LETTER TO THE EDITOR

HRAS is a therapeutic target in malignant chemo-resistant adenomyoepithelioma of the breast

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Abstract

Malignant adenomyoepithelioma (AME) of the breast is an exceptionally rare form of breast cancer, with a significant metastatic potential. Chemotherapy has been used in the management of advanced AME patients, however the majority of treatments are not effective. Recent studies report recurrent mutations in the HRAS Q61 hotspot in small series of AMEs, but there are no preclinical or clinical data showing H-Ras protein as a potential therapeutic target in malignant AMEs. We performed targeted sequencing of tumours’ samples from new series of 13 AMEs, including 9 benign and 4 malignant forms. Samples from the breast tumour and the matched axillary metastasis of one malignant HRAS mutated AME were engrafted and two patient-derived xenografts (PDX) were established that reproduced the typical AME morphology. The metastasis-derived PDX was treated in vivo by different chemotherapies and a combination of MEK and BRAF inhibitors (trametinib and dabrafenib). All malignant AMEs presented a recurrent mutation in the HRAS G13R or G12S hotspot. Mutation of PIK3CA were found in both benign and malignant AMEs, while AKT1 mutations were restricted to benign AMEs. Treatment of the PDX by the MEK inhibitor trametinib, resulted in a marked anti-tumor activity, in contrast to the BRAF inhibitor and the different chemotherapies that were ineffective. Overall, these findings further expand on the genetic features of AMEs and suggest that patients carrying advanced HRAS-mutated AMEs could potentially be treated with MEK inhibitors.

Keywords: Adenomyoepithelioma, HRAS, PDX, MEK inhibitor

To the Editor,

Adenomyoepithelioma (AME) of the breast is a rare biphasic tumour of breast composed of epithelial and myoepithelial cells. It is generally a benign disease and cases of malignant AME are rare [1]. Importantly, however, metastases have been documented even in cases lacking a histologically overt malignant component [2]. The epithelial component may express estrogen receptor (ER) and progesterone receptor (PR) [1]. Given the rarity of the disease, most of the literature consists of individual case reports or studies with a few patients. A specific treatment for metastatic AME has not been determined, and the prognosis of malignant AME with distant metastases is very poor [3, 4].

In the present study we analyzed the mutational profile of 13 AMEs (9 benign and 4 malignant forms), whose histo-pathological characteristics are summarized in Table 1. These cases were diagnosed as AMEs based on the criteria defined by 2019 World Health Organization Classification of the Breast Tumours [5]. Nine AMEs (69%) expressed estrogen receptor (ER) and progesterone receptor (PR) [1]. The mutational
| Case | Age  | Size (mm) | Category     | Architecture                                      | Myoepithelial cells | Mitosis / mm² | Cytologic atypia | Necrosis          | Metaplasia/ Associated findings | HER2 | ER, PR | Follow-up (mo) | Recurrence/ metastasis | HRAS | PIK3CA | AKT1 |
|------|------|-----------|--------------|--------------------------------------------------|---------------------|---------------|------------------|------------------|-------------------------------|-------|--------|----------------|--------------------------------|------|--------|------|
| T1   | 64   | 9         | Benign       | Tubular                                          | Clear               | 0             | Mild             | Present          | Squamous, sebaceous             | 0     | ER+PR+ | NA             | NA                             | Q61R | w.t    | w.t |
| T2   | 53   | 15        | Benign       | Tubular                                          | Clear               | 1             | Mild             | Absent           | 0                             | ER+PR+ | NA     | NA             | w.t                             | H1047R | w.t    | w.t |
| T3   | 38   | 24        | Benign       | Tubular                                          | Clear               | 1             | Moderate         | Absent           | Squamous                      | 0     | ER+PR+ | 15            | No                              | NA   | w.t    | E17K|
| T4   | 62   | 15        | Benign       | Tubular                                          | Clear               | 1             | Mild             | Present          | 0                             | ER+PR+ | 36     | No             | w.t                             | w.t   | w.t    | w.t |
| T5   | 63   | 12        | Benign       | Tubular, lobulated, papillary, cystic            | Clear               | 1             | Mild             | Absent           | Squamous, chondroid and myxoid matrix | 0     | ER+PR- | 9              | No                              | w.t   | w.t    | E17K|
| T6   | 37   | 18        | Benign       | Tubular, lobulated, papillary, cystic            | Clear               | 1             | Mild             | Absent           | 0                             | ER+PR- | 91     | Recurrence     | w.t                             | w.t   | E17K   |      |
| T7   | 70   | 9         | Benign       | Tubular                                          | Clear               | 1             | Moderate         | Absent           | 0                             | ER+PR+ | 91     | Recurrence     | w.t                             | H1047R| w.t    |      |
| T8   | 36   | 20        | Benign       | Tubular                                          | Clear, spindle      | 0             | Mild             | Absent           | 0                             | ER-PR- | 6      | No             | w.t                             | H1047R| w.t    |      |
| T9   | 66   | 16        | Benign       | Tubular, lobulated                               | Clear               | 0             | Mild             | Absent           | 0                             | ER-PR- | 6      | No             | w.t                             | w.t   | w.t    |      |
| T10  | 84   | 25        | Malignant    | Tubular, lobulated                               | Clear               | 3             | Severe           | Present          | 0                             | ER+PR+ | 12     | Recurrence     | G13R                            | w.t   | w.t    |      |
| T11  | 76   | 18        | Malignant    | Tubular, lobulated                               | Clear               | 3             | Moderate         | Present          | 0                             | ER-PR- | NA     | NA             | G13R                            | H1047R| w.t    |      |
| T12  | 60   | 19        | Malignant    | Tubular, spindle, cystic                         | Clear, spindle      | 6             | Severe           | Present          | 0                             | ER-PR- | 75     | No             | G13R                            | H1047R| w.t    |      |
| T13  | 55   | 55        | Malignant    | Tubular                                          | Clear               | 10            | Severe           | Absent           | 0                             | ER+PR- | 11     | Metastasis     | G12S                            | w.t   | w.t    |      |

*ER* estrogen receptor, *PR* progesterone receptor, *NA* not available, *w.t* wild-type
analysis revealed recurrently mutated genes, including \(HRAS\) (5/13, 38%), \(PIK3CA\) (4/13, 31%), and \(AKT1\) (4/13, 31%) (Table 1). The \(HRAS\) mutations affected the following mutation hotspots: three p.G13R, one p.G12S and one p.Q61R hotspot mutations. Mutations in the \(AKT1\) gene (E17K) were exclusively found in benign ER+ AMEs, while three out of four \(PIK3CA\) mutations (H1047R) were detected in ER-negative AMEs. \(HRAS\) was mutated in the four malignant AMEs (three in the G13R and one in the G12S hotspots), suggesting that these mutation hotspots may represent important driver of malignant AMEs. To our knowledge, only one case of malignant AME mutated for the \(HRAS\) G12 hotspot was previously identified (G12D) [6]. The low frequency of \(HRAS\) Q61R/K mutation hotpsot was in agreement with two studies [6, 7], while a third study published by Geyer et al. reported recurrent mutations of the \(HRAS\) Q61R mutation [8].

Mutations in the \(AKT1\) and \(PIK3CA\) genes were mutual exclusive in our series, while 2 out of four malignant AMEs harboured mutations in both \(HRAS\) and \(PIK3CA\) genes. These findings are concordant with those previously reported [7, 8] and underline the co-occurrence of two cancer driver genes in a fraction of malignant AMEs.
From one of the four malignant AMEs patients (T13), whose clinical history is summarized in Fig. 1a, we could generate two PDX, HBCx-120 and HBCx-121, established from the engraftment of the breast tumour and the axillary lymph node metastasis, respectively. The histological analysis of xenografts tumors showed that tumor morphology and immunohistochemistry profile was concordant with patient's samples (Fig. 1b). Both patient's nodal metastasis and HBCx-121 PDX show loss of ER expression, as compared to the matched breast tumour and HBCx-120 PDX. This phenotypic discordance between the primary tumor and the metastasis is frequent in breast cancer progression and metastasis, is generally associated to a worse survival and could be a consequence of intra-tumour heterogeneity and sub-clonal evolution of ER negative cells in the nodal metastasis [9, 10].

Patient's tumour samples including the two mastectomies (partial and total), the lymph node and the lung metastasis, and PDX samples carried the HRAS p.Gly12Ser mutation hotspot. As HRAS mutations are associated to activation of RAF/MEK/ERK signaling in different cancers [11], we treated the PDX HBCx-121 by a combination of dabrafenib (a RAF inhibitor) and trametinib (a MEK1/2 inhibitor). In parallel, we determined the response to different chemotherapies: AC (Adriamycin and cyclophosphamide), capecitabine and eribulin, three standard of care currently used for breast cancer treatment. PDX HBCx-121 responded with stable disease to trametinib (tumour growth inhibition of 82%), while dabrafenib had no effect on tumor growth (Fig. 1c). The combination of trametinib with dabrafenib did not increase the anti-tumour activity, suggesting that the combination effects are mediated by the MEK inhibitor. The PDX was resistant to the three chemotherapies tested.

To our knowledge, there are no clinical nor preclinical evidence showing that patients or PDX models of HRAS mutated AMEs could respond to MEK inhibitors. Trametinib as a single-agent is approved for the treatment for metastatic melanoma in patients with BRAF V600E or V600K mutations [12]. Inhibition of MAPK and P-AKT signaling pathways in treated tumours was analysed by Western Blot (Fig. 1d). Phospho-p44/42 MAPK (Erk1/2) was strongly inhibited in the combination group, while in trametinib-treated tumours the inhibition was heterogeneous among the different xenografts. In tumours treated by the combination, expression of P-AKT was strongly inhibited and expression of P-S6, the downstream effector of the PI3K/AKT/mTOR pathway, was decreased. This indicates that targeting the MAPK pathway with inhibitors that act at different levels, leads to a more profound inhibition of both P-ERK and P-AKT pathways, although this was not associated to increased anti-tumour activity.

In summary, we report a new series of AMEs showing recurrent mutations in the HRAS G12 and G13 hotspots. The treatment of a HRAS-mutated AME PDX with a FDA-approved MEK inhibitor (trametinib) exhibited significant anti-tumour activity, demonstrating that HRAS mutation is a therapeutic target in malignant AMEs. MEK inhibitors could be an important new approach for the treatment of HRAS mutated AMEs patients.

**Supplementary Information**
The online version contains supplementary material available at https://doi.org/10.1186/s13045-021-01158-3.

**Additional file 1.** Material and Methods and References.

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**Authors' contributions**
IB and EMa supervised the study and wrote the manuscript. FC and SV analysed and interpreted the NGS data. ML, AVS and CM selected the AME tumors and interpreted morphological and IHC datas. AD, EMo and IHC performed the molecular analysis of the PDX. FC and FR treated the patients and provided clinical data. All authors read and approved the final manuscript.

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**Availability of data and materials**
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

**Declarations**
**Ethics approval and consent to participate**
All patients gave their consent for the use of their samples for research purposes, by signing an informed consent form. The establishment of PDX and the preclinical experiments were performed in accordance with institutional guidelines and the rules of the French Ethics Committee (project Authorization No. 02163.02).

**Consent for publication**
Not applicable.

**Competing interests**
The authors declare that they have no competing interests.
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