Recurrent fusion of the genes *FN1* and *ALK* in gastrointestinal leiomyomas

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Leiomyomas of the gastrointestinal tract are mostly found in the esophagus, stomach, and colon. Genetic information about them is very limited and no fusion genes have been described. We present herein cytogenetic and molecular genetic analyses of two gastrointestinal leiomyomas found in the esophagus and small intestine. The esophageal leiomyoma had the karyotype 45,Y,der(X)t(X;6)(p22;p21),inv(2)(p23q35),add(6)(p21), – 11[cp6]/46,XY[7]. The intestinal leiomyoma karyotype was 46,X,add(X)(q2?),der(2)add(2)(p23)add(2)(q33),add(4)(p14),add(14)(q22)[10]/47,XX,+12[2]/46,XX[1]. RNA-sequencing detected *FN1*–*ALK* fusion transcripts in both tumors. RT-PCR together with Sanger sequencing verified the presence of the *FN1*–*ALK* fusion transcripts. Fluorescence *in situ* hybridization using an *ALK* breakapart probe further confirmed the rearrangement of the *ALK* gene. Immunohistochemical investigation of *ALK* in the leiomyoma of the small intestine revealed positivity with strong granular cytoplasmatic staining in the tumor cells. This is the first ever *ALK* fusion reported in gastrointestinal leiomyomas. Our results are of potential clinical importance because crizotinib, a selective *ALK* inhibitor, has demonstrated effect in patients whose tumors harbor *ALK* rearrangements. Thus, *ALK* emerges as a possible therapeutic target in patients whose tumors, including gastrointestinal leiomyomas, carry *ALK* fusions.

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Leiomyomas of the gastrointestinal tract mostly involve the esophagus, stomach, small intestine, and colon.^{1–6} They are classified as superficial leiomyomas that arise from the muscularis mucosae and deep leiomyomas that arise in the muscularis propria.¹

Esophageal leiomyomas are rare benign intramural tumors with an incidence of 0.006–0.1%. They account for two-thirds of all benign esophageal tumors⁷ and may be found at any age, but mostly between 30 and 50 years of age.⁷ The tumors often remain stable in size for years and malignant transformation to leiomyosarcoma has been reported in only four cases.⁷ The leiomyomas arise from the smooth muscle in the muscularis propria or muscularis mucosae, and most tumors are solitary involving only a localized area of the esophageal wall. Tumors with diameter < 5 cm are asymptomatic, but larger

tumors can cause epigastric discomfort, dysphagia, regurgitation, diarrhea, and weight loss.⁷ In the past, esophageal leiomyoma was considered part of the spectrum of mesenchymal tumors that also includes gastrointestinal stromal tumors (GIST). However, later studies have shown that the two are distinct entities with regard to ultrastructure, histology, and genetic and immunohistochemical markers.^{5,8}

Leiomyomas are rare in the small intestine.⁶ In a series of 1091 smooth muscle tumors of the small intestine, only 13 were diagnosed as leiomyomas.⁶ They nevertheless represent the most common symptomatic benign tumors of this location.⁹ The main clinical manifestations are hemorrhage, anemia, and/or abdominal pain/discomfort.⁹ The tumors may occur at any age with a peak incidence between 50 and 60 years in both males and females.¹⁰ They may be solitary or multiple and are mostly found in the jejunum, followed by the ileum and duodenum.¹⁰ Also, these leiomyomas arise from smooth muscle in the muscularis propria or mucosae and four growth patterns have been identified: intraluminal, which is the most common, intramural, extraluminal (subserosal), and dumbbell-shaped tumors.^{1,3,6,10}

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Macroscopically, leiomyomas are firm, rubbery, well-encapsulated masses with a smooth cut surface. Their cross section is white, gray, tan, or yellow in color and often has a whorled appearance. On histologic examination, leiomyomas are characteristically composed of uniform spindle cells arranged in fascicles or whorls with eosinophilic cytoplasm and surrounding hypovascular connective tissue. There are few or no mitotic figures, the nuclei are bland and cigar-shaped, and there is minimal to no cellular atypia.^{5,7,8}

Immunohistochemically, leiomyomas are typically positive for desmin and smooth muscle actin (SMA) but negative for CD34 and CD117 (which correspond to the KIT protein).^{2–8} In a recent study, however, the majority of esophageal and stomach leiomyomas were found to have scattered cells immunophenotypically positive for DOG-1 (discovered on GIST-1) and KIT.¹ These cells were indistinguishable from interstitial cells of Cajal and were absent from superficial leiomyomas of the colon and rectum.¹

Although a lot is known about the genetics of uterine leiomyomas,¹¹ corresponding information on gastrointestinal leiomyomas is very limited. A deletion at the *COL4A5/COL4A6* locus in Xq22 was reported in an esophageal leiomyoma.¹² A comparative genomic hybridization study showed genomic imbalances in three of nine esophageal leiomyomas. Gains of chromosome 5 were seen in all three tumors with less common gains being detected in or for chromosomes 3, 4, 8, and 17. Losses were detected in 16p.¹³ Screening of four esophageal leiomyomas for mutations of *PDGFRA* and *KIT* showed no mutations.¹

Only three cytogenetically analyzed gastrointestinal leiomyomas have been reported.^{14–16} An esophageal leiomyoma had a simple karyotype with trisomy for chromosomes 7 and 8 as the only changes,¹⁶ a leiomyoma of the small intestine had the karyotype 45,XX,del(1)(p12–13),–15,¹⁵ whereas a leiomyoma of the stomach showed more complex changes giving the karyotype 56,XY,+X,+5,+5,+7,+7,+8,+8,del(9)(p12p21),+del(9)(q12),+12,+12,dic(14;19)(p11;p11) or dic(14;19)(p11;q11),+19,–21.¹⁴ These three leiomyomas were reported in the ‘pre-KIT’ era, before the recognition of GIST as a distinct entity, a time when virtually all published series of leiomyomas and leiomyosarcomas of the gastrointestinal tract included a substantial number of GISTs.¹

We present here the cytogenetic and molecular genetic analyses of two gastrointestinal leiomyomas found in the esophagus and small intestine. In both cases, the tumor cells were found to carry an *FN1-ALK* fusion gene.

Materials and methods

Ethics Statement

The study was approved by the regional ethics committee (Regional komité for medisinsk forskningsetikk Sør-Øst, Norge, <http://helseforskning.etikk.no>).

no), and written informed consent was obtained from the patients to publication of the case details. The ethics committee’s approval included a review of the consent procedure. All patient information has been de-identified.

Presentation of Patients

Patient 1. After an 18 months history of increasing dysphagia, an otherwise healthy 38-years-old male was diagnosed with a 94 × 82 × 45 mm tumor in the distal esophagus. The tumor extended 20 mm proximal to the Z-line. A GIST was the primary suspicion based on the radiologic findings. Trans thoracic resection was performed. The tumor had a yellow, fleshy cut surface. Microscopically, the tumor was composed of bundles of spindle cells without atypia or increased mitotic figure (< 1/10 HPF) (Figure 1a and b). Immunohistochemical investigation revealed positivity for actin, caldesmon, desmin, and SMA (Figure 1c–f). The immunoreactivity was diffuse with the cells showing a strong cytoplasmic staining reaction (Figure 1c–f). CD117 and DOG-1 were negative. On the basis of morphology and immunohistochemistry, the tumor was diagnosed as a leiomyoma.

Patient 2. A 60-years-old woman with back pain underwent CT-urography. As an incidental finding, a 55 × 35 × 50 mm large tumor in the jejunal wall was found. Radiologically, GIST was the number one consideration. The tumor was resected. It had a white, whorled cut surface. Microscopically, the tumor was composed of bundles of spindle cells without atypia or increased mitotic figure (< 1/10 HPF) (Figure 3). Immunohistochemical investigation revealed positivity for desmin and SMA (Figure 3c and d). The immunoreactivity was diffuse with the cells showing a strong cytoplasmic staining reaction (Figure 3c and d). CD117 and DOG-1 were negative. On the basis of morphology and immunohistochemistry, the diagnosis was leiomyoma. Because of the molecular data obtained (see below), the tumor was also tested for expression of the ALK fusion protein (clone D5F3, Ventana /Roche) for which it was found positive with strong granular cytoplasmic staining of the cells (Figure 2e and f).

G-Banding and Karyotyping

Fresh samples of the tumors were received and processed for cytogenetic analysis as a part of our diagnostic routine. The samples were disaggregated mechanically and enzymatically with collagenase II (Worthington, Freehold, NJ, USA). The resulting cells were cultured and collected using standard techniques. Chromosome preparations were G-banded with Wright stain and examined. The karyotype was written according to The International System for Human Cytogenetic Nomenclature (ISCN) 2013 guidelines.¹⁷

Figure 1 Microscopic examination of the esophageal leiomyoma. (a) H&E-stained section showing the tumor's relation to the epithelium, $\times 100$. (b) H&E-stained section showing the tumor spindle cells, $\times 100$. (c) Immunoexpression of actin, $\times 200$. (d) Immunoexpression of caldesmon, $\times 200$. (e) Immunoexpression of desmin, $\times 200$. (f) Immunoexpression of SMA, $\times 200$. SMA, smooth muscle actin.

Fluorescence *In Situ* Hybridization (FISH)

FISH was performed on both metaphase spreads and interphase nuclei. For the esophageal leiomyoma (patient 1), the BAC CTD2242D5 (chr6: 34131452-34315125, UCSC Genome Browser on Human Feb.

2009, GRCh37/hg19 Assembly) was used in order to detect possible rearrangement of the *HMGA1* gene in 6p21 (see below). BAC CTD2242D5 spans the *HMGA1* locus. DNA was extracted and the probe labeled and hybridized according to Abbott Molecular recommendations (<http://www.abbottmolecular.com/home.html>).

For both gastrointestinal leiomyomas, the *ALK* Breakapart probe, purchased from CytoCell (<http://www.cytoCELL.co.uk>), was used in order to detect rearrangement of the *ALK* gene in 2p23. Fluorescent signals were captured and analyzed using the CytoVision system from Leica Biosystems (<http://www.leicabiosystems.com/pathology-imaging/cytogenetics/>).

High-Throughput Paired-End RNA-sequencing

Total RNA was extracted from frozen (-80°C) tumor tissue adjacent to that used for cytogenetic analysis and histologic examination. The miRNeasy Mini Kit was used according to the manufacturer's instructions (Qiagen, Hilden, Germany). Tumor tissues were disrupted and homogenized in Qiazol Lysis Reagent

Figure 2 Cytogenetic, molecular cytogenetic, and RT-PCR data on the esophageal leiomyoma. (a) Partial karyotype showing the chromosomes der(X), 6, der(6), and 11. Breakpoint positions are indicated by arrows. (b) FISH using an *HMGA1* breakapart probe. (c) FISH using an *ALK* breakapart probe showed splitting of *ALK*. The 3'-end part of the *ALK* gene (red probe) moved to 2q35 of the *inv(2)*, whereas the 5'-end part of the gene (green probe) remained on 2p23 of the *inv(2)*. (d) FISH using an *ALK* breakapart probe showed splitting of *ALK* in interphase nuclei. (e) Partial karyotype showing the *inv(2)(p23q35)* and the normal chromosome 2 together with the corresponding ideograms. Breakpoint positions are indicated by arrows. (f) Gel electrophoresis of the 847 bp amplified cDNA *FN1-ALK* fragment of the esophageal leiomyoma (lane L) using the primer combination FN1-6842F1 and ALK-3965R1. M: 1Kb DNA ladder (GeneRuler, ThermoFisher Scientific). (g) Partial sequence chromatogram of the amplified cDNA fragment showing the fusion point in the *FN1-ALK* fusion transcript. Exon 42 of *FN1* (nt 7284 of sequence with accession number NM_212482.1) was fused to exon 16 of *ALK* (nt 3585 of sequence with accession number NM_004304.4). FISH, fluorescence *in situ* hybridization.

(Qiagen) using a 5 mm stainless steel bead and TissueLyser II (Qiagen). Subsequently, total RNA was purified using QIAcube (Qiagen). The RNA quality was evaluated using the Experion Automated Electrophoresis System (Bio-Rad Laboratories, Oslo, Norway). Overall, 3 µg of total RNA were sent for high-throughput paired-end RNA-sequencing at the Norwegian Sequencing Centre, Ullevål Hospital (<http://www.sequencing.uio.no/>). Detailed information about the high-throughput paired-end RNA-sequencing was given elsewhere.¹⁸ The software FusionCatcher¹⁹ (<https://github.com/ndaniel/fusioncatcher>) was used for discovery of fusion transcripts.

Molecular Genetic Analyses

cDNA was synthesized from 2 µg of total RNA in a 20 µl reaction volume using iScript Advanced cDNA Synthesis Kit for RT-qPCR according to the manufacturer's instructions (Bio-Rad Laboratories). PCR assays were performed in 25 µl reaction volumes, which contained 12.5 µl of Premix Taq (Takara Bio Europe/SAS, Saint-Germain-en-Laye, France), 2 µl of cDNA, and 0.4 µM of each of the forward and reverse primers, and run on a C-1000 Thermal cycler (Bio-Rad Laboratories). The quality of the cDNA synthesis was assessed by amplification of a cDNA fragment of the *ABL1* gene using the primers ABL1-91F1 (5'-CAGCGGCCAGTAGCATCTGACTTTG-3') and ABL1-404R1 (5'-CTCAGCAGATACTCAGCGGCATTGC-3').²⁰ To detect possible FN1-ALK fusion transcripts, the primers FN1-6842F1 (5'-CGGTCCGGGACTCAATCCA AATG-3') and ALK-3965R1 (5'-CGTCCCGTGGTCACA GAAGCAGAT-3') were used for the esophageal leiomyoma. The primers FN1-7021F1 (5'-GCCTCACCAG AGGTGCCACCTACA-3') and ALK-3661R1 (5'-ATGG CCTGGGGCAGGAATGT-3') were used for the leiomyoma of small intestine. PCR cycling for the amplifications was: initial denaturation at 94 °C for 30 s followed by 35 cycles of 7 s at 98 °C, 30 s at 58 °C, 1 min at 72 °C, and a final extension for 5 min at 68 °C.

Overall, 3 µl of the PCR products were stained with GelRed (Biotium), analyzed by electrophoresis through 1% agarose gel, and photographed. DNA gel electrophoresis was performed using lithium borate buffer.²¹ The remaining PCR products were purified using the Qiaquick PCR purification kit (Qiagen) or the QIAquick Gel Extraction Kit (Qiagen) and direct sequencing was performed using the light run sequencing service of GATC Biotech (<http://www.gatc-biotech.com/en/sanger-services/ligthrun-sequencing.html>). The BLAST software (<http://www.ncbi.nlm.nih.gov/BLAST/>) was used for computer analysis of sequence data.

Results

Cytogenetic Analysis

Esophageal leiomyoma (patient 1). The initial G-banding analysis yielded the karyotype 45,Y,der(X)t(X;6)

(p22;p21),add(6)(p21),-11[cp6]/46,XY[7] (Figure 3a). Thus, our primary goal was to identify putative fusion gene(s) generated by these rearrangements. FISH with the BAC probe CTD2242D5, which covers the *HMGA1* locus, showed that the region covered by the probe was moved in its entirety to the derivative chromosome X. Hence, *HMGA1* was not rearranged (Figure 2b).

Analysis of the RNA-Seq raw data (see below) by the program FusionCatcher showed a fusion of *FN1* (from 2q35; chr2:216225179-216300791, UCSC Genome Browser on Human Feb. 2009, GRCh37/hg19 Assembly) with *ALK1* (from 2p23). Taking into consideration that an *FN1-ALK* fusion gene had been reported before in a stromal sarcoma²² and in inflammatory myofibroblastic tumors,^{23,24} we hypothesized that the *FN1-ALK* fusion gene in the present case might be the result of a cytogenetically cryptic t(2;2)(p23;q35) or inv(2)(p23q35). We therefore performed FISH using an *ALK* breakapart probe (Figure 2c and d) hybridized onto metaphase spreads and interphase nuclei and reevaluated the preparations. This FISH analysis showed that the *ALK* locus was indeed rearranged and that the chromosome aberration was an inversion, namely inv(2)(p23q35) (Figure 2e). Reassessment of the G-banding karyotypes in light of the FISH, RNA-sequencing, and other molecular genetic data thus yielded the karyotype 45,Y,der(X)t(X;6)(p22;p21),inv(2)(p23q35),add(6)(p21),-11[cp6]/46,XY[7] for the esophageal leiomyoma.

Leiomyoma of small intestine (patient 2). The G-banding analysis yielded the karyotype 46,X,add(X)(q2?),der(2)add(2)(p23)add(2)(q33),add(4)(p14),add(14)(q22)[10]/47,XX,+12[2]/46,XX[1].

Because the RNA-Seq showed a fusion of *FN1* (from 2q35) with *ALK1* (from 2p23) (see below), FISH was performed on metaphase spreads and interphase nuclei using the *ALK* breakapart probe. The analysis showed rearrangement of the *ALK* locus (Figure 4a and b). On the metaphase spreads, the 3'-end part of the *ALK* gene (red probe) had moved to 2q35 of the der(2), whereas the 5'-end part of the gene (green probe) had moved to 4p14 showing that the add(4)(p14) had material from chromosome 2 (Figure 4a and b). As expected, on interphase nuclei the *ALK* breakapart probe showed a split signal.

High-Throughput Paired-End RNA-Sequencing Analysis

Analysis of the fastq files obtained from the Norwegian Sequencing Centre, Ullevål Hospital (<http://www.sequencing.uio.no/>) with the FusionCatcher software revealed *FN1-ALK* fusion transcripts in both leiomyomas (Supplementary Table S1). Because of this and the fact that the *FN1-ALK* fusion gene has been reported previously in a stromal sarcoma²² and inflammatory myofibroblastic tumors,²²⁻²⁴ we decided to investigate further the *FN1-ALK* fusion transcripts.

Figure 3 Microscopic examination of the leiomyoma of small intestine. (a) H&E-stained section showing the tumor (100 $\times$). (b) H&E-stained high magnification (200 $\times$). (c) Immunohistochemistry of desmin, 200 $\times$. The tumor cells were positive for the intermediate filament desmin, consistent with a muscular origin. (d) Immunohistochemistry of SMA, 200 $\times$. Tumor cells were positive for SMA. (e) Immunohistochemistry of ALK, 200 $\times$. Note the consistent granular staining in the tumor cells. (f) Immunohistochemistry of ALK, high magnification (400 $\times$) showing strong, granular cytoplasmic staining. SMA, smooth muscle actin.

Molecular Genetic Confirmation of Fusions

PCR with the primers ABL1-91F1 and ABL1-404R1 amplified cDNA fragments of *ABL1* indicating that the

synthesized cDNAs were of good quality (data not shown).

RT-PCR using the primers FN1-6842F1 and ALK-3965R1 amplified an 847 bp cDNA fragment

Figure 4 Cytogenetic and RT-PCR confirmation of the presence of an *FN1-ALK* fusion transcript in the leiomyoma of small intestine. (a) G-banding of a metaphase spread. (b) FISH using the *ALK* breakapart probe on the metaphase spread shown in a. The 3'-end part of the *ALK* gene (red probe) moved to 2q35 of the der(2), whereas the 5'-end part of the gene (green probe) moved to 4p14 showing that the add(4)(p14) had received material from chromosome 2. (c) Gel electrophoresis of the 196 bp amplified *FN1-ALK* cDNA fragment in the leiomyoma (lane L2) using the primer combination FN1-7021F1 and ALK-3661R1. M: 1kb DNA ladder (GeneRuler, ThermoFisher Scientific). (d) Partial sequence chromatogram of the amplified cDNA fragment showing the fusion point in the *FN1-ALK* fusion transcript. Exon 41 of *FN1* (nt 7119 of sequence with accession number NM_212482.1) was fused to exon 16 of *ALK* (nt 3585 of sequence with accession number NM_004304.4). FISH, fluorescence *in situ* hybridization.

from the esophageal leiomyoma (Figure 2f). Subsequent direct Sanger sequencing verified the presence of an *FN1-ALK* chimeric transcript (Figure 2g) in which exon 42 of *FN1* (nt 7284 of sequence with accession number NM_212482.1) was fused to exon 16 of *ALK* (nt 3585 of sequence with accession number NM_004304.4).

Similarly, RT-PCR with the primer combination FN1-7021F1 and ALK-3661R1 amplified a 196 bp cDNA fragment from the leiomyoma of small intestine (Figure 4c). Sanger sequencing again verified the presence of an *FN1-ALK* chimeric transcript (Figure 2d) in which exon 41 of *FN1* (nt 7119 of sequence with accession number NM_212482.1) was fused to exon 16 of *ALK* (nt 3585 of sequence with accession number NM_004304.4).

Discussion

We describe *ALK* rearrangement and the generation of an *FN1-ALK* fusion in two gastrointestinal leiomyomas, one in the esophagus and the other in the small intestine. The *FN1-ALK* fusion was found by RNA-sequencing, whereupon FISH analysis confirmed the rearrangement and showed that it was the result of a cytogenetically hard-to-see inv(2)(p23q35) in the esophageal leiomyoma. Subsequent re-evaluation of the metaphase spreads indeed found the inv(2). In the leiomyoma of small intestine, FISH analysis showed that the 3'-end part of the *ALK* gene (red probe) had moved to 2q35 of the der(2), whereas the 5'-end part of the gene (green probe) had moved to 4p14. Thus, the FISH data indicated that the *FN1-ALK* fusion gene is located on chromosome band 2q35.

This is the first time that *ALK* involvement has been demonstrated in these rare, benign gastrointestinal smooth muscle tumors. On the other hand, *ALK* rearrangement has never been seen in uterine smooth muscle tumors, indicating a systematic difference in the pathogenesis of leiomyomas of these two sites.¹¹

The *ALK* gene, which codes for a receptor tyrosine kinase, has been found rearranged, mutated, or amplified in several tumors including anaplastic large cell lymphoma, diffuse large B-cell lymphoma, inflammatory myofibroblastic tumor, neuroblastoma, non-small cell lung cancer, ependymoma, and benign fibrous histiocytoma.^{25–28} The *ALK* fusions always include exons 20–29, which code for the cytoplasmic part of the *ALK* protein that harbors the tyrosine kinase domain whose activation promotes cell proliferation and survival.²⁶ Most of the fusion partners contain coiled-coil or leucine-zipper domains that drive the oligomerization, which appears to be necessary for ligand-independent activation of the fusion kinase.²⁶

The *FN1* gene codes for fibronectin, a glycoprotein present in a soluble dimeric form in plasma, and in a dimeric or multimeric form at the cell surface and in extracellular matrix (<http://www.ncbi.nlm.nih.gov/gene/2335>). Fibronectin is involved in cell adhesion and migration processes including embryogenesis, wound healing, blood coagulation, host defense, and metastasis (<http://www.ncbi.nlm.nih.gov/gene/2335>).

FN1-ALK fusion was previously reported in a stromal sarcoma of the ovary²² and in three inflammatory myofibroblastic tumors of the bladder.^{22–24} In the stromal sarcoma and two of the inflammatory myofibroblastic tumors, exon 23 of *FN1* was fused to exon 19 of *ALK*.^{22,23} In the third inflammatory myofibroblastic tumor, exon 20 of *FN1* was fused to exon 19 of *ALK*.²⁴ Thus, *FN1-ALK* in these tumors retains the transmembrane domain of *ALK* encoded by exons 19–20. Retention of this domain may lead to membrane/cytoplasmic localization of *ALK*.

The pattern of *ALK* expression immunohistochemically may reflect attributes of the *ALK* gene

partner. For example, myofibroblasts that express the *RANBP2-ALK* fusion gene exhibit nuclear membrane-associated ALK staining that is unique compared to the subcellular localization observed with other ALK fusions in IMT, presumably attributable to heteroassociation of the fusion with normal RANBP2 at the nuclear pore.²⁹ Immunofluorescence analysis showed that, in 3T3 cells, FN1-ALK had intracellular reticulum/Golgi localization, probably due to a defect of glycosylation of these over-expressed proteins.²²

In the esophageal leiomyoma *FN1-ALK* chimeric transcript, the fusion points were different from those of the previously reported *FN1-ALK* fusions in that exon 41 or exon 42 of *FN1* was fused to exon 16 of *ALK*. The putative FN1-ALK fusion protein thus obtained would contain the majority of the FN1 peptide, 2340 amino acids (aa) out of 2477 (FN1 protein sequence with accession number NP_997647.1), and the last 742 aa of ALK (aa 879-1620 in the ALK protein sequence with accession number NP_997647.1). The FN1 portion of FN1-ALK retains a diverse set of domains (<http://www.ncbi.nlm.nih.gov/protein/47132557>), which are involved in fibronectin self-association and interaction with other ECM components, potentially providing a strong activation signal to ALK.³⁰ The ALK portion of the chimeric protein retains the coagulation Factor Xa inhibitory site (found in region 987-1021 aa in NP_997647.1) together with the trans-membrane domain and the catalytic domain of the protein tyrosine kinase (http://www.ncbi.nlm.nih.gov/protein/NP_004295.2).

Ren *et al*²² showed that subcutaneously injected 3T3 cells expressing FN1-ALK induced more aggressive tumors in nude mice than those that developed after injection of 3T3 cells expressing wild-type ALK. The tumorigenic potential of the *FN1-ALK* fusion gene, as of most *ALK* fusions, probably depends on constitutive activation of ALK due to homodimerization of the fusion protein through the FN1 domain. Because FN1 is ubiquitously and abundantly expressed in human cells, the tumorigenicity of the fusion could also be due to high-level expression of ALK, which has come under the control of the strong FN1 promoter. Indeed, Ouchi *et al*²⁴ showed that the *FN1-ALK* fusion resulted in overexpression of *ALK*.

Ren *et al*²² also showed that the ALK inhibitors crizotinib and TAE684 markedly suppressed growth of FN1-ALK-induced tumors and abolished phosphorylation of the FN1-ALK protein. Given the sensitivity of FN1-ALK-induced tumors to ALK inhibitors,²² the recent promising results obtained with crizotinib^{31,32} and the development of a new generation of ALK inhibitors,^{33,34} we predict that patients whose tumors carry *FN1-ALK* would respond well to therapy targeted against ALK in at least a subset of patients, although the primary treatment for leiomyoma is likely to remain surgery.

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Disclosure/conflict of interest

The authors declare no conflict of interest.

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