HHS Public Access

Author manuscript

Curr Opin Urol. Author manuscript; available in PMC 2020 July 01.

Published in final edited form as:

Curr Opin Urol. 2019 July ; 29(4): 344–349. doi:10.1097/MOU.0000000000000642.

Genetics of Testicular Germ Cell Tumors

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Abstract

Purpose of review: Understanding the molecular basis underlying testicular germ cell tumors (TGCTs) may help improve patient outcomes, particularly for patients with poorer risk or chemoresistant disease. Here, we review the major contemporary advances in elucidating TGCT genetics by discussing patterns of TGCT inheritance, recent genomic and transcriptomic discoveries in TGCT, and the role of genetics in predicting therapeutic resistance and in guiding treatment.

Recent findings: In the absence of a major high-penetrance TGCT susceptibility gene, inheritance is likely driven by a complex polygenic model with considerable variation. The most common genomic alterations found in TGCTs include gains in chromosome 12p and mutations in *KIT*, *KRAS*, and *NRAS*, particularly in seminomas. Sensitivity to cisplatin-based chemotherapy likely relies on intact *TP53*, reciprocal loss of heterozygosity, and high mitochondrial priming. Targetable mutations are uncommon in TGCTs, however, posing a challenge for the development of effective personalized therapies. Consistent with the characteristically low tumor mutational burden, immune checkpoint inhibitors do not appear to be effective for most TGCTs.

Summary: Refinements in next-generation sequencing techniques over the last few years have enabled considerable advances in elucidating the genomic, transcriptomic, and epigenetic landscape of TGCTs. Future efforts focused on developing novel treatment modalities are needed.

Keywords

testis cancer; germ cell tumor; genetics; inheritance; chemoresistance

INTRODUCTION

Testicular germ cell tumors (TGCTs) represent the most common malignancy in young men, with a projected incidence of 9,560 new diagnoses in the U.S. in 2019.(1) TGCTs exhibit a heterogeneous clinical and pathologic spectrum, broadly classified into seminomatous and

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Conflicts of interest: none

non-seminomatous (NSGCT) groups. NSGCT is further characterized by various histologic subtypes, which may be pure or mixed. Understanding pathologic differences between TGCT subtypes is an important distinction given the implications both for prognosis and in guiding treatment strategies. Depending on stage and histology, patients may be effectively managed with surveillance, surgery, chemotherapy, radiation, or some combination thereof. As a whole, TGCT patients tend to do well clinically, even in the advanced setting, as evidenced by >80% 5-year overall survival and fewer than 500 deaths from TGCT projected for 2019 in the U.S.(1, 2)

A better understanding of the molecular and genetic basis underlying these heterogeneous tumors may help further improve upon patient outcomes, particularly for those with poorer risk or chemoresistant disease. It is this very heterogeneity, however, that makes it particularly difficult to study genetic correlates in TGCTs. Nevertheless, with the refinement of next-generation sequencing (NGS) techniques, the last few years have witnessed considerable advances in elucidating the genomic, transcriptomic, and epigenetic landscape of TGCTs. Herein, we review the major contemporary advances and future directions in understanding TGCT genetics by discussing patterns of TGCT inheritance, recent efforts to elucidate the genomic landscape of TGCT, and the role of genetics in predicting therapeutic resistance and in guiding treatment. Rather than provide a comprehensive overview of the genetics of TGCT, the present review will focus primarily on novel developments that emerged over the last couple years.

COMPLEX GENETIC PATTERNS UNDERLYING TGCT INHERITANCE

A thorough understanding of the genomic and environmental factors contributing to the rising incidence of TGCTs may shed light on the reasons for this trend and potentially identify approaches to counteract it.(3) Identified risk factors for developing TGCT include cryptorchidism, prior GCT, subfertility, disorders of sexual differentiation, and family history.(3-8) Among these, family history represents the strongest risk factor and may increase risk by up to 10-fold.(8-11)

Causative germline mutations for TGCTs remain largely undetected, though several single nucleotide polymorphisms (SNPs) have been associated with familial TGCT risk. Proposed candidate genes have included those linked to germ cell differentiation pathways (including those affecting the androgen receptor(12, 13)), cilia-microtubule function (e.g., *DNAAF1* inactivation(14)), and the receptor tyrosine kinase *KIT*/*KITLG* signaling pathway (e.g., rare deleterious variants in the phosphodiesterase *PDE11A*(15)). In an earlier genome-wide association study (GWAS) for TGCT, Rapley et al. found strong evidence for susceptibility loci on chromosomes 5, 6, and 12 accounting for 7% of the risk to siblings and 10% of the risk to offspring of TGCT patients.(16) They also noted that *KIT*/*KITLG* involvement, largely found on chromosome 12, may account for the strongest association. In a subsequent multistage GWAS of over 25,000 individuals, Litchfield et al. identified 4 additional susceptibility loci for TGCT including 3q23, 11q14.1, 16p13.13, and 16q24.2, supporting a polygenic model of TGCT.(17) It is important to note, however, that the genetic defects identified are rare and together account for only a minority of TGCT cases.

The role and interaction of genetic and non-genetic factors in driving familial TGCT were explored more extensively in two recent studies.(**18, **19) In one study, Litchfield et al. analyzed germline whole-exome (WES) data for 919 TGCT patients (306 familial cases) and 1,609 healthy controls, encompassing nearly one million rare gene variants that included 114 cancer susceptibility genes and 49 TCGT susceptibility loci from prior GWAS.(**19) They were unable to find a major high-penetrance TGCT susceptibility gene, again supporting a polygenic model of inherited susceptibility with considerable variation.

In another larger-scale study Loveday et al. performed polygenic risk score analysis of 37 TGCT susceptibility SNPs in 3,931 sporadic and 236 familial TGCT cases and 12,368 controls.(**18) In contrast to their prior work,(**19) they found a clear enrichment for TGCT susceptibility alleles in familial compared to sporadic cases, and they noted that many SNPs would not have been previously detected as they mapped to noncoding regions of DNA. The majority of familial TGCT cases were attributable to polygenic enrichment, suggesting that in the absence of a major high-penetrance TGCT susceptibility gene, familial clustering is likely driven by aggregate effects of polygenic variation.

Together, these studies highlight the complex nature of the genetic basis underlying inheritance patterns in TGCT. Although there is presently no defined role for genetic testing of family members of affected individuals, clinicians must maintain a high degree of vigilance, as these members are at an increased baseline risk of developing TGCT.

RECENT EFFORTS TO ELUCIDATE THE GENETIC LANDSCAPE OF TGCT

Chromosome 12p gains constitute the most common copy number alteration seen in TGCTs.(20-22) Single-gene point mutations are generally uncommon, though mutations in *KIT*, *TP53 (*for mediastinal primary tumors), *KRAS/BRAF*, and *NRAS* have been found to be recurrently mutated.(23) The *KIT* proto-oncogene in particular is the most commonly mutated gene in TGCTs, though such mutations are seen in only 19% of seminomas and 2% of NSGCTs.(24-27) Notably, different frequencies of chromosome 12q gains and *KITLG* variants between Caucasians and African-Americans may account for some of the ethnic variation seen in TGCTs.(16, 28-30)

In multiple NGS studies, although the common genomic alterations identified in TGCTs were recurrently identified, including 12p gains and KRAS and KIT mutations, targetable alterations were not as well-defined.(20, 22, **31-34) For example, in a recent comprehensive study of the mutational profile of 42 TGCT patients, Litchfield et al. noted a relatively low total mutational burden (TMB) compared to other malignancies and found common copy number alterations to include 12p gains and amplifications of the spermatocyte development gene FSIP2 and Xq28, along with recurrent mutations in KIT and the tumor suppressor gene CDC27.(22) Likewise, in another clinically integrated molecular analysis of tumors from 47 patients with TGCTs and 2 additional patients with primary mediastinal GCTs, Taylor-Weiner et al. used WES and RNA sequencing to identify highly recurrent chromosome arm-level amplifications and reciprocal deletions (reciprocal loss of heterozygosity (LOH)) as the primary somatic feature of GCTs.(**31) They also noted that

KRAS and RPL5 were significantly mutated, with KRAS mutations likely occurring phylogenetically after chromosome 12p gains.

In 2018, The Cancer Genome Atlas (TCGA) Research Network published their comprehensive analysis of 137 primary TGCTs using genomic, epigenomic, transcriptomic, and proteomic analysis.(**35) As previously identified, Shen et al. similarly noted that recurrent somatic mutations were rare with low overall TMB, and activating mutations were found in just 3 genes: KIT (18%), KRAS (14%), and NRAS (4%). These gene mutations were found primarily in seminoma patients, and enhanced KIT-PI3K-RAS signaling appeared to play a significant role in most seminomas. The authors also found that the seminoma, embryonal carcinoma, yolk sac tumor, and teratoma histologic subtypes harbored distinct DNA methylation, miRNA expression, immune infiltration, and copy number aberration profiles. Furthermore, they reported potential biomarkers for risk stratification, including microRNA (miRNA) expressed in teratoma, and methylation at non-canonical cytosine sites (CpH sites), observed in the setting of embryonal carcinoma. Together, their findings may provide biomarkers for studying TGCTs, risk-stratifying patients, and identifying potential therapeutic targets.

MICRORNAS: EMERGING BIOMARKERS FOR TGCT

The role of miRNA as an emerging serum biomarker for TGCT has been explored in several contemporary studies. miRNAs are small noncoding regions of RNA that are involved with epigenetic regulation of gene expression.(36) Several groups have identified a group of miRNAs that are secreted into circulation by testicular cancer tissue, specifically the miRNA-371–3 and miRNA-302/367 clusters.(37) These miRNA clusters, particularly miRNA-371a-3p, have been shown to exhibit higher sensitivity and specificity for GCT compared to traditional serum tumor markers in GCT (alpha-fetoprotein, beta-human chorionic gonadotropin, lactate dehydrogenase), and over 85% of patients with seminoma notably express miRNA-371a-3p.(38-41) The levels of these markers seem to be associated with both clinical stage and tumor bulk,(42, 43) supporting their role as potentially useful biomarkers in monitoring therapeutic response.

Recently, Dieckmann et al. conducted the largest prospective study to examine the performance characteristics of serum miRNA-371a-3p in the diagnosis and monitoring of patients with testicular cancer.(**44) Across an entire cohort of 616 patients with testis cancer, they noted that the miRNA-371a-3p test had 90% sensitivity, 94% specificity, 97% positive predictive value, 83% negative predictive value, and an area under the curve of 0.966 on receiver operating characteristic analysis for the primary diagnosis of GCT. The test outperformed the combination of classic serum tumor markers and was additionally associated with clinical stage, primary tumor size, and response to treatment. Notably, the marker was expressed in all histologic subtypes except teratoma. Their findings provide encouraging data to further support the clinical implementation of an miRNA-based test for patients with TGCT pending further validation.

THERAPEUTIC IMPLICATIONS OF TGCT GENETICS AND FUTURE DIRECTIONS

Platinum-based chemotherapy constitutes the first-line systemic regimen for advanced TGCT, and while most patients are cured with this approach, 10–20% of patients with metastatic TGCT will ultimately succumb to their disease.(3) Unfortunately, attempts to develop noncytotoxic targeted therapies for TCGT have been largely unsuccessful.(45) Indeed, there is a strong need to better understand molecular determinants of chemoresistance and identify actionable targets that may lead to the development of effective therapies in this setting.

In their integrated molecular analysis of TGCT, Taylor-Weiner et al. used BH3 profiling to functionally measure apoptotic signaling in tumors and suggested that chemosensitivity in TGCTs may rely on intact TP53, reciprocal LOH, and high mitochondrial priming.(**31) In contrast, chemoresistant tumors appeared to accumulate copy number events, including additional reciprocal LOH, and lose expression of pluripotency markers and apoptosis regulators (NANOG and POU5F1).(46, 47)

To explore genetic determinants of cisplatin resistance in GCTs further, Bagrodia et al. performed WES or targeted exon-capture-based sequencing on 180 tumors and, in line with the findings from Taylor-Weiner et al.,(**31) reported TP53 alterations, along with MDM2 amplifications, to be present exclusively in cisplatin-resistant tumors and prevalent in patients with primary mediastinal NSGCTs.(*48) Furthermore, actionable alterations, including RAC1 mutations, were found in over half of cisplatin-resistant GCTs, which may hold therapeutic implications for patients in the cisplatin-resistant space.

More recently, Necchi et al. performed NGS on 107 TGCTs (23 seminoma, 84 NSGCT) in patients who relapsed despite receipt of cisplatin-based chemotherapy, with most samples derived from chemorefractory metastatic lesions.(**49) RAS-RAF pathway (mostly KRAS alterations at the single-gene level) and cell-cycle pathway (largely non-targetable) alterations were the most common genomic alterations found in both seminomas and NSGCTs, while KIT and PI3K pathway gene alterations were more frequently seen in seminomas (22% and 26% of seminomas, respectively). Aside from KIT and PI3K pathway genes, only very few potentially targetable mutations were found, including BRAF (<5% of NSGCTs), ERBB2 (<4% of NSGCTs), and DNA repair genes (13% of TGCTs).

Immune checkpoint inhibitors (ICI) targeting programmed death-1 (PD-1), its ligand (PD-L1), or cytotoxic T-lymphocyte associated protein 4 (CTLA-4) have emerged as a promising class of systemic therapy in the frontline or chemorefractory setting for multiple malignancies, including lung cancer, bladder cancer, melanoma, and several others. The role of ICIs has been studied to a limited extent in TGCTs as well, but unfortunately results have not been encouraging.(50-52) This is not surprising in light of the low TMB and low degree of microsatellite instability found in TGCTs.(**49) Hence, without additional preclinical rationale and improved patient selection, the use of ICIs for TGCT will unlikely gain the same prominence as in other cancers.

Alternative targets in TGCT are under active investigation. In a small study of 7 patients, 5 with TGCTs, treatment with the antibody-drug conjugate brentuximab vedotin targeting CD30 resulted in a durable complete response (>6 months) in one patient and a brief partial response in another patient.(*53) The uncommon nature of targetable mutations in TGCTs remain a challenge in developing therapies for chemoresistant patients, and future efforts focusing on preclinical models and novel treatment modalities are much needed.

CONCLUSION

Considerable advances have been made over the last couple years to elucidate the complex genetic basis underlying TGCTs. Without an identifiable major high-penetrance TGCT susceptibility gene, familial clustering is likely driven by polygenic variation. Most commonly, gains in chromosome 12p and mutations in *KIT* and *KRAS* can be found in TGCTs, particularly among seminoma patients, though the uncommon nature of targetable mutations in TGCTs remains a challenge in developing therapies for patients resistant to cisplatin-based systemic chemotherapy. Nonetheless, chemosensitivity appears to rely on intact *TP53*, reciprocal LOH, and high mitochondrial priming. Consistent with the low TMB seen in TGCTs compared to other malignancies, ICI has not proven to be an effective management strategy for these patients. Serum miRNA is emerging as a promising biomarker for both diagnosis and therapeutic monitoring in patients with TGCT, and additional validation concerning its clinical utility is warranted. Indeed, future efforts focusing on developing novel, personalized treatment modalities are much needed.

ACKNOWLEDGEMENTS

Financial support and sponsorship: This work was supported by the Ruth L. Kirschstein National Research Service Award T32 CA136515–09 (N.S.), the University of Texas Southwestern Medical Center Physician Scientist Training Program (N.S.), and Dedman Family Scholarship in Clinical Care (A.B).

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KEY POINTS:

- Refinements in next-generation sequencing techniques over the last few years have enabled considerable advances in elucidating the genomic, transcriptomic, and epigenetic landscape of TGCTs.
- A major high-penetrance TGCT susceptibility gene has not been identified. Multiple studies support a complex polygenic model of inherited susceptibility to TGCTs with considerable variation.
- The most common alterations in TGCTs, particularly seminomas, include gains in chromosome 12p and single-gene point mutations in *KIT*, *KRAS*, and *NRAS*. It appears that enhanced *KIT-PI3K-RAS* signaling may play a significant role in most seminomas.
- Sensitivity to cisplatin-based chemotherapy appears to rely on intact *TP53*, reciprocal loss of heterozygosity, and high mitochondrial priming.
- Serum microRNAs (miRNA), particularly miRNA371–3, are an emerging candidate biomarker for both the diagnosis and therapeutic monitoring of patients with TGCT, and contemporary studies have demonstrated early promise.
- Consistent with the relatively low tumor mutational burden and microsatellite instability, immune checkpoint inhibitors do not appear to be effective for most TGCTs. The uncommon nature of targetable mutations in TGCTs remain a challenge in developing therapies for chemoresistant patients, and future efforts focused on developing novel treatment modalities are needed.