Recurrent Involvement of 2p23 in Inflammatory Myofibroblastic Tumors

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Abstract

Inflammatory myofibroblastic tumor (IMT) is a relatively rare soft tissue tumor. The reactive versus neoplastic pathogenesis of this tumor is unresolved. We found clonal chromosome aberrations involving 2p23 upon metaphase analysis of two IMTs. Fluorescence in situ hybridization with a probe flanking the ALK gene at 2p23 demonstrated rearrangement of the probe in both of these cases and in a third case, and immunohistochemistry revealed ALK expression in all three cases. 2p22–24 involvement has been reported previously in four additional cases of IMT. We suggest that chromosomal rearrangements involving 2p23 near or within ALK are recurrent alterations in IMT and that ALK may have a novel role outside its previously recognized realm of lymphoid neoplasms.

Introduction

IMTs are rare yet distinctive pseudosarcomatous inflammatory lesions that primarily occur in the soft tissue and viscera of children and young adults (1–3). Histologically, this lesion is composed of fascicles of bland myofibroblasts in a variably collagenous stroma admixed with a prominent inflammatory component of lymphocytes, eosinophils, and plasma cells. Its distinctive histological appearance has given this lesion a wide variety of names, including inflammatory pseudotumor, pseudosarcomatous myofibroblastic proliferation, inflammatory sarcoma, plasma cell granuloma, and inflammatory myohistiocytic proliferation (1, 3, 4). The diagnosis of IMT is often difficult, depending on the relative proportion of the inflammatory and myofibroblastic components. Some lesions can be difficult to distinguish from a reactive process, whereas other lesions can appear sarcomatous. Whether the entire histological spectrum represents a single entity and, consequently, whether consistent chromosomal or gene abnormalities are found throughout the spectrum are, therefore, important questions in determining the pathogenesis of IMT.

Materials and Methods

Histopathology

Case 1. Case 1 was a mediastinal mass from a 5-month-old girl. Histology revealed an IMT. Immunohistochemical stains were positive for muscle-specific actin and Kp1 in the large and spindled cells of the lesion. This patient had been 8 months without recurrence.

Case 2. Case 2 was an abdominal mass from a 9-year-old boy. Histology revealed a spindle cell lesion with central abundant inflammatory infiltrate, central collagenization, and calcification, consistent with IMT. Removal of the large mass required a partial small and large bowel resection. Immunohistochemical stains were positive for actin and desmin, with a prominent vascular network noted by CD31 staining. Epithelial markers were negative. The tumor recurred 3 months after the initial resection, requiring resection of three additional mesenteric nodules.

Case 3. Case 3 was an abdominal mass from a 4-year-old boy. Histology revealed a multinodular IMT involving mesentery and muscularis propria of the small intestine, with extension to the mesenteric margin and foci of venous invasion. No recurrence had been detected in 3 years of follow-up.

Cytogenetics

An analysis of metaphase cytogenetics was performed on cases 1 and 2. Tissue was minced; exposed to 400 units/ml collagenase type II for 3.5 h; and then cultured in RPMI 1640 medium with 15% FCS, penicillin, streptomycin, and glutamine at 37°C in a 5% CO2 humidified incubator for 2–4 days. Cells were harvested, slides were made, and chromosomes were G-banded following standard protocols. Ten to 20 metaphases were analyzed, and karyotypes were prepared. Chromosome abnormalities were described according to International System for Human Cytogenetic Nomenclature (1995) guidelines (5).

For case 1, an additional slide was prepared for SKY analysis according to the protocol supplied with the SKY Probe Kit (Applied Spectral Imaging, Carlsbad, CA). Metaphase images were acquired using a standard epifluorescence microscope equipped with a 150-W xenon lamp, a ×63 oil-immersion objective, and the ASI SpectraCube SD200 system. Counterstained images were captured with 100-W mercury illumination and inverted by SkyView software (ASI, Carlsbad, CA) to provide banded images. Ten cells were analyzed. Bands were assigned using the inverted 4',6-diamidino-2-phenylindole function.

FISH

FISH was performed using a biotinylated probe for NMYC (located at 2p24.1; Oncor, Gaithersburg MD). Metaphase preparations from case 1 and a normal lymphocyte control were hybridized, washed, and detected per manufacturer’s protocol. FISH was also performed with a probe purchased from Vysis, Inc. (Downer’s Grove, IL), which was designed to detect ALK rearrangements in non-Hodgkin’s lymphoma. This probe set contains a 250-kb probe for detecting t(2;5)(p23;q35) translocations involving ALK, with the telomeric side of the chromosome 2 breakpoint directly labeled in Spectrum Orange and a 300-kb probe centromeric to the breakpoint directly labeled with Spectrum Green. FISH was performed following manufacturer’s instructions using cytogenetic preparations from cases 1 and 2 and a touch preparation from case 3. One hundred or more interphase nuclei were scored from each case, and 20 and 5 metaphases were scored from cases 1 and 2, respectively.

ALK Immunohistochemistry

ALK1 antibody staining was performed on formalin-fixed, paraffin-embedded tissue from all three cases using monoclonal mouse antihuman ALK antibody (DAKO, Carpentaria, CA). Staining was detected using the avidin-biotin complex method.

Results

Cytogenetics

Case 1. Twenty cells were analyzed from the G-banded slides. The karyotype of case 1 was 52–54,XX,t(2;17)(p23;q23),+der(2)t(2;17),+3,+9,+11,+12,+13,+18,+20[5p10]/52–53,idem,–8 [5]/52–54,idem,del(X;10)[p22:p15]/21[52–53],idem,del(X;8)[p22:p23][2].

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The abbreviations used are: IMT, inflammatory myofibroblastic tumor; SKY, spectral karyotyping; FISH, fluorescence in situ hybridization.
Other dicentric chromosomes involving chromosomes 8, 10, and X were observed in single cells only. The translocation between chromosomes 2 and 17 is illustrated in Fig. 1. One normal cell confirmed a constitutionally normal karyotype.

Ten cells with an abnormal karyotype were analyzed by SKY. As with the G-banded metaphases, every abnormal SKY metaphase contained the balanced 2;17 translocation, with a second copy of the derivative 2 (Fig. 2b). The main clone seen by SKY (7 of 10 cells) was the same as that seen by G-banding (10 of 20 cells). Two cells were observed to contain a dicentric t(8;10)(q2?3;p15) in addition to the t(2;17). These dicentric rearrangements were not identical to those seen in the G-banded cells. Nonclonal structural abnormalities involving chromosome 8, including deletions, translocations, and duplications, were also seen.

Case 2. Ten metaphases were analyzed. The karyotype was 43–46.X.,−Y,i(8)(q10),−22,+r,+mar,inc[cp10]/92,ident[cp2]. Insufficient material was available to completely characterize the clonal chromosome abnormalities or to perform SKY analysis.

FISH

NMYC. FISH with NMYC probe to metaphases of case 1 showed signal on the normal chromosome 2 and translocation of signal to the der(17) in 8 of 10 cells. These results confirmed that the translocation breakpoint on chromosome 2 is proximal to band p24. The remaining cells were diploid and had two signals on apparently normal chromosome 2, assumed to be normal cells. The normal control verified the signal present on 2p24. No signals were seen on any other chromosome.

ALK. The dual-color ALK probe on metaphases from a normal control showed two immediately adjacent or fused red/green signals on interphase nuclei (98% of cells) and on chromosome 2p (100% of metaphase cells), as expected. In case 1, analysis of metaphase cells showed that the breakpoint of the t(2;17) splits the two-color signal, indicating a breakpoint within or very near the ALK gene. Both copies of der(2)t(2;17) were retained in the proximal probe signal (green), whereas the distal signal (red) went to the der(17) (Fig. 2a). Thirty-six percent of interphase nuclei showed the normal fusion signal, indicating one normal copy of chromosome 2, plus one red signal and two green signals, indicating the translocation chromosomes plus the additional der(2). Metaphase chromosomes from case 2 showed overlapping red and green signals on both normal chromosomes 2. The ring chromosome showed two copies of the distal probe (red), with only occasional signals from the proximal probe (green), suggesting that the ring contains partially deleted and duplicated sequences from chromosome 2p (Fig. 2c). Interphase cells showed normal signal (two fusions) in 86%. The remaining 14% were abnormal, with the most common pattern being two fusion with two red signals (4%). This corresponds to the two normal chromosomes 2 and the ring observed in metaphase spreads. Other cells were polyploid, with more than two fusion signals and more than two red signals. An additional 100 large nuclei were scored, excluding small round nuclei from lymphocytes with normal signals. Forty-two % of the large nuclei had two fusion signals and one to four red signals, 33% had polyploid versions of the same signal, and 25% had incomplete signals. Small green signals were only occasionally seen, suggesting that the proximal end of the probe was almost completely deleted in formation of the ring chromosome. In case 3, 33% of interphase cells showed the normal fusion signal plus one red signal and one green signal, again showing a split of the ALK probe. The simplest explanation would be a balanced translocation, although metaphase analysis was not available to confirm this interpretation.

ALK Immunohistochemistry

Immunohistochemical staining for ALK showed positive cytoplasmic staining in the myofibroblastic cells for all cases; however, the intensity and degree of staining were quite variable. The tumor from patient 2 showed diffuse, strong cytoplasmic positivity (Fig. 3), whereas the positive staining for cases 1 and 3 was focal and weak.

Discussion

Controversy exists as to the reactive versus neoplastic nature of IMT. Those in support of the reactive classification note that this lesion is often associated with antecedent pulmonary disease and infection, frequently regress with antibiotic and corticosteroid therapy, and rarely recurs with conservative surgical treatment (1, 3, 6).
Fig. 2. a, metaphase chromosomes from case 1 hybridized with two-color (red and green) ALK probe. Normal chromosome 2 (small arrow) shows overlapping signals from probes surrounding the ALK breakpoint identified in the t(2;5) translocation of lymphomas. In this IMT, the breakpoint of the t(2;17) splits the two-color signal, suggesting a breakpoint within or very near the ALK gene on 2p23. Large arrows, two copies of der(2)t(2;17) retaining the proximal probe (green) signal. Arrowhead, der(17)t(2;17) with the distal probe (red) signal. b, partial karyotypes from case 1 after classification by SKY confirm that the translocation involves only chromosomes 2 and 17. c, metaphase chromosomes from case 2, hybridized with two-color ALK probe. Normal chromosomes 2 (small arrows) show overlapping red and green signals. Ring chromosome (arrowhead) shows two copies of the distal probe (red), suggesting the ring contains partially deleted and duplicated sequences from chromosome 2p. d, interphase cells (touch preparation) from case 3 hybridized with two-color ALK probe. One normal signal (small arrow) with overlapping red and green signal is seen in most nuclei. Separate red (arrowhead) and green (large arrow) signals indicate that chromosome 2p is split at the breakpoint detected by the ALK probe.
contrast, some IMTs are locally aggressive and recurrent and may also be invasive and, rarely, distantly metastatic (4, 7, 8). However, recent cytogenetic reports have shown that at least some of these tumors contain various chromosomal aberrations that are clonal in origin, thus supporting the contention that this lesion is neoplastic in nature.

Here, we report three cases of IMT in which a consistent clonal chromosomal abnormality was found and suggest that a specific chromosomal region, 2p23, and, specifically, alterations near or within the ALK gene are consistently involved in this neoplasm. Recurrent involvement of 2p was found in four of the eight IMTs with clonal chromosomal changes that have been reported previously (Table 1; Refs. 3, 4, and 8–10). An apparent deletion of 2p beginning at band p23 was the only abnormality in one tumor (10); a t(1;2)(q21;p23) was reported as a sole abnormality in one of two related clones in another (8); and addition of unidentified material in two cases, at 2p22 and 2p24, respectively, was seen in the setting of very complex karyotypes (3). Thus, including the three cases in this report, 7 of 11 IMTs in which cytogenetics or FISH has been performed show alterations in the 2p22–24 region. Resolution of the breakpoints in the tumors reported previously was limited to G-banding, and the level of banding resolution achieved was not reported. Therefore, it is very possible that all tumors involve the same breakpoint in 2p.

These findings suggest that a crucial gene or regulatory region at 2p23 may be involved in IMT. The anaplastic lymphoma kinase gene (ALK) at 2p23 was discovered when the breakpoint of the t(2;5)(p23;q35) translocation found in some anaplastic large cell lymphomas was cloned (11). Fusion of the 3' end of ALK to the 5' end of the nucleolar phosphoprotein gene (NPM) at 5q35 in CD30+ lymphomas results in the formation of an activated tyrosine kinase with strong oncogenic activity (11, 12). Reliable detection of ALK expression by immunohistochemistry, especially in formalin-fixed, paraffin-embedded material, can be problematic. However, ALK expression has been detected by immunohistochemical staining with a monoclonal antibody in normal cells only in the central nervous system (scattered neurons, glial cells, and endothelial cells; Ref. 13). In tumors, expression has been found only in large cell lymphomas usually bearing the t(2;5) translocation and in lymphomatoid papulosis (12–14). ALK expression has also been reported in one lymphoma with inv(2)(p23q35) (14); an engineered hybrid TRP/ALK protein transformed rodent fibroblasts and produced lymphomas in mice (15). These results suggest that genes other than NPM may deregulate ALK expression. In the three cases reported here, FISH narrowed the breakpoint to within the region spanned by the ALK probe. The proximal and distal

### Table 1

<table>
<thead>
<tr>
<th>Case no.</th>
<th>Age/sex</th>
<th>Location</th>
<th>Tumor size (cm)</th>
<th>Immunohistochemistry</th>
<th>Karyotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3 yr/M</td>
<td>Omental-mesenteric</td>
<td>5 × 4 × 4.2</td>
<td>smooth muscle actin (+), vimentin (+), S100 (+), keratin (+)</td>
<td>47,XX,del(2)(p23)</td>
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<tr>
<td>2</td>
<td>6 yr/F</td>
<td>Uterus</td>
<td>4 × 4 × 4.2</td>
<td>smooth muscle actin (+), vimentin (+)</td>
<td>46,XXq13.12del(2)(p23)</td>
</tr>
<tr>
<td>3</td>
<td>30 yr/F</td>
<td>Lung</td>
<td>1.8</td>
<td>smooth muscle actin (+), vimentin (+)</td>
<td>46,XXq13.12del(2)(p23)</td>
</tr>
<tr>
<td>4</td>
<td>3 yr/F</td>
<td>Distal femur</td>
<td>2.0</td>
<td>smooth muscle actin (+), vimentin (+)</td>
<td>46,XXq13.12del(2)(p23)</td>
</tr>
<tr>
<td>5</td>
<td>27 yr/F</td>
<td>Distal femur</td>
<td>4.0</td>
<td>smooth muscle actin (+), vimentin (+)</td>
<td>46,XXq13.12del(2)(p23)</td>
</tr>
<tr>
<td>6</td>
<td>30 yr/F</td>
<td>Lung</td>
<td>1.23</td>
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<td>46,XXq13.12del(2)(p23)</td>
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<tr>
<td>7</td>
<td>8 yr/M</td>
<td>Thymus</td>
<td>1.23</td>
<td>smooth muscle actin (+), vimentin (+)</td>
<td>46,XXq13.12del(2)(p23)</td>
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<tr>
<td>8</td>
<td>14 yr/F</td>
<td>Abdomen</td>
<td>0.001</td>
<td>smooth muscle actin (+), vimentin (+)</td>
<td>46,XXq13.12del(2)(p23)</td>
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<tr>
<td>9</td>
<td>30 yr/M</td>
<td>Mediastinum</td>
<td>4 × 3 × 1.8</td>
<td>smooth muscle actin (+), vimentin (+)</td>
<td>47,XXq13.12del(2)(p23)</td>
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<td>10</td>
<td>9 yr/M</td>
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<td>20 × 12 × 12</td>
<td>smooth muscle actin (+), vimentin (+)</td>
<td>47,XXq13.12del(2)(p23)</td>
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<tr>
<td>11</td>
<td>10 yr/F</td>
<td>Abdomen</td>
<td>2 × 12 × 1.1</td>
<td>smooth muscle actin (+), vimentin (+)</td>
<td>47,XXq13.12del(2)(p23)</td>
</tr>
</tbody>
</table>

*NR, not repeated; ND, not determined.*

Fig. 3. Immunohistochemical staining for ALK performed on the tumor of case 2 showing diffuse, strong cytoplasmic staining confined to the myofibroblastic cells of the tumor.
portions of the ALK probe surround but do not span the 2p23 break-
point of the t(2;5) translocation and are separated by no more than 90
kb;3 we do not know whether another gene may reside within the area
contained between the two portions of this probe. However, detectable
ALK expression in these cases supports involvement of the ALK gene.
The gene(s) on chromosome 17 (case 1), the ring chromosome (case
2), and the presumed reciprocal translocation chromosome (case 3)
causing activation of the ALK gene in the IMTs that we report here are
unknown.

In conclusion, the clonal chromosomal findings described in this
report support the theory that at least some tumors with histology
interpreted as IMTs are neoplastic rather than reactive. In addition,
there now appears to be evidence of a consistent chromosomal aber-
tation associated with this lesion, namely, breakage in band p22–24 of
chromosome 2, with specific involvement of 2p23 and with ALK
expression demonstrated in the three cases reported here. IMT could,
therefore, represent yet another mesodermally derived neoplasm with
consistent chromosome abnormalities, joining the t(11;22) transloca-
tion of Ewing’s sarcoma, t(2;13) of alveolar rhabdomyosarcoma,
and the recently described t(12;15) of synovial sarcoma (16), and the recently described t(12;15)
infantile fibrosarcoma (17). Additional cytogenetic studies of this
highly enigmatic and minimally studied tumor are warranted to con-
firm the breakpoint on chromosome 2p and the role of the ALK gene.

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