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TPM3-ALK and *TPM4-ALK* Oncogenes in Inflammatory Myofibroblastic Tumors

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Inflammatory myofibroblastic tumors (IMTs) are neoplastic mesenchymal proliferations featuring an inflammatory infiltrate composed primarily of lymphocytes and plasma cells. The myofibroblastic cells in some IMTs contain chromosomal rearrangements involving the *ALK* receptor tyrosine-kinase locus region (chromosome band 2p23). ALK—which is normally restricted in its expression to neural tissues—is expressed strikingly in the IMT cells with 2p23 rearrangements. We now report a recurrent oncogenic mechanism, in IMTs, in which tropomyosin (TPM) N-terminal coiled-coil domains are fused to the ALK C-terminal kinase domain. We have cloned two *ALK* fusion genes, *TPM4-ALK* and *TPM3-ALK*, which encode ~95-kd fusion oncoproteins characterized by constitutive kinase activity and tyrosylphosphorylation. Immunohistochemical and molecular correlations, in other IMTs, implicate non-TPM ALK oncoproteins that are predominantly cytoplasmic or predominantly nuclear, presumably depending on the subcellular localization of the ALK fusion partner. Notably, a *TPM3-ALK* oncogene was reported recently in anaplastic lymphoma, and *TPM3-ALK* is thereby the first known fusion oncogene that transforms, *in vivo*, both mesenchymal and lymphoid human cell lineages. *(Am J Pathol 2000, 157:377–384)*

The inflammatory myofibroblastic tumor (IMT)[1–4] is a clinicopathologically distinctive but biologically controversial entity that was defined originally as a nonneoplastic lesion. IMTs arise usually in the soft tissues, most often in the abdomen of children and adolescents, and they are composed of myofibroblastic spindle cells admixed with a prominent inflammatory infiltrate of lymphocytes, plasma cells, and, less often, acute inflammatory cells. Notably, many patients with IMTs present with constitutional symptoms of fever and weight loss, and these same patients are often anemic and thrombocythemic. However, it is unclear whether the constitutional symptoms and inflammatory infiltrate are induced by tumor-cell factors or, alternately, whether the constitutional symptoms are secondary to the inflammatory process.

The uncertain pathogenesis of IMTs, and the ongoing question of its neoplastic *versus* reactive nature, is reflected in the large number of names which have been bestowed on this disorder. IMTs arising in the lung, particularly those that are well circumscribed, are generally cured by surgical excision. Until recently, these were referred to as "plasma cell granulomas," "inflammatory pseudotumors," or "pseudosarcomatous myofibroblastic proliferations." Intra-abdominal IMTs may pursue a somewhat more aggressive clinical course with multiple recurrences and with potential for metastatic spread in rare cases. Such tumors have often been referred to as "inflammatory myofibroblastic tumor" or "inflammatory fibrosarcoma."[1,2] However, most IMTs have overlapping histological characteristics and it is difficult to distinguish those with neoplastic potential from the potentially reactive subset that belong under the umbrella category of "inflammatory pseudotumor." Another complicating feature, with respect to classification, is that differences in clinical behavior, between different IMT subcategories, do not exclude a common pathogenesis.

Cytogenetic banding studies were the first assays to demonstrate unequivocal clonal mutations—indicative of a neoplastic pathogenesis—in IMTs.[5–10] Approximately 50% of soft-tissue IMT karyotypes contain clonal rearrangements of the chromosome 2 short arm,[5–10] and each of two cytogenetically characterized IMTs arising in bone contained rearrangements of the *HMGIC* region on chromosome band 12q15.[8] Recently, Griffin et al[10] showed that IMT 2p rearrangements fall within an ~100-kb region containing the *ALK* receptor tyrosine-kinase locus, on chromosome band 2p23. These rearrangements were associated with striking ALK expression in the IMT myofibroblastic spindle cells.[10] ALK is a

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receptor tyrosine kinase that was originally characterized as a component of the anaplastic large-cell lymphoma NPM-ALK fusion oncoprotein.[11] ALK expression is normally restricted to the central nervous system,[12–14] and expression of constitutively activated ALK, in anaplastic large-cell lymphoma, seems to be a pivotal transforming event. High-level ALK expression, in IMTs, is notable because ALK is expressed at low or undetectable levels in nonneoplastic fibroblasts. These findings suggest that IMTs might be neoplasms in which the myofibroblastic component is transformed, in some cases, by chromosomal mechanisms targeting *ALK*.

In the present study we show that chromosome 2p rearrangements in IMTs create *ALK* fusion genes. We characterize two such genes, tropomyosin 4 (*TPM4*)-*ALK* and tropomyosin 3 (*TPM3*)-*ALK*, and provide evidence for at least two additional fusion mechanisms that remain to be characterized. These studies also identify a diagnostically useful molecular marker in IMTs.

Materials and Methods

Eleven IMTs were obtained as either frozen or paraffin-embedded specimens. The cytogenetic features of cases 1, 2, 6, and 11 have been described previously.[6,8,9] Cytogenetic banding analyses had not been performed in the remaining seven cases.

Immunostaining

All cases were re-evaluated histologically by three of the authors (APA, BPR, and CDMF). ALK immunostaining was performed using monoclonal antibody ALK-1 (DAKO, Carpinteria, CA) at a dilution of 1:50 after antigen retrieval, by pressure steaming, in an ethylenediaminetetraacetic acid solution (pH 8.0). Antibody detection was accomplished using a labeled streptavidin-biotin LSAB+ (DAKO) method.

5′-Rapid Amplification of cDNA Ends (RACE) and DNA Sequencing

Total RNA was extracted from frozen tissue using Trizol (Life Technologies, Inc., Gaithersburg, MD), according to the manufacturer's protocol. One μg of RNA was then used for RACE using the Marathon Kit (Clontech, Palo Alto, CA) according to the manufacturer's protocol. First round polymerase chain reaction (PCR) was with the Marathon kit AP1 adapter primer and *ALK* reverse primer ALK/4436/R (5′-CTGTTGAGAGACCAGGAGAGGAGGAA) at 94°C for 30 seconds and 68°C for 8 minutes for 25 cycles. Second round PCR was with the Marathon kit AP2 adapter primer and *ALK* reverse primer ALK/4161/R, (5′-ACTTCCTGGTTGCTTTTGCTGGGGTAT) at 94°C for 30 seconds and 68°C for 8 minutes for 30 cycles. A single RACE product band was observed and was gel purified using the Qiaquick gel extraction kit (Qiagen, Valencia, CA), and the RACE fragment was then cycle sequenced in forward and reverse directions using the AP2 primer and *ALK* reverse primer ALK/3638/R (5′-AATCCAGTTCGTCCTGTTCAGAGCACA), respectively. Cycle sequencing was performed using ABI BigDye terminators, and the sequences were analyzed using an ABI Prism 310 automated sequencer (Applied Biosystems, Foster City, CA).

Fluorescence in Situ *Hybridization*

Interphase cells from IMT case 1 were spread on glass slides and denatured according to standard protocols. Hybridization and washing steps were performed as described.[15] ALK and *TPM4* region rearrangements were evaluated using a dual-color *ALK* split-apart probe (Vysis, Downers Grove, IL) and a yeast artificial chromosome clone, 766_E_7, mapping to chromosome band 19p13.1, which was labeled 50:50 biotin:digoxigenin by random octamer priming.[15] Detection was with streptavidin-fluorescein isothiocyanate (Zymed Laboratories, South San Francisco, CA) and rhodamine anti-digoxigenin (Zymed), and nuclei were counterstained with 0.4 mg/ml 4,6-diamidino-2-phenylindole-dihydrochloride. Images were captured using a charge-coupled device camera (Photometrics, Tucson, AZ).

RT-PCR and DNA Sequencing

Total RNAs were extracted from frozen tissue specimens using Trizol (Life Technologies, Inc.) and from paraffin-embedded tissues after a 5-day sodium dodecyl sulfate-proteinase K digestion.[16] One μg of the RNAs were reverse transcribed (GeneAmp Kit; Perkin Elmer, Norwalk, CT) using the ALK/3638/R primer. *TPM3-ALK* and *TPM4-ALK* fusion genes were evaluated using a nested PCR method that was optimized for detection of either *TPM3* or *TPM4* fusions in the extensively degraded RNAs isolated from paraffin sections. The TPM primers correspond to regions of near sequence identity in *TPM3* and *TPM4* and, hence, anneal effectively to both genes. First-round PCR was with TPM3/249/F (5′-ACTGATAAACCCAAGGAGGCAGAGA) and ALK/3455/R (5′- AGGTCTTGCCAGCAAAGCAGTAGTT) at 94°C for 30 seconds, 67 to 62°C (touchdown) for 30 seconds, and 72°C for 30 seconds for 10 cycles, and then 94°C for 30 seconds, 62°C for 30 seconds, and 72°C for 30 seconds for 25 cycles. Second-round PCR was with TPM3/269/F (5′-AGAGACCCGTGCTGAGTTTGCTGA) and ALK/3399/R (5′- CGGAGCTTGCTCAGCTTGTACTC) at 94°C for 30 seconds, and 68°C for 30 seconds for 30 cycles. Five microliters of the second-round PCR product were gel purified and cycle sequenced using the ALK/3399/R primer to determine whether the tropomyosin-derived sequence was contributed by *TPM3* or *TPM4*. TPM-ALK fusions were also subtyped using first-round *TPM-ALK* reverse transcriptase (RT)-PCR template (as described above) in a second-round PCR with a *TPM3*-specific forward primer, TPM3/284/F (5′-GTTTGCTGAGCGATCGGTAGCCAAGC) and ALK/3399R at 94°C for 30 seconds, and 68°C for 30 seconds for 30 cycles. Nested RT-PCR was required for consistent detection of the *TPM-ALK*

fusion transcripts, irrespective of whether RNAs were isolated from frozen or paraffin specimens.

Integrity of both frozen tumor and paraffin-derived RNAs was evaluated by nested RT-PCR for the ubiquitously expressed glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene. Reverse transcription was performed using random primers (GeneAmp Kit, Perkin Elmer), and first-round PCR was performed using GAPDH/177/F (5′-CCTCAACTACATGGTTTACATGTTC) and GAPDH/488/R (5′-CTGTTGTCATACTTCTCATGGTTCA) at 94°C for 30 seconds and 70 to 60°C (touchdown) for 1 minute for 10 cycles, and then 94°C for 30 seconds, and 60°C for 1 minute for 25 cycles. Second-round PCR was with GAPDH/250/F (5′-AACGGGAAGCTTGTCATCAAT) and GAPDH/427/R (5′-CAGAGATGATGACCCTTTTGG) at 94°C for 30 seconds, and 62°C for 30 seconds for 30 cycles.

In Vitro *Kinase Assay and Western Blotting*

Protein lysates were isolated from frozen tumor tissue in IMT cases 1 and 2 and from a non-IMT spindle-cell sarcoma. Three-mm^3 tumor pieces were minced in ice-cold lysis buffer (1% Nonidet P-40, 50 mmol/L Tris, pH 8.0, 100 mmol/L sodium fluoride, 30 mmol/L sodium pyrophosphate, 2 mmol/L sodium molybdate, 5 mmol/L ethylenediaminetetraacetic acid, 2 mmol/L sodium vanadate, 10 μg/ml aprotinin, 10 μg/ml leupeptin, and 100 μg/ml phenylmethylsulfonyl fluoride), homogenized further by 10 to 20 strokes of a Dounce homogenizer, then rocked for 45 minutes at 4°C. Residual cell debris was removed by centrifugation, and supernatant protein concentrations were determined using the BioRad MMT assay. Cell lysates (1.5 mg) were precleared with 20 μl of Protein G Sepharose (Zymed Laboratories) for 1 hour at 4°C, followed by sequential additions of 50 μl of anti-ALK hybridoma supernatant (ALK1; DAKO) and 20 μl of Protein G Sepharose with end-to-end rotation for 2 hours at 4°C after each addition. The immunoprecipitates were then washed three times in lysis buffer and twice in kinase buffer (20 mmol/L HEPES, pH 7.4, 10 mmol/L sodium fluoride, 10 mmol/L magnesium chloride, 10 mmol/L manganese chloride, 1 mmol/L sodium vanadate) before incubation in 20 μl of fresh kinase buffer containing 5 μCi ^{32}P-(γ)-ATP for 15 minutes at 25°C. Kinase reactions were stopped by addition of sodium dodecyl sulfate-polyacrylamide gel electrophoresis loading buffer, and 15 μl of each reaction were resolved on 4 to 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis gradient gels followed by electrophoretic transfer to polyvinylidene difluoride membranes (Millipore, Bedford, MA) and autoradiography. Tyrosylphosphorylation was evaluated by staining the membranes with PY99 anti-phosphotyrosine monoclonal antibody (Santa Cruz Biotechnology, Santa Cruz, CA). The membranes were then stripped and restained with a rabbit anti-ALK polyclonal antiserum (Sanbio, Uden, The Netherlands). Both PY99 and anti-ALK were detected by chemiluminescence (ECL; Pierce, Rockford, IL).

Table 1. Clinicopathological Data, *ALK* Expression, and *ALK* Fusion Events in 11 IMTs

Case no.	Age/sex	Tumor location	*ALK* expression	*ALK* fusion*
1	1/M	Abdomen	Cytoplasmic	*TPM4-ALK*
2	30/F	Lung	Cytoplasmic	*TPM3-ALK*
3	23/F	Abdomen	Cytoplasmic	*TPM3-ALK*
4	8/M	Abdomen	Cytoplasmic	–
5	1/F	Abdomen	Cytoplasmic	–
6	27/M	Femur	Cytoplasmic	–
7	4/M	Abdomen	Nuclear	–
8	40/M	Paratesticular	–	–
9	50/M	Abdomen	–	–
10	50/F	Lung	–	–
11	40/M	Femur	–	–

IMT, Inflammatory myofibroblastic tumor; –, not detected.
*By RT-PCR screening for *TPM4-ALK* and *TPM3-ALK*.

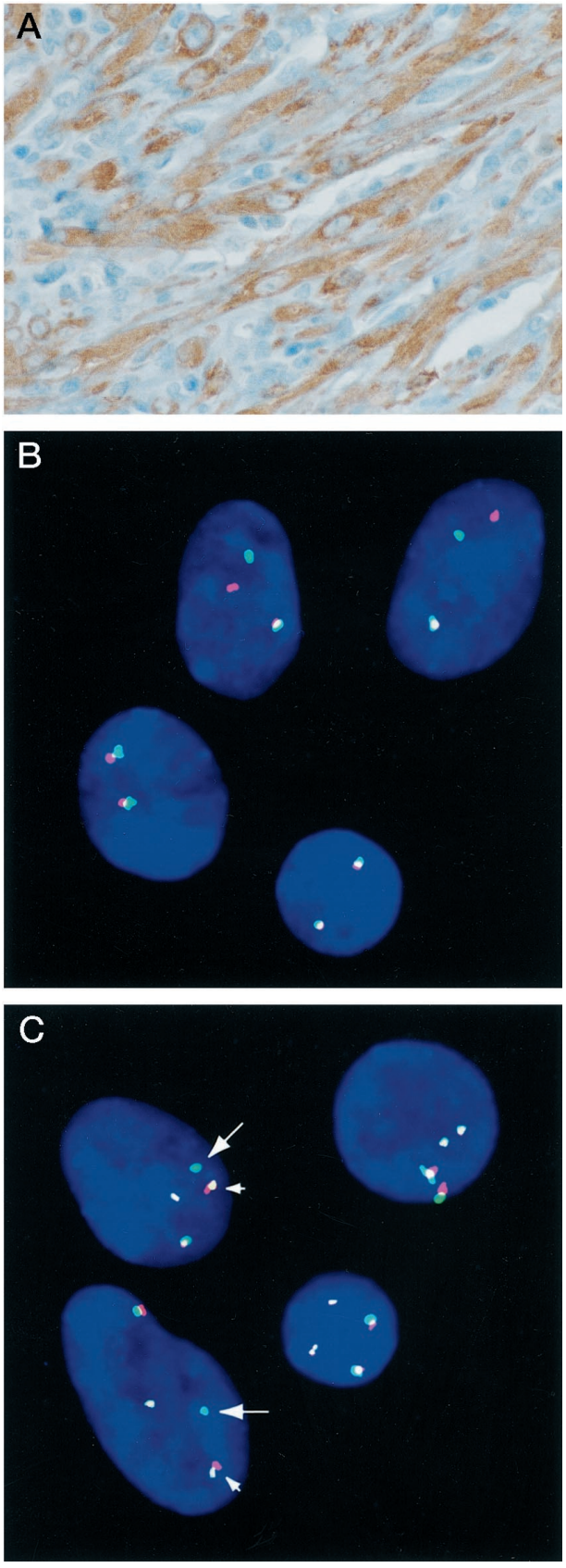
Results

Histology and ALK Expression

The 11 cases showed typical histomorphological features of IMTs, being composed of a variably cellular proliferation of plump, spindle-shaped fibroblasts/myofibroblasts set in a collagenous or myxoid stroma containing numerous lymphocytes and plasma cells. The IMT fibroblasts/myofibroblasts showed mild to moderate variation in nuclear size but there was no significant nuclear hyperchromasia or pleomorphism. Seven of 11 IMTs were ALK immunopositive, all having strong staining of 50 to 90% of the lesional spindle cells (Table 1, Figure 1A). The ALK immunostaining was cytoplasmic in six cases and nuclear in one, the latter being concentrated in the nuclear membrane with no nucleolar accentuation. ALK immunostaining was not detected in the IMT stromal inflammatory cells (Figure 1A).

Identification of a TPM4-ALK Fusion Gene

Cytogenetic analysis of IMT case 1 demonstrated a translocation involving the chromosome band 2p23 *ALK* locus region and an unidentified partner chromosome. FISH analysis demonstrated that the 2p23 rearrangement was within 200 kb of the *ALK* locus (Figure 1B). 5′-RACE was then performed to evaluate the possibility of a translocation-related *ALK* fusion transcript. A single RACE product of ~1,650 bp was obtained using *ALK*-specific primers corresponding to the ALK kinase domain. Sequence analysis demonstrated that the 1,650-bp RACE product was an in-frame fusion of the *TPM4* and *ALK* genes. The predicted fusion protein contains all but the 27 most C-terminal amino acids of TPM4, joined (TIDDLE::VYRRKH) to the ALK juxtamembrane and kinase domains. The *ALK* fusion breakpoint is identical to those in *NPM-ALK* and *TPM3-ALK* fusions in anaplastic large-cell lymphoma.[11,17] The *TPM4* locus maps to chromosome band 19p13.1, and FISH confirmation of *TPM4-ALK* fusion was obtained by co-hybridization of a dual-color *ALK* FISH probe and a yeast artificial chromosome clone,



766_E_7, mapping centromeric to the chromosome band 19p13.1 *TPM4* locus (Figure 1C).

The first 186 bp of the *TPM4-ALK* 5′-RACE sequence differs from the *TPM4* sequence published previously. Both the variant and previously published *TPM4* transcripts contain three TPM signature sequences, and virtually the entire protein sequence, in each case, is expected to form a coiled-coil structure. However, the variant *TPM4* transcript lacks the first two in-frame ATG codons found in the published *TPM4*, and hence has a smaller open reading frame. Alternate *TPM4-ALK* fusion transcripts containing the variant (designated type 1 hereafter) and published (designated type 2 hereafter) 5′ ends were evaluated by RT-PCR using type 1- (5′-CGAGGCTCCCCCGCCTCGTC-3′) or type 2- (5′-GCCATGGCCGGCCTCAACTCC-3′) specific forward primers and a common reverse primer, ALK/3369/R (5′-TGCAGCTCCATCTGCATGGCTTG-3′). *TPM4-ALK* type 1 (609 bp) and *TPM4-ALK* type 2 (716 bp) RT-PCR products were detected reproducibly on each of three separate PCR runs (Figure 2) and confirmed by sequencing (*TPM4-ALK* type 1 = GenBank AF186110, and *TPM4-ALK* type 2 = GenBank AF186109).

ALK Is Fused to Another TPM Family Member in IMT

The coding sequences for *TPM4* and *TPM3* are highly homologous, and the *TPM3* locus maps to chromosome bands 1q22-23. Notably, IMT case 2 contained a balanced translocation, t(1;2)(q21;p23), suggestive of a *TPM3-ALK* fusion event. We directed primers to homologous and nonhomologous regions of *TPM3* and *TPM4*, to establish RT-PCR screening approaches detecting both *TPM3-ALK* and *TPM4-ALK* fusions (method 1) *versus* *TPM3-ALK* fusion only (method 2). Evaluation of 11 IMTs confirmed detection of a *TPM-ALK* fusion in case 1 (*TPM4-ALK*) by RT-PCR method 1 but not by method 2 (Figure 3). By contrast, fusion sequences, consistent with *TPM3-ALK*, were identified by methods 1 and 2 in IMT cases 2 and 3 (Figure 3). Sequence analysis confirmed *TPM3-ALK* fusions in the latter two cases. The *TPM3-ALK* fusion breakpoints were identical to those reported recently in anaplastic large-cell lymphoma,[17] and the *TPM3* and *ALK* breakpoints were homologous and identical, respectively, to the *TPM4* and *ALK* breakpoints in IMT case 1 (Figure 4).

Figure 1. ALK subcellular localization (**A**) and FISH evaluations (**B–C**) in IMT case 1: Strong cytoplasmic ALK staining is evident in the spindle cells, whereas ALK is undetectable in the intervening inflammatory cells (**A**). FISH probes centromeric and telomeric in relationship to *ALK* are detected with Spectrum Green and Spectrum Orange, respectively (**B-C**). **B:** Centromeric and telomeric ALK probes are translocated apart in the top two cells. Bottom two cells, presumably inflammatory, have normal FISH signals. **C:** A chromosome band 19p13 yeast artificial chromosome clone, 766_E_7, centromeric to the TPM4 gene, is detected with 50:50 Texas Red: fluorescein isothiocyanate. **Small arrows** indicate colocalized yeast artificial chromosome 766_E_7 (yellow) and telomeric/red *ALK* probe, whereas **large arrows** indicate corresponding centromeric/green *ALK* probe. The two cells at right have normal FISH signals.

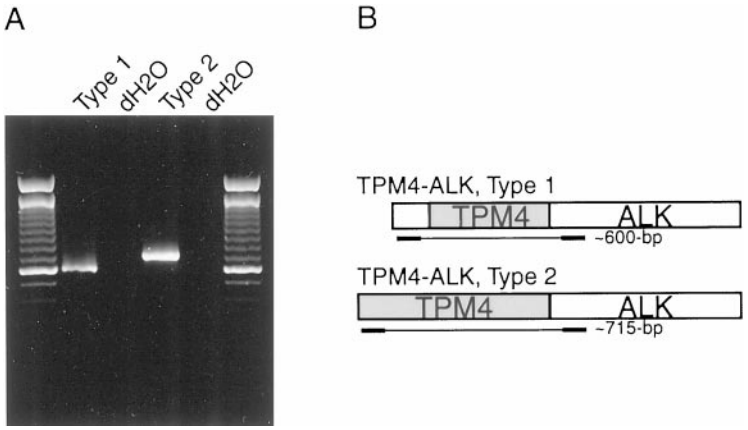


Figure 2. Expression of alternate *TPM4-ALK* transcripts in IMT case 1. Type 1 *TPM4-ALK* was identified by RACE, whereas the type 2 transcript was predicted based on published TPM4 sequence. Both type 1 (609 bp) and type 2 (716 bp) transcripts were identified by RT-PCR (**A**). Primer locations are as schematized (**B**).

TPM4-ALK and TPM3-ALK Are Constitutively Activated

Detection of putative ALK fusion proteins, and evaluation of tyrosine kinase activities, was accomplished by anti-ALK immunoprecipitation in protein lysates from IMT case 1 (*TPM4-ALK*) and case 2 (*TPM3-ALK*). *In vitro* kinase assays revealed similar patterns of putative ALK fusion proteins in the IMT but not in a non-IMT spindle cell sarcoma (Figure 5A). Both IMTs contained three kinase-active proteins ranging in size from 80 to 95 kd. Confirmation of tyrosylphosphorylated ALK forms was then obtained by sequential restaining with anti-phosphotyrosine and a polyclonal anti-ALK antibody (Figure 5, B–C). Notably, anti-ALK staining also revealed a 200-kd protein, consistent with native ALK, which was expressed strongly and weakly in IMT cases 1 and 2, respectively, and which lacked *in vitro* kinase activity and tyrosylphosphorylation.

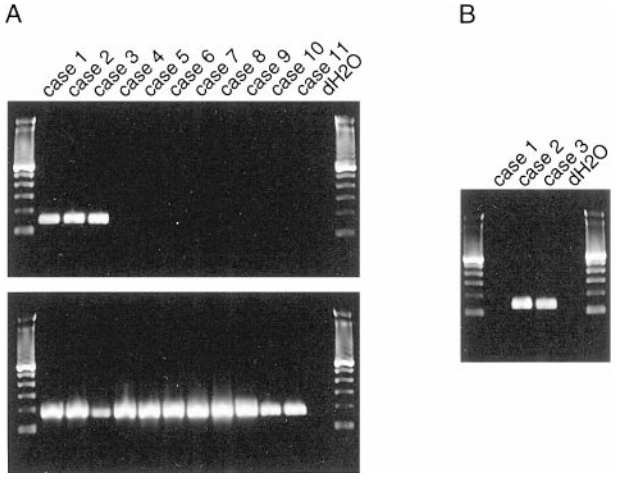


Figure 3. A: Expression of *TPM4-ALK* and *TPM3-ALK* in 11 IMT. RT-PCR with universal TPM4/TPM3 primers identifies 147-bp transcripts in IMT cases 1, 2, and 3 (**top**), whereas positive control (177 bp) GAPDH transcripts are amplified from all cases (**bottom**). **B:** RT-PCR with primers specific for *TPM3-ALK* reveals fusion transcripts (130 bp) in cases 2 and 3. The identity of all transcripts was confirmed by sequencing (data not shown).

Discussion

IMT are clinicopathologically distinctive tumors of disputed pathogenesis.[1–4] The studies described herein show that *ALK* activation is a recurrent oncogenic event in IMTs. *ALK* activation, in some cases, is accomplished by chromosomal fusion with the *TPM4* or *TPM3* loci. In other cases, *ALK* is likely activated by fusion with other genes.

Receptor tyrosine-kinase oncogenes have been described in many tumors, and the usual mechanisms of activation are point mutations or gene fusions that result in constitutive, ligand-independent, receptor oligomerization. Fusion receptor tyrosine-kinase oncoproteins are typically composed of an N-terminal sequence, encoded by the non-RTK member of the fusion pair, containing one or more oligomerization domains and replacing the RTK transmembrane and/or extracellular domains. Our studies indicate that either TPM3 or TPM4 can contribute oligomerization domains to ALK fusion oncoproteins in IMTs. Notably, the TPM3 sequences contained in IMT ALK oncoproteins are identical to those found in TPM3-NTRK1 oncoproteins in papillary thyroid carcinoma.[18] TPM3 contributes a coiled-coil self-association domain to the TPM3-NTRK1 oncoprotein and consequently enables constitutive oligomerization and NTRK1 kinase activation. Moreover, while our studies were in progress, *TPM3-ALK* oncogenic fusions, containing the same *TPM3* sequence, were reported in a subset of anaplastic large-cell lym-

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TPM4-GAGGCTGAGACCCGTGCTGAATTTGCAGAG
TPM3-GAGGCAGAGACCCGTGCTGAGTTTGCTGAG

TPM4-AGAACGGTTGCAAAACTGGAAAAGACAATTG
TPM3-AGATCGGTAGCCAAGCTGGAAAAGACAATTG

TPM4-ATGACCTGGAAGTGTACCGCCGGAAGCACCA
TPM3-ATGACCTGGAAGTGTACCGCCGGAAGCACCA
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Figure 4. Homology between *TPM4-ALK* and *TPM3-ALK* cDNA fusion regions. Sequence mismatches, used to distinguish the *TPM4-ALK* and *TPM3-ALK* RT-PCR products, are set in boxes. *ALK* sequence is shown in a gray box.

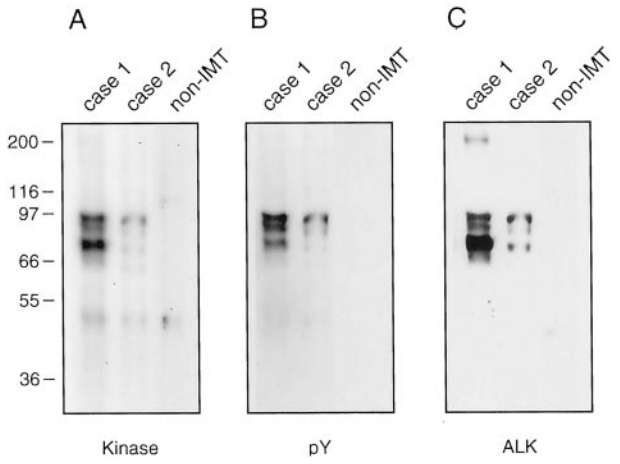

Figure 5. *In vitro* kinase (**A**), phosphotyrosine (**B**), and ALK (**C**) evaluations in IMT case 1 (TPM4-ALK), IMT case 2 (TPM3-ALK), and a non-IMT spindle-cell sarcoma. Three kinase-active, tyrosylphosphorylated ALK forms (80 to 95 kd) are seen in the IMT. Native ALK (200 kd), seen in IMT case 1 and also demonstrable in case 2 at longer exposures, lacked constitutive kinase activity and tyrosylphosphorylation.

phomas.[17] The *TPM3-ALK* and *TPM4-ALK* IMT fusion genes are highly homologous in that the TPM gene sequences in each case encode a related series of coiled-coil domains. In addition, the fusion breakpoints are found in the corresponding location for both genes (Figure 4). TPM3-ALK and TPM4-ALK are constitutively active oncoproteins, as evidenced by high-level, ligand-independent, kinase activity and resultant tyrosylphosphorylation (Figure 5).

TPM3 and *TPM4* are highly expressed in mesenchymal cell lineages: hence, both genes are expected to contribute active promoter regions to the IMT *ALK* fusion genes.[19–21] Physiological ALK expression, by contrast, is primarily restricted to the central nervous system and is low, specifically, in nonneoplastic mesenchymal cells.[12] Nonetheless, our studies reveal 200-kd ALK, consistent with expression of a native, nonfusion, ALK protein and encoded presumably by the nontranslocated allele, in neoplastic IMT myofibroblasts (Figure 5C). Therefore, ALK signaling pathways might be important biologically in IMT nonneoplastic progenitor cells. These pathways, when activated constitutively, might contribute to neoplastic transformation. It will be intriguing to determine whether other myofibroblastic neoplasms, particularly those arising in children and young adults, result from ALK activation. A speculative, but intriguing, aspect of the TPM-ALK oncogenic mechanism is the possible interference, through heterodimerization, with normal TPM cellular functions. Although there is no direct evidence for a dominant-negative TPM-ALK mechanism, several observations indicate a tumor suppressor role for certain TPM isoforms. It is known that TPM expression can suppress the transformed phenotype in both src- and ras-transformed fibroblasts.[22] Furthermore, TPMs are downmodulated, in part through decreased synthesis, in various retrovirus-induced neoplasms.[23,24] These findings suggest that interference with normal TPM function, which would potentially result from heterodimerization with TPM-ALK oncoproteins, might contribute to neoplastic transformation of IMT progenitor cells.

Both *TPM4-ALK* and *TPM3-ALK* fusions were identified in this series of IMTs (Table 1). Myofibroblasts with *TPM4-ALK* and *TPM3-ALK* fusions featured intense, predominantly cytoplasmic, ALK staining patterns (Figure 1A), in keeping with that reported for the TPM3-ALK oncoprotein in anaplastic large-cell lymphoma.[17] Four other IMTs expressed ALK at high levels but did not seem to contain *TPM-ALK* (cases 4 to 7; Table 1 and Figure 3) or *NPM-ALK* (data not shown) fusions. These cases included three in which ALK localization was cytoplasmic (cases 4, 5, and 6) and one in which ALK localization was seen mainly in the nuclear membrane (case 7). None of the cases demonstrated the nucleolar localization typical of NPM-ALK fusion oncoproteins. Previous studies, in anaplastic large-cell lymphoma, have shown that cellular sublocalization patterns, for the various ALK fusion oncoproteins, are consistent and distinctive.[25–27] In some cases, eg, NPM-ALK, a characteristic localization results from shuttling of fusion oncoprotein heterodimers to those cellular structures with which the ALK fusion partner is normally associated.[28] The subcellular localization patterns establish morphological signatures for the different ALK fusion proteins, notwithstanding the fact that the major site of oncogenic action—at least with respect to activation of ALK signaling pathways—is undoubtedly cytoplasmic.[26–28] Therefore, our data imply the existence of at least two alternative ALK fusion mechanisms in IMTs, and these putative fusions likely involve proteins that are predominantly cytoplasmic (cases 4, 5, and 6) and predominantly nuclear (case 7) in their native localization.

The studies described herein are of clinical relevance inasmuch as the clinical behavior of IMT is often difficult to predict. IMTs can be confused with various benign and malignant spindle-cell neoplasms or, alternatively, with various inflammatory nonneoplastic processes. Consequently, evaluation of *ALK* rearrangement and ALK expression will likely be useful in distinguishing IMTs from other myofibroblastic proliferations. Our preliminary studies show that ALK is not expressed in a variety of non-IMT lesions, including nodular fasciitis, inflammatory liposarcoma, inflammatory leiomyosarcoma, and angiomatoid malignant fibrous histiocytoma, which—akin to IMT—are characterized by neoplastic spindle cells admixed with an inflammatory component (CDM Fletcher, unpublished data). Based on our initial data, it seems that a substantial subset of IMTs will contain TPM-ALK fusions, whereas other cases will likely contain *ALK* fusions involving genes other than *TPM3* and *TPM4*. Still other IMTs, as evidenced by the absence of immunohistochemical ALK staining in a subset of cases (Table 1, cases 8 to 11), probably lack *ALK* fusion genes. This pathogenetic heterogeneity is reminiscent of that in anaplastic large-cell lymphomas, where tumors are increasingly divided into ALK-positive and ALK-negative categories. Indeed, there is a growing sense that ALK status (rearranged and expressed *versus* wild-type and nonexpressed) discriminates between two fundamentally different types of anaplastic large-cell lymphomas.[29–31] Notably, the four ALK-

negative IMTs in this series (Table 1) were diagnosed in 40- to 50-year-old patients, whereas the seven ALK-positive IMTs were diagnosed in patients younger than 30 years old. These data suggest that IMTs in children and young adults might depend generally on ALK activation, whereas IMTs in older adults might represent a different disease and/or have different transforming mechanisms. However, additional studies are required to determine whether ALK status correlates with histopathological features and clinical behavior in IMT.

It is intriguing that *ALK* fusion genes, including an identical *TPM3-ALK* fusion, are found in both anaplastic large-cell lymphoma and IMTs.[11,17,32–34] However, the preferred *ALK* fusion partners differ in anaplastic large-cell lymphoma and IMT. *NPM-ALK* fusions, found in 80% of anaplastic large-cell lymphomas, have not yet been identified in IMTs, and *TPM3-ALK* fusions are rare in anaplastic large-cell lymphoma. Anaplastic large-cell lymphoma and IMT are associated classically with systemic signs, particularly fever, and one wonders whether this is a direct consequence of unregulated and/or unscheduled ALK signaling. Recent studies show that specific RTK activating mechanisms can bring about transformation of different human cell lineages. For example, activating *KIT* point mutations are found in human mast cell neoplasms, myeloid leukemia, germ cell tumors, and gastrointestinal stromal tumors,[35–38] and *ETV6-NTRK3* fusion oncogenes are found in both myeloid leukemia and congenital fibrosarcoma.[39,40] Irrespective, our studies reveal the first example of an identical RTK fusion oncogene in lymphoid and mesenchymal neoplasia. This finding is in keeping with the observation that activated RTKs are generally transforming in both lymphoid (Ba/F3 cell) and mesenchymal (3T3 cell) models, *in vitro*. In summary, we have demonstrated a recurring oncogenic mechanism, involving ALK activation, in IMTs. These findings highlight the substantial relevance of RTK mechanisms in human mesenchymal neoplasia, and they underscore that such mechanisms are not lineage-specific.

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