Review

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129/Sv mice—a model system for studying germ cell biology and testicular cancer

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Abstract. Some forms of testicular germ cell tumors (TGCTs) arise from primordial germ cells (PGCs) during fetal development. In both humans and mice, genetic control of susceptibility is complex, involving both Mendelian and polygenic factors. Identification and characterization of TGCT genes will provide insight not only into the basis for inherited susceptibility, but also into the genetic control of the development of the PGC lineage. Recent work has revealed the identity of several susceptibility genes that are inherited as Mendelian traits, the chromosomal location of yet-to-be identified TGCT susceptibility genes, as well as clues to the nature of developmental pathways involved in tumorigenesis. In this review we summarize current understanding of the biology and genetics of TGCTs in mice and discuss the relevance of this work to testicular cancer in humans.

The 129/Sv inbred strain was the foundation for establishing embryonic stem (ES) cell cultures. The ability to manipulate these cells in vitro revolutionized mouse genetics by permitting intentional mutations in specific genes through genetic engineering (Baribault and Kemler 1989; Bradley et al. 1992, 1998). L.C. Stevens' pioneering work on embryonic carcinoma (EC) cells found in testicular germ cell tumors (TGCTs) in 129/Sv mice contributed importantly to methodologies for working with ES cells (Stevens and Hummel 1957; Stevens 1967a; Martin 1981). Although considerable information is available about the biology of ES cells (Robertson 1987; Hogan et al. 1994), much less is known about susceptibility to spontaneous testicular germ cell tumors. TGCTs originate during critical transitions in the development of primordial germ cells (PGCs), the embryonic precursors of the germline. These tumors are composed of a disorganized collection of various cell and tissue types derived from all three primary germ layers and at various stages of differentiation (Stevens 1967b). In humans, 96% of tumors in the testis are testicular germ cell tumors (Buetow 1995; Bosl and Motzer 1997). Genetic factors influence susceptibility to testicular cancer in both humans and mice (Stevens 1967a; Forman et al. 1992; Heimdal et al. 1997; Bishop et al. 1998; Matin et al. 1999; Rapley et al., 2000), but the identity of these genes has remained elusive. The 129/Sv inbred strain is an outstanding model to study inherited susceptibility to TGCTs, as well as development of the germline. In this review, we summarize the current understanding of the biology of TGCTs in mice, recent progress toward dissecting the genetic and molecular control of TGCT susceptibility, and the relevance of these studies to germline development in mammals and testicular cancer in humans.

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Biology of TGCTs. Testicular germ cell tumors arise spontaneously with an incidence of 1–10% in 3-week-old 129/Sv males (Stevens and Hummel 1957). The composition of these tumors changes during development (Stevens 1967a). In fetal and newborn mice, TGCTs are composed primarily of undifferentiated embryonal carcinoma (EC) cells; in mice that are about 5 days of age, tumors contain both undifferentiated and differentiated cell types; and in most adult mice, tumors are completely differentiated and lack EC cells (Fig. 1). The composition of differentiated TGCTs is highly variable. Some contain little more than neuronal tissues, while others contain a variety of cell and tissues types such as neuroepithelium, bone with marrow, cartilage, teeth, muscle, skin with hair and sebaceous glands, and glandular epithelia. These cells and tissues are derived from all three germ layers.

The remarkably variable composition of these tumors strongly suggests that EC cells are pluripotent. In vivo cloning experiments provide direct evidence for this hypothesis (Kleinsmith and Pierce 1964). Single EC cells transplanted into ectopic sites in adult 129/Sv mice differentiate into diverse cell and tissue types. In each mouse, differentiation can be biased towards particular cell and tissue types, depending presumably on unique cellular and environmental factors at the transplantation site.

L.C. Stevens used a series of genital ridge grafting experiments to identify the developmental stage at which TGCTs originate and the cell of origin for TGCTs (Stevens 1967b). Located along the midline in the lumbar region, the genital ridges are the primordium of embryonic gonads into which PGCs migrate at E11. Genital ridges from embryonic day 11 (E11) to E13 wild-type fetuses that were grafted into adult 129/Sv mice develop into TGCTs. The ability of a ridge to form TGCTs peaks in grafts from E11 to E12.5 fetuses and decreases in grafts from fetuses after E12.5 (Stevens 1966). Therefore, the onset of tumorigenesis probably occurs between E11 and E12.5. The essential nature of PGCs in tumorigenesis was demonstrated in experiments with genital ridge grafts from E12 fetuses homozygous for the Steel mutation (*Sl/Sl*), which are devoid of PGCs and which failed to develop into tumors when grafted into 129/Sv mice (Stevens 1967b).

An important question is whether PGCs in 129/Sv mice are pluripotent when TGCTs are initiated or whether pluripotency is an acquired property after PGCs are transformed to EC cells. As the only lineage that passes from generation to generation, the developmental potential of the PGC lineage is tightly regulated. Fertilized eggs are totipotent; early in development the germline is set aside, and development is restricted to gametes; at fertilization, totipotency is restored. Studies with aggregation chimeras suggest that PGCs from E10.5 embryos are already restricted to germline development (Donovan 1994). In TGCT-prone mice, PGCs may not be restricted to germline development until later developmental stages, so that at the onset of tumorigenesis PGCs remain pluripotent. Alternatively, PGCs may regain pluripotency under certain conditions and be transformed into EC cells at E11–E12.5, the critical period in tumorigenesis.

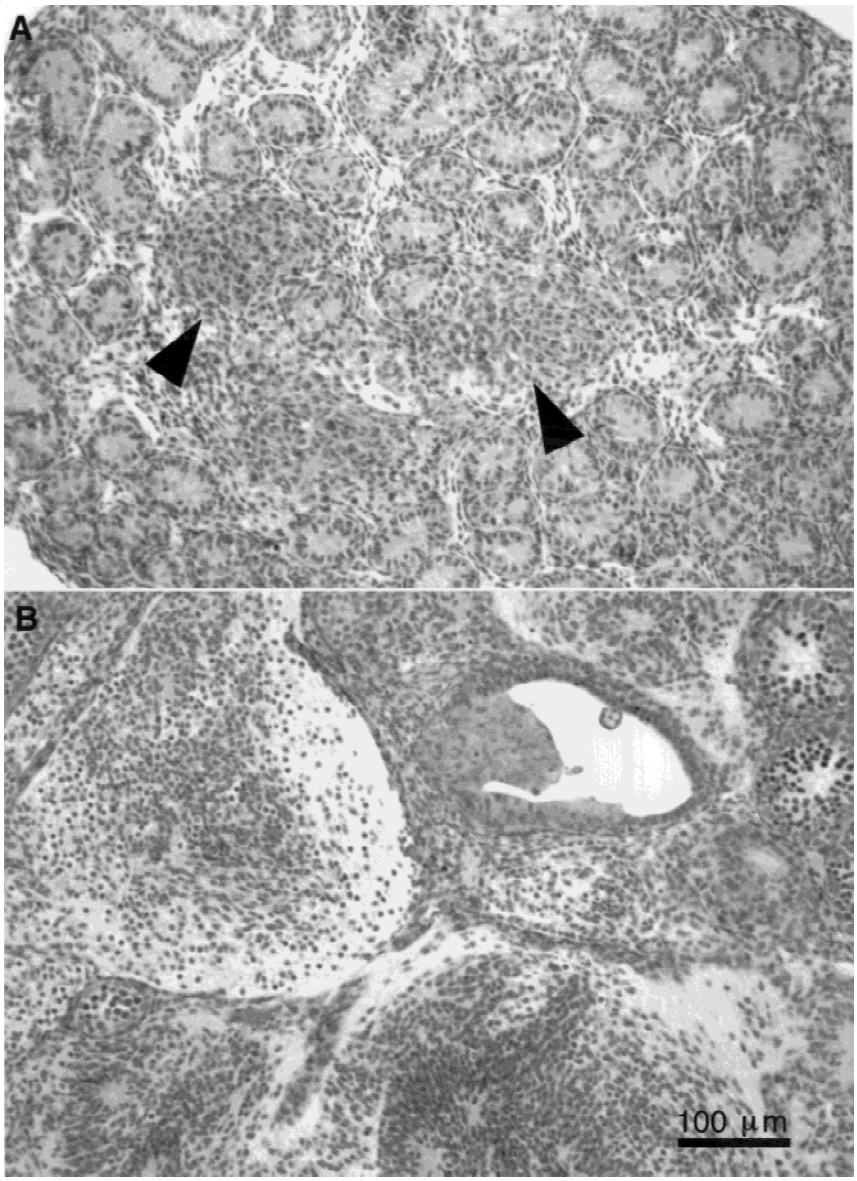

Fig. 1. Hematoxylin and eosin staining of tumor-bearing testes from 1-day and 10-day-old 129.MOLF–Chr19 mice. In newborn males, TGCTs are composed mainly of undifferentiated EC cells. At 10 days of age, TGCTs are composed of differentiated cell and tissue types. Scale bar indicates 100 μm.

TGCTs and PGC development. During mouse embryogenesis, PGCs are first recognized around E7 in the extra-embryonic mesoderm just posterior to the definitive primitive streak (Ginsburg et al. 1990). Around E7.5, PGCs leave the yolk sac, migrate through the hindgut and dorsal mesentery, and arrive at genital ridges by E11.5. During migration, PGCs undergo extensive mitotic proliferation, with the number of PGCs increasing from about 100 at E7 to about 25,000 at E13.5 (Tam and Snow 1981). Shortly after PGCs arrive in the genital ridges, their mitotic activity decreases and sexual differentiation of the fetal gonad begins (Lin 1997). In females, PGCs begin meiosis and then undergo meiotic cell cycle arrest at E13. In males, PGCs undergo mitotic G1 cell cycle arrest at E13.5. They resume mitotic activity shortly after birth and complete gonadal differentiation and spermatogenesis (Lin 1997).

The development of TGCT tumorigenesis parallels normal PGC development (Fig. 2). The onset of TGCT tumorigenesis occurs around E11–E12.5, when germline development undergoes critical transitions. During this period, the primordial germ cells arrive at the genital ridges, decrease their mitotic activity, and begin sexual differentiation. Cell cycle regulation is likely to play a role in TGCT tumorigenesis. The PGCs are still mitotically active around the time of TGCT onset, yet their mitotic activity decreases dramatically after that time point. It is possible that if the PGCs fail to enter mitotic G1 arrest and continue to divide, they may receive other developmental cues and become tumorigenic. Supporting this argument is the prolonged mitotic activity of PGCs in the 129/Sv-*Ter* strain—a strain that is highly susceptible to TGCTs (see below).

Sexual differentiation also influences the tumorigenic activity of the PGCs. In 129/Sv mice, germ cell tumors arise only in males, but not in females. This sex-specific effect may be due to hormonal regulation associated with sexual differentiation, or it could be simply explained by cell cycle regulation, since PGCs undergo mitotic G1 arrest in males and meiotic cell cycle arrest in females.

Recent studies have begun to reveal the molecular controls of PGC development (Donovan 1998; Wylie 1999). *Bmp4* and *Oct4* are required for the generation and totipotency of the germline lineage, respectively (Lawson et al. 1999; Nichols et al. 1998). Mast cell growth factor (*Mgf*), the KIT oncogene, leukemia inhibitory factor (LIF), basic fibroblast growth factor (bFGF), tumor necrosis factor-α (TNF-α), and interleukin-4 (IL-4) play important roles in PGC survival and proliferation (Dolci et al. 1991; Godin

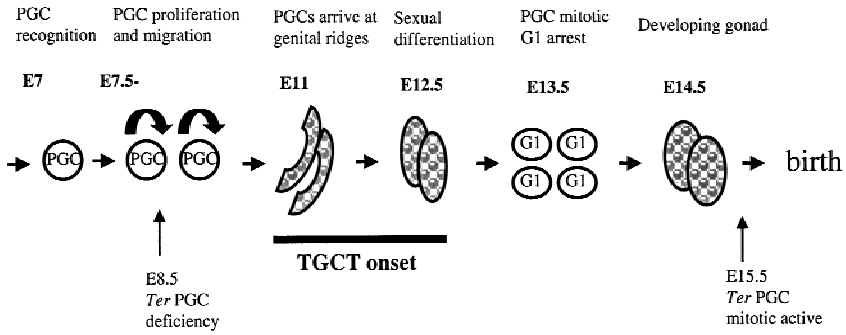

Fig. 2. PGC development and testicular cancer (after Stevens 1961, 1981).

et al. 1991; Matsui et al. 1991, 1992; Redsnick et al. 1992; Han et al. 1993; Kawase et al. 1994; Cooke et al. 1996). TGFβ1 controls PGC migration as well as PGC cell cycle arrest (Godin and Wylie 1991). Integrin β1 is required in PGCs to ensure colonization of the fetal gonad (Anderson et al. 1999). Desert hedgehog and cyclin D2 are expressed in the Sertoli cells and regulate germ cell function (Bitgood et al. 1996; Sicinski et al. 1996). TGCT susceptibility may result from dysfunction in various aspects of PGC development, including regulation of PGC proliferation, migration, cell cycle control, pluripotency, and interactions with somatic cells. It would be interesting to determine the functions of those genes controlling PGC development with respect to TGCT tumorigenesis.

Genetic control of TGCTs in 129/Sv mice. Occurrence of spontaneous TGCTs primarily in the 129/Sv inbred strain and rarely in other inbred strains or in mice with mixed genetic backgrounds implies that susceptibility to TGCTs is a genetic trait. Genetic analysis of segregating populations suggests that at least 3–5, and perhaps as many as 10–15, genes control susceptibility (Stevens and Mackensen, 1961, 1981; Matin et al. 1998, 1999). QTL studies to identify these genes have not yet been successful, although linkage of two of these genes, one on mouse Chr 13 and the other on Chr 19, was recently reported (Matin et al. 1999; Muller et al. 2000). Studies of gene mutations that have been made congenic on the 129/Sv background suggest that Mendelian factors are also involved. Mutant genes that modulate TGCT susceptibility include *Ter, Steel* and *Steel-J, Tgct1, Trp53,* and A^y. Mice with these gene mutations provide insights into the genetic basis for susceptibility and are being used to characterize genes and pathways involved in tumorigenesis.

Ter. The *Ter* mutation dramatically increases TGCT susceptibility (Stevens 1973). It has the strongest effect on susceptibility of any mutant that has been studied, with most males having a spontaneous tumor by 3 weeks of age (Noguchi and Stevens 1985). *Ter* has a semi-dominant effect on the incidence of TGCTs; the incidence is 94% in *Ter/Ter* homozygous males and 17% in *Ter*/+ heterozygous males (Noguchi and Stevens 1985; Asada et al. 1994). Another important phenotype resulting from the *Ter* mutation is germ cell deficiency. *Ter/Ter* mice show PGC deficiency, regardless of genetic background (Noguchi and Noguchi 1985). PGC deficiency is observed as early as E8.5 (Sakurai et al. 1995). In *Ter* homozygotes, the number of PGCs remains unchanged from E7.5 to E12.5, whereas PGCs normally proliferate more than 250-fold during this period (Tam and Snow 1981). In addition, PGCs continue to divide until E15.5 in *Ter* homozygotes, whereas they normally undergo mitotic G1 cell cycle arrest at E13.5 (Noguchi and Noguchi 1985). The prolonged mitotic activity of PGCs in *Ter* mutant mice suggests that the TER protein may be required for mitotic G1 arrest. Finally, the substantially increased TGCT risk in PGC-deficient mice argues that somatic events do not contribute significantly to tumorigenesis.

Steel and Steel-J. The Steel (*Sl*) and White-spotting (*W*) mutants were originally identified as coat color mutations (Silvers 1979). Mutant mice were subsequently shown to be defective in melanogenesis, gametogenesis, and hematopoiesis. *Sl* and *W* mutant homozygotes show PGC deficiency, and homozygous males are sterile. L.C. Stevens transferred various *Sl* and *W* alleles onto the 129/Sv background to test their effects on tumorigenesis (Stevens and Mackensen 1961; Stevens 1967a). Among the mutants tested, results for the Steel and Steel-J (Sl^J) allele were particularly striking. Despite partial PGC deficiency, Sl^J/+ heterozygous males show increased TGCT susceptibility on the 129/Sv background with Sl^J/+ males having more than twice as many TGCTs as their +/+ littermates. This phenotype is also observed in the *Sl* but not in the Sl^d mutant alleles. Moreover, none of the *W* mutants show increased tumor susceptibility on the 129/Sv background.

The molecular identity of the *Sl* and *W* mutants provides a possible explanation for Stevens' results with the *Sl* and *W* congenic strains. Steel mutations have molecular lesions in the *Mgf* gene, which encodes an EGF-like growth factor (Copeland et al. 1990; Zsebo et al. 1990), whereas *W* mutations have molecular lesions in the c-*kit* gene, which encodes a receptor tyrosine kinase and is the receptor for the MGF ligand (Chabot et al. 1988; Geissler et al. 1988). The *Mgf*/c-*kit* signaling pathway is required for PGC survival and proliferation during germline development (Dolci et al. 1991; Godin et al. 1991). Sl^J is a ~650-kb deletion that includes the *Mgf* gene (Bedell et al. 1996), the *Sl* allele is a larger lesion with ~810 kb deleted, whereas Sl^d is an intragenic point mutation within the *Mgf* gene (Bedell et al. 1996). The molecular lesions in *W* mutants range from point mutations to deletions (Nocka et al. 1990). Based on Stevens' observation and the molecular studies of the *Sl* and *W* mutants, it is unlikely that inactivation of the *Mgf*/c-*kit* pathway leads to increased TGCT susceptibility. Instead, an additional gene probably resides in the Sl^J and *Sl* deletions and is responsible for the tumor phenotype. This critical region on mouse Chr 10 is homologous to 12q22 in humans, which is deleted in many adult germ cell tumor cells (Murty et al. 1996). *Mgf* was originally thought to be the key gene within the deletion region, but recent studies exclude *Mgf* gene, suggesting instead a gene(s) closely linked to *Mgf* (Murty et al. 1996), which is consistent with the 129/Sv-*Sl* mouse models.

Tgct1. To identify TGCT susceptibility genes, a genome-wide survey was conducted in a sensitized *Ter*/+ background (Collin et al. 1996; Matin et al. 1999). Progeny of a backcross between (129/Sv–*Ter*/+ × MOLF/Ei)F_1–*Ter*/+ hybrids and 129/Sv–*Ter*/+ were examined for TGCTs. QTL analysis of tumor-bearing progeny, including both bilateral and unilateral cases, did not reveal evidence for linkage for TGCT susceptibility genes. However, when data from mice with bilateral tumors were analyzed separately from those with unilateral tumors, linkage near *D19Mit5* on the central portion of Chr 19 was detected, with a bias for MOLF-derived alleles in mice with bilateral TGCTs (Collin et al. 1996). Mice with unilateral tumors showed a modest excess of 129-

derived alleles on distal Chr 19. These results suggest that there are at least one, and perhaps two, TGCT susceptibility genes on Chr 19. These results also raise the possibility that the development of bilateral and unilateral tumors may be under distinct genetic control. Additional evidence supporting this notion was recently found in a genetic study of TGCTs in humans where a susceptibility gene for bilateral but not unilateral tumors was mapped to the Xq27 (Rapley et al. 2000).

To further characterize the TGCT susceptibility loci on Chr 19, a novel approach was used by constructing a Chromosome Substitution Strain (CSS, which is also known as a consomic strain; Nadeau et al. 2000). The 129.MOLF–Chr19 CSS was made by transferring the entire Chr 19 from the MOLF/Ei strain onto 129/Sv inbred background through repeated backcrossing and selection (Matin et al. 1999). As predicted, 129.MOLF–Chr19 CSS males showed dramatically increased TGCT susceptibility. Homosomic animals show ~80% tumor incidence and heterosomic animals show ~20% tumor incidence. Homosomic males are equally susceptible to unilateral and bilateral tumors, whereas tumors in heterosomic males are primarily unilateral (97%–98%) and rarely bilateral (2%–3%). Linkage studies, which involved crosses between the 129.MOLF–Chr19 CSS and 129/Sv in which only Chr 19 segregates, revealed a susceptibility gene named *Tgct1* near *D19Mit5*, confirming the previous genome scan results. Remarkably, despite frequent TGCTs, 129.MOLF–Chr19 homosomic males are fertile and breed well. As a result, this CSS provides new opportunities for many genetic and molecular experiments. Gene expression analysis in genital ridges during fetal development can be examined in homozygous animals, interactions between *Tgct1* and other susceptibility genes can be tested, and ENU mutagenesis can be used to find new mutants that control TGCT susceptibility.

Trp53. *Trp53* is a tumor suppressor gene that controls cell cycle and apoptosis in many different cell types (Levine 1997). TRP-deficient (*Trp53*$^{-/-}$) mice were generated by using gene knockout technology (Donehower et al. 1992). Homozygous mutant mice develop normally but are highly susceptible to various tumors. The spectrum of tumors in these mice depends on genetic background (Harvey et al. 1993). On a mixed (C57BL/6 × 129/Sv) background, 75% of the homozygous mutants develop lymphomas and 9% develop testicular tumors; as a congenic on the 129/Sv background, TGCT incidence is increased to 35% and lymphoma incidence is about 65%, and as a congenic on the C57BL/6J background, homozygous mutant mice have a <2% incidence of TGCTs. Although the identity of the genes that modulate TGCT susceptibility is not known, the chromosomal location (Chr 13) for one of these genes (*pgct1*) was recently determined in mice with bilateral tumors (Muller et al. 2000).

The effect of the *Trp53* pathway on TGCT susceptibility may be indirect, acting through other pathways to contribute to TGCT susceptibility. Two lines of evidence support this notion. First, although loss of *Trp53* function is found in about 50% of types of tumors in humans, *Trp53* mutations are rarely found in TGCT cases (Levine 1997). Second, although *Trp53* is often overexpressed in human TGCTs, its transcriptional activity seems to be inhibited because downstream genes that are regulated by *Trp53* are normally expressed in human TGCTs (Lutzker 1998).

A^{y}. In contrast to other TGCT models, mice bearing the *A*^{y} (lethal yellow) allele of the agouti locus show reduced TGCT incidence (Stevens 1967a; Noguchi and Stevens 1982). *A*^{y}/+ mice show increased susceptibility to several types of tumors, such as pulmonary, mammary, and skin tumors (Stevens 1967a). However, when Stevens transferred the *A*^{y} allele onto the 129/Sv background, he found that *A*^{y}/+ males have one tenth as many TGCTs as their wild-type littermates. *A*^{y} is a dominant mutation of the agouti gene, which results in ectopic expression of the agouti gene product and leads to chronic inhibition of signal transduction through the MC1-R and MC4-R melanocortin receptors (Bultman et al. 1992; Miller et al. 1993; Dinulescu and Cone 2000). It is not obvious how aberrant signaling through the melanocortin receptor pathway relates to TGCT tumorigenesis, however. Stevens' result is currently being verified by reintroducing the *A*^{y} allele onto 129/Sv background and evaluating the effect of the *A*^{y} mutant gene on TGCT incidence.

Gene interactions. Of the TGCT susceptibility genes, only the molecular identities of *Trp53* and *A*^{y} are known. Given the distinct functions of these genes, it is difficult to generalize about the nature of pathways that contribute to tumorigenesis. Identification of the *Ter* and *Tgct1* genes, as well as the TGCT susceptibility gene within the Steel deletion, should provide clues to the nature of these pathways. Studies of tumorigenesis in double-mutant mice will help dissect the hierarchy of interactions that leads to TGCTs. Preliminary evidence indicates that *Sl* and *Tgct1* act together to increase the incidence of bilateral tumors (L. Jiang and J.H. Nadeau, unpublished), suggesting that these genes act in the same pathway.

The propensity of these Mendelian factors to contribute to TGCTs on the 129/Sv inbred background but not on other strain backgrounds argues that interactions between these factors and 129/Sv-derived TGCT genes control susceptibility. Although it remains to be determined whether these genes act in an additive, epistatic, or threshold manner (or a combination of these models), sensitized linkage crosses that have been used to map TGCT genes have also begun to reveal evidence for gene interactions. Interestingly, the nature of the interactions seems to depend on the particular mutant gene that is used to sensitize the linkage cross. Linkage crosses involving the *Ter* gene revealed a gene (*Tgct1*) on Chr 19 (Collin et al. 1996; Matin et al. 1999), whereas linkage crosses using the *Trp53* mutant detected a gene (*pgct1*) on Chr 13 but not on Chr 19 (Muller et al. 2000). These results suggest that *Ter* and *Trp53* act through different genetic partners to promote bilateral tumorigenesis.

TGCTs in mice and humans. Depending on the age of tumor onset, there are three forms of testicular cancer: infantile testicular cancer, which occurs in fetus and infants; adult testicular cancer, which occurs in the second to fourth decades of life, when other types of cancer are rare; and spermatocytic cancer, which occurs in older men (Looijenga and Oosterhuis 1999). There has been a long-standing controversy about whether mice with TGCTs are valid models of human testicular cancer. The greatest similarities involve infantile testicular cancer. They both arise from PGCs during fetal development. Histologically they are both non-seminomatous, displaying embryonal and extra-embryonal differentiation patterns. The karyotype of tumor cells appears to be normal in both species (Stevens 1967a; Looijenga and Oosterhuis 1999), although tumors in the mouse were studied many years ago before the advent of modern cytological methods.

For the major form of testicular cancer, adult testicular cancer, mouse models may not be valid. Adult TGCTs are believed to arise from carcinoma in situ (CIS). Tumors are found in the second to fourth decades of life. They are composed of both non-seminomas and seminomas, which retain the morphology of spermatogonial germ cells. Consistent chromosomal abnormalities such as iso-12p are often found in these tumors (Murty and Chaganti 1998). It appears that somatic events are required for CIS to become invasive. Mouse TGCTs do not show these various traits. Clearly, the pathogenesis of these kinds of adult TGCTs in humans is different from that of mouse TGCTs. Tumor cells differentiate very early in mice, long before sexual maturation, perhaps explaining why

mouse TGCTs do not contain seminomas. However, it is not clear when and how CIS cells arise. CIS cells may be similar to undifferentiated EC cells found in fetal gonads. Both CIS and EC cells are pluripotent stem cells and arise early in development, suggesting that the formation of CIS and EC cells may be under similar genetic control. Mouse TGCT models may, therefore, be suitable for dissecting the genetic predisposition to some, but perhaps not all aspects of adult forms of testicular cancer.

Genetic predisposition has long been suspected to play a role in the susceptibility to testicular cancer. TGCTs occur predominantly in Caucasian populations with an incidence ratio for Caucasian-to-African American populations of about 5 to 1 (Bosl and Motzer 1997). Studies of familial testicular cancer cases indicate an 8- to 10-fold increased risk among brothers and a 4-fold increased risk in father-to-son transmission (Forman et al. 1992; Heimdal et al. 1997). It is estimated that genetic predisposition contributes to about a third of testicular cancer cases. However, because of the rarity of families with testicular cancer, it has been difficult to identify the critical genetic regions responsible for TGCT predisposition. Recently the first strong evidence for a TGCT susceptibility gene was found on Xq27 (Rapley et al. 2000). This study is based on 99 families with two or more cases of TGCTs. When all the cases were pooled, a relatively low heterogeneity lod score (HLOD) of 1.91 was obtained. But when data were analyzed from families with at least one bilateral case, a significant HLOD of 4.7 was obtained. Similar results were found in mice where regions specific for bilateral but not unilateral tumors were found (Collin et al. 1996; Matin et al. 1999; Muller et al. 2000). Several autosomal regions have been identified with relatively weak evidence, such as 3q, 18q, and loci on Chrs 4, 5, 11, and 22 in humans (Bishop et al. 1998) and on Chr 7 in mice (Collins et al. 1996).

Urogenital, testicular, and spermatogenic anomalies. Unlike most other types of cancer, testicular germ cell tumors arise during fetal development. TGCTs are congenital anomalies, as well as spontaneous tumors. Several other testicular abnormalities are associated with TGCT cases. *Ter* and *Sl* homozygous mice are PGC deficient, and adult mice are sterile (Silvers 1979; Noguchi and Noguchi 1985). 129.MOLF–Chr19 males show various spermatogenic abnormalities ranging from degenerate testes to testes with multinucleated giant cells, abnormal meioses, and germ cell deficiency (Matin et al. 1999). In humans, susceptibility to undescended testes is also linked to the Xq27 region (Rapley et al. 2000). These observations suggest that genetic pathways controlling TGCT predisposition may also play important roles in other aspects of gonadal development. Mouse TGCT models provide important experimental systems to study the etiology and pathogenesis of these developmental anomalies.

Perspectives. Mouse models as experimental systems have made many contributions to disease gene discovery and gene function studies. In the case of testicular cancer, studies on mouse TGCTs pioneered by Stevens established an important experimental system. These models provide a unique opportunity to dissect the genetic predisposition to TGCTs, as well as to study mammalian germline development. Progress in QTL analysis as well as the use of single-gene mutations such as *Ter* and the *Trp53* knockout mouse in sensitized crosses has begun to reveal the chromosomal location of TGCT susceptibility genes (Matin et al. 1999; Muller et al. 2000). Establishing the molecular identity of *Ter*, *Tgct1*, *pgct1* and the TGCT gene near *Sl* will provide important clues about the genes and pathways that modulate TGCT susceptibility. Genetic interactions between different mutants can be used to evaluate how different genes and pathways work together to cause tumor formation. The 129.MOLF–Chr19 strain revealed a novel and powerful way to dissect the genetic control of polygenic traits and can be used for many developmental, molecular, and genetic experiments. For example, a sensitized genetic screen based on the 129.MOLF–Chr19 strain is being used to identify new ENU-induced mutants with increased or decreased TGCT susceptibility (L. Jiang and J.H. Nadeau, unpublished). These forward-genetic approaches will reveal new components of genetic pathways and establish the hierarchy of interactions, as has been demonstrated in *C. elegans*, *Drosophila*, *Arabidopsis*, and zebrafish. With the sequence of the human genome soon to be completed, candidate genes in the Xq27 region will be identified and validated in familial and sporadic cases. It will be extremely interesting to determine whether the same gene affects TGCTs in mice. An important caveat is that, although the same genes may not be involved in modulating TGCT susceptibility in humans and mice, it is likely that the same developmental pathways are involved in both species, as is the case with colon cancer (Beazer-Barclay et al. 1996; Baron and Sandler 2000). We expect that mouse TGCT models will, therefore, continue to provide insights into the genetic and biological control of TGCTs susceptibility and PGC development.

TGCTs raise many interesting questions, including the control of cell cycle in PGC, as well as the regulation of pluripotency and differentiation of germ cells. Although many factors have been identified that control PGC survival, proliferation, and migration, little is known about how mitotic G1 arrest of PGCs is controlled in males. We suspect that failure of mitotic G1 arrest leads to TGCTs. 129/Sv mice provide a model for studying the control of cell cycle arrest in PGCs. These mouse models also permit studies of the transition of PGCs to EC cells; this will shed light on the regulation of developmental potency and differentiation of germ cells as well as stem cells in general.

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