Commentary

Somatic Genetic Changes in Association with Testicular Germ Cell Tumor Prognosis

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Testicular germ cell tumor (TGCT) is the most common cancer in men aged 20–40. Approximately 8980 new cases of TGCT were diagnosed in the United States in 2003.1 TGCT is relatively rare, accounting for only 2% of male malignancies. However, the incidence of TGCT in the United States has steadily risen from 3.2 in 100,000 in 1973 to 5.7 per 100,000 in 2000 (54%).2 mainly limited to the white population. TGCT has been described as “a model for a curable neoplasm,” since most patients will be cured and become long-term survivors.

The prognostic and treatment of patients with TGCT is based upon the histology of the tumor and the stage at presentation. There are two major histologic subtypes of TGCT: seminomas and non-seminomas. For patients with metastatic disease, cisplatin-based chemotherapy is administered with curative intent. Patients with metastatic disease are classified into good-, intermediate- and poor-prognosis categories based upon a number of clinical factors including the TGCT subtype (seminoma versus non-seminoma), the site of the primary (gonadal/retroperitoneal versus mediastinal), the degree of serum tumor marker elevation, and whether visceral disease is present.4 The International Germ Cell Consensus (IGCCCG) classification of the individual patient with metastatic disease predicts prognosis and determines the choice of chemotherapy regimen and the number of chemotherapy cycles administered.

While somatic genetic changes within TGCTs have been investigated extensively (see ref. 5), few studies have been done which correlate those changes with prognosis or response to therapy. The lack of studies is in part due to the paucity of tumors that do not respond to treatment, so that accruing enough tumors to examine the question of prognosis is difficult. However, there is strong rationale and interest in understanding the genetic changes important in TGCTs and correlating these changes with clinical outcome. If there are genetic changes that differentiate which patients with metastatic disease likely will not be cured with standard cisplatin-based chemotherapy, more intensive and/or novel therapies could be targeted specifically for this poor prognosis population.

CHROMOSOMAL CHANGES IN TGCT

The most common genetic change observed in TGCT is isochromosome 12p (i(12p)). For a comprehensive review of the cytogenetics of TGCT, see reference six.6 Other chromosomal changes have been observed in TGCT, albeit at lower frequency. Increases in copy number at 7p21-pter, 7q21-q33, 8q12-23 (seminoma), 12p11-pter, 17q11-q21 (non-seminoma), 21q21-qter, 22q11-qter (seminoma) and Xq have been seen, as well as decreases at 4q21-qter, 5q14-qter, 11p11-p15, 11q14-q24, 13q14-q31 and 18q12-qter.6 i12p is a universal change in TGCTs and has not been linked to drug resistance or prognosis.7,8 In one study of 17 tumors from relapse-free patients and 17 chemotherapy-resistant tumors, using standard comparative genomic hybridization, high level amplifications were not detected (outside of 12p) in the chemosensitive tumors, but were detected in the chemotherapy-resistant tumors.9 However further studies of large chromosomal changes in relationship to prognosis and sensitivity to chemotherapeutic agents have not been done.

MICROSATELLITE INSTABILITY AND TGCT PROGNOSIS

Microsatellite instability in tumors is caused by deficiency of any of the mismatch repair (MMR) enzymes, including hMSH2, hMLH1, hMSH6, hPMS1, hPMS2, and hMSH3. Normal MMR is directed at excising nucleotides that are incorrectly paired with the nucleotide on the opposite DNA strand. Mismatched base pairing most frequently happens during the process of DNA replication. Reduction in MMR efficiency leads to genetic instability, in particular the expansion or contraction of short repetitive stretches of DNA sequence such as dinucleotide repeat sequences (microsatellites). Microsatellite instability has been observed in sporadic human tumors due to somatic loss of function of
MMR genes; tumors remain diploid but have many deleterious mutations, particularly changes in the length of repeated nucleotides. Germline mutations in the MMR genes are the cause of Hereditary Non-Polyposis Colorectal Cancer (HNPCC); TGCT is not a component tumors of HNPCC.

Limited microsatellite instability (MSI) previously has been observed in a subset of TGCTs ranging from 0–29% in several small studies of TGCTs. In these studies of TGCT that did find evidence of MSI, it was not observed as a wide spread phenomenon but rather limited to only a one or a few microsatellites. The studies all concluded that MSI does not play a significant role in TGCT pathogenesis. Several studies have reported higher rates of instability in tri- and tetranucleotide than dinucleotide repeat sequences. In the largest previous study, Mayer et al. examined MSI in 111 TGCTs, eleven of which they characterized as chemo-resistant, using eight mono- or dinucleotide markers. Similar to previous studies, in the control or chemo-sensitive TGCTs, six TGCTs had one locus of MSI. However, in the 11 chemo-resistant tumors, they found MSI in five cases, four of which had two or more loci of MSI. The authors also examined Kaplan-Meier survival curves for progression free survival and found that the chemo-resistant tumors that were MSI positive had a longer median progression free survival (p = 0.05), however the numbers are quite small. As in previous papers, the authors found consistent positive immunohistochemistry staining for the MMR proteins MLH1, MSH2 and MSH6, even in tumors that exhibited loci of MSI.

In the current study, Velasco et al. examined 118 TGCTs for MSI using ten markers, and found MSI in 30 TGCTs (25%) at three or more markers, a much higher rate of MSI than has been previously reported. The authors also report a correlation with low MSH2 or MSLH1 staining. The authors suggest that the differences in their findings from previous studies could be due to their selection of markers, distribution of tumor histology, and number of tumors. However, while they identified MSI in 19%, 10% and 21% of tumors in BAT25, BAT26 and D2S123 respectively, two previous studies also had examined these markers and found much lower levels or no MSI. In addition, Velasco et al. comment specifically that these markers are useful for detecting MSI in seminomas; however they comprise over 50% of the samples in previous studies. The remaining explanation for the difference in rates of MSI are differences in stage and rate of recurrence between the sample set, and one that would be supported by the paper accompanying this article, also by Velasco et al. The authors identify a correlation between MSI tumors and low MSH2 immunohistochemistry staining as would be expected. MSI and low MSH2 staining were associated with an increased risk of recurrence when compared to TGCTs with loss of heterozygosity only (LOH) and high MSH2 staining, independently (p = 0.017, p = 0.0003 respectively) and together (p = 0.0027). The authors do not present an analysis of the MSI and low MSH2 tumors as predictors of recurrence over the whole sample set, suggesting that they are not predictors of recurrent disease overall. Thus, the presence of MSI is unlikely to be useful in the clinical setting (based on this study) to distinguish between the TGCTs likely to recur and those that will not. However, the association of MSI and TGCT recurrence clearly deserves more study, particularly as there are some inconsistencies across studies, and may emerge as a valuable tool in the long run.

SINGLE GENE MUTATIONS IN TGCT PROGNOSIS

Multiple studies have examined whether changes in p53 are present in TGCT, either though immunohistochemistry (IHC) of protein levels or through mutation screening of tumors, as reviewed in reference 20. IHC of p53 shows a high level of wild-type protein in TGCTs, however many studies report less than 10% of nuclei within the TGCTs as staining positive. As recorded in the IARC p53 database, TP53 mutations are infrequent as compared to other tumor types, only identified in 11% of TGCTs overall. Houldsworth et al. suggested that TP53 mutations may correlate with resistance to chemotherapy, based on their identification of four TP53 mutations (17%) in 23 tumors associated with chemo-resistance and a decreased apoptotic response to cisplatin in a TGCT cell line with a p53 mutation. However, Kersemakers et al. examined 18 tumors associated with chemo-resistance, and did not identify any decrement in p53 staining by IHC; none of the tumor had mutations in TP53. The same group examined a large group of proteins, including p53, using IHC in unselected patients, metastatic patients who had achieved remission and chemoresistant TGCTs for correlation with disease outcome, and did not find any differences among the tumors. Using IHC of p53 and Ki67, apoptosis (assayed by TUNEL), HCG and AFP, Mazumdar et al. used cluster analysis to differentiate prognostic subgroups within non-seminomas. They identified a cluster that was associated with better prognosis at five years: 94% (95% CI 86%–100%) that included good, intermediate and poor-risk patients. Cluster affiliation was an independent predictor of outcome (p = 0.04), but not as powerful as IGCCCG risk status (p = 0.005). In general, the clustered TGCTs had lower p53 expression, apoptotic indexes, HCG levels and higher Ki67 expression and AFP levels. While this study did not examine p53 mutation status as a marker of prognosis, the necessity of including multiple markers in the cluster analysis to predict outcome suggest that even if they play a role, it is not a definitive one.

OTHER BIOMARKERS STUDIED IN ASSOCIATION WITH PROGNOSIS

Several other cancer biomarkers recently have been studied in conjunction with tumor stage and prognosis. Using IHC, Kollmannsberger et al. stained for the presence of c-KIT, the EGFR family and HER-2/neu amplification in 22 patients with chemoresistant TGCTs and compared them to 12 chemo-sensitive tumors, with the goal of determining whether patients chemo-resistant TGCTs might be candidates for currently available targeted therapies. In addition, c-KIT regulates primordial germ cell migration, proliferation and apoptosis. c-KIT mutations have been observed in TGCTs, principally seminomas, and mediastinal germ cell tumors. Kollmannsberger and colleagues did not observe any difference in IHC staining patterns between the chemo-resistant and sensitive tumors, and positive staining was limited in both cases. Madani and colleagues examined c-KIT expression in 23 chemotherapeutic-refractory non-seminomas (15 late relapse and eight transformed non-seminomas). In contrast to the findings by Kollmannsberger, they found c-KIT and EGFR expression in 48% and 65% of patients respectively. Madani observed c-KIT expression outside the non-seminomatous elements of the tumor, which may imply that targeted therapy would be useful for transformed TGCTs based on the cellular origin of the transformed elements. They did not observe any c-KIT mutations, consistent with earlier observations of mutations in...
hCG may act to increase apoptosis are elucidated in TGCTs.

TGCT stage and prognosis, there is considerable interest in defining studies examining the genetic basis of prognosis in TGCTs. Mandokoly et al. also examined LRP expression in 70 TGCTs using IHC, and found positive staining in 29 (41%) tumors. LRP is a major vault protein that may mediate multidrug-resistance by the compartmentalization of drugs away from the intracellular drug targets.4,5 The authors substantiated the IHC findings using RT-PCR and Western blotting to examine mRNA and protein levels, respectively. The authors did not identify any relationship between LRP positivity and stage. However, more patients with LRP negative tumors achieved complete response (p = 0.015) and those with expression of LRP had a shorter overall survival (p = 0.043). A recent study by Hatekeyama et al. identified trophinin expression by IHC in 158 TGCTs.36 Trophinin is a membrane protein that facilitates the invasion of the endometrium by trophoblasts during implantation. The frequency of trophinin staining increased with increasing stage (p<0.001) and was positive in all patients with lung metastasis, across tumor types and stages. Trophinin staining correlated with β-hCG levels (p = 0.004), suggesting that β-hCG may act to increase trophinin levels. While somatic mutations were not directly assessed in findings from the previous study, including examination of the primary vs metastatic tissue, use of different anti-EGFR antibodies and threshold for considering a sample positive. Mandoky and colleagues recently have examined HER-2/neu and lung resistance-related protein (LRP) expression in TGCTs.31-33 They assessed the genetic changes that lead to sensitivity or conversely resistance to the invasion of the endometrium by trophoblasts during implantation. The frequency of trophinin staining increased with increasing stage (p<0.001) and was positive in all patients with lung metastasis, across tumor types and stages. Trophinin staining correlated with β-hCG levels (p = 0.004), suggesting that β-hCG may act to increase trophinin levels. While somatic mutations were not directly assessed in these studies, they may provide guidance about a further level of studies examining the genetic basis of prognosis in TGCTs.

Beyond understanding what genetic changes may be linked to TGCT stage and prognosis, there is considerable interest in defining the genetic changes that lead to sensitivity or conversely resistance to cisplatin based therapy.34 Understanding what makes TGCTs sensitive to cisplatin may allow us to sensitize other tumors types to cisplatin and improve their cure rate. Thus, it is very important that further genetic changes predictive of prognosis and response to chemotherapy are elucidated in TGCTs.

References