Modern Pathology (2011) 24, 974–982

A potential role for targeted therapy in a subset of metastasizing adnexal carcinomas

Dora Dias-Santagata[1], Quynh Lam[1], Kristin Bergethon[1], Gabrielle M Baker[1], A John Iafrate[1], Dinesh Rakheja[2] and Mai P Hoang[1]

[1]*Department of Pathology, Massachusetts General Hospital and Harvard Medical School, Boston, MA, USA and* [2]*Department of Pathology, University of Texas Southwestern Medical School and Children's Medical Center, Dallas, Texas, USA*

Metastasizing adnexal carcinomas are rare; thus, currently there is no uniform treatment guideline. Chemotherapeutic drugs that selectively target cancer-promoting pathways may complement conventional therapeutic approaches. We performed immunohistochemistry (epidermal growth factor receptor (EGFR), HER2, and CD117), *EGFR* and *ERBB2* fluorescence *in situ* hybridization (FISH), and multiplexed SNaPshot® genotyping (testing for recurrent mutations in 15 cancer genes including *BRAF*, *EGFR*, *KRAS, PIK3CA*, and *TP53*) on primary tumors and corresponding metastases of 14 metastasizing adnexal carcinomas (three apocrine, six eccrine, two hidradenocarcinomas, two porocarcinomas, and one aggressive digital papillary adenocarcinoma). Metastasis to regional lymph node was most common, followed by skin and then lungs. Follow-up was available in 12 patients (5 months to 8 years) with 1 died of widespread metastases. Although EGFR overexpression was a prevalent feature in this cohort, seen in 7/11 (64%) primary tumors and 10/14 (71%) metastases; FISH for *EGFR* gene amplification was negative in 9 tested primary tumors and 12 metastases. FISH of the one primary tumor and three metastases with 2+ HER2 overexpression revealed a low level of *ERBB2* gene amplification in one apocrine carcinoma and corresponding metastasis. CD117 expression was seen only in rare cases. *PIK3CA* (2/12, 17%) and *TP53* (3/12, 25%) mutations were detected in two (one hidradenocarcinoma, one porocarcinoma) and three (one eccrine, one hidradenocarcinoma, and one aggressive digital papillary adenocarcinoma) cases, respectively. The role of EGFR inhibitor therapy in metastasizing adnexal carcinomas with protein overexpression remains unclear. Targeted therapy including PI3K pathway inhibitors might be a potential treatment for rare cases of adnexal carcinomas with metastases.
Modern Pathology (2011) **24,** 974–982; doi:10.1038/modpathol.2011.48; published online 18 March 2011

Keywords: adnexal neoplasm; *EGFR*; gene mutation; metastasis; *PIK3CA*; *TP53*

Adnexal carcinomas are rare and associated with poor prognosis. Currently, there is no uniform guideline concerning their treatment, especially for those with metastases. Wide surgical excision remains the treatment of choice; but treatment success has been documented only in isolated case reports.[1–3] Three of nine cases of clear cell eccrine carcinomas reported by Wong *et al*[4] developed metastases despite local excision, radiation, and chemotherapy.

Targeted therapy may be a potential treatment option in patients whose tumors are characterized by a relevant oncogene mutation. In a growing number of tumor types including breast and colorectal and lung cancer, selective agents that target critical cancer-promoting pathways are now the treatment of choice for those patients carrying the genetic changes recognized by the drugs.[4–7]

ERBB2 (v-erb-b2 erythroblastic leukemia viral oncogene homolog 2) gene amplification and response to trastuzumab were documented in a case of metastasizing hidradenocarcinoma.[2] Members of the ERBB receptor tyrosine kinase family, including epidermal growth factor receptor (EGFR), HER2, HER3, and HER4, present possible targeted therapeutic options.[8] The membranous expression of

Correspondence: Dr MP Hoang, MD, Dermatopathology Unit, Massachusetts General Hospital, 55 Fruit Street, Warren 820, Boston, MA 02114, USA.
E-mail: mhoang@partners.org
The results have been presented in part at the 31st Symposium of the International Society of Dermatopathology, Barcelona, Spain on October 2010.
Received 16 November 2010; revised 3 January 2011; accepted 4 January 2011; published online 18 March 2011

these markers has therapeutic implications and second-generation EGFR tyrosine kinase inhibitors such as HKI-272, XL647, and BIBW2992 have dual activity, inhibiting both EGFR and HER2 receptors.[9,10]

Another case of metastasizing clear cell hidradenocarcinoma was reported to be stabilized over 8 months with Sunitinib treatment, an oral multitargeted tyrosine kinase inhibitor.[3] KIT (CD117) is known to be expressed in normal adnexal tissue.[11] Moreover, *KIT*-activating (v-kit Hardy–Zuckerman 4 feline sarcoma viral oncogene homolog) mutations are reported to be associated with a variety of malignant neoplasms and specific chemotherapeutic agents (imatinib and sorafenib) are available.[12,13] Clinical experience in patients with gastrointestinal stromal tumor indicates that the presence and location of specific *KIT* mutations can predict sensitivity and resistance patterns to KIT kinase inhibitors.[14]

To investigate whether metastasizing adnexal carcinomas possess activation of oncogenic pathways that can be targeted by available chemotherapeutic agent, we performed immunohistochemistry for EGFR, HER2, and CD117 (KIT) expression; fluorescence *in situ* hybridization (FISH) for *EGFR* and *ERBB2* gene amplification; and molecular analyses for recurrent mutations in 15 cancer genes including *BRAF*, *EGFR*, *KRAS, PIK3CA*, and *TP53* on cases of adnexal carcinomas with metastases.[15]

Materials and methods

The study has been approved by the Massachusetts General Hospital institutional review board (IRB No. 2009P001738). Archival materials of all metastasizing adnexal carcinomas including aggressive digital papillary adenocarcinoma, apocrine carcinomas, eccrine ductal carcinoma, hidradenocarcinoma, and porocarcinoma, diagnosed between 1987 and 2010 were retrieved from the pathology files of the Massachusetts General Hospital, Boston, MA, USA. Age, gender, tumor site, tumor size, and clinical follow-up information (such as local recurrence or metastasis) were extracted from the patients' medical records. All patient data were de-identified. The histological sections of all cases were re-examined and the diagnoses were confirmed.

Immunohistochemistry

Immunohistochemical studies were performed on 5-μm-thick sections of formalin-fixed, paraffin-embedded tissue, using the standard techniques, heat-induced epitope retrieval buffer, and primary antibodies against CD117 (1:200, Dakocytomation, Carpinteria, CA, USA), EGFR (3C6, 1:2, Ventana Medical Systems, Tucson, AZ, USA), and HER2 (4B5, predilute, Ventana Medical Systems). Appropriate positive and negative controls were included.

Evaluation of the membranous EGFR expression was performed using a combined scoring system based on both the staining intensity (0 = no staining, 1 = weak, 2 = moderate, and 3 = strong staining) as well as the percentage of positive cells (0% = 0, <25% = 1, 26–50% = 2, 51–75% = 3, and >75% = 4), similar to that outlined by Janisson-Dargaud *et al*.[16] The sum of these two scores yielded a total score from 0 to 7 (1–3 = weak and 4–7 = strong).

Overexpression of HER2 was defined as positive membranous staining in >10% of the neoplastic cells. Partial and faint, weak or thin, and intense or thick circumferential membrane staining in >10% of the tumor cells were scored as 1+ (negative), 2+ (equivocal), and 3+ (positive), respectively. Evaluation of CD117-positive cells were recorded as <10% = negative, 10–50% = 1+, 51–75% = 2+, and >75% = 3+.[17]

Mutational Analysis and *EGFR* and *ERBB2* FISH

A SNaPshot® genotyping assay recently developed by our group was performed on primary tumors and corresponding metastases of 14 metastasizing adnexal carcinomas with available archival materials.[15] This assay consists of multiplexed PCR followed by a single-base extension reaction and uses the commercially available SNaPshot platform (Applied Biosystems). The original tumor genotyping panel described by Dias-Santagata *et al*[15] was expanded to include three additional assays (AKT1.49, testing for the *AKT1* E17K mutation; IDH1.394 and IDH1.395, testing for hotspot mutations in *IDH1*, which affect codon R132). The full panel and tests for common mutations in 15 cancer genes are outlined in Table 1. SNaPshot® genotyping was performed using previously described conditions,[15] and included the following additional primers for *AKT1* and *IDH1* (PCR: *AKT1* exon 3 Forward, 5′-ACGTTGGATGGGTAGAGTGTGCGTGG CTCT-3′; *AKT1* exon 3 Reverse, 5′-ACGTTGGATGA GGTGCCATCATTCTTGAGG-3′; *IDH1* exon 4 Forward, 5′-ACGTTGGATGGGCTTGTGAGTGGATGGGTA-3′, *IDH1* exon 4 Reverse 5′-ACGTTGGATGgcaaaatcaca ttattgccaac-3′. Extension: *AKT1*.49 extR 5′-CTGACT GACTGACTGACTGACTGACTGACTGACTGACTG ACTGACTGACTGACTGACTGACTGACTGACTGA GCCAGGTCTTGATGTACT-3′*IDH1*.394 extR 5′-GA CTGACTGGACTGACTGACTGACTGACTGGACTG ACTGACTGAGATCCCCATAAGCATGAC-3′, *IDH1*.395 extR 5′-TGATCCCCATAAGCATGA-3′). *EGFR* gene copy number was assessed in 9 primary tumors and 12 metastases by FISH as previously published.[18] *ERBB2* gene copy number was also assessed in four cases with HER2 IHC overexpression.

Results

A total of 14 cases were identified: 3 apocrine carcinomas, 6 eccrine ductal carcinomas, 2 hidradeno-

Table 1 SNaPshot® mutational assays[15]

Gene	*Amino acid–cDNA residue*	*Gene*	*Amino acid–cDNA residue*
v-akt murine thymoma viral oncogene homolog1 (AKT1)	E17–49G	NOTCH1	L1575–4724T
Adenomatous polyposis coli (APC)	R1114–3340C		L1601–4802T
	Q1338–4012C	Neuroblastoma RAS viral (v-ras)	G12–34G
	R1450–4348C	oncogene homolog (NRAS)	G12–35G
	T1556fs–4666_4667insA		G13–37G
v-raf murine sarcoma viral oncogene homolog B1 (BRAF)	V600–1798G		G13–38G
	V600–1799T		Q61–181C
Catenin (cadherin-associated protein), β 1, 88 kDa (CTNNB1)	D32–94G		Q61–182A
	D32–95A		Q61–183A
	S33–98C	Phosphoinositide-3-kinase,	R88–263G
	G34–101G	catalytic, α polypeptide (PIK3CA)	E542–1624G
	S37–109T		E545–1633G
	S37–110C		Q546–1636C
	T41–121A		Q546–1637A
	T41–122C		H1047–3139C
	S45–133T		H1047–3140A
	S45–134C		G1049–3145G
Epidermal growth factor receptor (EGFR)	G719–2155G	Phosphatase and tensin homolog	R130–388C
	T790–2369C	(PTEN)	R173–517C
	L858–2573T		R233–697C
	E746_A750–2235_2249del		K267fs–800delA
	E746_A750–2235_2250del	Tumor protein 53 (TP53)	R175–524G
Isocitrate dehydrogenase 1 (NADP+), soluble (IDH1)	R132–394C		G245–733G
	R132–395G		R248–742C
Fms-related tyrosine kinase 3 (FLT3)	D835–2503G		R248–743G
Janus kinase 3 (JAK2)	V617–1849G		R273–817C
v-kit Hardy–Zuckerman 4 feline sarcoma viral oncogene (KIT)	D816–2447A		R273–818G
v-Ki-ras2 Kirsten rat sarcoma	G12–34G		R306–916C
viral oncogene homolog (KRAS)	G12–35G		
	G13–37G		
	G13–38G		

carcinomas, 2 porocarcinomas, and 1 aggressive digital papillary adenocarcinoma (Table 2). The age of the patients ranged from 38 to 85 years (median, 68.5 years). The male to female ratio was 6:1. The majority of the patients received excision and regional lymph node dissection. Three patients received chemotherapy and one received radiotherapy. One patient received both radiotherapy and chemotherapy. Follow-up was available in 12 patients ranging from 5 months to 8 years (Table 2). Recurrences developed in two patients. Metastasis to regional lymph node is most commonly observed. Widespread metastases were observed in two patients that resulted in death in one patient, 7 years status post diagnosis. Metastases to both lymph node and skin were observed in four cases. Metastases to both lymph node and lung were observed in two cases.

Immunohistochemistry

Immunohistochemistry was performed on 11 primary tumors and 14 metastases. The immunohistochemical results are summarized in Table 3. Overexpression of EGFR was seen in 7/11 (64%) primary tumors and 10/14 (71%) metastases (Figure 1a–c). All cases exhibited strong membranous EGFR expression (Figure 1c). Overexpression of HER2 (2+) was seen in 2/11 (18%) apocrine carcinomas and 3/14 (21%) metastases (from two apocrine carcinomas and one aggressive digital papillary adenocarcinoma) (Figure 1d–f). Weak (1+) CD117 expression was noted in 1/9 (11%) primary tumors and 1/14 (7%) metastases (Figure 1g–i).

Mutational Analysis and FISH

The molecular results are summarized in Table 3. We have recently developed a multiplexed tumor genotyping clinical assay that uses the SNaPshot platform from Applied Biosystems.[15] This assay performs well with archived tissue and tests for recurrent mutations in 15 cancer genes, including *BRAF*, *EGFR*, *KRAS*, *PIK3CA*, and *TP53* (Table 1). The genes included in this panel were selected

Table 2 Summary of clinical information

	Age/sex	*Site*	*Treatment*	*Recurrence*	*Follow-up*	*Site of metastases*
Apocrine carcinoma						
1	38/M	L axilla	Excision, axillary LN dissection	NA	NA	L axillary lymph node
2	69/F	R vulva	Radical vulvectomy, bilateral inguinal LN dissection	No	6 years NED	R groin lymph node
3	81/M	Axilla	Excision, R axillary LN dissection, radiotherapy	Yes	2 years 7 months NED	Axillary lymph node
Eccrine carcinoma						
4	68/F	L foot	Excision	No	6 months NED	R groin lymph node
5	62/M	L leg	Amputation, LN dissection, lung resection	No	7 years DOD	Lungs, liver, bilateral adrenals, thoracic LNs
6	62/M	Groin	Radical excision, bilateral LN dissection, chemotherapy (5-FU, cisplatinum), radiotherapy	No	9 months NED	Skin
7	58/M	L neck	Excision	No	8 years NED	Lymph node
8	80/M	L clavicular	Excison	Yes	2 years AWD	Parotid LN, skin, thoracic spine, brain, spleen
9	66/M	L leg	Excision, LN dissection	No	1 year 2 months NED	L inguinal lymph node
Porocarcinoma						
10	78/M	L calf	Excision, L groin LN dissection	No	NA	L inguinal lymph node, skin
11	85/M	L anterior auricular	Excision, L neck dissection	No	11 months NED	Intraparotid lymph nodes
Aggressive papillary adenocarcinoma						
12	51/M	L index finger	Excision, L axillary LN dissection, L lung resection, chemotherapy (6 cycles of Cabo/Taxol)	No	3 years NED	L axillary lymph node, L lingular lung
Hidradenocarcinoma						
13	81/M	Groin	Excison, LN dissection	No	9 months NED	Lymph node, skin
14	78/M	R shoulder	Excision, R axillary LN dissection	No	5 months AWD	R axillary lymph node, skin

Abbreviations: AWD: alive with disease; DOD: died of disease; F: female; LN: lymph node; M: male; NA: not available; NED: no evidence of disease.

based on their clinical significance and on the availability of therapeutic agents (either FDA-approved or under clinical testing) targeting these cancer pathways.[15] SNaPshot genotyping identified somatic mutations in 42% ($n=5$) of the 12 available paired tumor samples (Table 3). Activating mutations in *PIK3CA* mutations were detected in one hidradenocarcinoma (c.1624G>A; p.Glu542Lys) and one porocarcinoma (c.1633G>A; p.Glu545Lys) (Figure 2). *TP53* mutations were detected in one eccrine (c.743G>A; p.Arg248Gln), one hidradenocarcinoma (c.817C>T; p.Arg273Cys), and one aggressive digital papillary adenocarcinoma (c.818G>A; p.Arg273His) (Figure 2). The PIK3CA tumor mutations were found in two older males of this cohort (Tables 2 and 3), but given the sample size correlative analysis is limited.

FISH for *EGFR* gene amplification was negative in 9 tested primary tumors and 12 metastases, including 7 primaries and 7 metastases with strong EGFR expression. Low level of *ERBB2* gene amplification was seen in one apocrine carcinoma and its corresponding metastasis (Table 3).

Discussion

Adnexal carcinomas and breast carcinomas are analogous tumors often with similar histology. It is interesting that in this study we detected mutations in PIK3CA and TP53 in a subset of metastasizing adnexal carcinomas, as both of these genes are known to be frequently mutated in breast carcinomas. *TP53* mutations have been previously described in adnexal carcinomas (Table 4), but to our knowledge this is the first report of PIK3CA mutations in these tumors, which is a therapeutically relevant finding. There was concordant detection of *PIK3CA* and *TP53* mutations in both the primary tumors and the corresponding metastases from patients with metastasizing adnexal carcinomas with the exception of one case (case 11). One possible explanation is that the metastasis had a distinct primary origin. Alternatively, it is conceivable that the primary tumor was heterogeneous and the component without *PIK3CA* mutation gave rise to the metastasis. Larger studies will be needed to determine whether loss of an activating *PIK3CA*

Table 3 Summary of immunohistochemistry, mutational analysis and fluorescence *in situ* hybridization

	Primary			*Metastasis*			*Primary*		*Metastasis*	
	EGFR	*HER2*	*CD117*	*EGFR*	*HER2*	*CD117*	*SNaPshot® results*	*EGFR FISH*	*SNaPshot® results*	*EGFR FISH*
Apocrine carcinoma										
1	NA	NA	NA	0	2+ (FISH −)	0	NA	NA	NA	NA
2	0	2+ (**FISH+**)[a]	0	0	2+ (**FISH+**)[a]	0	WT	No amplification	WT	No amplification
3	0	2+ (NA)	NA	0	0	0	NA	NA	WT	No amplification
Eccrine carcinoma										
4	4	0	0	7	0	0	WT	No amplification	WT	No amplification
5	0	0	1+	0	0	0	WT	No amplification	WT	No amplification
6	6	0	0	5	0	0	*TP53* c.743G>A (p.Arg248Gln)	No amplification	*TP53* c.743G>A (p.Arg248Gln)	No amplification
7	0	0	0	5	0	0	NA	NA	NA	NA
8	4	0	0	7	0	0	WT	No amplification	WT	No amplification
9	7	0	0	7	0	0	WT	No amplification	WT	No amplification
Porocarcinoma										
10	NA	NA	NA	7	0	0	NA	NA	WT	No amplification
11	6	0	0	7	0	0	*PIK3CA* c.1633G>A (p.Glu545Lys)	No amplification	WT	No amplification
Aggressive digital papillary adenocarcinoma										
12	NA	NA	NA	4	2+ (FISH−)	1+	NA	NA	*TP53* c.818G>A (p.Arg273His)	No amplification
Hidradenocarcinoma										
13	5	0	0	5	0	0	*TP53* c.817C>T (p.Arg273Cys)	No amplification	*TP53* c.817C>T (p.Arg273Cys)	No amplification
14	6	0	0	6	0	0	*PIK3CA* c.1624G>A (p.Glu542Lys)	No amplification	*PIK3CA* c.1624G>A (p.Glu542Lys)	No amplification

Abbreviations: WT: wild type; NA: not available.
[a]The ratio between the *ERBB2*-specific probe and a control centromere probe in chromosome 17 was 2.2 for the primary tumor and 2.3 for the metastasis (50 nuclei count).
Bold indicates positive FISH results.

mutation during tumor progression and metastasis is a common occurrence. Such a finding would have therapeutic implications for patients with metastatic disease and argue for molecular testing of the metastasis before clinical decision-making.

The phosphatidylinositol 3 kinase (PIK3) signaling pathway is an important regulator of cell growth, proliferation, cell motility, angiogenesis, and survival and it has been shown that *PIK3CA* (phosphatidylinositol 3-kinase, catalytic, α polypeptide) is the most frequently mutated gene in breast cancer.[19,20] It is thought that in breast cancer, oncogenic mutations in *PIK3CA* or low levels of *PTEN* expression may confer resistance to treatment with trastuzumab, a monoclonal antibody that targets the HER2/NEU receptor.[21] *ERBB2* amplification and *PIK3CA* mutation were validated as biomarkers for sensitivity to the single-agent PIK3 inhibitor, GDC-0941, in breast cancer models.[22] Other studies have shown that cancers with *PIK3CA* mutations were sensitive to single-agent PI3K inhibitors and dual PI3K-mammalian target of rapamycin inhibitors.[23,24]

In breast carcinomas, the majority of mutations have been identified in the helical domain (exon 9, 37%) and in the kinase domain (exon 20, 63%) of *PIK3CA*.[25] All mutations were single-based substitutions.[25] Similarly, we detected two mutations, c.1624G>A:p.Glu542Lys and c.1633G>A:Glu545Lys, in exon 9 of *PIK3CA,* in one hidradenocarcinoma and one porocarcinoma, respectively. These two mutations are among the three most frequently reported mutations in breast cancer.[25] Mutations in codon 545 are mutational hotspots reported in ovarian and colorectal carcinomas as well.[26,27] The clustering of mutations within *PIK3CA* may prove useful for therapeutic purpose.

Tumor suppressor gene, *TP53*, located on the short arm of chromosome 17p13 has been implicated in the regulation of cell growth, DNA repair, and apoptosis. The *TP53* gene is frequently (14–52%) altered in human breast carcinomas and is the most commonly mutated gene in human tumors.[28–30] Mutations are usually clustered within the most conserved regions of exons 4, 5, 7, and 8.[31] *TP53* mutations have been previously documented in nine cases of adnexal carcinomas (Table 4).[32–35] Similar to findings reported by Biernat *et al*,[32] we found *TP53* mutations within codon 248 of exon 7

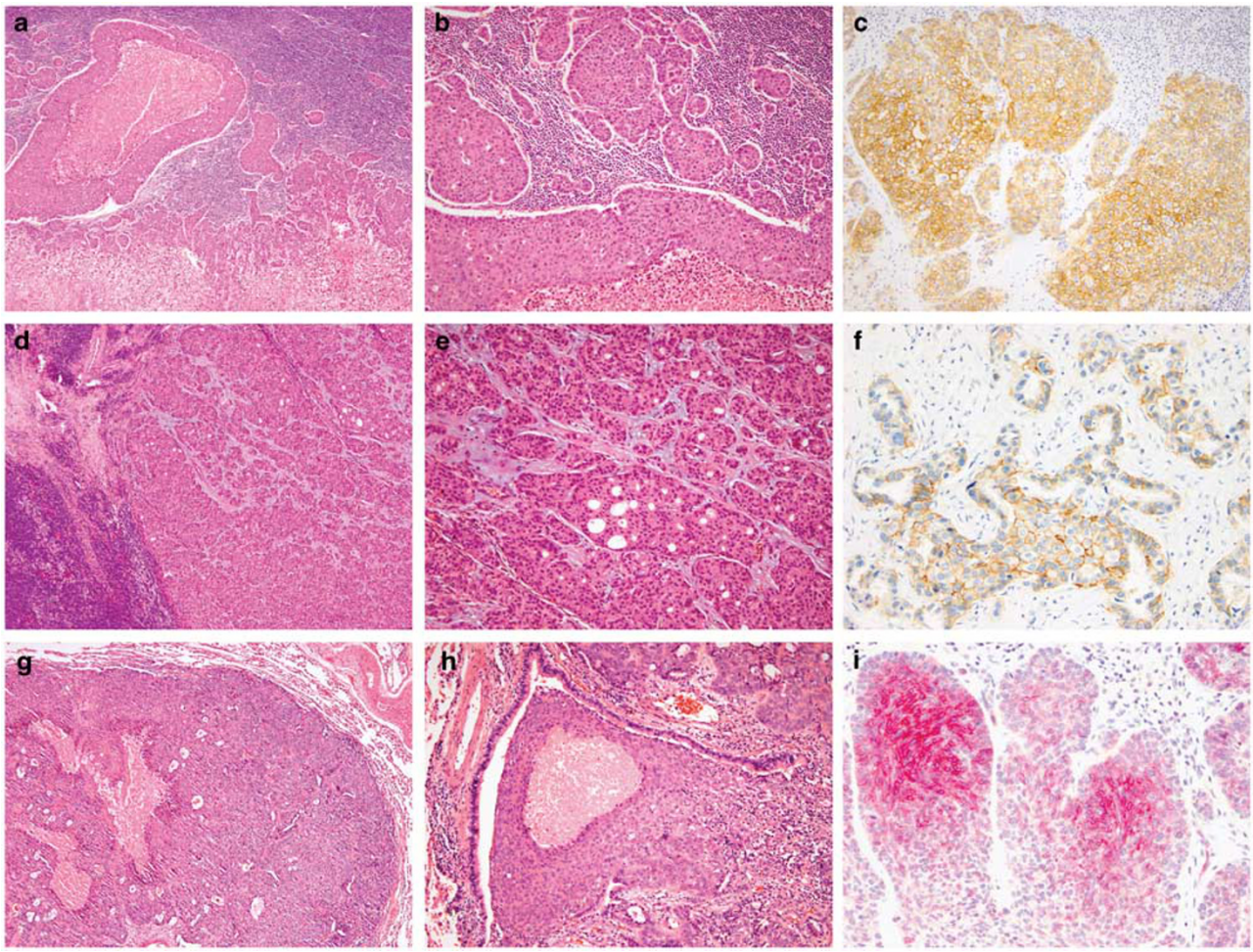

Figure 1 Histologic and immunohistochemical features. (**a**, **b**) In a case of metastasizing eccrine carcinoma, nodules and nests of neoplastic cells with comedonecrosis and duct formation are seen within a lymph node (× 40, × 100). (**c**) Strong EGFR membranous staining is seen in the majority of the neoplastic cells (× 100). (**d**, **e**) In a case of metastasizing apocrine carcinoma, there is diffuse replacement of the lymph node by neoplastic cells with ample eosinophilic cytoplasm (× 4, × 100). (**f**) Weak membranous HER2 expression is noted (× 200). (**g**, **h**) In a case of metastasizing aggressive digital papillary adenocarcinoma, a glandular carcinoma is seen within pulmonary parenchyma. (**i**) CD117 expression is focally noted (× 200).

and codon 273 of exon 8 in 25% of our cases. Interestingly, these mutations have also been reported in breast carcinomas.[36–38]

EGFR/erbB-1 belongs to a receptor family with tyrosine kinase activity whose gene is located on chromosome 7p12. The EGFR signaling that mediates proliferation, migration, invasion, and suppression of apoptosis, can be blocked by a growing number of drug inhibitors. The role of EGFR inhibitor therapy in metastasizing adnexal carcinomas with protein overexpression remains unclear. Although 64% of the primary tumors and 71% of the metastases in our series demonstrated EGFR protein overexpression, the EGFR FISH studies were negative. This is not an unexpected finding; as similar results were noted in our recent study of hidradenocarcinomas.[39] In addition, increased EGFR expression has been shown in various epithelial malignancies with or without gene amplification and the underlying mechanisms of EGFR protein overexpression are still unclear at this time.[40] The lack of gene amplification does not entirely exclude the role of EGFR inhibitors in the treatment of metastasizing adnexal carcinomas; as there are conflicting results in the literature regarding the role of IHC, FISH, and/or PCR in treatment decision of various tumors.[41–43] There is frequent discrepancy between the EGFR protein expression and gene amplification in other tumors in the literature.[44] Furthermore, inconsistent results have also been reported regarding the correlation between *EGFR* gene mutations and *EGFR* gene amplification/polysomy detected by FISH (Bell *et al*, 2005; Sone *et al*, 2005). In a recent series of 100 patients with advanced pulmonary adenocarcinoma, Gupta *et al*[41] proposed that tumors should be evaluated by more than one method to identify patients that may benefit from tyrosine kinase inhibitors therapy.

One of three metastasizing adnexal carcinomas in our series with (2 +) HER2 overexpression had low

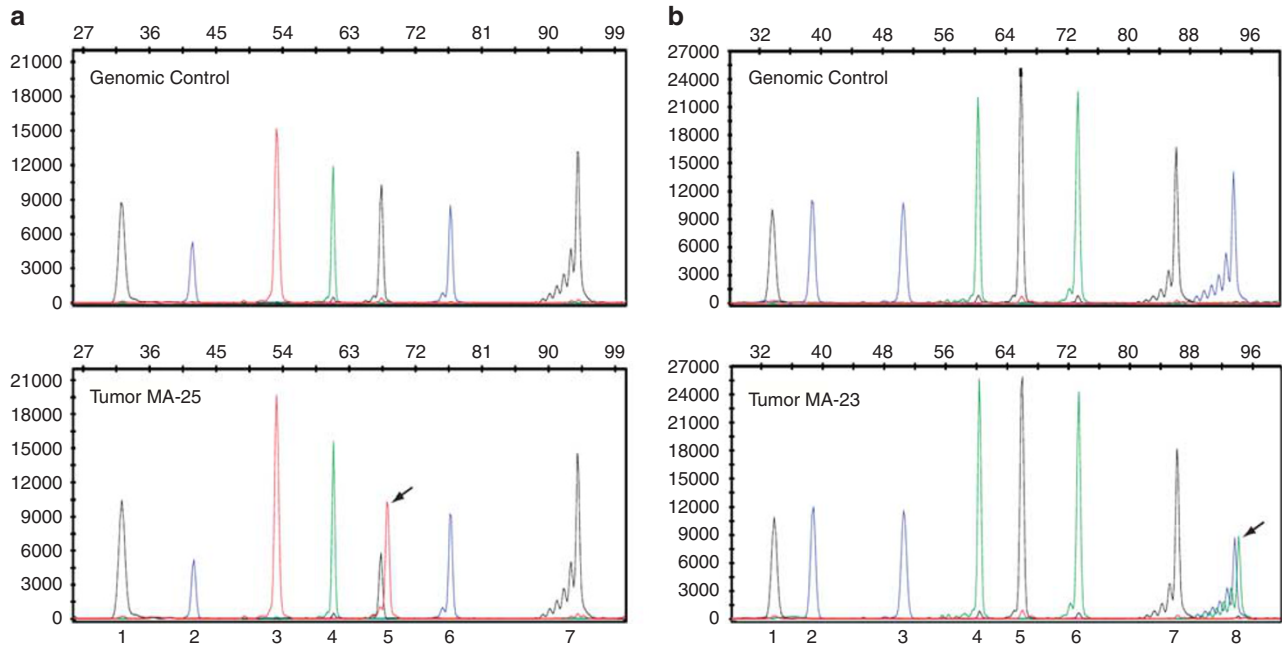

Figure 2 Mutational profiling of metastasizing adnexal carcinoma using SNaPshot® genotyping. In both cases, the top panel shows genotyping data obtained with normal male genomic DNA (Promega, Madison, WI, USA) and the lower panel illustrates mutation detection (arrows) in tumor DNA derived from formalin-fixed paraffin-embedded specimens. (**a**) Identification of the *PIK3CA* Glu542Lys (c.1624G>A) mutation in case 14 (hidradenocarcinoma) (please note that the PIK3CA 1624 assay is designed in the reverse orientation). Assays: (1) *IDH1* 395; (2) *EGFR* 2236_50del F; (3) *EGFR* 2573; (4) *CTNNB1* 133; (5) *PIK3CA* 1624; (6) *NRAS* 35; and (7) *AKT1* 49. (**b**) Identification of the *TP53* Arg273His (c.818G>A) mutation in case 12 (aggressive papillary adenocarcinoma). Assays: (1) *CTNNB1* 98; (2) *KRAS* 37; (3) *EGFR* 2155; (4) *KIT* 2447; (5) *PIK3CA* 3145; (6) *PIK3CA* 1637; (7) *APC* 4012; and (8) *TP53* 818.

Table 4 Summary of *TP53* mutations in adnexal carcinomas[32–35]

Case	*Histological type*	*TP53 mutation*	*Reference*
1	Spiradenocarcinoma	Exon 8, Glu285Lys	Biernat *et al*[32]
2	Spiradenocarcinoma	Exon 7, Arg248Gln	Biernat *et al*[32]
3	Porocarcinoma	Exon 8, codons 273-275, 9 bp deletion	Biernat *et al*[32]
4	Hidradenocarcinoma	Exon 5, Cys176Tyr	Biernat *et al*[32]
5	Hidradenocarcinoma	Exon 7, Arg248Gln	Biernat *et al*[32]
6	Trichilemmal carcinoma	Exon 8, codon 306, C→T	Takata *et al*[33]
7	Eccrine carcinoma	Exon 5, Cys176Arg	Takata *et al*[34]
8	Hidradenocarcinoma	Exon 8, Arg273His	Kazakov *et al*[35]
9	Hidradenocarcinoma	Exon 6, Arg196X and Arg213X	Kazakov *et al*[35]
10	Eccrine carcinoma with metastasis	Exon 7, Arg248Gln	Current study
11	Hidradenocarcinoma with metastasis	Exon 8, Arg273Cys	Current study
12	Aggressive digital papillary adenocarcinoma with metastasis	Exon 8, Arg273His	Current study

level of *ERBB2* gene amplification. This is consistent with prior published results indicating that high level *ERBB2* gene amplification is unlikely in the setting of 2+ HER2 overexpression.[35] Strong (3+) overexpression of HER2 (3+) and gene amplification have been documented in one case of metastasizing malignant hidradenoma.[2] These findings suggested that *ERBB2* may be a relevant therapeutic target in rare cases of adnexal carcinoma.

KIT (CD117) is a transmembrane tyrosine kinase whose alterations are of interest as it is a target for STI571 (Glivec) therapy. In our study, only one case weakly overexpressed CD117. *KIT* mutational analyses have not been reported in adnexal carcinomas. *KIT* mutations were found in none of 10 CD117-positive breast carcinomas and in none of 30 adenoid cystic carcinomas of the major and minor salivary glands.[45,46] These findings suggest that *KIT* activation may not be a major driving mechanism in adnexal carcinomas and their analogous tumors (breast and salivary gland).

In summary, we report mutations in *PIK3CA* and *TP53* in a subset of metastasizing adnexal carcinomas. The role of EGFR inhibitor therapy in metastasizing adnexal carcinoma cases with protein overexpression remains unclear. The lack

of correlation between the protein expression and polysomy/gene amplification suggests that molecular mechanisms other than gene amplification have a role in EGFR overexpression in metastasizing adnexal carcinomas. Targeted therapy including PI3K pathway inhibitors, which is currently in clinical testing, might be a potential treatment for rare cases of adnexal carcinomas with metastases. Actual therapeutic trials will be of interest in seeing whether these results will translate to therapeutic response.

Disclosure/conflict of interest

A patent application for the SNaPshot® genotyping methods used in this study was submitted by DDS and AJI and is currently pending.

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