

ENVIRONMENTAL CARCINOGENESIS

Environmental and Host Factors in Testicular Germ Cell Tumors

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GLOSSARY

- Cryptorchism:** Undescended testis or testes
Hydrocele: A circumscribed collection of fluid in the tunica vaginalis of the testicle or along the spermatic chord
Hypospadias: A developmental anomaly in which the urethra opens on the underside of the penis or on the perineum
Orchiectomy: Excision of one or both testes
Orchiopexy: Surgical fixation in the scrotum of an undescended testis

INTRODUCTION

Recent reports on cancer incidence in the United States have been generally favorable, noting declining or plateauing trends for many cancers (1). The incidence of testicular cancer, however, has not been part of the general trend. The rate of testicular cancer has continued to increase during much of the 20th century and is particularly of concern because the disease primarily affects young men. Testicular germ cell cancer is the most common cancer among U.S. males in the age group 25-34

years (2). The American Cancer Society estimates that there will be approximately 7200 testicular tumors diagnosed in 2001, and the great majority of these will occur in young adults (3). Unlike most adult cancers, the incidence does not increase markedly with age. Testicular cancer incidence peaks at ages 25-39 years and declines thereafter. The great majority of testicular tumors (95%) that occur at the peak ages are germ cell tumors (GCTs), which can be grouped histologically into seminomas and nonseminomas.

GEOGRAPHIC PATTERNS

The highest rates of testicular cancer in the world are in seen in populations of European ancestry. The Scandinavian populations have rates 5-10 times higher than the rates in populations of African and Asian descent (4). A notable exception to this is the high rate seen among the Maori of New Zealand (5).

In addition to experiencing high absolute rates, men of European ancestry have seen the greatest increase in incidence over the last 50-70 years. For example, between 1973 and 1995, testicular cancer incidence increased in European American men by 55% in those

younger than age 65 years (6). Similar increases in incidence among men of European heritage were seen in Ontario (7), Norway (8), Denmark, Sweden, the former East Germany, Poland (9), and Australia (10). Northern European rates began to increase among men born after 1920 (9). Data from the Connecticut Tumor Registry mirror this observation among European American men, whose rates began to increase by the mid-1950s (11). Recent reports suggest that the rates may have begun to stabilize in the United States, although there is no evidence of decline (12). In contrast, rates among African American men are one-fifth as high and have remained stable over the same interval (11). In several studies, the increase in rate has been found to be more consistent with a birth-cohort effect than with a calendar time effect (9,13). An anomaly to this finding is that the cohort of men born in Denmark, Norway, and Sweden during the years surrounding World War II (1939–1945) did not experience an increase in risk (9). In contrast, the cohort of men born in Poland, the former East Germany, and Finland (countries that, arguably, were even more affected by the war) saw a continued increase in risk during the war years (13). The 20th century's striking increase in incidence that has not equally affected all ethnic groups suggests that there has either been an ethnic-specific change in a risk factor or there has been a global change in a risk factor that only affects genetically susceptible ethnic groups. At this time, however, the etiology of the tumor remains largely unknown.

CRYPTORCHISM

The only well-established risk factors for GCT are cryptorchism (i.e., undescended testis) and family history of GCT. The relative risk of testicular cancer in men with cryptorchism has been estimated to be approximately 10 (2). However, only 10% of testicular cancers develop in men with cryptorchism (14). Whether cryptorchism itself predisposes to cancer or whether the two outcomes share common risk factors is not well understood. Evidence suggesting that the two conditions may simply share a common etiology is the fact that 10–25% of men with unilateral cryptorchism develop testicular cancer in the contralateral gonad (15). In addition, both conditions have been associated with low birthweight and/or premature birth as well as with the presence of other gonadal anomalies such as hypospadias, hydroceles, and atrophic testes (2). Several reports have noted no ethnic discrepancy in the incidence of cryptorchism among newborns,

despite that fact that the rate of GCT is five times as great in European American men as in African American men (16). In addition, Kallmann syndrome, a condition of congenital hypogonadotropic hypogonadism, is characterized in males by cryptorchism, but not by testicular cancer (17). Suggesting, however, that the condition of cryptorchism is itself risk producing is the observation that orchiopexy (i.e., surgical repair of cryptorchism) prior to age 10 substantially reduces the risk of GCT (14). The effect of orchiopexy on reducing risk has also supported the theory that increased temperature in the testes may be a risk factor for testicular cancer (18).

Whatever the relationship between the two outcomes, it is clear that cryptorchism itself cannot explain the increase in GCT. Even though cryptorchism was reported to have increased between the 1950s and the 1980s, the proportion of testicular cancer patients with cryptorchism appears to have remained constant at approximately 10% of men with GCT (19).

PERINATAL RISK FACTORS

A growing body of evidence suggests that perinatal factors are related to the risk of developing GCT (20). The perinatal factors suggested to increase risk by some reports include: being born with a variety of testicular anomalies such as hypospadias, inguinal hernia, hydrocele, and testicular atrophy (14,21); prematurity (22); low birth weight (23, 24); low birth order (25,26); being a member of a small sibship (25,26); neonatal jaundice (24); being a dizygous twin (27); and having Down syndrome (28). Maternal factors include advanced maternal age (25), bleeding during pregnancy (23), extreme nausea in pregnancy (29), hormonal use during pregnancy (30), increased maternal body weight (30), and high maternal socioeconomic status (24).

LIFESTYLE FACTORS

Not all hypothesized risk factors are related to the perinatal period. Testicular trauma (31), personal history of GCT (31), infertility (14), increased adult height (21), increased socioeconomic status (32), breast cancer in the mother (33), a history of sexually transmitted diseases (31), and prior testicular biopsies (34) have all been found by some studies to increase risk. Factors that have been reported by some studies to reduce risk include later age at puberty (14,21, 31,35), reported mononucleosis

(33), adolescent acne, as well as increased body mass and baldness (29).

The relationship of physical activity to risk has been examined by several studies and has been found to be both risk reducing (14,21) and risk enhancing (36). In addition, at least one study has found no association (37). Although physical activity could reduce risk by delaying puberty (38) and mediating hormone levels (39), testicular trauma from physical activity might conceivably increase risk (36).

Occupation

Occupational associations have been suggested for GCT in some studies, although the most consistent finding has been a generalized increased risk among white-collar employees (2). Although an early review of occupational associations with GCT found increased risk among military servicemen (40), this association was not universally supported by later studies (41). Similarly, an association with farming occupations has been supported by some studies (42), but not by others (43). GCT clusters have been reported among leather workers (44), aircraft repairmen (45), podiatrists (46), and policemen holding hand-held radar guns (47). Regardless of the attributable risk of any of the studied occupational exposures, however, it is unlikely that they can explain much of the increase in risk.

Nutrition

A nutritional etiology of GCT has not been extensively examined, however, associations have been reported for diets high in fat and total calories (48), and high in milk consumption in adolescence (49). Although changing dietary patterns in the 20th century could conceivably explain a change in GCT incidence rates, the data to support such an association do not currently exist.

ETHNIC AND MIGRANT STUDIES

The striking ethnic discrepancy in risk of GCT suggests the importance of studying specific ethnicities and immigrants for clues to etiology. Unfortunately, studies of these types have been rarely conducted. Most studies of ethnic comparisons have been exclusively studies of rates. Moul et al. (50) studied the medical records of 66 African Americans with GCT. Although they reported that the tumors took a similar clinical course to tumors in European Americans, no information on risk factors

was available. One speculation concerning ethnic differences in the United States is that of Henderson et al. (51), who reported that African American mothers had higher testosterone levels in pregnancy. The authors hypothesized that higher levels of testosterone would protect the developing male fetus from the feminizing effects of maternal estrogens. Gallagher et al. (21) included 89 ethnic minorities in their case group of GCT in a Canadian case control study. The heterogeneity of the minority group, however, did not permit ethnic-specific analysis. In a study of GCT incidence in New Zealand, Wilkinson et al. (52) reported that the Maori had higher rates of GCT than men of European descent. In speculating on the discrepancy, the investigators noted that the Maori tended to have lower birth weights and higher maternal obesity rates than the non-Maori. Puberty in Maori boys, however, tended to be at a later age than in non-Maori boys. In a comparison of ethnic rates among South Africans, the investigators reported that, like the situation in the United States, South Africans of European ancestry had significantly higher rates than those South Africans of African ancestry, indicating that true ethnic differences in risk may exist. In contrast is the report of a migrant study that examined the risk of GCT among men of British ancestry who were born in India but migrated to Great Britain (53). The migrants experienced lower mortality rates of GCT than did men born in Great Britain, indicating the possible importance of early life experience. In another migrant study, GCT rates among migrants to Israel and their sons were examined (54). The researchers concluded that the marked variation in risk of migrants tended to persist into the next generation, although the high risk among migrants from Europe was somewhat reduced. As yet, there are no reported studies of risk factors among ethnic minorities.

HISTOLOGIC DIFFERENCES IN RISK FACTORS

Several studies have attempted to stratify their risk factor analysis on histology of the tumor. As noted by Moller et al., it is unlikely that huge differences in risk factors exist between seminomas and nonseminomas simply because of the similarity in incidence trends (55). In addition, mixed tumors composed of both seminoma and nonseminoma elements are not uncommon.

Nevertheless, a number of studies have found a higher risk of seminoma than nonseminoma in cryptorchid men (10,31,56,57). In contrast, other studies have found no differences in risk of either histologic type conferred by

cryptorchism (21,25,33,35,58). Swerdlow et al. (25) and Prener et al. (57) found a higher risk of seminoma in men with inguinal hernia diagnosed prior to age 15 years. Morrison (56), Haughey et al. (58), and Gallagher (21) found no difference in histologic type conferred by inguinal hernia at a young age, whereas Coupland et al. (31) found an increased risk of nonseminomas in men with inguinal hernias diagnosed after age 15 years. Coupland et al. (31) and Stone et al. (10) reported an increased risk of nonseminoma with testicular trauma, whereas Haughey et al. (58) found no difference in risk of either histological type. Coupland et al. (31) found that a history of sexually transmitted diseases was more significantly related to nonseminoma than seminoma. The three studies that examined age at puberty by histology (31,33,35) all reported a stronger protective effect of late puberty for nonseminomas.

ENVIRONMENTAL ENDOCRINE MODULATORS

Arguably the most hotly debated topic in testicular cancer at present is the effect of endocrine modulators on sperm count, sex ratio, fertility, and testicular cancer (59). Specifically, it has been theorized that certain organochlorine and other compounds (e.g., dichlorodiphenyltrichloroethane [DDT] and metabolites, polychlorinated biphenyl [PCB] congeners, aldrin, dieldrin, and endrin) act as either weak estrogens or antiandrogens by binding to the estrogen and androgen receptors, thereby increasing the risk of infertility, cryptorchism, and testicular cancer as well as breast and prostate cancers. Although the early focus of attention was on the ability of endocrine modulators to act as weak estrogens, recent reports have highlighted their capability to act as antiandrogens. As noted by Kelce et al. (60), the persistent metabolite of DDT, *p,p'*-dichlorodiphenyldichloroethylene (DDE), is a potent androgen receptor antagonist. Consistent with this finding is the observation that most of the endocrine modulator effects noted in animals have affected the males of the species rather than the females.

Although studies that directly examine the relationship between the levels of endocrine modulators in humans and the risk of GCT have not yet been reported, an ecologic study has been completed. Cocco et al. (61) reported no association between either adipose tissue levels of *p,p'*-DDE in the general population or *p,p'*-DDE levels in tree bark and testicular cancer mortality rates in the United States. Whether a correlation exists between

p,p'-DDE levels and GCT incidence, however, remains to be reported.

Assessment of the biological plausibility of a relationship between endocrine modulators and GCT may be inferred from the data on the sons of diethylstilbestrol (DES)-exposed women. It is estimated that DES is several times more potent than 17β -estradiol and hundreds to thousands of times more potent than environmental endocrine modulators. Therefore, it has been argued that it would be unlikely to see outcomes associated with the endocrine modulators that were not associated with DES (59).

In mice, in utero DES exposure results in numerous testicular effects including cryptorchism, inflammation, hyperplasia, and adenocarcinoma of the rete testis (62). In human males, several case reports have noted the occurrence of testicular cancer in sons of DES-exposed mothers (63), and a National Cancer Institute (NCI) multicenter study found a nonsignificant 2–2.5-fold risk (64). Other researchers have reported conflicting results (22,30,33) and reviews of the literature have concluded, in general, that the data are equivocal (65). In addition, the Danish experience of only low exposure to DES in the 1950s, yet greatly increasing GCT rates, suggests that DES exposure alone is unlikely to explain the increase (66).

Even though the effect of DES on testicular cancer risk in the population remains unclear, it is conceivable that other hormonal exposures may have an effect. Indirect evidence suggests that the intrauterine hormonal milieu may affect the risk of GCT. For example, excessive nausea early in pregnancy, reported to increase the risk of GCT, is believed to be due to increased estrogen levels. Similarly, the increased risk reported among firstborn children and dizygotic twins may be related to higher maternal estrogen levels in these pregnancies (67). Maternal obesity, a condition consistent with decreased sex hormone binding globulin (SHBG) levels and increased serum free estrogen levels, has also been associated with GCT risk. A complementary hypothesis has suggested that African American men may be at decreased risk of GCT because their mothers have higher testosterone levels in pregnancy than do European American mothers (51). If these exposures to endogenous hormones can influence risk, so might exposures to exogenous environmental hormones. None of the environmental endocrine modulators appear to bind to SHBG, and thus, the unbound environmental endocrine modulators may increase total estrogen activity. It can also be argued that the net combined estrogenic effects of environmental endocrine modulators may exceed those of DES. In support of an

effect independent of the estrogen and androgen receptors, it was recently reported that two organochlorine pesticides, toxaphene and chlordane, were capable of binding to the estrogen-related receptor α -1 orphan receptor and modulating aromatase activity (68). If endocrine modulator exposure is capable of producing the postulated spectrum of effects (from cryptorchism to infertility, decreased sperm count, and GCT), it seems likely that the exposure would have to be in utero, and very likely in the first few weeks of gestation (69).

ENDOGENOUS HORMONES

The suggestion that exogenous endocrine modulators may affect risk of GCT raises the question of whether endogenous hormones might also affect risk. This has been a difficult question to address because of the retrospective nature of most GCT studies. Several studies, however, compared endogenous hormone levels of men prior to orchiectomy with levels in control men (70). In general, these studies found that men with GCT have higher follicle-stimulating hormone (FSH) levels and somewhat lower testosterone levels than do control men. Studies in cryptorchid men have reported a similar hormonal milieu (71). The associations of reduced body muscle mass and reduced baldness among men with GCT have also suggested that testosterone levels in men with GCT may be in the lower end of the spectrum (29). These observations of high FSH and low testosterone have suggested that GCT arises in a state of "gonadotropin overdrive" in which the gonads have lost the ability to respond to gonadotropins (72). Arguing the importance of hypersecretion of gonadotropins in GCT is the observation that men with low levels of gonadotropins (e.g., men with hypogonadotropic hypogonadism) rarely develop GCT, despite their high rate of cryptorchism.

VIRUSES

An infectious etiology of testicular cancer was first suggested based on epidemiologic similarities with Hodgkin's disease (73). Using paralytic polio as a model, the authors hypothesized that viral infections in late childhood or adolescence might induce adverse tissue responses that would lead to these cancers in young adulthood. Further support for a viral etiology has been the observation that human immunodeficiency virus (HIV)(+) men have an increased risk of testicular cancer and that other cancers over-represented in HIV(+) individuals are linked to viral infections (e.g., Kaposi's sar-

coma and human herpes virus 8 [HHV-8], non-Hodgkin's lymphoma, and Epstein-Barr virus [EBV]).

Although a number of studies have examined viral antibody titers in GCT, few have had adequate power to test the hypotheses (74-76). Although infection with the mumps virus is known to cause orchitis in 20-30% of postpubertal males and sterility in a smaller subset, a relationship with testicular cancer has not been clearly demonstrated (75,77). The most promising candidate viruses implicated by several studies have been EBV and cytomegalovirus (CMV) (74-76). Both viruses are members of the herpes family and are known to cause p53 overexpression, a common finding in GCT (78). In addition, both viruses have been demonstrated to have oncogenic potential (79), and CMV infection during pregnancy has been associated in case reports with cryptorchism in the newborn.

Most recently, the DNA of parvovirus B19, a member of the parvoviridae family, has been found in testicular GCTs (80). In a small study of 39 cases and 12 controls, the investigators demonstrated that the tumors of 33 of the 39 cases were positive for parvovirus B19 sequences. Seventy percent of the cases were also seropositive for parvovirus B19, whereas none of the controls were positive in tissue or serum. A prospective evaluation of parvovirus B19 infection and GCT has not been reported.

ENDOGENOUS RETROVIRUSES

One of the most intriguing recent findings in GCT has been the observation that many GCTs express endogenous retroviruses. Human endogenous retroviruses (HERVs), with similarities to exogenous retroviruses known to cause disease in animals, constitute approximately 0.1-0.6% of the human genome (81). Although most HERVs are defective due to multiple stop codons, recent research indicates that some members of the class II HERV family retain some of their original retroviral functions. For example, HERV sequences are expressed in several tissues and cell lines (82) and some encode particles released from teratocarcinoma cell lines (83). Recently, antibodies specific for the HERV-K10 *gag* and *env* proteins were identified in patients with seminomas and other GCTs (84). HERV-K transcripts have now been detected in most types of testicular GCTs, as well as in carcinoma in situ (CIS) and in the gonocytes of dysgenetic gonads (85). The reason that HERV-K is turned on and the significance of HERV-K expression in GCT are not understood. It may simply be an epiphenomenon, as suggested by the stimulation of HERV expres-

sion by female steroid hormones in a breast cancer cell line (86). Immunodeficiency of the host does not appear to be adequate to induce HERV-K10 expression, however, because HIV(+) men are no more likely to have HERV-K antibodies than are HIV(-) men (87). As has been demonstrated with other GCT tumor markers (i.e., human chorionic gonadotropin [HCG] and alpha-fetoprotein [AFP]), HERV-K expression resolves on removal of the tumor. Whether the 60% of GCT patients who have antibodies to HERV-K10 (87) have different risk factors than the GCT patients who do not have antibodies has not been previously examined.

FAMILIAL AND HEREDITARY FACTORS

Genetic susceptibility to testicular cancer has not been widely investigated to date. An early association with human leukocyte antigen (HLA) loci was reported (88), however, subsequent studies have reached conflicting conclusions. Dieckmann et al. (89) found evidence of an association between HLA Bw41 and seminoma in a German population. Studies in Austrian (90) and Japanese (91) populations also reported associations, but not with the same loci. Conversely, no HLA associations were found in either an Israeli (92) or a British (93) population.

Researchers from the Norwegian Radium Hospital have examined several genetic susceptibility loci in a Norwegian case control population (94-96). Although the investigators reported the association of rare *Ha-ras1* alleles with bilateral testicular cancer (94) and the association of a polymorphism in the Wilms' tumor 1 locus with both bilateral disease and metastatic disease (95), they found no significant differences in three polymorphisms in the 5' end of the estrogen receptor 1 gene (96).

In a report from Denmark, no association was found between *GSTM1* genotype and testicular cancer (97). Studying another member of the glutathione-*s*-transferase family, Harries et al. found that both seminoma and nonseminoma were associated with a polymorphism in the locus encoding glutathione-*s*-transferase π (*GSTP1*) in a Scottish population (98). The allele associated with lower enzyme activity was significantly over-represented in the testicular cancer cases versus controls. Because *GST* π is a major enzyme involved in the inactivation of cigarette smoke carcinogens, such as benzo[*a*]pyrene and acrolein (99), its association with testicular cancer has yet to be explained. Overexpression of *GST* π , however, is common in many tumor types, including tumors of the testis (100).

Study of the familiarity of testicular cancer has been limited by the relative infrequency of the disease. Nevertheless, it has been reported that the relative risk of developing testicular cancer in first-degree male relatives of testicular cancer patients is between 6 and 10 (93,101). Although most researchers have found that the risk is greater if the first-degree relative is a brother (relative risk [RR] = 8-10) rather than a father (RR = 4) (102), it is not clear that the difference in risk is real. Estimates of risk conferred by an affected father are complicated by reduced fertility associated both with the cancer itself and with its treatment in prior generations (14). In addition, prior to the use of cisplatin as a chemotherapeutic agent in the 1970s, the poor prognosis for metastatic disease made it likely that affected individuals would not live long enough to reproduce. Regardless of whether a risk difference exists between affected brothers and fathers, a relative risk of 6-10 is consistent with the involvement of predisposing genes (103). As with other familial cancer syndromes, there is evidence that both the age of onset and the laterality of the tumor differ in familial vs. sporadic cases. Forman et al. (93) and Heimdal et al. (101) found that testicular cancer cases with a family history had a significantly earlier age of onset (29 years) when compared with cases that reported no family history (32.5 years). The same researchers also found that the incidence of bilateral disease was 7.3% in the familial cases versus 2.6% in the nonfamilial cases.

Linkage studies of GCT have been particularly hampered by the lack of GCT families. To increase the ability to localize a GCT gene, the International Testicular Cancer Linkage Consortium was formed in 1994 by investigators in the United Kingdom, Australia, Norway, and Canada. The group has reported evidence for linkage on chromosomes 3, 5, 12, and 18 (104). Most recently, the Consortium reported localization of a GCT susceptibility gene to Xq27 among families that did not demonstrate a male-to-male transmission pattern (105). The data also suggested that the locus conferred an increased risk of bilateral GCT and undescended testis.

Like the family studies, twin studies of GCT have been somewhat limited by numbers. Braun et al. (27) reported that, in contrast with the general Swedish population, dizygotic twins in the Swedish Twin Registry had a significantly higher risk of testicular cancer. In contrast, the risk in monozygous twins was not increased. The authors noted that their results were consistent with the hypothesis that elevated levels of maternal hormones might be related to GCT risk in that higher levels of FSH, luteinizing hormone (LH), and estradiol had all been reported in mothers of dizygous twins. In a study that only

included twins, Swerdlow et al. (106) also found that dizygous twins were at increased risk when compared with monozygous twins. In commenting on these studies, Lambalk and Boomsma (107) suggested that the findings of increased testicular cancer risk in dizygous twins were most consistent with a genetic tendency in mothers toward hypersecretion of FSH. Their sons would then inherit this tendency. In support of this postulate, it has been demonstrated that men undergoing surgery for testicular cancer have higher FSH levels than normal (28). In addition, men with Down syndrome, a condition with an increased risk of GCT, have higher than normal FSH levels (28). Recently, it was reported (108) that mothers of Down syndrome children also have higher FSH levels than mothers of non-Down syndrome children. The fact that many testicular tumors overexpress cyclin D2 is also consistent with this line of reasoning in that cyclin D2 has been demonstrated to be a FSH-responsive gene (109).

A segregation analysis of GCT in Norwegian and Swedish families favored a recessive mode of inheritance, although a dominant mode could not be conclusively ruled out (110). An analysis of bilateral disease (111) also favored a recessive mode and, like the Scandinavian study, estimated the risk of disease among homozygotes to be approximately 45%. These findings in favor of a recessive mode of inheritance may simply reflect that vertical transmission was hampered in prior generations. Search for the location of the predisposing gene has, most recently, reported evidence for regions on chromosomes 3, 5, 12, and 18 (104).

TUMOR GENETICS

Both seminomas and nonseminomas are suggested to arise from a CIS lesion. In addition, both seminomas and nonseminomas are nearly always hyperdiploid. The seminomas exhibit chromosome numbers in triploid to tetraploid range and the nonseminomas in diploid to hypertriploid range. With respect to their level of ploidy, most cytogenetic studies have found that certain chromosomes tend to be either over-represented (e.g., chromosomes 8, 21, and X) or underrepresented (e.g., chromosomes 18, 13, 11, 5, 4, and 9). On the basis of this hyperdiploidy, it has been suggested that polyploidization occurs as an initial cytogenetic change resulting in CIS. Further evolution then leads to seminomas, and yet further nondisjunctive loss of chromosomes leads to the development of nonseminomas (112). Eighty percent of all GCTs and CIS, regardless of histology, have an isochromosome of the short arm of chromosome 12 [i(12p)] (113). Nearly

all tumors that lack i(12p) have tandem duplication of 12p, thus the vast majority of GCTs have increased copies of 12p (114). Attempts to identify which genes on 12p are highly overexpressed in GCT have pointed toward the CCND2 cell cycle regulation locus, which codes for cyclin D2 (115). The investigators reported a marked increase in mRNA levels of CCND, in contrast to lower levels of mRNA of other candidate genes. The germ cells of normal testes had no detectable cyclin D2, whereas both CIS and seminomas had high levels of expression. On the basis of these data, it has been suggested that deregulated cyclin D2 plays an initial role in transformation of germ cells. Supporting this hypothesis is the observation that cyclin D2-deficient mice have hypoplastic testes (109). The same investigators also reported that cyclin D2 was an FSH-responsive gene, which may further explain its high level of expression, because men with GCT have been demonstrated to have high FSH levels at diagnosis (70).

In addition to the i(12p), 20% of GCTs have consistent deletion or rearrangement breakpoints at 12q12 to q24 (116). Other regions commonly found to be deleted or rearranged include 6q13-25, 1p31-36, and 7q (112). Several loss-of-heterozygosity (LOH) studies of GCTs have been conducted, but usually with few polymorphic markers and relatively few tumors. These studies have suggested a high frequency of loss at 3p, 11p, 12q, 1p, 5p, and 5q (114,116). In addition, frequent LOH has been observed in several known tumor suppressor genes, i.e., retinoblastoma 1 (13q14), deleted in colon cancer (18q21), Wilms' tumor 1 (11p13), and nonmetastatic protein 23 (17q21-25) (117,118). In contrast to these findings, the tumor suppressor gene *p53*, which is mutated in about 50% of solid tumors, is very rarely mutated in GCT (119). In fact, most GCTs express high-levels of wild-type *p53* protein, a factor that is thought to explain the exquisite sensitivity of the tumor to chemotherapy (120). Studies of oncogenes, to date, have found that most known oncogenes are not amplified in GCT (112).

CONCLUSIONS

Despite the increasing rates of testicular GCTs seen during much of the 20th century, GCT etiology remains poorly understood. Large geographic and ethnic discrepancies in rates suggest that both environmental and genetic factors may contribute to causing testicular GCT. The association with perinatal risk factors such as maternal nausea, and congenital anomalies such as cryptorchidism, as well as young age of onset, all suggest that the

tumor may be in utero in origin. The challenge in testicular cancer epidemiology will be to obtain accurate information about events surrounding the perinatal period of adults. A second challenge will be determining, if the tumor is initiated in utero, whether lifestyle choices, such as diet and physical activity, can decrease the risk of developing the tumor.

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