A Case of Metastatic Malignant Breast Adenomyoepithelioma With a Codon-61 Mutation of *HRAS*

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Clinical Practice Points

- Malignant mammary adenomyoepithelioma is a rare subtype of breast cancer, the biological characteristics of which are largely unknown.
- A recent study showed that codon-61 mutations of V-Ha-Ras Harvey Rat Sarcoma Viral Oncogene Homolog (*HRAS*) are frequently associated with breast adenomyoepithelioma.
- We report the case of a 41-year-old premenopausal woman with metastatic malignant adenomyoepithelioma of the right breast harboring a Q61R mutation of *HRAS*.
- This is, to our knowledge, the first case of malignant breast adenomyoepithelioma with distant metastases found to harbor a codon-61 mutation of *HRAS*.
- Clinical sequencing might prove informative for diagnosis of malignant mammary adenomyoepithelioma.

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Introduction

Malignant mammary adenomyoepithelioma (epithelial-myoepithelial carcinoma) is a rare subtype of breast cancer characterized by dual composition of luminal and myoepithelial compartments.[1,2] Malignant adenomyoepithelioma is distinguished from benign adenomyoepithelioma by the presence of nuclear atypia, increased mitotic activity, and necrosis. Although its biological characteristics are largely unknown, recurrent mutations of V-Ha-Ras Harvey Rat Sarcoma Viral Oncogene Homolog *(HRAS)* and of genes related to the Phosphatidylinositol-3-Kinase and Protein Kinase B (PI3K-AKT) signaling pathway such as phosphatidylinositol 3-kinase, catalytic, alpha polypeptide (*PIK3CA)* and phosphatidylinositol 3-kinase, p85 alpha regulatory subunit (*PIK3R1)* were recently shown to be associated with breast adenomyoepithelioma.[3] Herein we describe the case of a 41-year-old woman with metastatic malignant breast adenomyoepithelioma, for which targeted next-generation sequencing (NGS) analysis revealed the presence of a Q61R mutation of *HRAS*.

Case

Routine screening of a 41-year-old premenopausal Japanese woman using breast ultrasonography revealed a mass with calcification in the right breast. A core needle biopsy was performed, and the tumor was suspected as being intraductal papilloma, which had been decided to be followed-up. A year later, the patient noticed a mass growing rapidly in her right breast, and a lumpectomy was undertaken. Three months after the lumpectomy, local recurrence was observed and mastectomy was performed. After another 9 months, recurrence became apparent in the subcutaneous tissue of the right chest wall and lumpectomy was again performed. Because of the repeated recurrence, contrast-enhanced computed tomography (CT) was performed and revealed multiple lung metastases. The patient was then referred to our hospital.

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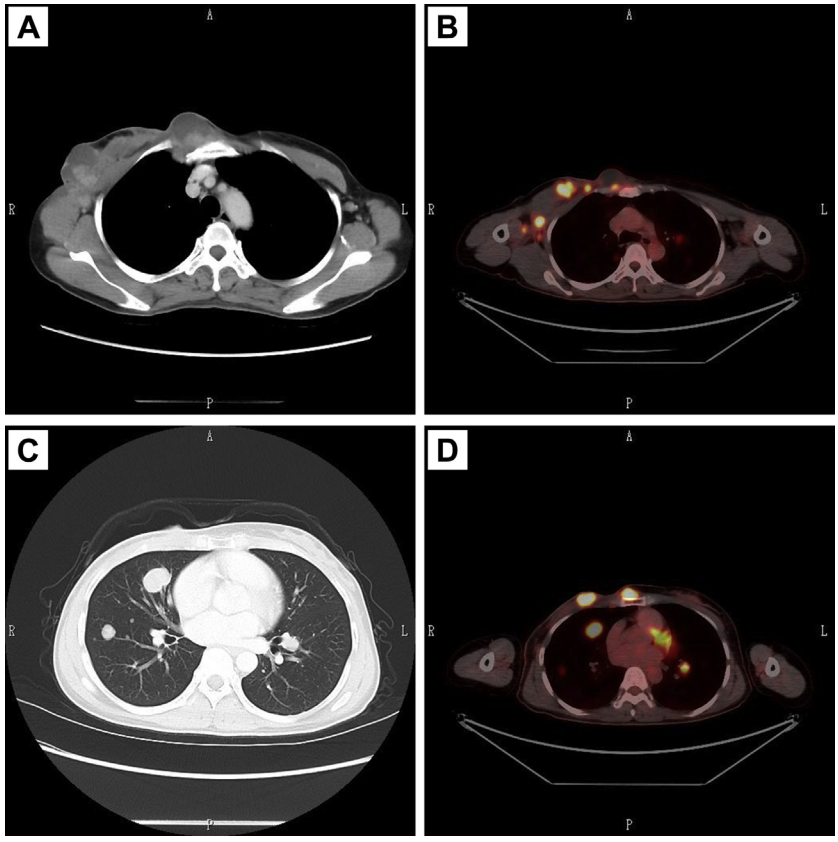
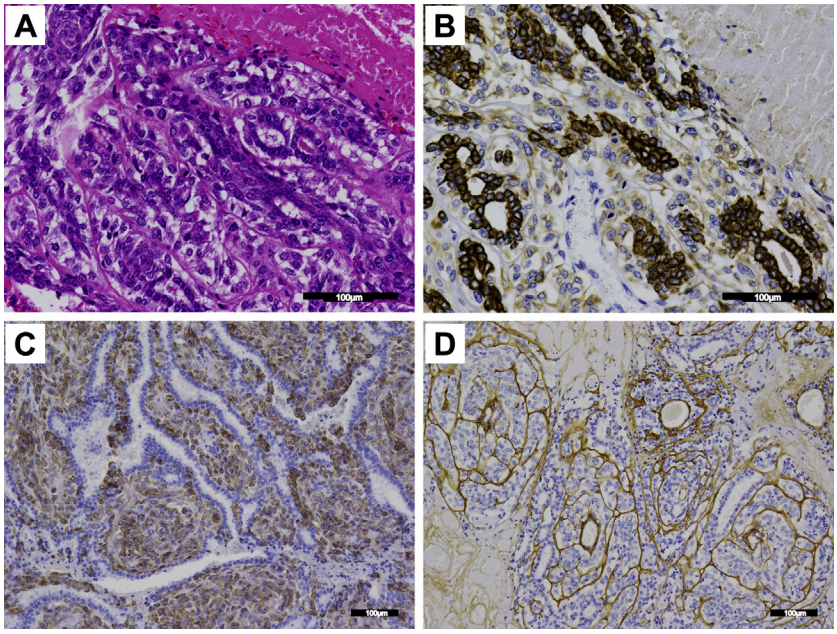
Figure 1 Chest Computed Tomography (CT) Scans (A) and (C) and Positron Emission Tomography-CT Scans With [^{18}F] fluorodeoxyglucose (B) and (D) Performed After the Patient Was Referred to Our Hospital



The CT and positron emission tomography-CT scans were performed in our hospital and showed multiple subcutaneous cystic recurrent tumors with fluid accumulation in the right chest, bilateral axial lymph node metastases, and multiple lung metastases, all of which manifested increased [^{18}F]fluorodeoxyglucose uptake (Figure 1). Pathological examination revealed that the primary tumor was a grossly well circumscribed nodular lesion, although microscopic examination revealed a histologically ragged margin. The tumor showed dual differentiation, better appreciated in immunohistochemistry. Cytokeratin (CK) 7-positive epithelial cell-lined tubules and compressed spaces were shown, whereas another population of cells positive for CK14, p63, CD10, and S100 surrounded or underlaid the CK7-positive cells (Figure 2A-C). The cytoplasm of this second population of cells was often clear, consistent with myoepithelial differentiation, and the presence of collagen IV-positive basement membrane material between these cells further supported this notion (Figure 2D). However, these cells did not stain with antibodies to α-smooth muscle actin or to calponin, as would be expected for myoepithelial cells. Some epithelium-lined spaces were cystically dilated, and the neoplastic cells were slightly pleomorphic. The mitotic count was 8 per 10 high-power fields, and areas of infarct or necrosis were present. Both populations of neoplastic cells were immunohistochemically negative for the estrogen receptor (ER), the progesterone receptor, and HER2. A small proportion of epithelial cells was positive for ER, which might have reflected entrapment of non-neoplastic ducts in the tumor. The recurrent tumors showed frank invasive growth and slightly more pronounced cytological atypia, with increased mitotic counts. Tubular architecture was more obscure, and CK7-positive epithelial cells lined collapsed slit-like spaces and cystically dilated spaces. The lymph node metastases also manifested biphasic differentiation. The pathological findings suggested a diagnosis of malignant adenomyoepithelioma, but the failure to detect immunohistochemical signals for smooth muscle antigens in any part of the tumor was deemed problematic for such a diagnosis.

We obtained informed consent from the patient for targeted NGS analysis with an Ion Ampliseq Cancer Hotspot Panel v2 (ThermoFisher Scientific, Waltham, MA). This analysis revealed the presence of a Q61R mutation of *HRAS*, which is uncommon in invasive ductal carcinoma but was recently shown to be associated with breast adenomyoepithelioma.[3] No other clinically relevant somatic variant was detected using NGS. On the basis of the combination of the histological and molecular evidence, we therefore made a diagnosis of malignant adenomyoepithelioma. The tumor was thus identified as metastatic triple-negative breast cancer, classified as malignant adenomyoepithelioma harboring the *HRAS*(Q61R) mutation. Genetic

Figure 2 Microscopic Findings of Malignant Adenomyoepithelioma. (A) Tubular Structures Composed of Epithelial Cells Were Surrounded by Cells That Showed Clear Cytoplasm, Suggestive of Myoepithelial Differentiation, as Well as Slight Nuclear Pleomorphism. Necrosis or Infarct Is Apparent at the Upper Right. Hematoxylin and Eosin Staining; Original Magnification ×200. (B) and (C) Immunohistochemical Staining Revealed That the Former Population of Cells Was Positive for CK7 (B) Whereas the Latter Was Positive for CK14 (C) as Well as for p63, CD10, and S100 (Not Shown). Original Magnification ×200 (B) or ×100 (C). (D) Immunohistochemical Staining for Collagen IV Revealed Deposition of Basement Membrane Material Between Cells, Suggestive of Myoepithelial Differentiation. Original Magnification ×100



testing using BRACAnalysis (Myriad Genetics, Salt Lake City, UT) did not detect any Breast Cancer 1 mutation (*BRCA1*) or Breast Cancer 2 (*BRCA2)* mutation.

The patient started first-line chemotherapy with a taxane-based trial regimen after providing written informed consent. After 2 cycles of the therapy, a CT scan revealed marked shrinkage of the multiple lung metastases, but the subcutaneous tumors had slightly increased in size. The response to the treatment was evaluated as stable disease according to Response Evaluation Criteria in Solid Tumors version 1.1 (RECIST). The patient completed 4 cycles of treatment, after which another CT scan revealed progression of the subcutaneous recurrent tumors, which was evaluated as progressive disease (RECIST). Palliative radiation therapy (40 Gy in 16 fractions) was administered for the subcutaneous tumors and resulted in a partial tumor reduction. Combination therapy with adriamycin (40 mg/m^2) and cyclophosphamide (500 mg/m^2) is planned as a second-line treatment.

Discussion

We presented a patient diagnosed with metastatic malignant adenomyoepithelioma of the breast for which NGS analysis revealed the presence of the Q61R mutation of *HRAS*. Malignant adenomyoepithelioma is a rare subtype of breast cancer that is classified under "epithelial-myoepithelial tumors" according to the fourth edition of the World Health Organization Classification of Tumors of the Breast.[4] Indeed, little is known about malignant adenomyoepithelioma because of its rarity.

Standard therapy for breast cancer according to its status for hormone receptors and HER2 is applied for malignant adenomyoepithelioma, but its response to chemotherapy is not known. A case of malignant adenomyoepithelioma was reported to respond well to multiple chemotherapy regimens including taxane-based chemotherapy, anthracycline, and eribulin.[5] Another case was reported to respond to imatinib, which is not routinely administered for breast cancer.[6] In the present case, taxane-based therapy did not result in a durable response and tumor progression was aggressive. However, radiotherapy induced regression of the subcutaneous tumors. Further studies are thus warranted to elucidate the best treatment regimen for malignant adenomyoepithelioma.

V-Ha-Ras Harvey Rat Sarcoma Viral Oncogene Homolog mutations are rare in breast cancer, being present in 0.62% of all breast tumors according to the Catalogue Of Somatic Mutations In Cancer v87 database (https://cancer.sanger.ac.uk/cosmic).[7] A recent study of 43 cases of mammary adenomyoepithelioma including some malignant cases identified recurrent mutations affecting codon-61 of *HRAS* and genes related to the PI3K-AKT signaling pathway.[3] All of the cases with an *HRAS* mutation at codon 61 (n =

8) were ER-negative and had a concomitant mutation in *PIK3CA* or *PIK3R1*. Mutations of *HRAS* were associated with aggressive clinical features and lymph node metastasis. Another case of early-stage breast cancer diagnosed as malignant adenomyoepithelioma harboring *HRAS* and *PIK3CA* mutations was also recently reported, but the *HRAS* mutation in this instance was G12D.[8] Our present study revealed a codon-61 mutation of *HRAS* in mammary malignant adenomyoepithelioma with distant metastases. No mutation was detected in *PIK3CA*, but the possibility of *PIK3R1* mutation positivity remains because this gene was not included in the NGS panel used in our analysis.

Because *HRAS* mutation is uncommon in breast cancer and adenomyoepithelioma often presents with *HRAS* mutation as discussed previously, the analysis of *HRAS* mutation would support the diagnosis of malignant adenomyoepithelioma.

Conclusion

To our knowledge, our study is the first to report a case of malignant mammary adenomyoepithelioma with distant metastases harboring a codon-61 mutation of *HRAS*. Because malignant adenomyoepithelioma is a rare histological subtype of breast cancer, its diagnosis might cause hesitation. However, the additional information provided by NGS analysis regarding the mutational activation of *HRAS* in the present case strengthened the basis for such a diagnosis. Our report thus supports the notion that mutational analysis of *HRAS* might help in the diagnosis of malignant adenomyoepithelioma. Further study is required to clarify the relation between codon-61 mutations of *HRAS* and the pathogenesis of malignant adenomyoepithelioma.

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Disclosure

The authors have stated that they have no conflicts of interest.

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