Rb1 GENE MUTATIONS IN OSTEOSARCOMA

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Genetic alternations, such as mutations caused inactivities of tumor suppressor gene, have been identified in a wide variety of tumors, including osteosarcoma. Osteosarcoma is the most frequent primary malignant bone tumor that occurs in the extremities of young adolescents in most cases. Because of the high frequent occurrence of this type of tumor in hereditary retinoblastoma patients, involvement of the Rb1 gene mutations was suspected in the development of osteosarcoma, and a few reports have shown alternations of the Rb1 gene in osteosarcoma. We studied Rb1 gene mutations in 9 osteosarcoma samples and one cell line (OS 732) to explore the types and mechanism of Rb1 gene mutations in osteosarcoma.

MATERIALS, METHODS AND RESULTS

Tissue samples were obtained from osteosarcoma patients diagnosed by clinic and pathobiology from 1994 to 1995. The control samples were normal peripheral blood obtained from normal persons without immediate family or past history of malignant disease. Genomic DNAs from above samples were extracted according to the convention method, and the 27 exons of Rb1 gene were amplified by PCR respectively. The diluted PCR products were denatured and then loaded on 8% nondenatured polyacrylamide gels. Electrophoresis were performed with constant power of 30W for 3 to 6 h at room temperature. Silver staining was used to develop the gels. There were four samples appeared abnormal band shifts compared with control samples. Direct sequencing of the PCR fragments from all the samples were performed. There is a mutation found in one of the samples. The mutation occurred in the position of Rb1 cDNA 1804 which is a C to T single base substitution. The mutation leads the arginine codon CGA changed into stop codon TGA.

DISCUSSION

Recently, the results from osteosarcoma study showed that the abnormal expression of Rb1 gene or alterations of Rb1 protein’s function played a very important role in the development of osteosarcoma. There are no “hot spots” of mutation in the Rb1 gene in retinoblastomas as well as in other malignancies. Based on the optional condition of PCR-SSCP, we have found 4 samples had abnormal bandings compared with control samples, and they distributed in four exons. One of these four samples was demonstrated to exite a C→T single base substitution. This mutation results in a codon CGA changed into stop codon TGA.

Mutations caused premuted stop codon in Rb1 gene is a common reason that leads inactivity of Rb1 protein. The mutation of C→T is usually considered to be due to the higher mutation rate of 5-methylcylosine in CpG. And the characterized flanking sequence around mutation point may be another important factor causing mutation. Previous studies have indicated that almost 50% mutations in Rb1 gene in retinoblastomas involved the CGA coden. Our finding is similar to that of Bunichiorowadayama et al.. According to our result and others, we concluded that exons with CGA code involved should be considered as relative hot spots when detecting mutation in Rb1 gene. We will adjust the experimental conditions for sequencing in order to look for higher mutation detection rate to do the further molecular mechanism study for the osteosarcom.

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