistical methods. Furthermore, gene-profiling technology relies on the principle of comparing neoplastic tissue with control tissue, but this highly sensitive technology can be easily contaminated, especially in TGCT where normal testicular tissue contains a mixture of cell types and tumors often represent varied histologies.⁹

Nevertheless, several interesting expression findings are reported. Researchers from Memorial Sloan Kettering Cancer Center performed gene expression profiling on NSGCT from cisplatin-treated patients to identify a 10-gene model that was independently predictive of survival after clinical risk stratification was included in a multivariate model. Interestingly, genes involved in immune-response pathways were more highly expressed in patients with favorable clinical outcomes, while genes associated with active differentiation were more highly expressed in patients with poorer clinical outcome.⁶⁷ As a follow-up, the authors performed comparative genomic hybridization to identify regions associated with survival and then identified individual genes associated with survival but were distinct from their previous expression-only analysis.⁶⁸

Certain gene expression levels appear to correlate to cisplatin-resistance. Seladin-1, a multi-functional protein involved in inhibition of apoptosis through regulation of caspase-3, had highest expression in teratoma (considered chemo/radiotherapy-resistant), and lowest expressions in chemo/radiotherapy-sensitive seminoma.⁶⁹ In another study, 3 TGCT cell lines made resistant to cisplatin via long-term exposure to the drug had marked increase in

CCND1 expression, a cell cycle regulatory gene implicated in cisplatin-resistance.⁷⁰ Other gene expression patterns and transcriptome sequencing (complete set of RNA transcripts produced) show promising early results in differentiating metastatic from nonmetastatic tumors.^{71,72}

Epigenetics

Epigenetic modifications in DNA or associated proteins beyond the DNA sequence itself are recognized to play a role in the development of many malignancies, including TGCT development and associated with chemotherapy resistance.⁷³ There are three main epigenetic mechanisms identified: DNA promoter methylation, chromatin remodeling, and microRNA (miRNA) regulation.⁷⁴ While chromatin remodeling and miRNA analyses are nascent for TGCTs, DNA promoter methylation differences are more extensively studied in TGCTs.⁷⁵ Seminomas are extensively hypomethylated compared to nonseminomas which show higher DNA methylation frequencies on the level of other solid tumors.^{76–78}

In NSGCT patients, Koul et al. found that *RASSF1A* and *HIC1* promoter hypermethylation was associated with cisplatin-resistance, while *MGMT* and *RARB* promoter hypermethylation was associated with cisplatin sensitivity. Furthermore, cisplatin treatment appeared to induce *de novo* promoter hypermethylation *in vivo*, potentially explaining acquired cisplatin-resistance in some patients.⁷⁹ Cheung et al. studied the DNA methylation profile of 6 embryonal carcinomas and identified 40 genes/non-coding RNAs with hypermethylated promoters, including several sex-linked genes and *RBMY1A*, a testis-specific gene for spermatogenesis not previously implicated in TGCT.⁸⁰ Honecker reported promoter methylation of *hMLH1* and resultant reduced protein expression was associated cisplatin-resistance.⁵³ In a provocative study, Wermann et al. demonstrated a relationship between 5-methyl cystosine and chemotherapy sensitivity in TGCT whereby cisplatin-resistant seminomas showed a hypermethylated pattern not otherwise noted in seminomas. Furthermore, a cell line from this tumor specimen demonstrated cisplatin sensitivity after demethylation with 5-azacytidine.⁸¹

Although the functions of the majority of miRNAs that regulate gene expression are yet to be elucidated, miRNA-372 and –373 are identified to suppress the p53 pathway in TGCTs, allowing cellular proliferation in the presence of *wild-type* p53.⁸² miRNA 449a and 449b, which are believed to suppress cell proliferation through various p53 dependent and independent mechanisms, are decreased in TGCT specimens compared to normal testes.⁸³ Promoter hypermethylation of miRNA 199a has been implicated in the upregulation of PODXL, an anti-adhesion protein expressed in TGCT and implicated in development of invasiveness and metastasis.⁸⁴

Cellular differentiation can occur through epigenetic alterations, and TGCTs are no different. The pluripotency and transcription factor Oct4 appears to play an important role in maintaining an apoptotic response to DNA damage.⁸⁵ The relationship between Oct4 and cisplatin responsiveness is complex and several studies show no direct correlation with Oct4 expression and treatment response.^{86,87}

Conclusion

TGCT development is a complex, multistep process that likely begins *in utero* during the embryo stage with GWAS identifying a strong inheritable component. The precursor lesion to invasive tumors is ITGCN, which has not been thoroughly genetically characterized but likely involves KIT/KITLG signaling in a proportion of patients. While believed to have a common etiology, precursor lesion (ITGCN), and chromosomal aberration profile (gain of 12p), modern genetic analyses demonstrate significant differences between tumor types that are currently being investigated to explain their distinct biology and provide clues in responsiveness to treatment. Broad chromosomal abnormalities are features of TCGT while somatic mutation rates are low and recurrent mutations are infrequent. Mechanisms of cisplatin-resistance are not well characterized but our understanding is increasing with large scale sequencing efforts. Molecular characteristics of TCGT are currently not used for predicting clinical behavior or response to treatment. Nevertheless, while still in the early phases, elucidation of the genetic and epigenetic characteristics of TGCT will very likely have significant clinical implications regarding prevention, treatment strategy, and prognosis.

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References of Special Interest:

**Litchfield K, Thomsen H, Mitchell JS, et al. Quantifying the heritability of testicular germ cell tumour using both population-based and genomic approaches. *Sci Rep* 2015;5:13889.

These authors performed a population-based analysis in Sweden and estimated heritability of TGCT at 48.9%. GWAS estimated heritability by all common SNPs at 37.4%, a complimentary finding using a different technique.

**Litchfield K, Summersgill B, Yost S, et al. Whole-exome sequencing reveals the mutational spectrum of testicular germ cell tumours. *Nat Commun* 2015;6:597

These authors performed whole-exome sequencing of TGCTs and found a uniformly low mutational rate compared to other common somatic tumors. Furthermore, they confirmed the previous findings of gain of chromosome 12p, *KIT* mutations and also found novel mutations implicated in cisplatin-resistance.

**Korkola JE, Heck S, Olshen AB et al. Development and validation of a gene-based model for outcome prediction in germ cell tumors using a combined genomic and expression profiling approach. *PLoS One* 2015;10:e0142846.

The authors attempted to improve upon their previous work correlating gene-expression profiling to survival in patients with NSGCT treated with cisplatin. In this study they used comparative genomic hybridization to first identify regions of differential expression to find specific gene expression levels that were a significant independent prognostic marker of outcomes.

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Key Points

- Adult TGCTs have frequent chromosomal anomalies, including ubiquitous gain of isochromosome 12p, and low rates of somatic mutations.
- Recent molecular studies have confirmed well-described TGCT-associated genetic mutations (i.e. *KIT* and *KRAS*) and also identified novel mutations.
- High rates of alterations within the *TP53-MDM2* axis are seen in patients with platinum-resistant disease as are potentially actionable targets, including *TP53-MDM2*, PI3 Kinase, and MAPK signaling pathway alterations.
- Increasing genomic and epigenomic characterization of TGCT may shed light on the biology these tumors, including prognosis and mechanisms of cisplatin-resistance.