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

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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-limiting 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 MAPK-ligase signaling is the most frequent driver of histiocytes, we treated each patient with the MEK-inhibitor trametinib.

Aims:

- To determine the mechanisms by which GAB2-BRAF and TFG-RET fusion genes induce histiogenesis 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 MSCP expression vector, transformed into E. coli, and sequenced validated by Sanger sequencing. Primers were either transcribed into HEK293T cells or retrovirally transduced into BaF3 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. Endosomal 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 μg protein was run by SDS-PAGE. Antibodies used are indicated.

Cytokine-independent growth assay
- BaF3 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 cytokina-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.

- BRAF-MRI demonstrated enhancing lesions in the brainstem, left temporal lobe, and neocortex. Sequencing on the biopsy revealed a novel GAB2-BRAF fusion. Patient has shown continuous clinical improvement over the past two years, being treated with trametinib and desmopressin, with resolution of cutaneous and CNS lesions.

Clinical Data (continued)

Cutaneous Manifestations and Pathology:

- Hypotheses: Novel gene fusions and sustained response to targeted therapy.

Experimental Data

- Clinical Data (continued)

- 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 effective in cases of JXG and other histiocytoses requiring treatment.

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Graphic representation of GAB2-BRAF and TFG-RET fusion products with annotated functional domains. Breakpoint regions were determined by targeted sequencing of histiocytic lesions.