Human Pathology (2016) **53**, 137–141

Human PATHOLOGY

www.elsevier.com/locate/humpath

Case study

Translocation t(7;12) as the sole chromosomal abnormality resulting in *ACTB-GLI1* fusion in pediatric gastric pericytoma

Eumenia Castro MD, PhD [a,*], Nahir Cortes-Santiago MD, PhD [b], Lizmery M. Suarez Ferguson MS [b], Pulivarthi H. Rao PhD [c], Rajkumar Venkatramani MD, MS [c], Dolores López-Terrada MD, PhD [b]

[a]*Department of Pathology and Immunology, Texas Children's Hospital, Baylor College of Medicine, Houston, TX 77030*
[b]*Department of Pathology and Immunology, Molecular Oncology Laboratory, Houston, TX 77030*
[c]*Department of Pediatrics, Texas Children's Cancer and Hematology Center, Texas Children's Hospital, Baylor College of Medicine, Houston, TX 77030*

Received 4 December 2015; revised 1 February 2016; accepted 10 February 2016

Keywords:
Pediatric;
Gastric;
Tumor;
Rare;
Translocation;
Pericytoma

Summary We hereby report an unusual gastric tumor arising from the pyloric wall of the stomach in a 9-year-old child harboring the exceptionally rare translocation t(7;12) resulting in *ACTB-GLI1* gene fusion. This tumor has been previously classified as pericytoma with t(7;12) and described in 6 patients, 2 of them children. We discuss the challenges in recognizing this rare entity and the importance of the molecular studies in establishing the correct diagnosis. Our case is the first report of this type arising in the stomach of a child.

1. Introduction

Gastric neoplasms are extremely rare in children. Given their infrequent occurrence, information regarding their clinical presentation and histopathology is very limited. Pediatric gastric tumors are most commonly associated with familial predisposition syndromes such as succinate dehydrogenase–related gastric stromal tumors, germline mutations of E-cadherin/*CDH1* gene, *BRCA1*/*BRCA2*, *APC*, and *TP53*. Mutations of *BMPR1A*, *SMAD4*, and *PTEN* genes (responsible for juvenile polyposis syndrome, juvenile intestinal polyposis, and Cowden disease), and DNA mismatch repair genes, have also been associated with the development of epithelial tumors [1]. Sporadic gastric tumors are even rarer.

Recently, a newly defined soft tissue tumor characterized by the presence of a recurrent t(7;12)(p22;q13) translocation resulting in the fusion of *ACTB* (β-actin) and *GLI* (GLI family zinc finger 1) genes, was reported in 6 patients and classified as pericytoma with t(7;12). Two of the reported cases were identified in the tongue of children [2,3]. We report a third pediatric case of this entity, this time arising in the pyloric wall of the stomach in a 9-year-old patient.

2. Case report

A 9-year-old previously healthy girl, one of nonidentical twins, developed one episode of abdominal pain and vomiting. The father felt an abdominal mass and took her

* Corresponding author.
E-mail addresses: ecastro@bcm.edu (E. Castro), CortesSantiago@bcm.edu (N. Cortes-Santiago), lmsuare1@texaschildrens.org (L. M. S. Ferguson), PHRAO@texaschildrens.org (P. H. Rao), venkatra@bcm.edu (R. Venkatramani), doloresl@bcm.edu (D. López-Terrada).

http://dx.doi.org/10.1016/j.humpath.2016.02.015
0046-8177/

to the pediatrician. Ultrasonographic examination of the abdomen revealed a cystic mass in the right upper quadrant. Computed tomography scan of the abdomen showed a 6 × 7 × 6–cm solid and cystic mass arising from either the distal stomach or the proximal duodenum. There were no other lesions in the abdomen or chest. The child underwent a diagnostic laparoscopy followed by exploratory laparotomy, which revealed a mobile mass attached to the anterior portion of pylorus. The mass was completely resected, and a Heineke-Mikulicz pyloroplasty was performed.

3. Material and methods

3.1. Morphology and immunohistochemical analysis

The specimen was received fresh for gross examination. Portions of the tumor were snap frozen for molecular studies and submitted in RPMI for cytogenetic analysis. A small portion was fixed in glutaraldehyde and submitted for electron microscopy. The slides were stained with hematoxylin-eosin (H&E) stain and immunohistochemical stains for DOG1 (SP31, Abcam, Cambridge, MA ,USA), CD99 (O13, Signet, Dedham, MA, USA), Kit (c-Kit, Dako, Carpinteria, CA , USA), chromogranin A (DAK-A3, Dako), CD45 (PD7/26&2B11, Dako), synaptophysin (Snp88, BioGenex, Fremont, CA USA), CD56 (BC56C04, Biocare Medical, Concord, CA 94520, USA), inhibin (R1, Leica, Buffalo Grove, IL USA), CD10 (56C6, Leica), β-catenin (17C2, Leica), CD34 (QBEnd-10, Dako), smooth muscle actin (aSM-1, Leica), vimentin (VIM3B4, Dako), E-cadherin (2.40E + 11, Cell Signaling, Boston, MA , USA), pancytokeratin (AE1/AE3, Chemicon, Dublin, OH , USA), epithelial membrane antigen (E29, Dako), progesterone receptor (16, Leica Microsystems), WT-1 (WT-1, Leica), desmin (O33, Biogenex), cytokeratin 7 (OV-TL 12/30, Dako), INI-1 (25, BD Biosciences, Menlo Park, CA, USA), S-100 (I5E2E2 + 4C4.9, Biocare), PAX-8 (MRQ-50, Ventana Medical, Tucson, AZ, USA), Ki-67 (MIB-1, Dako), and CD 68 (KP-1, Dako). The immunohistochemical stains were performed using routine steps of deparaffinization, dehydration, and antigen-retrieving technology. Commercially available primary and secondary antibodies were used. Pertinent positive and negative controls were run with each sample. 3,3′-Diaminobenzidine staining was carried out with utilization of the avidin-biotin-peroxidase system. All sections were counterstained with hematoxylin.

3.2. Cytogenetic analysis and fluorescence in situ hybridization

Tumor tissue was finely minced into small pieces and cultured in Dulbecco modified Eagle medium (Gibco Invitrogen, Carlsbad, CA) containing 15% fetal calf serum, 1% L-glutamine, and 1% penicillin-streptomycin (Gibco Invitrogen), and Primocin (Sigma, Invivogen, San Diego, CA). The cultures were harvested, and G-banding was performed using standard protocols. Clonal chromosomal abnormalities identified by G-banding were described according to the International System for Human Cytogenetic Nomenclature (2013). A tumor clone was identified by 2 or more cells with the same chromosomal gain or structural aberration and/or loss of the same chromosome in 3 or more cells.

Fluorescence in situ hybridization (FISH) was performed using *EWSR1* break-apart probe (Abbott Molecular, Abbott Park, IL) on interphase nuclei. The slides were counterstained with 4,6-diamidino-2-phenylindole, and the images were captured using CytoVision 7.3.1 (Leica Biosystems Richmond Inc, Richmond, IL). A total of 200 interphase nuclei were analyzed for *EWSR1* rearrangement FISH analysis.

3.3. Reverse transcription, genomic polymerase chain reaction, and sequencing confirmation

The presence of ACTB-GLI1 fusion transcripts was confirmed by reverse-transcription polymerase chain reaction (RT-PCR) and genomic DNA amplification. cDNA was prepared from RNA and amplified on a GeneAmp 9700 (Applied Biosystems, Foster City, CA, USA) using 13.875 μL of DEPC H_2O (Ambion, Waltham, MA, USA), 2.0 μL of 5× RT buffer (Invitrogen), 1.75 μL of dNTPs (Applied Biosystems), 1.25 μL of 0.1 mol/L DTT (Applied Biosystems), 0.50 μL of 10 μmol/L forward and reverse primers (Sigma-Aldrich, St Louis, MO), 0.125 μL of Ampli Taq Gold Polymerase (Applied Biosystems), and 5 μL cDNA. Two primer pairs were used for the amplification of the ACTB-GLI fusion transcript. ACT61F-GLI868R and ACT61F-GLI1477R were used in cDNA amplification, whereas ACT80F-GLI720R primer pair was used to detect the transcript with genomic DNA [2,3].

DNA amplification was carried out on a GeneAmp 9700 (Applied Biosystems) using 14.4 μL of nuclease-free H_2O, 2.5 μL of 10× PCR buffer with $MgCl_2$ (Applied Biosystems), 1 μL of 25 mmol/L $MgCl_2$ (Invintrogen), 1 μL of 10 mmol/L dNTPs, 0.50 μL of 10 μmol/L forward and reverse primers (Sigma-Aldrich, St Louis, MO), 0.125 μL of 5 U/μL AmpliTaq Gold LD DNA polymerase (Applied Biosystems), and 5 μL of 25 ng/μL patient DNA.

Gel electrophoresis and sequencing confirmation were performed for all amplicons. PCR products generated by GeneAmp 9700 thermocycler were visualized on a 2% agarose gel using the Imager Documentation System (Alpha Innotech Corporation, Santa Clara, CA, USA), purified by USB ExoSAP-IT (Affymetrix, Santa Clara, CA, USA) according to the manufacturer's protocol, and cycle sequenced using Big Dye v1.1 reagents (Applied Biosystems) according to the manufacturer's protocol. Products from the sequencing reaction were purified with CleanSEQ Sequencing Purification System (Agencourt Bioscience Corp, Beverly, MA) and sequencing performed by capillary electrophoresis on an ABI3130 (Applied Biosystems).