KRAS and *GNAS* Co-Mutation in Metastatic Low-Grade Appendiceal Mucinous Neoplasm (LAMN) to the Ovaries: A Practical Role for Next-Generation Sequencing

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
Literature Search F
Funds Collection G

BCDEF 1,2 Daniel R. Matson
A 1,2 Jin Xu
B 3 Laura Huffman
B 3 Lisa Barroilhet
BCD 2 Molly Accola
ABCD 1 William M. Rehrauer
ABCDEFG 1 Paul Weisman

1 Department of Pathology and Laboratory Medicine, University of Wisconsin School of Medicine and Public Health, Madison, WI, U.S.A.
2 University of Wisconsin Hospitals and Clinics, Madison, WI, U.S.A.
3 Department of Obstetrics and Gynecology, Division of Gynecologic Oncology, University of Wisconsin School of Medicine and Public Health, Madison, WI, U.S.A.

Corresponding Author: Paul Weisman, e-mail: pweisman@wisc.edu
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Patient:	**Female, 49**
Final Diagnosis:	**Metastatic LAMN**
Symptoms:	**Abdominal discomfort**
Medication:	**—**
Clinical Procedure:	**Laparoscopic total abdominal hysterectomy • bilateral salpingo-oophorectomy • complete supracolic omentectomy • appendectomy**
Specialty:	**Oncology**

Objective: Unknown ethiology

Background: Low-grade appendiceal mucinous neoplasms (LAMNs) are cytologically low-grade tumors of the appendix and are a frequent cause of pseudomyxoma peritonei. They can become a diagnostic challenge when they metastasize to the ovaries, where they may mimic primary ovarian mucinous tumors.

Case Report: We report the case of a patient with very large bilateral ovarian mucinous tumors and a concurrent minute LAMN incidentally discovered in a grossly normal appendix. A primary ovarian tumor was suspected, but histological analysis of the ovaries suggested an appendiceal origin. Immunohistochemical studies were not informative and a consensus regarding the source of the ovarian tumors could not be reached within our department. Subsequent next-generation sequencing of tumors from the right ovary, left ovary, appendix, and matched normal tissue demonstrated identical somatic point mutations in *KRAS* and *GNAS* present in all tumors. The patient was diagnosed with metastatic LAMN and did not receive further treatment. She remains disease-free after 15 months of close observation.

Conclusions: Determining the tissue of origin in low-grade mucinous tumors of the ovaries can be challenging when a concurrent LAMN is identified in the appendix. In cases where histology and immunohistochemistry are insufficient to render a diagnosis, the presence of concurrent *KRAS* and *GNAS* mutations in both tumors strongly favors a diagnosis of metastatic LAMN. We emphasize the utility of targeted next-generation sequencing to establish tissue of origin in challenging cases when LAMN is suspected as the source of mucinous ovarian tumors.

MeSH Keywords: Appendiceal Neoplasms • Ovarian Neoplasms • Sequence Analysis, DNA

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Background

Low-grade appendiceal mucinous neoplasms (LAMNs) are cytologically low-grade tumors of the appendix, which despite their indolent appearance, are a frequent cause of pseudomyxoma peritonei (PMP) [1,2]. LAMNs can represent a diagnostic challenge when they metastasize to the ovaries, where they can be difficult to distinguish from primary ovarian mucinous tumors [3–5]. Here, we present a case involving metastatic LAMN to the ovaries and describe how next-generation sequencing was utilized to support the diagnosis of metastatic disease. We discuss the use of next-generation sequencing as a practical diagnostic tool to resolve the origin of mucinous ovarian tumors when a concurrent LAMN is identified in the appendix.

Case Report

A 49-year-old woman with hypertension and no previous history of malignancy presented with several months of abdominal pain and distension, which had worsened during the previous 3 weeks. A CT scan of the abdomen was read as a single large septated mass (27.0×22.6×12.6 cm) involving both ovaries. Labs revealed elevated serum levels of CA 125, CA 19-9, and CEA. She underwent laparoscopic total abdominal hysterectomy, bilateral salpingo-oophorectomy, complete supracolic omentectomy, and appendectomy. Intraoperatively, there were bilateral ovarian tumors and no mucinous ascites. The appendix was normal in appearance.

The left ovary was sent for intra-operative frozen section analysis. Grossly, the ovary was enlarged (5640 g, 30.0 cm), with a focal surface defect. A diagnosis of "mucinous neoplasm, suspicious for borderline tumor" was rendered. The right ovary was also enlarged (866 g, 16.0 cm) but intact. Both ovaries had rough patches on their surface suspicious for possible surface involvement, and their cut surfaces showed numerous septations without solid areas. The appendix (2.5 cm in length ×0.5 cm in diameter) exuded a minimal amount of luminal mucin upon sectioning but was otherwise grossly unremarkable. The uterus, cervix, and fallopian tubes were also unremarkable.

Microscopic examination of the appendix (entirely submitted) revealed a 0.5-cm tumor in the body which exhibited a low-grade mucinous epithelial lining and no frankly infiltrative growth (Figure 1A). Focal deposits of acellular mucin were visible within the wall, although the wall was intact. The lamina propria and muscularis mucosae were absent at the periphery of the tumor (Figure 1B). Both ovarian tumors showed similar histologic findings including mucinous morphology with low-grade cytological features, focally complex architecture, abundant pseudomyxoma ovarii, and extensive sub-epithelial clefting (Figure 2).

Immunohistochemical stains were performed on sections of tumor from the right ovary, left ovary, and appendix (Figure 3). The ovarian tumors were strongly and diffusely positive for CK7 and CK20. However, the tumor in the appendix was only focally immunoreactive for both markers. In contrast, all 3 tumors were diffusely and strongly positive for CDX2, while PAX8 was diffusely negative.

In an effort to support the histological impression of metastatic LAMN, DNA isolated from the ovarian tumors, the appendiceal tumor, and matched normal tissue were subjected to an
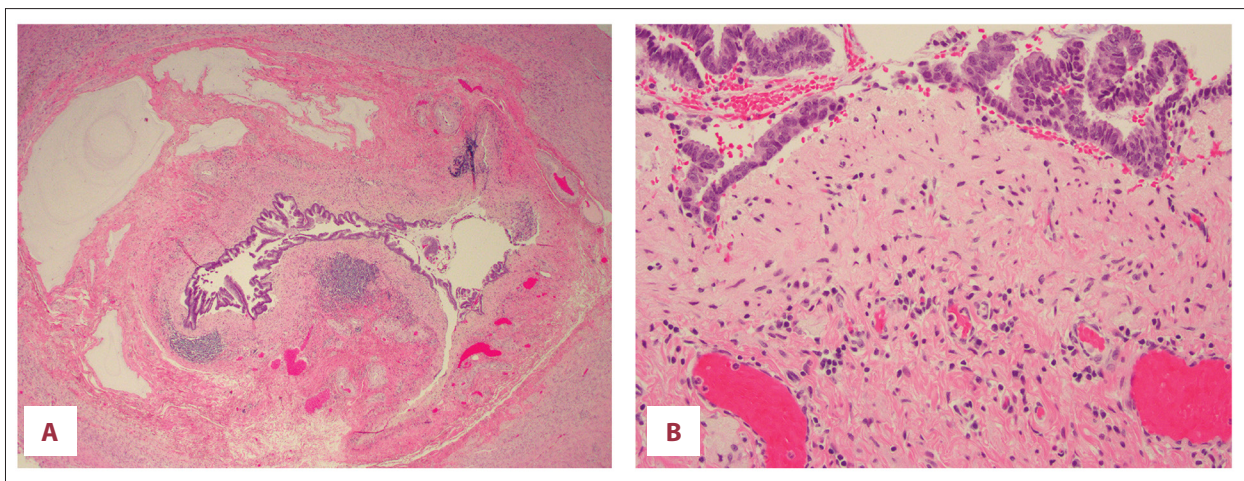

Figure 1. Sections of the appendix demonstrating the histologic appearance of LAMN. (**A**) Section of appendix showing LAMN with focal acellular mucin dissecting through the wall of the appendix. Frankly infiltrative growth and rupture are absent. (**B**) High-power view showing epithelium directly overlying fibrous tissue with corresponding loss of the lamina propria and muscularis mucosae. All images are H & E. (**A**): 40×; (**B**): 200×.
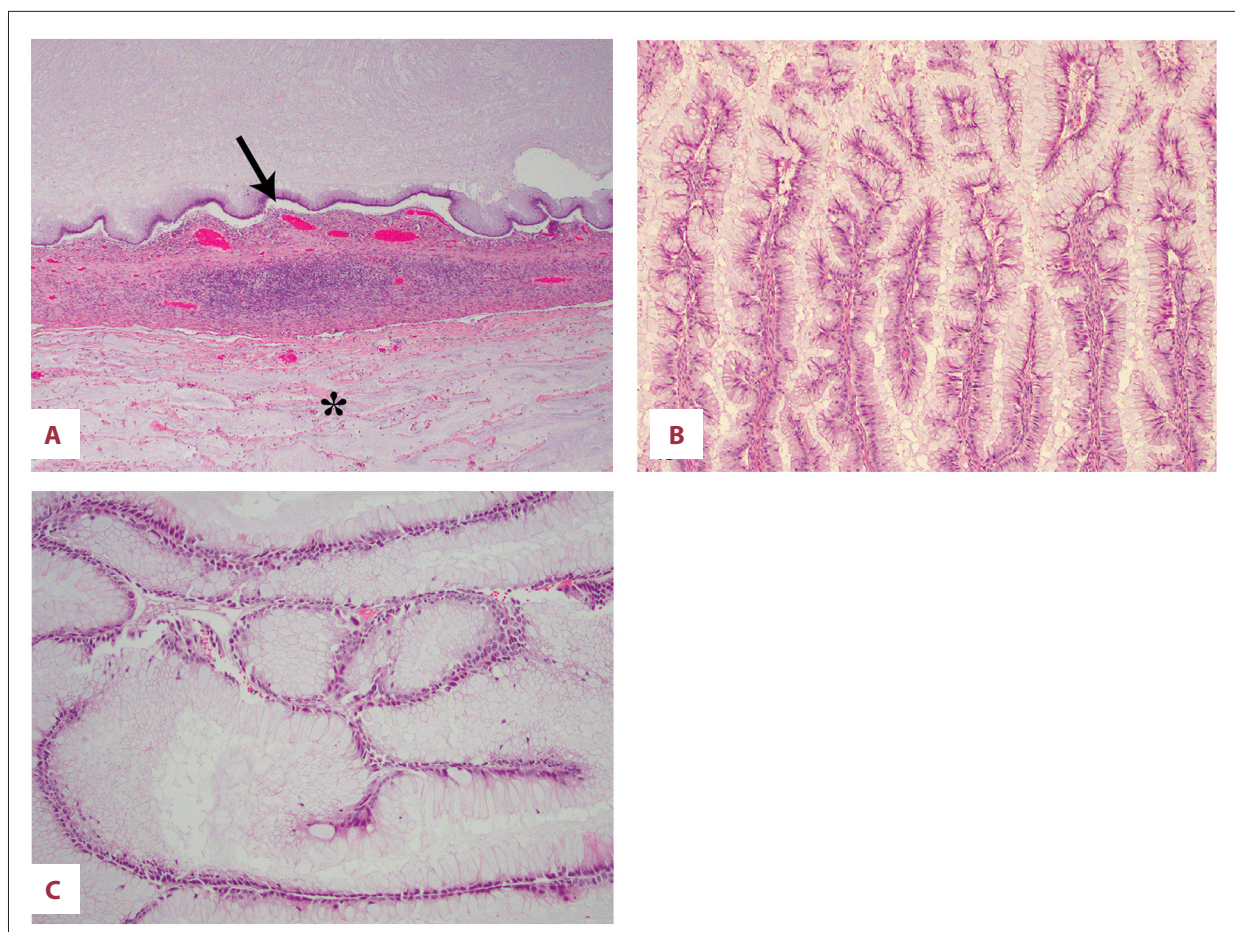
Figure 2. Sections demonstrating the histologic appearance of the ovarian tumors. (**A**) Sections of the left ovary showing sub-epithelial clefting (arrow) and pseudomyxoma ovarii (asterisk). (**B**) Additional section of the left ovary showing a complex, interanastomosing glandular arrangement. (**C**) Section of left ovary showing hypermucinous glands. All images are H & E. (**A**): 40×; (**B, C**): 200×.

in-house next-generation targeted sequencing panel. This panel is validated to identify more than 2800 genetic variants in 50 genes commonly mutated in cancer [6]. It includes genes that are mutated frequently in primary ovarian mucinous neoplasms and primary colon carcinomas, such as *KRAS*, *TP53*, *BRAF*, *CDKN2A*, *PIK3CA*, *FBXW7*, *SMAD4*, and *NRAS* [7,8]. Our panel does not include *BRCA1* or *BRCA2*; however, *BRCA* mutations including familial mutations are reported to be exceptionally rare in primary ovarian mucinous tumors [7,9]. Samples from all 3 tumors harbored identical point mutations in *KRAS* (c.35G>T p.G12V) and *GNAS* (c.602G>A p.R201H), which were absent in the matched normal tissue. No additional clinically significant somatic mutations were identified in the panel.

The patient received a final diagnosis of LAMN metastatic to the ovaries. Because she had both ovarian tumors and the appendix removed at surgery, her disease was considered to have been optimally debulked and she began a period of close observation. She remains free from disease 15 months after surgery.

Discussion

Our case highlights the difficulty in distinguishing metastatic LAMN from concurrent primary mucinous ovarian tumors. Determining the tissue of origin in low-grade mucinous tumors involving the ovaries and appendix can be challenging. However, establishing the tissue of origin is important because metastatic LAMN may run an aggressive clinical course, while bilateral low-grade mucinous ovarian tumors and an incidental LAMN represent surgically curable disease.

These 2 scenarios often can be resolved on histologic grounds, but this is not always the case. LAMNs may be grossly unremarkable, with microscopic evidence of invasion limited to a subtle, pushing type of invasion with loss of the lamina propria and muscularis mucosae (Figure 1B) [10]. The presence of acellular mucin within the wall of the appendix also can be a helpful feature [11]. Ovarian tumors can display subtle histologic features which favor a diagnosis of metastatic LAMN.
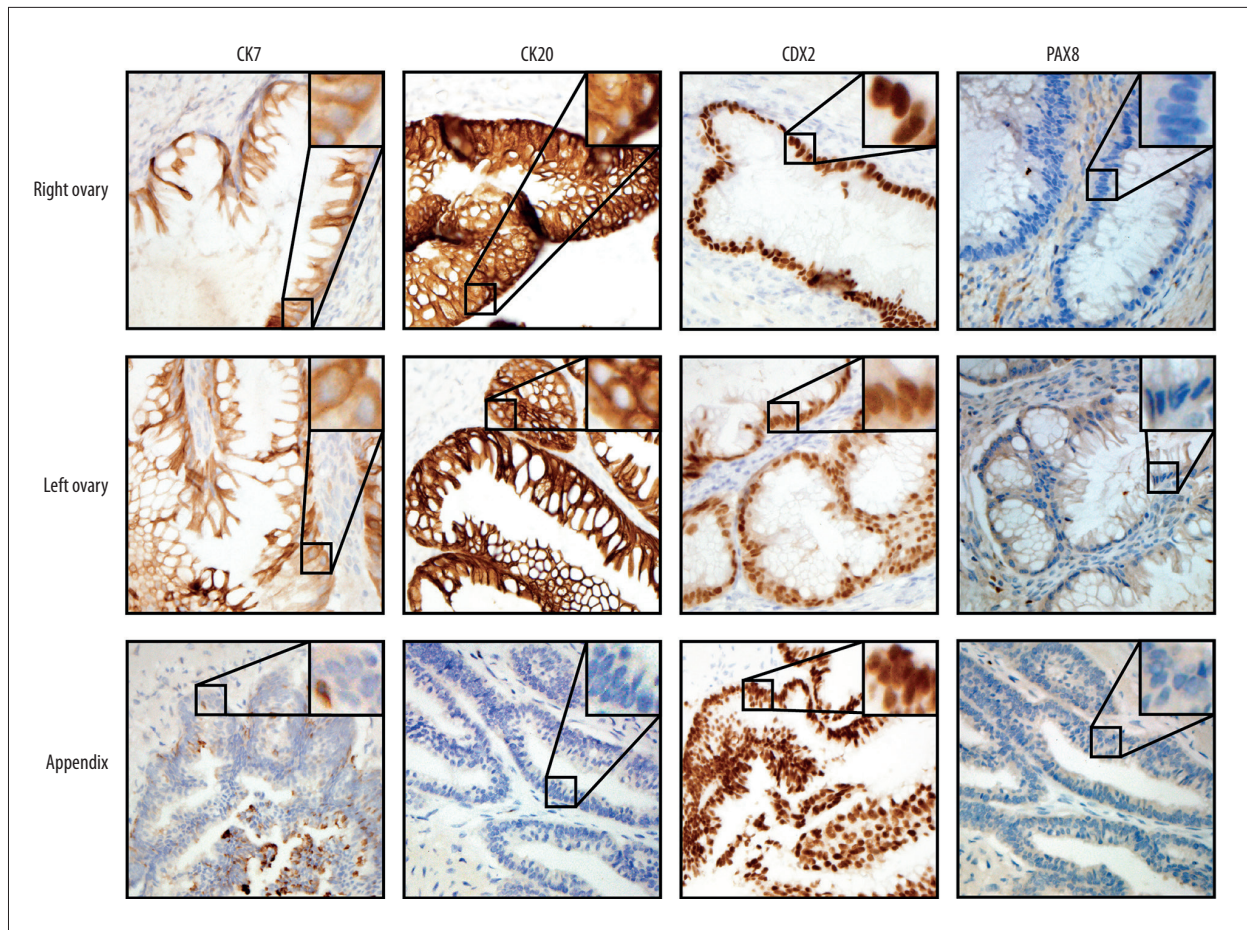
Figure 3. Representative images demonstrating the discordant immunohistochemical (IHC) staining pattern of the tumors in the appendix, left ovary, and right ovary. All tumors were positive for CDX2, while only the ovarian tumors were strongly and diffusely positive for CK7 and CK20; the appendix tumor showed only very focal staining for CK7 and CK20. All tumors were negative for PAX8. All images are DAB with hematoxylin counterstain. 600×.

These features include abundant pseudomyxoma ovarii without an exuberant inflammatory response (Figure 2A), prominent sub-epithelial clefting (Figure 2A), and a complex glandular architecture with tall mucinous cells (Figure 2B, 2C) [5]. Notably, overt mucinous ascites is not the only manifestation of pseudomyxoma peritonei (PMP), because ovarian involvement by LAMN also is a form of PMP [11]. While these features were present in our case, the prevailing opinion in our department was that the ovarian tumors represented bilateral primary mucinous borderline tumors and the minute appendiceal LAMN was an incidental finding. This idea was supported by the absence of overt mucinous ascites and discordant immunohistochemical staining between the tumors in the ovaries and the appendix (Figure 3).

In cases where 2 or more concurrent tumors are identified at different anatomic sites, DNA sequencing has been utilized to determine whether the masses represent metastatic disease or synchronous primary tumors [12–14]. We believe this approach

may have particular utility for low-grade tumors such as LAMNs which tend to be genetically stable. Our institution favors next-generation sequencing (NGS) for this purpose. However, since genetic variants associated with LAMN are limited primarily to several hot-spot regions in *KRAS* and *GNAS*, Sanger sequencing also would be a viable alternative. NGS is currently available at most academic medical centers and accessible to community hospitals through reference laboratories. To date, the expense of NGS has limited its use primarily to the molecular characterization of treatment-refractory tumors. In these cases, it often identifies actionable therapeutic targets and may help match patients with available clinical trials. However, as the cost of sequencing continues to fall, NGS will be viewed increasingly as a practical tool to resolve common diagnostic dilemmas in pathology rather than as an assay of last resort. Its utility will include identifying the tissue origin of tumors.

In the present case, we submitted samples of tumor from both ovaries, the appendix, and matched normal tissue for analysis

by our in-house cancer gene mutation panel. The NGS was completed at no cost to the patient and we did not seek reimbursement from the patient's insurance. The results identified clinically significant somatic point mutations in *KRAS* and *GNAS* in the appendix, which also were identified in both ovarian tumors. Both of these mutations are associated with oncogenesis. *KRAS* is a proto-oncogene encoding a GTPase that acts as a molecular switch to control cellular pathways of proliferation and differentiation [15]. Mutation of *KRAS* at G12 greatly reduces GTP hydrolysis, leading to constitutive *KRAS* activity [15]. *GNAS* encodes the stimulatory alpha subunit of G-protein-coupled receptors [16]. Mutation of *GNAS* at R201 has been reported to result in loss of GTPase activity, leading to constitutive downstream pathway activation [17].

LAMNs have related but demonstrably different genetic signatures compared to primary mucinous ovarian tumors. While most LAMNs are defined by co-mutation of *KRAS* and *GNAS*, primary mucinous ovarian tumors exhibit more varied genetics with mutations in multiple known tumor drivers [7,18,19]. In a series of 82 mucinous ovarian tumors, fewer than 3% contained

KRAS and *GNAS* co-mutations [7]. Thus, we propose that when identical *KRAS* and *GNAS* mutations are identified concurrently in a LAMN and ovarian mucinous tumor, the diagnosis of metastatic LAMN can be made with a high degree of certainty.

Conclusions

Careful attention to morphologic features can be coupled with the practical application of next-generation sequencing to resolve challenging cases involving concurrent appendiceal and ovarian mucinous tumors of unclear etiology. In our case, next-generation sequencing provided a rapid and practical solution to distinguish between metastatic disease and concurrent primary tumors.

Acknowledgements

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