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Generalized verrucosis and abnormal T cell activation due to homozygous *TAOK2* mutation

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ABSTRACT

Background: Generalized verrucosis (GV) is a chronic and progressive cutaneous human papillomavirus (HPV) infection resulting in multiple warts and associated with acquired or genetic immune defects. We identified a consanguineous Arab family manifesting GV and recurrent bacterial and viral infections, in association with inflammatory bowel disease (IBD).
Objective: To identify the mutated gene responsible for GV, recurrent infections and IBD, in this family.
Methods: Flow cytometry of peripheral blood mononuclear cells was performed, as well as proliferation and cell cycle assays of T cells. Whole exome sequencing was utilized to detect candidate mutated genes, assuming an autosomal recessive mode of inheritance. Skin fibroblasts from a patient, the mother and control were incubated with sorbitol to detect the phosphorylation ability of TAOK2, and a clonogenic assay was performed to assess the survival and proliferative capacity of fibroblasts' colonies.
Results: Despite normal immunophenotyping of T and B cells, T cell proliferation upon activation was impaired in a patient compared to a heterozygous family member and a control. Genetic analyses identified a rare homozygous missense variant, c.2098C>T (p.R700C) in the *TAOK2* gene, segregating with the disease phenotype in the family. *TAOK2* encodes the TAO2 kinase, a mitogen activated protein kinase kinase kinase (MAP3K) in the p38-MAPK cascade. The mutation is predicted to disrupt its normal folding and molecular interaction; however, no impairment was observed in TAOK2 kinase activity toward its downstream target, MEK3/6, in patient's fibroblasts. Despite this normal kinase activity, a noticeably higher survival/proliferation of patient's skin fibroblasts was found.
Conclusions: A mutation in *TAOK2* appears to cause a novel form of primary immunodeficiency, characterized by an impaired T cell proliferation upon activation. This novel cause of GV gives further support to the importance of the p38-MAPK pathway in the immune response against HPV, and possibly also in the pathogenesis of IBD.

1. Introduction

Generalized verrucosis (GV) is defined as a chronic and progressive cutaneous human papillomavirus (HPV) infection presenting with greater than 20 lesions and exhibiting marked resistance to conventional therapies [1]. Host defense against HPV relies on intact and functioning cellular immunity including T cell and natural killer (NK) cell cytotoxicity. Therefore, GV should raise a concern for an underlying immune defect [2]. GV can result from genetic disorders such as epidermodysplasia verruciformis (EV) or other inherited immunodeficiency disorders, as well as acquired malfunctioning of the immune system [1].

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EV is a rare autosomal recessive disorder caused by mutations in the genes *TMC6* or *TMC8*, encoding the EVER proteins. The latter form a complex with the Zinc (Zn) transporter ZnT1 in the endoplasmic reticulum. Their disruption leads to a defect in cell mediated immunity due to interrupted Zn transport between cell compartments, causing keratinocyte proliferation and oncoprotein overexpression in infected keratinocytes [3,4]. As opposed to other causes of GV, EV is characterized by infection with ubiquitous β-HPV types (EV-HPV types), although skin tropic α-HPV genotypes may also be involved [2]. It is also associated with a tendency to develop skin malignancies, mainly squamous cell carcinoma [5].

Several other genetic defects, such as those due to mutations in *RHOH*, *MST1* and *TPP2*, resulting in T cell dysfunction, can also manifest with cutaneous infection with EV-HPV types [6–8]. GV due to non EV-HPV types has been described in numerous primary and acquired immunodeficiency disorders, such as the recently identified interleukin (IL)-7-deficiency disorder [9]. Various other defects in cellular and cytotoxic immunity provided by T and NK cells, as well as defects in humoral immunity have been associated with widespread and recalcitrant non-EV-HPV infection [2].

In this report, we describe a consanguineous Arab family manifesting GV, recurrent bacterial and viral infections in association with inflammatory bowel disease (IBD), due to a homozygous missense mutation in the *TAOK2* gene.

2. Materials and methods

2.1. Patients

The current study includes a family of an Arab descent. The study was approved by the Institutional Review Board of Hadassah Hebrew University Medical Center, and written informed consent was obtained from the subjects. Blood samples and skin biopsies for fibroblast cultures were collected from 2 patients and 8 unaffected family members available for the study.

2.2. Flow cytometry

Human peripheral blood mononuclear cells (PBMCs) after lymphoprep purification (according to the manufacturing protocol) were surface labeled with the following antibodies: CD4-APC (OKT4); CD3-Af700 (OKT3); CD8-Af700 (RPA-18); CD19-biotin (H1B19). The cells were then washed with fluorescence-activated cell sorting buffer, and the biotin labeled cells were labeled with a second reagent-streptavidin conjugated BV510 and washed. Samples were analyzed by flow cytometry on a Beckman Coulter Gallios flow cytometer.

2.3. Proliferation

Human PBMCs after lymphoprep purification (according to the manufacturing protocol) were activated with anti CD3 (OKT3) at 1 μg/ml concentration for 96 h. The cells were labeled prior to activation with CellTrace–Violet (Invitrogen) according to the manufacturing protocol, except for the reagent concentration of 1 μM/1 ml/5×10^6 cells.

2.4. Cell cycle

Cell cycle analysis was preformed using a BD BrdU FITC Assay kit, according to the manufacturing protocol. Briefly, 1×10^6 human PBMCs per point were activated for 96 h as previously described, let to incorporate bromo-2-deoxyuridine (BrdU) for 1.5 h and then cell surface labeled with CD4-APC (OKT4); CD3-Af700 (OKT3) antibodies. The cells were analyzed by flow cytometry on a Beckman Coulter Gallios flow cytometer.

2.5. Genetic analyses

To detect candidate mutated genes, whole exome sequencing was performed at Otogenetics corporation using Roche NimbleGen V2 (44.1 Mbp) paired-end sample preparation kit and Illumina HiSeq2000 at a 50× coverage with the DNA sample of patient III-1. Sequence reads were aligned to the human genome reference sequence (build hg19) and variants were called and annotated using the DNAnexus software package. The mutation was verified in all family members by direct sequencing.

2.6. Cell culture

Patient derived fibroblasts and BJ human cells were grown in minimal essential media supplemented with 10% (v/v) fetal calf serum, penicillin and streptomycin.

2.7. Immunoblotting

Cells were lysed with Laemmli buffer and total protein concentration was determined. 15 μg of total protein from each sample was separated by SDS–PAGE and transferred onto a polyvinylidene difluoride membrane (Invitrogen). The membranes were probed with the following primary antibodies: α + β tubulin I + II (1:1000 Sigma), total MEK3 (1:1000 Santa Cruz), phosphorylated MEK3/6 (1:1000 Cell Signaling), phosphorylate TAOK2 (1:1000 Abcam). Secondary antibodies: horseradish-peroxidase-conjugated goat anti-mouse and goat anti-rabbit (H + L) (1:10,000, Jackson Laboratories).

2.8. Clonogenic assay

1000, 500 and 250 cells/well were seeded in 6 well plates. After 10–21 days cells were fixated with 2.5% glutaraldehyde solution for 10 min and stained with 1% methylene blue solution [10].

3. Results

We investigated a consanguineous Arab family, with two affected siblings (Fig. 1A). Patient III-1 is a 12-year-old female, who suffers from numerous warts on the hands, feet and face since the age of six. The lesions were highly resistant to a wide array of treatments, including various topical agents, cryotherapy, and recurrent procedures of electrodessication and curettage. Her past medical history included perianal abscess at the age of 40 days, necessitating surgical intervention, pyelonephritis due to *Escherichia coli* infection at the age of two, left lower lobe pneumonia at the age of five, and recurrent episodes of impetiginized eczema. Following one of the surgical interventions for her warts she developed osteomyelitis of the proximal interphalangeal joint of the 3rd digit of her right hand due to *Pseudomonas aeruginosa* infection.

Patient III-3 is a 5-year-old female, who developed cutaneous warts on her face and hands since the age of two years. Treatments with cryotherapy and curettage were ineffective, and additional warts continued to appear. The patient was diagnosed with ileocolonic Crohn's disease at the age of 3 and had recurrent episodes of cytomegalovirus (CMV) colitis. The patient has been treated with ganciclovir, prednisone, azathioprine and recently also with infliximab with clinical and laboratory improvement of her Crohn's disease. It should be noted that the warts appeared prior to commencement of the immunosuppressive treatment, and persisted even when the patient was not receiving corticosteroids.