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NOTES

Integration of Friend Murine Leukemia Virus into Both Alleles of the p53 Oncogene in an Erythroleukemic Cell Line

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The Friend virus-transformed erythroleukemic cell line DP16-9B4 has undergone a complex rearrangement of the p53 oncogene and lacks any detectable expression of the p53 protein. We report here characterization of both p53 alleles in this cell line and identify independent integrations of Friend murine leukemia virus sequences into the coding region of both alleles.

Several leukemia and tumor cell lines with rearrangement of the p53 oncogene have been described (2, 16, 18, 19, 27, 32, 33). These rearrangements result in loss of p53 protein or production of truncated forms of the p53 protein. We have described several Friend virus-induced erythroleukemic cell lines that have undergone rearrangement of the p53 oncogene (2, 18, 27). One cell line, which did not make p53 protein, showed a complex rearrangement of both alleles of the p53 oncogene (18). To understand the mechanism for these rearrangements we have molecularly cloned both p53 alleles from this cell line. We show in this study that the helper virus Friend murine leukemia virus (F-MuLV) has integrated independently into both alleles of the p53 gene.

DP16-9B4 is an erythroleukemic cell line previously described as not making p53 protein and being less tumorigenic when injected into syngeneic mice, compared with a normal p53-expressing erythroleukemic cell line, DP16-9B3, isolated from the same mouse (18). Southern blots (25, 30) of DP16-9B4 genomic DNA digests, using a p53 cDNA as probe (10), revealed a loss of the normal 17.4-kilobase-pair (kbp) *Eco*RI fragment and the appearance of a novel *Eco*RI fragment greater than 23 kbp (Fig. 1). Digestion with *Bgl*II revealed four bands of 16.6, 14.3, 8.4, and 6.1 kbp, compared with 18 kbp for the normal p53 gene, suggesting that both alleles are rearranged. The 3.1-kbp *Eco*RI and the 2.3-kbp *Bgl*II fragments represent a processed pseudogene which is inactive (36).

To understand in more detail the molecular mechanisms which led to the rearrangement of the p53 gene in DP16-9B4, we chose to clone both rearranged alleles. Genomic DNA from DP16-9B4 was digested with *Eco*RI, and DNA greater than 23 kbp was enriched for by preparative gel electrophoresis (15). This DNA was ligated with purified phage λL47.1 arms (14), packaged in vitro (Promega Biotec, Mississauga, Ontario, Canada), and plated on *Escherichia coli* WA803 (34). The lambda library was screened with the p53 cDNA clone 27.1a (10) as previously described (15). In the preliminary restriction analysis of 14 clones, two common banding patterns emerged, indicating that both rearranged alleles were cloned. A pair of recombinant phage clones from each group were randomly chosen. Lines D and E of Fig. 2 show the restriction maps of clones 713/533 and 711/523, respectively. The exploded view reveals the new cleavage sites introduced by the foreign DNA sequences. The last known restriction sites or p53-hybridizing sequences compared with the normal p53 gene are indicated above. Both rearrangements appear to be the result of an integration of foreign sequences: in clones 713/533, an insert of ≈3.5 kbp with an apparent loss of a region containing exon 10, and in clones 711/523, an insert of ≈4.8 kbp with an apparent loss of a region containing exons 5 to 7. Although there is hybridization of the p53 cDNA probe to at least exon 9 of clones 711/523, it is not clear whether a portion or all of exon 8 remains intact.

To test whether these rearrangements may be virus mediated, we subjected a duplicate set of Southern blots to a panel of endogenous and exogenous viral probes. These

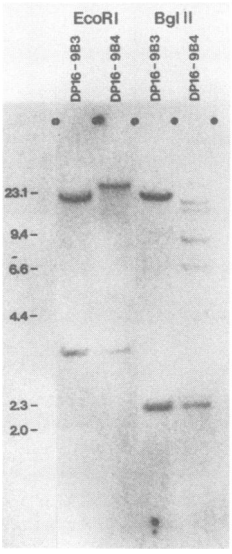

FIG. 1. Southern blot analysis of the p53 gene in Friend cell lines from mouse DP16. Genomic DNA from cell lines DP16-9B3 and DP16-9B4 was digested with *Eco*RI or *Bgl*II, fractionated by electrophoresis in 0.8% agarose gels, transferred to nylon membranes, and probed with nick-translated murine p53 cDNA 27.1a (10).

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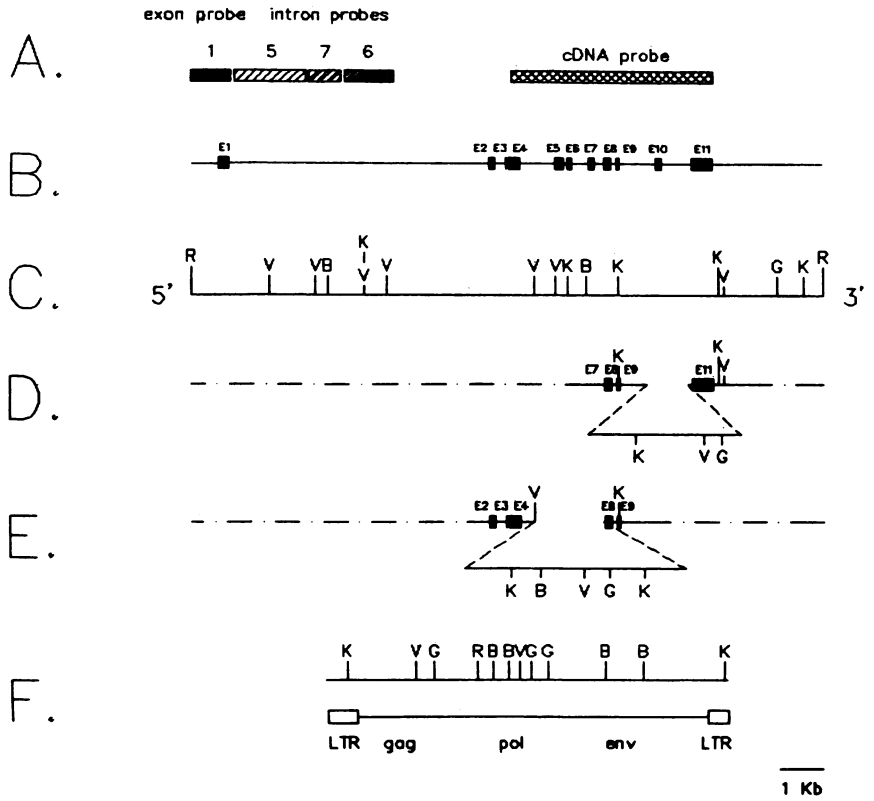

FIG. 2. Restriction enzyme maps of normal and rearranged p53 genes. (A) Regions of the p53 probes used. (B and C) Restriction map of the mouse p53 gene, taken from published data (1, 22, 33); exons as determined by Bienz et al. (1) are depicted as solid boxes. (C) Cleavage sites for enzymes *Eco*RI (R), *Bam*HI (B), *Bgl*II (G), *Kpn*I (K), and *Pvu*II (V) for the genomic clone of the murine erythroleukemia cell line CB7 p53 gene (27). (D and E) The genomic clones 713 and 533 have an insert of ≈3.5 kb (D), while the genomic clones 711 and 523 have an insert of ≈4.8 kb (E). The approximate sites of integration are indicated by the last known restriction sites or p53-hybridizing sequences. The exploded view indicates the size and restriction pattern of the inserted foreign sequences. (F) Physical map of the F-MuLV genome, derived from the published data of A. Oliff, W. Koch, and R. Friedrich (12, 13, 21).

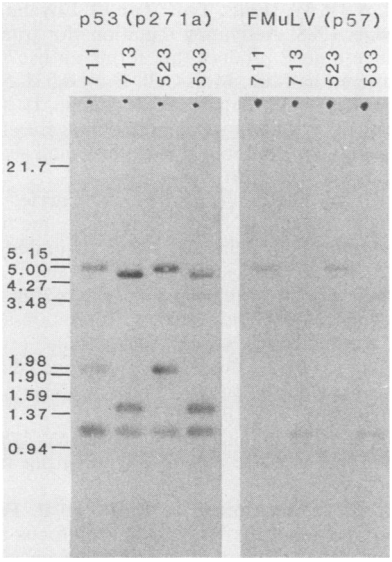

FIG. 3. Southern blot analysis of the λL47.1 recombinant clones 711, 713, 523, and 533. The recombinant inserts were isolated from λL47.1 vector arms by *Eco*RI digestion followed by two rounds of preparative gel electrophoresis. The purified insert DNA was digested with *Kpn*I, fractionated on duplicate 1.0% agarose gels, transferred to nylon membranes, and probed with either nick-translated murine p53 cDNA 27.1a (10) or F-MuLV viral DNA derived from p57-FMuLV (21), as indicated.

were comprised of the intracisternal A particle long terminal repeat (8) and the spleen focus-forming virus (35), the spleen focus-forming virus gene *env* (35), F-MuLV (21), and F-MuLV *env* (20) probes. p57-FMuLV contains the entire viral genome of the helper virus clone 57 (21). It was the only member of this panel which specifically hybridized to the recombinant clones. Southern blots of *Kpn*I digests of the clones are shown in Fig. 3. The blot shown in the left panel of Fig. 3 was probed with p27.1a, which contains the cDNA sequences of exon 4 through exon 11 (10). In the right panel of Fig. 3, a duplicate Southern blot was probed with p57-FMuLV (21). The 1.2-kbp hybridizing fragment of clones 713/533 and the 5.0-kbp fragment of clones 711/523 indicate the inclusion of F-MuLV sequences into the respective *Kpn*I fragments. A restriction map of the F-MuLV is shown in Fig. 2F for comparison.

The 713/533 clones have an inserted sequence which hybridizes to the entire helper virus probe but not specifically to the envelope region. From the restriction map of the helper virus and that of the insert, it seems likely that these sequences represent the first 2.0 kbp of the 5′ end of F-MuLV and loss of the envelope and 3′ long terminal repeat region. The rearrangement of the second allele, represented by clones 711/523, is considerably more complex. The first 600 bp of the insert contains a new *Kpn*I site and it also hybridizes to the helper virus probe. From this hybridization and the restriction map of the helper virus, these sequences

are likely to represent a portion of either the 5′ or 3′ long terminal repeat, both of which contain the only *Kpn*I sites. The remaining 2.8 kbp of the insert 3′ to this does not hybridize to either the helper virus or p53 cDNA probes; it may therefore represent 3′ p53 intron sequences.

It is clear that these rearrangements occurred in vivo during the progression of Friend virus-induced erythroleukemia and are not artifacts of molecular cloning. First, the p53 rearrangements in DP16-9B4 have also been found in other cell lines independently isolated from the same spleen (18). Second, Southern blot analysis of DNA from DP16-9B4 using an F-MuLV-*env*-specific probe revealed only a single *env*-hybridizing fragment, which did not comigrate with the rearranged alleles (2). This indicates that internal viral deletions existed in the p53 gene of the original DNA and that there are at least three F-MuLV integration sites in the DP16-9B4 genome. Finally, the phage library was not amplified, and several clones with the same restriction pattern were isolated. Consistent with our finding is the previously published example of Moloney leukemia virus integrating into the p53 gene of an Abelson virus-induced lymphoma cell line. The integrated provirus also contained a 3-kb insertion of foreign sequences into the Moloney murine leukemia virus *pol* and *env* regions (32). The significance of the insertions or deletions within viral sequences remains to be understood.

There have been other examples of inactivation of the p53 gene by rearrangements. The human myeloid leukemia cell line HL60 had undergone major deletions in both alleles of p53 (33). One acute promyelocytic leukemia patient showed rearrangement of one p53 allele and loss of the other (24). Recently, rearrangement of the p53 gene has been reported in three of six osteogenic sarcoma tumors and three of five osteogenic sarcoma cell lines, as well as one Hodgkin's lymphoma cell line (16).

At the moment it is not known what selective advantage inactivation of the p53 gene confers on cells. A possible advantage the p53-negative cells may have over cells expressing p53 is the ability to escape the host immune response. The p53 protein was originally identified as a tumor antigen (4) and is found in the plasma membrane (17) and on the cell surface (28). Lung and breast carcinoma patients (3) and tumor-bearing animals have been shown to make antibodies against p53 protein (4, 29). It is possible that cells expressing large amounts of an altered p53 protein on their cell surface could be subject to an immune response. Hence, cells that do not express the altered p53 protein would then have a selective advantage.

Alternatively, if p53 were acting as a "tumor suppressor" then inactivation of the gene may play a role in transformation. This argument is met with opposition from gene transfer experiments, in which the p53 gene can act as a dominant oncogene (5, 6, 9, 11, 23, 26), and experiments in which cell lines not making p53 protein are less tumorigenic than p53-positive cells when injected subcutaneously (18, 31). However, it has recently been shown that p53 genes capable of immortalizing primary cells have undergone activating mutations (7). This results in p53 proteins which then bind heat-shock protein hsp70 and lose an epitope to the monoclonal antibody PAb246 (7). Further, Munroe and Benchimol have suggested that these mutations may allow the mutant p53 gene to act in a dominant negative manner. The mutant p53 protein may act to inactivate the wild-type p53 protein or gene function (D. G. Munroe and S. Benchimol, submitted for publication). The dominant-acting p53 oncogene in these situations may therefore be similar to a loss of p53 activity by retrovirus insertion. Experiments are now under way to test these hypotheses.

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ADDENDUM

Y. Ben-David et al. have recently described an erythroleukemic cell line that shows integration of the spleen focus-forming virus into one allele of the p53 gene (Oncogene **3**:179–185, 1988).

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