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Case report

A case of alveolar rhabdomyosarcoma with a chromosomal translocation, t(2;13)(q37;q14)

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Summary. We report on an alveolar rhabdomyosarcoma occurring in a 17-year-old girl. Histologically, the tumour exhibited a proliferation of small, round cells and formed alveolar structures. Immunohistochemical studies of the tumour cells showed desmin and creatine kinase MM positivity. In electron-microscopic studies, the tumour cells showed an abundance of glycogen granules, and myofilaments were recognized in the cytoplasm. Chromosome analysis revealed a translocation, t(2;13)(q37;q14), which is thought to be common in this subtype of rhabdomyosarcoma. Conventional ultrastructural and immunohistochemical investigations and chromosome analysis thus appear to be a highly promising combination of methods for improved pathological diagnosis of alveolar rhabdomyosarcoma.

Key words: Rhabdomyosarcoma, alveolar type – Karyotyping – Pathological diagnosis

Introduction

Rhabdomyosarcoma is a high-grade malignant tumour occurring mainly in the soft tissue of the upper and lower extremities. This rare, non-epithelial neoplasm may be divided into three subtypes, embryonal, alveolar, and pleomorphic, according to its histological features (Enzinger and Weiss 1988). However, it is often difficult to distinguish subtypes and to differentiate the disease from other soft-tissue tumours, particularly the small-round-cell tumours of children. In the present report, we describe a case of alveolar rhabdomyosarcoma in which light-microscopic, electron-microscopic and immunohistochemical studies were performed. Furthermore, chromosome analysis performed in parallel revealed an abnormality, t(2;13)(q37;q14), which is thought to be specific to alveolar rhabdomyosarcoma (Seidal et al. 1982; Turc-Carel et al. 1986).

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Case report

The patient was a 17-year-old girl. In about July 1986, she was found to have a mass in the distal and volar portion of the right forearm. Later, she noticed numbness on the dorsal side of both thighs, and she came to the Hokkaido University Hospital for consultation. The size of the tumour on her right forearm was 8×5 cm and gallium scintigrams displayed a remarkable uptake by the lesion. Computerized tomography showed a tumour with an irregular border, accompanied by a low-density area in the inner muscle of the forearm. In the left iliopsoas muscle, a uniform low-density tumour shadow was also present. There was extensive destruction from the third lumbar vertebra to the sacrum, and the tumour had invaded the vertebral canal. Blood chemistry examination showed that the lactate dehydrogenase level was 1268 IU/l and the creatine kinase level was 292 IU/l. Therefore, a biopsy of the right forearm was performed in January 1987. The tumour was solid and showed a connection with the fourth and fifth flexor digitorum profundus, but no connection was seen with the ulnar nerve or the median nerve. The pathological diagnosis was alveolar rhabdomyosarcoma. At the same time, a needle biopsy of the low back tumour was performed, and metastases to this area were confirmed. Chemotherapy and radiation therapy were administered. However, pulmonary metastases occurred and the patient died in June 1988.

Results

On the cut surface of the biopsy sample, the tumour was greyish-white and soft, with indications of necrosis. Under the light microscope, it was seen to consist of uniform small, round cells in a diffuse pattern of proliferation. Groups of dozens of tumour cells were divided by septa which were composed of collagen fibres and small capillaries. The tumour cells were adherent to the margins of the septa (Fig. 1). The nuclei were generally round, and mitotic figures were frequent. The cytoplasm was scanty, and no large cells with eosinophilic cyto-

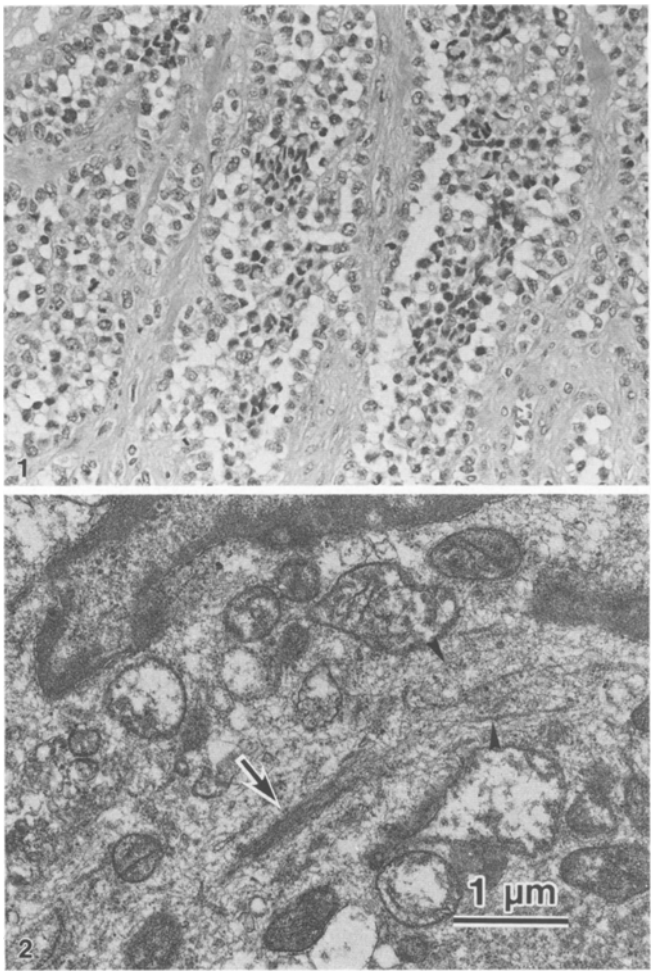

Fig. 1. Micrograph of biopsy specimen of the tumour. Aggregates of small, round tumour cells are separated by irregularly shaped fibrous septa. H&E, ×250

Fig. 2. Electron micrographs. Alternating thick and thin myofilaments (*arrows*) and irregularly aligned ribosomes along filaments (*arrowhead*) are present. ×20500

plasm or cross-striations were recognized. Some tumour cells exhibited degeneration and necrosis. Immunohistochemical studies by using the standard three-step indirect avidin-biotin-peroxidase method (Hsu et al. 1981) showed that the cells stained positively for desmin (Dako, Denmark, at a dilution of 1:100), creatine kinase MM (Merck, 1:200), neuron-specific enolase (Dako, 1:200), and S-100 protein (Dako, 1:400), but not for myoglobin (Dako, 1:400). Electron-microscopically, tumour cells exhibited abundant glycogen granules, as well as well-developed mitochondria and Golgi apparatus. In some of the cells, parallel arrays of myofilaments and aligned ribosomes were seen in the cytoplasm; however, Z-bands were not observed (Fig. 2).

Chromosome analysis. At biopsy, some of the tumour cells were sampled under sterile conditions. Tissue was minced with scissors into small fragments of less than 0.5 mm^3, and these were inoculated into the subcutaneous space on the back of BALB/c AJc-nu mice (Nihon Clea, Tokyo, Japan). The cells grown in mice were transplanted serially, and these cells were used for chromosome analysis. Isolated cells suspended in RPMI-1640 medium supplemented with 10% heat-inactivated fetal calf serum were inoculated in plastic culture flasks and were incubated at 37° C in humidified 5% CO_2 in air. The culture medium was replaced with fresh medium every 3 days, and colcemide was added to the cultures at a cellularity of 90% confluency. Conventionally air-dried chromosome slides were stained for G-banding, and the karyotype was described according to the International System for Human Cytogenetic Nomenclature (1985). The modal chromosome number of the tumour cells was 86. A characteristic translocation involving chromosomes 2 and 13, t(2;13)(q37;q14) was seen in all tumour cells analysed. The karyotype was interpreted as 86, XXX, −X, −1, −2, −3, −4, −5, −7, −13, −13, −14, −18, t(2;13)(q37;q14), +i(1q), +der(13)t(2;13)(q37;q14), +3mar (Fig. 3).

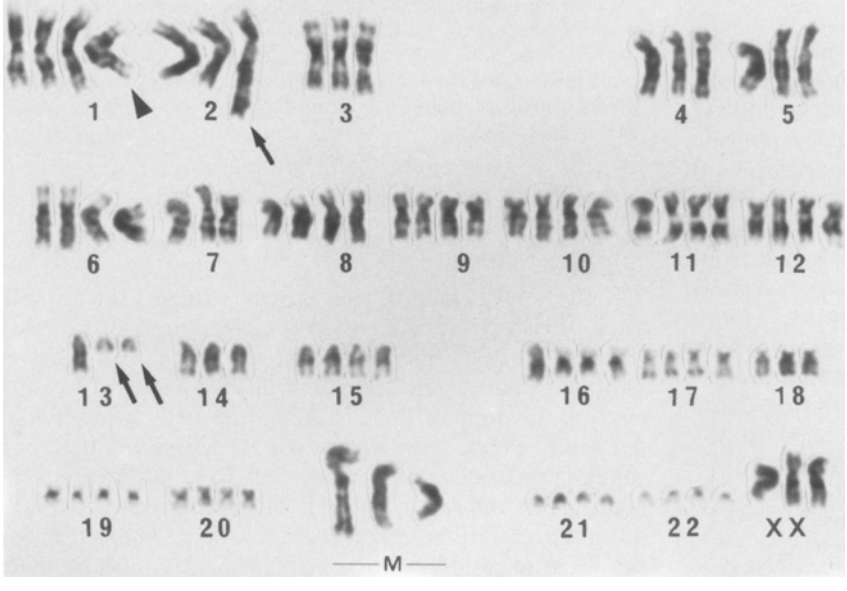

Fig. 3. Karyotype (G-banding) showing 86, XXX, −X, −1, −2, −3, −4, −5, −7, −13, −13, −14, −18, t(2;13)(q37;q14), +i(1q), +der(13)t(2;13)(q37;q14), +3mar. *Arrows* indicate t(2;13)(q37;q14), and *arrowhead* shows +i(1q)

Discussion

Because soft-tissue tumours can be of many different types, the diagnosis of such tumours by microscopic examination is difficult. It is necessary to distinguish rhabdomyosarcoma from other small-round-cell tumours of childhood, including Ewing's sarcoma, non-Hodgkin's lymphoma, and neuroblastoma. In our case the following diagnostic characteristics were present: the tumour developed in the muscles of the forearm, as determined from roentgenographs and biopsy; light-microscopic examination showed alveolar structures with a proliferation of small round cells; desmin and creatine kinase MM were present; and electron-microscopically, abundant glycogen granules and myofilaments were seen in the cytoplasm. These features satisfy the diagnosis of alveolar rhabdomyosarcoma.

Reports on cases in which chromosome analysis of alveolar rhabdomyosarcoma was performed are rare. Trent et al. (1985) have previously described a specific structural abnormality of chromosome 3 (3p14-13) in rhabdomyosarcoma. Seidal et al. (1982) and Turc-Carel et al. (1986) reported that they saw the translocation of t(2;13)(q37;q14) in their alveolar cases. Following their publications, there have been several cases of alveolar rhabdomyosarcoma that had the t(2;13) (Lai et al. 1987; Lizard-Nacol et al. 1987; Rowe et al. 1987; Douglass et al. 1987). In the embryonal and pleomorphic types, however, no consistent chromosomal aberration has been reported. Douglass et al. (1987) found t(2;13) in 5 of 11 cases of rhabdomyosarcoma, including the embryonal type and the undifferentiated type. They stressed that the t(2;13) may be associated with advanced rhabdomyosarcoma rather than with a single subtype of the tumour. However, this should be confirmed by studies of chromosomes in additional cases.

In cytogenetic studies on several patients with rhabdomyosarcoma, we found that some cases of the alveolar form showed a translocation of chromosome 2 and chromosomes other than 13 (unpublished data). This may indicate that the breakpoint at 2q37 is more important than that at 13q14 in the development of alveolar rhabdomyosarcoma.

We conclude that in the pathological diagnosis of rhabdomyosarcoma, microscopic and immunocytochemical investigations should be combined with chromosome analysis, which seems to be a useful supplementary method. As the number of cases increases, the relationship between the breakpoints of chromosomes and the mechanism of carcinogenesis can be studied.

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