Review

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Genomic Evaluation of Testicular Germ Cell Tumors and its Role in Treatment Planning

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Abstract

The incidence of testicular germ cell tumors (TGCT) has significantly increased over the last 40 years and continues to increase among the young male population. The geographical differences show that a combination of genetic and environmental factors play a role in the pathogenesis of TGCT. However, since the actual mechanisms involved in the development of this disease have still not been fully explained, studies at the genomic level have gained importance. Although TGCTs respond well to chemotherapy, 20-30% of patients display classical chemotherapy resistance. There exists a need for detailed investigation of the molecular mechanisms responsible for such resistance. In this review, we briefly discussed the current information on some risk factors and genomic factors involved in the development of TGCTs.

Keywords: Testicular germ cell tumors, genomics, epigenomics, risk factors

Introduction

Testicular germ cell tumors (TGCTs) have an incidence of up to 10.5 in 100,000 men and are the most common solid tumors affecting the young male population (1). In fact, although testicular cancers are a rare cancer type, it is worrying that the incidence of testicular cancer has doubled in the past 30 years according to statistical analyses in countries such as the USA and UK (1,2).

Almost all primary testicular tumors (95%) are TGCT, while the remaining minority includes nongerminal neoplasms such as Leydig cell tumors, Sertoli cell tumors, or lymphomas. These tumors are comprised of seminomas, which account for approximately 50% of cases and are derived from undifferentiated primordial germ cells, and nonseminomas, which constitute approximately 40% of cases and exhibit different stages of differentiation. The other 10% of cases show mixed histology (3). Seminomas and nonseminomas are derived from both gonadal and extragonadal anatomic sites and have distinct biological and metastatic properties. Nonseminoma germ cell tumors include embryonic and extraembryonic components and are classified as embryonal cell carcinoma, choriocarcinoma, yolk sac tumor, and teratoma (3,4).

Mortality due to testicular cancer is very low. The main reason for this is the high success rates in the treatment of low- and moderate-risk testicular cancer. Treatment success depends on careful grading during diagnosis, initiation of chemotherapy, as well as effective early treatment with radiotherapy and surgery if necessary, followed by fairly good follow-up. TGCT patients are treated with radiotherapy or chemotherapy depending on histological type and tumor stage after surgery. Nonseminoma germ cell tumors are more aggressive in terms of early spread, have poorer prognosis in advanced stage disease, and are sensitive to platinum-based combination chemotherapy but less responsive to radiation, except for teratomas. In contrast, seminomas have a good prognosis and are highly sensitivity to both chemotherapy and radiation (5).

Despite their differences in morphology and time of appearance, recent genome-wide association studies (GWAS) have shown that seminomas and nonseminomas share a common molecular pathogenetic process. Both seminoma and nonseminoma tumors are believed to be derived from carcinoma in situ (CIS), also known as intratubular germ cell neoplasia (ITGCN), where the generation and expansion of tumor cells is restricted in the seminiferous tubules. ITGCN is a precursor lesion often visualized close to invasive TGCT (6,7).

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Epidemiology, Etiology, and Risk Factors

The incidence of TGCT displays marked variation between different countries and ethnic groups, with significantly more cases in Scandinavia than in Africa, Asia, and Latin America, where a very low incidence has been reported. The higher incidence of this disease in Western countries has been attributed to greater exposure to disease-causing factors such as intrauterine exposure to endocrine disruptors like hormone use during pregnancy and fetal development. Since TGCT is a hormone-dependent cancer, variable estrogen levels increase the risk of disease during fetal development. Numerous studies on TGCT have associated disease development with a variety of factors such as cryptorchidism, family history of testicular cancer, maternal lifestyle, genetic factors, hypospadias, and low fertility (4,8).

Family history is an important risk factor for the development of TGCT. Studies performed in large populations showed that sons of men with TGCT have a 4-6 times higher risk of developing TGCT, while brothers have a 8-10 times higher risk (9).

Patients with Klinefelter syndrome (47, XXY), which is a germ-line chromosomal abnormality, rarely develop TGCT. However, approximately one-third of patients with extragonadal germ cell tumors have an increased risk of testicular CIS. In addition, a 1.6-Mb deletion in the azoospermia factor region of the Y chromosome in patients with infertility doubles the risk of developing TGCT (10,11).

Age is another important risk factor in TGCT development. The most common histological type, seminoma, is seen most commonly in men between 30 and 35 years of age. Embryonal carcinoma and teratocarcinoma are common between the ages of 25 and 35, and choriocarcinoma is common in the ages of 20-30. Although yolk sac tumors and benign teratomas are common in early childhood, these 2 histologic types appear in combination with other histologic types in older ages (12,13).

Although the results of some studies are variable due to small sample sizes, additional risk factors such as low maternal parity, twin birth, birth order, and young maternal age are all considered TGCT risk factors (14).

Genetic and Epigenetic Anomalies in TGCT Development and Their Relevance to Clinical Presentation

Different genetic abnormalities play an important role in the development of TGCTs. In recent years, studies on TGCT have taken important steps toward elucidating the mechanism of this disease when genetic and epigenetic factors are considered together. ITGCN, a noninvasive lesion believed to occur during fetal development, is a precursor for the development of TGCT. ITGCN precursor cells, which are inactive until adolescence, reproduce with hormonal effects. After that, isochromosome 12p [i(12p)] formation causes invasive growth as a possible trigger event and turns into ITGCN, seminoma or nonseminoma TGCT histology (15). The association between TGCT and various anomalies (polyploidization, amplification, etc.) in chromosome 12 has been known for many years. Gains in chromosome 12 and formation of i(12p) on karyotype analysis in many TGCT specimens (nearly 80%) was the first genetic marker discovered in TGCT. The remaining 20% also have 12p gains, but these occur through chromosomal rearrangements such as amplification of small chromosomal regions (16,17).

Other genes in the 12p region include *NANOG*, *STELLA*, and *GDF3*, which are stem-cell associated genes that play an important role in the embryonic stem cell self-renewal and pluripotent character. CCND2 controls the cell cycle and is known to maintain the proliferative advantage in early transformed cells. The *KRAS* oncogene is responsible for malignant transformation, while the *GLUT3*, *GAPDH*, and *TPI1* genes play a role in energy metabolism and give tumor cells proliferative advantage. DNA repair genes *MGST1* and *RAD52* may also contribute to the development of TGCT (18).

In TGCTs it is often possible to encounter aneuploidic chromosomal gains. In other studies investigating chromosomal anomalies, it has been shown that copy numbers of chromosomes 7, 8, 17, and X are increased and copy numbers of chromosomes 4, 11, 13, and 18 are decreased. Gains in chromosomes 7, 8, 21, and X are common in both seminomas and nonseminomas (16,17,19).

The only locus identified in a linkage analysis study by Rapley et al. (6) was Xq27. In addition, there was an X-linked inheritance pattern in the history of the investigated families. However, this relationship was not confirmed in a comprehensive independent analysis (9). In another study, Lutke Holzik et al. (20) detected a gene on Xq27 that moderately increased the risk of developing sporadic TGCT but not familial TGCT.

The *KIT* gene is located at the 4q12 chromosomal region and belongs to the receptor tyrosine kinase family. It plays crucial roles in cell survival and proliferation and is well characterized in TGCT. This gene encodes the human homolog of the proto-oncogene *c-KIT* (4). According to current data in the Catalogue of Somatic Mutations in Cancer (COSMIC) database, approximately 22% (80/366) of the KIT mutations are identified in seminomas and approximately 3% (3/104) are identified in nonseminomas.

Single nucleotide polymorphisms (SNPs) in the *KITLG* gene, which is the ligand for the KIT receptor tyrosine kinase, are associated with a 2.5-fold increased TGCT risk. Seven SNPs showing the strongest linkage to TGCT susceptibility, according to the data obtained in GWAS, have been identified for the *KITLG* gene and adjacent areas. This gene is located in the 12q21.3.2 chromosomal region and is responsible for primordial germ cell development, survival, and migration. KITLG is involved in proliferation and survival by performing c-KIT dimerization and autophosphorylation in the c-KIT-KITLG signaling pathway. These results indicate that KITLG plays a critical role in TGCT tumorigenesis. Interestingly, variations in KITLG sequence are more common in European populations than in African populations. Moreover, KITLG has been associated with pigmentation level. This may explain the significant differences in TGCT incidence between ethnic groups (4,6).

The 4 major GWAS performed to date have demonstrated the association of testicular cancers with genes such as *KITLG*, *SPRY4*, *BAK1*, *TERT*, *ATF1IP*, and *DMRT1*, which are generally recognized as important in cancer pathogenesis (6,7,21,22). Susceptibility locus studies in GWAS revealed over 40 risk loci associated with TGCT, and KITLG was found to be the strongest genetic risk factor for TGCT in these studies. *SPRY4* and *BAK1*,

involved in *KITLG-KIT* signaling, are other susceptibility genes for TGCT. While SPRY4 inhibits KITLG-KIT signaling, KITLG KIT signaling suppresses BAK1, which encodes pro-apoptotic proteins and promotes apoptosis by inhibiting the function of anti-apoptotic proteins. For these reasons, KIT signaling is very important for TGCT (23,24).

GWAS for TGCT have revealed high telomerase activity in seminoma due to reactivation of *TERT* gene, which encodes the catalytic subunit of telomerase reverse transcriptase and protects from shorten the chromosomal ends in somatic cells, while this activity was low in teratomas. It is especially noteworthy that TERT is associated with TGCT because testicular germ cells are one of the rare cell types with high levels of telomerase expression in adults. It is also known that TERT is frequently reactive in cancer cells and in this way increases capacity to divide relative to normal healthy cells. The amplification of the 5q15 region observed in many cancer types also supports this (23,25).

In the COSMIC database for the KRAS and NRAS proto-oncogene receptor tyrosine kinase proteins activating Raf/MEK/ERK and PI3 kinase pathways, mutation rates in seminoma cases are 9% and 5%, respectively, while there are no mutations in nonseminoma cases. Hacioglu et al. (26) demonstrated that both K-RAS and N-RAS mutations coexist in 2 patients with seminomatous tumors and another with nonseminomatous tumors. They also suggested that the analysis of K-RAS and N-RAS mutations in TGCTs may provide more treatment options, especially in platinum-resistant tumors (26).

Other studies in patients with impaired sexual development have shown that mutations in genes such as *SRY*, *SOX9*, *NR5A1*, *GATA4*, and *DMRT1*, which are involved in processes related to sex differentiation, can pose a risk for TGCT development (23,27).

The p53 protein plays an important role in cell cycle regulation, cellular stress response, apoptosis induction, and maintenance of genomic integrity by DNA repair. Loss of p53 function results in impaired cell cycle regulation and increased tumor aggressiveness and chemotherapy resistance. Although p53 mutations are common in somatic tumors, the COSMIC database shows that these mutations are very rare in TGCT cases. Moreover, wild-type p53 is frequently overexpressed in TGCTs. However, p53 overexpression occurs in association with overexpression of murine double minute-2 (MDM-2), the oncoprotein that inhibits p53 through ubiquitination, and MDM-2 amplification has been reported at a rate of about 25% in cisplatin-resistant patients (4,28,29).

In addition to genetic factors, epigenetic mechanisms play an important role in the etiopathogenesis of TGCT. All epigenetic regulatory processes are responsible for the initiation and maintenance of pluripotency in embryonic stem cells and the continuity of the characteristics of differentiated cell types. The best known regulatory epigenetic mechanism is DNA methylation. Changes in gene methylation known as important contributors to tumorigenesis include hypomethylation and increased expression of oncogenes, and hypermethylation of CpG islands in the promoter and decreased expression of tumor suppressor genes. In terms of the DNA methylation status of different TGCT types, seminoma cases have very low DNA methylation levels, whereas nonseminomatous teratomas, yolk sac tumors, and choriocarcinomas show hypermethylation due to overexpression of DNA methyltransferases (DNMT) (4). In studies to clarify epigenetic mechanisms in TGCTs, promoter methylation was shown in genes such as tumor suppressors *APC*, *ARF*, *TP53*, *RARB2*, *BRCA1*, *RASSF1A*, cell cycle regulator CCNA1, DNA repair genes such as *MGMT* and *hMLH1*, transcription factor HOXA9, and PRSS21, which encodes the protein testisin in testis cell maturation (30).

Although TGCT is generally curable, resistance to cisplatin is observed in a considerable proportion of patients. Unfortunately, there is currently no effective treatment method for this subset of patients. Therefore, it is important to investigate the molecular mechanisms causing cisplatin resistance. Cisplatin-based chemotherapy has an important role in the treatment of TGCT (31). Cisplatin therapy induces apoptosis induction by creating DNA damage in tumor cells. In TGCT, apoptosis is induced by proapoptotic proteins such as Noxa and Puma because of the low mutation rates of tumor suppressors such as p53 and Rb. These p53-mediated cellular responses also occur with OCT4, a regulator of pluripotency which is highly expressed in seminomas and embryonal cell carcinomas. Studies have shown that depletion of OCT-4 causes a significant decrease in cisplatin hypersensitivity and may account for acquired cisplatin resistance in refractory tumors. OCT4 is a transcription factor that regulates the expression of Noxa. In an *in vitro* study by Gutekunst et al. (32), RNA interference-mediated OCT4 suppression was associated with reduced Noxa expression as well as decreased cisplatin sensitivity. These findings indicate that the sensitivity of OCT4 to chemotherapy depends on the control of Noxa expression (4,32).

Loss of the mismatch repair system, which is required to recognize base pairing errors in DNA, is related to the chromosomal anomaly known as microsatellite instability. Studies have shown that microsatellite instability is associated with BRAF V600E mutation and this mutation is associated with treatment failure in TGCT patients. Honecker et al. (33) showed that mutated BRAF is associated with a decrease in hMLH1 protein, which plays an important role in DNA repair and promoter hypermethylation, in chemoresistant GCT samples. Therefore, these results indicate that genetic testing may be useful for estimating resistance in TGCT (33).

Albany et al. (34) showed that the second generation DNA methylation inhibitor guadecitabine reduced cisplatin resistance even at low doses in embryonic carcinoma (EC) cell lines and the cisplatin-resistant EC xenograft mouse model. These investigators indicated that these preclinical results are promising for treating refractory TGCT patients with guadecitabine alone or with cisplatin, because the sensitivity of EC cells to guadecitabine depends on the high level of DNMT3B expression in EC cells (34).

Aurora B, a serine/threonine protein kinase, is a regulatory protein with important roles in the cell cycle. Aurora B overexpression has been observed in a variety of tumor types, including TGCT, and is associated with poor prognosis in cancer patients. Some studies have shown that inhibition of Aurora kinases led to significantly reduced proliferation. Therefore, therapeutic Aurora kinase inhibition is thought to be important as a possible anticancer regimen due to its important role in cell division (17,35,36).

In an *in vitro* study, Schaffrath et al. (37) tested the effect of kinase inhibitors such as the mammalian target of rapamycin inhibitor RAD001, the endothelial growth factor receptor and vascular endothelial growth factor receptor inhibitor AEE788, and IGF-1R inhibitor AEW541 alone or in combination with cisplatin in cisplatin-sensitive and resistant TGCT cell lines. The researchers demonstrated the inhibitory activity of these kinase inhibitors on cell proliferation at single doses in cisplatin-sensitive and resistant TGCT cell lines. However, when these agents were combined with cisplatin, no promising results were observed in the cisplatin-resistant cell lines. For this reason, they indicated that these targeted drugs have no potential for the treatment of cisplatin-resistant nonseminomatous germ cell tumors (37).

As discussed above, although there are many known molecular characteristics of TCGT, they are not currently used for clinical prediction. However, elucidation of the genetic and epigenetic properties of TGCT has important clinical implications in terms of prevention, treatment strategy, and prognosis in early disease stages.

Conclusion

Much research has been conducted and is still being done to define the molecular mechanisms that can lead to the development of TGCT, which is a complex and multistage disease. In the process of TGCT development, presence of ITGCN, which is known as a precursor lesion with high KIT/KITLG signaling, and chromosomal gains in 12p are the generally known basic profile. However, several advanced genetic analyses aiming to explain TGCT biology and provide insight into treatment response have demonstrated significant differences between tumor subtypes. Therefore, genomic approaches are not currently being used to predict clinical behavior or treatment response in patients with TCGT, which is a heterogeneous tumor group. However, elucidation of the genetic and epigenetic properties of TGCT, while still in early stages, is thought to have significant clinical benefits in terms of disease prevention in the young male population, chemotherapy response, survival after chemotherapy, and mortality reduction in treatment-resistant disease. In addition, especially nonseminoma patients may be classified as having low-, moderate-, and high-risk of treatment resistance according to clinically demonstrated molecular differences. This would allow treatment to be individualized based on predicted chemotherapeutic response according to patients' genomic characteristics. In terms of TGCT detection, since testicular biopsy is an invasive procedure, identifying the precursor lesion ITGCN together with the aforementioned signaling pathways via noninvasive semen testing may facilitate TGCT screening.

Cytotoxic chemotherapy is the mainstay of treatment for advanced disease. Unfortunately, initial therapeutic trials for molecular targets such as receptor tyrosine kinases have been disappointing. TGCT is a relatively low-frequency cancer that is generally susceptible to platinum-based therapies. However, the molecular mechanisms of cisplatin resistance, which is an important clinical issue, have not been well defined. Understanding the molecular mechanisms of cisplatin resistance in TGCT and considering how new therapeutic targets can be discovered in chemoresistant TGCT are necessary to further improve clinical care for this patient group.

Ethics

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: İ.K., E.K., S.S., Design: İ.K., E.K., S.S., Data Collection or Processing: İ.K., E.K., S.S., Analysis or Interpretation: İ.K., E.K., S.S., Literature Search: İ.K., E.K., S.S., Writing: İ.K., E.K., S.S.

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