Novel Gene Fusions and Sustained Response to Targeted Therapy in Children with Systemic Juvenile Xanthogranuloma

Emma Dewey[1], Bradley Wilson[1], Emily Fogler[1], Lynn Lee[1], Jason Clark[1], Justin Ferrell[1], Esther Knapp[2], Somak Roy[1], A. Carl Merrow[1], Robert Lorsbach[1], Jennifer Picarsic[1], Adam Nelson[1], Ashish Kumar[1]

[1]Cincinnati Children's Hospital Medical Center and University of Cincinnati College of Medicine, Cincinnati, OH, USA, [2]University of Louisville, Louisville, KY, USA

Introduction

- Histiocytic disorders include a variety of conditions similarly involving the overproduction of histiocytes.
- Juvenile xanthogranuloma (JXG) is a non-Langerhans cell histiocytic disease, most commonly affecting children, manifesting as self-resolving skin lesions.
- While uncommon, systemic and central nervous system (CNS) involvement in JXG can lead to significant morbidity and mortality. Surgical excision, chemotherapy, and radiation have been reported as somewhat effective, but side effects of treatment can be severe and may not cure the underlying disease.
- We describe two children with systemic JXG, in whom novel gene-fusions (GAB2-BRAF and TFG-RET) were identified. Since increased MAP-kinase signaling is the most frequent driver of histiocytoses, we treated each patient with the MEK-inhibitor trametinib.

Aims:

- To determine the mechanisms by which GAB2-BRAF and TFG-RET fusion genes induce histiocytosis with the goal of eliciting a more direct, efficient treatment for patients with JXG, specifically those with more severe systemic and CNS involvement.

Hypotheses:

- GAB2-BRAF and TFG-RET constitutively activate the MAPK pathway.
- This activation can be blocked with available MEK inhibitors.

Methods

Cloning and expression of fusion proteins

- Total RNA from HEK293T cells was converted to cDNA. PCR primers were designed to amplify the exons contained in the GAB2-BRAF and TFG-RET fusion genes. PCR products were assembled into the MSCV expression vector, transformed into *E. coli*, and sequence validated by Sanger sequencing. Plasmids were either transiently transfected into HEK293T cells or retrovirally transduced into Ba/F3 cells to generate stable cell lines.

MAPK pathway analysis by western blot

- HEK293T cells were transfected with expression vectors for 24 hours, followed by serum starvation overnight. Epidermal growth factor (EGF) was added at 50 ng/mL for five minutes followed by cell lysis. Protein concentration was determined by BCA assay and 15 ug protein was run by SDS-PAGE. Antibodies used are indicated.

Cytokine-independent growth assay

- Ba/F3 cells expressing empty vector (EV), GAB2-BRAF, TFG-RET, or BCR-ABL were plated in triplicate at a concentration of 100,000 cells/ml in cytokine-free RPMI. Viable cell counts were determined using Trypan Blue exclusion.

Clinical Data

Patient 1 (GAB2-BRAF)

History: 6-year-old male presenting with widespread rash and symptoms of diabetes insipidus. Biopsy of the rash revealed features consistent with JXG. Brain MRI demonstrated enhancing lesions in the brainstem, left temporal lobe, and neurohypophysis. Sequencing on the biopsy revealed a novel GAB2-BRAF fusion. Patient has shown continuous clinical improvement over the past two years of being treated with trametinib and desmopressin, with resolution of cutaneous and CNS lesions.

Cutaneous Manifestations and Pathology:

Figure 1. Left panel: Photographs of xanthomatous lesions of eyelid (A) and thigh (B) **Middle panel:** (A) H&E staining (40x) demonstrating JXG infiltrate on the skin with superficial dermal expansion of bland small histiocytes with finely vacuolated cytoplasm; scattered eosinophils are noted. (B) H&E (200x) demonstrating detail of bland small histiocytes and eosinophils pictured in (A). (C) CD163 immunohistochemistry (200x) highlighting histiocytes. (D) p-ERK immunohistochemistry (200x) highlighting a subset of the plump lesional histiocytes. Background endothelial and stromal cells are noted. **Right panel:** Resolution of skin rash after treatment with trametinib.

Radiology: Cranial MRI demonstrated CNS lesions

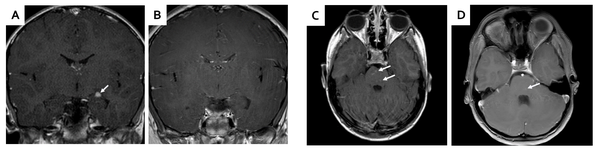

Figure 2. Coronal post-contrast T1-weighted MRI shows an enhancing lesion in the medial left temporal lobe (arrow) (A) and MRI obtained two years later showing interval resolution of the previously identified lesion (B). Axial post-contrast T1-weighted MRI shows multiple small poorly-defined lesions in the pons (arrows) (C) and MRI obtained two years later showing faint residual enhancement in the posterior pons (arrow) with resolution of the other previously identified lesions (D).

Patient 2 (TFG-RET)

History: Patient 2 is a male infant noted to have thrombocytopenia at birth, which progressed to pancytopenia, and hepatosplenomegaly. Bone marrow biopsy showed histiocytic hyperplasia but was overall nonspecific. He remained transfusion dependent. Extensive diagnostic investigations failed to identify a diagnosis. By 4 months of age, he developed skin lesions, and biopsy revealed features consistent with JXG. Sequencing showed a novel TFG-RET fusion. Trametinib was initiated, with prompt resolution of pancytopenia, organomegaly, and flattening of the skin lesions. At three months since initiation of trametinib therapy, the child is asymptomatic and thriving.

Clinical Data (continued)

Cutaneous Manifestations and Pathology:

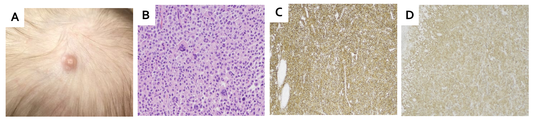

Figure 3. Photograph of xanthomatous lesion on scalp (A). H&E staining (200x) of biopsy demonstrating plump histiocytes and Touton giant cells. Immunohistochemistry showing the histiocytes staining positive for CD163 (C) and factor XIIIa (D).

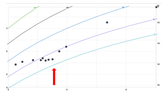

Figure 4. Growth curve of patient 2 with weight on vertical axis (Kg on left, lbs on the right) and age (in months) on the horizontal axis. The red arrow indicates initiation of treatment with trametinib. After failing to grow for several weeks, rapid weight gain was observed upon initiation of trametinib.

Experimental Data

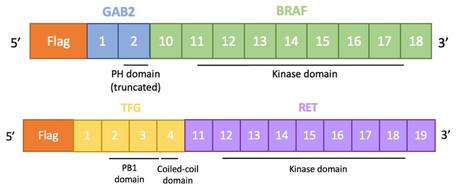

Figure 5. Graphic representation of GAB2-BRAF and TFG-RET fusion products with annotated functional domains. Breakpoint regions were determined by targeted sequencing of histiocytic lesions. Numbers indicate respective exons of each gene.

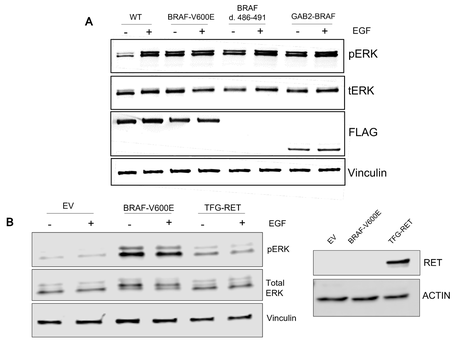

Figure 6. MAPK pathway activation in HEK293T cells following transfection with plasmids expressing fusion genes. (A) Western blot analysis of HEK293T cells transfected with plasmids expressing wild-type (WT) BRAF, BRAF-V600E, BRAF with 486-491 deletion (d.486-491), and GAB2-BRAF fusion, with (+) or without (-) EGF treatment. (B) 293T cells transfected with empty vector (EV), BRAF-V600E, and TFG-RET.

Experimental Data (continued)

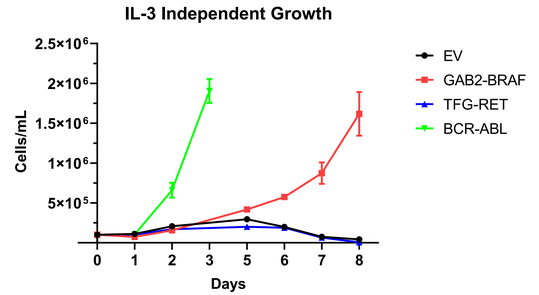

Figure 7. Stable expression of GAB2-BRAF in Ba/F3 cells (red line) induces IL-3 independent growth while TFG-RET expression does not (blue line). Ba/F3 cells expressing BCR-ABL (green line) were used as a positive control for cytokine-independent growth.

Conclusions

- Systemic JXG is associated with novel gene fusions that result in activation of MAPK signaling.
- Treatment with MEK inhibitor trametinib resulted in rapid and sustained disease control in both GAB2-BRAF and TFG-RET associated JXG.
- Experimental data demonstrate that GAB2-BRAF and TFG-RET fusions activate the MAPK pathway in a constitutive, RAS-independent manner.
- GAB2-BRAF expression induces cytokine-independent growth in BaF3 cells while TFG-RET expression does not.
- Treatment with trametinib and other MEK inhibitors should be similarly explored in cases of JXG and other histiocytoses requiring treatment.

Acknowledgments and Disclosures

We thank the patients and their families for participating in research and allowing us to share these data.

AK is a consultant and speaker for SOBI.