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Spotlight on Germ Cells

Genetic aspects of testicular germ cell tumors

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Testicular Germ Cell Tumor (TGCT) is the most common malignant tumor in young Caucasian men with an annual increase of 3–6% in the past 50 years. Data in the literature indicate that both environmental and genetic factors acting on the primordial gonocyte/gonocyte are implicated in the etiology, progression and treatment sensitivity of TGCTs. This review focuses on genetic factors involved in the etiology, progression and treatment sensitivity of TGCTs.

Introduction

Testicular Germ Cell Tumor (TGCT) is the most common malignant tumor—accounting for up to 60% of all malignancies—in young Caucasian men aged between 20 and 40 years. Clinically and histologically they are divided into seminomas and nonseminomas. The worldwide incidence of TGCTs is between 6–11 per 100,000, with significant variation. It has more than doubled in the past 50 years with an annual increase of 3–6%. Epidemiological studies showing striking geographical and ethnic differences largely support the TDS hypothesis. Observations on populations migrating from countries with high incidence (Denmark) and with markedly lower incidence (Finland) to Sweden provided evidence for the importance of environmental factors acting at prenatal/perinatal period. The first generation immigrants retained the incidence as in their country of origin, whereas the second generation (born in Sweden) had a similar risk to native Swedes. The most likely environmental chemicals acting in utero able to provoke TDS seem to be endocrine disruptors (xeno-estrogens), although direct evidence is lacking so far.

Concerning ethnic differences, it is well known that Asian and African populations have a low risk of TGCTs and in contrast to what was observed in the Scandinavian migrating populations, men with African or Asian descent maintains their low risk even inhabiting in an area of high risk. This has been observed in African-Americans living in the USA for many generations indicating the importance of inherited susceptibility factors as well. In fact, it demonstrates the dominance of genetics over environment in these cases. This review focuses on genetic factors involved in the etiology, progression and treatment sensitivity of TGCTs.

Inherited Susceptibility Factors

TGCTs can occur in a sporadic or familial manner (about 2%). Family history is among the strongest risk factors for TGCTs with a relative risk to a brother of a TGCT case of 8–10 and between fathers and sons of 4–6. These figures are much higher than those reported for most other cancer types, which rarely exceed 4. Other evidences for strong hereditary component come from twin studies showing a greater concordance for disease in monozygotic than in dizygotic twins and from segregation analysis of familial cases suggesting a recessive mode of inheritance. The link between bilateral TGCTs and genetic factors has been further strengthened by demonstrating that brothers and fathers of bilateral cases have a greater risk to develop a TGCT than those of monolateral cases. These data clearly indicate that susceptibility genes do play a role in the etiology of TGCTs and bilateral and familial cases are the most obvious candidates for searching inherited susceptibility factors.
Genetic Linkage Analysis

Thanks to genetic linkage analysis a number of cancer susceptibility alleles have been identified. Concerning TGCTs a major effort was made by the International Testicular Cancer Linkage Consortium (ITCLC) in collecting a sufficiently large set of multiple-case families for genetic linkage studies. The first set of pedigrees (n = 134) provided strong evidence of a susceptibility locus at Xq27 in a subset of families with disease distribution compatible with X linkage (HLOD = 2.01) and history of at least one bilateral case (HLOD = 4.7). The high Heterogeneity LOD score (HLOD) was not confirmed when subsequently 163 additional pedigrees were studied. Although a relevant gene in this region cannot be excluded, if it exists it would account only for a small proportion of TGCT susceptibility, possibly related to cryptorchidism.

The same Consortium published the results of a genome-wide analysis on 237 TGCT pedigrees indicating six “regions of interest” at different autosomal chromosomes. However, simulation analysis suggested that neither of these loci is likely to explain a sibling relative risk of 4. It is therefore clear that susceptibility to TGCTs cannot be due to one major gene effect and multiple loci must contribute.

The Precursor Lesion of TGCTs

The most likely precursor of TGCT is a gonocyte or primordial germ cell that escaped normal maturation. This lesion is known as carcinoma in situ (CIS) or the Intratubular Germ Cell Neoplasia Unclassified (ITGCU) according to the World health Organization (WHO). The counterpart of CIS in the dysgenetic gonad (see below) is known as gonadoblastomas. These precursors can progress to invasive components of different histologies, including seminoma as well as various types of nonseminomas (i.e., embryonal carcinoma, teratoma, yolk sac tumor and choriocarcinoma). OCT3/4 is diagnostic to identify CIS, gonadoblastoma, seminoma and embryonal carcinoma. In line with this origin of TGCTs, individuals with severe abnormalities of gonadal development associated with the intersex syndrome, currently referred to as Disorders of Sex Development (DSD) show an increased risk for this kind of cancer, in which the Y chromosome genetic material is of crucial importance.

The Y Chromosome

The commonest structural abnormalities of the Y chromosome are microdeletions of the long arm of the Y chromosome (Yq). The Yq contain three AZF (AZoospermia Factor) regions, AZFa, b and c in which are mapping genes that are most likely to be involved in germ cell differentiation and spermatogenesis. The deletion of one or more AZF regions is strictly associated with impaired sperm production and also distinct Y chromosome lineages (Y haplogroups) have been reported as predisposing factors for oligo/azoosperma. Recently, a novel deletion designated “gr/gr”, which removes half of the gene content of the AZFc region, has been reported as genetic risk factor for TGCTs by Nathan et al., (2005).

Figure 1. Schematic representation of the Y chromosome showing (A) the deletion intervals AZFa, AZFb and AZFc regions. AZFb deletions overlap with the AZFc region. (B) The AZFc region with the indication of the location of multicopy genes and transcription units in the reference sequence published by Skaletsky et al., (2003). This region contains a number of repeated sequences with the same orientation (matching arrows) which through intrachromosomal recombination may lead to deletions. (C) gr/gr deletions which remove half of the AZFc gene content, but can vary in breakpoints. This deletion has been reported as genetic risk factor for TGCTs by Nathan et al., (2005).
between Y haplogroups and TGCTs. Similarly, classical AZF deletions (removing all STSs located in a given AZF region) are not associated with TGCT and were not found at all in a total of 575 cancer patients. Single STS deletions on the Y chromosome are likely to be due to PCR artefacts or to polymorphisms in the primer sequence binding site which implies that they must be confirmed by sequencing the deletion breakpoints. Since confirmed single STS deletions have not been described in the literature the appealing link between Y chromosome instability (expressed as mosaic or single STS deletions) and TGCT remains to be established.

On the contrary of classical AZF deletions, “gr/gr” deletion has been reported as a significant risk factor for TGCT. A two fold increased risk of TGCT for “gr/gr” deletion carriers in 1807 affected subjects has been observed with an even higher—three fold—increase in familial cases. In contrast to what would be expected, the authors found that probands from TGCT families exhibiting maternal lineage were at greater risk than those exhibiting paternal lineage. The association between “gr/gr” deletion and TGCT was not confirmed in a separate UK study and an Italian study. In general, the analysis of a very large group of subjects might be more appropriate for detecting genetic predispositions acting at low penetrance than smaller studies, however the situation is more complex for the Y chromosome than for the rest of the genome because of potential biases such as population stratification. Ethnic and geographic matching of cases and controls is fundamental when searching for Y related factors and the multicenter study, although very large, contained only 15% of cases and 23% of unaffected males from designed epidemiologic case-control studies. Moreover, since both gr/gr deletions and familial cases are rare the strength of the large multicenter study is still limited and would require further confirmation.

The above discussed data indicate an unlikely role for AZF deletions and genes or gene families in TGCT formation. However assuming that only a subgroup of patients will have a Y related etiology, the most likely candidates are those who present both impaired spermatogenesis and a TGCT. Only a small proportion of published studies were able to collect data on semen analysis, therefore, final conclusions are awaited.

An interesting candidate to explain the role of the Y chromosome in the development of TGCTs is the Testis Specific Protein on the Y chromosome (TSPY). This multicopy gene lies within the so called Gonadoblastoma on the Y chromosome (GBY) region, related to malignant transformation of primordial germ cell/gonocytes in patients with specific forms of DSD, especially hypopituitarism and gonadal dysgenesis, and is highly expressed in CIS and GB. Although the mechanism is still unknown, it might be related to cell cycle control.

Other Polymorphisms

Only a few association studies are available in the literature. The two polymorphic microsatellites of the androgen receptor (AR) gene (CAG and GGN repeats in exon 1) have been studied in relationship with infertility, cryptorchidism as well as TGCTs. The rational for the analysis of AR polymorphisms derives from a proposed—not yet elucidated—role for androgen function in early foetal testicular development. While androgen insensitivity is an important risk factor for TGCTs, especially demonstrated in patients with DSD, an increased androgen signalling during development may, in theory, decrease the risk. Given that the length of the polymorphic polyglutamin stretch (encoded by CAG repeats) influences the transactivation capacity of the receptor, variations in its length, still in the polymorphic range, may confer stronger or weaker androgenicity. Studies which focused on TGCT patients failed to find an association between CAG repeat length and cancer risk. The observation by one single group about an association between long CAG tracts and tumor progression to nonseminomas and a clinically more aggressive disease awaits confirmation.

A role in genetic susceptibility to endocrine disruption can be hypothesized for polymorphisms in those genes involved in pathways related to the action and metabolism/detoxification of endocrine disruptors or endogenous estrogens. According to the intrauterine origin of TGCTs a logical target for susceptibility factors is represented by maternal genetic polymorphisms. A recent case-parent study investigated a number of genes involved in the oestrogen metabolism and found an association between cytochrome P450 gene polymorphisms (both maternal and in the index subjects) and increased risk of TGCTs. Given the small sample size, this highly promising finding needs to be replicated by independent studies.

The Role of Somatic Genetic Changes

Chromosomal constitution. Many studies investigated the chromosomal constitution of TGCTs, including its precursor lesion CIS. Besides a consistent aneuploidy, specific chromosomal gains and losses are identified, but the only recurrent structural imbalance is the gain of the short arm of chromosome 12, mostly as isochromosomes. The majority of studies indicate that gain of 12p is progression related. In spite of various attempts, there is no single 12p-target gene identified. A number of genes have been suggested to be relevant, including KRAS2, NANO2, although the actual proof is lacking so far.

Single nucleotide polymorphism analysis in TGCTs demonstrated the presence of so called uniparental disomies, especially in nonseminoma. Although of putative interest, the biological relevance remains to be shown.

Currently a number of integrated analyses of expression of genes and proteins as well as DNA copy changes are performed. Overall, the data suggest a close correlation between the two, in which the expression drives the chromosomal imbalances or vice versa.

Mutational status. Various studies with the goal to identify pathogenetic mutations have been performed on TGCTs. Although mutations have been identified, these seem to be limited in frequency, with the possible exceptions of c-KIT and KRAS-2, and more recently BRAF. c-KIT is a kinase receptor relevant for a number of crucial processes during normal development, including survival and migration of PGCs from the epiblast to the genital ridge. In normal development of germ cells, c-KIT is downregulated upon arrival of the PGCs in the genital ridge, although it can still be detected at a relatively low level in human spermatogonia. c-KIT is also present at a high level in CIS and gonadoblastoma and is overall downregulated upon invasive growth. Activating mutations, leading to a stem cell factor (SCF) independent active receptor, have been found predominantly in bilateral TGCTs but some studies found them mainly in primary unilateral nonseminomas. The sensitivity of the mutation detection may be responsible for the conflicting data, as well as tumor progression-related loss. That indeed c-KIT has
an important role in the pathogenesis of TGCTs, is supported by the observation that this gene can be overexpressed due to a highly restricted genomic amplification only including this gene. The most recent identification of SCF being a diagnostic marker for early malignant germ cells is of interest in this context.28 The c-KIT signaling pathway has been linked to PI3K, both in mouse PGCs as well as TGCTs. It is of interest that activating KRAS2 mutations are found in TGCTs. Activation of a mutated KRAS2 results in an increased in vitro survival of seminoma cells, which are normally not able to survive outside the patient, as well as an earlier age at clinical presentation of the tumor.

The proto-oncogene BRAF has been shown to be mutated in a variety of cancers, including TGCTs. The affected pathway is the MEK pathway, in which RAS also acts. Activating mutations of KRAS and BRAF are mutually exclusive in TGCTs. A correlation between BRAF mutation and hypermethylation of the promoter of hMLH1 has been reported. Since hMLH1 is involved in mismatch repair, absence or mutations in this gene results in microsatellite instability (MSI). Indeed, MSI instability has been reported to be related to treatment resistance (i.e., cisplatin-based) in multiple studies. However, the exact link between BRAF status, MSI and treatment sensitivity of TGCTs has to be clarified.

An overall low mutation frequency is rather exceptional for solid cancers, although it seems to be the rule for TGCTs. That this is indeed not due to the pre-selection of genes under investigation, but an overall phenomenon is supported by the results of a high throughput investigation on the mutation status of the genome. This might again be related to the embryonic origin of the tumors. In fact, embryonic stem cells have a unique mechanism which is one of the two DNA strands is kept protected against any form of mutations. This protects the DNA from anomalies to be transmitted to the next generation.

TP53 and microRNAs. One of the intriguing observations is that also TP53 is hardly mutated in TGCTs for. It is however, interesting that TP53 target genes have been found to be frequently hypermethylated in TGCTs. The explanation for the wild type TP53 status in TGCTs was obtained, amongst others, as a result of the expression analysis of certain microRNAs. The miRNA cluster 371–373 (mapped to chromosome 19) is specifically expressed in the seminomas and embryonal carcinomas and as expected in human embryonic stem cells. This cluster of microRNAs was before found to be able to mimic the presence of a mutated TP53 in overlying cellular senescence in a high throughput in vitro model system. The miRNAs interact with the 3’ UTR of the mRNA encoding the tumor suppressor gene protein LATS-2, which is involved in the regulation of G1-S transition in the cell cycle. LATS-2 is indeed a downstream target of TP53, and inactivation of TP53 results in absence of LATS-2 protein, and thereby overruling cellular senescence.

Conclusions

The analysis of familial cases did not provide evidence for linkage to any locus possibly because there are several moderate-risk genes involved in development of TGCTs. This implies that alternative approaches not based on linkage analysis should be taken into consideration for future investigations. One option could be the analysis of an enriched study population in specific subgroups (for example TGCT patients with impairment of spermatogenesis) versus unaffected controls. Given that spermatogenic dysfunction is more common in TGCTs than can be explained by either local tumor or general cancer effect, it is likely that mutations in spermatogenic candidate genes will be involved only in those patients who are associated with reduced sperm count. Up to now no specific screening for mutations in spermatogenesis candidate genes has been performed in large enough group of selected cases with impaired spermatogenesis. An other even more promising alternative is a genome wide association study which could provide susceptibility locus mapping even for low penetrance disease loci. In the era of International Hapmap project (2003) and of appropriate technology platforms such studies became now a realistic way to approach multifactorial diseases. In addition, introduction of (high throughput) mutation and genome imbalance analyses and expression profiling has advanced our understanding of the pathogenetic mechanisms involved in TGCTs. However, this has just begun to unravel the complex interaction of multiple pathways involved, for which an integrated approach would be most informative.

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