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Immunohistochemistry and FISH on Tissue Microarrays in the Evaluation of Inflammatory Myofibroblastic Tumor

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Abstract

Inflammatory myofibroblastic tumor (IMT) is a rare soft tissue tumor composed of myofibroblasts with an inflammatory infiltrate. Various gene fusions involving the anaplastic lymphoma kinase (ALK) gene at chromosome 2p23 have been described in IMT, suggesting a neoplastic etiology. The ALK rearrangement is reportedly more common in children and there are only rare IMTs in adults with genetic information. This study represents the largest series of lesions designated as IMT on morphologic grounds with comparative ALK IHC and FISH analysis. Sixteen IMTs were retrieved from the UNM and UVa archives. Inclusion criteria required spindle cells, inflammatory cells and thorough clinical, histological and immununohistochemical exclusion of other diagnostic possibilities. IHC was performed on each of the sixteen cases using a monoclonal ALK1 antibody and evaluated for cytoplasmic and nuclear stain intensity and distribution. FISH was performed on tissue microarray sections using the ALK breakapart genomic marker. MetaSystems automated FISH analyzer detected signal patterns on interphase nuclei and results were interpreted on a Meta workstation. Patients ranged in age from 2-70 years (mean 48; median 43). IHC and FISH were successful on 16/16 and 10/16 cases, respectively. One case was positive for ALK rearrangement by FISH. This case also demonstrated diffusely strong cytoplasmic and focally strong nuclear immunopositivity. One case showed diffusely strong cytoplasmic ALK immunopositivity without gene rearrangement by FISH. ALK overexpression (IHC) and genomic rearrangements (FISH) are uncommon in our series of IMTs. This may be related to the older age of patients in our study. ALK IHC negative cases did not show ALK gene rearrangement by FISH. Further investigation is warranted to determine if ALK positive lesions represent a different, more biologically aggressive entity, necessitating a distinct designation.

Introduction

Inflammatory myofibroblastic tumor (IMT) is a rare soft tissue tumor arising predominately in children and young adults.[1-2] IMTs are composed of spindle cell myofibroblastic proliferations with a prominent infiltrate of plasma cells, lymphocytes and eosinophils. The entity of IMT arouse in 1995[1] from the recognition of similarities among a variety of lesions with myofibroblasts and inflammatory cells including: inflammatory pseudotumor of the lung, plasma cell granuloma, postoperative spindle cell nodule, pseudosarcomatous myofibroblastic proliferation, and atypical fibromyxoid tumor.[3] There has been ongoing debate over the reactive versus neoplastic nature of the lesion. However, the discovery of a genetic translocation involving the ALK gene (anaplastic lymphoma kinase) on chromosome 2p23 argues for a neoplastic process in at least a subset of lesions.[4]

ALK was first identified as an important translocated gene in anaplastic large cell lymphoma (ALCL) in 1994. The main translocation in ALCL is t(2;5)(p23;q35) where the ALK gene located on 2p23 is fused to the NPM (nucleophosmin) gene at 5q35. ALK encodes for a tyrosine kinase and NPM for a nucleolar phosphoprotein. The resulting fusion gene encodes a chimeric protein which is a constitutively active tyrosine kinase.[5] The discovery of ALK translocations in a subset of IMTs indicates that inappropriate activation of the ALK signaling pathway may be a critical step in the neoplastic transformation of myofibroblasts.[3]

Several ALK fusion genes have been reported in IMTs. Molecular studies have identified ALK fusion involving tropomyosin-3 and -4 (TPM-3 and -4), the clathrin heavy chain (CLTC), cysteinyl-tRNA synthetase (CARS), and Ran-Binding Protein 2 (RANBP2).[6-9] Lawrence et al has demonstrated that the TPM3-ALK and TPM4-ALK fusion proteins in IMT are phosphorylated and activated.[6] It is postulated that the other fusion partners also result in a

constitutively active tyrosine kinase conferring tumorgenesis. The discovery of ALK translocations in IMTs was an important step in understanding the pathophysiology of the tumor; however, only 30-50% of IMTs are found to have detectable ALK rearrangements. In addition, those with ALK rearrangements are almost exclusively in children. Coffin et al reported 11 of 40 cases positive by immunohistochemistry (IHC) with ALK antibody and all patients with ALK positivity were younger than 10 years.[10] Lawrence et al reported a slightly higher rate using cytogenetic methods with approximately 50% (7 out of 11) containing ALK rearrangements. All the negative cases were in adults older than 40 years.[6]

Along with genetic heterogeneity, IMTs also exhibit morphologic and clinical variation. The various morphologic differences led to the definition of three different histological patterns including: nodular fasciitis-like, fibrous histiocytoma-like and desmoid-like. According to Coffin et al, histological pattern does not have a clear correlation with clinical behavior.[1] In addition, thus far ALK immunoreactivity has not been linked with specific histological features.

Given that most data on ALK gene rearrangement in IMTs involves children and young adults we investigated IMTs in a predominately adult population. In addition, most studies require ALK immunopositivity as a prerequisite to perform genetic studies for ALK gene mutations, even though there is extreme variability in staining in cases with known ALK gene rearrangements. Griffen et al described three cases with known ALK gene rearrangement where ALK immunopositivity ranged from focally weak to diffusely strong.[11] We investigated ALK IHC and ALK gene rearrangement by FISH on all cases of IMT to further assess the sensitivity of ALK IHC in predicting genetic abnormalities. Lastly, we assessed the efficacy of utilizing a tissue microarray (TMA) for detecting ALK gene rearrangements on interphase nuclei using a

FISH probe. TMAs allow for the evaluation of multiple lesions on one slide, thus having the potential to greatly decrease study costs and time.

Materials and Methods

Selection

The University of New Mexico and University of Virginia surgical pathology files were searched for archival, formalin-fixed, paraffin-embedded cases of inflammatory myofibroblastic tumor. Inclusion criteria required spindle cells and inflammatory cells along with thorough clinical, histological and immunohistochemical exclusion of other diagnostic possibilities. A total of sixteen cases were retrieved.

ALK Immunohistochemistry

ALK1 antibody staining was performed on 4-μm sections of formalin-fixed, paraffin-embedded tissue for all sixteen cases using monoclonal mouse antihuman ALK antibody (Dako, Carpinteria, CA). Staining was detected using the avidin-biotin immunoperoxidase technique. The primary antibody was omitted for the negative control. Sections of appropriate tissues containing the targeted antigen served as positive controls. Antigenic reactions were interpreted semiquantitatively for both distribution and intensity. Distribution was scored as rare cells (0-5%), focal (6-50%), diffuse (51-95%), or complete (>95%). Intensity was scored subjectively by multiple authors (KR, LC, KC) as 0, +1, +2, or +3.

FISH

FISH was performed on tissue microarray (TMA) sections. To prepare the TMA, core tissue biopsies (2mm diameter; height 2-4 mm) were taken from each of the sixteen "donor" paraffin-embedded blocks containing IMT. The cores were obtained by punching the area of the donor block with a thin-walled stainless steel tube shaped like a cork borer. A hematoxylin and eosin-

stained section overlying the surface of the donor block was used as a guide to identify regions positive for IMT. The core taken from each donor block was transferred into a recipient tissue microarray block capable of holding 21 cases, including a positive control. Tissue microarray sections were cut at 4-5 micron intervals for FISH.

FISH was performed using the Vysis LSI ALK dual color break apart rearrangement probe. The probe contained two differently labeled probes on opposite sides of the breakpoint of the ALK gene. An approximately 250 kb probe for the telomeric side of the ALK breakpoint was labeled with SpectrumOrange and the centromeric probe was approximately 300 kb and labeled with SpectrumGreen. The sections were pretreated with a Vysis VP-2000 tissue processor following standard protocol. Ten ml of the ALK rearrangement probe was then applied to each tissue section, covered with a 22 mm^2 coverslip and sealed. The slides were placed on a Vysis Hybrite, co-denatured with the probe at 73 degrees C for 6 minutes and allowed to hybridize at 37 degrees C for 16-20 hours. Hybridized slides were then washed and counterstained with DAPI. The fluorescent signals in interphase nuclei on the hybridized slides were then examined and analyzed on the MetaSystems computer and subsequently validated by the investigators (KC, KR).

Results

Patients ranged in age from 2-70 years (median 48; mean 43). Lesions were located in the cervix, lung, bladder, soft tissue, breast, liver and esophagus.

ALK Immunohistochemistry

Immunohistochemical staining for ALK demonstrated positive nuclear staining in the myofibroblastic cells in one case and cytoplasmic staining in two of the sixteen cases. The case with nuclear staining (focal, +3) was located in the cervix and also demonstrated diffusely strong

(+3) cytoplasmic staining. The second case showing solely cytoplasmic staining was located in the bladder was also diffusely strong (+3). The remaining fourteen cases did not show any evidence of cytoplasmic or nuclear staining.

FISH

FISH was successful in ten of the sixteen cases. The six unsuccessful cases either experienced tissue drop-out during TMA processing or exhibited marked background nonspecific fluorescence prohibiting adequate evaluation. One of the ten successful cases was positive for ALK break apart gene rearrangement. The positive FISH case was located in the cervix and also demonstrated diffusely strong (+3) cytoplasmic and focally strong (+3) nuclear staining. Please refer to Table 1.

Discussion

Inflammatory myofibroblastic tumor is a relatively rare soft tissue tumor composed of a spindle cell myofibroblastic proliferation with infiltrating inflammatory cells including lymphocytes, plasma cells and eosinophils. It was recognized as a distinct entity in 1995[1], however, the uncertainty regarding its neoplastic versus reactive nature remains a topic of debate. The discovery of a clonal abnormality involving the ALK gene on 2p23 in several cases of IMTs favors a neoplastic origin in a subset of lesions.[6-9] In addition to a clonal population of cells, some IMTs are locally aggressive, recurrent, invasive, and rarely metastatic further favoring a neoplastic process.[11] The clinical behavior varies among individuals but has been termed as a tumor with indeterminate or low malignant potential.[12]

ALK overexpression (IHC) and genomic rearrangements (FISH) are uncommon in our series of IMT. This may be a result of the older age of patients in our study (mean 48; median 43). Our findings correlate with previous reports stating that most cases of IMT with genetic

abnormalities are found in children and young adults[6,10]. IMTs in children with ALK gene rearrangements may represent a separate entity from ALK negative lesions found in adults. Further research into the clinical progression of IMTs found in children and adults is warranted in order to further understand and better classify IMTs.

In our study ALK immunopositivity correlated with ALK gene status, except for in one case. This case was located in the bladder and demonstrated diffusely strong cytoplasmic staining without evidence of ALK gene rearrangement by FISH. ALK overexpression may be a result of a genetic mutation unable to be detected by the ALK break apart probe used in the study. Such findings bring up the possibility of additional ALK gene mutations, other than gene fusion, resulting in IMT tumorgenesis. Further molecular studies may be indicated to determine the existence of other ALK gene mutations in IMTs.

All ALK immunonegative cases were also negative for ALK gene rearrangements by FISH. In addition, the one case positive for gene rearrangement by FISH also had diffusely strong cytoplasmic and focally strong nuclear ALK immunopositivity. Thus, ALK IHC is a sensitive marker for detecting ALK gene rearrangement in IMTs. This is useful since IHC is much cheaper, more accessible and has a shorter turn around time than FISH at many institutions.

Two problems experienced in our study limiting the amount of FISH data available for analysis included TMA tissue drop-out and non-specific binding of probe to stromal tissue. Tissue drop-out occurs when a core falls out of the recipient block during sectioning. One possible remedy for this situation is warming of the recipient block once all cores have been inserted. This allows for the paraffin of the recipient block and cores to slowly melt and congeal producing a more cohesive and less fragile block during sectioning. Non-specific binding of

probe to stromal tissue caused inappropriate fluorescence and inability to adequately evaluate for ALK break apart in a few cases. This has the potential to be a recurring problem since many cases of IMT have a large stromal tissue component. By selecting areas of the lesion with a high myofibroblast population the amount of non-specific binding is likely to be reduced. Additionally, by placing more than one core from different locations in the same lesion into the recipient block increases the likelihood of producing analyzable results. In the cases where the ALK breakapart probe was adequately hybridized MetaSystems was effective in detecting signals.

Lastly, it is well known that IMT is a heterogeneous tumor. One question that arises is whether the 2 mm core in the TMA is representative of the entire lesion. It can by hypothesized that IMTs containing an ALK fusion gene should exhibit this mutation in all myofibroblasts present. As a result, a 2 mm core from the lesion should be adequate to detect the ALK gene rearrangement by FISH. As mentioned above, in those cases where a large amount of stromal tissue is present more than one core from each lesion can be placed into the recipient block to guarantee adequate representation of the lesion. Even by placing up to four cores from each lesion into the recipient block, five lesions can be represented. By doing this, the cost of FISH reagents is still markedly decreased.

In conclusion, the results of this study support the theory that the majority of IMTs with ALK gene rearrangement are found in children and young adults. Our findings also support the idea that a subset of lesions with histology described as IMT are neoplastic rather than reactive. More research into the clinical progression and behavior of ALK positive IMTs found in children and ALK negative lesions found in adults may help further understand and subclassify IMTs. In our study ALK IHC was a sensitive marker for identifying lesions positive for ALK gene

rearrangement by FISH. This finding is useful for diagnostic purposes since IHC costs less and is more accessible at most institutions. Additional studies linking histology, gene status, and clinical behavior are needed in order to better understand this complicated lesion.

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Table 1

ALK IHC and FISH

	Cervix (1)	Lung (5)	Bladder (2)	Soft Tissue (5)	Breast (1)	Liver (1)	Esophagus (1)
ALK IHC cytoplasm	1/1 3+ D	0/5	1/2 3+ D	0/5	0/1	0/1	0/1
ALK IHC nuclear	1/1 3+ F	0/5	0/2	0/5	R 1+	0/1	0/1
ALK FISH status	Positive	Negative	Neg (1) ND (1)	Neg (3) ND (2)	ND	ND	ND

D = diffuse; F = focal; R = rare; ND = no data

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