Published in final edited form as:

Genes Chromosomes Cancer. 2016 October ; 55(10): 814–817. doi:10.1002/gcc.22382.

Expanding the Spectrum of *ALK*-Rearranged Renal Cell Carcinomas in Children: Identification of a Novel *HOOK1-ALK* Fusion Transcript

Mariana M. Cajas¹, Lawrence J. Jennings¹, David George¹, and Elizabeth J. Perlman¹

¹Department of Pathology and Laboratory Medicine - Ann & Robert H. Lurie Children's Hospital of Chicago and Northwestern University Feinberg School of Medicine – Chicago, IL

To the Editor

We have recently characterized the morphologic, immunohistochemical and molecular features of a distinctive group of renal cell carcinomas (RCC) occurring in children and characterized by *ALK* gene rearrangements (Cajas et al., 2016). These tumors manifested either *VCL-ALK* (3 patients) or *TPM3-ALK* (3 patients) fusion transcripts. The histopathological, ultrastructural and immunohistochemical features seen in these 6 cases were unique and no differences were noted among cases with the two different *ALK* fusion partners. All tumors were composed of cells with eosinophilic cytoplasm and high grade nuclear features arranged in a predominantly solid pattern with a prominent vascular background, showed frequent intracytoplasmic vacuoles, were associated with a striking lymphoplasmacytic infiltrate, and showed a similar immunohistochemical profile with expression of epithelial markers, TFE3 and retention of INI-1. Based on these shared features, as well as their overall prevalence within a large series of pediatric RCC, we proposed that these neoplasms belong to a distinct subgroup of RCC frequently occurring in pediatric patients, which we have referred to as *ALK*-rearranged RCC.

Following the publication of our study, we have identified an additional example of *ALK*-rearranged RCC in a pediatric patient. This patient was enrolled in the AREN03B2 Children's Oncology Group protocol with a diagnosis of RCC, and a full set of H&E slides and the institutional pathology report were submitted for central pathology review. The patient, a 16 years old Caucasian male, presented with hematuria and a renal mass, and underwent right radical nephrectomy. There was no history of sickle cell disease or trait, which was present in 3/6 of the previously reported cases. Gross examination revealed a 5.5 cm well-circumscribed, soft and friable tan mass with areas of hemorrhage. Histological sections demonstrated a neoplasm entirely surrounded by a fibrous pseudocapsule and predominantly composed of papillary architecture (Figure 1A) alternating with solid areas. The cells were round to polygonal with variable amounts of eosinophilic cytoplasm (Figure 1B and C). Frequent intracytoplasmic lumina were noted, and the presence of dense eosinophilic cytoplasm in a subset of cells was reminiscent of intracytoplasmic inclusions

(Figure 1B and C). Scattered mucin-filled glandular structures were also appreciated (Figure 1B and D). Most of the nuclei were irregular with vesicular chromatin and small nucleoli, consistent with an ISUP grade 3, and a small subset of cells showed greater degrees of nuclear pleomorphism, hyperchromasia and more prominent nucleoli consistent with an ISUP grade 4. A patchy mild lymphoplasmacytic inflammatory infiltrate permeated the tumor cells throughout the neoplasm, and occasional dystrophic calcifications were appreciated. Immunohistochemical stains showed diffuse and strong expression of EMA, vimentin and TFE3 (nuclear), focal expression of cytokeratin 7, retained INI-1 expression and lack of p53 accumulation. A diagnosis of MiT family translocation RCC was initially considered, however, FISH studies for a *TFE3* rearrangement were negative. Despite the extensive papillary architecture, features overlapping with our previously reported *ALK*-rearranged RCC were noted, prompting us to investigate for the presence of an *ALK* fusion transcript in this neoplasm.

RNA was extracted from archival formalin-fixed paraffin-embedded (FFPE) tissue as previously described, and in-depth molecular analysis using next generation sequencing (NGS) methodology was then performed in this case in an attempt to identify an *ALK* fusion transcript, using previously described methodology (Cajaiba et al., 2016). The results demonstrated the presence of a *HOOK1-ALK* fusion transcript spanning exon 20 of *HOOK1* and exon 21 of *ALK*. Using the results of the above sequencing data as references for *HOOK1-ALK* fusion breakpoint sites, two different primer sets were generated for both *HOOK1-ALK* fusion transcripts and the control *GUSB* transcript (sequences available upon request). Human mRNA sequences for *HOOK1* (NM_015888.4), *ALK* (NM_004304.4) and *GUSB* (NM_000181.2) were downloaded from the NCBI Nucleotide database (NCBI, <http://www.ncbi.nlm.nih.gov/nucleotide/>). The *HOOK1-ALK* fusion was confirmed by RT-PCR and Sanger-dideoxy sequencing (Figure 2).

The *HOOK1* gene (located in chromosome band 1p32) encodes a protein involved in endocytosis and sorting of other cytoplasmic proteins, including proteins involved in cytoskeleton assembly (Weimer et al., 2005; Maldonado-Baez and Donaldson, 2013). The *HOOK1* protein has been indirectly implicated in the pathogenesis of Batten disease (juvenile neuronal ceroid lipofuscinosis) and Alzheimer disease through mediation of abnormal protein accumulation (Weimer et al., 2005; Herrmann et al., 2015). According to the COSMIC (Catalogue Of Somatic Mutations In Cancer) database, over 70 missense mutations involving *HOOK1* have been documented in solid tumors (http://cancer.sanger.ac.uk/cosmic/gene/samples?coords=AA%3AAA&src=gene&end=729&mut=substitution_missense&ln=HOOK1&all_data=&id=54894&seqlen=729&start=1), most notably in a small subset of gastrointestinal adenocarcinomas (Dulak et al., 2013; Giannakis et al., 2014), and also in an isolated case of papillary RCC (Durinck et al., 2015). Other than a probably artifactual fusion with the *FAF1* gene (also located in chromosome band 1p32) described in a breast carcinoma (Yoshihara et al., 2015), to our knowledge no gene fusion involving *HOOK1* as the 3' partner has been described in the literature to this date.

In our previous study, we raised the possibility that additional, unknown partner genes could be involved in the pathogenesis of *ALK*-rearranged RCC, and the identification of additional

pediatric and adult cases might expand the spectrum of *ALK*-rearranged RCC. Indeed, besides the case reported here, two additional cases of *ALK*-rearranged RCC have been reported in adult patients since the publication of our study, both corresponding to *STRN-ALK* fusions (Kusano et al., 2016). In addition to our pediatric cases, seven adult cases have been reported (Sugawara et al., 2012; Sukov et al., 2012; Lee et al., 2013; Kusano et al., 2016). Within the total of 13 reported cases, the fusion partners include *TPM3* (4 cases, 3 pediatric and 1 adult), *VCL* (3 pediatric cases, all with sickle cell disease or trait), *STRN* (2 adult cases) and *EML4* (1 adult case). Those involving *STRN*, the adult case involving *TPM3* (Sugawara et al., 2012) and our case involving *HOOK1* share a predominantly papillary architecture, mucin deposition and calcifications, which were absent in our original six cases (Cajaiba et al., 2016). These observations add to the notion that the morphological spectrum of *ALK*-rearranged RCC may indeed be wider than we originally thought. However, features consistently identified in all *ALK*-rearranged RCC remain, including abundant eosinophilic cytoplasm, intracytoplasmic lumina, and TFE3 immunohistochemical expression, and may represent unifying features. Recognition of these neoplasms may have therapeutic implications in view of the possibility of targeted therapy with *ALK* inhibitors.

Acknowledgments

Supported by: This Study was supported by grants from the National Institutes of Health to the Children's Oncology Group (U10CA180886, U24CA114766, U10CA180899, and U10CA098543).

REFERENCES

1. Cajaiba MM, Jennings LJ, Rohan SM, Perez-Atayde A, Marino-Enriquez A, Fletcher JA, Geller J, Leuer K, Bridge JA, Elizabeth J, Perlman EJ. *ALK*-rearranged pediatric renal cell carcinomas in children. *Genes Chromosomes Cancer*. 2016; 55:442–451. [PubMed: 26773439]
2. Dulak AM, Stojanov P, Peng S, Lawrence MS, Fox C, Stewart C, Bandla S, Imamura Y, Schumacher SE, Shefler E, McKenna A, Carter SL, Cibulskis K, Sivachenko A, Saksena G, Voet D, Ramos AH, Auclair D, Thompson K, Sougnez C, Onofrio RC, Guiducci C, Beroukhi R, Zhou Z, Lin L, Lin J, Reddy R, Chang A, Landrenau R, Pennathur A, Ogino S, Luketich JD, Golub TR, Gabriel SB, Lander ES, Beer DG, Godfrey TE, Getz G, Bass AJ. Exome and whole-genome sequencing of esophageal adenocarcinoma identifies recurrent driver events and mutational complexity. *Nat Genet*. 2013; 45:478–488. [PubMed: 23525077]
3. Durinck S, Stawiski EW, Pavia-Jimenez A, Modrusan Z, Kapur P, Jaiswal BS, Zhang N, Toffessi-Tcheuyap V, Nguyen TT, Pahuja KB, Chen YJ, Saleem S, Chaudhuri S, Heldens S, Jackson M, Pena-Llopis S, Guillory J, Toy K, Ha C, Harris CJ, Holloman E, Hill HM, Stinson J, Rivers CS, Janakiraman V, Wang W, Kinch LN, Grishin NV, Haverty PM, Chow B, Gehring JS, Reeder J, Pau G, Wu TD, Margulis V, Lotan Y, Sagalowsky A, Pedrosa I, de Sauvage FJ, Brugarolas J, Seshagiri S. Spectrum of diverse genomic alterations define non-clear cell renal carcinoma subtypes. *Nat Genet*. 2015; 47:13–24. [PubMed: 25401301]
4. Giannakis M, Hodis E, Mu XJ, Yamauchi M, Rosenbluh J, Cibulskis K, Saksena G, Lawrence MS, Qian ZR, Nishihara R, Van Allen EM, Hahn WC, Gabriel SB, Lander ES, Getz G, Ogino S, Fichs CS, Garraway LA. *RNF43* is frequently mutated in colorectal and endometrial cancers. *Nat Genet*. 2014; 46:1264–1267. [PubMed: 25344691]
5. Herrmann L, Wiegmann C, Arsalan-Werner A, Hilbrich I, Jager C, Flach K, Suttikus A, Lachmann I, Arendt T, Holzer M. Hook proteins: association with Alzheimer pathology and regulatory role of Hook3 in amyloid beta generation. *PLOS One*. 2015; 10:e0119423. [PubMed: 25799409]
6. Kusano H, Togashi Y, Akiba J, Moriya F, Baba K, Matsuzaki N, Yuba Y, Shiraishi Y, Kanamaru H, Kuroda N, Sakata S, Takeuchi K, Yano H. Two cases of renal cell carcinoma harboring a novel *STRN-ALK* fusion gene. *Am J Surg Pathol*. 2016 EPUB.

7. Lee C, Park JW, Suh JH, Nam KH, Moon KC. *ALK*-positive renal cell carcinoma in a large series of consecutively resected Korean renal cell carcinoma patients. *Korean J Pathol.* 2013; 47:452–467. [PubMed: 24255633]
8. Maldonado-Baez L, Donaldson JG. Hook1, microtubules and Rab22. Mediators of selective sorting of clathrin-independent endocytic cargo proteins on endosomes. *BioArchitecture.* 2013; 3:141–146. [PubMed: 24284901]
9. Sugawara E, Togashi Y, Kuroda N, Sakata S, Hatano S, Asaka R, Yuasa T, Yonese J, Kitagawa M, Mano H, Ishikawa Y, Takeuchi K. Identification of anaplastic lymphoma kinase fusions in renal cancer. *Cancer.* 2012; 118:4427–4436. [PubMed: 22252991]
10. Sukov WR, Hodge J, Lohse CM, Akre MK, Leibovich BC, Thompson RH, Cheville JC. *ALK* alterations in adult renal cell carcinoma: frequency, clinicopathologic features and outcome in a large series of consecutively treated patients. *Mod Pathol.* 2012; 25:1516–1525. [PubMed: 22743654]
11. Weimer JM, Chattopadhyay S, Custer AW, Pearce DA. Elevation of Hook1 in a disease model of Batten disease does not affect a novel interaction between Ankyrin G and Hook1. *Biochem Biophys Res Comm.* 2005; 330:1176–1181. [PubMed: 15823567]
12. Yoshihara K, Wang Q, Torres-Margia W, Vegesna R, Kim H, Verhaak RGW. The landscape and therapeutic relevance of cancer-associated transcript fusions. *Oncogene.* 2015; 34:4845–4854. [PubMed: 25500544]

Figure 1. Morphological features of a renal cell carcinoma bearing a *HOOK1-ALK* fusion transcript. Predominant papillary architecture (A) with variable amounts of eosinophilic cytoplasm (B and C), intracytoplasmic lumina (C) and occasional mucin-filled glandular spaces (B and D).

Figure 2.
Sequencing analysis showing fusion of *HOOK1* exon 20 to *ALK* exon 21.