Genetics and Biology of Adult Human Male Germ Cell Tumors

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Introduction

Adult male germ cell tumors (GCTs) provide a unique opportunity to study the generation of a transformed pluripotent cell from a totipotential GC in lineage differentiation and on the path to gametogenesis. The pluripotentiality of the tumor cells manifests as histological differentiation into GC-like undifferentiated (SE), primitive zygotic (EC), embryonal-like somatically differentiated (TE), and extra-embryonally differentiated (CC, YST) phenotypes. The tumors and cell lines derived from them comprise exceptional model systems for the molecular analysis of human embryonal cell fate and lineage differentiation. The majority of GCTs show exquisite sensitivity to cisplatin-based treatment and have served as models for the development of chemotherapy for solid tumors. Until recently, the molecular mechanisms of GC transformation, GCT differentiation, or GCT chemotherapy sensitivity and resistance were understood poorly. Very recent studies of GCTs have suggested that: (a) overexpression of cyclin D2 is a very early, possibly the oncogenic, event in GC tumorigenesis; (b) differentiation in GCTs may be governed by several possibly interacting pathways, such as loss of regulators of GC totipotentiality and of embryonic development, and genomic imprinting; and (c) chemotherapy sensitivity and resistance may be rooted in part in a p53-dependent apoptotic pathway. In this review, these new data are discussed in the context of GC and GCT biology, and several novel testable genetic models are proposed.

Predisposing Factors and Pathobiology of GCTs

Although male GCTs comprise only ~2% of all human malignancies, they are the most common cancers in young males (ages 20–40 years; Ref. 1). Over the past several decades, the incidence of GCTs has been steadily increasing in the Western world (2, 3). Several risk factors for GCT development have been identified, which include cryptorchidism, spermatogenic or testicular dysgenesis, Klinefelter’s syndrome, prior history of a GCT, and a positive family history (4). Positive family history indicates the involvement of inherited predisposing factors and hence is of importance in identifying novel genes that may play a role in GCT development. Overall, a 6–10-fold increase in risk for development of a GCT has been estimated for the first-degree male relative of an affected individual (5, 6). Approximately 2% of GCT patients have another affected family member. Most of such reported families in the literature have two affected members, although rare families with three or more affected members have been identified (7). This pattern of affection suggests either a low penetrant dominant or a recessive mode of inheritance (8). To identify the candidate predisposing gene(s) by linkage analysis, an International Testicular Cancer Linkage Consortium was formed in 1994, and its efforts by screening polymorphic DNA markers in families thus far revealed only suggestive linkage with several chromosomal regions (7).

GCTs are divided into two broad groups, SE and NSE. SE-GCTs retain the morphology of spermatogonial GCs and are exquisitely sensitive to treatment by radiation as well as chemotherapy (Fig. 1A; Refs. 9 and 10). NSE-GCTs display embryonal and extra-embryonal differentiation patterns which include primitive zygotic (EC), embryonal-like somatically differentiated (TE), and extra-embryonally differentiated (CC, YST) phenotypes (Fig. 1, B–H; Ref. 9). They are, as a group, sensitive to chemotherapy, although they are less sensitive to radiation treatment than are SE-GCTs (10). NSE-GCTs usually occur as mixed tumors, with both differentiated and undifferentiated elements (9). Among tumors with differentiated elements, mature TE elements exhibit the most complete differentiation, often presenting such cell types as cartilage, neural tissue, and mucinous and nonmucinous glands (Fig. 1, E–H; Ref. 9). These tissue elements within a TE, however, develop in an unorganized fashion. On occasion, mature cell types in TE lesions undergo malignant transformation into neoplastic elements that show histological features characteristic of de novo tumors affecting multiple cell lineages (11). GCTs of all types are frequently associated with CIS (9). In nearly all cases, CIS lesions progress to invasive lesions. Both SE- and NSE-GCTs are suggested to arise from cytologically identical CIS lesions, indicating a common cell of origin of all GCTs.

A possible murine model for human GCTs has been identified by Stevens and colleagues (12, 13), who discovered that spontaneous testicular teratomas develop in the inbred strain 129/Sv. In addition, regulation of sensitivity and resistance of GCTs to DNA-damaging agents. In addition, a number of genetic models will be presented that may serve as starting points for experimental approaches to the unraveling of the biological paradox that these tumors represent.
Testicular teratomas developed when genital ridges from day 12–13 embryos were transplanted into adult testes (14). These tumors have been studied extensively over the past four decades. They have been shown to be derived from PGCs, comprised of apparently normal and unmutated genomes, transplantable, and able to participate in normal murine development (15). Recent studies have shown that mutations at other loci, such as Ter and Trp53, significantly enhance the susceptibility of the 129/Sv strain to develop testicular teratomas (16, 17). In contrast, adult human male GCTs arise from preexisting CIS, their genomes are heteroploid and aberrant, and as discussed above, no predisposing genetic factors are confirmed as yet. Therefore, murine teratomas appear not to be good models for adult male GCTs in terms of their genesis. However, the human and murine tumors share certain features reflecting their GC origin, i.e., pluripotentiality, expression of wild-type p53, and sensitivity to DNA-damaging agents (15, 18). These features have made them, along with human GCTs, an attractive model system for the analysis of these phenomena.

**Mechanism of GC Transformation**

The fate and development of PGCs in the mammalian embryo have been well described (19–21). They are first recognized in the epiblast of the gastrulating embryo. They migrate to the primitive streak mesoderm, move on to the endoderm via the allantois, and passing through the hindgut, reach the genital ridges. In the human, they are incorporated in the developing gonad by the seventh or eighth week of fetal life, when they are sometimes termed the gonocytes, which differentiate into spermatogonia during the second and third trimesters of pregnancy. In the postnatal testis, the spermatogonial cells in the seminiferous tubules undergo a series of mitotic divisions leading to the development, successively of type A, intermediate, and type B spermatogonia. The type B spermatogonium undergoes premeiotic replication and enters meiosis as the primary spermatocyte. A protracted prophase comprising the leptotene, zygotene, pachytene, diplotene, and diakinesis stages is followed by mitoses I and II, culminating in four haploid cells that develop into spermatids and spermatozoa. Extensive cell death is a striking feature of spermato genesis (22). Apoptotic cell death plays an important role during development by regulating the size of a lineage in relation to its local environment, survival itself being dependent upon availability of growth factors and their regulatory stimuli (23). In the postnatal murine testis, apoptosis is detected in type A spermatogonia through to meiotic spermatocytes (24). The spermatogenous cells in the adult human testis similarly undergo apoptosis (Fig. 2). For a GCT to develop, transformation has to occur in a GC at some point during these highly complex proliferation and differentiation programs regulated by apoptosis.

Cytogenetic and molecular genetic analysis of male GCTs has yielded important data relevant to the understanding of the mechanism of GC transformation (26). Virtually 100% of tumors show increased copy number of 12p, as one or more copies of i(12p) or as tandem duplications of 12p, in situ or transposed elsewhere in the genome (Ref. 27; Fig. 3). Notably, this chromosomal marker has been observed as early as CIS, suggesting that this abnormality is among the
earliest, if not the earliest, genetic change associated with the genesis of these tumors (26, 28). Recent CGH analysis of GCTs confirmed universal overrepresentation of 12p DNA. It also identified, in a small proportion of tumors with 12p overrepresentation, mostly SEs, a further amplification of the proximal 12p11.2–12 region (Refs. 29–31; Fig. 3). Although the \textit{RASK2}, \textit{SOX5}, and \textit{JAW1} genes have been shown to be contained within this region, their functional roles in GCT development remain to be determined (30). It is conceivable that other, as yet unidentified, genes mapped to this region may be involved in GCT development.

To identify the possible driver gene on this chromosomal arm whose deregulated expression may lead to GCT development, a candidate gene approach was undertaken using GCT-derived cell lines and tumor biopsies. These studies indicated that the \textit{CCND2} gene, mapped at 12p13, may be the best candidate for the 12p driver gene (32). Cyclin D2 is one of the D-type cyclins which, along with the cdks cdk4 and/or cdk6, regulate the phosphorylation of pRB and control the G1-S cell cycle checkpoint (33). Disruption of this checkpoint through amplification/overexpression of D-type cyclins is known to be one of the important pathways in human tumor development (33). Cyclin D2 is expressed by murine day 7–day 13 (postnatal) testicular spermatogonia, suggesting that this cyclin may normally regulate spermatogonial mitotic G1-S transition in the postnatal testis (Ref. 34; Fig. 4).

In an analysis of established GCT cell lines, the \textit{CCND2} mRNA and steady-state levels of cyclin D2 showed comparable abundance, whereas immunoprecipitation/immunoblotting analyses showed that in all cyclin D2-expressing cell lines, cdk4 and/or cdk6, were complexed with it and that the complexes were active by exhibiting Rb kinase activity (32). In an immunohistochemical analysis of paraffin-embedded tissue sections, GCs in the normal testis showed occasional cyclin D2-positive spermatogonia, whereas the majority of aberrant GCs in the CIS lesion expressed cyclin D2 (Fig. 5, A and B). In tumor specimens, SEs expressed cyclin D2, whereas ECs exhibited more focal, predominantly nuclear expression of cyclin D2 (Ref. 32; Fig. 5, C and D). Among TEs, cyclin D2 expression appeared to correlate with a particular pathway of somatic differentiation (Ref. 32; Fig. 5E). These data suggested that cyclin D2 expression may be an early event in GCT development with a subsequent down-regulation in certain lineages of differentiation (32).

Although the CIS cell is generally regarded as the precursor of all adult male GCTs, the target stage of GC development at which transformation occurs is not known. Two models of origin of CIS cells have been put forward. One was proposed by Skakkebaek et al. (21, 35). This model suggested that fetal gonocytes, which have escaped normal development into spermatogonia, may undergo abnormal cell division mediated by a kit receptor/SCF paracrine loop, leading to the origin of CIS cells. The kit receptor is normally expressed by GCs during the first trimester and postnatally during meiosis, whereas SCF is expressed by the Sertoli cells (36). Gonocytes derailed in their normal development have been postulated to be susceptible to subsequent invasive growth through the mediation of postnatal and pubertal gonadotrophin stimulation. This hypothesis is based mainly on a consideration of immunophenotypic markers expressed by gonocytes and CIS cells, types of abnormal germ cells seen in developmental disorders that predispose to germ cell tumors, and epidemiology of...
GCT incidence (21). A second model proposed by us took into account four established genetic properties of GCTs, i.e., increased 12p copy number, expression of cyclin D2 in CIS, consistent near triploid-tetraploid chromosome numbers, and abundant expression of wild-type p53 (37). We have postulated that the most likely target cell for transformation during GC development may be one with replicated chromosomes that expresses wild-type p53, harbors DNA breaks, and may be prone to apoptosis. Such a stage is represented by the zygotene-pachytene spermatocyte, at which a “recombination checkpoint” appears to operate (38), which can provide an apoptotic trigger in the presence of unresolved DNA double-strand breaks. This stage is temporally the longest phase during spermatogenesis with the cell cycle machinery halted to permit recombinational events to complete. It also contains replicated DNA, and based on murine data, wild-type p53 protein is temporally expressed at this stage (38). According to this model, aberrant chromatid exchange events associated with crossing-over during zygotene-pachytene may lead to increased 12p copy number and overexpression of cyclin D2. Such a cell may escape recombination checkpoint-associated apoptotic response through the oncogenic effect of cyclin D2, leading to aberrant reinitiation of cell cycle and genomic instability (Fig. 6; Ref. 37). A role for cyclin D2 in the development of GCTs has also been suggested by Sicinski et al. (39) through studies of mice homozygously inactivated (mutant) for the Ccnd2 gene and expression of CCND2 mRNA in ovarian granulosa cell tumors and testicular GCT cell lines. Cyclin D2 is normally induced during ovarian granulosa cell development by FSH. Absence of cyclin D2 in mutant mice led to female sterility because of the inability of granulosa cells to proliferate normally in response to FSH (39). Although the testis weight of mutant mice was the same as that of normal mice at birth, mutant testes suffered growth retardation during sexual maturation, resulting in small testes weights and reduced sperm counts. From these observations, the authors concluded that cyclin D2, as a D-type cyclin expressed in mitotically active postnatal GCs, plays an important role in the development of FSH-responsive ovarian and testicular cells, and its subversion may play an oncogenic role (39).

In GCs that have reentered the cell cycle after cyclin D2 activation, downstream events, such as loss of tumor suppressor genes brought about by genomic instability, may lead to neoplastic progression. Extensive molecular genetic analysis has identified genomic and/or functional (expression) loss of several known tumor suppressor genes, such as RB1, DCC, and NME, and genomic loss at several previously recognized as well as novel chromosomal sites (reviewed in Ref. 26, 40). The genetic model proposed by us can be tested experimentally by producing transgenic mice overexpressing cyclin D2 in spermatocytes.

**Origin of Extragonadal GCTs**

The majority of male GCTs are gonadal, whereas a minority occur in extragonadal sites such as the anterior mediastinum (thymus), retroperitoneum, and the pineal gland (10). Retroperitoneal tumors are generally considered to be metastases of primary gonadal lesions, whereas the origin of primary mediastinal and pineal lesions has been a matter for speculation. A view that has been current for some time suggested that mediastinal and pineal GCTs are derived from local transformation of PGCs misplaced during embryogenesis (41). This view itself was derived from the early concepts that tumors, tumor-like malformations, and ectopic tissues arise from abnormal or vagrant PGCs held back in their embryonal migration (42, 43). Thus, if such PGCs were to develop into tumors, then the midline, the anterior mediastinum especially, would be the most common site of their occurrence. However, Witschi (44) studying human embryos and Chiquoine (45) studying murine embryos failed to find evidence of misplaced GCTs. More recent studies have indicated that ectopically placed PGCs detected in sections of murine embryos undergo apop-
tosis (46). Notwithstanding these negative embryological data, the concept of extragonadal primary GCTs continues to persist (47). This proposition was tested in one study by comparing the clonal cytogenetic changes in a group of primary extragonadal tumors with those in a group of primary gonadal tumors with the hypothesis that if these tumors truly represented de novo transformations of PGCs in a non-gonadal environment, then their genetic (cytogenetic) profiles may be different from those of gonadal GCTs (48). Although the two groups differed significantly in the patterns of differentiation that they exhibited, neither specific chromosome aberrations, including increased 12p copy number, nor incidence of recurring breakpoints were significantly different between the two groups (48). On the basis of these observations, an alternative suggestion was made, according to which primary mediastinal presentation of GCTs may represent some form of reverse migration of occult CIS lesions in the gonad, and hence they may also be gonadal in origin (48).

**Embryonal-like Differentiation in GCTs**

Male GCTs are true experiments of nature. They display, albeit in a spatially and temporally abnormal manner, patterns of differentiation that mimic stages normally undergone by the developing zygote (Fig. 1). By their very nature, normal GCs are truly totipotential, a faculty that is normally activated upon fertilization. Murine studies have suggested that proliferation, survival, and maintenance of mammalian GCs in a totipotential state is regulated by the local environment, through the actions of various factors such as SCF, bone morphogenetic protein 8B, and the cytokine/leukemia inhibitory factor and fibroblast growth factor families (49, reviewed in Ref. 50). Gene knock-out studies in mice have shown that a variety of receptors (e.g., kit for SCF and gp130 for leukemia inhibitory factor) and their downstream effectors (e.g., OCT4) affect GC development (36, 51, 52). Isolated human GCs also appear to require similar factors for survival and pluripotentiality (53). In the case of transformed GCs, SE cells can be viewed as mitotically dividing, transformed GCs that have retained the inhibitory mechanism for zygotic-like differentiation, a feature of GCs prior to fertilization. The in vivo expression patterns of kit and SCF in GCTs are consistent with such a view. Thus, the kit receptor, which normally is expressed by spermatogonia and primary spermatocytes (36), is expressed mainly by CIS and SEs (54). On the other hand, NSEs (EC, TE) appear to down-regulate kit and up-regulate SCF, consistent with their loss of germ cell phenotype and acquisition of somatic fates (54). The key developmental difference between SEs and NSEs thus is the loss of ability to retain GC-like totipotentiality by the latter. Such a loss could result from loss of function of receptor(s) or downstream effector(s), either by nondisjunctional loss of chromosomes or by subregional losses/mutations concurrent with tumor evolution. Few molecular genetic studies have been directed at determining the role of genes associated with loss of GC totipotentiality in GCTs.

Mammalian embryonal differentiation is perhaps the most complex biological process that has evolved. Much effort has recently been directed toward understanding the mechanistic basis of decisions that determine the nature and regulation of proliferation and differentiation signals in the developing zygote (23, 55). In this context, GCTs provide an unique opportunity to study embryonal (TE) versus extra-embryonal (YST, CC) pathways of differentiation, as well as development of somatic lineages. Analysis of genome-wide allelic loss in GCTs showed an overall higher loss in the highly differentiated TEs compared with the less differentiated ECs (56). These studies identified chromosomal sites that may harbor effector genes, such as transcription factors whose loss may prompt either induction of differentiation or lineage decision. Notable among the genes deleted in TEs were NME1 and NME2, for which there is evidence of function as transcription factors to negatively regulate differentiation (57). The levels of Nm23 proteins encoded by these genes were also found to be 4–5-fold lower in TEs compared with ECs (58). Further studies are clearly required to clarify the role of NME genes in GCT differentiation.

In the developing normal embryo, embryonic and extra-embryonic differentiation programs would require spatial and temporal coordination of cellular proliferation with cell fate/lineage decisions. The isolation and maintenance in vitro of tumor-derived EC cell lines has provided powerful cellular tools in which the coordination of such molecular events can be studied (reviewed in Ref. 59). These multipotential EC cell lines can undergo spontaneous or morphogen-induced differentiation along multiple pathways (59). Some EC cell lines display the ability to differentiate along somatic and extra-embryonic endodermal lineages, placing them as equivalents of cells derived from the inner cell mass in the developing embryo (60, 61). Others exhibit the additional ability to differentiate into trophoblastic cells, placing them at an earlier stage in embryogenesis prior to trophectodermal fate decision (62, 63). In contrast, yet other EC cell lines lack the ability to undergo differentiation (64). The genetic lesion(s) that underlie the refractiveness to differentiation exhibited by the latter cell lines are unknown; their identification is important to an understanding of the master regulators of differentiation induction in the developing zygote.

The EC cell lines represent an in vitro system wherein the functional involvement of candidate genes in human cell fate/lineage decisions can be determined. Although this potential use remains to be fully exploited, the multipotential EC cell line, NT2/D1, has recently been used to analyze the role of differing retinoid receptors in all-trans-retinoic acid-induced neuronal differentiation (65, 66). From a different perspective, the same cell line is being used as a source of differentiated neuronal cells that otherwise would be unavailable or difficult to obtain for experimental analysis of molecular mechanisms involved in disorders such as Alzheimer’s disease (67). Further characterization of cell fates induced in the multipotential EC cell lines by differing morphogens may lead to the development of resources for studies of other developmentally related disorders.

The ability of GCTs to undergo an embryonal-like developmental program without the contribution of a maternal complement has obvious implications to genomic imprinting. Parental imprints are erased in the PGCs, and new imprinting patterns are laid down during gametogenesis and again during embryogenesis (Fig. 6; Refs. 68 and 69). Therefore, the target cell for transformation proposed in our model, the meiotic spermatocyte (Fig. 6), would be imprint erased, which is consistent with recent observations of biallelic expression in GCTs of IGF2 and H19 genes, which normally show monoallelic expression in postfertilization somatic tissues (70, 71). A possible mechanism by which embryonal and extra-embryonal types of major differentiation pathways are initiated in imprint-erased transformed germ cells may be differential methylation of critical chromosomal regions.

The postulated multiple pathways and their underlying mechanisms of differentiation in GCTs discussed above clearly represent first attempts at constructing testable hypotheses. They also emphasize the value of GCTs and GCT-derived EC cell lines as powerful experimental models for the analysis of human embryonal differentiation. Expression profiles of the various GCT elements in vivo and EC cell lines induced to undergo lineage differentiation in vitro, assessed by microarray hybridization analysis, would undoubtedly lead to identification of candidate genes involved in the developmental programs elicited by these tumors. The relevance of such analyses to the understanding of embryonal cell fate regulation is obvious.
Malignant Transformation in TEs

Among all GCTs, TEs show the most vivid patterns of somatic differentiation and exhibit lineages derived from two or more germinal layers (ectoderm, mesoderm, and endoderm) with mature (adult-type) or immature (fetal-type) elements (9). On rare occasions, TEs undergo a further “malignant transformation” characterized by aggressive proliferation and histological differentiation into various non-GC malignancies such as hematopoietic (leukemia, lymphoma), mesenchymal (sarcoma, especially rhabdomyosarcoma), epithelial (carcinoma), and neurogenic (primitive neuroectodermal tumor, neuroblastoma; Ref. 11). The histological similarity of some of these transformed malignancies to their spontaneous counterparts is so complete that their GC origin can be determined only by the detection of the i(12p) marker (72, 73). On the other hand, these transformed malignancies exhibit cytogentic or genetic lesions characteristic of specific types of spontaneous tumors, e.g., +8 and del(5q) in myeloid leukemia (74), 2q37 rearrangements in rhabdomyosarcoma (74), t(11; 22)(q24;q12) translocation in primitive neuroectodermal tumor (74), and TP53 gene mutations in sarcoma (75). The patterns of malignant transformation and the genetic changes displayed by the transformed lesions underscore the similarity between the differentiation of the transformed GCs into TE lineages and normal embryonal differentiation of the same lineages. Often, the TE and the transformed lesion coexist as discrete entities in the same testis. Therefore, these transformed malignant lesions can serve as excellent models for the study of tumorigenesis in diverse cell types.

Chemotherapy Resistance of GCTs

GCTs have served as a model for the treatment of adult solid tumors since the identification, during the mid-1970s, of the curative potential of cisplatin-based chemotherapy in 70–80% of tumors with advanced disease (10). Because of the high cure rate, increased attention was paid to the morbidity and mortality of treatment. As a result, distinguishing between patients most likely to be rendered free of disease and cured (good-risk) and those least likely to be cured (poor-risk) was the subject of intensive clinical investigation in the early and mid-1980s. Clinicopathological features that have been associated with a poor outcome include mediastinal NSE presentation and hepatic, osseous, and/or brain metastases (10). In addition, patients with high serum levels of lactate dehydrogenase, α-fetoprotein, or human chorionic gonadotrophin also are considered to have a low likelihood of complete remission (10). Surgical resection of residual masses after chemotherapy has often revealed the presence of TE, consistent with the relative resistance of this histology to cisplatin-based chemotherapy (10). Malignant transformation of TE elements can result in a tumor that does not respond to cisplatin-based chemotherapy but responds to treatment appropriate for the transformed histology (11).

Recent molecular genetic studies of GCTs that are clinically resistant to cisplatin-based chemotherapy have identified a subset that harbors TP53 gene mutations (76), a molecular alteration not normally associated with GCTs (6, 77). Evaluation of the cellular response to cisplatin in one GCT-derived cell line with a TP53 gene mutation indicated a relative resistance to cisplatin, in contrast to the extreme sensitivity of another GCT-derived cell line with wild-type TP53 (76). The simplest explanation for the cisplatin resistance of this subset of GCTs is their inability to mount an apoptotic response after drug exposure because of an inactivating TP53 gene mutation. Curiously, TP53 gene mutations were detected mainly in TEs, including one that had undergone malignant transformation to sarcoma (76). Immuno-histochemical analyses of GCTs have shown that, on the whole, these tumors display higher than normal levels of wild-type p53 (78, 79), with somewhat lower levels in mature TEs (80). Thus, somatic differentiation associated with a decline in p53 levels may well comprise a cellular setting for selective pressure for TP53 gene mutation to occur.

One recent study has analyzed a cohort of cisplatin-resistant GCTs for the presence of amplified DNA sequences (31), a genetic abnormality often associated with tumor progression and resistance to therapy (81). In this study, CGH was performed on a panel of GCTs comprising 17 resistant and 17 sensitive tumors (31). High-level amplification of eight chromosomal regions (other than 12p) was detected in five resistant tumors but in none of the sensitive group (31). For four of these regions, good candidate genes have been identified (31). Thus, these data suggest that gene amplification of chromosomal regions other than 12p may be associated with resistance of GCTs to cisplatin-based chemotherapy. Once the identity and function of the amplified genes are determined, they may become relevant to the understanding of chemotherapy resistance of other tumor types.

From a biological viewpoint, male GCTs offer a unique system in which the cellular factors potending their exquisite sensitivity to chemotherapy can be studied. A few studies have described a reduced ability of GCT cell lines to repair DNA lesions induced by cisplatin (82). The mechanism of repair of DNA lesions induced by cisplatin and other similar agents is well understood (83). It has been suggested that the reduced repair capacity of GCTs results from the shielding of cisplatin-induced lesions in DNA to repair by high-mobility group domain proteins specific to GCs, such as the product of the testis-determining factor gene, SRY (84). Other recent in vitro studies have indicated that GCT cell lines have a low level of XPA activity, a protein intimately involved in DNA damage recognition and facilitation of DNA repair complex assembly (85). However, whether the low level of DNA repair in the tumor cells is inherent to GCs or represents a tumor-associated phenomenon is unknown. The precise biochemical link between the induction of physical damage in DNA and the cellular response to it also is unclear as yet. In general, after exposure to DNA-damaging agents, cells respond either by induction of a delay in the cell cycle at the G1-S phase boundary (to permit DNA repair prior to replication) or by the induction of apoptosis, both of which are thought to be mediated by p53 (86). The former response is suggested to be effected via a transactivation function of p53, whereby p21, the product of the p53-responsive gene WAF1, acts as a cell cycle inhibitor by complexing and inactivating the function of cyclin-cdk complexes required for cell cycle progression (86). The precise mechanism whereby p53 induces an apoptotic response remains unknown, although this function of p53 appears to be independent of its transactivation activity (87). A number of reports have indicated that the in vitro cellular response of GCT cell lines to direct DNA-damaging agents such as cisplatin as well as indirect DNA-damaging agents such as etoposide is apoptosis (76, 88, 89), consistent with the effectiveness of such treatment in vivo (10). In these cell lines, exposure to the respective agents resulted in an induction in both the levels and activity of p53, induction of WAF1, absence of G1-S phase delay, and a dramatic increase in the number of cells undergoing apoptotic cell death (76, 88, 89). Recent studies indicate that p53 activity can be regulated by both covalent (phosphorylation and acetylation) and noncovalent (mdm2 interaction) modifications (90). The mechanisms by which modified or unmodified p53 interacts with and regulates cell cycle components such as pRb and p19ARF and members of apoptosis pathways are being actively studied (91). The relevance of these mechanisms to the apoptotic response in GCTs needs to be examined both prior to and after exposure to chemotherapeutic agents.

It has also been suggested that the rapid apoptotic response of
GCTs upon exposure to chemotherapeutic agents may be attributable to a high ratio of the proapoptotic bax protein to the antiapoptotic bcl-2 protein. In such a cellular setting, apoptosis would be favored (88). This has been substantiated in a limited number of GCT cell lines and needs to be further investigated at the in vivo level (88). However, other in vitro studies have suggested that bcl-X<sub>L</sub>, a bcl-2-related antiapoptotic protein, may act as the regulator of DNA damage-induced cell death in GCTs, rather than bcl-2 (92). In these studies, exogenous overexpression of bcl-2 in a GCT cell line resulted in sensitization of the cell line to DNA damage-induced cell death. Associated with this sensitization was a decreased endogenous expression of bcl-X<sub>L</sub>, with little or no change in the level of bax expression. A much attenuated apoptotic response to cisplatin has been observed in somatically differentiated GCT cell lines, reflective of the relative resistance of TE elements of GCT specimens described above (89). Thus, the elucidation of the molecular mechanisms whereby the unique apoptotic response to chemotherapeutic agents is achieved in GCTs will not only contribute toward the understanding of how such a response is achieved but also may illuminate how resistance may be circumvented in GCTs and possibly other tumor systems as well.

In this review, we have attempted to summarize the current state of knowledge of genetic and molecular biological mechanisms that regulate GC and GCT transformation, GCT migration and differentiation, and GCT chemotherapy sensitivity and resistance. We have also discussed several novel hypotheses bearing on these aspects. Elucidation of mechanisms of GCT biology is important for a fuller understanding of these tumors as well as to the understanding of human embryonal cell fate determination. Such knowledge of GCTs may also be of significance to the understanding of the biological behavior and response to treatment of other tumor types because, being derived from totipotential GCs, GCTs are capable of eliciting the fates of all cell lineages, normal as well as neoplastic. They are an outstanding model system.

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Note Added in Proof

While the manuscript was in review, Rapley et al. (Nature Genetics, 24: 197–200, 2000) reported evidence for a possible GCT susceptibility locus at Xq27.

References


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