Advances in Brief

Analysis of a Metastasizing Testicular Mixed Gonadal Stromal Tumor with Osteosarcoma Components Suggests That a Malignant Tumor with the Histology of Osteosarcoma May Develop without Primary Involvement of RBl and TP53

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Abstract

A malignant stromal tumor of the testis with an osteosarcoma component and five of its metastases mainly containing osteosarcoma have been analyzed for RBl and TP53 abnormalities. Whereas in the primary tumor and in some of the metastases loss of heterozygosity could not be detected for RBl or for the 17p13 region in which TP53 is located, other metastases showed such losses of heterozygosity. By polymerase chain reaction analysis an 18-base pair deletion from exon 5 of the TP53 gene was found in a small proportion of primary tumor cells and in one of the metastases, but not in the other metastases. Therefore, in this case neither RBl nor TP53 seems to play an essential role in the initiation of osteosarcoma.

Introduction

In osteosarcoma a frequent loss of heterozygosity of chromosome 13 including the region of the RBl gene has been observed (1-4). Moreover, structural abnormalities of the RBl gene in osteosarcoma are not uncommon (4-8) and expression studies have shown altered expression in osteosarcoma (9, 10). The same holds true for the TP53 gene. In osteosarcoma loss of heterozygosity of chromosome 17 occurred at least as frequently as that of chromosome 13 (5, 11). The common region of loss is 17p13, where TP53 is located (11). In some cases of osteosarcoma TP53 mutations have been characterized (12-15) and altered expression has also been shown to occur (12-14). Thus, although far from exclusively in osteosarcoma, RBl and TP53 may play a role in the development of this type of tumor. Therefore, we analyzed a mixed gonadal stromal tumor of the testis with heterologous mesenchymal elements mainly containing osteosarcoma for abnormalities of these genes.

Materials and Methods

The primary tumor as well as its metastases have been described previously (16). All the metastases had osteosarcoma as their only tumor component present in 70-80% of the cells.

Southern analysis was carried out on DNA isolated from the primary tumor and remaining metastases. Southern analysis of the tumor and its metastases was performed as described previously (5). The probes used were the intragenic RBl marker p68, p144D6 at 17p13, the RBl cDNA clones pG3.8M and pG62, and the p53 cDNA clone pR4.2. Quantitative densitometric analysis of the autoradiographs was carried out by automated scanning densitometry (LKB Gelscan XL).

Genomic sequences of TP53 were amplified by PCR. The primers used allowed the coding sequences of the TP53 gene to be derived from the TP53 sequence (17). The numbers in the code indicate the exons or pairs of exons amplified between the 5' and 3' primers (denoted L and R, respectively). The TP53 primer sets were: 23L (CCTTTTGGCACTTACCAGAA), 23R (CCTTTTGGCACTTACCAGAA); 4L (TTTTCACCATCCTACAGGG), 4R (ACGGCGCAACTGACGTCGGA); 5L (TTCCTTTCCTGCACTTACCC), 5R (GTGATCCTAAACTCCACAG); 78L (CCTTTGACGTTACCACCTCA), 78R (GTGAGGCTCCCCCTTTCTTG); 89L (CCTATCCTGATTAGGGTGA), 89R (CCAACTCTTTGACTCTCGAG); 10L (CCTTGTTGCTGAGATGCTGT), 10R (CTGAAGTTCTCCACTGTTG); 11L (GCTTCTGCTCTTACAGCCA), 11R (TGCTTCTGACGACACATCT).

The amplified sequences were subjected to a SSCP analysis (18). Abberrant PCR products were directly sequenced using the same primers as for the PCR.

Results

The result of a Southern analysis of RsaI-digested tumor DNA hybridized with p68 is shown in Fig. 1 A. For the inguinal metastasis and three of the four lung metastases loss of the longer allele can be seen. For the primary tumor and remaining lung metastasis (Fig. 1 A, Lane d) no reduction of the hybridization signal was observed. Southern analysis of HindIII-digested DNA of the tumor components with the RBl cDNA probes pG3.8M and pG62 did not result in any aberrant pattern (results not shown). To analyze loss of heterozygosity for 17p13.1, the region of the TP53 gene, the variable number of tandem repeats probe p144D6 was hybridized to RsaI digested tumor DNA. The results are shown in Fig. 1 B. A clear loss of the longer allele can be seen for the inguinal metastasis. The primary tumor and two lung metastases (Fig. 1 B, Lanes e and d) retained this allele. A clearly reduced signal for the longer allele is visible in Lanes e and f containing DNA from the other lung metastases. Southern analysis of the tumor and its metastases with TP53 cDNA did not result in any aberrant pattern (results not shown).

The DNA was also subjected to a PCR analysis with the TP53 primers (Fig. 2). With the exception of the inguinal metastasis, all tumor material gave amplified products of the TP53 gene.
length expected. The inguinal metastasis, however, appeared with a shorter product in addition to the normal one upon PCR with primers 56L and 56R, indicating the presence of a deletion. Finding the mutation only in the inguinal metastasis which consisted mainly of osteosarcoma raises the question of its origin. The primary tumor also contained osteosarcoma elements; therefore it cannot be excluded that a proportion of its cells also contained the mutation. A minority of cells containing a mutation may escape detection by PCR since in the competition for primers they will be competed out by the cells containing the normal alleles. Fig. 3 shows how we tried to address this problem. After PCR of DNA from the primary tumor and gel electrophoresis of the amplified products part of the agarose gel at the position where the shorter product if present should be expected was cut out. This material was used in a second PCR. Gel electrophoresis of the product revealed indeed the presence of the shorter product as well as the normal one (of which also evidently enough molecules were present in the gel piece to give a visible product after amplification) and heteroduplexes of normal and deleted products (Fig. 3). The mutant TP53 allele was therefore present in only a small proportion of cells of the primary tumor. It was not observed in any of the lung metastases upon application of the same procedure to them.

Sequencing revealed a deletion of 18 base pairs in exon 5 of the TP53 gene, corresponding to a sequence of 6 amino acid residues deleted including an arginine, a cysteine, a proline, two histidines and a glutamic acid. From Fig. 4 the different alternatives for such a deletion can be inferred. To detect possible further mutations of TP53 we carried out SSCP analysis of all exons. Normal patterns were revealed for all tumor material with the exception of the inguinal metastasis analyzed with primers 56L and 56R. The aberrant pattern shown by this metastasis was also faintly visible together with a dominating normal pattern upon SSCP analysis of the primary tumor with the 56L and 56R primers (results not shown).

Discussion

The results of our analysis of loss of heterozygosity for the RB1 and TP53 regions of this tumor give a heterogeneous picture. For RB1 one lung metastasis did not reveal loss of heterozygosity in contrast to the inguinal metastasis and the other three lung metastases, which all showed clearly loss of one allele. The inguinal metastasis also reveals a clear loss of heterozygosity for the 17p13 region. Two lung metastases showed no allelic loss for this region. The two remaining lung metastases, however, partially had loss of one allele. In the primary tumor loss of heterozygosity could not be detected for RB1 or for the 17p13 region. It might be obscured by the presence of a large majority of non-osteosarcoma tumor cells that do not have a loss or partial deletion of one of the chromosomes 13 or 17. This argument cannot hold true, however, for the metastases that showed retention, because they contained osteosarcoma as their only tumor component in 70–80% of the cells.
The TP53 deletion was found in the primary tumor and in the inguinal metastasis, but not in the lung metastases. SSCP analysis did not reveal any further mutations in the TP53, gene. Because two of the lung metastases partially had loss of heterozygosity for the 17p13 region, there is no correlation between loss of heterozygosity at 17p13 and the occurrence of a TP53 mutation. The finding of loss of 17p13 without a concomitant occurrence of a TP53 mutation may be indicative of the presence of another tumor suppressor gene in the 17p13 region, as also suggested earlier (19).

The sequences flanking the 18-base pair deletion show a 7-base pairs direct repeat. The deletion might thus well be a consequence of a slipped mispairing of these two direct repeats during DNA replication. The same mutation has also been found in a human breast tumor (20).

We have already suggested earlier that the RB1 gene alone cannot play a crucial role in the initiation of osteosarcoma (5). If RB1 and TP53 genes would play an essential role in the development of the osteosarcoma component of this gonadal tumor one would expect to find in all metastases the same aberrations as in the primary tumor. Therefore, our present results can be taken as an indication that neither RB1 nor TP53 are essential for the development of a malignant tumor with the histology of osteosarcoma. In general, caution is in order when claiming a tissue-specific involvement of oncogenes and tumor suppressor genes in tumorigenesis.

References

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