Original Article

Subsets of Salivary Duct Carcinoma Defined by Morphologic Evidence of Pleomorphic Adenoma, *PLAG1* or *HMGA2* Rearrangements, and Common Genetic Alterations

Simion I. Chiosea, MD[1]; Lester D. R. Thompson, MD[2]; Ilan Weinreb, MD[3]; Julie E. Bauman, MD[4]; Alyssa M. Mahaffey, BS[1]; Caitlyn Miller, BS[1]; Robert L. Ferris, MD, PhD[5]; and William E. Gooding, MS[6]

BACKGROUND: The authors hypothesized that histogenetic classification of salivary duct carcinoma (SDC) could account for de novo tumors and those with morphologic or molecular evidence (pleomorphic adenoma gene 1 [*PLAG1*], high-mobility group AT hook 2 [*HMGA2*] rearrangement, amplification) of pleomorphic adenoma (PA). **METHODS:** SDCs (n = 66) were reviewed for morphologic evidence of PA. *PLAG1* and *HMGA2* alterations were detected by fluorescence in situ hybridization (FISH). *PLAG1*-positive tumors were tested by FISH for fibroblast growth factor receptor 1 (*FGFR1*) rearrangement. Thirty-nine tumors were analyzed using a commercial panel for mutations and copy number variations in 50 cancer-related genes. **RESULTS:** On the basis of combined morphologic and molecular evidence of PA, 4 subsets of SDC emerged: 1) carcinomas with morphologic evidence of PA but intact *PLAG1* and *HMGA2* (n = 22); 2) carcinomas with *PLAG1* alteration (n = 18) or 3) *HMGA2* alteration (n = 12); and 4) de novo carcinomas, without morphologic or molecular evidence of PA (n = 14). The median disease-free survival was 37 months (95% confidence interval, 28.4-45.6 months). Disease-free survival and other clinicopathologic parameters did not differ for the subsets defined above. Combined Harvey rat sarcoma viral oncogene homolog/phosphatidylinositol-4,5-biphosphate 3-kinase, catalytic subunit α (*HRAS/PIK3CA*) mutations were observed predominantly in de novo carcinomas (5 of 8 vs 2 of 31 tumors; $P = .035$). Erb-B2 receptor tyrosine kinase 2 (*ERBB2*) copy number gain was not observed in de novo carcinomas (0 of 8 vs 12 of 31 tumors; $P = .08$). Tumor protein 53 (*TP53*) mutations were more common in SDC ex pleomorphic adenomas than in de novo carcinomas (17 of 31 vs 1 of 8 tumors; $P = .033$). **CONCLUSIONS:** The genetic profile of SDC varies with the absence or presence of pre-existing PA and its cytogenetic signature. Most de novo SDCs harbor combined *HRAS/PIK3CA* mutations and no *ERBB2* amplification. ***Cancer* 2016;000:000–000**. © *2016 American Cancer Society.*

KEYWORDS: malignant transformation, next-generation sequencing, pleomorphic adenoma, salivary, salivary duct carcinoma.

INTRODUCTION

Salivary duct carcinoma (SDC) is one of the most aggressive salivary malignancies and most patients present with advanced disease.[1-5] The current management of patients with SDC includes surgical resection followed by radiotherapy and/or chemotherapy. With conventional therapy, more than one-half of patients with SDC die of disease in 3 to 5 years.[4,5]

SDC is the most common histologic type of carcinoma ex pleomorphic adenoma (ex-PA), and at least one-half of SDCs arise from pleomorphic adenoma (PA).[6-9] PA was the first benign human epithelial neoplasm to to be shown to harbor recurrent cytogenetic abnormalities involving pleomorphic adenoma gene 1 (*PLAG1*) and high-mobility group A2 (*HMGA2*).[10,11] Several cytogenetically defined subsets of PA have been recognized, including those with *PLAG1* or *HMGA2* rearrangements, other rearrangements, and cytogenetically intact PA. Comparable cytogenetic abnormalities were reported in SDC[7,12,13] as a reflection of PA diversity.

Currently used targeted therapeutic modalities, including anti-erb-B2 receptor tyrosine kinase 2 (anti-ERBB2) approaches, androgen-deprivation therapy, and vemurafenib, are characterized by variable clinical benefits.[14-17] Recently,

Corresponding author: Simion I. Chiosea MD, University of Pittsburgh Medical Center, Presbyterian University Hospital, A610-2, 200 Lothrop Street, Pittsburgh, PA 15213, Fax: (412) 647-7799; chioseasi@upmc.edu

[1]Depatment of Pathology, University of Pittsburgh Medical Center, Pittsburgh, Pennsylvania; [2]Department of Pathology, Southern California Permanente Medical Group, Woodland Hills, California; [3]Department of Pathology, University Health Network, Toronto, Ontario, Canada; [4]Department of Medicine, University of Pittsburgh Medical Center, Pittsburgh, Pennsylvania; [5]Division of Head and Neck Surgery, Department of Otolaryngology, University of Pittsburgh Cancer Institute, Pittsburgh, Pennsylvania; [6]Biostatistics Facility, University of Pittsburgh Cancer Institute, Pittsburgh, Pennsylvania

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additional potentially targetable genetic abnormalities in SDC were identified, including mutations of the gene encoding the p110α catalytic subunit of phosphoinositide 3-kinase (*PIK3CA*), either alone or combined with Harvey rat sarcoma viral oncogene homolog (*HRAS*) mutations.[14,18-20]

The objective of this study was, first, to correlate *PLAG1* and *HMGA2* alterations with clinicopathologic features in a large cohort of patients with SDC. For instance, determining the frequency of *PLAG1* and *HMGA2* rearrangements in SDC may help to better understand its origin (de novo vs ex-PA). By the time of clinical presentation, SDC may overrun the remaining evidence of pre-existing PA. Practically, *PLAG1* and *HMGA2* alterations may help to distinguish salivary and nonsalivary high-grade adenocarcinomas on small biopsies, especially those with an occult primary.

Second, to describe genetic events that may be associated with malignant transformation of PA into SDC, we wanted to characterize the relation between the presence of pre-existing PA (as determined by a combination of morphology and *PLAG1* and *HMGA2* status) and mutations and copy number variations in 50 cancer-related genes.

MATERIALS AND METHODS

Patients and Histologic Review

This study was approved by our institutional review board (IRB991206). Patients whose samples satisfied the following eligibility criteria were included: surgical resection of primary SDC; sufficient formalin-fixed, paraffin-embedded material for *PLAG1* and *HMGA2* fluorescence in situ hybridization (FISH) studies; and availability of clinical information to characterize disease-free survival (DFS). In total, 66 patients were included from the authors' institutions from 1996 to 2015. Thirty-nine of these tumors were used to achieve the second objective using next-generation sequencing.

Tumors were staged according to the seventh edition of the American Joint Committee on Cancer *Cancer Staging Manual*.[21] Chondroid or myxoid stroma with benign ductal elements and hyalinized hypocellular nodules were accepted as histologic evidence of pre-existing PA.

Immunohistochemistry

Immunohistochemistry (IHC) for tumor protein 53 (p53) (DO-7 monoclonal mouse, 1:100 dilution; Dako, Carpinteria, Calif) was performed according to the manufacturer's recommendations. IHC for p53 was interpreted according to the methods described by Boyle et al.[19,22]

FISH

PLAG1 and *HMGA2* rearrangements were detected using break-apart FISH probes (Empire Genomics, Buffalo, NY). From 60 to 100 cells per tumor were analyzed using the Leica Biosystems FISH Imaging System (CytoVision FISH Capture and Analysis Workstation, Buffalo Grove, Ill). Hyperploidy or amplification (centromeric enumeration probes were not used) was defined as the presence of >2 *PLAG1* or *HMGA2* signals in >75% of cells. The *PLAG1* and *HMGA2* rearrangement status of 27 tumors was previously determined and reported.[7] Fibroblast growth factor receptor 1 (*FGFR1*) FISH was performed as previously described.[23]

Library Preparation, Sequencing, and Data Analysis

DNA extraction and targeted next-generation sequencing analysis were performed as previously described.[19] Library concentrations and amplicon sizes were determined using the TapeStation System (Agilent Technologies, Santa Clara, Calif). Subsequently, the multiplexed, barcoded libraries were enriched by clonal amplification using emulsion polymerase chain reaction on templated Ion Sphere Particles and loaded on an Ion 318 Chip. Massively parallel sequencing was carried out on a Ion Torrent Personal Genome Machine sequencer (Life Technologies, Carlsbad, Calif) using the Ion Personal Genome Machine Sequencing 200 Kit version 2 according to the manufacturer's instructions. After a successful sequencing reaction, the raw signal data were analyzed using the Ion Torrent platform-optimized Torrent Suite version 4.0.2 (Life Technologies). The short sequence reads were aligned to the human genome reference sequence (GRCh37/hg19). Variant calling was performed using the Variant Caller version 4.0 plugin (integrated with Torrent Suite), which generated a list of detected sequence variations in a variant calling file (VCF v4.1; available at http://www.1000genomes.org/wiki/analysis/variant percent20-call percent20format/vcf-variant-call-format-version-41; accessed September 1, 2015). The variant calls were annotated, filtered, and prioritized using SeqReporter,[24] an in-house knowledge base, and the following publically available databases; COSMIC v68 (http://cancer.sanger.ac.uk/cancergenome/projects/cosmic/; accessed September 1, 2015), dbSNP build 137 (http://www.ncbi.nlm.nih.gov/SNP/; accessed September 1, 2015), in silico prediction scores (polymorphism phenotyping 2 [PolyPhen-2] and sorting intolerant from tolerant [SIFT]) from dbNSFP light version 1.3.[25] Sequence variants with at least 300× depth of coverage and a mutant allele frequency of >5%

of the total reads were included for analysis. Copy number variations were identified by next-generation sequencing as previously described.[26] *ERBB2* gain was defined as >8 copies, and cyclin-dependent kinase inhibitor 2A (*CDKN2A*) loss was defined as <0.5 copies.

Statistical Analysis

The SDC 4 subgroups were compared using an exact (permutation based) 2-tailed chi-square test. *P* values were adjusted using the method of Benjamini and Hochberg.[27] Demographic and clinical comparisons between SDC groups were conducted using the Wilcoxon test for continuous data and the Fisher exact test or chi-square test for discrete data. DFS survival was compared between Pittsburgh/Southern California Permanente Medical Group and Toronto cohorts using a log-rank test.

RESULTS

The clinicopathologic parameters of 66 patients with SDC are summarized in Table 1 and Supporting Table 1 (see online Supporting Information). Eighty percent of patients were males, and most patients presented with clinical stage IV disease arising in the parotid gland. In 3 patients, unremarkable PA was resected 10, 31, and 33 years before the diagnosis of SDC. The estimated median DFS was 37 months (95% confidence interval, 28.4-45.6 months).

None of the demographic or clinicopathologic parameters of interest (sex, age, tumor site, pathologic or clinical stage, p53 by immunohistochemistry) differed by origin of SDC (de novo vs ex-PA, as defined by morphology), and none were associated with DFS ($P = .37$). DFS was comparable among patients diagnosed at different institutions ($P = .49$).

Four Subsets of SDC Defined by the Morphologic Evidence of PA and PLAG1 or HMGA2

Of 39 SDCs, 13 had *PLAG1* alterations, including 7 with rearrangement only (Fig. 1A,B), 4 had rearrangement and hyperploidy, and 2 had hyperploidy only. Five were characterized by *HMGA2* rearrangement, including 4 with rearrangement only (Fig. 1C,D) and 1 with rearrangement and hyperploidy.

On the basis of the morphologic evidence of PA and *PLAG1* and *HMGA2* status, SDCs can be categorized into 4 subsets (Fig. 2), including 27 cases with previously reported *PLAG1* and *HMGA2* alteration status.[7] Overall, based on morphologic appearance and *PLAG1* and *HMGA2* status, 52 of 66 SDC (78%) in the current study arose ex-PAs.

TABLE 1. Demographic and Clinicopathologic Features of Patients with Salivary Duct Carcinoma (n = 66)

Clinicopathologic Feature	No. of Patients/Total (%)
Men	53/66 (80)
Age: Mean [range], y	66 [33-81]
Site	
Parotid	58 (88)
Submandibular	8 (12)
Tumor size: Mean [range], cm	3.4 [0.8-7.2]
Origin[a]	
De novo	20 (30)
Ex-PA	46 (70)
Pathologic tumor classification	
pT1	9 (14)
pT2	12 (18)
pT3	22 (33)
pT4	23 (35)
Pathologic lymph node classification[b]	
pN0	6 (9)
pN1	10 (15)
pN2	46 (70)
p53 IHC	
Extreme positive	20 (30)
Extreme negative	14 (21)
Normal	32 (48)
PLAG1 positive[c]	18 (27)
HMGA2 positive	12 (18)

Abbreviations: Ex-PA, ex pleomorphic adenoma; *HMGA2*, high-mobility group AT hook 2; IHC, immunohistochemistry; PA, pleomorphic adenoma; *PLAG1*, pleomorphic adenoma gene 1.
[a] Origin based on morphologic evaluation.
[b] Pathologic lymph node status was unknown for 4 patients.
[c] Positive accounts for tumors with rearrangement alone, rearrangement accompanied by copy number gain, or copy number gain alone.

The list of potential PLAG1 fusion partners includes *FGFR1*.[11] Therefore, 13 SDCs with *PLAG1* alterations were tested for *FGFR1* rearrangement. One tumor with an *FGFR1* rearrangement was identified (tumor 8) (Fig. 3), suggesting that, in this tumor, *FGFR1* was the fusion partner of *PLAG1*. In tumor 2, *FGFR1* FISH failed. In the remaining 11 tumors with *PLAG1* alteration, *FGFR1* was intact. Patients' DFS, sex, age, tumor site and size, pathologic tumor classification, pathologic lymph node classification, and clinical stage did not correlate with the 4 SDC subsets.

Correlations Between the 4 Subsets of SDC and Genetic Alterations in 50 Cancer-Related Genes

Correlations between the 4 SDC subsets and mutations and copy number variations of *PIK3CA*, *HRAS*, *ERBB2*, v-Raf murine sarcoma viral oncogene homolog B (*BRAF*), *FGFR3*, *TP53*, *CDKN2A*, phosphatase and tensin homolog (*PTEN*), and ataxia telangiectasia mutated serine/threonine kinase (*ATM*) are illustrated in Figure 3.

The following genes were negative for mutations and copy number alterations: ABL proto-oncogene 1

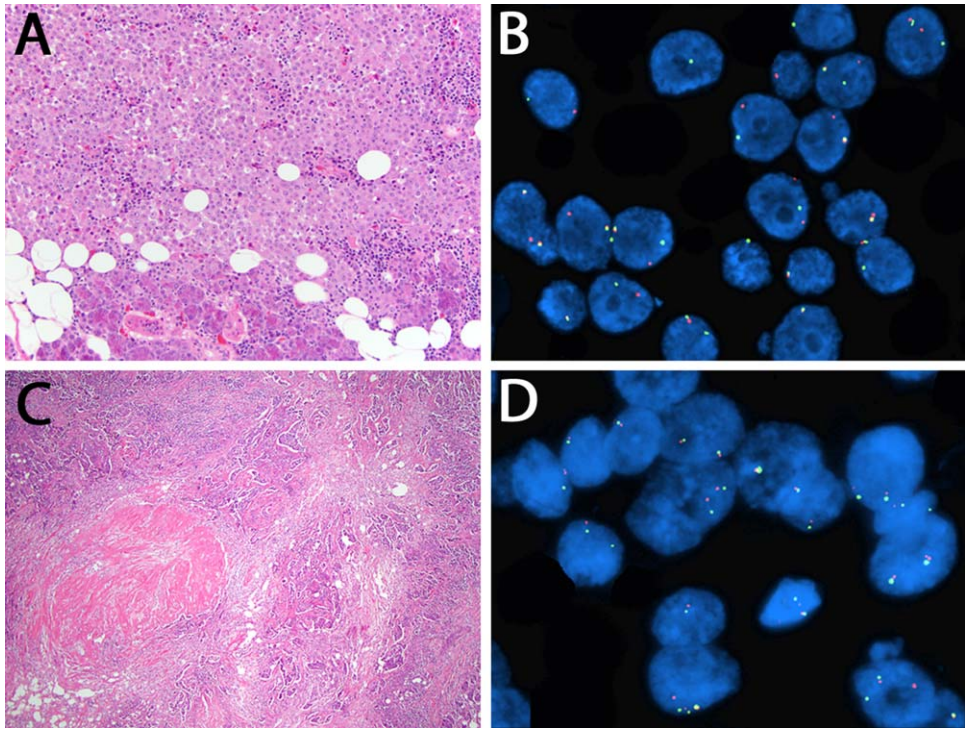

Figure 1. (A,B) Salivary duct carcinoma, 5.5 cm, involving submandibular gland, without morphologic evidence of pleomorphic adenoma (1 tissue section per 1 cm of the tumor was examined microscopically; H&E stain, original magnification ×200). (B) On fluorescence in situ hybridization using a break-apart probe, tumor cells with the rearrangement exhibit green and red split signals in addition to the normal fused yellow signal. Eighty-one percent of cells had pleomorphic adenoma gene 1 (*PLAG1*) rearrangements. (C,D) Salivary duct carcinoma, 3 cm, entirely submitted for microscopic examination and a 0.1-cm hyalinized nodule was identified (H&E stain, original magnification ×40). (D) Eighty percent of cells had high-mobility group AT hook 2 (*HMGA2*) rearrangements. On fluorescence in situ hybridization using a break-apart probe, tumor cells with the rearrangement exhibit green and red split signals in addition to the normal fused yellow signal.

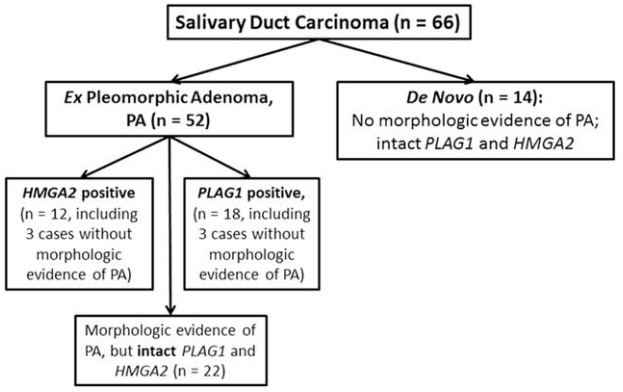

Figure 2. Four subsets of salivary duct carcinoma based on morphologic evidence of pleomorphic adenoma (PA) and alterations in pleomorphic adenoma gene 1 (*PLAG1*) or high-mobility group AT hook 2 (*HMGA2*) identified by fluorescent in situ hybridization.

nonreceptor tyrosine kinase (*ABL1*); AKT serine/threonine kinase 1 (*AKT1*); anaplastic lymphoma receptor tyrosine kinase (*ALK*); adenomatosis polyposis coli (*APC*); cadherin 1 (*CDH1*); colony-stimulating factor 1 receptor (*CSF1R*); catenin β1 (*CTNNB1*); epidermal growth factor receptor (*EGFR*); erb-b2 receptor tyrosine kinase 4 (*ERBB4*); enhancer of zeste 2 polycomb repressive complex 2 subunit (*EZH2*); *FGFR1*; *FGFR2*, F-box and WD repeat domain containing 7 (*FBXW7*); fms-related tyrosine kinase 3 (*FLT3*); G protein subunit α 11 (*GNA11*); GNAS complex locus (*GNAS*); G protein subunit α q (*GNAQ*); HNF1 homeobox A (*HNF1A*); isocitrate dehydrogenase 1 (*IDH1*); *IDH2;* Janus kinase 2 (*JAK2*); *JAK3;* kinase insert domain receptor (*KDR*); kit proto-oncogene receptor tyrosine kinase (*KIT*); Kirsten rat sarcoma proto-oncogene GTPase (*KRAS*); MET proto-oncogene receptor tyrosine kinase (*MET*); mut-L homolog 1 (*MLH1*); myeloproliferative leukemia virus proto-oncogene, thrombopoietin receptor (*MPL*); neuroblastoma RAS viral oncogene homolog (*NRAS*); notch 1(*NOTCH1*); nucleophosmin 1 (*NPM1*); platelet-derived growth factor receptor α (*PDGFRA*); protein tyrosine phosphatase, nonreceptor type 11 (*PTPN11*); retinoblastoma transcriptional corepressor (*RB1*); ret proto-oncogene (*RET*);

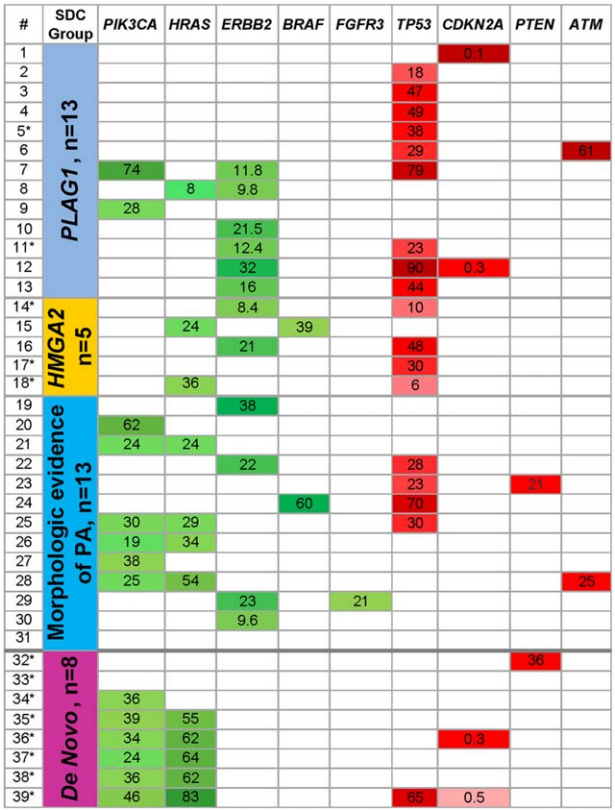

Figure 3. Correlations between the 4 subsets of salivary duct carcinoma (SDC) (second column) and mutations and copy number variations in 50 cancer-related genes are illustrated. Only genes with mutations or copy number alterations are shown. In the first column, an asterisk identifies tumors that were without morphologic evidence of pleomorphic adenoma (PA). Mutations and copy number gains in oncogenes are highlighted in green. Mutations and deletions in tumor suppressor genes are highlighted in red. For tumors with mutations, the mutant allelic frequency is indicated, and stronger color intensity (eg, darker green) indicates higher mutant allele frequency. Copy numbers are indicated for erb-b2 receptor tyrosine kinase 2 (*ERBB2*) and cyclin-dependent kinase inhibitor 2A (*CDKN2A*). *ATM* indicates ataxia telangiectasia mutated serine/threonine kinase; *BRAF*, v-Raf murine sarcoma viral oncogene homolog B; *FGFR3*, fibroblast growth factor receptor 3; *HMGA2*, high-mobility group AT hook 2; *HRAS*, Harvey rat sarcoma viral oncogene homolog; *PIK3CA*, phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit α; *PLAG1*, pleomorphic adenoma gene 1; *PTEN*, phosphatase and tensin homolog; *TP53*, tumor protein 53.

SMAD family member 4 (*SMAD4*), SWI/SNF related, matrix associated, actin dependent, regulator of chromatin, subfamily b, member 1 (*SMARCB1*); smoothened, frizzled class receptor (*SMO*); SRC proto-oncogene, non-receptor tyrosine kinase (*SRC*); serine/threonine kinase 11 (*STK11*); and von Hippel-Lindau tumor suppressor (*VHL*). The coverage of some hotspots for the following genes was below the laboratory cutoff of 300× reads, which is of insufficient quality to make an accurate call: *TP53* (n = 4), *HRAS* (n = 1), *EGFR* (n = 3), *APC* (n = 3), and *p16* (n = 16).

TP53 Mutations Occurred Predominantly in SDC Ex-PA

TP53 mutations were the most common genetic abnormality (18 of 39 tumors; 46%). *TP53* mutation details and the results of p53 immunohistochemistry are summarized in Supporting Table 2 (see online supporting information). All *TP53* mutations were in exons 4 through 8. *TP53* mutations were more common in SDC ex-PA than in de novo carcinoma (17 of 31 vs 1 of 8 tumors; $P = .033$). For instance, 9 of 13 tumors (69%) with *PLAG1* rearrangements and 4 of 5 (80%) with *HMGA2* rearrangements harbored *TP53* mutations.

ERBB2 *Copy Number Gain Occurred Predominantly in SDC Ex-PA*

In this study, *ERBB2* copy number gain was not observed in de novo SDC (0 of 8 vs 12 of 31 tumors; $P = .08$; when de novo status was defined by morphology and *PLAG1* and *HMGA2* status). *ERBB2* copy number gain was observed in SDC de novo tumors when de novo status was defined by morphology alone (2 of 13 vs 10 of 26 tumors; $P = .5$).

Combined HRAS/PIK3CA Mutations With Higher Mutant Allelic Frequency of HRAS Were Observed Predominantly in de Novo Salivary Duct Carcinoma

PIK3CA exon 9 (p.E542K [n = 4] and p.E545K [n = 3]) or exon 20 (p.H1047R, n = 7) mutations were identified in 14 of 39 SDCs (36%). *HRAS* mutations were identified in 13 of 38 successfully tested SDCs (32%), including p.Q61R (n = 8), p.Q61K (n = 2), p.G13R (n = 1), p.G13V (n = 1), and p.T20I (n = 1). In addition to tumors that had isolated *PIK3CA* or *HRAS* mutations, 9 revealed co-occurring mutations in these 2 genes. None of the tumors with the combination of *PIK3CA* and *HRAS* mutations carried *PLAG1* or *HMGA2* rearrangements. Unsupervised clustering revealed that combined *HRAS*/*PIK3CA* mutations with mutant allelic frequencies higher in *HRAS* than in *PIK3CA* were observed predominantly in de novo SDCs (5 of 8 vs 2 of 31 tumors; $P = .035$).

Other Mutations

Two tumors harbored *BRAF* mutations: p.V600E and p.G466V (tumor 15) (Fig. 3). Two tumors had *ATM* mutations: p.V410A (tumor 6) and p.F858L (tumor 28). An *FGFR3* p.F384L mutation was identified in 1 tumor (tumor 29).

DISCUSSION

Various salivary gland carcinomas arise from PA (eg, epithelial-myoepithelial carcinoma, myoepithelial carcinoma, SDC). The true prevalence of malignant transformation of PA into carcinoma is best assessed according to the specific histologic type of salivary carcinoma. On the basis of morphologic evidence alone, in the current multiinstitutional series, 46 of 66 SDCs (70%) were ex-PAs. This is higher than the previously reported rate.[8] This discrepancy is perhaps best explained by referral bias and a more extensive sampling of tumors included in the current study: the need for abundant material and exclusion of samples with a failed next-generation sequencing library may have inadvertently lead to the inclusion of more recently diagnosed cases. Over the last few years, we tended to examine salivary tumors microscopically in their entirety. This may have enriched the current series for more extensively sampled cases. Anecdotally, it was previously demonstrated that, to identify pre-existing PA, up to 100 tissue blocks might have to be examined.[28]

When morphologic evidence of PA is complemented by the knowledge of *PLAG1* and *HMGA2* status, additional cases of SDC ex-PA are identified. The overall prevalence of SDC ex-PAs in this series was 79% (52 of 66 tumors). Studying SDCs with molecular evidence of PA may help to fine tune our understanding of morphologic signs of pre-existing PA. For instance, before the knowledge was available about *HMGA2* rearrangement in 1 of the tumors (Fig. 1C), a 0.1-cm hyalinized nodule devoid of bland ducts was not considered sufficient evidence for a pre-existing PA.

Previously, a cytogenetic study characterized basic clinicopathologic features of 220 PAs with *PLAG1* and *HMGA2* rearrangements.[10] Although the technical performance of conventional cytogenetics may be distinct from that of FISH, it is tempting to compare the age of patients with PAs and SDCs harboring *PLAG1* or *HMGA2* alterations (Table 2). The reported average age of patients with *PLAG1*-positive PA was 39 years,[10] whereas the average age of patients with *PLAG1*-positive SDC in the current study was 61 years. This difference in patients' average age suggests that it may take 22 years for a *PLAG1*-positive PA to transform into an SDC. Perhaps a better understanding of genetic alterations leading to malignant transformation of PAs may lead to the earlier identification of "high-risk" PAs. The prevalence of *PLAG1* and *HMGA2* alterations is similar in PAs and SDCs (Table 2), suggesting that neither *PLAG1* nor *HMGA2* alteration predisposes a PA to malignant transformation.

One of the technical limitations of this study is its reliance on FISH, which is unlikely to identify intrachromosomal rearrangements. Also, break-apart probe designs preclude the identification of specific *PLAG1* or *HMGA2* fusion partners. For instance, the list of potential *PLAG1* fusion partners includes *CTNNB1* (β-catenin), leukemia-inhibitory factor receptor, transcription elongation factor A, coiled-coil-helix domain containing 7, and *FGFR1*.[11] One tumor with a likely *PLAG1-FGFR1* fusion was identified in this study. On the basis of the prevalence of *TP53* mutations and *ERBB2* copy number gain, there may be at least 2 more subgroups of SDCs with *PLAG1* rearrangement: those with *TP53* mutation only and those with *TP53* mutation and *ERBB2* amplification. Perhaps specific *PLAG1* fusion partners (other than *FGFR1*) are associated with one of the above combinations of genetic abnormalities. Even when the status of *PLAG1* and *HMGA2* is accounted for, the genetic landscape of SDC remains very complex, with overlapping genetic alterations. Considering that most patients with SDC present with disease at an advanced clinical stage, the lack of a correlation between molecular findings and DFS is not surprising.

ERBB2 copy number gain and *TP53* have been associated with malignant transformation of PAs.[29,30] The findings of this study appear to reconcile some of the prior seemingly discrepant reports on the role of *TP53* in malignant transformation of adenomas. *TP53* abnormalities were associated with malignant transformation of PAs in some[31-33] but not all[34] studies. Here, we have demonstrated that *TP53* mutations are more common in SDCs with *PLAG1* or *HMGA2* alterations. It is plausible that studies that did not identify an association between *TP53* abnormalities and carcinoma ex-PA inadvertently focused on carcinomas arising in PAs with intact *PLAG1* and *HMGA2*. Studies of salivary duct carcinomas de novo would have identified few, if any, cases with *ERBB2* amplification or *TP53* mutation.

One of the most distinct characteristics of de novo SDC is coexisting mutations in *HRAS* and *PIK3CA*. *PIK3CA* is one of the most studied effectors of *HRAS*. For instance, *HRAS*/*PIK3CA* cooperation is crucial to *HRAS*-induced skin cancer formation.[35,36] In other solid tumors, *PIK3CA* mutations are believed to occur in invasive tumors, whereas upstream mutations in *RAS* genes occur at comparable rates at early and late stages, suggesting that *PIK3CA* mutation is a later event that may boost earlier activation of the PI3K pathway. In human mammary epithelial cells, the addition of PIK3CA p.H1047R greatly increased the anchorage-independent growth of an HRAS

TABLE 2. Prevalence of *PLAG1* or *HMGA2* Alteration and Average Age of Patients With Pleomorphic Adenoma (Literature Review) and Salivary Duct Carcinoma

Patient Group	Average Patient Age [range], y[a]		Prevalence of Alterations: No. of Patients/Total (%)	
	Pleomorphic Adenoma[b]	Salivary Duct Carcinoma	Pleomorphic Adenoma[b]	Salivary Duct Carcinoma
Patients with tumors carrying *PLAG1* alteration	39	61 [33-75]	56/220 (25.5)	18/66 (26)
Patients with tumors carrying *HMGA2* alteration	45.9	64.6 [37-81]	29/220 (13.2)	12/66 (18)

Abbreviations: *HMGA2*, high-mobility group AT hook 2; *PLAG1*, pleomorphic adenoma gene 1.
[a] Age ranges are indicated for patients in the current study.
[b] Data on patients with pleomorphic adenoma are from Bullerdiek et al, 1993.[10]

TABLE 3. Potential Therapeutic Implications of Common Genetic Alterations in Salivary Duct Carcinoma

Molecular Alteration	Potential Targeted Therapies	References
Androgen receptor positivity, IHC	Androgen-deprivation therapy	Locati 2016[39]
ERBB2 copy number gain	ERBB2 targeting therapy (eg, trastuzumab)	Limaye 2013[17]
ERBB2 copy number gain co-occurring with *PIK3CA*, *HRAS*, or *FGFR3* mutation	Based on recent trials in breast carcinoma, dual targeting of *ERBB2* and *PIK3CA* may be needed	Majewski 2015,[40] Loibl 2014[41]
PIK3CA or *HRAS* mutation alone	Mammalian target of rapamycin or mitogen-activated protein kinase/extracellular signal-regulated kinases inhibitors; everolimus, trametinib	Janku 2014,[42] Infante 2012[43]
Combined *PIK3CA* and *HRAS* mutations	Dual mitogen-activated protein kinase/extracellular signal-regulated kinases and PI3K inhibition	Mazumar 2014[44]
TP53 mutations	Targeted approaches to restoring the TP53-MDM2 loop (eg, nutlin-3 analogs); in studies of breast carcinoma, the presence of *TP53* mutations was associated with better response to docetaxel chemotherapy	Stenman 2014,[45] Gluck 2012[46]
Combined *TP53* and *ATM* alteration	Observed in 1% of breast carcinomas; DNA-damaging chemotherapy and ATM inhibition are likely most effective in this subset of cancers	Jiang 2009[47]
ATM mutation	Olaparib; most likely, ATM inhibitors are contraindicated in treatment of cancer with normal TP53	Jiang 2009,[47] Weston 2010[48]

Abbreviations: *ATM*, ataxia telangiectasia mutated serine/threonine kinase; *ERBB2*, erb-b2 receptor tyrosine kinase 2; *FGFR3*, fibroblast growth factor receptor 3; *HRAS*, Harvey rat sarcoma viral oncogene homolog; IHC, immunohistochemistry; MDM2, MDM2 proto-oncogene; PI3K, phosphatidylinositol-4,5-bisphosphate 3-kinase; *PIK3CA*, phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit α; *TP53*, tumor protein 53.

p.G12V mutated cell line.[37] The presence of *HRAS* mutations at higher allelic frequency is consistent with *HRAS* mutation being the earlier event: the presence of mutant allele in clinical tumor samples at a frequency >50% is consistent with mutant allele-specific imbalance.[38] Practically speaking, the complexity of the genetic landscape of SDC confounds implementation of targeted therapy. Potential therapeutic approaches are summarized in Table 3.[17,39-48]

In conclusion, the profile of mutational findings and copy number alterations vary with morphologic evidence of PA and *PLAG1* or *HMGA2* status. *PLAG1*-positive and *HMGA2*-positive tumors tend to have *TP53* mutations or *ERBB2* copy number gain, whereas de novo SDCs frequently harbor *HRAS* and *PIK3CA* mutations. These findings elucidate the process of malignant transformation of PA into an SDC and provide a list of potential predictive biomarkers for the investigation of (dual) targeted therapies.

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AUTHOR CONTRIBUTIONS

Simion I. Chiosea: Conceptualization, visualization, design, acquisition of data, provision of study materials, interpretation of data, formal analysis, writing–original draft, writing–critical revision, final approval of the version to be published, supervision, agreement to be accountable for all aspects of the work, and funding

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