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Histological and cytogenetic findings in a malignant tumor of the chest wall and lung (Askin tumor)

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Summary. This report describes a histological and cytogenetic study of a malignant tumor involving the chest wall and lung (Askin tumor) of a young girl. Although initially considered to represent a variant of Ewing's sarcoma, immunocytochemical studies disclosed neuron-specific enolase in neoplastic cells. Ultrastructural study revealed rare cells which contained microtubules and/or dense core neurosecretory type granules. Cytogenetic analysis of neoplastic cells disclosed a reciprocal translocation (11;22) (q24;q12) and occasional extrachromosomal structures interpreted as double minute chromosomes. The latter finding, an indication of gene amplification, is commonly identified in neural crest-derived neoplasms. These ultrastructural, immunocytochemical, and karyotypic data provide evidence in support of a neuroepithelial histogenesis for the Askin tumor.

Key words: Primitive neuroectodermal tumor – Neuroepithelioma – Ewing's sarcoma – Translocation – Double minute chromosomes – Askin tumor

Introduction

In 1979, Askin and colleagues (Askin et al. 1979) described a series of twenty malignant small cell neoplasms arising in the chest wall or peripheral lung tissue of children. The tumors tended to recur locally, rather than metastasize widely, yet the median survival from the time of diagnosis was only eight months. Although the precise histogenesis of these neoplasms was not established, some of the clinical and pathologic features resembled those present in the osseous and extraosseous variants of Ewing's sarcoma. In contrast to Ewing's sarcoma, however, these neoplasms arose principally

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in girls, were devoid of intracellular glycogen, and, in three cases, contained ultrastructural features suggestive of a neuroepithelial origin.

The matter rested until 1983, when a series of eight similar chest wall neoplasms was found to contain intracellular neuron-specific enolase, a marker for neural tissue and tumors of neural crest origin (Linnoila et al. 1983). This report was noteworthy since all cases lacked light microscopic features of neuroepithelial neoplasms and only rare dense core granules were identified by electron microscopy. The authors concluded that this immunocytochemical study served to confirm a neural origin for the "Askin tumor". This sanguine interpretation might be challenged today, since immunocytochemical reactivity for neuron-specific enolase has been documented in neoplastic conditions for which a neuroectodermal histogenesis is lacking (Vinores et al. 1984).

Within the past two years several reports have described a reciprocal translocation involving band q12 of chromosome 22 in neoplastic cells and cell lines from Ewing's sarcomas (Aurias et al. 1983; Turc-Carel et al. 1983) and peripheral neuroepitheliomas (Whang-Peng et al. 1984). That identical cytogenetic aberrations should occur in these neoplasms is striking, suggesting either a common histogenesis and/or mechanism of oncogenesis. Against this background, we report a case study of a malignant tumor of the chest wall (Askin tumor) in which the neoplastic cells demonstrated microtubules, dense core neurosecretory-type granules, neuron-specific enolase, a reciprocal translocation (11;22) (q24;q12), and occasional extrachromosomal structures interpreted as double minute chromosomes. These collective findings suggest a neuroepithelial histogenesis for the "Askin tumor". A brief description (letter) of this observation has been published (de Chadarévian et al. 1984).

Case history

An 11-year-old girl presented with a three week history of mild dyspnea, low-grade fever, and weakness. Chest radiographs revealed a massive pleural effusion, intrathoracic mass, and periosteal reaction of the left fifth rib. Computerized tomography and ultrasound studies demonstrated that the mass was solid and homogeneous. Bone scan disclosed increased uptake in the left fifth rib. Skeletal survey was negative. Exploratory thoracotomy revealed a non-resectable intrathoracic neoplasm which invaded the posterior chest wall, lung, diaphragm, and pericardium. Combinational chemotherapy and radiotherapy (4500 rads) were administered over the ensuing months. Although an initial reduction of tumor size was noted, the neoplasm recurred and extended to involve the thoracic vertebrae, sufficient to induce paraplegia. She died eight months after the onset of symptoms.

Pathologic findings

The surgical specimen was soft, flesh-like, and extensively necrotic. Histological sections revealed a cellular neoplasm with both diffuse and lobular growth patterns, the latter imparted by broad fibrovascular septae. The neoplastic cells generally featured round nuclei, fine chromatin, rare small nucleoli, and scant, often indistinct cytoplasm. In many fields, clusters of neoplastic cells demonstrated intense nuclear hyperchromasia and distinct, intensely acidophilic cytoplasm (Fig. 1). Mitoses were numerous, as were extensive areas of coagulative necrosis. Scant amounts of glycogen were present in but a few cells. Occasional rosette-like structures (Fig. 2)

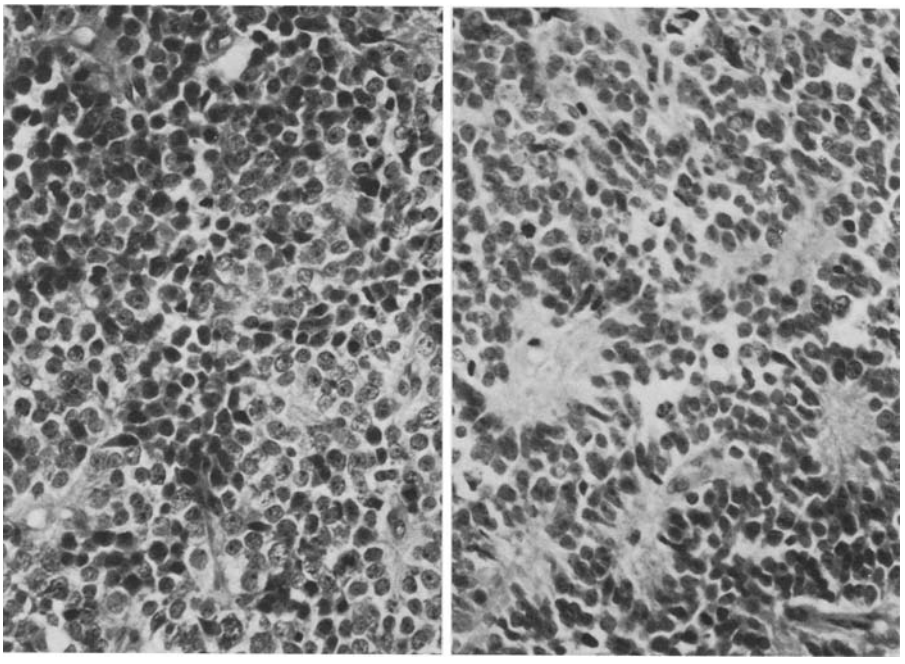

Fig. 1. Photomicrograph of neoplasm illustrating two cell types. HPS × 200

Fig. 2. Photomicrograph of neoplasm illustrating rosette-like structures. HPS × 200

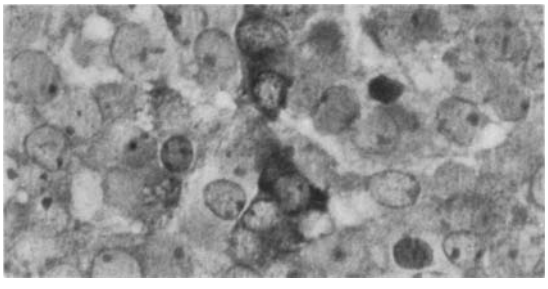

Fig. 3. Photomicrograph of neoplasm illustrating several cells with cytoplasmic staining for neuronspecific enolase × 600

were noted in scattered histological fields. Immunocytochemical studies were undertaken employing neuron-specific enolase antiserum (DAKO PAP KIT, Cedarlane Laboratories, Hornby, Ontario). Cytoplasmic immunoreactive staining was observed in isolated cells in scattered areas of the neoplasm (Fig. 3). Electron microscopy revealed modest numbers of mitochondria, polyribosomes, and occasional membrane systems. In rare cells, centrioles, microtubules (Fig. 4A), and dense core granules (120–200 nm) (Fig. 4B) were identified. Neither desmosomes, basement membrane material, nor aggregates of cytofilaments were noted.

Cytogenetic findings

Direct chromosomal preparations of minced tumor tissue were GTG banded (ISCN, 1981). The modal number was 40, showing a number of numerical changes which could not be fully analyzed (few well GTG-banded metaphases were found and there was some karyotypic

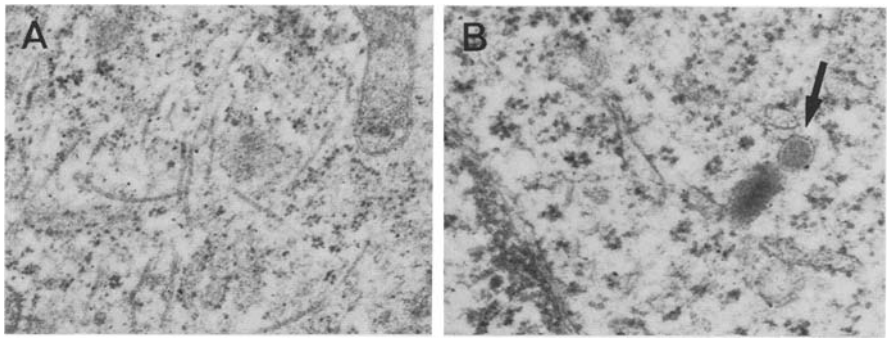

Fig. 4. Electron micrograph (a) of microtubules (×10,000) and (b) electron dense granule (*arrow*) (×15,000) in cytoplasm of neoplastic cells

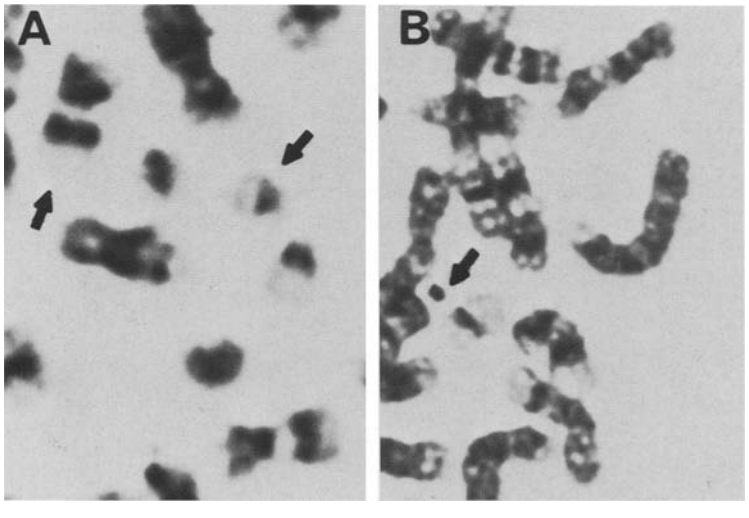

Fig. 5. Photograph (a) of 11;22 translocation and (b) extrachromosomal material interpreted as double minute chromosomes

variability). However, a reciprocal translocation involving the long arms of chromosomes 11 and 22 was consistently found (Fig. 5A). Furthermore, extrachromosomal material interpreted as double minute chromosomes were present in 5% of analyzable cells (Fig. 5B). Unfortunately, the CBG banding technique used to exclude centromeric heterochromatin in these structures was inconclusive on these aged cytogenetic preparations.

Discussion

This case study describes a malignant neoplasm of the chest wall (Askin tumor) in which neuron-specific enolase, a reciprocal translocation (11;22) (q24;q12), and occasional extrachromosomal material interpreted as double minute chromosomes were identified in neoplastic cells. These findings in association with the ultrastructural demonstration of microtubules and neurosecretory-type granules in neoplastic cells collectively suggest a neuro-

epithelial histogenesis for this neoplasm. Thus, this report both confirms and extends the finding of a recent study of three cases of the Askin tumor which provided cytochemical and ultrastructural support for a neuroectodermal origin for the entity (Gonzales-Crussi et al. 1984).

Primitive neuroectodermal tumors are uncommon malignant neoplasms of soft tissue and/or bone, presumed to be derived from primitive neuroectoderm (Seemayer et al. 1975; Jaffe et al. 1984). In most instances, those tumors are not associated with catecholamine production, neither are neurosecretory granules present in significant numbers. In recent years, a conceptual tendency developed to regard such neoplasms as neuroblastomas which present in older children and young adults at peripheral, rather than central (adrenal, autonomic ganglia) sites (Mackay et al. 1976; Hashimoto et al. 1983). More recent studies which demonstrate ultrastructural, immunocytochemical, and cytogenetic differences between primitive neuroectodermal tumors and neuroblastoma suggest that the entities are distinct (Jaffe et al. 1984; Potluri et al. 1984; Tsokos et al. 1985).

The relation between Ewing's sarcoma and neuroepithelial neoplasms has long been a matter of discussion (Triche et al. 1983). For some time, the Ewing tumor was believed to be laden with glycogen, such that its demonstration was a requisite for the diagnosis (Schajowicz 1959). In recent years, variants of Ewing's sarcoma have been described which contain little, if any, glycogen (Kissane et al. 1983). To compound matters, intracytoplasmic glycogen has been demonstrated in neuroblastomas (Yunis et al. 1979) and peripheral neuroepitheliomas (Linnoila et al. 1983). Equally vexing, variants of Ewing's sarcoma have been described to contain true rosettes (Kissane et al. 1983) and neurosecretory granules and microtubules (Schmidt et al. 1982), the putative hallmarks of neuroepithelial tumors. The presence or absence of neuron-specific enolase in such neoplasms may constitute one morphological differentiating feature. In a recent study by Jaffe and colleagues, the interesting and challenging notion was raised that Ewing's sarcoma may represent the most undifferentiated form of neuroectodermal tumor (Jaffe et al. 1984). This hypothesis has received support to some extent by the demonstration of antigenic determinants previously associated with tissues of neuroectodermal origin on cell lines of Ewing's sarcoma (Lipinski et al. 1984).

An interesting finding in this study is the occasional presence of extrachromosomal material interpreted to represent double minutes in an "Askin tumor". Whether after establishing a permanent cell line, these cells with extrachromosomal material would have found an appropriate milieu for multiplying remains unknown and deserves further study. Double minute chromosomes (DM) and homogeneously staining regions of chromosomes (HRS) are two karyotypic features which imply gene amplification (Barker 1982). DMs are paired chromatin bodies devoid of centromeres which segregate randomly at cell division. HSRs are domains within chromosomes devoid of the normal banding pattern. While first recognized in tumor cells resistant to cytotoxic drugs (Numberg et al. 1978), diverse human and murine cancers have been found to contain DMs and/or HSRs (Barker 1982;

Alitalo et al. 1983; Little et al. 1983; Schwab et al. 1983). Of particular note, DMs are commonly observed in permanent cell lines established from neuroectodermal tumors, such as neuroblastoma, retinoblastoma, malignant melanoma, peripheral neuroepithelioma, and primitive neuroectodermal tumors (Potluri et al. 1984). The gene amplified has been shown to be homologous to *v-myc*, either *N-myc* in neuroblastoma or *c-myc* in peripheral neuroepithelioma and primitive neuroectodermal tumor. Thus, this report strengthens the possible association of the Askin tumor with neural crest-derived neoplasms. Although studies were not attempted to probe for oncogene amplification in our neoplasm, prospective studies of similar neoplasms, as well as those which arise in neurocristopathic (Bolande 1974) settings might demonstrate oncogene amplification, in addition to DMs, HSRs, and/or chromosomal translocations.

At this writing, four reciprocal translocations involving chromosome 22 are associated with neoplasia. The first described, t (9;22) (q34;q11), the Philadelphia chromosome of chronic myelogenous leukemia (Rowley 1973), represents a reciprocal translocation involving *c-sis* and *c-abl* oncogenes (de Klein et al. 1982). Although *c-abl* oncogene is not amplified, its expression is altered (Canaani et al. 1984). In the second described, a variant of Burkitt's lymphoma, t (8;22) (q24;q22), *c-myc* remains on chromosome 8, yet the oncogene is deregulated and transcribed at high levels (Croce et al. 1983). Ewing's sarcoma and peripheral neuroepithelioma constitute the third and fourth neoplasms associated with reciprocal translocations involving chromosome 22. Although the oncogene *c-sis* is located on the long arm of chromosome 22, *c-sis* oncogene is not activated in Ewing's sarcoma (Bechet et al. 1983). Interestingly, hybridization with the *c-myc* gene probe reveals active transcription of the *c-myc* locus in Ewing's sarcoma (Bechet et al. 1983) and in peripheral neuroepithelioma and primitive neuroectodermal tumor (Potluri et al. 1984).

In conclusion, given the morphologic difficulties in distinguishing Ewing's sarcoma from neural crest-derived neoplasms, prospective histochemical, cytogenetic, and DNA studies of such neoplasms might resolve some of the contentious issues concerning these entities.

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