

HRAS Mutations in Epithelial–Myoepithelial Carcinoma

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Abstract The molecular profile of epithelial–myoepithelial carcinomas (EMCa) has not been well studied, though a recent association with Harvey rat sarcoma viral oncogene homolog (*HRAS*) mutations has been noted. To confirm and validate this, we surveyed fifteen EMCa for *HRAS* codon 61 mutations and correlated *HRAS* status with clinicopathologic parameters. There were 11 females and 4 males and mean patient age was 64 (range 49–90). Parotid gland was most commonly involved ($n = 10$) and the most common histologic appearance was that of a ‘classic’ EMCa (7/15). Four of fifteen (26.7 %) cases demonstrated local recurrence, while 2/15 (13.3 %) demonstrated distant metastases. Other variant morphologies included EMCa arising from pleomorphic adenoma (3/15), and high grade EMCa (2/15). *HRAS* exon 3, codon 61 mutations, p.Q61R ($n = 3$) and p.Q61 K ($n = 1$) were identified in 4 of 15 successfully tested EMCAs (14 patients). Two cases were classic type, while the other cases consisted of one oncogenic variant, and one tumor with myoepithelial overgrowth, the latter of which showed the same mutation in both the primary and recurrence. Of note, the high grade EMCa and EMCa ex pleomorphic adenoma were negative for mutations. Given the small number of cases, there were no significant differences between mutation positive and mutation negative cases in terms of age, gender and outcome.

Keywords Epithelial–myoepithelial carcinoma · *HRAS* · Mutation · Oncogenic variant

Introduction

RAS proteins are encoded by three proto-oncogenes: *HRAS*, *KRAS*, and *NRAS* for which activating mutations were implicated in many human tumor types. The relative frequency of the three mutant isoforms as well as the distribution of codons involved varies considerably by organ site and tumor type. For instance, while *KRAS* mutations predominate in lung and colon adenocarcinomas, *HRAS* (Harvey rat sarcoma viral oncogene homolog) mutations are overrepresented in salivary gland tumors as per the COSMIC dataset [1] and present in about 15 % of salivary cancers as a whole [1]. *HRAS* has been implicated in salivary tumorigenesis as early as the 1990s: transgenic mice expressing a *HRAS* p.G12 V mutation developed “adenosquamous” carcinomas of submandibular glands [2]. Since then a variety of common tumor types including pleomorphic adenoma (PA), carcinoma ex pleomorphic adenoma (CAxPA), mucoepidermoid carcinoma, and adenocarcinoma not otherwise specified have been reported to have *HRAS* mutations or protein (p21) overexpression in as many as 1 out of 3 tumors [3–7]. Given the relative frequency of *HRAS* mutations in common tumor types, particularly pleomorphic adenoma and its transformed malignant counterpart, applicability of *HRAS* mutational analysis in less common tumor types may provide insights into their molecular pathogenesis.

Among these rarer tumor types is epithelial–myoepithelial carcinoma (EMCa), a low grade biphasic salivary gland malignancy initially described by Donath et al. [8]. This tumor often has a deceptively bland appearance and in

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many ways resembles PA, though it is rather rare comprising ~2 % of salivary gland malignancies. In some cases EMCa is the actual carcinoma type arising from PA in CAXPA. To date, the tumorigenesis for this rare carcinoma is unclear and there is very little in the literature regarding relevant genetic alterations. However, recently, up to 33 % rate of *HRAS* codon 61 (exon 3) mutations in EMCa was suggested in a preliminary report by Cros et al. [4]. Thus, in order to confirm/validate these findings, we herein survey fifteen EMCa for *HRAS* codon 61 mutations and correlate *HRAS* status with clinicopathologic parameters.

Methods

Case Selection

This study was approved by our Institutional Review Board (IRB# PRO07050360). Sixteen EMCa in fifteen patients (1980–2013) had sufficient and available formalin fixed paraffin embedded tissue for mutational analysis, and were included in the study. Clinicopathologic parameters were reviewed. The parameters for cases 1–10 were previously reported [9, 10], but follow-up was subsequently updated for this study. Tumors were categorized as follows: classic, ex pleomorphic adenoma, high grade, with myoepithelial overgrowth, oncocytic and apocrine variants. Classic tumors fulfilled the WHO 2005 requirement for having a clear cell myoepithelial component [11]. EMCa ex pleomorphic adenoma, EMCa with myoepithelial overgrowth, oncocytic and apocrine variants fulfilled criteria previously described [9, 10]. High grade transformation of either myoepithelial or epithelial components qualified as high grade for this study [12]. Tumors were staged according to 7th edition AJCC rules.

HRAS Mutation Analysis

Tissue cores from tumor targets were obtained as previously described [13]. DNA was isolated from tissue cores using the DN easy tissue kit (Qiagen, Valencia, CA) according to the manufacturer's instructions. For the detection of mutations, DNA was amplified with primers flanking exon 3 of the *HRAS* gene (forward primer 5'-GTC CTC CTG CAG GAT TCC TA-3' and reverse primer 5'-CGG GGT TCA CCT GTA CT-3'). PCR products were sequenced in both sense and antisense directions using the BigDye Terminator v3.1 cycle sequencing kit on ABI 3730 (Applied Biosystems, Inc., Foster City, CA) according to the manufacturer's instructions. The sequences were

analyzed using Mutation Surveyor software (SoftGenetics, LLC., State College, PA).

Results

Clinicopathologic parameters and mutational status are summarized in Table 1. This set of tumors had a female predilection (11:4), and mean patient age was 64 (range 49–90). Site distribution was as follows: Parotid—10, Submandibular—2, Maxillary—2, Lung—1. The mean tumor size was 3 cm (range 0.8–6.5 cm). The most common histologic appearance was that of a 'classic' EMCa (7/15). Other variant morphologies included EMCa arising from pleomorphic adenoma (3/15), high grade EMCa (2/15), oncocytic EMCa (1/15), apocrine EMCa (1/15), and EMCa with myoepithelial overgrowth (1/15). All cases were node negative. Four of fifteen (26.7 %) cases demonstrated local recurrence, while 2/15 (13.3 %) demonstrated distant metastases. Overall, four patients had died, but only one patient specifically died of disease, within a month of diagnosis. Mean follow up on patients without recurrence or death from disease was 4.6 years.

Of the tumors tested, twelve were primary, while four were recurrences. One recurrence was paired to a primary. One case failed, and *HRAS* exon 3, codon 61—p.Q61R c.182A>G and p.Q61 K, c.183C>A mutations were identified in 4 of 15 successfully tested EMCAs (thus in 4/14 patients) (Fig. 1). Two cases were classic type (Fig. 2a), while the other cases consisted of one oncocytic variant (Fig. 2b), and one tumor with myoepithelial overgrowth (Fig. 2c) the latter of which shows the same mutation in both the primary and recurrence. Of note, the high grade EMCa and EMCa ex pleomorphic adenoma were negative for mutations. Given the small number of cases, there were no significant differences between mutation positive and mutation negative cases in terms of age, gender and outcome.

Discussion

Our findings validate the prior abstract report [4] on the frequency of *HRAS* mutations in EMCa and provide further clinicopathologic correlates. Almost 1/3 of our cases are positive for the mutation thus adding to the list of salivary gland tumors reported to show *HRAS* mutations. However, the significance of this finding is not particularly clear in the context of EMCa given the small number of cases. As the initial findings by Cros et al. [4] were reported in abstract form, detailed features of the EMCa in that study are not available for comment. Aside from the small size of this

Table 1 Clinicopathologic parameters and mutation status

Case	Gender	Age	Case type	Site	Size (cm)	Histologic subtype	Nodal status	HRAS mutation status	HRAS mutation type	TNM stage	Recurrence	Distant metastasis	Site of metastasis	DFS (years)	OS (years)	Status
1	F	61	Primary	Parotid	0.8	Classic		Negative		T1Nx	N	N		6.7	6.7	DOC
2	F	55	Primary	Submandibular	2.8	High grade		Negative		T3NxM1	N	Y	Pelvic	0.1	0.1	DOD
3	F	66	Primary	Parotid	2	Classic		Positive	p.Q61R	T1N0	N				9.5	DOC
4	M	90	Primary	Parotid	2.4	Classic	Negative	Positive	p.Q61R	T2N0	N	N		5.4	5.4	DOC
5	F	62	Recurrence	Parotid	6.5	Classic		Negative		T3Nx	Y					LTF
6	F	71	Recurrence	Maxillary		Classic		Negative		TxNx	Y	N		1.0	7.3	Alive
7	M	78	Primary	Parotid	2.2	Classic	Negative	Failed		T2N0	N	N		7.4	7.4	Alive
8	F	51	Primary	Parotid	4.7	Ex pleomorphic adenoma		Negative		T3Nx	N	N		1.1	1.1	Alive
9	F	82	Primary	Parotid	4.3	Oncocytic		Positive	p.Q61 K	T3Nx	N	N		8.5	8.5	Alive
10	M	49	Primary	Parotid	3	Apocrine		Negative		T2Nx	N	N		6.0	6.0	Alive
11	M	61	Recurrence	Lung	3.5	High grade	Negative	Negative		T2N0M1	Y	Y	Brain	2.7	4.6	Alive
12	F	59	Primary	Parotid	1.2	Ex pleomorphic Adenoma		Negative		T1Nx	N	N		0.3	0.3	Alive
13	F	68	Primary and recurrence	Submandibular	2.7	Myoepithelial overgrowth	Negative	Positive	p.Q61R	T2N0	Y	N		1.2	1.2	Alive
14	F	58	Primary	Maxillary	4.1	Classic	Negative	Negative		T3Nx	N	N		1.2	1.2	Alive
15	F	55	Primary	Parotid	1.5	Ex pleomorphic adenoma	Negative	Negative		T1Nx	N	N		0.1	0.1	Alive

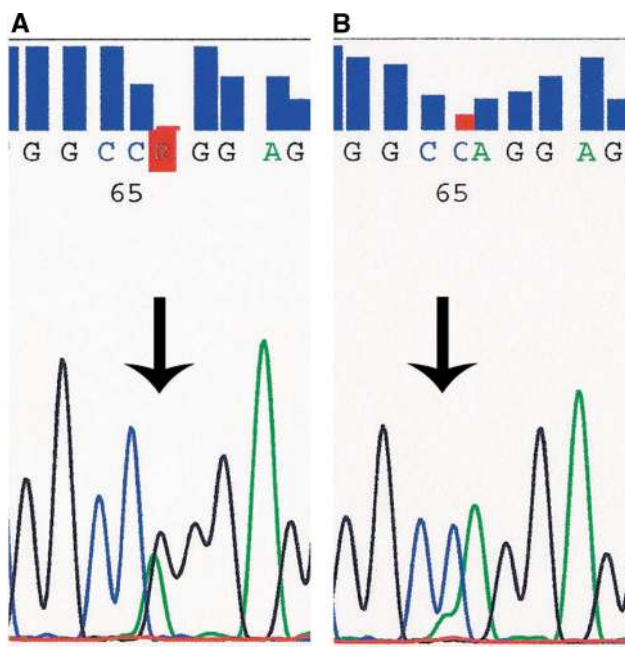


Fig. 1 Segments of exon 3 *HRAS* sequencing electropherogram, forward. **a** Arrow indicates c.182A>G substitution, resulting in p.Q61R missense heterozygous mutation. **b** Arrow indicates c.181C>A substitution, resulting in p.Q61 K missense heterozygous mutation

study, another limitation is the restriction of our evaluation of exon 3 codon 61 of *HRAS*. This limit was based on testing availability, but it is plausible that other EMCa may harbor *HRAS* mutations in exon 2, codons 12 or 13.

In some salivary carcinoma types, *HRAS* mutations may be linked to tumor progression. For instance, the *HRAS* experience in mucoepidermoid carcinomas (MEC) [14, 15] suggests that, in MEC, the frequency of *HRAS* mutations was greatest in high grade tumors, implying a role in progression. Parenthetically, this draws parallels to the

transgenic *HRAS* p.G12 V murine models showing ‘adenosquamous’ carcinomas. Similarly, Halteren et al. [16] reported 3 cases of adenocarcinomas of the parotid gland, not otherwise specified, with *HRAS* p.Q61R (of 17 adenocarcinomas and 2 pleomorphic adenomas). Of note, adenocarcinomas with *HRAS* mutation were moderately-to-poorly differentiated, with regional lymph node metastases and perineural invasion.

However, our findings do not seem to show a link to aggressiveness in EMCa. Though only four cases showed mutation, we have found *HRAS* mutations in classic, indolent and aggressive variants of EMCa, and have found no correlation with outcome measures. Additionally, both the primary and paired recurrence in one case harbored a mutation, suggesting the mutation to be an early event. Furthermore, one lethal EMCa showed no mutation. It appears that these alterations do not necessarily portend a more aggressive course.

Interestingly, this is somewhat similar to the cumulative findings in pleomorphic adenomas and carcinoma ex pleomorphic adenoma. For instance, while *HRAS* protein was shown to be overexpressed (admittedly not entirely analogous) in carcinomas arising in pleomorphic adenomas [5, 6], Milasin et al. [3] reported that 35 % (6/17) of pleomorphic adenomas of salivary glands harbored exon 2 codon 12 *HRAS* mutation suggesting that *HRAS* mutations or overexpression are not necessarily required for malignant transformation. Of note, we had 3 EMCa ex PA, and none showed transformation.

Thus in summary, we confirm that a subset of EMCa demonstrate *HRAS* codon 61 mutations. These mutations seem to be dispersed across classic and variant morphologies with no correlation with outcome measures based on this small series, suggesting that it does not necessarily denote an aggressive phenotype.

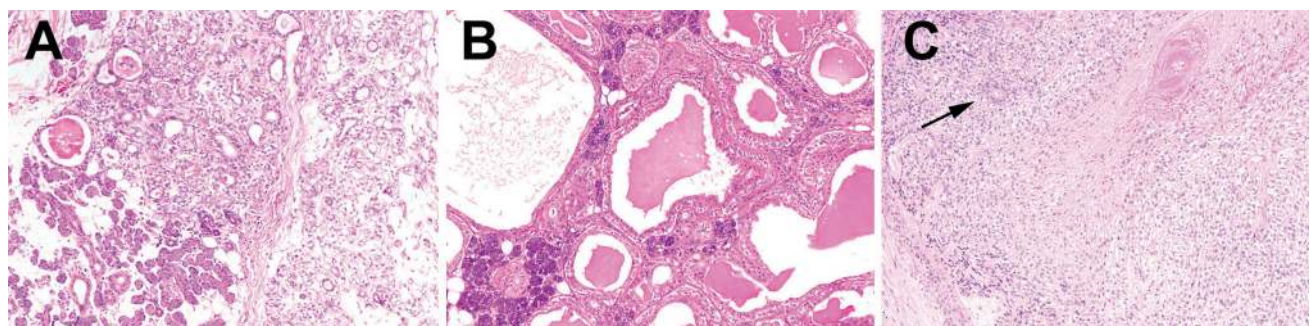


Fig. 2 Morphologic subtypes of EMCa with *HRAS* mutations. **a** This classic EMCa from the parotid (100×) harbored p.Q61R mutation. **b** This oncocytic EMCa (100×) of parotid on the other hand showed a p.Q61 K mutation. **c** This submandibular tumor with a p.Q61 K

mutation is an aggressive tumor showing myoepithelial overgrowth and necrosis (100×). Arrow indicates the rare compressed ductal component

Conflict of interest None.

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