SHORT COMMUNICATION

Microsatellite instability in human testicular germ cell tumours

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Summary DNA samples obtained from 29 testicular germ cell tumours have been screened for instability at nine different microsatellite sequences consisting of dinucleotide, trinucleotide and tetranucleotide loci. Overall, in tumours from six (21%) patients we found abnormalities in at least one of the loci examined. Mutation was most frequently found in tetranucleotide and trinucleotide repeats with only a low proportion of alterations in dinucleotide repeats. This pattern of instability is distinct from that reported in colorectal cancer and other cancers that have a high level of alterations in dinucleotide repeats.

Keywords: testicular germ cell tumours; microsatellite instability

Testicular germ cell tumours (TGCTs) form a histologically heterogeneous group of neoplasms that are thought to arise from primordial germ cells. Despite receiving considerable attention in recent years, very little is known about the molecular mechanism of TGCT development. Cytogenetic studies have identified isochromosome 12p as a consistent karyotypic abnormality that is present in the majority of tumours (Atkin and Baker, 1982). Loss of heterozygosity studies have identified multiple chromosomal regions that may be the sites of tumour-suppressor genes (Murry et al., 1994a) while H-RAS, N-RAS and DCC abnormalities have all been implicated in the aetiology of these tumours (Ganguly et al., 1990; Moul et al., 1992; Murty et al., 1994b).

Several studies have implicated defects in DNA mismatch repair in the pathogenesis of hereditary non-polyposis colorectal cancer (HNPPC). Widespread alterations in genomic DNA, as indicated by changes in microsatellite sequences consisting of dinucleotide and trinucleotide repeats and in other simple repeat sequences, were discovered in colorectal tumours (Armour et al., 1989; Aaltoinen et al., 1993; Ionov et al., 1993; Thibodeau et al., 1993). Recognition that the types of genetic alterations observed in these studies were similar to those associated with abnormalities of bacterial mismatch repair genes such as mutS and mutL initially led to the discovery that the human homologues of these genes, hMSH2 and hMLH1 (Fishel et al., 1993; Leach et al., 1993; Bronner et al., 1994; Papadopoulos et al., 1994), were mutated in HNPPC kindreds. More recently mutations in two human homologues of yeast repair genes designated PMS1 and PMS2 have also been found in HNPPC families (Nicolaides et al., 1994). In addition to the observed abnormalities in colorectal cancer, a high frequency of genetic instability of microsatellites (the mutator phenotype) has also been observed in other classes of human cancer, including endometrial (Risinger et al., 1993; Tucker Burks et al., 1994) pancreatic (Han et al., 1993), gastric (Han et al., 1993) oesophageal (Meltzer et al., 1994) and small-cell lung cancer (Merlo et al., 1994; Shridhar et al., 1994). In contrast, lower frequencies of instability have been observed in breast cancers, ovarian cancers, soft-tissue sarcomas and tumours of the nervous system (Wooster et al., 1994). In the present study we have assessed the stability of microsatellite sequences in TGCTs.

Materials and methods

Tumour and blood samples were collected from testicular cancer patients at the Royal Marsden Hospital, Sutton, UK. DNA was prepared from tumour and matched lymphocyte specimens as described previously (Sambrook et al., 1989; Lahiri and Nurnberger, 1991). Short tandem repeats were amplified using polymerase chain reaction (PCR). In each case one primer was radiolabelled with \(^3^P\) at the 5'-end using T4 polynucleotide kinase and \([\gamma\rbracket^3^P\rbracket\PiATP.\ PCR was performed in at 25 μl volume containing 50 ng of DNA, 2.5 μl of 10 x reaction buffer (Advanced Biotechnology), 0.2 μM of each dNTP, 0.1 unit of Taq polymerase and 20 pmol of each primer. PCR was performed using cycle conditions of 94°C for 60 s, 55–60°C for 60 s and 72°C for 60 s. Following amplification for 35 cycles the products were denatured in formamide–EDTA loading buffer and subjected to electrophoresis in denaturing polyacrylamide gels (6%, w/v). Visualisation was by autoradiography for 5–72 h. Nine polymorphic loci were examined. Seven of these markers were the same as used by Wooster et al. (1994), to allow comparison between tumour types. D1S216 was substituted for chromosome 16 markers to widen the number of chromosomes examined and to examine an area where instability had been previously reported (Murry et al., 1994c). TFIID was included as a trinucleotide marker which we had found to be useful in screening for a low rate of instability in other tumours. Primers used to amplify the myotonic dystrophy CAG repeat and the androgen receptor CAG repeat are reported by Wooster et al. (1994). The primers that amplify the von Willebrand’s factor tetranucleotide repeats vWFα (Kimpston et al., 1992) and vWFb (van Amstel and Reisman 1990) and to amplify four dinucleotide repeats corresponding to the loci D1S216, D2S123, D16S303 and D17S5796 (Thompson et al., 1992; Weissenbach et al., 1992) are found in the relevant references. The primers used to amplify the TFIID trinucleotide repeat are 5'-TGCCAC-TGGACTGACC-3' and 5'-GCTGCCACTGCCTGTTT-3'.

Results

In the present study a series of 29 testicular germ cell tumours were examined for instability in a series of nine tandem repeats. The tumour group consisted of 12 seminomas, 12 non-seminomatous germ cell tumours (NSGCTs) and four tumours with combined seminoma–NSGCT histology. The loci examined included two tetranucleotide repeats, vWFα and vWFb, located within introns of the gene.
encoding von Willebrand's factor (vWF) and three trinucleotide repeats present in genes for the androgen receptor (AR), myotronic dystrophy (DM) and the TFIID transcription factor. Four dinucleotide repeats (D1S216, D2S123, D16S303 and D17S796) were also examined. Alleles amplified by PCR frequently exhibited a characteristic stutter appearing as doublets with the bands of different or, in some cases, similar intensity. Abnormal sized bands were detected in six different tumours (Table I, Figure 1). To exclude technical artefacts or specimen contamination these abnormalities were reproduced in independent PCR amplifications and separate gel loadings. The consistent occurrence of identical alleles with the same size in normal and tumour DNA at other microsatellite loci excluded errors such as incorrect numbering or tissue contamination.

Three of the tumours exhibited a single abnormality with two tumours containing two and a single tumour containing four alterations (Table I). Notably the highest frequency of abnormality was observed for tetranucleotide repeats and trinucleotide repeats (10 of 145 typings, 7%) while there was a relatively low level of alteration in dinucleotide repeats (1 of 116 typings, 0.9%). There was no clear association with particular histological type as abnormalities were detected in seminomas, in a NSGCT and in a tumour with combined histology.

### Discussion

In an unselected group of primary testicular cancers six (21%) tumours showed microsatellite instability detected as mobility shift in tumour DNA compared with paired lymphocyte DNA. The highest level of abnormalities was found in tetranucleotide and trinucleotide repeats with only a low level of alteration in dinucleotide repeats. This situation contrasts with that found for colorectal and other classes of human cancer where a high level of mutations are found in dinucleotide repeats. Indeed the spectrum of mutation resembles more closely that reported by Wooster et al. (1994) who, in a study of 196 breast cancers, ovarian cancers, soft tissue sarcomas and tumours of the nervous system, also detected the majority of alterations in tri- and tetranucleotide

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**Figure 1** Analysis of microsatellite repeats in paired lymphocyte (B) and tumour (T) DNAs from the same patient. The GCT number assigned to each patient with a testicular germ cell tumour is also shown. Instabilities were observed in the repeat found within the TFIID transcription factor gene (TFIID), the CAG repeat within the androgen receptor gene (AR), the TCTA tetranucleotide repeats found within introns of the von Willebrand's factor gene (vWFa, vWfb) and the CA dinucleotide repeat at the D16S303 locus. Alleles amplified by PCR are frequently associated with a characteristic stutter and appear as doublets with bands of different or similar intensity. Additional alleles detected in tumours are indicated by small arrows.
repeats. The mutation rate in tri- and tetranucleotide repeats in TGCT (10 in 145 typings, 7%) was, however, somewhat higher than that reported by Wooster et al. (1994), who found 15 alterations in 194 typings (15.5%). Although the stability of tri- and tetranucleotide repeats has in general been studied less thoroughly than dinucleotide repeats, instability of trinucleotide repeats has been detected in colorectal tumours which have the mutator phenotype (Aaltonen et al. 1993). Abnormalities in trinucleotide repeats have also been detected in a variety of human genetic conditions. For example, CAG repeats or CTG repeats are expanded in myotonic dystrophy. X-linked spinocerebellar ataxia type 1, Huntington's disease and spinocerebellar ataxia type 2 (Willems, 1994).

Our results are in agreement with those obtained by Lothe et al. (1993), who failed to find replication errors in a series of 86 germ cell tumours, including familial cases, using seven dinucleotide repeat markers mapping to lp, lp, lp, lp, lp, lp, lp. A high level of replication error type genetic instability was observed in germ cell tumours by Murty et al. (1994a). However, the instability was restricted to markers at chromosome lp42–43 and the abnormalities included loss of heterozygosity, dinucleotide repeat replication errors as well as alterations of variable number of tandem repeat regions. In parallel experiments in the same study alterations were not observed in dinucleotide repeats mapping to 12q, 17p and 18q. In contrast, in the present study we show that replication errors are not restricted to chromosome 1 but can occur in tri- and tetranucleotide repeats mapping to chromosomes 6 (TFIID), 12 (vWF), 16 (D16S303) and X (AR). The significance of the microsatellite instability found in TGCTs needs further investigation. The rarity of dinucleotide repeat abnormalities would argue against involvement of the hMSH2 and hMLH1 genes. However it is possible that mutation in other repair genes could be responsible for the abnormalities in tri- and tetranucleotide repeats. As more information becomes available on these genes it would therefore be of interest to investigate their role in the aetiology of TGCT. In this regard it is worthy of note that the karyotypes of TGCTs are frequently complex with a DNA index commonly in the hypotriploid–hypertriploid range while high levels of loss of heterozygosity are observed for many chromosomal arms including 2p, 3p, 3q, 5p, 9p, 9q, 10q, 11p, 12q, 13q, 17p, 17q, 18q and 20p (Murty et al., 1994a). Though other mechanisms may also be acting both of these findings would be consistent with the presence of mutations in repair genes that predispose to larger genetic changes in TGCT.

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