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Advances in Brief

Replication Error-Type Genetic Instability at 1q42–43 in Human Male Germ Cell Tumors[1]

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Abstract

The replication error phenotype, recognized as microsatellite sequence alterations, has recently been suggested to be associated with hereditary nonpolyposis colorectal cancer and other types of sporadic tumors. We examined paired tumor-normal DNAs from 69 human male germ cell tumors for somatic instability at the 1q42–43 region. Analysis of a variable number of tandem repeats marker (*D1S74*) and 3 $(CA)_n$ type microsatellite loci (*D1S235*, *D1S180*, and angiotensinogen) revealed genetic alterations in tumor DNAs of 26 (38.2%) cases. The changes observed comprised rearrangements with *D1S74* detected by Southern blot analysis in 4 of 55 (7%) cases; replication error-type alterations with *D1S235*, *D1S180*, and angiotensinogen in 12 of 66 (18.2%) cases; and loss of heterozygosity in 12 of 67 (17.9%) cases with the same probes. The microsatellite sequence alterations were more common in histological subsets other than teratomas, while the loss of heterozygosity was significantly more frequent in teratomas compared to other histologies. These results suggest that microsatellite instability and loss of heterozygosity at 1q42–43 may be unrelated genetic events which may play a role in germ cell tumor development.

Introduction

Widespread genomic instability detected as microsatellite variations has been identified as a novel genetic mechanism associated with tumor development (1–3). Frequent gene mutations caused by microsatellite instability leading to DNA RERs[3] have recently been identified in many tumor types (1, 4–8). This common somatic instability at multiple unrelated microsatellite loci in cancer suggested a role for genomic instability in tumor development. A gene responsible for such a high rate of mutations in hereditary nonpolyposis colon cancer, sporadic colorectal cancer, and the associated Muir-Torre syndrome has been shown to map to 2p (9, 10).

Microsatellite instability represents a pathway in tumor development distinct from loss of tumor suppressor gene function, recognized as LOH, exhibited by tumor suppressor genes. A previous study of human male GCTs, assaying for polymorphisms at 7 microsatellite loci located on 1p, 5q, 8p, 10p, 11p, 13, and 17q, did not reveal instability (11). During an allelotype analysis of human male GCTs, we noted DNA rearrangements in 4 tumors when assayed with a probe for the VNTR locus, *D1S74*. This observation prompted us to evaluate microsatellite instability at the 1q42–43 region. We show here that RER-type genetic instability characterizes this region in 18.2% of cases of GCTs.

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[3] The abbreviations used are: RER, replication error; RER+, replication error positive; GCT, germ cell tumor; LOH, loss of heterozygosity; VNTR, variable number of tandem repeats; *AGT*, angiotensinogen.

Materials and Methods

Tumor Specimens, Normal Mononuclear Cells, and Cell Lines. Fifty-seven tumor tissues and the corresponding peripheral blood specimens from 52 GCT patients were obtained at the Memorial Sloan-Kettering Cancer Center during 1988–1993. In addition, 12 GCT cell lines, which included 8 from the panel of GCT tumors above and 4 additional tumor cell lines (833KE, 577MF, 577Mlu, and 2061H) and the corresponding lymphoblastoid cell lines (derived by D. L. B.), were included in the present investigation. The following histologies were represented in the tumor biopsy panel: 5 seminomas; 19 teratomas; 15 embryonal carcinomas; 5 yolk sac tumors; 12 mixed tumors; and 1 combined tumor. The tumors from which the cell lines were derived comprised 9 teratomas, 2 embryonal carcinomas, and 1 mixed tumor.

DNA Analysis of Polymorphic Markers. High molecular weight DNA was isolated from tumor tissues, peripheral blood mononuclear cells, and tumor and lymphoblastoid cell lines by proteinase K digestion, phenol extraction, and ethanol precipitation following standard methods. Southern blots were prepared using 6–10 μg of tumor-normal DNAs digested with the restriction enzymes *Taq*I, *Pst*I, *Xba*I, and *Hind*III and were hybridized with ^{32}P-labeled probe. Polymerase chain reaction was carried out in a 25-μl reaction volume containing 50–100 ng of genomic DNA under standard conditions, with 20 pmol of primers in which one-fifth of 1 primer was end-labeled with [γ-^{32}P]ATP. The amplification reactions were carried out for 25 cycles at 94°C, 56°C, and 72°C for 60 s. The amplified polymerase chain reaction products were denatured in sequencing stop solution and electrophoresed on 6% denaturing polyacrylamide gels. The dried gels were autoradiographed for 5–48 h.

Probe and $(CA)_n$ Microsatellite Primers. A VNTR marker, *D1S74* (*cYNA13*), obtained from the American Type Culture Collection, was used to assay allelic alterations. The dinucleotide repeat markers *D1S235*, *D1S180* and *AGT* mapped to 1q42–43 were obtained from Research Genetics (Huntsville, AL).

Fluorescence *in Situ* Hybridization. The probe *D1S74* in vector was biotin labeled and hybridized to bromodeoxyuridine-pulsed normal human metaphase chromosomes for subregional mapping by previously described methods (12).

Results

Rearrangements at 1q42 by Southern Analysis Using *D1S74*. The VNTR probe *cYNA13* hybridized to 56 paired normal-tumor DNA samples digested with restriction enzymes *Taq*I or *Pst*I revealed new fragments in addition to the constitutional alleles in 4 tumor DNAs (Fig. 1). In addition, 3 informative tumors showed LOH in tumor DNAs. Thus, 7 of 56 (12.5%) tumors showed evidence of genetic alterations at this locus. The additional fragments in the 4 cases with DNA rearrangements suggested either specific gene alterations or genetic instability at this locus. The novel fragments in the 4 tumors were also confirmed after digestion with the restriction enzymes *Xba*I and *Hind*III (data not shown). The blots containing the 4 tumor DNAs with rearrangements, sequentially hybridized with more than 15 probes mapped to different chromosomal arms, showed no evidence of rearranged bands or partially digested DNA (data not shown). The *cYNA13* probe was previously mapped to the 1q32–44

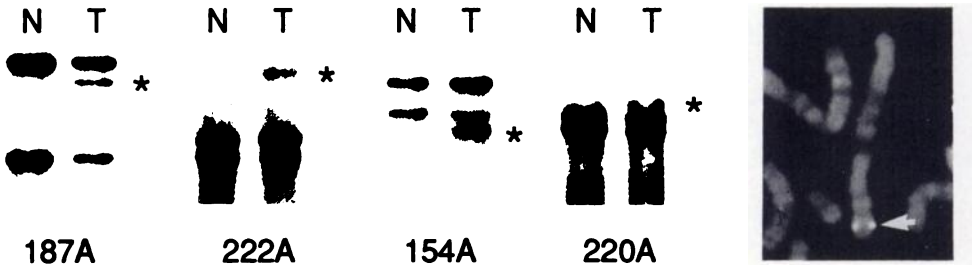

Fig. 1. Molecular alterations of VNTR locus *D1S74* in male GCT and its chromosomal localization to 1q42. Rearrangements of *D1S74* in *Taq*I-digested tumor DNA. *Bottom abscissa*, tumor numbers; *, extra fragments; *N*, normal; *T*, tumor. *Right*, partial normal metaphase showing localization of *D1S74* at 1q42. *Arrow*, hybridization signal of biotin-labeled probe after counterstaining with propidium iodide.

region (13, 14). In order to further investigate these genetic changes, we determined the map position of *cYNA13* by the fluorescence *in situ* hybridization assay. Of 51 metaphases with hybridization signals at 1q42, 21 showed doublets (signals on both chromatids) on both homologues, 19 showed doublets on 1 homologue, and the remaining 7 showed combinations of 1 or 2 signals on 1 or both homologues. This analysis localized *cYNA13* at 1q42 (Fig. 1).

Analysis of Microsatellite Instability. To further investigate the genetic alterations observed with *cYNA13*, we evaluated $(CA)_n$ microsatellite polymorphisms at 3 loci, which included *D1S180* (mfd126), *AGT* (15), and *D1S235* (16), all mapped to the 1q42–43 region. We found microsatellite instability (RER+), observed as mobility shift, resulting in decreased or increased sizes of alleles or as a new allele in 12 of 66 (18.2%) cases. The changes observed were noted at all 3 loci: 6 of 65 (9.2%) at *D1S235*; 5 of 66 (8%) at *D1S180*; and 9 of 66 (13.6%) at *AGT* in 20 instances of 197 comparisons (Fig. 2). Of the 12 tumors showing RER+, 2 had instability at all 3 loci, 4 had instability at 2 loci, and 6 had instability at 1 locus. Similar genetic alterations were not seen in any of the corresponding normal DNAs. All RER+ results were confirmed by replicate analysis.

Table 1 *Frequency of RER+ in histological subsets of male GCT*

Histology	RER+/no. of cases (%)			
	D1S235	*D1S180*	*AGT*	Total
Teratoma	0/26 (0.0)	0/27 (0.0)	1/27 (3.7)	1/27 (3.7)
Embryonal carcinoma	1/16 (6.3)	3/16 (18.8)	4/16 (25)	6/16 (37.5)
Yolk sac	2/5 (40)	0/5 (0.0)	2/5 (40)	2/5 (40)
Mixed/combined	2/13 (15.4)	1/13 (7.7)	2/13 (15.4)	2/13 (15.4)
Seminoma	1/5 (20)	1/5 (20)	0/5 (0.0)	1/5 (20)

Table 2 *Frequency of LOH in histological subsets of GCT*

Histology	LOH/no. of informative cases (%)				
	D1S74	*D1S235*	*D1S180*	AGT	Total
Teratoma	1/20 (5)	3/19 (15.8)	5/21 (23.8)	1/20 (5)	8/27 (29.6)
Embryonal carcinoma	1/14 (7.1)	0/8 (0.0)	1/14 (7.1)	0/15 (0.0)	1/17 (5.9)
Yolk sac	0/5 (0.0)	0/1 (0.0)	0/4 (0.0)	0/5 (0.0)	0/5 (0.0)
Mixed/combined	1/11 (9.1)	1/9 (11.1)	1/11 (9.1)	2/12 (16.7)	3/13 (23.1)
Seminoma	0/4 (0.0)	0/2 (0.0)	0/3 (0.0)	0/4 (0.0)	0/5 (0.0)

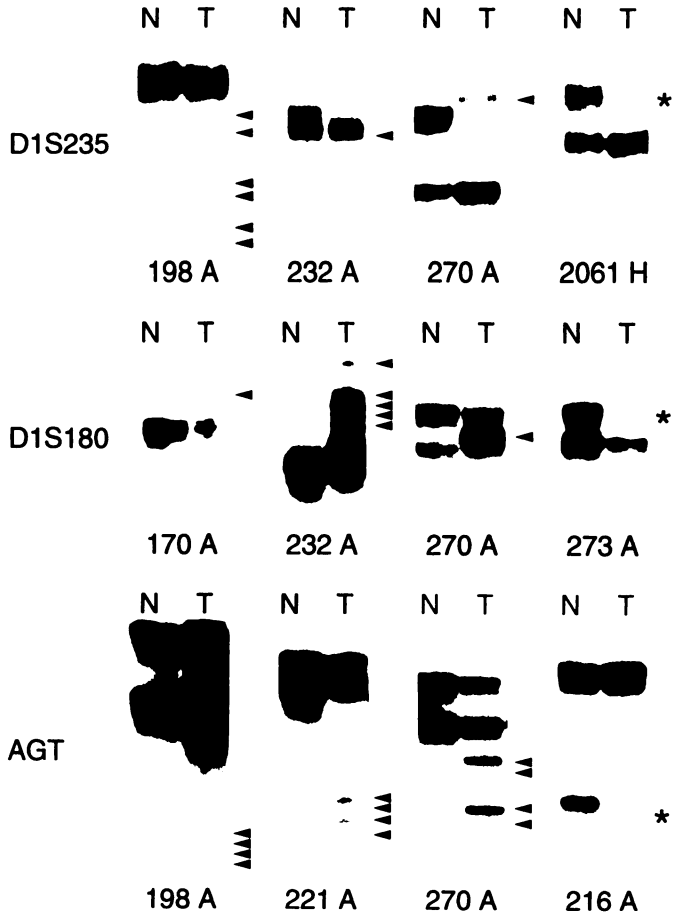

Fig. 2. Dinucleotide repeat polymorphisms in normal and tumor tissues in male GCT. *Ordinate*, microsatellite loci; *bottom abscissas*, tumor numbers. *Arrowheads*, decreased or increased size of repeats in tumor DNA; *, LOH; *N*, normal; *T*, tumor.

LOH at 1q42. In addition to the 3 of 54 (5.6%) informative cases which showed LOH at *D1S74*, allelic losses (absent allele or alleles with decreased signal intensity) were detected at all 3 microsatellite loci. The frequency of LOH observed in informative cases was: 4 of 39 (10.3%) at *D1S235*; 7 of 53 (13.2%) at *D1S180*; and 3 of 56 (5.4%) at *AGT*. In all, 12 (17.9%) cases showed LOH in at least 1 of the loci among 67 informative tumors.

Histological Associations of $(CA)_n$ Instability, Rearrangement, and LOH. We also explored the relationship between RER+ phenomenon and LOH with histological differentiation. The RER+ phenomenon was noted in all histological types of tumors other than teratomas but most commonly in embryonal carcinomas and yolk sac tumors (Table 1). The exception was an extra allele found with the *AGT* gene-associated $(CA)_n$ repeat in a cell line derived from a teratoma (T-240A). However, the primary tumor from which this cell line was derived did not have a similar extra allele, suggesting that the cell line represents a clonal alteration within the tumor. Both mixed histology tumors which showed RER+ had embryonal carcinoma as one of the components. These results suggest that the RER+ phenomenon may be a common feature of nonteratomatous GCTs, which included embryonal carcinomas, yolk sac tumors, and seminomas. The additional bands observed with the hybridization of *D1S74* probe were seen in 2 teratomas, 1 mixed embryonal carcinoma/yolk sac tumor, and 1 seminoma. The distribution of LOH at 1q42–43 also varied among histological subsets, with the highest incidence in teratomas (Table 2). Of 12 of 67 informative cases which showed LOH at least 1 locus, 8 of 27 (29.6%) were teratomas, 1 of 17 (5.9%) was an embryonal carcinoma, and 3 of 13 (23.1%) were of mixed histology, while no LOH was found in 5 each of informative yolk sac

tumors and seminomas. The differences between teratomas and embryonal carcinomas were statistically significant ($P < 0.01$). Thus, these results suggest that RER+ and LOH at 1q42–43 may be unrelated genetic events.

Discussion

The high frequency of microsatellite instability caused by the *hMSH2* gene linked to chromosome 2 (9, 17, 18) was initially implicated in hereditary nonpolyposis colorectal cancer (1). Subsequently, a high incidence of $(CA)_n$ sequence instability has been found in several sporadic tumor types, which included colorectal (12–28%) (1–3), endometrial (17%) (6), pancreatic (67%), ovarian (16%) (5), gastric (22.7–39%) (5, 7, 19), and breast (10.6%) (8) carcinomas. Thus, the 18.2% microsatellite instability noted by us in GCTs is similar in incidence to previous reports in sporadic tumor types. These data suggest that microsatellite instability is an important phenomenon in GCT development. A previous study investigating $(CA)_n$ markers at 7 different chromosomal arms other than 1q in 86 GCT, including 19 bilateral and familial cases, did not reveal any instability (11). The presence of an instability locus in the 1q42–43 region is further supported by the observation of rearrangements with *D1S74* in 4 cases of GCT, although its relationship to $(CA)_n$ microsatellite instability is not clear. $(CA)_n$ instability, however, was not observed in GCT at 6 other loci mapped to 12q, 17p, and 18q (data not shown). A detailed analysis of 1q42–43 region, and possibly also other regions, is required to further understand the role of microsatellite instability in male GCTs.

Of the 12 RER+ cases observed at 1q42–43, the majority were in embryonal carcinomas (37.5%) and yolk sac tumors (40%) (Table 1). This clearly demonstrated that the RER+ phenomenon in GCT may be more prevalent in embryonal carcinomas and yolk sac tumors than in teratomas and seminomas. LOH at 1q42–43, however, was more frequent in teratomas (29.6%) compared to embryonal carcinoma (5.9%) (Table 2). We have previously demonstrated by allelotype analysis involving the entire genome (20) and detailed analysis of LOH on chromosome 1[4] that teratomas exhibit a >2-fold increase in LOH compared to embryonal carcinomas. Embryonal carcinomas in the human male are pluripotential tumors which may progress to extraembryonic (yolk sac), trophoblastic (choriocarcinoma), or somatic (teratoma) lineages. Teratomas usually present highly differentiated somatic tissue types. The association of high incidence of somatic instability in embryonal carcinomas and yolk sac tumors suggests a relationship to the undifferentiated and proliferative nature of these tumors. The high frequency of LOH in teratomas has been suggested to be associated with somatic differentiation by loss or suppression of certain controlling loci (20). The nature of the genetic instability observed here and its relationship to the origin of male GCTs and histological differentiation phenotypes need to be investigated further.

References

1. Aaltonen, L. A., Peltomaki, P., Leach, F. S., Sistonen, P., Pylkkanen, L., Mecklin, J-P., Jarvinen, H., Powell, S. M., Jen, J., Hamilton, S. R., Petersen, G. M., Kinzler, K. W., Vogelstein, B., and de la Chapelle, A. Clues to the pathogenesis of familial colorectal cancer. Science (Washington DC), *260:* 812–816, 1993.
2. Thibodeau, S. N., Bren, G., and Schaid, D. Microsatellite instability in cancer of the proximal colon. Science (Washington DC), *260:* 816–819, 1993.
3. Ionov, Y., Peinado, M. A., Malkhosyan, S., Shibata, D., and Perucho, M. Ubiquitous somatic mutations in simple repeated sequences reveal a new mechanism for colonic carcinogenesis. Nature (Lond.), *363:* 558–561, 1993.
4. Gonzalez-Zulueta, M., Ruppert, J. M., Tokino, K., Tsai, Y. C., Spruck, C. H III., Miyao, N., Nichols, P. W., Hermann, G. G., Horn, T., Steven, K., Summerhayes, I. C., Sidransky, D., and Jones, P. A. Microsatellite instability in bladder cancer. Cancer Res., *53:* 5620–5623, 1993.
5. Han, H-J., Yanagisawa, A., Kato, Y., Park, J-G., and Nakamura, Y. Genetic instability in pancreatic cancer and poorly differentiated type of gastric cancer. Cancer Res., *53:* 5087–5089, 1993.
6. Risinger, J. I., Berchuck, A., Kohler, M. F., Watson, P., Lynch, H. T., and Boyd, J. Genetic instability of microsatellites in endometrial carcinoma. Cancer Res., *53:* 5100–5103, 1993.
7. Mironov, N. M., Aguelon, M. A. M., Potapova, G. I., Omori, Y., Gorbunov, O. V., Klimenkov, A. A., and Yamasaki, H. Alterations of $(CA)_n$ DNA repeats and tumor suppressor genes in human gastric cancer. Cancer Res., *54:* 41–44, 1994.
8. Wooster, R., Cleton-Jansen, A-M., Collins, N., Mangion, J., Cornelis, R. S., Cooper, C. S., Gusterson, B. A., Ponder, B. A. J., von Deimling, A., Wiestler, O. D., Cornelisse, C. J., Devilee, P., and Stratton, M. R. Instability of short tandem repeats (microsatellites) in human cancers. Nat. Genet., *6:* 152–156, 1994.
9. Peltomaki, P., Aaltonen, L. A., Sistonen, P., Pylkkanen, L., Mecklin, J-P., Jarvinen, H., Green, J. S., Jass, J. R., Weber, J. L., Leach, F. S., Petersen, G. M., Hamilton, S. R., de la Chapelle, A., and Vogelstein, B. Genetic mapping of a locus predisposing to human colorectal cancer. Science (Washington DC), *260:* 810–812, 1993.
10. Hall, N. R., Murday, V. A., Chapman, P., Williams, M. A. T., Burn, J., Finan, P. J., and Bishop, D. T. Genetic linkage in Muir-Torre syndrome to the same chromosomal region as cancer family syndrome. Eur. J. Cancer, *30:* 180–182, 1994.
11. Lothe, R. A., Peltomaki, P., Meling, G. I., Aaltonen, L. A., Nystrom-Lahti, M., Pylkkanen, L., Heimdal K., Andersen, T. I., Moller, P., Rognum, T. O., Fossa, S. D., Haldorsen, T., Langmark, F., Brogger, A., de la Chapelle, A., and Borrensen, A-L. Genomic instability in colorectal cancer: relationship to clinicopathological variables and family history. Cancer Res., *53:* 5849–5852, 1993.
12. Mathew, S., Murty, V. V. V. S., Hunziker, W., and Chaganti, R. S. K. Subregional mapping of 13 single-copy genes on the long arm of chromosome 12 by fluorescence *in situ* hybridization. Genomics, *14:* 775–779, 1992.
13. Dracopoli, N. C., O'Connell, P., Elsner, T. I., Lalouel, J-M., White, R. L., Buetow, K. H., Nishimura, D. Y., Murray, J. C., Helms, C., Mishra, S. K., Donis-Keller, H., Hall, J. M., Lee, M. K., King, M-C., Attwood, J., Morton, N. E., Robson, E. B., Mahtani, M., Willard, H. F., Royle, N. J., Patel, I., Jeffreys, A. J., Verga, V., Jenkins, T., Weber, J. L., Mitchell, A. L., and Bale, A. E. The CEPH consortium linkage map of human chromosome 1. Genomics, *9:* 686–700, 1991.
14. Cuticchia, A. J., Pearson, P. L., and Klinger, H. P. (eds.). Chromosome coordinating meeting, 1992. Genome Priority Reports, Vol 1. Basel: S. Karger AG, 1993.
15. Kotelevtsev, Y. V., Clauser, E., Corvol, P., and Soubrier, F. Dinucleotide repeat polymorphism in the human angiotensinogen gene. Nucleic Acids Res., *19:* 6978, 1991.
16. Weissenbach, J., Gyapay, G., Dib, C., Vignal, A., Morissette, J., Millasseau, P., Vaysseix, G., and Lathrop, M. A second-generation linkage map of the human genome. Nature (Lond.), *359:* 794–801, 1992.
17. Fishel, R., Lescoe, M. K., Rao, M. R. S., Copeland, N. G., Jenkins, N. A., Garber, J., Kane, M., and Kolodner, R. The human mutator gene homolog *MSH2* and its association with hereditary nonpolyposis colorectal cancer. Cell, *75:* 1027–1038, 1993.
18. Leach, F. S., Nicolaides, N. C., Papadopoulos, N., Liu, B., Jen, J., Parsons, R., Peltomaki, P., Sistonen, P., Aaltonen, L. A., Nystrom-Lahti, M., Guan, X-Y., Zang, J., Meltzer, P. S., Yu, J-W., Kao, F-T., Chen, D. J., Cerosaletti, K. M., Fournier, R. E. K., Todd, S., Lewis, T., Leach, R. J., Naylor, S. L., Weissenbach, J., Mecklin, J-P., Jarvinen, H., Petersen, G. M., Hamilton, S. R., Green, J., Jan, J., Watson, P., Lynch, H. T., Trent, J. M., de la Chapelle, A., Kinzler, K. W., and Vogelstein, B. Mutations of a *mutS* homolog in hereditary nonpolyposis colorectal cancer. Cell, *75:* 1215–1225, 1993.
19. Rhyu, M-G., Park, W-S., and Meltzer, S. Microsatellite instability occurs frequently in human gastric carcinoma. Oncogene, *9:* 29–32, 1994.
20. Murty, V. V. V. S., Bosl, G. J., Houldsworth, J., Meyers, M., Mukherjee, A. B., Reuter, V., and Chaganti, R. S. K. Allelic loss and somatic differentiation in human male germ cell tumors. Oncogene, *9*, in press, 1994.

[4] S. Mathew, V. V. V. S. Murty, G. J. Bosl, and R. S. K. Chaganti, unpublished results.

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