Identification of a novel *NRG1 fusion with targeted therapeutic implications in locally advanced pediatric cholangiocarcinoma*

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Abstract

Locally advanced cholangiocarcinoma has a poor prognosis, with long-term survival observed only in patients where complete surgical resection is achieved. Pediatric cholangiocarcinoma is exceedingly rare, with an estimated 15-22 cases reported in the last 40 years. As such, no standard therapeutic regimen exists. Novel strategies combining conventional chemotherapy and radiotherapy followed by targeted agents can lead to durable treatment responses and are applicable to pediatric cholangiocarcinoma management. We present a case of a 17-year-old patient with unresectable cholangiocarcinoma whose tumor genetic sequencing revealed a novel, actionable NRG1 translocation, providing an opportunity to utilize combination treatment in the pediatric setting.

Introduction

Locally advanced cholangiocarcinoma has a generally poor prognosis, and complete surgical resection is crucial for long-term survival.[1,2] Median progression free survival and overall survival with chemotherapy alone has been documented as 8 months and 11.7 months, respectively.[3] Pediatric cholangiocarcinoma is exceedingly rare, with an estimate of 15-22 literature-reported cases in the last 40 years. Of these pediatric patients, the vast majority have an underlying gastrointestinal disorder predisposing them to malignancy.[4] We report a rare case of a previously healthy teenager who presented with obstructive jaundice, and was subsequently diagnosed with locally advanced perihilar cholangiocarcinoma. Whole exome and whole transcriptome sequencing of tumor tissue and a paired normal sample identified a novel *NRG1* fusion. The patient maintained stable disease for over two years after an initial partial response to standard cytotoxic chemotherapy and proton beam radiotherapy followed by oral personalized targeted therapy.

Case Description

A previously healthy 16-year-old Hispanic girl presented with abdominal pain and jaundice. She reported a 4-week history of intermittent, sharp, right upper quadrant pain that was worse after eating. At the same time, scleral icterus was noted by her friends. When her stools became acholic and urine dark in color, she presented for evaluation at a local clinic. Bloodwork revealed transaminitis (AST 322 U/L [normal 17-33 U/L], ALT 360 U/L [normal 8-24 U/L]) with a direct hyperbilirubinemia (total bilirubin 7.2 mg/dL [normal 0.2-1 mg/dL], direct bilirubin 5.2 mg/dL [normal <0.3 mg/dL]) and elevated GGT (1,346 U/L [normal 10-21 U/L]). The patient was advised to seek additional care at an outside emergency room where computed tomography (CT) of the abdomen and pelvis revealed a large, infiltrative central liver mass. She was then transferred to Children's Healthcare of Atlanta for further evaluation and management. Magnetic resonance imaging (MRI) confirmed a poorly defined, T1 hypointense, T2 hyperintense mass at the porta hepatis measuring 6 cm x 6.7 cm x 6.9 cm with distal biliary ductal dilatation. The left portal vein was not

visualized in its course through the mass; the right portal vein demonstrated normal enhancement (Figs. 1A and 1B). A chest CT was notable only for a left lower lobe calcified granuloma, and a PET scan revealed FDG avidity solely in the known hepatic mass. The patient underwent percutaneous biliary decompression and drain placement along with biopsy of the mass by interventional radiology. Pathologic evaluation of the biopsied tissue demonstrated immunohistochemical staining positive for CK7 (strong, diffuse), mucicarmine, CK19 (focal), CK56 (focal), and galectin-3 (focal); negative staining for CK20, S100P, TTF-1, and CDX-2, which, in conjunction with morphologic features, confirmed the diagnosis of cholangiocarcinoma (Figs. 1C and D)**.** Her liver tumor was deemed unresectable due to its locally advanced nature. The patient therefore received chemotherapy treatment for unresectable cholangiocarcinoma with eight cycles of cisplatin-gemcitabine (cisplatin 25mg/m2 followed by gemcitabine 1000mg/m2 on days 1 and 8 every 3 weeks).[5]A partial response to therapy was documented. She then underwent consolidation with proton beam radiation therapy (58.05 Gy; 3.87 Gy per fraction for a total of 15 fractions) with stable disease on follow-up imaging.

Due to the rare nature of this adolescent's cancer and her limited response to standard therapy, she was enrolled on our institution's precision medicine study. Tumor tissue from the diagnostic biopsy was sent for comprehensive genomic sequencing (whole exome/paired normal and transcriptome sequencing) utilizing the GEM ExTra®assay (Ashion Analytics, Phoenix, Arizona). Details of the test methodology and clinical reporting have been published previously.[6] Tumor tissue sequencing identified a novel, presumed oncogenic *AGRN-NRG1* fusion between chromosome 1 (*AGRN*) and chromosome 8 (*NRG1*) with breakpoints within exon 2 of *AGRN* and intron 1 of *NRG1* (Fig. 2A). Based on our institution's molecular tumor board discussion, the patient was started on the oral pan-*ERBB* family inhibitor, afatinib, as a form of maintenance therapy. She continued on afatinib 40mg daily with stable disease until two years after diagnosis with the main side effect being acneiform rash. The patient then experienced a localized progression with development of three new, subcentimeter, satellite liver lesions. Biopsy of the largest lesion by interventional radiology confirmed cholangiocarcinoma, and sequencing identified persistence of the *AGRN-NRG1* fusion along with the development of a *MCL1*amplification. Despite detection of 66 new genomic alterations, none were classified as driver events (Fig 2B). The patient chose to enroll on a clinical trial with seribantumab, an anti-*ERBB3* monoclonal antibody, and continues on this therapy at present.

Discussion

Cholangiocarcinoma is a highly aggressive malignancy that arises from epithelial cells of the intrahepatic and extrahepatic bile duct system, often presenting in an advanced stage. Risk factors for the disease include chronic hepatitis and cirrhosis, biliary inflammatory diseases, and hepatobiliary fluke infection. However, in the majority of cases, no risk factor is identified.[1] In the pediatric population, cholangiocarcinoma is exceedingly rare, with only 15 cases identified from 1973 to 2013 based on SEER18 data and 22 cases reported in the literature. Ninety percent of these patients had an underlying gastrointestinal comorbidity, with a poor three-year overall survival at 35.3%.[4]

Surgery is the mainstay of curative therapy for cholangiocarcinoma, though only ˜35% of patients present with early-stage disease amenable to upfront resection.[7] Standard treatment for patients with unresectable cholangiocarcinoma includes eight cycles of cisplatin and gemcitabine. However, median progression free survival and overall survival with chemotherapy alone has been documented as 8 months and 11.7 months, respectively.[3]The role of radiation in the treatment of liver tumors is evolving, and proton therapy is an attractive local control strategy, providing a theoretical clinical benefit over photon-based treatment by allowing for safer dose-escalation in larger tumors.[8]

Precision medicine and personalized therapies are becoming more common in the treatment of cholangiocarcinoma, with a goal of providing precise therapy with improved efficacy and safety profiles.[9]To date, a number of mutated oncogenes have been identified in cholangiocarcinoma, including *IDH1* , *KRAS, BRAF, ARID1A, PBRM1, EGFR/ERBB1, HER2/ERBB2* among others.[10,11] Inhibitors of key oncogenic pathways have been evaluated as single agents, combined targeted therapies, and in combination with standard chemotherapy.[9] Neuregulin-1 (*NRG1)* gene fusion events are rare, yet potentially actionable driver mutations that have been detected across multiple solid tumor types at a frequency of 0.2% with significant fusion partner

heterogeneity.[12] They are largely mutually exclusive with other oncogenic drivers. In cholangiocarcinoma, *NRG1* fusions have been observed in approximately 0.8% of cases.[12] These fusions promote tumorigenesis via an *EGF*- like domain within *NRG1* that binds *ERBB3* in a para/juxtacrine or autocrine fashion, resulting in *ERBB2/ERBB3* heterodimerization and increased downstream signaling. Hence, targeted agents are often utilized. Clinical activity in *NRG1* fusion-positive malignancy has been reported with combined inhibition of *ERBB* and *ERBB2* with erlotinib and pertuzumab, other dual-inhibition of multiple *ERBB* receptors such as afatinib, and with anti-*ERBB3* monoclonal antibodies.[13,14] Additional clinical trials are underway.

Conclusion

Pediatric cholangiocarcinoma is exceedingly rare, and in the case described here, identification of our patient's novel *AGRN-NRG1* fusion not only helped to further define the genetic landscape of pediatric cholangiocarcinoma, it also provided a molecular target for a personalized, multimodal therapeutic regimen and disease control with good quality of life for over two years, 18 months longer than previously documented median progression free survival rates.

Conflict of Interest statement: The authors declare no conflicts of interest.

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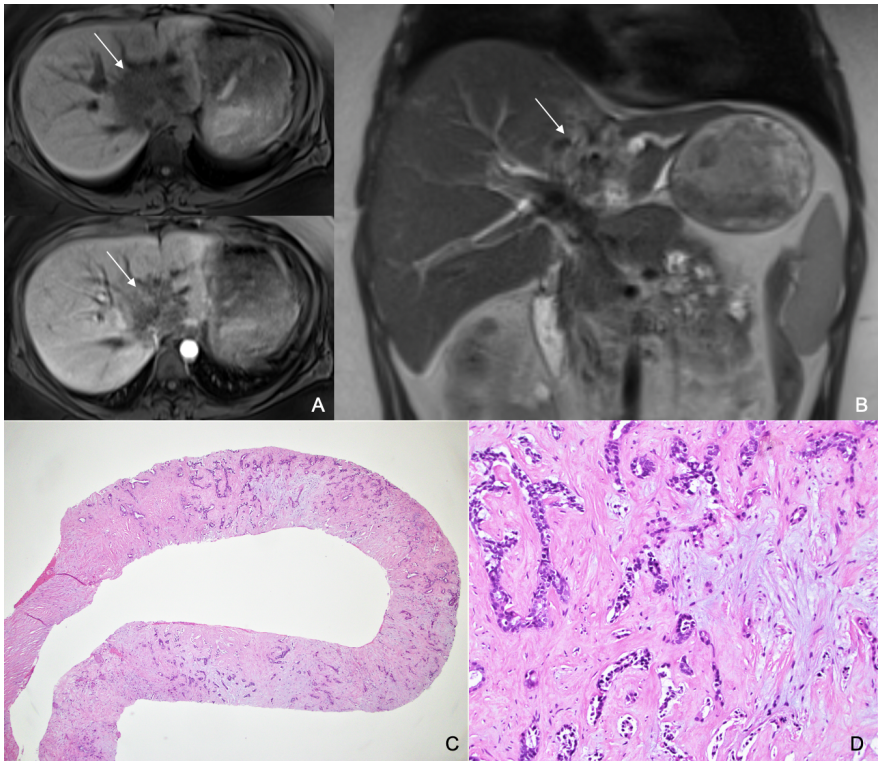
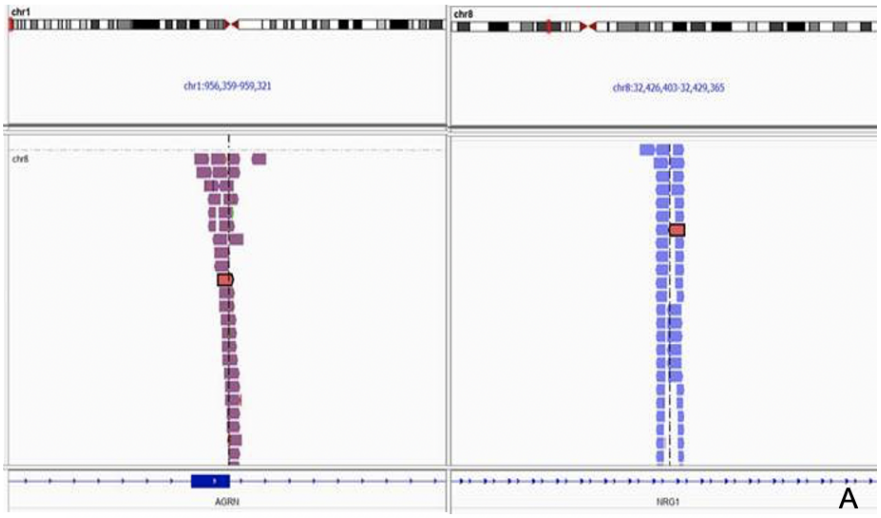
Figure 1A-D.

MRI images at diagnosis confirm a T1 hypointense (A), T2 hyperintense (B) ill-defined 6cm x 6.7cm x 6.9cm mass in the central liver. Needle core biopsies of the tumor demonstrated infiltrative, irregular cords and glands resembling cholangioles or canals of Hering in a dense fibrous desmoplastic stroma (C, H&E stain, x40). The tumor cells show cuboidal mucin production with low-grade cytologic atypia (D, H&E stain, x200).

Figure 2A-B.

Screen capture from the Integrative Genomics Viewer (IGV) showing breakpoint spanning sequencing reads for the *AGRN* and *NRG1*loci (A). Below, a Venn diagram comparison of the sequencing reports from diagnosis (11 genomic alterations detected in biopsy number one) and progression (66 new genomic alterations detected in biopsy number two). Further sequencing did not reveal obvious driver genomic events to indicate the mechanism of progression (B).





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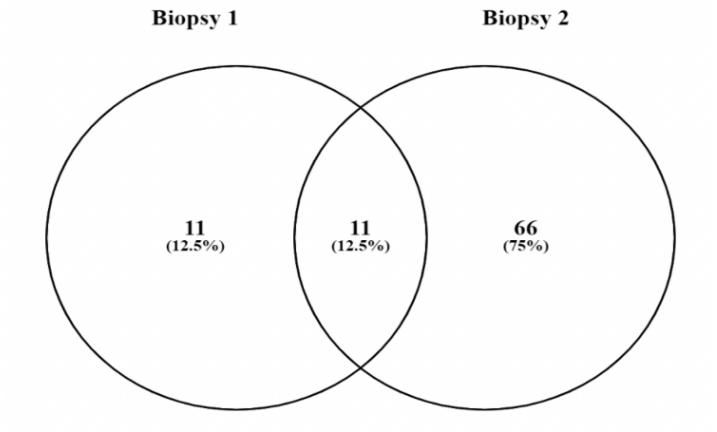

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