Original Article
Recurrent inflammatory myofibroblastic tumors harboring PIK3CA and KIT mutations

Cheng-Fang Li1*, Chun-Xia Liu12*, Bing-Cheng Li1, Yao-Yuan Shen1, Xiao-Bin Cui12, Wei Liu1, Hong-Chao Dong1, Li-Juan Pang12, Wei-Hua Liang1, Feng Li12

1Department of Pathology, Shihezi University School of Medicine, Shihezi, Xinjiang 832002, China; 2Department of Pathology, The First Affiliated Hospital, Shihezi University School of Medicine, Shihezi, Xinjiang 832002, China. *Equal contributors.

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Abstract: Inflammatory myofibroblastic tumour (IMT) is a relatively rare soft tissue malignancy. It exhibits locally aggressive behavior with a tendency for local recurrence and rare metastasis, and rare recurrent IMTs may show histological progression. The genetic hallmark of IMT is ALK rearrangement from chromosome arm 2p, but gene mutations involved in IMT remain poorly understood. The aim of the present study was to perform a pairwise comparison of the gene mutations occurring in primary and recurrent IMT from the same patient. We conducted a high-throughput analysis of 238 known mutations of 19 oncogenes in pairwise comparison primary and recurrent samples from 2 patients of IMT using Sequenom MassARRAY technology. Our results revealed 2 mutations in 2 recurrent lesion samples, including one in exon 11 of the KIT gene, resulting in a T-C substitution at position 1727 (L576P), the recurrent sample underwent histologic progression with “pleomorphic undifferentiated sarcoma-like” transformation; the other mutation was in exon 19 of the phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit alpha (PIK3CA) gene, resulting in a G-A substitution at position 1624 (E542K). Moreover, no any mutation was found in the primary lesion samples from 2 patients. Our findings suggest that variable genome changes might be present in IMT, especially during the progression from a primary tumour to recurrence. To the best of our knowledge, no such longitudinal study of IMT has been undertaken previously.

Keywords: Inflammatory myofibroblastic tumour, gene mutation, recurrent tumour, MassARRAY

Introduction

Inflammatory myofibroblastic tumour (IMT) is a characteristic neoplasm composed of myofibroblastic and fibroblastic spindle cells accompanied by an inflammatory infiltration of plasma cells, lymphocytes, and/or eosinophils. IMT primarily occurs in the soft tissue and viscera of children and young adults. In general, these tumors are clinicopathologically distinctive but biologically not characteristic. Rarely, IMTs may undergo histological evolution to a morphologically higher grade lesion after recurrences [1]. Recently, the World Health Organization has defined IMT as an intermediate- or low-grade malignant myofibroblastic neoplasm. The genetic hallmarks of IMT are the rearrangements of the anaplastic lymphoma kinase (ALK) locus on chromosome 2p23, which are documented in approximately 50% of all IMT cases by FISH [2]. Since ALK protein expression reliably correlates with ALK rearrangement [3], it essentially serves as a surrogate marker for ALK translocation. However, 50% of IMT cases are negative for ALK expression on immunohistochemistry, and thus, whether these ALK-negative IMTs should be considered to have the same pathogenesis as ALK-positive IMTs is not clear. Moreover, the other genetic factors, such as gene mutations contributing to IMT development and progression remain poorly understood.

Furthermore, very limited information is available on point mutations in IMT. Missense mutations of the p53 gene have been reported in IMT [4]. Another recent report has identified a secondary mutation of ALK, F1174L, in a case of IMT harboring a Ras-related nuclear protein binding protein 2 (RANBP2)-ALK translocation [5]. However, other potentially important mutations might exist in IMT, but no relevant pub-
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Figure 1. MRI view. A. Coronal T1-weighted image. B. Sagittal T1-weighted images showed unclear boundaries mass within an intramuscular mass in the right upper extremity (white arrow).
Sequenom MassARRAY technology employs a mass spectrometry-based genotyping approach, which is a more sensitive methodology for mutation analysis than traditional Sanger sequencing, although it is highly concordant with Sanger sequencing [6, 7]. The OncoCarta Panel v 1.0 based on Sequenom technology, which includes 238 alleles of 19 common oncogenes, has been used to explore the mutation profile of many malignant tumors [8-10]. The present study aimed to detect clinically relevant hotspot mutations in primary and recurrent IMTs from same patient using OncoCarta Panel v 1.0. This report is the first to describe IMTs with mutations in the KIT and PIK3CA genes. In fact, to our knowledge, there are even no any reports of group wise comparisons between primary and recurrent IMT with regard to their gene mutation status.

Materials and methods

Case description

Case 1: A 57-year-old Chinese woman presented to us with pain and discomfort due to an intramuscular mass in the right upper extremity...
ty, which she had experienced for 10 days. Laboratory tests indicated no significant abnormality. T1-weighted MRI image (coronal and sagittal scans) showed a diffuse high signal soft tissue mass centered in deep muscular of the right upper extremity (Figure 1). Complete and extended surgical resection was performed with negative microscopic margins, and no adjuvant therapy was administered. The macroscopic specimen showed a myxoid tan cut surface. The histologic diagnosis was IMT with spindle cells of myofibroblastic differentiation accompanied by numerous inflammatory cells, such as plasma cells, lymphocytes, and eosinophils (Figure 2A, 2B). The patient experienced local recurrence a year after surgery. After complete surgical resection, radiation therapy was performed for a total of 35 treatment sessions. Microscopic examination revealed histological progression with compact spindle cell proliferation (Figure 2C), focally “pleomorphic undifferentiated sarcoma-like” transformation (Figure 2D), scattered distribution of ganglion-like cells, tumour cells invading the surrounding muscles, and mitotic figures. To date, the patient has been continuously followed up, with 12 months of progression-free survival.

Case 2: A 37-year-old Chinese man presented to us with a swollen mass with a multinodular growth pattern in the right thigh, which was present for 1 month. Complete resection was performed, and microscopic examination showed classic spindle cells with inflammatory cells in the dense collagenous background (Figure 3A, 3B), which revealed a histologic diagnosis of IMT. No adjuvant therapy was administered after resection. The patient experienced local recurrence 6 months after surgery, and the tumour was again surgically
Figure 5. Immunohistochemical staining of inflammatory myofibroblastic tumour of case 2. A. ALK revealed a scattered cytoplasmic staining pattern (×200). B. SMA staining is positive (×200). C. Vimentin is positive (×200). D. P53 staining is scattered positive (×200).

Figure 6. Spectrum of the recurrent lesion samples on mutation profiling using the Sequenom MassARRAY platform. A. The figure shows a T-C substitution, resulting in an amino acid change from leucine to proline at position 576 of the KIT gene in the recurrent lesion sample of case 1. B. A PIK3CA mutation in the recurrent lesion sample of case 2 with a substitution of GAA for AAA, resulting in a change from glutamate to lysine at codon 542.

removed. Histological examination demonstrated loosely arranged spindle cells (Figure 3C), spindle to polygonal cells transformation of the tumour cells with plasma cells present in a myx-
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Table 1. ALK fusion partners and protein expression site in well-documented IMT cases

<table>
<thead>
<tr>
<th>partner</th>
<th>locus</th>
<th>Method</th>
<th>ALK IHC</th>
<th>age (year)/sex</th>
<th>site</th>
<th>Year, first author</th>
</tr>
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<td></td>
<td>RT-PCR, FISH</td>
<td>NM+</td>
<td>6/M</td>
<td>Omentum and mesentery</td>
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<td>NM+</td>
<td>39/M</td>
<td>Mesentery</td>
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<td>CP+</td>
<td>34/F</td>
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CP: Cytoplasmic; NM: nuclear membrane.

oid background in recurrent sample (Figure 3D). To date, no signs of recurrence or metastasis have been observed for 45 months.

**Immunohistochemistry**

Immunohistochemical staining was performed on 4 μm thick FFPE tissue sections. ALK immunostaining was performed using mouse monoclonal antihuman antibody ALK-1 (CD246; Dako, Carpentaria, CA; clone ALK1) with a 1:5 dilution. A commercial Envision+ kit (DAKO) was used for IHC. Briefly, slides were deparaffinized and pretreated with 1 mM EDTA, pH 8.0 and heat-mediated antigen retrieval in a steam pressure cooker, then sections were incubated for 10 minutes with 3% hydrogen peroxide to block endogenous peroxidase activity. All further steps were performed according to the manufacturer’s instructions. Other antibodies was performed using standard laboratory protocols and automated processing (LEICA).

**Extraction of genomic DNA from archived formalin-fixed, paraffin-embedded (FFPE) tissues**

FFPE IMT tissue samples were obtained from the archives of the Department of Pathology at the First Affiliated Hospital, Shihezi University School of Medicine (Xinjiang, China). Total DNA was extracted from 10 consecutive 5-μm FFPE tissue sections using the DNeasy® FFPE kit (QIAGEN, Germany). The extraction was performed as recommended by the manufacturer. However, protease K digestion was extended overnight, and an additional digestion step was performed for samples incompletely digested after the overnight treatment. DNA samples were collected from the primary and matching recurrent tumour tissue in case 1 and case 2.

**Mutation analysis using MassARRAY (Sequenom) genotyping**

Sequenom technology is based on matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS), a simple methodology for the detection of clinically relevant hotspot mutations in malignancy. The prevalence of mutant alleles was estimated by calculating the ratio of the area of the raw spectra of the mutant allele to that of the corresponding wild type. The OncoCarta panel v1.0 was developed by Sequenom to include 238 alleles of 19 common oncogenes (ABL1, JAK-2, CDK4, KRAS, HRAS, NRAS, AKT1, AKT2, KIT, EGFR, MET, ERBB2, PDGFA, BRAF, FGFR1,
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In case 1, cytoplasm staining for ALK protein was present in recurrent lesion sample from case 1 (Figure 4A), staining for vimentin, smooth muscle actin (SMA), calponin, cluster of differentiation (CD)-68, PTEN, CD34, P53 and Ki-67 (40%) was positive, whereas that for CD117, desmin, AE1/3, cyclinD1, MDM2, S-100, Human Herpesvirus 8 (HHV-8) and Epstein-Barr virus (EBV) was negative in the primary lesion sample. In the recurrent lesion, staining for HHV-8 (Figure 4B), vimentin, calponin (Figure 4C), SMA (Figure 4D), CD68, desmin and Ki-67 (40%) was diffusely positive, and P53 was positive, whereas that for CD117, PTEN, CD34, AE1/3, cyclinD1, MDM2, S-100 and EBV was negative.

For case 2, it was scattered positive for ALK staining (Figure 5A). Furthermore, SMA, vimentin (Figure 5B, 5C), CD68, P53 (Figure 5D), PTEN and Ki-67 (10%) was positive, cyclinD1 was focal positive, whereas that for AE1/3, calponin, CD34, MDM2, CD117, desmin, S-100, HHV-8 and EBV was negative in the primary lesion. In the recurrent sample, vimentin, SMA, CD68, CD34, cyclinD1 (focal), PTEN, P53 and Ki-67 (10%) was positive, yet AE1/3, calponin, desmin, MDM2, CD117, S-100, HHV-8 and EBV was negative in the recurrent lesion.

Mutation analysis

Mutation screening using Sequenom MassARRAY revealed that a KIT mutation was present in the recurrent lesion sample of case 1, resulting in a T-C substitution at position 1727, which led to a leucine to proline substitution (L576P) (Figure 6A). Such a mutation was detected in exon 11 of the gene. No mutation was found in the primary lesion sample of case 1. Moreover, a PIK3CA mutation in exon 19 was detected in the recurrent lesion sample of case 2, involving a GAA→AAA transition that resulted in a glutamate to lysine substitution at codon 542 (E542K, Figure 6B), no any mutation was also found in the primary.

Discussion

IMT is also known as inflammatory pseudotumour, plasma cell granuloma, plasma cell pseudo-tumour, myofibroblastic proliferation, myofibroblastoma, or inflammatory fibrosarcoma, which reflects the disease’s uncertain histogenesis. The tumour exhibits characteristic histological features with three distinct patterns present in the same lesions: (a) a myxoid, vascular, and inflammatory pattern; (b) a compact spindle cell pattern; and (c) a dense fibrotic pattern [12]. The diagnosis of IMT cannot be based on clinical findings alone, and supplemental histopathological and immunohistochemical studies are usually required. However, immunohistochemistry does not play a major role in confirming the diagnosis owing to the variable expression and lack of specificity of myofibroblastic markers. ALK positivity is often helpful if present, but its absence does not exclude the diagnosis of IMT, especially in adults. The compact spindle cell pattern of IMT is particularly difficult to distinguish from a variety of conditions with similar histology, such as spindle cell sarcomas, spindle cell melanomas, sarcomatoid carcinomas, rhabdomyosarcoma, and leiomyosarcoma. Additionally, IMT may present with only mild cytological atypia [13]. Further, inflammatory infiltration may also be seen in gastrointestinal stromal tumors (GISTs), and IMTs are consistently negative for c-kit [12, 14]. Our finding of immunohistochemistry showed CD117 was negative in all IMT samples. One report identified human herpesvirus-8 (HHV-8) DNA in both pulmonary and extra-pulmonary IMT, but expression of HHV-8-associated antigens was not investigated [12]. In our case, HHV-8 expression was positive in recurrence of case 1, whereas HHV-8 were all negative in our other cases. So the evidence for HHV-8 infection in IMT needs to further proof.
With regard to molecular characterization, ALK gene rearrangement has been reported in a half to two-thirds of all IMT cases, more commonly in younger and pediatric patients [15]. ALK protein expression was served as a surrogate marker for ALK translocation. ALK is a receptor tyrosine kinase that belongs to the insulin receptor family. Its expression normally promotes cell proliferation, survival, and differentiation in the nervous system by activating the PI3K/AKT, MAPK/ERK, and STAT3 pathways [16]. In IMT, several molecules have been reported to fuse with ALK, including RANBP2, CARS, TPM3, TPM4, ATIC, CLTC, SEC31L1, and PPFIBP1 (Table 1). Protein fusion due to chromosomal translocation is the most common mechanism of ALK overexpression and ALK kinase domain activation. These features render ALK fusion oncokine an ideal molecular target [16]. Such agents (e.g. crizotinib) have been tested in IMTs with ALK rearrangements, demonstrating a promising response, whereas patients without the ALK rearrangement showed no response [17], suggesting that ALK fusion products are a relevant target in sensitive cases. Our study showed ALK was positive in both cases, it may suggest ALK rearrangement. The similar reports displayed that the expression of ALK immunostaining may be associated with the specific gene fusion [12], for example the expression of TPM3/4-ALK was associated with smooth cytoplasmic staining, CLTC-ALK expression correlated with granular cytoplasmic staining, a RANBP2-ALK fusion was shown to express nuclear membrane staining of ALK. But the relationship between fusion genes and ALK immunostaining has needed to be validated in large series of ALK-positive cases.

Many different tumour types harbor somatic gene mutations, which contribute to tumour progression and can also serve as therapeutic targets. However, such point mutations are so rare in IMT that there is limited information on the genes involved. To date, there is only 1 report on p53 missense mutations in 2 of 15 IMT cases, determined using direct sequencing [4]. A secondary ALK mutation, F1174L, has been reported as the cause of crizotinib resistance in a patient with the RANBP2-ALK translocation. F1174L RANBP2-ALK has been shown to promote the growth of Ba/F3 cells (a neuroblastoma cell line) in vitro [5]. Thus, mutation screening using the Sequenom MassARRAY in this study revealed a KIT L576P and a PIK3CA E542K mutation in IMT recurrent lesion samples for the first time.

KIT is a type III tyrosine kinase receptor. The KIT L576P mutation is commonly found in GISTs [18] and melanoma [19]. Patients with KIT L576P mutation are often responsive to imatinib, and it is a low-risk indicator in GISTs [18]. Similarly, melanoma patients with such a mutation are sensitive to KIT inhibitors such as nilotinib and imatinib [20]. L576P-expressing melanocytes exhibit increased proliferation and migration in vitro [21]. Furthermore, L576P mutation has been reported in extra-gastrointestinal malignancy [22], breast cancer [23], and thymic carcinoma [24]. The KIT L576P mutation was more sensitive to imatinib than other KIT mutations in cases of thymic carcinoma [24]. From these published data, we can speculate that KIT L576P may play a similar role in many types of lesions. Our results showed a KIT mutation in recurrent sample and no mutation was found in the primary lesion from case 1. Moreover, it showed local muscles aggressive and the histological progression in recurrence one. We therefore hypothesize that this mutation may contribute to the local aggressive behavior, recurrence and transformation of the spindle cells to pleomorphic undifferentiated sarcoma of IMT, and may as well be an undeveloped therapeutic target, suggesting that the morphology and biological behavior might change with time in the primary IMT and that genomic abnormalities may be involved in this process.

PIK3CA, which encodes the catalytic subunit of phosphatidylinositol 3-kinase, a major downstream signalling component of growth factor receptor tyrosine kinases, is a common oncopgene in human cancers, including colorectal cancer [25, 26], breast carcinomas [27, 28], and sarcoma [29]. Mutations of PIK3CA often cluster in two domains, the helical domain (E542K and E545K) and the kinase domain (H1047L and H1047R). The presence of PIK3CA mutation (including E542K) has been shown to be associated with a significant increase in cancer-specific mortality [25]. As previously observed in breast cancers [28], patients with helical-domain PIK3CA mutations often have worse outcomes than those with kinase-
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domain mutations. E542K mutation might increase the risk of ductal carcinoma in situ progression [27]. However, another study showed that only patients with concomitant PIK3CA mutations in exons 9 and 20 had poor prognosis in colorectal cancer [26]. Our mutation screening revealed the hotspot E542K mutation, which involved exon 9 of PIK3CA or its helical domain. Although we evaluated primary and recurrent lesion samples, its morphology was slightly different from that of the primary lesion. Additionally, no evidence of progression has been observed to date (45 months later). The PIK3CA mutation therefore may not play an important role or its role may be not obvious in the lesion.

As we all know, the pathogenesis of IMT is still unknown. We can speculate that various genes and pathways involved in development, such as ALK, P53, KIT, PIK3CA, which will be expected to serve as potential therapeutic targets, but their actual roles need to be further validated. Currently, the optimal treatment method is surgical resection for resectable cases. In our cases, the anatomic sites were the extremities, and therefore, complete resection was easy. An ALK-negative IMT has previously shown no response to crizotinib therapy [17]. Non-steroidal anti-inflammatory agents may therefore be useful for tumours without ALK rearrangements [30, 31]. Steroids, radiotherapy, and chemotherapy have shown limited success in the treatment of advanced metastatic tumors [16]. So, specific targeted therapies are to be developed in the future, and more studies are required to investigate IMTs at the genomic level. Furthermore, strict and careful follow-up of these cases is mandatory as the behavior of these tumors is unpredictable.

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Disclosure of conflict of interest

None.

Address correspondence to: Dr. Feng Li, Department of Pathology, Shihezi University School of Medicine, Shihezi, Xinjiang 832002, China. Tel: +86-0993-205-7136; E-mail: lifeng7855@126.com

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