EML4::ALK Fusions In Complex Lymphatic Malformations

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Abstract

Gorham-Stout disease (GSD) and generalized lymphatic anomaly (GLA) are subtypes of complex lymphatic malformations (CLMs) with osseous involvement that cause significant complications in children, including pain and pathologic fractures. Mutations in cellular growth pathways are common, and the mTOR inhibitor sirolimus alleviates symptoms in some, but not all, patients. We describe two patients, one with GSD and one with GLA, who were found to have EML4::ALK fusions. This report of a targetable, oncogenic fusion in vascular malformations expands our understanding of the genetic basis for CLMs and suggesting additional targeted therapies could be effective.

Introduction

Complex lymphatic malformations (CLMs) result from anomalous lymphatic development. They are present at birth and can expand during childhood and adolescence.[1] Significant morbidity and mortality results from disfigurement, overgrowth, impact on vital structures, and abnormal lymph drainage.[2] The impacted tissues vary, including skin, bone, mediastinum, pleura, pericardium, liver, spleen and gastrointestinal tract. Gorham-Stout disease (GSD) and generalized lymphatic anomaly (GLA) are CLM subtypes with osseous involvement that present with skeletal pain and pathologic fractures.[3]In GSD, normal bone is replaced by rapidly expanding lymphangiomatous tissue causing progressive osteolysis.[4] With GLA, extensive bone lesions can progress but do not cause osteolysis.[3]

Genetic sequencing of CLMs have revealed somatic mutations in genes in the receptor tyrosine kinase (RTK), RAS/MAPK, and PI3K growth pathways (Fig. 1A).[5-11] PI3K mutations have been found in over half of patients with GLA[6], and an activating KRAS mutation was reported in a patient with GSD.[9] The critical role these mutations play in driving CLMs is evidenced by the efficacy of sirolimus, an inhibitor of mTOR, which is a central downstream effector in these pathways.[6,12] Despite efficacy in many patients, some do not respond, and there is a need for more widespread sequencing of CLMs to identify alternate targeted therapies.

Therapies targeted to genetic alterations are highly desirable. In a retrospective study describing 19 patients with *PIK3CA*- related overgrowth spectrum (PROS) treated with the PI3Ka inhibitor alpelisib, all patients had functional benefit (e.g. decreased pain and/or increased mobility).[13] Many patients also had decreased size of their malformations.[13] Based on these results and results of an ongoing trial, alpelisib was approved for PROS in the United States in 2022. Other mutation-specific targeted therapies show efficacy in preclinical

studies, providing hope that as more molecular targets are uncovered in vascular malformations, we will be able to offer more effective, individualized therapies.[8,11] Since vascular malformations are mosaic disorders, mutation-specific therapies can be particularly effective by maintaining efficacy while bypassing toxicity on non-effected tissue.

In this report, we describe a targetable kinase fusion, the EML4::ALK fusion, commonly found in non-small cell lung cancer (NSCLC), in one patient with GSD and one patient with GLA. Given that neither kinase fusions nor ALK mutations have been previously reported in vascular malformations, this report may have a significant impact on genetic testing and treatment options for patients with vascular malformations.

Case reports/Results

Patient 1: 13 year old male with GLA.

At 2 weeks old this patient was noted to have a large macrocystic lymphatic malformation of his neck. He had several admissions in early childhood for superinfection, with pain and swelling. At age 5, he underwent sclerotherapy and surgical resection, but continued to have flares treated with short courses of steroids and/or antibiotics. MRI findings were compatible with GLA with diffuse lymphatic malformations involving the neck, spleen, retroperitoneum, and osseous structures (Fig. 1B). At age 10 he had worsening bone involvement at the clivus and occipital condyles, with increasing pain. Sirolimus was started, with mixed response on imaging. He had improved pain, but residual back pain correlating with a spinal lesion. Genetic testing on archived tissue showed the EML4::ALK fusion; immunohistochemistry demonstrated ALK expression on lymphatic tissue, but not neighboring tissue, consistent with somatic mosaicism (Figs. 1C and 1D).

Patient 2: 20 year-old female with GSD.

This patient presented with an extensive lymphatic malformation in the lumbar spine and sacrum, involving the paraspinal muscles, subcutaneous soft tissues, and extending into the lower lumbar neural foramina, with diffuse lytic changes throughout the lumbar spine and pelvis (Figs. 1E and 1F). She was treated with interferon alpha and zoledronic acid from age 7-8, followed by several years of alendronate. Her symptoms and scans were stable during that time, but it was unclear whether that was therapy response or the waxing and waning course of the disease. She then developed worsening headaches, and a myelogram demonstrated a fistulous connection between the thecal sac and the lymphatic malformation. A lumbar drain and multiple blood patches were placed for treatment of chronic lumbar CSF leak.[14] She started sirolimus at age 18, but subsequently discontinued due to hyperlipidemia and lack of effect. Due to ongoing symptoms and worsening back pain she underwent bone biopsy of a lytic lesion. Genetic testing of the lesion showed the EML4::ALK fusion.

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Discussion

Anaplastic lymphoma kinase (ALK) is a RTK first discovered as part of an oncogenic fusion in anaplastic large cell lymphoma.[15]ALK has an intracellular kinase domain that can be constitutively activated by mutations, causing persistent, dysregulated cell growth.

In ALK fusions, the C-terminal portion of the ALK gene, which includes the kinase domain, is fused with another gene, leading to a chimeric protein with pathogenic kinase activation. ALK fusions have been described in a variety of tumors, from rare tumors such as inflammatory myofibroblastic tumors[16], to more common cancers such as NSCLC.[17] In NSCLC, 3-5% of tumors harbor ALK fusions, with EML::ALK fusions being most common.[17]ALK fusions are validated drug targets with three generations of ALK inhibitors available (first generation: crizotinib, second generation: ceritinib, alectinib, brigatinib and ensatinib, third generation: lorlatinib). After several studies showed equivocal efficacy and significantly improved side effects

and quality of life for patients on ALK inhibitors compared to chemotherapy, these medications became frontline therapy for ALK-fusion positive NSCLC.[18]

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Figure 2. ALK::EML4 patient specific breakpoints for (A) Patient 1, and (B) Patient 2. Notably, breakpoints ALK in both patients are intronic. The ALK domains colored are the basic domain, the WD (tryptophan-aspartic acid) domain, and the kinase domain.

ALK fusions can be treated with ALK-targeted therapies. Dozens of ALK fusion partners have been described and fusion breakpoints vary by individual tumor.[17] For our patients, the EML4::ALK fusion was the result of an inversion of the short (p) arm of chromosome 2, with breakpoints in the *ALK* and *EML4* genes (Figs. 2A and B). For both patients, the *ALK* breakpoint lies within intron 19, the most frequent breakpoint, while the breakpoints in EML4, which are typically quite variable (PMID: 18593892), lie in intron 6 and intron 2. Amplicon-based cancer gene panels which are commonly sent for vascular malformations may miss targetable fusions because standard primers may not cover breakpoints. In contrast, capture-based cancer gene panels that target known oncogenic fusions are more likely to identify targetable fusions. Alternatively, RNA-based fusion panels can be used to capture more oncogenic fusions.[19] A recent multi-omic analysis of a patient with GSD reported large chromosomal events resulting in multiple gene fusions.[20] None of the fusions involved known oncogenes, however this result supports the idea that structural rearrangements and gene fusions may be more common in CLMs than previously appreciated. In addition, the commonality of the EML4::ALK fusion in GLA and GSD suggests that they may share common pathogenesis, and may even be part of a disease spectrum rather than truly distinct entities. Whether ALK-inhibitors are clinically effective in these patients remains a question under investigation.

Based on this report, clinicians caring for patients with CLMs should consider sending not only panels that evaluate for missense mutations and small insertions/deletions, but also cancer fusion panels. This report of a well characterized, targetable oncogenic kinase fusion in two patients with CLM is likely the first of many reports of kinase fusions leading to CLMs. Just as the landscape of kinase fusions in cancer has exploded over the last two decades[21], followed by improved targeted therapy options, we hope the same will be true of CLMs and other vascular malformations in the coming years.

Conflict of interest statement:

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