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REVIEW

Testicular germ cell tumor: a comprehensive review

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Abstract

Testicular tumors are the most common tumors in adolescent and young men and germ cell tumors (TGCTs) account for most of all testicular cancers. Increasing incidence of TGCTs among males provides strong motivation to understand its biological and genetic basis. Gains of chromosome arm 12p and aneuploidy are nearly universal in TGCTs, but TGCTs have low point mutation rate. It is thought that TGCTs develop from premalignant intratubular germ cell neoplasia that is believed to arise from the failure of normal maturation of gonocytes during fetal or postnatal development. Progression toward invasive TGCTs (seminoma and nonseminoma) then occurs after puberty. Both inherited genetic factors and environmental risk factors emerge as important contributors to TGCT susceptibility. Genome-wide association studies have so far identified more than 30 risk loci for TGCTs, suggesting that a polygenic model fits better with the genetic landscape of the disease. Despite high cure rates because of its particular sensitivity to platinum-based chemotherapy, exploration of mechanisms underlying the occurrence, progression, metastasis, recurrence, chemotherapeutic resistance, early diagnosis and optional clinical therapeutics without long-term side effects are urgently needed to reduce the cancer burden in this underserved age group. Herein, we present an up-to-date review on clinical challenges, origin and progression, risk factors, TGCT mouse models, serum diagnostic markers, resistance mechanisms, miRNA regulation, and database resources of TGCTs. We appeal that more attention should be paid to the basic research and clinical diagnosis and treatment of TGCTs.

Keywords Spermatogenesis · Teratoma · Risk factors · Platinum resistance · Serum makers · MiRNA · Mouse models · Database

Introduction

TGCTs are rare tumors in the general population, but are the most commonly occurring malignancy among males between ages 15 and 44 years [1]. The diagnosis of TGCTs primarily depends on physical examination, ultrasonography, magnetic resonance imaging, measurement of serum tumor markers and pathological examination. Standard treatment for TGCTs is radical orchiectomy and/or combination with chemotherapy or radiotherapy or retroperitoneal lymph node dissection. Furthermore, small interfering RNA therapy [2, 3], microRNA therapy [4, 5] and immunotherapy [6] are suggested to be potential therapeutic strategies; however, there is a long road ahead for such treatments to prove their clinical value. Significant parameters, such as angiolymphatic invasion, degree of extra testicular invasion, rete testis invasion and serum tumor marker levels [7], oncogenes [8], promoter methylation [9], polymorphism [10] and tumor-infiltrating immunocytes [11] are suggested as potential prognostic factors for TGCT patients.

TGCTs are characterized by frequent chromosomal anomalies and low rates of somatic mutations. Chromosome arm 12p amplification, such as isochromosome 12p and chromosome 12p overrepresentation, is the most common genetic hallmark that accounts for many types of TGCTs [12, 13]. The exact mechanisms of 12p gain in TGCTs are unclear, but the ubiquitous gain of 12p-derived sequences implies a significant role for some genes on 12p, such as *CCND2*, *KRAS*, *TNFRSF1A*, *GLUT3*, *REA*, *NANOG*, *DPPA3*, and

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GDF3 [14–17], in the development, pluripotency maintenance and/or progression of TGCTs.

It is not accurate to say that TGCTs are completely curable malignancies. According to the European Association of Urology (EAU) testis cancer guidelines, approximately 15–30% of TGCT patients will relapse after first-line chemotherapy and will require additional salvage therapies [18, 19]. Primary TGCTs have been reported to metastasize to the retroperitoneal lymph nodes [20], brain [21, 22], neck [23, 24], heart [25, 26], pulmonary arteries [27], inferior vena cava and aorta [28], lung [29], liver [30], stomach [31, 32] and cartilage [33]. Long-term relative survival after diagnosis of TGCTs generally continued to decline with increasing follow-up time, particularly beyond 15–30 years [34]. The side effect of TGCT therapy on other organs also offers insight into the long-term risks of TGCT survivorship. Kidney disease [35], cerebrovascular accidents, secondary leukemia, internal carotid artery occlusion and stroke associated with chemotherapy in TGCT patients have been occasionally described in the literature [36–42].

More recently, focus has expanded beyond survival to emphasize the quality of life issues when optimizing treatment algorithms. Attention should be paid toward persisting physical symptoms and psychosocial needs. Patients with TGCTs and azoospermia, submitted to onco-testicular sperm extraction and sperm cryopreservation, had the delivery of a healthy baby after intracytoplasmic sperm injection, which emphasizes the importance of fertility preservation in oncology patients [43, 44]. For patients with bilateral TGCTs, testis-sparing surgery provides a better quality of life (e.g., sufficient endogenous testosterone production) and may be considered a safe, feasible alternative treatment [45, 46]. Testicular self-examination (TSE) practices are found to be inadequate and efforts should be made to develop programs that can increase knowledge related to testicular cancer as well as the practice of TSE [47].

Herein, we present a comprehensive review on origin, progression, histological types, risk factors, TGCT mouse models, serum diagnostic markers, resistance mechanisms, miRNA regulation and database resources of TGCTs.

Origin, progression and histological types of TGCTs

Spermatogenesis is fundamental to the establishment and maintenance of male fertility. Given that TGCTs are believed to arise from failure of normal maturation of gonocytes, understanding the process and regulatory controls of spermatogenesis will provide valuable insights into the occurrence and features of TGCTs. It is generally considered that mammalian spermatogenesis is a complex sequential process of germ cell differentiation from primordial germ cells (PGCs) or spermatogonial stem cells (SSCs) to functional haploid sperm [48, 49] (Fig. 1, left). Spermatogenesis further requires intricate interaction between germ cells (spermatogonia, spermatocytes, round/elongating spermatids) and supporting somatic cells (Sertoli cells, Leydig cells,

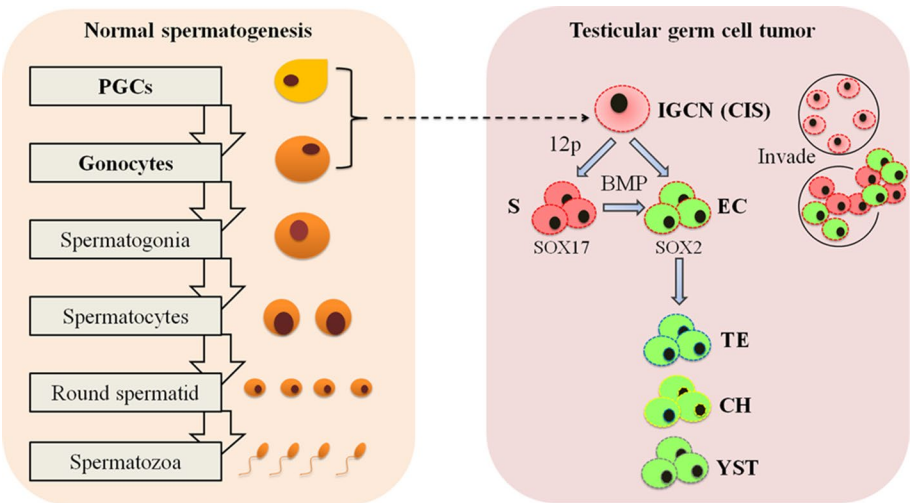

Fig. 1 Model of normal spermatogenesis and occurrence of TGCTs. Spermatogenesis is a tightly regulated process of the continuous supply of spermatozoa. Differentiation of primordial germ cells (PGCs) into gonocytes, self-renewal and differentiation of spermatogonial stem cells, and subsequent commitment to meiotic spermatocytes and haploid round/elongating spermatids are the key events of spermatogenesis. Under pathological conditions, gonocytes that fail to undergo correct spermatogenic differentiation, but develop into intratubular germ cell neoplasia (IGCN) or carcinoma in situ (CIS) represent the precursor cells for TGCTs during early stage of germline development. CIS can further progress into invasive seminoma (S) and (or then) nonseminoma, including undifferentiated EC, as well as differentiated teratoma (TE), choriocarcinoma (CH) and yolk sac tumor (YST)

peritubular myoid cells, endothelial cells, macrophage and newly discovered innate lymphoid and mesenchymal cells) [50–54]. Recent single-cell RNA sequencing of murine or human spermatogenesis reveals a continuous developmental trajectory of germ cells from spermatogonia to spermatids (12 or 14 germ cell states) and identifies cell type-specific markers and candidate transcription regulators in each cell component [55, 56], representing a community resource and foundation to in-depth study of spermatogenesis.

The histogenesis of TGCTs is complex. It is thought that TGCTs develop from premalignant intratubular germ cell neoplasia (IGCN), also known as carcinoma in situ (CIS), that are believed to arise from failure of normal maturation of fetal germ cells from PGCs into pre-spermatogonia [57]. Expression profiling studies reveal that IGCN cells closely parallel PGCs and maintain their genome in a demethylated and undifferentiated state [58, 59]. IGCN progresses toward invasive TGCTs then after puberty, when IGCN cells begin to proliferate, likely involving the influences of hormones. TGCTs are classified broadly into two major histologic groups: seminoma and non-seminoma germ cell tumors. Non-seminoma can be further subdivided into undifferentiated embryonal carcinoma (EC), as well as differentiated teratoma, choriocarcinoma and yolk sac tumor [60] (Fig. 1, right). Both IGCN and TGCT cells are typically aneuploid, but premalignant IGCN does not gain chromosomal material from 12p, which are pathognomonic for malignant IGCN and TGCTs [61, 62]. Transition from IGCN to invasive TGCTs is associated with the loss of *PTEN* and *P21* as well as gain of *MDM2* expression [63, 64]. *KRAS* mutations are exclusive to the primary TGCT tumors and not in the patient-matched pre-invasive IGCN [65]. Seminoma and EC present significant differences in clinical features, therapy and prognosis, and they show characteristics of the PGCs and embryonic stem cells (ESCs), respectively [66]. For proper diagnosis of the different histological subgroups of TGCTs, immunological staining is required using distinctive molecular markers. POU class 5 homeobox 1 (POU5F1, also known as OCT3/4), is positive in IGCN, seminoma and EC, but not in any choriocarcinoma, teratoma or YST [67]. KIT proto-oncogene receptor tyrosine kinase (KIT, also known as CD117), is positive in IGCN and seminoma and negative in EC [68]. TNF receptor superfamily member 8 (TNFRSF8, also known as CD30) expression helps pathologists to identify sites of EC in the tumors [69]. Furthermore, Glypican 3 (GPC3) is useful as an immunohistochemical marker for TGCTs differentiated to extraembryonic tissue, especially YST [70, 71]. Moreover, Sal-like protein 4 (SALL4) is a more sensitive marker than α-fetoprotein (AFP) and GPC3 for YST [72]. Integration of tumor characteristics and high-dimensional assays of genomic, epigenomic, transcriptomic and proteomic features [73] recently reveals novel distinctive molecular landscapes of TGCT histologic types and identifies previously unappreciated diversity within each component, including a separate subset of seminoma defined by *KIT* mutations.

The precise mechanism of the progression from premalignant IGCN to subtypes of invasive tumors is not completely understood. IGCN gives rise to seminoma and EC separately or seminoma is the intermediate stage between IGCN and EC remains a matter of debate. The first hypothesis is supported by the observation that IGCN is a phenotypically heterogeneous lesion containing cells in different stages of progression [74, 75], and *KIT* mutations are observed in a subset of seminoma, but not in EC [73]. The latter model is set up mainly on the basis of the phenotypic resemblance of seminoma to IGCN, learning from the studies of ploidy [76], cytogenetics [77] and pathomorphology [78]. Several studies support the common clonal origin of metastatic mature teratoma with other components of a mixed germ cell tumor [79, 80]. Intriguingly, transplantation of seminoma-like cell line TCam-2 into the seminiferous tubules results in the formation of an IGCN/seminoma, while transplantation into the flank or corpus striatum will trigger TCam-2 cells to adopt an EC-like fate. This model suggests that transition of seminoma to ECs relies on signals from the tumor microenvironment [81]. During this reprogramming, the microenvironment inhibition of bone morphogenetic protein (BMP) signaling is the initial event, resulting in activation of NODAL signaling, upregulation of pluripotency factors (e.g., SOX2) and downregulation of seminoma markers (e.g., SOX17) [82, 83]. It will be interesting to further investigate whether EC can transit into seminoma upon interference with microenvironment factors.

Risk factors of TGCTs

The increased (4- to 10-fold) risk of TGCTs among brothers and sons of affected men together with findings in twin studies supports a strong genetic component contributing to TGCT susceptibility [84–88]. Genome-wide association studies (GWAS) have been particularly effective in identifying multiple common variants with strong contribution to TGCT risk. Initial GWAS studies identified allele variation within the c-KIT ligand (*KITLG*) on 12q22 as the strongest genetic risk factor for TGCTs (per-allele OR > 2.6) [89, 90]. Approximately, 40 identified allele variations on chromosomes conferring TGCT susceptibility [91–101] are summarized in Fig. 2. Pathway-based analysis of GWAS data reveals the association of PGC formation, sex determination/differentiation, spermatogonial maintenance genes (e.g., *KITLG* [102], *PRDM14* [103], *DMRT1* [104], *GATA4* [50] and *DAZL* [105]) with susceptibility to TGCTs. TGCT GWAS to date have been fairly small compared with those seen for other diseases, and multiple additional TGCT

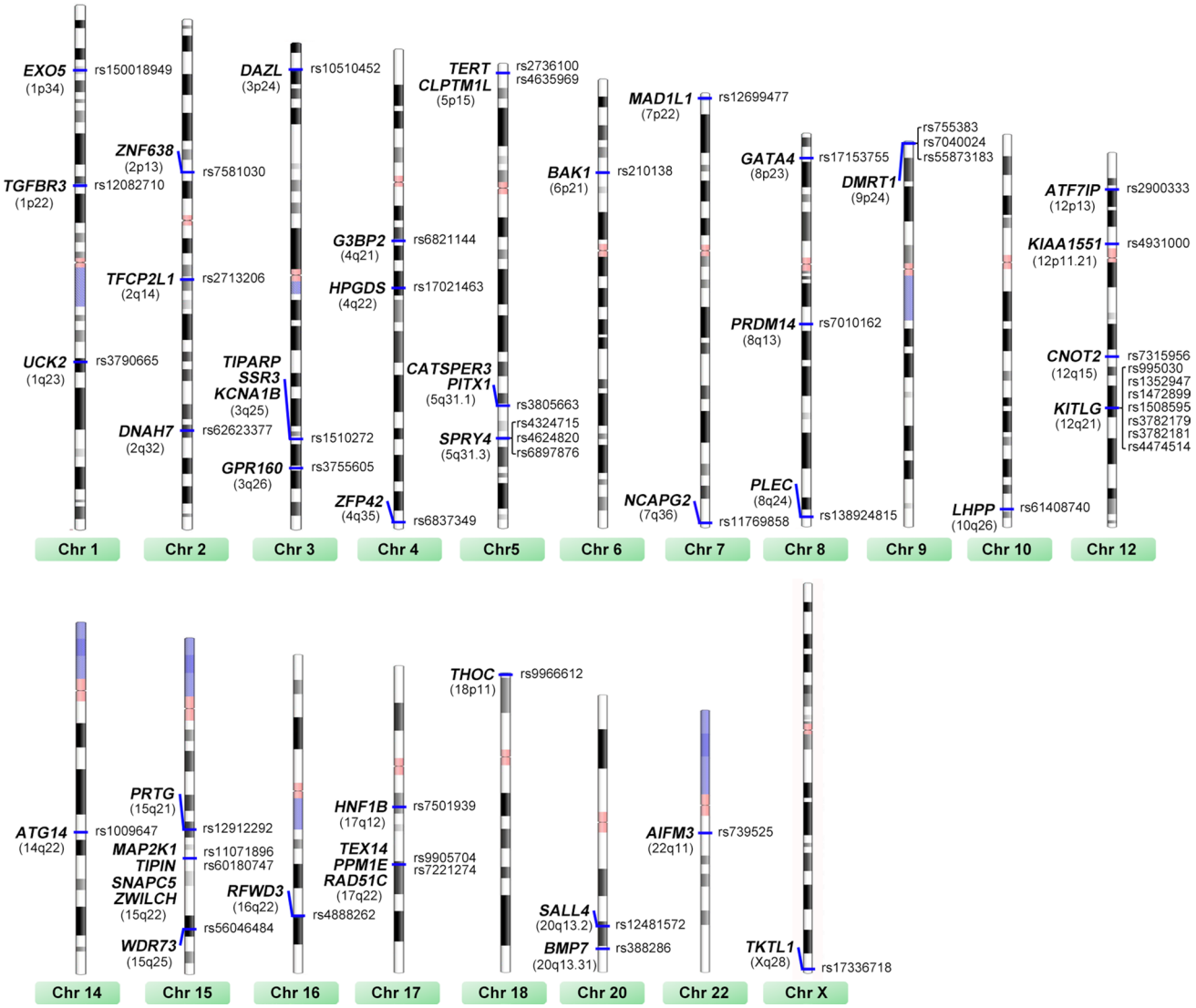

Fig. 2 The current known TGCT susceptibility loci that have been identified through GWAS. Single nucleotide polymorphisms, locus on chromosome (Chr) and candidate gene or genes are listed

susceptibility loci and their functional characteristics remain to be identified.

In addition to genetic susceptibility loci, significate risk factors for the development of TGCTs include cryptorchidism [106], disorders of sex development [107], hypo/infertility [108], contralateral germ cell tumors [109] and endogenous and exogenous hormones [110]. Although the sample size is insufficient and reliable correlation should be established, environmental risk factors are currently estimated to account for half of TGCT predisposing reasons. If necessary, patients with high risks of TGCT occurrence such as family history of TGCTs, infertility and environmental risk exploration may be notified to take physical and genetic screening for the genetic susceptibility loci, credible prognosis markers or premalignant IGCN lesion.

Mouse models for TGCT study

Animal models, such as mouse models, provide novel insights into the molecular mechanisms underlying the origin, progression and development of TGCTs. Mouse strains with low versus high teratoma incidence (129 versus 129.MOLF-Chr 19 (M19)) provide original in-depth research of TGCTs in mice. M19 carries chromosome (Chr) 19 from the MOLF, whereas all other chromosomes are from the 129 strain. Approximately, 70% of M19 males develop TGCTs in contrast to approximately 5% in the 129 strain, suggesting that Chr 19 contains susceptibility loci of TGCTs [111, 112]. However, potential loci on Chr 19 have not been identified by GWAS yet.

The origin of TGCT cells predicts that developmental pathways that control germ cell pluripotency or differentiation may be involved in the malignant transformation of these cells. Using mouse strains of 129, M19 and FVB (resistant to teratoma formation), a previous study suggests that ectopic germ cell proliferation and dysexpression of germ cell pluripotency and differentiation-associated factors at a specific developmental time point, E15.5, are directly correlated with increased teratoma risk [113]. *Nodal*-knockout mice show premature differentiation and reduced pluripotency marker expression, and NODAL signaling components are overexpressed in human TGCT samples [114]. In contrast, germ cell expression of male sex determination gene *Nanos2* is relatively low in teratoma-susceptible mouse strains and deficiency for *Nanos2* increases teratoma incidence in 129 mice [115]. Furthermore, DMRT1 controls the mitosis–meiosis switch in mice and humans and loss of *Dmrt1* in 129 strain mice results in a > 90% incidence of testicular teratomas [104, 116]. These genetic studies in mouse models further advance our understanding that delayed male germ cell specification and retained pluripotency may cause gonocytes to form IGCN, EC foci and teratoma on a susceptible genetic background.

An increase in tumor incidence in mice has proven to be relevant to understanding genetic risk factors for TGCTs in humans. One good example is that loss of the transmembrane Kit ligand (*kitl*) increases TGCT susceptibility in 129 mice [117]. GWAS have identified *KITLG* as a solid TGCT risk gene in humans accordingly [89]. TGCT occurrence is observed at higher incidence in mice mutant for *Dnd1* [118] or *Pten* [119] or *A1cf/Ago2* [120] in 129 inbred strains. Furthermore, spindle-associated Rhamm acts as a gatekeeper preventing IGCN initiation, because seminoma occurred in 3.7% of *Rhamm* mutant male mice [121]. Pierpont et al. recently developed a novel mouse TGCT model by germ cell-specific *Kras* activation and *Pten* inactivation in 129 backgrounds that developed malignant and metastatic TGCTs composed of teratoma and EC [122]. Mouse models provide biological insight into TGCT development, but their relevance to human tumorigenesis is limited, as no mutations of genes such as *RHAMM and ALCF/AGO2* have been shown in human TGCTs. Whether mouse TGCT models precisely reflect the biology of human TGCTs requires to be determined.

Serum diagnostic tests for TGCTs

Compared to other solid organ malignancies, the role of serum tumor markers in TGCTs is unprecedented; these markers are fully used in the diagnosis, staging, risk stratification and surveillance of patients with TGCTs [123]. The most common serum tumor markers for TGCTs include α-fetoprotein (AFP) and human chorionic gonadotropin (hCG) [123]. They are relatively sensitive, specific and clinically useful tumor markers for TGCTs, providing value on diagnosis, classification, staging and prediction. Some progresses have been achieved to find novel serum biomarkers with good sensitivity and accuracy. MicroRNAs (miRNAs) are short non-coding RNAs that show exciting promise as a new-style biomarker of TGCTs [124]. The sensitivity and specificity of miR-371a-3p alone is ~ 90% for the diagnosis of malignant TGCTs [125], but that value can be increased further by using a combination of other miRNAs, including miR-372-3p, miR-373-3p and miR-367-3p [126–128]. Furthermore, miR-371a-3p serum level is increased in recurrence of TGCT patients, indicating its additional value as a biomarker for detecting disease relapse in TGCT patients [129]. The value of miRNA serum markers needs to be validated in more studies or a prospective clinical trial. Moreover, patients in the yolk sac seminoma subgroup have the poorest clinical outcome, tending to undergo somatic transformation and chemoresistance [130]; however, identification of subtype-specific serum biomarkers is still a big challenge.

Resistance of TGCTs

TGCTs are highly curable tumors in most cases, because of the exquisite sensitivity of seminoma and EC to DNA damaging agents; however, teratomas are mostly resistant to chemotherapeutic drugs [131]. Given that TGCTs are unique in their responsiveness to platinum-based chemotherapy, they are considered as a model for exploring the molecular mechanisms behind the exceptional sensitivity of TGCT cells to DNA damaging chemotherapeutics. At present, various hypotheses on the platinum hypersensitivity of TGCT cells have been reported. An easily activated apoptotic response and the deficiency of the DNA damage response/repair activation may account for this behavior [132, 133]. The tumor-suppressor gene *TP53* commonly mutated in solid tumors is rarely mutated in TGCTs (~ 1.29%) [134] and silencing of *TP53* is sufficient to abrogate the hypersensitivity of TGCT cells to cisplatin [135]. A recent clinical whole-exome and transcriptome sequencing study proposes that the basis of chemosensitivity in TGCTs with a wild-type *TP53* genomic background is a result of a fundamental apoptotic propensity caused by increased mitochondrial priming [65]. HMGB4, a protein preferentially expressed in testes, uniquely blocks excision repair of cisplatin–DNA adducts, 1,2-intrastrand cross-links, to potentiate the sensitivity of TGCT cells to cisplatin therapy [136]. Furthermore, chemoresistant teratomas or transformed carcinomas are associated with continued progression of reciprocal loss of heterozygosity (RLOH) copy number and reduction of

pluripotency markers (*NANOG* and *OCT3/4*) [65]; however, it is uncertain whether the loss of pluripotency markers is a driver of chemoresistance.

Although the majority of TGCTs will respond with excellent cure rates, some (more than 10%) patients will relapse or demonstrate refractory disease after operation and chemotherapy. Limited options exist for patients with platinum refractory disease [137]. Exploring the mechanisms underlying platinum resistance and identifying novel treatment options that are effective in the platinum-refractory patients require an urgent priority. Both activation of the PDGFRβ-AKT pathway [138] and overexpression of MAD2γ [139] or cytoplasmic p21 [140] are explored to contribute to cisplatin-acquired resistance in TGCT cells. In contrast, disrupting MDM2-TP53 interaction [141] or stimulating expression of miR-302a [142] or miR-383 [143] increases the sensibility of TGCT cells to cisplatin exposure. Notably, compound HP-14 and poly(ADP-ribose) polymerase (PARP) inhibitor restrain the growth of cisplatin-resistant TGCT cells [144, 145].

The mutation rate is uniformly low in TGCTs [73] and no significant difference is observed in the mutational rate between seminoma and non-seminoma cases [146]. Intriguingly, several clinical studies of TGCT patients with different response to chemotherapy indicate that some gene mutations exhibit discrepancy between resistance and sensitivity. There is a significantly higher incidence of *BRAF* [147] and *XRCC2* [146] mutation in chemotherapy-resistant TGCTs compared with sensitive controls. Furthermore, mutations in *AKT1* and *PIK3CA* are observed exclusively in cisplatin-resistant tumors [148]. Polymorphisms of *BLMH* [149], *PAI-1* [150], *GSTP1* [151], *ARVCF* [152], *TPMT* and *COMT* [153] are associated with reduced survival, higher prevalence of early relapses, platinum refractory and chemotherapy-related organ toxicity after chemotherapy for TGCT patients. Although *TP53* mutations rarely occur in TGCTs, a recent study of whole-exome and targeted sequencing of cisplatin-sensitive and -resistant TGCTs suggests that *TP53* alterations (16.3% vs. 0%) and combined *MDM2/TP53* alterations (24.0% vs. 2.6%) are more common among cisplatin-resistant TGCTs than sensitive ones [154]. Unlike testicular primary tumors, mediastinal primary non-seminoma has frequent *TP53* alterations (72.2% vs. 2.5%) and an increased rate of platinum-based therapy resistance, resulting in survival of only ~50% [154]. It is noteworthy that several studies support the common clonal origin of metastatic mature teratoma with other components of a mixed germ cell tumor [79, 80]. It's still an open question why different subtypes of a mixed TGCT show the diversity of the above identified mutations and how genetics determine cisplatin resistance. In phase II studies, the combination of gemcitabine, oxaliplatin and paclitaxel achieves long-term overall survival (> 2 years) in ~20% of patients with cisplatin-refractory or multiply relapsed TGCTs [137, 155]. By contrast, limited effects are reported in patients treated with sunitunib [156], oxaliplatin plus bevacizumab [157] or everolimus [158].

Emerging role of miRNAs

MiRNAs recently emerge as an important regulator of TGCT cells. MiRNA expression profiles of TGCTs and normal testis tissues using small RNA sequencing reveal numerous dysregulated miRNAs in TGCTs [159, 160]. Compared with normal testes, the expression of some miRNAs (e.g., miR-199a-5p/3p, miR-514a-3p) is downregulated, while others, such as miR-223-3p, is overexpressed in TGCT tissues. Recent research has confirmed the role of miRNAs as either tumor suppressors or activators (oncomiRs) in TGCT cells. Subsequent identification of functional miRNAs–mRNAs interactions in TGCT cells helps delineate post-regulatory mechanisms and may lead to new therapies.

Forced expression of miR-199a-5p/3p in TGCT cells leads to suppression of cell growth, cancer migration, invasion and metastasis [161], indicating that miR-199a-5p/3p may act as a tumor-suppressor miRNA. Tumor cell suppression activity of the miR-199a-5p is mediated by its target *PODXL* [161] and *MAFB* [4], while miR-199a-3p inhibits tumor cell growth and migration via targeting transcription factor SP1 and glucose metabolism [5]. Furthermore, miR-199a-5p/3p and miR-214 can form a self-regulatory network via PSMD10-TP53-DNMT1 in TGCT cells [162]. MiR-514a-3p induces apoptosis through direct regulation of *PEG3* and PEG3-mediated activation of the NF-kappa B pathway [159]. High miR-223-3p expression in TGCT cells targets *FBXW7* to promote cell growth and inhibit apoptosis in TGCT cell lines [163]. OncomiR miR-1297 promotes growth of TGCT cells via targeting tumor-suppressor gene *PTEN* [164] and long-noncoding RNA MEG3 contradicts the inhibitory effects of miR-1297 on *PTEN* [165]. In vivo evidence should be included in miRNA studies to validate the involvement of miRNAs in TGCT progression in future.

Database to stimulate TGCT study

Gene Expression Omnibus (GEO) is an international public repository that archives and freely distributes microarray, next-generation sequencing and other forms of high-throughput functional genomics data submitted by researchers. The GEO database can be searched using many different attributes including keywords (e.g., testicular germ cell tumor) and GEO accession (e.g., GSE1818). Furthermore, the GEO2R in GEO website allows us to compare two or more groups of samples in a GEO series to identify

differentially expressed items and thus provides a simple interface that allows users to perform analysis without R statistical expertise. Taking the advantage of the GEO database, we can obtain informative knowledge about TGCTs. For example, GSE1818 provides RNA profiling of normal testis ($n=3$), IGCN ($n=3$), seminoma ($n=3$), EC ($n=5$), yolk sac ($n=4$), teratoma ($n=4$) and choriocarcinoma ($n=1$). Using GEO2R to perform multiple comparisons, a series of predominant genes are observed for normal testis (e.g., *KIF2A*, *DDEF2*, and *TBPL1*), IGCN (e.g., *CYP17A1* and *ACE2*), seminoma (e.g., *CAPNS1*, *ZKSCAN3*, and *EIF3F*), EC (e.g., *GAL*, *BCAT1*, and *CALB1*), yolk sac (e.g., *APOA2* and *CYP26A1*), teratoma (e.g., *COL1A1*, *COL61*, and *MFAP4*) and choriocarcinoma (e.g., *INSL4*, *CRH*, and *HTRA4*) (Fig. 3a). It will be interesting to identify specific marker genes that mark the formation of IGCN and transition from IGCN to invasive TGCTs, because measures can be taken before IGCN progression toward malignant and invasive TGCTs or their relapse. Furthermore, RNA profiling of relapsed seminoma ($n=15$), non-relapsed seminoma

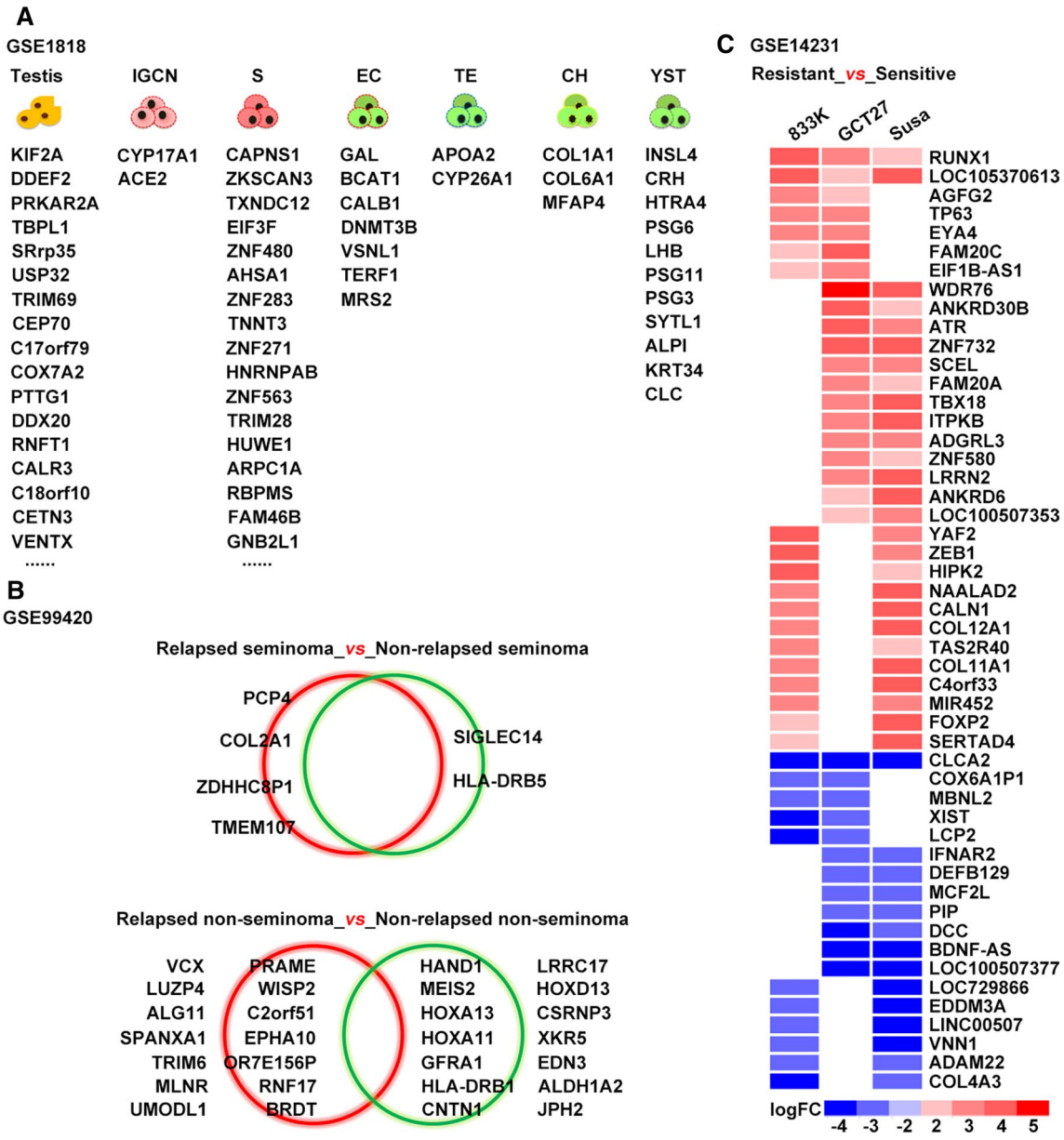

Fig. 3 GEO datasets representing a community resource to study TGCTs. **a** The dataset GSE1818 is particularly useful for comparisons between various histological subtypes of TGCTs versus each other or versus normal testis. **b** DEGs between relapsed and non-relapsed TGCTs are obtained by analyzing GSE99420. **c** Noel et al. provided an expression profiling of parental and cisplatin-resistant TGCT cell lines under accession no. GSE14231

($n=15$), relapsed non-seminoma ($n=12$) and non-relapsed non-seminoma ($n=15$) is included in GSE99420. Differentially expressed genes (DEGs) are obtained by GEO2R analysis between relapsed TGCTs and non-relapsed TGCTs (Fig. 3b). Moreover, GSE14231 identifies significant changes of RNA profiling in three human TGCT cell lines (833 K, GCT27 and Susa) and their cisplatin-resistant variants ($n=2$ each group), and these DEGs are considered to participate in cisplatin sensitivity or resistance of TGCT cells (Fig. 3c).

The Cancer Genome Atlas (TCGA) provides comprehensive and multi-dimensional maps of the key genomic changes in 33 types of cancer, including TGCTs. The genomic information of TCGA helps to improve the prevention, diagnosis and treatment of cancer. The cBioPortal for Cancer Genomics (http://cbioportal.org/) provides visualization, analysis and download of large-scale cancer genomics data sets, including TCGA [166, 167]. A recent systematic analysis of TCGA database concludes that TGCTs exhibit high aneuploidy and a markedly low rate of somatic mutation (mean 0.5 mutations per Mb) [73], consistent with previous exome-wide sequencing studies [146, 168, 169]. Somatic mutation of only three genes (*KIT*, *KRAS*, and *NRAS*) achieves significance in TGCTs, whereas large-scale copy number variation such as gain of chromosomal material from 12p is frequently observed [73]. Using TCGA and cBioPortal, a comparison of seminoma ($n=69$) and EC ($n=31$) samples obtains a list of differentially expressed miRNAs. Notably, miR-515 family that lists between *DPRX* and *NLRP12* gene in Chr 19 accounts for approximately 55% of predominantly expressed genes in EC as compared with seminoma (Fig. 4). The role of miR-515 family in the distinction of EC from seminoma deserves further investigation.

Concluding remarks

In summary, we described the developmental (origin and progression), genetic (susceptibility loci) and molecular (resistance mechanisms, miRNA involvement) aspects of TGCTs, and discussed the emerging TGCT mouse models, public database resources and serum diagnostic markers with application prospects.

TGCTs are histologically heterogeneous and distinctly curable with chemotherapy. One major challenge is the development of therapeutic approaches for cisplatin-refractory or multiply relapsed TGCTs. TGCTs are highly curable tumors in most cases, because of their exquisite sensitivity of seminoma and EC to DNA damaging agents; however, teratoma are mostly resistant to chemotherapeutic drugs [131]. In most cases, surgical resection is specifically required for teratomas and identifying ways

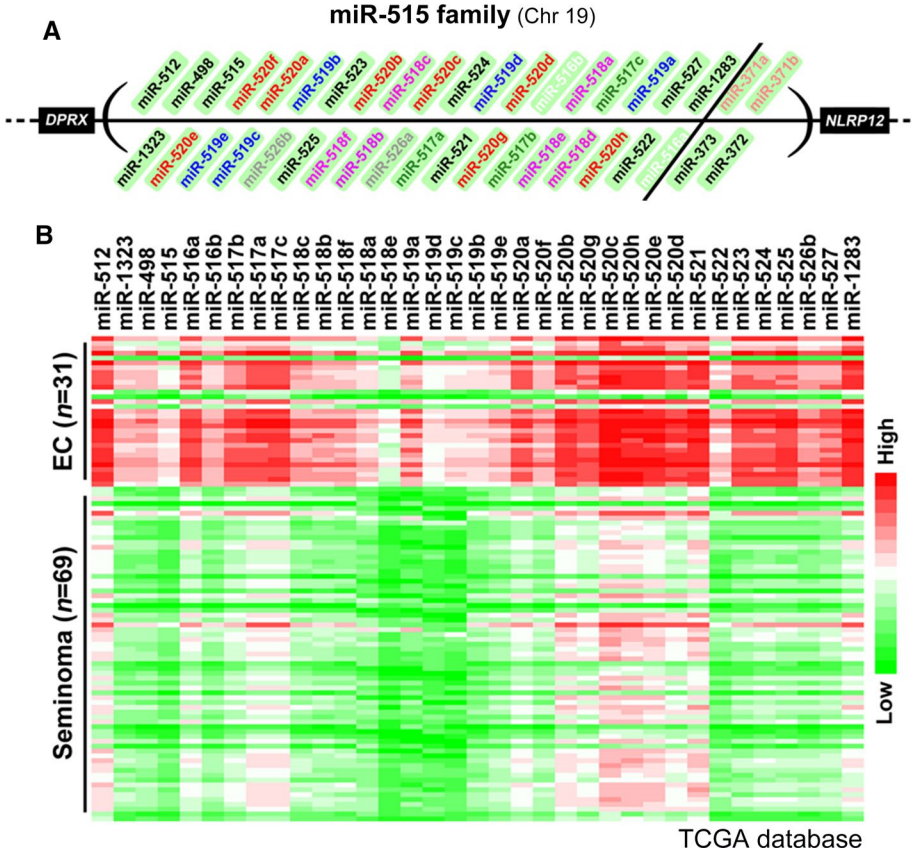

Fig. 4 An example of data analysis using TCGA and cBioPortal. MiRNA profiling data of 31 patients with EC and 69 patients with seminoma was extracted from the TCGA database and DEGs were analyzed. Notably, miR-515 family that lists between *DPRX* and *NLRP12* gene in Chr 19 accounts for approximately 55% of predominantly expressed genes in ECs

to discern teratomas from nonviable tissues after chemotherapy is important to avoid unnecessary invasive surgeries. Malignant transformation of TGCTs into somatic malignancy is uncommon [170]. Patients whose primary TGCTs contain yolk sac tumor and seminoma have a poor clinical outcome, tending to undergo chemoresistance and somatic transformation within their metastatic lesions after chemotherapy [130]. Similarly, teratoma with malignant transformation had a worse prognosis than other types of TGCTs [171].

Patient-derived xenografts (PDX) are models of cancer where the tissue or cells from a patient's tumor are implanted into immunodeficient or humanized mice. PDX provides unique opportunities for cancer research, treatment evaluation and drug discovery. However, understanding the limitations of PDX models and the difference between PDX and human tumors in their natural environment is required for optimal application. Firstly, it is vital to ensure that appropriate PDX tumor model is used, because several studies suggest that human tumors engrafted in immunodeficient mice are susceptible to the formation of lymphocytic neoplasms [172, 173]. Secondly, PDX undergo mouse-specific tumor evolution and show genomic instability; for instance, the copy number alteration landscapes of PDX change continuously and differ from those acquired in patients [174, 175]. Moreover, further development and use of mouse genetic TGCT models will provide novel insight into the underlying molecular mechanisms of TGCTs, as well as useful tools to test therapeutic strategies. Nevertheless, whether mouse TGCT models precisely reflect the biology of various subtypes of human TGCTs requires to be determined.

Targeting of tumor cells is not equivalent to targeting tumor tissues. Tumor cells display extensive and dynamic cross-talk with the microenvironment, mainly containing tumor-infiltrating lymphocytes, tumor-associated macrophages, cancer-associated fibroblasts, surrounding stroma and tumor vasculature [176]. TGCTs are frequently characterized by T lymphocyte infiltration [177]. Deep immune characterization of TGCTs shows that activated T cell infiltration is closely correlated with seminoma histology, early stage and good prognosis. Seminomas show increased T cell infiltration, decreased regulatory T cells, increased program death-ligand 1 (PD-L1) and increased program-death 1 (PD-1)/PD-L1 spatial interaction compared with non-seminoma [73, 178, 179]. EMMPRIN secreted by EC cells via membrane vesicles exerts its matrix metalloproteinase-inducing effect on fibroblasts within the tumor microenvironment to promote tumor invasion [180]; thus, EMMPRIN may predict an unfavorable prognosis in patients with TGCTs [181]. MiR-125b in TGCT tumor cells promotes TGCT xenograft growth through stimulating the recruitment of tumor-associated macrophages [182]. In addition to the intrinsic properties of tumor cells, more attention should be paid to tumor microenvironment and corresponding therapeutics directing against 'tumor' rather than 'tumor cells'.

Anti-PD-1 is standard immunotherapy for multiple cancers, and the expression of its ligand, PD-L1, has been described in TGCTs [6, 183]. Immunotherapy using PD-1/PD-L1 inhibitors (e.g., pembrolizumab) has been performed to treat platinum-refractory TGCTs [184, 185]. However, a phase II study of anti-PD-1 in refractory TGCTs (Clinicaltrials.gov, NCT02499952) was terminated due to lack of efficacy. Pembrolizumab is well tolerated, but does not appear to have clinically meaningful single-agent activity [186]. brentuximab vedotin (BV) is an antibody–drug conjugate consisting of the chimeric anti-CD30 antibody conjugated to an antimitotic drug monomethylauristatin E [187–189]. A phase II trial of BV in refractory CD30-positive TGCTs (ClinicalTrials.gov, NCT01851200) has been completed; however, the clinical outcomes have not been reported.

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Compliance with ethical standards

Conflict of interest The authors declare that there are no conflicts of interest.

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