Molecular Characterization of Apocrine Salivary Duct Carcinoma

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Abstract: Contemporary classification and treatment of salivary duct carcinoma (SDC) require its thorough molecular characterization. Thirty apocrine SDCs were analyzed by the Ion Ampliseq Cancer HotSpot panel v2 for mutations in 50 cancer-related genes. Mutational findings were corroborated by immunohistochemistry (eg, TP53, BRAF, β-catenin, estrogen, and androgen receptors) or Sanger sequencing/SNaPshot polymerase chain reaction. *ERBB2* (*HER2*), *PTEN*, *FGFR1*, *CDKN2A*/*P16*, *CMET*, *EGFR*, *MDM2*, and *PIK3CA* copy number changes were studied by fluorescence in situ hybridization. *TP53* mutations (15/27, 56%), *PTEN* loss (11/29, 38%, including 2 cases with *PTEN* mutation), *PIK3CA* hotspot mutations (10/30, 33%), *HRAS* hotspot mutations (10/29; 34%), and *ERBB2* amplification (9/29, 31%, including 1 case with mutation) represented the 5 most common abnormalities. There was no correlation between genetic changes and clinicopathologic parameters. There was substantial overlap between genetic changes: 8 of 9 cases with *ERBB2* amplification also harbored a *PIK3CA*, *HRAS*, and *TP53* mutation and/or *PTEN* loss. Six of 10 cases with *PIK3CA* mutation also had an *HRAS* mutation. These findings provide a molecular rationale for dual targeting of mitogen-activated protein kinase and phosphoinositide 3-kinase pathways in SDC. *FGFR1* amplification (3/29, 10%) represents a new potential target. On the basis of studies of breast carcinomas, the efficacy of anti-ERBB2 therapy will likely be decreased in SDC with *ERBB2* amplification co-occurring with *PIK3CA* mutation or *PTEN* loss. Therefore, isolated ERBB2 testing is insufficient for theranostic stratification of apocrine SDC. On the basis of the prevalence and type of genetic changes, apocrine SDC appears to resemble one subtype of breast carcinoma—"luminal androgen receptor positive/molecular apocrine."

Key Words: salivary duct carcinoma, next-generation sequencing, *TP53*, *ERBB2* (*HER2*), *PIK3CA*

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Salivary duct carcinoma (SDC) is one of the most aggressive salivary gland malignancies, with most patients presenting with cervical lymph node or distant metastases.[1–5] The current management of SDC includes surgical resection followed by radiotherapy and/or chemotherapy. With conventional therapy, more than half of patients with SDC die of disease in a relatively short time period,[4,5] highlighting a need for improved treatment strategies.

Presently used targeted therapeutic modalities, including anti-ERBB2 antibodies and androgen deprivation therapy, are characterized by variable results.[6–8] Recently, additional potentially targetable genetic abnormalities in SDC were identified, including mutations of the gene encoding the p110α catalytic subunit of phosphoinositide 3-kinase (*PIK3CA*),[9,10] *PTEN* deletion,[11] and occasional *BRAF* or *ERBB2* (*HER2*) mutations.[12–14]

Nonetheless, the prevalence of genetic changes in these and potentially other genes and the relationship between genetic changes and clinicopathologic features of SDC are unknown. As an example, it is possible that the origin of SDC (de novo vs. ex pleomorphic adenoma [PA]) or androgen receptor (AR) status may affect the prevalence of genetic alterations. As salivary gland tumors are relatively rare within the realm of human neoplasia, there remains an underrepresentation of genetic studies of these lesions. To this end, we analyzed 30 apocrine SDCs for mutations in 50 cancer-related genes and copy number changes of 8 potentially targetable genes.

MATERIALS AND METHODS

Patients

This study was approved by the Institutional Review Board (IRB# IRB991206). Thirty-seven cases of SDC (1989 to 2012) from the Department of Pathology, University of Pittsburgh Medical Center, satisfied the

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following inclusion criteria: confirmed diagnosis of SDC (malignant ductal proliferation of cells with high nuclear grade, abundant granular to vacuolated eosinophilic cytoplasm, with decapitation secretions, and solid and/or cribriform growth patterns) and availability of sufficient formalin-fixed paraffin-embedded tissue for next-generation sequencing (NGS), immunohistochemistry (IHC), and fluorescence in situ hybridization (FISH). Library preparation for NGS failed in 7 cases. Some aspects of 17 cases were described previously.[9,15,16] Tumors were staged according to the seventh edition of the American Joint Committee on Cancer.[17]

Immunohistochemistry

The antibodies used in this study are listed in Supplemental Table S1 (Supplemental Digital Content 1, http://links.lww.com/PAS/A267).

IHC was performed as per manufacturer's recommendations. Quantification of AR staining was performed using the Allred system.[18] Briefly, as per ASCO/CAP guidelines for interpretation of estrogen receptor (ER), cases with a total score of 3 or greater were considered positive.[19] IHC studies for TP53 and AR were performed using whole sections. TP53 IHC was interpreted as per Boyle et al.[20] Briefly, entirely negative staining was considered "extreme negative" expression, whereas strong diffuse confluent positivity was interpreted as "extreme positive." All intermediate results were considered to be "nonextreme" expression. IHC studies for ER, anti-BRAF-V600E, β-catenin (CTNNB1), anti-IDH1-R132H, and e-cadherin (CDH1) were performed on tissue microarray sections. Paraffin "donor" blocks were selected for each case by one of the coauthors (S.C.). Using a manual tissue arrayer (MTA-1; Beecher Instruments, Sun Prairie, WI) 1 mm cores were transferred from each donor block to a blank recipient paraffin block by one of the coauthors (R.R.S.).

Fluorescence In Situ Hybridization

FISH was performed for *CMET*, *EGFR*, *Fibroblast Growth Factor Receptor 1* (*FGFR1*), *ERBB2*, *MDM2*, *PIK3CA*, *PTEN*, and *CDKNA2/P16*. Sixty to 120 cells per case were analyzed using Leica Biosystems FISH Imaging System (CytoVision FISH Capture and Analysis Workstation, Buffalo Grove, IL). Technical details and the number of unsuccessfully tested cases are listed in Supplemental Table S2 (Supplemental Digital Content 1, http://links.lww.com/PAS/A267). Amplification was defined as *CMET*/chromosome (centromere) enumeration probe (CEP) *CEP7* > 2, *EGFR*/*CEP7* > 2, *FGFR1*/*CEP8* > 2, *ERBB2*/*CEP17* > 2.2, *MDM2*/*CEP12* > 2, and *PIK3CA*/*CEP3* > 2. *PTEN* FISH was interpreted as previously described.[9,21,22] *CDKN2A* loss was defined as *CDKN2A*/*CEP9* < 0.8.

Library Preparation and Sequencing

For targeted NGS analysis, DNA was extracted from tissue cores obtained by one of the coauthors (S.C.) as previously described[23] and amplified using the Ion AmpliSeq Library kit and Hotspot v2 primer pool. The Hotspot v2 cancer panel generated 207 amplicons targeting approximately 2800 hotspots across 50 cancer-associated genes. Briefly, the amplicons were partially digested, phosphorylated, and ligated to Ion adapters with the addition of Ion Xpress barcode sequences according to the manufacturer's instructions. After ligation, the amplicons underwent nick translation and additional library polymerase chain reaction (PCR) amplification cycles. Library concentration and amplicon sizes were determined using TapeStation System (Agilent Technologies, Santa Clara, CA). Subsequently, the multiplexed barcoded libraries were enriched by clonal amplification using emulsion PCR on templated Ion Sphere Particles and loaded on Ion 318 Chip. Massively parallel sequencing was carried out on an Ion Torrent Personal Genome Machine (PGM) sequencer (Life Technologies, Carlsbad, CA) using the Ion PGM Sequencing 200 Kit v2 according to the manufacturer's instructions.

Data Analysis

After a successful sequencing reaction, the raw signal data were analyzed using Ion Torrent platform-optimized Torrent Suite v4.0.2 (Life Technologies, Carlsbad, CA). The primary data analysis included signal processing, base calling, quality score assignment, adapter trimming, PCR duplicate removal, short sequence read alignment, mapping quality control, coverage analysis, and variant calling. The short sequence reads were aligned to the human genome reference sequence (GRCh37/hg19). Variant calling was performed using Variant Caller v4.0 plugin (integrated with Torrent Suite) that generated a list of detected sequence variations (single nucleotide variants and insertions or deletions [indels]) in a variant calling file (VCF v4.1; http://www.1000genomes.org/wiki/analysis/variant%20call%20format/vcf-variant-call-format-version-41). The variant calls were annotated, filtered, and prioritized using SeqReporter[24] with the help of an in-house knowledge base and the following publically available data bases: COSMIC v68 (http://cancer.sanger.ac.uk/cancergenome/projects/cosmic/), dbSNP build 137 (http://www.ncbi.nlm.nih.gov/SNP/), and in silico prediction scores (PolyPhen-2 and SIFT) from dbNSFP light v1.3.[25] Sequence variants with at least × 300 depth of coverage and mutant allele frequency of > 5% of the total reads were included for analysis. Integrated Genomics Viewer (IGV, Broad Institute, Cambridge, MA) was used to review the sequence read pileups as appropriate.

PTEN mutations were confirmed by Sanger sequencing (Supplemental Fig. S1, Supplemental Digital Content 1, http://links.lww.com/PAS/A267). *PIK3CA* mutations were confirmed by SNaPshot PCR as per the manufacturer's manual and as previously described.[22,26] The nomenclature change from exon 9 to 10 and from exon 20 to 21 (and corresponding HGVS nomenclature) for *PIK3CA* gene in this manuscript is based on the RefSeq Transcript ID (Accession.version NM_006218.2) mapped to the human genome reference primary assembly (GRCh37.p13) (available at: http://www.ncbi.nlm.

nih.gov/nuccore/54792081, accessed on 1/2/2014). TP53 and anti-BRAF-V600E IHC were used to corroborate the presence of *TP53* and *BRAF* mutations.

Statistical Analysis

χ^2 or Fisher exact tests were used to examine associations between categorical variables, and the *t* test was used to examine associations between continuous variables (Microsoft Excel, Redmond, WA).

RESULTS

The clinicopathologic parameters of 30 patients with SDC that were successfully tested by NGS are summarized in Table 1. As expected, most patients were elderly men presenting with locoregionally advanced disease affecting predominantly the parotid gland. Most cases showed evidence of preexisting PA (Table 1). All cases were ER negative and AR positive by IHC. The details of AR IHC analysis are listed in Supplemental Table S3 (Supplemental Digital Content 1, http://links.lww.com/PAS/A267).

Mutations

TP53

TP53 mutations were the most common genetic abnormality (15/27, 56%). *TP53* mutation details and the results of TP53 IHC are summarized in Table 2. All *TP53* mutations were in exons 5, 6, 7, and 8. Missense mutations were predominant over deletion/frame shift–type mutations. All *TP53*-mutated cases had only 1 mutation within this gene. In 4 of 15 (27%) *TP53*-mutated cases the mutant allelic frequency was >50%. Most mutations were unique, and only 1 recurring exon 6 mutation (c.626_627delGA; p.R209fs*6) was identified in 2 cases.

TABLE 1. Demographic and Clinicopathologic Features of 30 Patients With Apocrine AR-positive SDC

Clinicopathologic Feature	N (%)
Sex	
Male	23/30 (77)
Female	7/30 (23)
Age, mean (range) (y)	67 (45-80)
Site	
Parotid	26 (87)
Submandibular	4 (13)
Tumor size* (n = 29), mean (range) (cm)	3.5 (0.8-7.2)
Origin* (n = 29)	
De novo	12 (41)
Ex PA	17 (59)
pT* (n = 29)	
1	6 (21)
2	3 (10)
3	10 (34)
4	10 (34)
pN (n = 26)	
0	3 (12)
1	4 (15)
2	19 (73)

*In 1 case, the primary tumor site could not be determined and resection of the primary tumor was not performed.

Combining cases with either extreme positive or extreme negative TP53 IHC results[20] (Fig. 1), the sensitivity and specificity of TP53 IHC for *TP53* mutations were 87% and 92%, respectively. The correlation between *TP53* mutational results and TP53 IHC revealed 1 false-positive case (ie, strong to moderate TP53 IHC staining and wild-type *TP53* by sequencing). There were 2 false-negative cases with nonextreme TP53 IHC and *TP53* mutations (Table 2; cases 11 and 12).

PIK3CA, HRAS, and Other Mutations

The second and third most common genetic abnormalities involved the *PIK3CA* and *HRAS* genes. *PIK3CA* exon 10 (p.E545K [n = 2] and p.E542K [n = 3]) or exon 21 (p.H1047R [n = 5]) mutations were identified in 10 of 30 (33%) SDCs. All *PIK3CA* mutations are well-known activating hotspot mutations, confirmed by SNaPshot PCR. *HRAS* mutations were identified in 10 of 29 SDCs (34%), including p.Q61R (n = 7), p.G13R (n = 1), p.G13V (n = 1), and p.T20I (n = 1). Six SDCs harbored both *PIK3CA* and *HRAS* mutations (Table 3).

Both cases with *PTEN* mutation (exon 5, p.R130Q, and exon 6, p.Y177C [Supplemental Fig. S1, Supplemental Digital Content 1, http://links.lww.com/PAS/A267]) also had *PTEN* loss by FISH.

BRAF V600E mutation was identified in only 1 case and confirmed by anti-BRAF-V600E IHC. The SDC with BRAF mutation identified by NGS showed intense cytoplasmic staining (Fig. 2A). BRAF wild-type cases showed weak to moderate cytoplasmic staining and/or nonspecific nuclear staining (Figs. 2B, C).[27]

One *ERBB2* mutation in exon 19 (c.2301C > G, p.I767M) was identified in an SDC with *ERBB2* amplification. The mutant allelic frequency was at 92%, suggesting that the mutant allele was amplified. *ERBB2* mutations were previously reported in SDC.[12–14]

Other tested mutation hotspots in genes listed below were negative: *ABL1*, *AKT1*, *ALK*, *APC*, *ATM*, *CDH1*, *CSF1R*, *CTNNB1*, *EGFR*, *ERBB4*, *EZH2*, *FGFR1*, *FGFR2*, *FGFR3*, *FBXW7*, *FLT3*, *GNA11*, *GNAS*, *GNAQ*, *HNF1A*, *IDH1*, *IDH2*, *JAK2*, *JAK3*, *KDR*, *KIT*, *KRAS*, *MET*, *MLH1*, *MPL*, *NRAS*, *NOTCH1*, *NPM1*, *PDGFRA*, *PTPN11*, *RB1*, *RET*, *SMAD4*, *SMARCB1*, *SMO*, *SRC*, *STK11*, *VHL*. The coverage of some hotspots for the following genes was below the laboratory cutoff of ×300, which is of insufficient quality to make an accurate call: *TP53* (n = 3), *HRAS* (n = 1), *EGFR* (n = 3), *APC* (n = 3), *p16* (n = 15).

The presumably functional mutations in *APC* or *CTNNB1* would lead to nuclear translocation of β-catenin. In agreement with NGS results, IHC for β-catenin performed on all 30 SDCs showed no nuclear β-catenin staining. E-cadherin IHC showed preserved membranous staining in all SDCs, potentially supporting the absence of some functional *E-cadherin*/*CDH1* mutations. Also in agreement with NGS results, IHC with anti-IDH1-R132H antibody was negative in all cases.

TABLE 2. Summary of *p53* Mutations and p53 IHC

#	Ex.	cDNA	Protein	Type of Mutation	MAF (%)	p53 IHC
1	5	c.524G > A	p.R175H	Missense	79	EP
2	5	c.527G > T	p.C176F	Missense	70	EP
3	6	c.578A > G	p.H193R	Missense	90	EP
4	6	c.592G > A	p.E198K	Missense	6	EP
5	7	c.711G > T	p.M237I	Missense	23	EP
6	7	c.733G > A	p.G245S	Missense	48	EP
7	7	c.770T > C	p.L257P	Missense	23	EP
8	8	c.796G > C	p.G266R	Missense	47	EP
9	8	c.811G > A	p.E271K	Missense	18	EP
10	8	c.817C > T	p.R273C	Missense	30	EP
11	8	c.847C > T	p.R283C	Missense	30	NE
12	8	c.886C > T	p.H296Y	Missense	6	NE
13	6	c.626_627delGA	p.R209fs*6	Del.-fs	65	EN
14	6	c.626_627delGA	p.R209fs*6	Del.-fs	38	EN
15	7	c.718_719delAG	p.S240fs*23	Del.-fs	28	EN

# indicates patient number; del., deletion; EN, extreme negative; EP, extreme positive; Ex., exon; fs*, frameshift leading to premature stop codon; MAF, mutant allele frequency; NE, nonextreme staining.

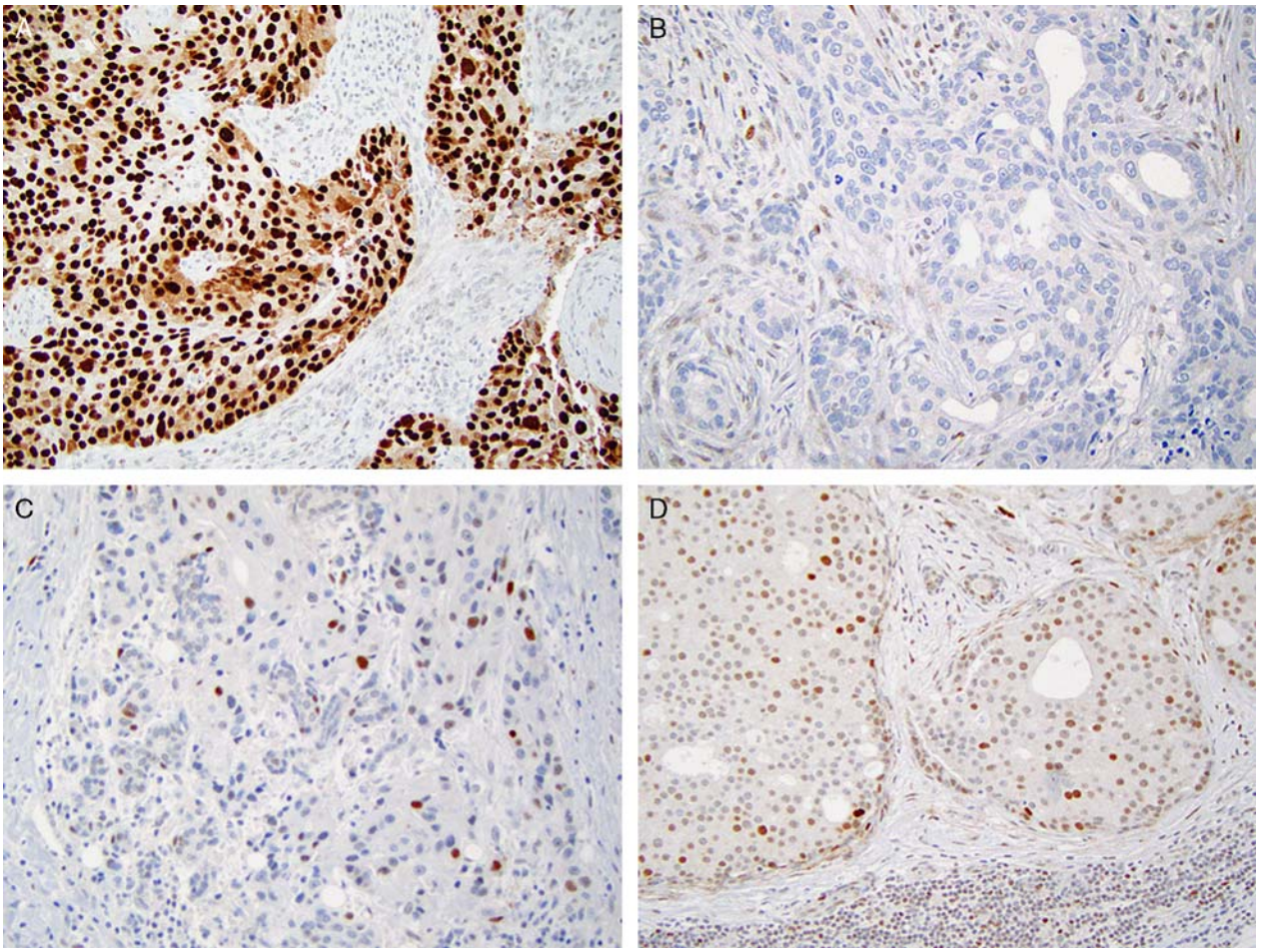

FIGURE 1. TP53 IHC. A, SDC with *TP53* p.E198K mutation with mutant allele frequency of 6% (extreme positive, IHC). B, SDC with extreme negative TP53 IHC staining and *TP53* p.R209fs* deletion/frame shift mutation. In the background, occasional nuclei of myofibroblasts of desmoplastic stroma show staining intensity reflective of the presumably wild-type TP53 (IHC). C and D, Examples of nonextreme staining. C, SDC with TP53 p.H296Y mutation and mutant allele frequency of 6% (IHC). D, SDC with TP53 p.R283C mutation and mutant allele frequency of 30% (IHC).

TABLE 3. Summary of and Relationship Between Identified Mutations and Gene Copy Number Changes

	Mutational Status				Copy Number Changes, FISH				
#	*p53*	*PIK3CA*	*HRAS*	*BRAF*	*PTEN*	*ERBB2*	*FGFR1*	*CDKN2A*	*MDM2*
10	Mut	WT	WT	WT	N	N	N	N	N
14	Mut	WT	WT	WT	N	N	N	N	N
8	Mut	WT	WT	WT	Loss	N	Ampl	N	N
5	Mut	WT	WT	WT	Loss*	N	N	N	N
3	Mut	WT	WT	WT	Loss	N	Ampl	N	Fail
15	Mut	WT	WT	WT	N	Ampl	N	Fail	N
9	Mut	WT	WT	WT	N	Ampl	N	N	N
7	Mut	WT	WT	WT	N	Ampl*	N	N	N
6	Mut	WT	WT	WT	N	Ampl	N	N	N
2	Mut	WT	WT	Mut	N	N	Ampl	N	N
4	Mut	WT	Mut	WT	Loss	N	N	N	N
12	Mut	Mut	Mut	WT	N	N	N	N	N
13	Mut	Mut	Mut	WT	N	N	N	N	N
11	Mut	Mut	Mut	WT	N	Ampl	N	Fail	N
1	Mut	Mut	WT	WT	N	Ampl	N	N	Ampl
16	WT	Mut	Mut	WT	N	N	N	Loss	N
17	WT	Mut	Mut	WT	N	N	N	Loss	N
18	WT	Mut	Mut	WT	Loss	N	N	Fail	N
19	WT†	Mut	WT	WT	N	N	N	Fail	N
20	WT	Mut	WT	WT	N	N	N	N	N
21	WT	Mut	WT	WT	N	N	N	N	N
22	WT†	WT	Mut	WT	Loss	N	N	Fail	Fail
23	WT	WT	Mut	WT	Loss	Ampl	N	N	N
24	WT	WT	Mut	WT	Fail	Fail	Fail	Fail	N
25	WT	WT	WT	WT	Loss*	N	N	N	N
26	WT	WT	WT	WT	N	Ampl	N	N	N
27	WT	WT	WT	WT	Loss	Ampl	N	Fail	Fail
28	WT	WT	Fail	WT	Loss	N	N	Fail	N
29	WT†	WT	WT	WT	Loss	N	N	N	N
30	WT	WT	WT	WT	N	N	N	Fail	N

Loss-of-function mutations/deletions in known tumor suppressors (eg, *TP53*, *PTEN*) are highlighted in pink; activating mutations in known oncogenes are highlighted in green; copy number gains/amplifications identified by FISH are highlighted in red.

*Presence of mutation in addition to copy number change.

†Failed sequencing, p53 status for these cases is based on p53 IHC.

# indicates patient number; Ampl., amplified. Mut., mutated; N, normal copy number, without loss or amplification; WT, wild type.

Copy Number Changes as Detected by FISH

FISH identified *PTEN* loss in 11 of 29 (38%) successfully tested SDCs. The mechanisms of *PTEN* loss included homozygous deletion (n = 2), chromosome 10 monosomy (n = 4), and hemizygous deletion (n = 5).

The second most common copy number change was that of *ERBB2* amplification: 9/29 (31%). Interestingly, in all but 1 case, *ERBB2* amplification was accompanied by other genetic alterations: *TP53*, *PIK3CA*, and/or *HRAS* mutation, *PTEN* loss, or *MDM2* amplification.

The third most common copy number change involved the *FGFR1* gene: 3 SDCs showed *FGFR1*/*CEP8* of 2.6, 3, and 4.1.

Two of 21 successfully tested SDCs showed *CDKN2A*/*P16* loss.

None of the successfully tested SDCs showed *PIK3CA*, *EGFR*, or *CMET* amplification.

MDM2 FISH

Prior studies have identified *HMGA2* and *MDM2* coamplification in a subset of carcinomas ex PA.[28,29] To validate the ability of *MDM2* FISH to serve as a surrogate marker for *HMGA2* amplification, 3 SDCs with previously identified *HMGA2* amplification[30] were tested and shown to have *MDM2* coamplification (Fig. 3A). Only 1 of 27 successfully tested SDCs in this cohort showed *MDM2* amplification (Fig. 3B). It was a case of SDC ex PA (Table 3, case #1).

The relationship between *TP53*, *PIK3CA*, *HRAS*, *PTEN*, *ERBB2*, *BRAF* mutations and *PTEN*, *ERBB2*, *FGFR1*, *CDKN2A*/*P16*, and *MDM2* copy number changes is shown in Table 3. There was no correlation between the origin of SDC (de novo vs. ex PA) and molecular alterations. There was only 1 SDC that showed no genetic abnormalities. It was a case of SDC ex recurrent PA.

DISCUSSION

SDCs are known to harbor genetic alterations in *TP53*, *PTEN*, *CDKN2A*/*P16*,[2] the Phosphoinositide 3-Kinase (PI3K) pathway,[9–12] *PLAG1*/*HMGA2*,[30] and *ERBB2*.[31] However, the relationship between these and other genetic alterations in SDC is not well understood, and translation to the clinical practice is limited. Relying only on techniques (IHC, FISH, Sanger sequencing, and NGS) currently routinely offered by our clinical CLIA-certified laboratories, we showed that most

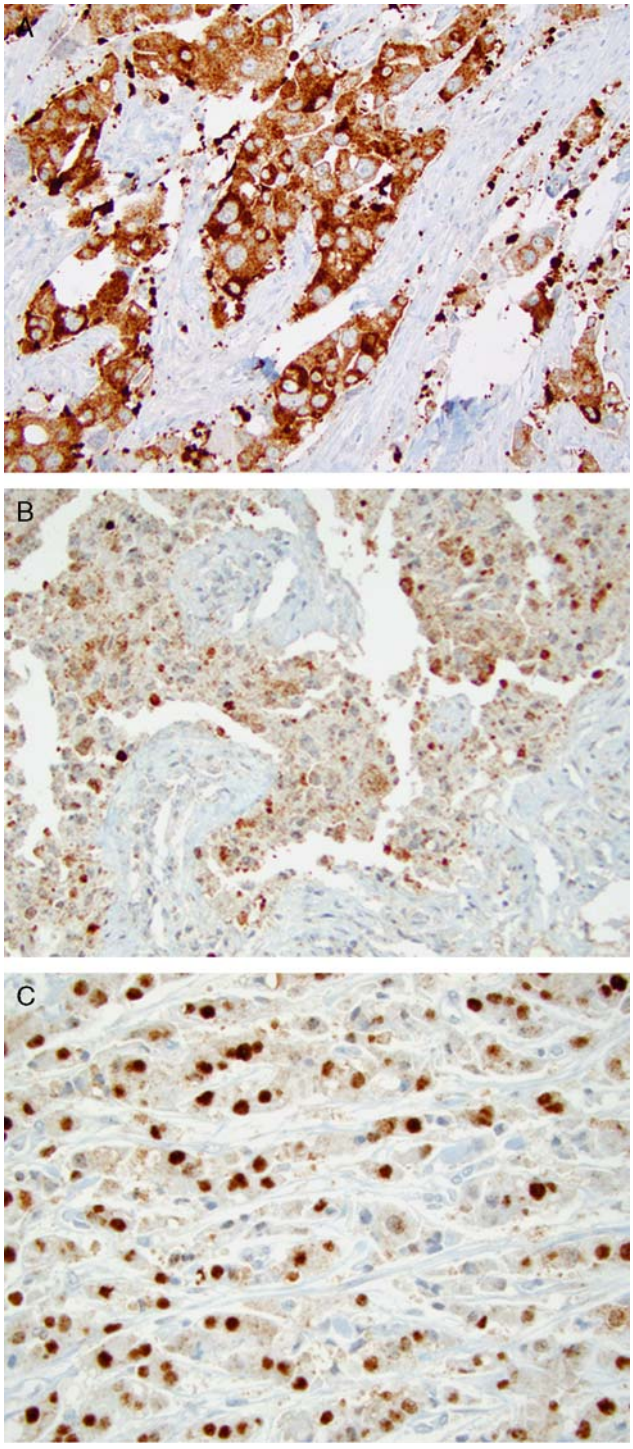

FIGURE 2. IHC for the BRAF V600E with VE1 monoclonal antibody. A, SDC with a BRAF V600E mutation identified by sequencing, showing diffuse cytoplasmic staining (IHC). This is the only case with BRAF V600E mutation by sequencing, and it showed the strongest cytoplasmic staining. B, BRAF wild-type SDC showing weak to moderate predominantly cytoplasmic staining. Thirteen of 29 BRAF wild-type SDCs showed such staining (IHC). C, Seven wild-type SDCs showed nonspecific nuclear staining (IHC).

SDCs harbor >1 genetic alteration, most of which are therapeutically actionable.

The finding of a frequent coexistence of *ERBB2* amplification with *PIK3CA* mutations or *PTEN* loss appears to be of more pressing clinical significance. Specifically, 2 of 9 cases with *ERBB2* amplification also carried *PIK3CA* mutation. In patients with breast carcinomas, anti-ERBB2 therapy was associated with a lower response rate of tumors with *ERBB2* amplification and *PIK3CA* mutation.[32] Similarly, *PTEN* loss (seen here in an additional 2 of 9 cases with *ERBB2* amplification) confers resistance to trastuzumab therapy.[33] Furthermore, 2 *ERBB2*-amplified SDCs also had *HRAS* mutation. As *HRAS* is downstream of *ERBB2*, the presence of *HRAS* mutation is also very likely to undermine the efficacy of anti-ERBB2 therapy.

One common approach to advance understanding of SDC is to extend current findings from breast oncology to SDC. To better the analogy between SDC and breast carcinoma, one has to understand how to apply the current classification of breast carcinomas to SDC. On the basis of expression of ER, progesterone receptor (PR), and ERBB2, breast carcinomas are now categorized into luminal (ER^+, $ERBB2^-$), *ERBB2*-amplified, and triple-negative breast carcinomas (TNBC; ER^-, PR^-, $ERBB2^-$).[34,35] TNBC themselves are a very heterogenous group of carcinomas.[36] Within the TNBC category, "luminal AR-positive/molecular apocrine" type[37] represents one of the better-defined subtypes with high prevalence of *TP53* and *PIK3CA* mutations and *PTEN* loss.[35,36,38]

Analogous to breast carcinoma classification, 2 teams have recently stratified SDC into several groups.[39,40] In both studies, the second most common group was defined by combined AR and ERBB2 positivity. However, it is unclear whether ERBB2 positivity truly defines a distinct subset of AR-positive SDC that may benefit from anti-ERBB2 therapy. Here we show that 4 of 9 cases of SDC with *ERBB2* amplification had *PIK3CA* mutation or *PTEN* loss, both of which have been shown to be associated with resistance to anti-ERBB2 therapy in breast carcinomas.[32,33] Therefore, it appears that isolated ERBB2 testing may be insufficient for theranostic stratification of AR-positive SDC.

PIK3CA mutations are an example of more recently discovered targetable abnormality in SDC. However, the coexistence of *PIK3CA* and *HRAS* mutations in 6 SDCs may explain potential resistance to PI3K pathway inhibition and highlights the need for combined mitogen-activated protein kinase/extracellular signal-regulated kinases (MEK) and PI3K inhibition. Such an approach was shown to be synergistic in head and neck squamous cell carcinoma cell lines.[41]

It has been previously shown that somatic missense *TP53* mutations cause nuclear accumulation of the TP53 protein, whereas truncated TP53 proteins resulting from frameshift mutations lead to the loss of TP53 protein.[42] Here, TP53 IHC was used to corroborate the *TP53* sequencing findings. Following the TP53 IHC scoring scheme proposed by Boyle et al,[20] the sensitivity and specificity of TP53 IHC for *TP53* mutations were 87%

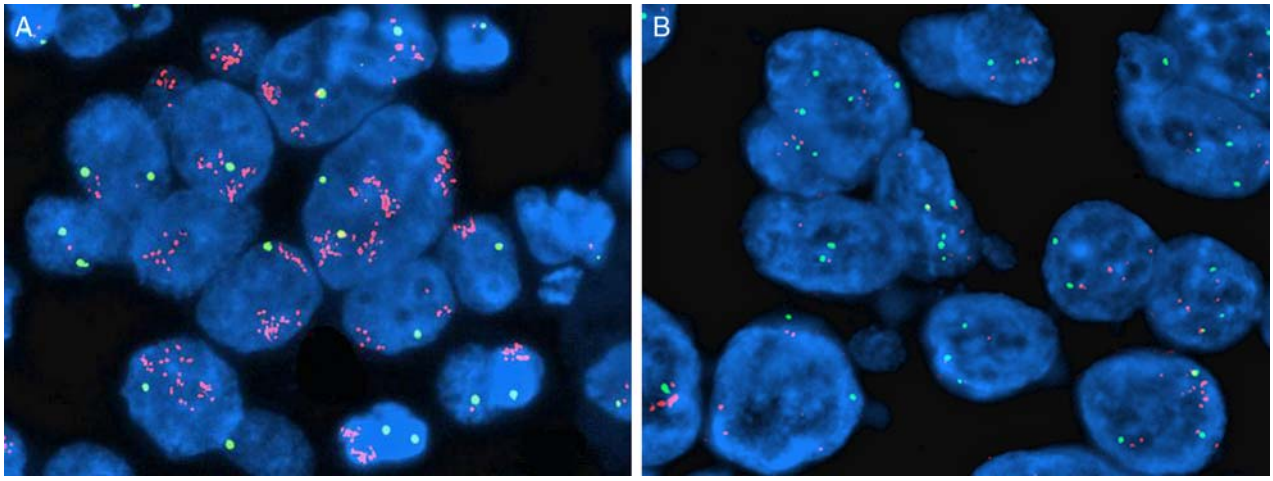

FIGURE 3. *MDM2* FISH. A, SDC with previously reported *HMGA2* amplification; *MDM2/CEP12* = 21.2. B, SDC, case #1, with *MDM2/CEP12* = 2.9.

and 92%, respectively. The nonextreme pattern of TP53 IHC was seen in just 1 *TP53*-mutated case. The IHC findings in this case may be explained by the low frequency of the mutated *TP53* allele (6%, case #12, Table 2). In contrast, case #4 with 6% allelic frequency of an exon 6 mutation was TP53 IHC positive.

Another case with nonextreme TP53 IHC (#11, Table 2) had p.R283C missense *TP53* mutation with 30% of mutant allele frequency. In prior studies of breast carcinomas, it was shown that this mutation was also negative by IHC using the PAb 1801 anti-TP53 antibody.[43]

Although various targeted approaches to restoring the TP53-MDM2 loop are under development (eg, nutlin-3 analogs[44]), *TP53* mutations appear to correlate with response to some types of conventional chemotherapy. In studies of breast carcinoma, the presence of *TP53* mutations (as determined by sequencing) was associated with better response to docetaxel chemotherapy.[45] This could be secondary to *TP53*-mutated cells being more susceptible to taxane-induced mitotic block and apoptosis.[46]

Two other studies have recently sequenced SDCs, using an Ion Torrent sequencer[14] or massively parallel sequencing (results available in abstract form only[13]). The number and type of mutations identified and validated in our report are closer to those in the preliminary report by Dogan et al.[13] For instance, unlike Ku and colleagues, we did not identify abnormalities in *AKT1*, *ALK*, *APC*, *ATM*, *CTNNB1*, *CDH1*, *CDKN2A*, *EGFR*, *ERBB4*, *FBXW7*, *FLT3*, *GNAS*, *IDH1*, *IDH2*, *KDR*, *KRAS*, *MET*, *MPL*, *NOTCH1*, *PDGFR, RET*, *SMAD4*, *SMARCB1*, *SMO*, *STK11*, or *VHL*. Genetic changes in *APC*, *CTNNB1*, and *CDH1* are believed to cause an abnormal IHC staining. In agreement with negative sequencing results in our study, none of our SDCs had nuclear β-catenin expression or change of membranous E-cadherin staining. The apparently higher frequency of mutations in the study by Ku and colleagues may be explained by technical issues and differences in tested SDC. Assuming that the prior study of SDC from this institution[47] is representative of cases that were sequenced,[14] SDCs studied by Ku and colleagues have one of the lowest AR expression rates reported in the literature (at about 56%). In addition, the authors do not share technical details on mutated allele frequency or cutoff values defining a reliable coverage and provide no validation studies. We chose conservative cutoffs usually used in clinical practice—$\times 300$ depth of coverage and mutant allele frequency of $> 5\%$.

A subset of SDC will now join the list of other carcinomas with *FGFR1* amplification—carcinomas of the breast, lung, and stomach.[48] Importantly, several FGFR1 inhibitors are now in phase II/III clinical trials.[48]

In conclusion, the degree of overlap between *ERBB2* amplification and other genetic changes has therapeutic implications for the use of anti-ERBB2 therapy, which is increasingly common in SDC but lacks an adequate predictive biomarker. The efficacy of anti-ERBB2 therapy will likely be decreased in SDC, wherein *ERBB2* amplification co-occurs with *HRAS* mutation, *PIK3CA* mutation, or *PTEN* loss. Therefore, we highlight the deficiency of isolated testing for *ERBB2* amplification for theranostic stratification of apocrine SDC. Moreover, in light of the frequently co-occurring actionable events observed in our cohort, dual targeting could be justified in specific cases. Our findings provide a molecular rationale for the investigation of combined therapeutic inhibition of ERBB2 and PI3K, or PI3K and MEK in specific cases of SDC. Finally, *FGFR1* amplification represents a new potential target. Overall, on the basis of the prevalence and type of genetic changes observed in our cohort (*TP53*, *PIK3CA* mutations, *PTEN* loss), apocrine SDC appears to resemble 1 subtype of breast carcinoma—"luminal AR^+/molecular apocrine." Thus, continued attention to the advance of molecular medicine in breast cancer may pave the way for similar personalized approaches in SDC.

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