Journal of Korean Medical Science
Vol. 11, No. 1, 94-98 February 1996

Deletion of Rb1 Gene in Late Osteosarcoma From Survivor of Unilateral Retinoblastoma

—A Case Report—

Soo-Yong Lee[1),3)], Dae-Geun Jeon[1)], Jong-Seok Lee[1)],
Chang-Seon Hwang[3)], Kwang Huh[2)], Tai-Won Lee[4)], Seok-Il Hong[2),3)]

Department of Orthopedic Surgery[1)], Clinical Pathology[2)], Laboratory of Cell Biology[3)], Department of Opthalmology[4)] Korea Cancer Center Hospital, Seoul, Korea

It has been well known that the survivors of retinoblastoma are prone to have osteosarcoma. But the secondary tumor usually occurs in bilateral, hereditary type of retinoblastoma. We report one case of osteosarcoma in a survivor of unilateral, sporadic retinoblastoma. A fourteen year old male presented with a painfully swollen distal forearm of 2 month duration. He had enucleated his left eye 10 years ago due to retinoblastoma with no other adjuvant therapy. We managed him with our conventional protocol and identified deletion of Rb gene from his pathological specimen by using the PCR-RFLP method. This result is unusual for unilateral nonhereditable retinoblastoma and may suggest gene level change even in sporadic cases. And Rb gene study may be helpful for unilateral, sporadic retinoblastoma patient in detecting the possiblity of late osteosarcoma.

Key Words : *Rb1 gene deletion, Osteosarcoma, Unilateral retinoblastoma*

INTRODUCTION

Retinoblastoma is one of the group of childhood tumors to which predisposition can be inherited as an autosomal dominant trait (30~40 %) and is supposed to develop according to Knudson's two-hit theory (Knudson, 1971 ; Cavenee et al., 1983 ; Dryja et al., 1984). The model specifies that familial retinoblastoma is due to a germline mutation and a subsequent somatic mutation. In contrast, sporadic type results from two somatic mutation. In bilateral retinoblastoma, incidence of late tumor is about 15 % and is exceptional in unilateral cases. We report a case of osteosarcoma with Rb gene deletion following unilateral retinoblastoma.

Address for correspondence : *Soo-Yong Lee, Department of Orthopedic Surgery Korea Cancer Center Hospital, 215-4, Gongneung-dong Nowon-gu, Seoul, 139-240, Korea.*
Tel : 970-1241, Fax : 977-2954.
This work was supported by part of a grant from the Ministry of Science and Technology, Korea.

CASE REPORT

Fourteen-year old male visited our outpatient clinic with a painfully swollen wrist of 2 months duration. Simple X-ray showed mixed osteoblastic and osteolytic lesion on the distal radius (Fig. 1). Chest CT and bone scan were done for staging and no metastasis was found.

MRI showed mixed high and low signal intensity and neurovascular structure was not clearly defined due to peritumoral edema (Fig. 2). On laboratory examination serum alkaline phosphatase level was

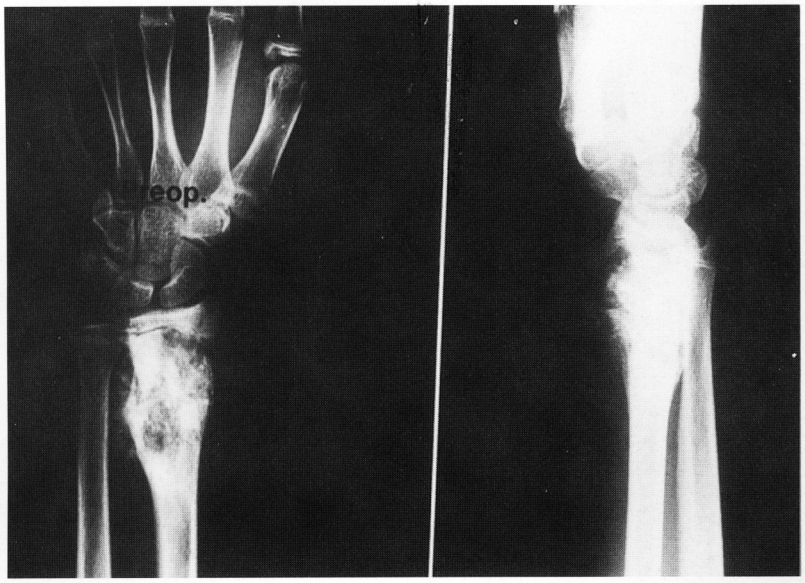

Fig. 1. Simple X-ray shows osteoblastic lesion on distal radius with margination of tumor due to chemotherapeutic effect.

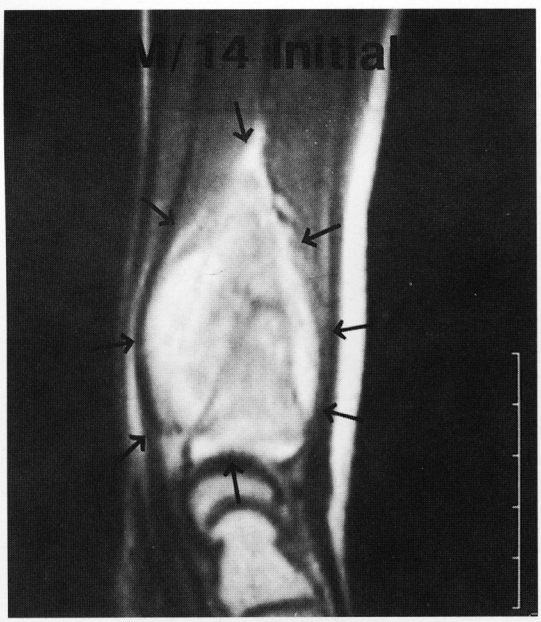

Fig. 2. MRI shows high signal intensity on T2 image and peritumoral edema.

227 U/L and lactic acid dehydrogenase level was 154 U/L. He have had retinoblastoma on his left eye at age 4 and taken enucleation (Fig. 3). At that time he received neither chemotherapy nor radiotherapy on the lesion site. His family history for retinoblastoma or other malignancy was all negative. We reexamined his opposite eye to detect occult retinoblastoma including a retinal photograph. For distal radius, open biopsy was done to confirm the osteosarcoma (Fig. 4) and two cycles of neoadjuvant chemotherapy were performed according to our protocol. One cycle consists of two doses of high dose methotrexate (8g/m^2) in a one week interval and another week later, adriamycin (60mg/m^2) and intraarterial cisplatin (120mg/m^2). Clinical and radiological response was good. After en bloc resection of tumor, the distal radius was reconstructed with proximal fibula (Fig. 5). Pathological response was 94 %. This was categorized as good responder and followed the same protocol as preoperative chemotherapy.

For detection of Rb-LOH in this case of osteosarcoma, we performed PCR-LOH analysis using three set of primers, originally reported by McGee el al. (1990) and Yandell and Dryja (1989). DNA was amplified with primer set 1 (upstream, TTCCATTGAAGAACAAATGG ; downstream, GCAATTGCACAATCCAAGTT) and set 2 (upstream, CTGCAGTCCCACCTCAGCCTCCTTAGTAGA ; downstream, GGATCCGCAGCTCTAGACTAATCCCAGCAC) for Xbal RFLP within intron 17. The size of PCR products was 945 base pairs (bp) for #1 and 190 bp for #2, respectively. Each product was digested with *Xba*I and electrophoresed in 1.5 % agarose gel and we did not find any evidence of LOH. We detected

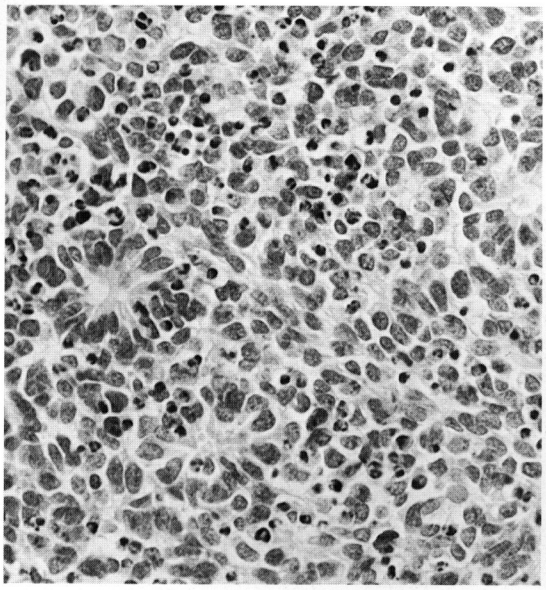

Fig. 3. Light microscopic finding of retinoblastoma composed of dense masses of small round hyperchromatic cells with typical rosettes (H&E, ×100).

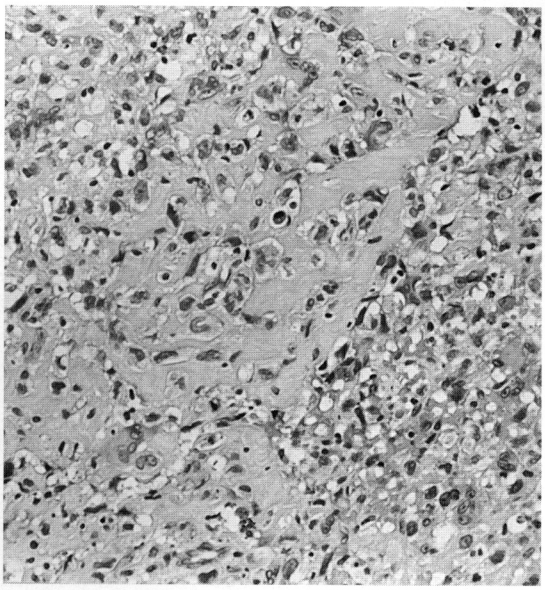

Fig. 4. Osteosarcoma demonstrating osteoid growing in sarcomatous stroma (H&E, ×100).

Rb-LOH in this case using primer set 3 (upstream, CTCCTCCCTACTTACTTGT ; downstream, AATTAACACAAGGTTGTGGTGG) for intron 20 of Rb gene. The products were two DNA bands with 260 bp and 300 bp in normal and only one DNA band with 260 bp in tumor (Fig. 6).

DISCUSSION

By a late extraocular tumor, we mean a malignant tumor which occurs anywhere in the body of a retinoblastoma survivor, and which does not constitute a metastasis, but a new primary malignant neoplasm. Second tumor (Abramson et al., 1984) is mostly an osteosarcoma whether it is radiation induced or not, is prevalent in bilateral retinoblastoma cases and only exceptionally in unilateral cases. It is beyond all doubt that sarcomas may arise in radiated bone and other apparently radiation-induced tumors have been reported too, e.g., rhabdomyosarcoma, reticulosarcoma, fibrosarcoma, and undifferentiated sarcomata. Children with bilateral disease who received radiotherapy had a nearly threefold higher mortality from second tumors than bilateral nonirradiated patients and radiotherapy appears to further enhance the inborn

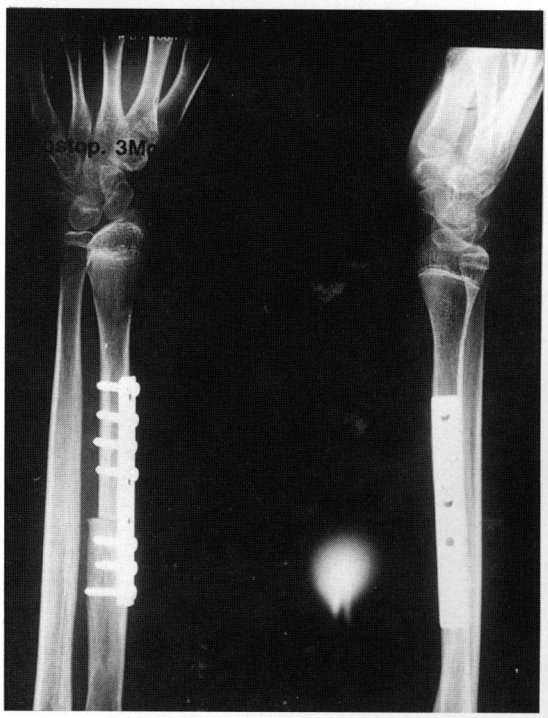

Fig. 5. Postoperative X-ray shows reconstruction of defect with ipsilateral proximal fibula with good alignment.

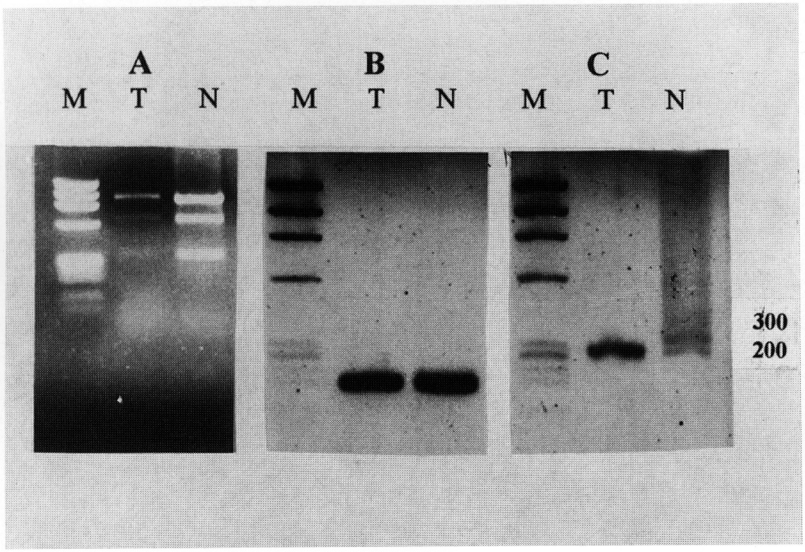

Fig. 6. LOH demostrated by PCR. M, X 174 Hae III marker ; N, normal tissue ; T, tumor. In A, DNA was amplified using PCR primer set 1 flanking a *Xba* I RFLP site in intron 17 of the Rb gene. A 945 base pair amplicon was created. PCR products were digested with *Xba*I and run on an agarouse gel. The cut allele resulted in 630 and 315 base pair bands. As shown, the intensity of the cut bands is not decreased in tumor. B, DNA was amplified using PCR primer set 2 flanking a *Xba* I RFLP site in intron 17 of the Rb gene. A 190 base pair of amplicon was created. PCR product was digested with *Xba*I and run on an agarouse gel. The cut allele was not detected. C, LOH at intron 20 variable number of tandom repeated site shown using primer set 3. The upper allele is partially lost in the tumor.

susceptibility to development of the second cancer (Eng et al., 1993).

However, the prevalence of osteosarcoma in retinoblastoma and its family (Shimke et al., 1974) (not affected case also rather high) may implicate that these two tumors share a common predisposing factor-probably a germ line mutation (Hansen et al.,1985 ; Dryja et al., 1986). Molecular genetic evidence has been reported, that the development of these disparate tumor types involves specific somatic loss of constitutional heterozygosity for the region of human chromosome 13 that includes the Rb1 locus (Hansen et al., 1985). But almost all conventional osteosarcoma occur sporadically and the clinical pattern of this tumor suggests this is a nonhereditary cancer. Araki et al. (1991) reported eight of 23 conventional sporadic osteosarcoma (35 %) showed structural alterations of Rb gene. This result was slightly lower than in retinoblastoma (40~60 %). Inactivation of Rb gene may be needed as the first and main tumorigenic factor in retinoblastoma. But in osteosarcoma, inactivation of Rb gene may be one of many tumorigenic factors and this may be the reason why its age of occurrence is higher and does not show any evidence of inheritable pattern.

Here we report one case of Rb gene deletion in late osteosarcoma in the unilateral and sporadic retinoblastoma survivor which is very rare and unusual.

RRFERENCES

Abramson DH, Ellsworth RM, Kitchin FD, Tung G. *Second nonocular tumors in retinoblastoma survivors. Ophthalmology 1984 ; 91 : 1351-5.*

Araki N, Uchida A, Kimura T, Yoshikawa H, Aoki Y, Ueda T, Takai SI, Miki T, Ono K. *Involvement of the retinoblastoma gene in primary osteosarcomas and other bone and soft tissue tumors. Clin Orthop 1991 ; 270 : 271-7.*

Cavenee WK, Dryja TP, Phillips RA, Benedict WF, Godbout R, Gallie BL, Murphree AL, Strong LC, White RL. *Expression recessive alleles by chromosomal mechanisms in retinoblastoma. Nature 1983 ; 305 : 779-84.*

Dryja TP, Cavenee WK, White RL, Rapaport JM, Petersen R, Albert DM, Bruns GAP. *Homozygosity of chromosome 13 in retinoblastoma. N Engl J Med 1984 ; 310 : 550-3.*

Dryja TP, Rapaport JM, Epstein J, Goorin AM, Weichselbaum R, Koufos A, Cavenee WK. *Chromosome 13 homozygosity in osteosarcoma without retinoblastoma. Am J Hum Genet 1986 ; 38 : 59-66.*

Eng C, Li FP, Abramson DH, Ellsworth RM, Wong FL, Goldman MB, Seddon J, Tarbell N, Boice JD. *Mortality from second tumors among long-term survivors of retinoblastoma. J Natl Cancer Inst 1993 ; 85 : 1121-8.*

Hansen MF, Koufos A, Gallie BL, Phillips RA, Fodstad O, Brogger A, Gedde-Dahl T, Cavenee WK. *Osteosarcoma and retinoblastoma : a shared chromosomal mechanism revealing recessive predisposition. Proc Natl Acad Sci 1985 ; 82 : 6216-20.*

Knudson AG. *Mutation and cancer : statistical study of retinoblastoma. Proc Natl Acad Sci 1971 ; 68 : 820-3.*

McGee TL, Cowley GS, Yandell DW, Dryja TP. *Detection of*

the XbaI RFLP within the retinoblastoma locus by PCR. Nucl Acid Res 1990 18 : 207.

Shimke RN, Lowman JT, Codman GAB. *Retinoblastoma and osteogenic sarcoma in siblings. Cancer 1974 ; 34 : 2077-9.*

Yandell DW, Dryja TP. *Detection of DNA sequence polymorphisms by enzymatic amplification and direct sequencing. Am J Hum Genet 1989 ; 45 : 547-55.*