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An analysis of genetic transmission in a father and son with osteosarcoma

Osteosarkomlu baba ve oğulda genetik geçişin irdelenmesi

Fusun TOKATLI,[1] Rusen COSAR ALAS,[1] Semsi ALTANER,[2] Funda PALA,[3] Kazim UYGUN,[4] Cem UZAL,[1] Erol YALNIZ[5]

Trakya University, Faculty of Medicine, [1]Department of Radiation Oncology, [2]Department of Pathology, [3]Department of Medical Biology, [4]Department of Medical Oncology, [5]Department of Orthopedics and Traumatology

Ailesel osteosarkom kalıtımsal sendromlar içinde nadir görülen bir hastalıktır. Otuz yedi yaşında bir babada ve, babaya tanı konmasından üç yıl sonra, 17 yaşındaki oğlunda sırasıyla sol ve sağ femur distalinde osteosarkom saptandı. İki olguya da kemoterapi ve cerrahi tedavi uygulandı. Her iki olguda da p53 tümör baskılayıcı gen ve HER-2/neu onkogeni immünhistokimyasal olarak pozitif bulundu. Ek olarak, çocukta retinoblastom 1 gen kaybı vardı. Babada tanı anında ve tedaviden 13 ay sonra akciğer metastazı saptandı. Çocukta uzak metastaz yoktu. İlk olgu, gelişen merkezi semptomlara bağlı olarak tanıdan 39 ay sonra kaybedilirken, oğul tedavinin tamamlanmasından sonraki birinci yılda hastalıksız olarak yaşamını sürdürmekteydi. Her iki olguda belirlenen genetik anormallikler osteosarkomun patogenezinde spesifik genetik değişikliklerin rol oynayabileceğini desteklemektedir.

Anahtar sözcükler: Kemik neoplazileri/genetik; erbB-2 geni; retinoblastoma geni; p53 geni; osteosarkom/genetik.

Familial osteosarcoma is a rare hereditary disease. We present a 37-year-old father and a 17-year-old son who developed osteosarcoma in the left and right distal femurs, respectively, at a three-year interval. They were treated with chemotherapy followed by surgery. Both had positive immunostaining for p53 tumor suppressor gene and HER-2/neu oncogene. The son also exhibited deletion of the retinoblastoma 1 gene. Pulmonary metastasis was detected in the father at the time of diagnosis and 13 months after primary treatment, whereas no distant metastasis was present in the child. The father died 39 months after the diagnosis from primary symptoms, but the son led a disease-free survival a year after completion of treatment. Genetic abnormalities documented in the father and son corroborate the presence of specific genetic alterations in the pathogenesis of osteosarcoma.

Key words: Bone neoplasms/genetics; genes, erbB-2; genes, retinoblastoma; genes, p53; osteosarcoma/genetics.

Osteosarcoma is the most common primary tumour of the bone. Chemotherapy and surgery are effective treatment modalities in this tumor. Histologic response (the degree of necrosis) to chemotherapy is currently the strongest prognostic factor. But reliable prognostic markers are yet not available.[1] Besides morphological and biochemical markers, genetic markers have also been investigated. Overexpression of HER-2/neu and the alterations of p53 and retinoblastoma 1 (RB1) gene have been correlated with osteosarcoma pathogenesis and clinical outcome.[2-6] Familial cases other than hereditary syndromes in osteosarcoma are rare and reported as case reports in the literature. [7-9] The following report describes two cases of osteosarcoma (father and son) with genetic alterations, and their clinical outcome.

Case reports

Patient 1: A 37 – year - old male patient was referred to the hospital for a painful swelling at his left distal femur. Magnetic resonance images showed a partially calcified tumoral mass which is approximately of 4cm thickness, in the bone along

Correspondence to: Dr. Fusun Tokatli. Trakya University, Faculty of Medicine, Department of Radiation Oncology, 22030 Edirne.
Phone: +90284 - 235 76 42 / 1065 Fax: +90284 - 236 10 74 e-mail: fusun_t@yahoo.com

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with medullary involvement.of the bone marrow. Another extraskeletal tumoral component in the parosteal muscle groups along the diaphisis was also noted on the magnetic resonance images. Classical osteosarcoma was diagnosed after a performed biopsy and p53 and HER-2/neu were stained positive on immunohistochemical staining (Figure 1a, b). Tumours were classified as p53 positive if more than 10% nuclei were stained (1:100, Clone DO7, DAKO Cytomation, Glostrup, Denmark). HER-2/neu (1:100, Clone e2-4001+3B5, NeoMarkers, Fremont, CA, USA) staining was scored on a scale of 0, 1+, 2+ or 3+. Positive HER-2/neu expression was defined by weak / moderate (2+) or moderate / strong (3+) complete membrane staining in more than 30% of the tumour cells. He was assessed as stage IV disease due to metastatic pulmonary nodules on his computed thorax tomography, and then PEI chemotherapy (every three weeks, cisplatin 100mg / m2 day 1, epirubicin 90mg / m2 day 1, ifosfamide 2gr / m2 days 2 - 4) was administered. Following three cycles of chemotherapy, metastasectomy and organ preserving surgery were applied. After detecting 80% necrosis in operation specimen, further three cycles of adjuvant chemotherapy was administered. Then he was assigned to follow-up. Thirteen months after the completion of initial therapy, six cycles of high dose ifosfamide chemotherapy (every three weeks, 10gr / m2 days 1 - 6) were administered because of disease progression. But the patient was lost due to central symptoms following the last cycle of chemotherapy, 39 months after the initial diagnosis.

Patient 2: A 17 – year - old male patient was referred to the hospital for swelling in his right distal femur three years after his father's diagnosis. The magnetic resonance imaging and bone scan revealed a mass which was correlated with osteosarcoma. Classical osteosarcoma was diagnosed after a performed biopsy and p53 and HER-2/neu were also positive on immunohistochemical staining as patient 1. RB1 gene was studied in his peripheral blood by fluorescence in situ hybridization (FISH) technique which was performed using directly Rhodamine labelled 13q14 probe (Cancer Genetics Inc, USA). One hundred cells were scored, and 11 (11%) cells with deletion of 13q14 were observed (Figure 2). The patient had no distant metastases, thus three cycles of alternated API chemotherapy (every three weeks, adriamycin 60mg / m2 days 1 - 2, cisplatin 120mg / m2 day 1, ifosfamide 3gr / m2 days 1 - 2) were given and then he was undergone to organ pre-

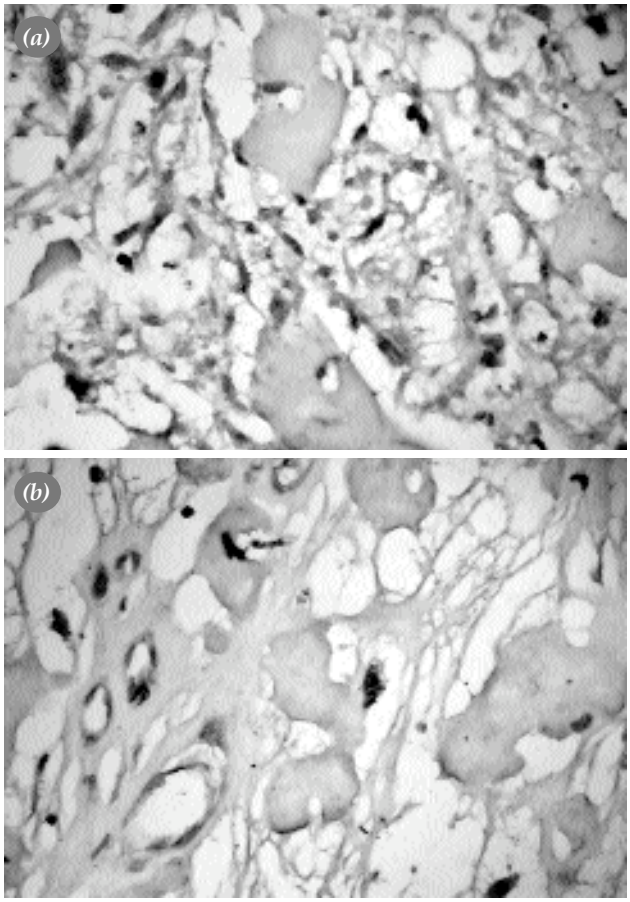

Figure 1. a) Positive immunostaining for p53 (immunoperoxidase x 200) b) for HER-2/neu in tumour cells (immunoperoxidase x 300)

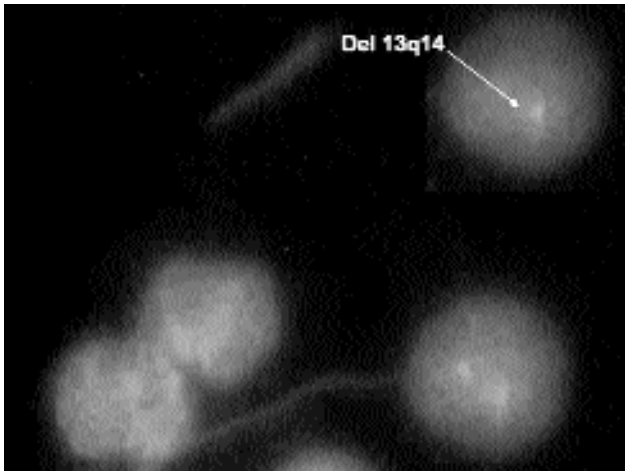

Figure 2. Deletion in retinoblastoma 1 (13q14) gene detected by fluorescence in situ hybridization technique

serving surgery. After detecting 90% necrosis in operation specimen, he was administered three more cycles of adjuvant AP and high dose ifosfamide (10gr / m2 days 1 - 5). One-year after the completion of therapy, the patient has remained disease-free.

Discussion

Familial cases other than hereditary syndromes are rare and generally reported as case reports in the literature. [7-9] In a review of literature including 25 reports up to year 2000, Hillmann et al.[7] reported totally 59 familial osteosarcoma patients and of these, only four patients were described as father to child case presentations Longhi et al. [9] also reported a father and son diagnosed osteosarcoma. Our present father and son cases are the sixth case report as father to child diagnosed osteosarcoma in the English literature. Longhi et al. [9] found that a total loss of RB protein expression and an inactive form of p53 together in their two patients. Toguchida et al. [10] shown that cytogenetically visible germline mutations are usually in the paternally derived gene. In our cases, RB1 gene was shown on the son, and p53 gene deletion and also a HER-2/neu overexpression were detected in both cases.

Studies done in the past 25 years proved that human cancer is primarily a genetic disease. Acquired abnormalities in genes that control the normal growth and differentiation of cells lead to neoplasia. The most common of which are the mutation of p53 suppressor gene and the overexpression of HER-2/neu oncogene.[11,12]

The p53 gene encodes a protein that is predominantly localized in the nucleus.[12] This gene is believed to be a critical determinant in the induction of cell-cycle arrest, programmed cell death, and possibly DNA repair in response to cellular stresses that may lead to DNA damage. p53 suppressor gene mutations plays a key role in the development of Li-Fraumeni syndrome which is characterized with bone and soft tissue sarcomas. [13] A second consequence of p53 protein is a change in the expressed protein, which results in its accumulation in tumour cell nuclei and enables its detection immunohistochemically. [12] In our cases, p53 protein was studied immunohistochemically and found more than 10% nuclei were stained for both cases.

The HER-2/neu oncogene located on human chromosome 17 encodes a 185 kDa transmembrane glycoprotein with tyrosine kinase activity.[11] The expression of the proto-oncogene HER-2/neu has been shown to be of predictive value in breast cancer outcome.[11] The HER-2/neu oncogene has also been considered as a prognostic marker for osteosarcomas, but reports of mainly immunohistochemical studies are controversial. Some studies demonstrated a significant correlation between HER-2/neu overexpression and a poor clinical outcome; others observed HER-2/neu overexpression irrespective of histologic subtype and grade that was not associated with response to preoperative chemotherapy and clinical outcome.[2,3,14] Further studies are needed before HER-2/neu oncogene expression can be accepted as a prognostic factor for survival. In our cases, both have overexpression of HER-2/neu. The first case that had lung metastases at diagnosis lived 39 months, while the second case is still alive and disease - free at a year after treatment. In the review of Hillman et al. 7 the mean survival of cases was 30 months.

Retinoblastoma 1 gene is one component in a cell-cycle control pathway and cell proliferation like p53 gene.[15] Both genes have been proposed to act as tumour suppressor genes, suggesting that, tumourigenesis is the result of the loss of gene functions. The presence of RB1 and p53 gene alterations was proved to indicate poor prognosis in the studies. [4-6] Retinoblastoma 1 gene mutations - a deletion in the 1-4 bands on the long arm of chromosome 13 - primarily predispose to retinoblastoma and a lesser extent to osteosarcoma. The Retinoblastoma 1 gene was altered in 40% of high-grade bone tumours, but not in low-grade tumours.[16] In this study, both of the cases are high-grade osteosarcomas, but because of the father was dead, the son's peripheral blood was studied for RB1 gene and the mutation was shown.

The identification of effective chemotherapy significantly improved the outcome last several decades in osteosarcoma.[1] Despite an increase in the disease-free survival, metastases develop in 30-40% of patients. Therefore, a need exists for new therapeutic strategies in osteosarcoma treatment. Besides the predictive value, HER-2/neu may also

be considered as a target for osteosarcoma treatment like for breast cancer. [11]

In order to clarify the role of genetic factors in the aetiology, the genetic characteristics of these types of families should be investigated carefully. Until a cost-effective program is developed to screen for osteosarcoma, a detailed family history should be obtained from every new patient with osteosarcoma and parents should be urged to schedule early evaluation of blood relatives with complaints of painful extremities.

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