Expression of hMLH1 and hMSH2 and assessment of microsatellite instability in testicular and mediastinal germ cell tumours

Mojgan Devouassoux-Shisheboran1,2, Claire Mauduit1, Raymonde Bouvier3, Françoise Berger4, Mourad Bouras1, Jean Pierre Droz5 and Mohamed Benahmed1,3

1Institut National de la Santé et de la recherche Médicale, INSERM U-407, Communication en Biologie de la Reproduction, Faculté de médecine Lyon-Sud, B.P. 12, F-69921 Oullins Cedex, 2Service d’Anatomie et de Cytologie Pathologiques, Hôpital de la Croix Rousse (Université Claude Bernard, Lyon 1), 103 Grande rue de la Croix Rousse, 69317 Lyon, Cedex 04, 3Service d’Anatomie et de Cytologie Pathologiques, Hôpital Edouard Herriot, 3, Place d’Arsonval, 69437 Lyon, Cedex 03, 4Service d’Anatomie Pathologique, JE 2267, Centre hospitalier Lyon-Sud, 69495 Pierre Bénite Cedex and 5Centre Léon Bérard, 28, rue Laennec, 69008 Lyon, France

3To whom correspondence should be addressed at: Institut National de la Santé et de la recherche Médicale, INSERM U-407, Communication en Biologie de la Reproduction, Faculté de médecine Lyon-Sud, B.P. 12, F-69921 Oullins Cedex, France. E-mail: benahmed@lsgrisn1.univ-lyon1.fr

The aim of this study was to investigate DNA mismatch repair deficiency in male germ cell tumours. We analysed the expression of two mismatch repair proteins, human mutL homologue 1 (hMLH1) and human mutS homologue 2 (hMSH2), and evaluated the frequency of microsatellite instability with 10 mononucleotide and two dinucleotide repeat sequences, in 39 paired tumour/normal DNA samples obtained from 17 testicular and two mediastinal germ cell tumours. In all 19 cases, hMLH1 and hMSH2 both showed nuclear immunolocalization in invasive and testicular in-situ tumours. In non-neoplastic seminiferous tubules, hMLH1 was expressed only in premeiotic germ cells, while hMSH2 was seen in all stages of spermatogenesis. Genetic analysis of dinucleotide markers revealed loss of heterozygosity in one of two testicular yolk sac tumours at D18S58 and an allelic shift at D2S123 in two of three testicular embryonal carcinomas, while none of the 12 seminomas exhibited a genetic abnormality at these loci. No abnormalities were demonstrated with the 10 mononucleotide markers. The two mediastinal germ cell tumours showed no genetic instability or allelic loss with all 12 markers. We suggest that genetic alterations as assessed by microsatellite analysis in germ cell tumours may reflect tissue maturation and phenotypic differentiation rather than tumour progression. In addition, we suggest that hMLH1 and hMSH2 genes may not be implicated in the genesis of germ cell tumours.

Key words: germ cell tumour/hMLH1/hMSH2/microsatellite instability/testis

Introduction

Genetic modifications in two major gene classes, proto-oncogenes and tumour suppressor genes, are known to contribute to tumour growth. Recently, a third class of genes involved in DNA repair, the mismatch repair genes, has been implicated in development and predisposition to cancer (Ionov et al., 1993). Mismatched DNA bases occur during the DNA replication process and are corrected by a DNA repair system that comprises at least six genes: human mutL homologue 1 (hMLH1), human mutS homologue 2 (hMSH2), human mutS homologue 3 (hMSH3), human mutS homologue 6 (hMSH6), and human post-meiotic segregation (hPMS1 and hPMS2) (Leach et al., 1993; Bronner et al., 1994; Nicolaides et al., 1994; Papadopoulos et al., 1994, 1995). In addition, MLH3, a novel DNA mismatch repair gene, has been recently cloned (Lipkin et al., 2000). Inactivation of mismatch repair genes leads to genetic alterations, characterized by small deletions or expansions within small repeat sequences in tumour DNA as compared with matching normal DNA, and this is known as microsatellite instability (MSI) (Loeb, 1994). Tumours with MSI have been classified as ‘microsatellite unstable’ or ‘replication error positive’ (RER+). MSI was first identified in the hereditary nonpolyposis colorectal cancer (HNPCC) syndrome, where a high frequency of germline mutations in mismatch repair genes was demonstrated in kindred, specifically hMSH2 (31% of cases) and hMLH1 (33% of cases), with hPMS1 (2%) and hPMS2 (4%) being less frequently mutated (Liu et al., 1996). Inactivating mutations of hMSH2 and hMLH1 and their lack of expression has been also demonstrated.
in a variety of sporadic epithelial tumours, albeit less frequently (Liu et al., 1995; Thibodeau et al., 1996). However, in germ cell-derived neoplasms, genetic instability has been rarely assessed. A few studies in testicular germ cell tumours (TGCTs) have been performed and the results are conflicting (Lothe et al., 1993; Peltomaki et al., 1993; Murty et al., 1994c; Huddart et al., 1995; Faulkner and Friedlander, 2000). The repetitive DNA sequences most commonly analysed in these tumours are dinucleotide repeats, widely distributed throughout the genome, on chromosomes 1, 2, 3, 5, 8, 10, 11, 13, 16, 17 and 18 (Lothe et al., 1993; Peltomaki et al., 1993; Huddart et al., 1995; Faulkner and Friedlander, 2000). The frequency of reported RER+ phenotype tumours is low, ranging from 0–18% (Lothe et al., 1993; Peltomaki et al., 1993; Murty et al., 1994c; Huddart et al., 1995; Faulkner and Friedlander, 2000), although tri- and tetranucleotide repeats have shown a much higher rate of instability (20% of cases) than dinucleotide repeats in testicular and ovarian germ cell tumours (GCTs) (Huddart et al., 1995; Faulkner and Friedlander, 2000). However, the microsatellite markers recommended by the National Cancer Institute Workshop for the detection of MSI in colorectal cancer, specifically mononucleotide repeats, have never been investigated in GCTs (Boland et al., 1998). Moreover, genetic instability of GCTs arising in the mediastinum has never been studied and the expression of hMLH1 or hMSH2 in germ cell tumours is unknown.

To investigate DNA mismatch repair deficiency in a series of testicular and mediastinal GCTs, we examined the immunohistochemical expression of hMLH1 and hMSH2 in 19 cases and characterized these cases for MSI, using selective tissue microdissection by a laser capture microdissection system and a set of microsatellite markers that have not been analysed to date in germ cell neoplasms.

**Materials and methods**

**Tumour samples**

A total of 17 testicular germ cell tumours from 16 patients were included in this study (Table I). One patient presented with a synchronous bilateral testicular seminoma. The mean age of the patients was 32.5 years (range: 20 – 42). The testicular tumours were classified according to World Health Organization recommendations (Mostof and Sesterhenn, 1998). They included 11 pure seminomas and six mixed germ cell tumours (MGCTs). One component from each of the six MGCTs (the component with available frozen tissue) was analysed. These components included embryonal carcinomas in three cases, yolk sac tumours in two cases and a seminoma in one case. Clinical information was available for each patient. Twelve patients (seven pure seminomas and five MGCTs) were stage I, four patients (pure seminomas) were stage II, and one patient with a MGCT had a stage III disease.

Two mediastinal yolk sac tumours from two male patients, aged 29 and 37 years old, were also included in the study.

**Immunohistochemistry**

One boun-fix, paraffin-embedded block was selected from each of the 19 cases for immunohistochemical study. In cases of MGCTs, the block selected was chosen from the same histological component used for genetic analysis. Six microns sections were deparaffinized and rehydrated through graded alcohols to water. The slides were immersed in 10 mmol/l citrate buffer, pH 6.0, and subjected to heat-induced antigen retrieval for pretreatment. Endogeneous peroxidases were quenched in 3% H2O2 for 15 min. Mouse monoclonal antibody to hMLH1 (clone G168-15, 10 µg/ml, Pharmingen-Becton Dickinson, France) and mouse monoclonal antibody to hMSH2 (clone FE11, 0.5 µg/ml, Oncogene Science, MA, USA) were applied and tissue sections were incubated overnight at 4°C. Immunodetection of hMLH1 was performed by the EnVision+ system using HRP (Dako Corporation, Grenoble, France) and the Catalysed Signal Amplification (CSA) system (Dako) was used for immunodetection of hMSH2, according to the manufacturer’s instructions and using diaminobenzidine as the chromogen. Sections were counterstained with haematoxylin and mounted. Negative controls were performed omitting the primary antibody. The normal staining pattern of both hMLH1 and hMSH2 was nuclear. The complete absence of nuclear staining in tumour cells was considered to be an abnormal pattern. Intact staining of adjacent non-neoplastic cells and lymphocytes served as internal positive controls.

**DNA extraction**

Frozen TGCTs and normal tissues (non-neoplastic testicular tissue or epididymis) and formalin-fixed, paraffin-embedded mediastinal GCTs and normal tissues (lymphocytes) were used for genetic analysis.

GCTs, especially seminomas, exhibit large numbers of non-neoplastic stromal cells and lymphocytes. However, tissue microdissection allows for selective analysis of tumour cells with little or no contamination by normal tissue (Bonner et al., 1997). Since MSI assessment is based on comparative analysis of tumour cells with normal tissue from the same individual, it is important to obtain tumour DNA that is as pure as possible.

For each case, multiple samples from different areas of the tumour and normal tissue (Figure 1) were microdissected from 7 µm frozen sections using a laser capture microdissection system (PixCell II®) as previously described (Bonner et al., 1997). In one case of pure seminoma, a subpopulation of intratubular germ cell neoplasia or carcinoma in situ was available on the frozen section and was microdissected separately. Procured films containing the cells were immediately resuspended and incubated for 48 h at 55°C in a 50 µl solution containing 50 mmol/l Tris-hydrochloric acid (pH 8.0), 1 mmol/l EDTA, 0.5% Tween 20 and 0.5 mg/ml proteinase K. The mixture was heated to 95°C for 5 min to inactivate the enzyme and was stored at −20°C until use.

**MSI analysis**

Using the polymerase chain reaction (PCR), paired tumour and normal tissues were independently evaluated for genomic instability using 12 microsatellite loci. Different types of mononucleotide repeats, used in the assessment of MSI in colorectal cancers, were chosen for this study. These included long (A)n tracts in an intron of the c-kit oncogene (BAT 25), in intron 5 of the hMSH2 gene (BAT 26), in an intron of the 3-ß hydroxysteroid dehydrogenase gene (BAT 40), and short mononucleotide repeats (<10 bp) within an intron of the amyloid-beta protein (APP) gene (BAT 10B) and an intron of dystrophin gene (BAT 10A) (Parsons et al., 1995). Short mononucleotide repeats within coding regions of genes implicated in carcinoma genesis were also studied and included the (A)10 tract of the Transforming Growth Factor beta Receptor II (TGFßR II) gene, (G)8 tracts of the Bax and Insulin Growth Factor II Receptor (IGFIIIR) genes, the (C)8 tract of the hMSH6 gene, and the (A)8 tract of the hMSH2 gene (Chung et al., 1997; Dietmaier et al., 1997). Two dinucleotide repeats, D18S58 at 18q22.3 and D2S123 at 2p16, were also analysed (Dietmaier et al., 1997) (Table II).
The PCR was performed in a 10 μl reaction solution containing 50–100 ng of DNA, 1.5 mmol/l MgCl₂, 1 μl of 10× PCR buffer (Perkin Elmer, Norwalk, CT, USA), 0.5 IU Taq DNA polymerase, 1.25 mmol/l of each of the four standard nucleoside triphosphates, 0.5 IU Taq DNA polymerase, to electrophoresis at 60W for an average of 100 min. Gels were washed and stained with 0.5 μg/ml ethidium bromide. DNA bands were visualized and photographed under ultraviolet illumination. DNA bands were excised from the gel and digested in situ with 1 unit of Hau I (New England Biolabs, Beverly, MA, USA) for 30 min. The DNA fragments were separated by electrophoresis, transferred onto nitrocellulose, and autoradiographed for 24 h.

The results were interpreted independently by two of the authors (M.D-S. and C.M.). PCR was repeated in cases of discrepancy in interpretation. MSI was defined as the presence of allelic shift or additional bands in tumour samples compared with normal tissue.

Results

Immunohistochemical analysis, performed in all 19 GCTs, revealed intact nuclear staining of tumour cells with antibodies to hMLH1 and hMSH2 (Figures 2 and 3). The carcinoma in situ was present on three sections from testicular neoplasms, and also exhibited intact nuclear staining with both antibodies. Non-neoplastic seminiferous tubules adjacent to the tumour were present in seven sections. With the hMLH1 antibody, spermatogonia and early spermatocytes showed an intense immunostaining while spermatocytes type II, spermatids and spermatozoa were negative (Figure 2). This pattern of staining contrasted with that of seminiferous tubules involved in the carcinoma in situ where all the cells in the tubules were positively stained. With the hMSH2 antibody, a positive nuclear staining was seen in all stages of spermatogenesis except spermatozoa (Figure 3). In seminomas, only lymphocytes within germinal centres demonstrated a nuclear staining. Leydig cells, rete testis and epididymis also stained with both antibodies.

The PCR-based amplification of 12 repetitive nucleotide sequences, used to characterize the genetic instability status of our 17 testicular and two mediastinal GCTs, included five intronic mononucleotide repeats, BAT 25, BAT 26, BAT 40, BAT 10A, BAT 10B, five mononucleotide repeat sequences

Table 1. Clinical information and PCR-based microsatellite analysis in 17 cases of testicular and two cases of mediastinal germ cell tumours with 12 markers

<table>
<thead>
<tr>
<th>Age</th>
<th>Tumour site</th>
<th>Stagea</th>
<th>Histology</th>
<th>IHC HMLH1/hMSH2</th>
<th>DiN markers</th>
<th>MonoN markers</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>D2S123</td>
<td>D18S58</td>
</tr>
<tr>
<td>29</td>
<td>L</td>
<td>T2</td>
<td>Seminoma</td>
<td>+/+</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>39</td>
<td>R</td>
<td>T2</td>
<td>Seminoma</td>
<td>+/+</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>42</td>
<td>L</td>
<td>T1</td>
<td>Seminoma</td>
<td>+/+</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>28</td>
<td>R</td>
<td>T1</td>
<td>Seminoma</td>
<td>+/+</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>41</td>
<td>L</td>
<td>T1</td>
<td>Seminoma</td>
<td>+/+</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>29</td>
<td>R</td>
<td>T1</td>
<td>Seminoma</td>
<td>+/+</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>20</td>
<td>L</td>
<td>T1</td>
<td>Seminoma</td>
<td>+/+</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>34</td>
<td>R</td>
<td>T1</td>
<td>Seminoma</td>
<td>+/+</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>42</td>
<td>R</td>
<td>T2</td>
<td>Seminoma</td>
<td>+/+</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>31</td>
<td>L</td>
<td>T2</td>
<td>Seminoma</td>
<td>+/+</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>24</td>
<td>L</td>
<td>T1</td>
<td>MGCT (YST)</td>
<td>+/+</td>
<td>NA/LOH</td>
<td>NA</td>
</tr>
<tr>
<td>27</td>
<td>R</td>
<td>T3</td>
<td>MGCT (EC)</td>
<td>+/+</td>
<td>NA/NI</td>
<td>NA</td>
</tr>
<tr>
<td>22</td>
<td>L</td>
<td>T1</td>
<td>MGCT (EC)</td>
<td>+/+</td>
<td>MSI</td>
<td>NA/NI</td>
</tr>
<tr>
<td>34</td>
<td>L</td>
<td>T1</td>
<td>MGCT (EC)</td>
<td>+/+</td>
<td>MSI</td>
<td>NA/NI</td>
</tr>
<tr>
<td>39</td>
<td>R</td>
<td>T1</td>
<td>MGCT (Sem)</td>
<td>+/+</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>37</td>
<td>M</td>
<td>YST</td>
<td>+/+</td>
<td>NA</td>
<td>NA</td>
<td>NA/NI</td>
</tr>
<tr>
<td>29</td>
<td>M</td>
<td>YST</td>
<td>+/+</td>
<td>NA</td>
<td>NA</td>
<td>NA/NI</td>
</tr>
</tbody>
</table>

aStage according to the American Joint Committee on Cancer and Union International Contre le Cancer. R = right testis; L = left testis; M = mediastinum; CIS = carcinoma in situ; IHC = immunohistochemistry; DiN = dinucleotide; MonoN = mononucleotide; LOH = loss of heterozygosity; MSI = microsatellite instability; NA = no alteration; NI = not informative for LOH analysis; MGCT = mixed germ cell tumour; MGCT ( ) = component used for genetic analysis and immunohistochemistry; YST = yolk sac tumour; EC = embryonal carcinoma; Sem = seminoma.

Figure 1. Testicular seminoma before (a) and after (b) laser capture tissue microdissection using PixCell II®. Only tumour cells are microdissected leaving the non-neoplastic stromal fibrous tissue and lymphocytes on the slide. Scale bar = 100 μm
Table II. Primers for the microsatellite markers (MM) used to assess genetic instability of testicular and mediastinal germ cell tumours in this study

<table>
<thead>
<tr>
<th>MM</th>
<th>Sequence Sense (5′–3′)</th>
<th>Antisense (5′–3′)</th>
<th>Product size (bp)</th>
<th>Annealing temp. (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BAT 25</td>
<td>TCGCCTCAAGAATGTAAGT</td>
<td>TCTGCATTTAACTATGGCTC</td>
<td>90</td>
<td>47</td>
</tr>
<tr>
<td>BAT26</td>
<td>TGA CACTTTTGGACTTACGCC</td>
<td>AACCACCTACATTTTTAAACC</td>
<td>80–100</td>
<td>47</td>
</tr>
<tr>
<td>BAT 40</td>
<td>ACACACCTGCTTTTGTTTCTCTT</td>
<td>GTAGAGCAAGACCACCTTTG</td>
<td>80–110</td>
<td>47</td>
</tr>
<tr>
<td>BAT 10A</td>
<td>GATATATAGC ATATAACACTG</td>
<td>GAAACACAAAGGAAGTGTCTG</td>
<td>126</td>
<td>55</td>
</tr>
<tr>
<td>BAT 10B</td>
<td>ATGTGGCTATTGACCACACAC</td>
<td>GGTCAAGGCTACAGTAAGC</td>
<td>121</td>
<td>55</td>
</tr>
<tr>
<td>TGFβRII</td>
<td>TTTTTTCTGGAGATGCTGC</td>
<td>GAAGAAAGTCTCACCAAGG</td>
<td>71</td>
<td>47</td>
</tr>
<tr>
<td>IGF RII</td>
<td>GCAGGTCTCTGAGCTCTGAGA</td>
<td>GAAGAAAGATGGCAGTGGAGC</td>
<td>111</td>
<td>55</td>
</tr>
<tr>
<td>Bax</td>
<td>ATCCAGAAGACGAGAGGCGGCG</td>
<td>ACCTCCTAGCCTTGGTGGT</td>
<td>156</td>
<td>60</td>
</tr>
<tr>
<td>hMSH3</td>
<td>AGATGCGATCCCTCTATCAAGC</td>
<td>ACTCCCCCCCTGGCTCAACCTA</td>
<td>153</td>
<td>60</td>
</tr>
<tr>
<td>hMSH6</td>
<td>GGGTGTGTTGCTCTTGATGGC</td>
<td>GCATGCAACAGGATGCGT</td>
<td>94</td>
<td>60</td>
</tr>
<tr>
<td>D2S123</td>
<td>CATTTGTCAGCTTGGTATACCA</td>
<td>CTCTTTTTCTGCTTGGGACCA</td>
<td>140</td>
<td>54</td>
</tr>
<tr>
<td>D18S58</td>
<td>GCTCCCGGCTGTCTTTTT</td>
<td>GCAGGAATCGACAGGAACCTT</td>
<td>144–160</td>
<td>54</td>
</tr>
</tbody>
</table>

within coding regions of genes for TGFβ RII, Bax, IGF RII, hMSH3 and hMSH6, and two dinucleotide repeat microsatellite markers, D18S58 and D2S123 (Table II).

Two different tumour areas were selected from each frozen sample of TGCT and paraffin-embedded slides of mediastinal GCTs, and microdissected separately. In one case, an area of carcinoma in situ was also microdissected. In another, synchronous bilateral seminomas were selectively dissected. A total of 35 normal/tumour area pairs from 17 TGCTs and four normal/tumour area pairs from two mediastinal GCTs were examined.

No altered lengths of novel alleles were detected with the 10 mononucleotide repeats in any of the paired normal/tumour samples (data not shown). In three cases of TGCTs, dinucleotide repeats revealed a different allelic pattern in tumour DNA as compared with normal DNA (Figure 4). In one case of MGCT, loss of heterozygosity (LOH) was detected
with D18S58. With this marker, selectively procured neoplastic tissues from a yolk sac tumour component were homozygous, demonstrating the presence of the lower allele only, while heterozygosity was retained in normal tissues. None of the other 14 informative cases with this marker, including another testicular and one of two mediastinal yolk sac tumours, showed an allelic loss. In another two cases of testicular MGCTs, allelic shifts were observed in the tumour DNA as compared with normal DNA, with D2S123. In both cases, the DNA was obtained from an embryonal carcinoma component. The rate of MSI in these two cases was low at 8% (1 of 12 markers).

Discussion

In the current study, using immunohistochemistry, we examined the expression pattern of hMSH2 and hMLH1 in a series of 17 TGCTs including seminomatous and non-seminomatous tumours at different clinical stages and in two mediastinal yolk sac tumours. Immunohistochemical detection of hMLH1 and hMSH2 has been found to be a practical test for identifying repair deficiency with 97% sensitivity and 100% specificity (Marcus et al., 1999). We demonstrated that the two genes whose disrupted expression is most frequently implicated in the genetic instability of HNPCC, hMSH2 and hMLH1, are expressed in these GCTs. Indeed, both proteins were detected by immunohistochemistry in the nuclei of tumour cells in all of our cases, regardless of histological type. In this context, MSI at tri- and tetranucleotide loci detected in a subset of TGCTs (Huddart et al., 1995; Faulkner and Friedlander, 2000) might implicate other DNA repair genes or be controlled by a mechanism unrelated to the mismatch repair system. Our immunohistochemical study exhibited increased expression of both hMSH2 and hMLH1 in invasive and in-situ testicular tumour cells. The intensity and extent of immunostaining was higher in tumour cells than in normal stromal lymphocytes. Using immunohistochemistry, increased expression of hMSH2 in sporadic tumours of the gastro-intestinal tract and in urothelial malignancies has been reported (Leach et al., 1996, 2000). However, the significance of the increased expression of mismatch repair proteins evaluated by immunohistochemistry alone is not clear.

The DNA mismatch repair system has been found to play a role in the meiotic recombination process during mouse spermatogenesis (Geeta Vani et al., 1999; Richardson et al., 2000), and MLH1-deficient males have been reported to be infertile (Baker et al., 1996). In contrast to rat spermatogenesis where the MSH2 gene is essentially expressed in mitotically proliferating spermatogonia and early in meiotic prophase in the spermatocytes (Richardson et al., 2000), we found that the hMSH2 protein in human non-neoplastic testis was expressed in all stages of spermatogenesis, except spermatogonia. On the other hand, MLH1 gene expression in rat testis peaks at day 30 post-partum, when round spermatids appear (Geeta Vani et al., 1999), while in our study the hMLH1 protein was immunolocalized in premeiotic germ cells, spermatogonia and early spermatocytes and was absent from round spermatids and spermatooza. The expression pattern of hMLH1 and hMSH2 in human seminiferous tubules, while somewhat different from that in rat testis, indicates their role during genetic recombination in human spermatogenesis.

To correlate the genetic stability of our GCTs with the immunohistochemical findings, we analysed different types of mononucleotide repeats (n = 10) and two dinucleotide markers in 39 paired tumour/normal DNA samples from 17 testicular and two mediastinal GCTs. A low frequency (8%, two of 19 tumours) of MSI was consistent with our immunohistochemical results in which the expression of mismatch repair genes was not altered. Deficiency in the mismatch repair system seems to prompt mismatched DNA in mononucleotide repeats and thus, in colon cancers mononucleotide repeats, in particular long (A)n tracts, are most often affected by MSI (Dietaimier et al., 1997). In addition, genetically unstable HNPCC-related cancers have demonstrated frameshift mutations in mononucleotide repeats within exons of genes for TGF βRII (Myeroff et al., 1995), IGFRII (Ouyang et al., 1997), Bax (Rampino et al., 1997), and hMSH3 and hMSH6 (Malkhosyan et al., 1996). However, in our study none of the 39 paired tumour/normal DNA samples exhibited MSI within intronic or exonic mononucleotide repeat sequences. Ovarian GCTs have shown a low rate of MSI, with only one tumour (2% of cases) demonstrating instability at 36% of loci assessed and 11 additional cases (26%) being unstable at <10% of loci (Faulkner and Friedlander, 2000). To our knowledge, this is the first study investigating MSI in mediastinal GCTs, and none of the two yolk sac tumours showed a RER+ phenotype. In contrast to tumours of epithelial origin and in particular the HNPCC-related cancers, MSI and altered expression of hMLH1 and hMSH2 genes seem not to be involved in the development of gonadic or extra-gonadic GCTs.

In the present study, analysis of two dinucleotide microsatellite markers (D2S123 and D18S58) revealed allelic alterations. In two testicular MGCTs, MSI was detected at D2S123. Although the MSI rate of these two tumours was low (8%, one of 12 loci), instability was demonstrated with dinucleotide markers (one of two loci), while none of the mononucleotide repeat sequences assessed showed evidence of RER. MSI was detected in two non-seminomatous tumours, both of which were the embryonal carcinoma component of MGCTs, while none of the seminomas showed a RER+ phenotype. The frequency of MSI seen in our embryonal carcinoma samples was high (66%, two of three cases). A higher frequency of RER+, specifically at lq42.43, has been reported in embryonal carcinomas and yolk sac tumours as compared with teratomas and seminomas (Murty et al., 1994c). Taken together, these results suggest that at specific chromosomal regions, the RER+ phenomenon in TGCTs may be more important in the development of embryonal carcinomas than in other histological types of germ cell tumours.

In addition, we demonstrated an allelic loss in 6.6% of informative cases at 18q22.3 with D18S58. With this marker, DNA from the yolk sac tumour component of a testicular MGCT showed the presence of the lower allelic band only, while the constitutional DNA was heterozygous. LOH at 18q21–22 has been reported in 38–55% of TGCTs (Murty et al., 1994a,b; Peng et al., 1995; Strohmeyer et al., 1997; Rothe et al., 1999). Of interest is the presence of allelic
deletion at 18q22 in one of our two testicular yolk sac tumours, while tumours of other histological types did not demonstrate LOH at this locus. However in the present study, one of two mediastinal yolk sac tumours that was informative at this locus did not show LOH. Markers at 18q22 were found to be most frequently lost in yolk sac tumours in one series of 76 TGCTs (44%), while in other histological types the rate of LOH at this locus varied from 0–30% (Rothe et al., 1999). The DCC (Deleted in Colon Cancer) tumour suppressor gene is located proximal to this region at 18q21.3. Frequent allelic deletions and loss of expression of DCC has been found in TGCTs, specifically in the teratomatous component (Murty et al., 1994a,b; Strohmeyer et al., 1997). However, the DCC locus was not found to be affected by LOH in yolk sac tumour samples that had allelic loss at 18q22 (Rothe et al., 1999).

D18S58 is mapped to DNA-M-1, which codes for an adhesion molecule (DNAX accessory molecule-1) involved in the cytotoxic function mediated by natural killer and T lymphocytes (Shibuya et al., 1996). A role for DNA-M-1 in tumorigenesis, however, has not been established. In the present study, the more frequent allelic deletion at 18q22 in testicular yolk sac tumours as compared with other subsets of germ cell tumours suggests that a gene located near this chromosomal region might promote differentiation of totipotent germ cells towards a yolk sac phenotype. Similarly, the high degree of genetic loss of the non metastatic protein 23 genes in teratomas as compared with other germ cell tumours, has suggested that these genes might be associated with somatic differentiation in TGCTs (Murty et al., 1994a).

In summary, our results reveal that in contrast to some epithelial neoplasms, GCTs do not show MSI in mononucleotide repeat sequences and that hMLH1 and hMSH2 are normally expressed in these tumours, suggesting that these DNA mismatch repair proteins might not be implicated in the genesis of gonadic and extra-gonadic GCTs. The demonstration of allelic loss in testicular yolk sac tumours at D18S58 and a higher frequency of MSI with dinucleotide markers in embryonal carcinomas suggests that microsatellite analysis in germ cell tumours might provide additional information on the histogenesis and phenotypic differentiation of these tumours, rather than their tumourogenesis.

Acknowledgements
This work was supported by the Institut National de la Santé et de la Recherche Médicale and by the Ligue Contre le Cancer, Comité Départemental de l’Ain, France.

References


Murty, V.V., Li, R.G., Houldsworth, J. et al. (1994b) Frequent allelic deletions and loss of expression characterize the DCC gene in male germ cell tumors. Oncogene, 9, 3227–3231.


Assessment of mismatch repair deficiency in male germ cell tumours


Received on April 17, 2001; accepted on September 25, 2001