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Consistent *PLAG1* and *HMGA2* abnormalities distinguish carcinoma ex-pleomorphic adenoma from its de novo counterparts☆

Nora Katabi, MD[a,*], **Ronald Ghossein, MD**[a], **Alan Ho, MD**[b], **Snjezana Dogan, MD**[a], **Lei Zhang, MS**[a], **Yun-Shao Sung, MS**[a], and **Cristina R. Antonescu, MD**[a,*]

Nora Katabi: katabin@makcc.org; Cristina R. Antonescu: antonesc@mskcc.org

[a]Department of Pathology, Memorial Sloan-Kettering Cancer Center, New York, NY, 10065

[b]Department of Oncology, Memorial Sloan-Kettering Cancer Center, New York, NY, 10065

Summary

Carcinoma ex-pleomorphic adenoma (CA ex-PA) is a malignant salivary gland tumor that arises in association with pleomorphic adenoma (PA). Both PA and CA ex-PA have a broad spectrum of histology, and distinction from their histologic mimics may be difficult based on morphology alone. *PLAG1* and *HMGA2* abnormalities are the most common genetic events in both PA and CA ex-PA; however, the use of *PLAG1* and *HMGA2* as adjunct molecular tests has not been well established. Fluorescence in situ hybridization for *PLAG1* and *HMGA2* was performed on 22 CA ex-PA (10 myoepithelial carcinomas [MECAs], 10 salivary duct carcinomas [SDCs], 1 carcinoma with squamoglandular features, and 1 mixed MECA-adenocarcinoma not otherwise specified), 20 de novo carcinomas (11 MECAs and 9 SDCs), 16 PAs, and 11 PA-histologic mimics. All except 3 CAs ex-PA (86%) were positive for *PLAG1* or *HMGA2* rearrangements/amplifications. In contrast, 18 (90%) of 20 de novo carcinomas lacked abnormalities in *PLAG1* or *HMGA2* ($P < .01$). *PLAG1* or *HMGA2* rearrangements were identified in 6 (67%) of 9 hypocellular myxoid PAs and in 2 (29%) of 7 cellular PAs. Furthermore, all morphologic mimics of PA were negative for *PLAG1* or *HMGA2*. *PLAG1* and *HMGA2* rearrangements are the most common genetic events in CA ex-PA regardless of the histologic subtype. Unlike CA ex-PA, de novo carcinomas were negative for *PLAG1* and *HMGA2*. Interestingly, rearrangements of *PLAG1*/*HMGA2* were identified in most hypocellular PAs but only in a small subset of cellular PAs. Fluorescence in situ hybridization for *PLAG1* or *HMGA2* can be used to distinguish between PA and CA ex-PA and their morphologic mimics.

Keywords

Carcinoma ex pleomorphic adenoma; Myoepithelial carcinoma; Salivary duct carcinoma; PLAG1; HMGA2

☆Competing interests: None.

*Corresponding authors. Memorial Sloan-Kettering Cancer Center, 1275 York Ave, New York, NY 10021.

Supplementary data

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1. Introduction

Carcinoma ex-pleomorphic Adenoma (CA ex-PA) is a rare malignant neoplasm that arises in association with primary or recurrent pleomorphic adenoma (PA) [1,2]. Typically, they are high grade and show similar histology to their de novo counterparts. Salivary duct carcinoma (SDC) is the most common histologic subtype of CA ex-PA, followed by myoepithelial carcinoma (MECAs) [2,3]. However, any histologic subtype of salivary gland carcinoma can arise in association with PA [2,3]. CA ex-PA is classified based on the degree of tumor invasion through the preexistent PA capsule into the surrounding tissue as noninvasive, minimally invasive, and widely invasive [1]. The extent of invasion has been found to correlate with the clinical outcome in many studies [1,3,4]. Typically, no metastases or recurrences are found in patients with noninvasive/minimally invasive CA ex-PA in contrast to widely invasive tumors [1,4].

At the molecular level, rearrangements of *PLAG1* gene (*pleomorphic adenoma gene 1*), a developmentally regulated zinc-finger proto-oncogene, located on 8q12 chromosomal region, are common abnormalities in PA and CA ex-PA [5-7]. The *PLAG1* fusion partner genes include *CTNNB1* (*β*-catenin), *LIFR* (leukemia inhibitory factor receptor), and *FGFR1* (fibroblast growth factor receptor 1) [5,7-10]. Moreover, *HMGA2* gene rearrangements with or without amplifications have been described in PA as well as in CA ex-PA [11]. Nevertheless, the diagnostic role of *PLAG1* and *HMGA2* abnormalities in salivary gland tumors has not been well established yet. The objectives of this study are to evaluate the morphologic spectrum of CA ex-PA in relation to its genetic features and examine the potential genetic link between CA ex-PA and its de novo counterparts. Furthermore, we sought to study *PLAG1* and *HMGA2* alterations in histologically different types of PAs and their morphologic mimics and investigate the presence of *EWSR1* rearrangements in the latter.

2. Materials and methods

2.1. Tumors characteristics

A total of 66 salivary gland tumors (19 CA ex-PAs, 20 de novo carcinomas, 16 PAs, and 11 histologic mimics of PAs) were selected from the Department of Pathology files of Memorial Sloan-Kettering Cancer Center. Tumors were assessed for their anatomical location and size. The histologic type of the malignant component in CA ex-PA was defined according to the World Health Organization classification of salivary gland tumors [1]. The tumor was considered as myoepithelial if it was composed of at least 90% myoepithelial cells, displaying the typical histology of MECA, and/or showing immunohistochemical evidence of myoepithelial differentiation, that is, positivity for a keratin marker (Cam5.2, CK7, AE1/3) and at least one of the myoepithelial markers (S100, calponin, p63, or smooth muscle actin). CA ex-PAs were classified into noninvasive, minimally invasive, and widely invasive based on the World Health Organization criteria [1]. The percentage of the residual parent PA in CA ex-PA was estimated. Histologic analysis of CA ex-PAs was performed to include the following: mitotic rate, nuclear pleomorphism, tumor necrosis, and morphologic features of the PA component. In addition, PAs were assessed for cellularity, ductal, and myoepithelial differentiation; cell type; and matrix formation.

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2.2. Fluorescence in situ hybridization

Fluorescence in situ hybridization (FISH) on interphase nuclei from paraffin-embedded 4-μm sections was performed using custom probes of bacterial artificial chromosomes (BACs), flanking *EWSR1* in 22q12, *PLAG1* on 8q12, *HMGA2* on 12q14, *CTNNB1* on 3p21, *LIFR* on 5p13, and *FGFR1* on 8p11 (Supplementary Table 1). BAC clones were chosen according to University of California, Santa Cruz genome browser (http://genome.uscs.edu). The BAC clones were obtained from BACPAC sources of Children's Hospital of Oakland Research Institute (Oakland, CA; http://bacpac.chori.org). DNA from individual BACs was isolated according to the manufacturer's instructions, labeled with different fluorochromes in a nick translation reaction, denatured, and hybridized to pretreated slides. Slides were then incubated, washed, and mounted with DAPI in an antifade solution, as previously described. Each BAC was validated for approximate cytogenetic location and specificity by hybridizing to normal human metaphases. Two hundred successive nuclei were examined using a Zeiss fluorescence microscope (Zeiss Axioplan, Oberkochen, Germany), controlled by Isis 5 software (ISIS) (Metasystems, Waltham, MA). A positive score was interpreted when at least 20% of the nuclei showed a break-apart signal. Nuclei with incomplete set of signals were omitted from the score.

3. Results

3.1. Clinicopathological characteristics

3.1.1. CA ex-PA cases—Table 1 shows the demographic and histologic features of CA ex-PA cases. The histologic types of the malignant component of the 22 CA ex-PA cases were MECA (10 cases), SDC (10 cases), carcinoma with squamoglandular features (1 case), and mixed MECA and adenocarcinoma not otherwise specified (NOS; 1 case). Detailed clinical and histologic data were available in all except 2 outside cases. All CA ex-PA tumors except 3 were located in the parotid gland. The nonparotid tumors were located in the base of tongue, the nasal cavity, and the lacrimal gland. In 2 cases, carcinoma was associated with a recurrent PA. The extent of invasion was classified as widely invasive in all cases except one, which was noninvasive. All SDCs were high grade showing increased mitotic activity and tumor necrosis (Fig. 1A). On the other hand, MECAs showed a wide range of mitotic activities ranging from 2 to 22 mitoses per 10 high-power fields (HPFs). Nuclear pleomorphism was noted in all CA ex-PA cases except 4. Tumor necrosis was identified in all MECAs (Fig. 1B), except for 2 cases, one of which showed low mitotic activity (2/10 HPFs) and bland nuclear features, whereas the second case had increased mitotic activity of 12/10 HPFs. The benign PA component was assessed in 19 cases, comprising a variable proportion of the entire neoplasm, ranging from less than 5% to 70% and being composed of benign epithelial and stromal/mesenchymal elements. The PA component was found either as a well-demarcated area (Fig. 1C) from the malignant component or intermixed with the malignant component. Extensive stromal hyalinization was noted in 61% (11/19) of the PA components. Among these, 3 were composed only of a discrete hyalinized stromal nodule without definite benign ductal elements (Fig. 1D).

3.1.2. De novo carcinomas—The de novo carcinoma group included 11 MECAs and 9 SDCs. There was no statistically significant difference in the frequency of nuclear

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pleomorphism, mitotic activity, and presence/absence of tumor necrosis between CA ex-PA and de novo carcinoma groups.

3.1.3. Pleomorphic adenomas—Typical PA morphology with hypocellular myxoid matrix and ductal/tubular differentiation was identified in 9 cases (Fig. 2A). Seven PAs were cellular composed of monomorphic myoepithelial/mesenchymal cells with occasional ductal structures and focal stromal matrix (Fig. 2B).

3.2. Immunohistochemical studies

Review of the immunohistochemical studies were performed on 18 of 21 MECAs (CA ex-PA and de novo), and the carcinomas were positive for a cytokeratin and at least one of the myoepithelial markers (S100, calponin, p63, and smooth muscle actin). The other 3 cases showed the typical morphologic appearance of MECA.

3.3. FISH results

3.3.1. Carcinoma ex-pleomorphic adenoma—Most CA ex-PA cases (19/22; 86%) showed abnormalities of either *PLAG1* or *HMGA2* by FISH analysis (Table 2). Most tumors showed greater than 50% break-apart signals (range, 30%-85%). The 3 negative CA ex-PAs were of SDC subtype and showed a clear-cut benign PA component. Sixteen (73%) CA ex-PA tumors showed *PLAG1* gene rearrangements with or without amplifications (Fig. 3A). Among the 16 *PLAG1*-rearranged CA ex-PA cases, 12 showed an unbalanced pattern, being associated with deletion of telomeric parts (n = 5), centromeric amplification (n = 3), centromeric amplification and deletion of telomeric parts (n = 1), amplifications of both centromeric and telomeric ends (n = 1), and deletion of centromeric parts (n = 2). *HMGA2* rearrangements were identified in 3 of 8 CA ex-PA tumors (Fig. 3B), all of which were associated with an unbalanced pattern, showing in one case each telomeric amplification, centromeric amplification, and amplification of both telomeric and centromeric regions. The 3 CA ex-PA tumors associated with a burned-out hyalinized nodule were positive for *PLAG1* rearrangements. In 2 cases, the carcinoma and the coexisting PA components were analyzed separately showing the following: one showed *PLAG1* rearrangements in both benign and malignant components, whereas the other showed *HMGA2* rearrangements and amplification only in the carcinoma component.

In addition, FISH was performed on 14 of 16 *PLAG1*-rearranged carcinomas to identify potential fusion partners, including *FGFR1*, *CTNNB1*, and *LIFR*. Two of the 11 tested tumors showed *CTNNB1* rearrangements with an unbalanced telomeric loss (Fig. 3C). *FGFR1* gene rearrangements were identified in 5 of 6 CAs ex-PA of MECA type (Fig. 3D), but in none of the 3 SDC ex-PA cases tested. All the rearranged *FGFR1* tumors showed an unbalanced pattern, which correlated with the unbalanced *PLAG1* rearrangements. The 4 examined CA ex-PA tumors for *LIFR* abnormalities were negative.

3.3.2. De novo carcinoma—All except 2 (90%) de novo carcinomas (10 MECAs and 8 SDCs) lacked abnormalities in *PLAG1* and *HMGA2* genes ($P < .01$; Table 3). One de novo carcinoma showed *PLAG1* rearrangement and 1 showed *HMGA2* rearrangement, and both carcinomas were salivary duct type. *FGFR1* gene rearrangements were investigated in 3 de

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novo MECAs and 1 myoepithelioma, but no gene abnormalities were found. Furthermore, none of the tested MECAs (8 CA ex-PAs and 4 de novo carcinomas) showed *EWSR1* gene rearrangements.

3.3.3. PA and histologic mimics—*PLAG1* or *HMGA2* rearrangements were identified in 6 (67%) of 9 typical PAs showing myxoid matrix and in 2 (29%) of 7 cellular PAs (Table 4). Eight (50%) of 16 PAs showed *PLAG1* or *HMGA2* gene rearrangements by FISH analysis (Table 4). All tumors showed a balanced gene rearrangement, except for 2 cases, which showed *PLAG1* rearrangement with associated centromeric amplification in one case, whereas the other showed *HMGA2* gene rearrangements with deletion of the telomeric parts. All tumors in the control group including 2 epithelial MECAs (EMCs), 2 basal cell adenocarcinomas, and 7 polymorphous low-grade adenocarcinomas (PLGAs) were negative for *PLAG1* and *HMGA2* abnormalities (Table 5). In addition, no *EWSR1* rearrangements were detected in 1 myoepithelioma and 6 PLGAs tested.

4. Discussion

In this study, we examined *PLAG1* and *HMGA2* gene alternations in different histologic subtypes of CA ex-PA, their de novo counterparts, and the entities that enter in their differential diagnosis. CA ex-PA is a carcinoma that arises in association with a coexisting PA component or a prior excised PA at the same site [1,2]. Occasionally, the carcinoma component might completely efface the preexistent benign PA. In some cases, only a hyalinized nodule without epithelial elements might be seen. This raises the possibility of a preexisting PA component, but pathologists are often reluctant to accept as enough evidence for a diagnosis of CA ex-PA in these cases. CA ex-PA is typically aggressive and might have worse prognosis compared with its de novo counterparts, especially in cases of MECA [12]. Moreover, CA ex-PA and, to a higher extent, PA have a broad spectrum of histologic features, and distinction from their histologic mimics such as EMC, PLGA, and basal cell adenoma/adenocarcinoma may be difficult based on morphology alone. Therefore, evaluation of genetic alterations by FISH ancillary test could represent a valuable tool in the diagnostically challenging cases.

Genetic alterations of *PLAG1* and *HMGA2* genes, including gene rearrangements and amplifications, have been previously described in PA [11,13,14]. These genetic abnormalities have been also identified in histologically similar soft tissue tumors [15,16] and CA ex-PA [6,17]. In the study by Bahrami et al [15], 12 (63%) of 19 CAs ex-PAs were positive for *PLAG1* rearrangements and 1 of 3 cases were positive for *HMGA2* rearrangements by FISH analysis. In accordance with the study by Bahrami et al, we found *PLAG1* and/or *HMGA2* rearrangements in 19 (86%) of 22 CAs ex-PAs. However, in contrast with the study by Bahrami et al, which covered a wider histologic spectrum of CA ex-PA types (adenocarcinoma NOS, SDC, MECA, EMC, squamous cell carcinoma, and carcinosarcoma), our work focused mainly on the 2 most common histologic types of CA ex-PA (SDC and MECA) [2,3,15]. The 3 *PLAG1*/ *HMGA2*-negative CA ex-PA cases in our study were of SDC type showing an overt benign PA component, suggesting either undetectable *PLAG1* abnormalities by FISH resolution or alternative oncogenic events. Interestingly, in 2 cases analyzed, *PLAG1* gene rearrangements were present in both the

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benign and malignant components in one case, whereas in the other, *HMGA2* gene rearrangement and amplification were present only in the malignant component. Similar to the results in Bahrami et al [18], *PLAG1* and/or *HMGA2* alterations were found in 4 (50%) of 8 CA ex-PAs with SDC morphology. Furthermore, *PLAG1* or *HMGA2* FISH abnormalities were detected in 8 (80%) of 10 SDCs containing a hyalinized nodule rather than a recognizable PA, in keeping with a preexisting, but burned-out, PA [18]. Our findings further support this assumption, with all 3 CA ex-PA tumors associated with a hyalinized nodule being positive for *PLAG1* rearrangements. This result has a diagnostic impact in the surgical pathology of salivary gland as indicated earlier. As reported previously, we found *CTNNB1* rearrangements in 2 SDC ex-PA cases, in keeping with a t(3;8)(p21;q12) [5,7,9,10]. In addition, the presence of t(8;8)(q12.1;p12), resulting in *FGFR1-PLAG1* fusion has been identified in a subgroup of PA [19]. Of interest and in keeping with our prior data, *FGFR1* rearrangements (often associated with telomeric deletion and centromeric amplifications) were identified in 5 of 6 *PLAG1*-rearranged MECAs ex-PA, but not in de novo myoepithelial tumors [16].

In this study, we investigated the potential genetic link between CA ex-PAs and their de novo counterparts. Also, in contrast to CA ex-PA, all but 2 de novo carcinomas (90%) lacked abnormalities in *PLAG1* or *HMGA2* genes ($P < .01$). These 2 latter cases might have had a PA component that was not sampled or was completely obscured by the malignant component. Recently, we found that myoepithelial CA ex-PA correlates with a worse clinical behavior compared with de novo MECA [12]. This highlights the valuable role of this FISH assay in separating CA ex-PA from de novo counterparts and therefore improves tumor stratification.

PA has a broad spectrum of morphologic features, and distinction from its histologic mimics may be challenging. Furthermore, a subset of PA, which is usually cellular and often showing one predominant uniform cell type, can morphologically resemble other salivary gland tumors, in particular PLGA. In this current study, we selected 9 cases of typical PA showing hypocellular myxoid stroma with ductal and myoepithelial elements and 7 cases of cellular PA with a dominant 1-cell type. Interestingly, *PLAG1* or *HMGA2* rearrangements were identified in most (78%) hypocellular myxoid PAs, but only in one-third (29%) of cellular PAs. This implies that cellular PA might represent a discrete genetic subgroup; however, additional molecular and immunohistochemical studies are required to characterize this further. In contrast, all morphologic mimics of PA and CA ex-PA, including PLGA, EMC, and basal cell adenoma/adenocarcinoma, were negative for *PLAG1* or *HMGA2* abnormalities. Given the positive predictive value, this assay can therefore be used to distinguish PA from its morphologic mimics.

In our previous study, we have found *PLAG1* gene rearrangements in a significant proportion of cutaneous and benign soft tissue myoepithelial tumors that are associated with tubuloductal differentiation and morphologically resemble salivary PA [16]. In addition to the shared *PLAG1* and *HMGA2* gene rearrangements between soft tissue myoepithelial tumors and PA of salivary gland, *EWSR1* gene rearrangements have been reported as a common event in myoepithelial tumors arising outside salivary glands and in clear cell hyalinizing carcinoma of salivary gland [20,21]. In this study, none of the tested salivary

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myoepithelial tumors (8 CA ex-PAs, 4 de novo carcinomas, and 1 myoepithelioma) showed *EWSR1* gene abnormalities, confirming previously reported findings [8,21].

Given the morphologic similarities between PLGA and myoepithelial tumors, we also examined *EWSR1* gene alterations by FISH in 6 PLGAs, but none were positive.

In summary, *PLAG1* and *HMGA2* gene abnormalities are the most common genetic events in different histologic subtypes of CA ex-PA. *PLAG1*/*HMGA2* rearrangements were identified in most hypocellular PAs, but only in a small subset of cellular PAs. In contrast to CA ex-PA, most de novo SDC and MECA were negative for *PLAG1* and *HMGA2* gene abnormalities. FISH ancillary tests for *PLAG1* or *HMGA2* gene alterations can be used to distinguish between CA ex-PA and its de novo counterparts as well as separate PA from its morphologic mimics.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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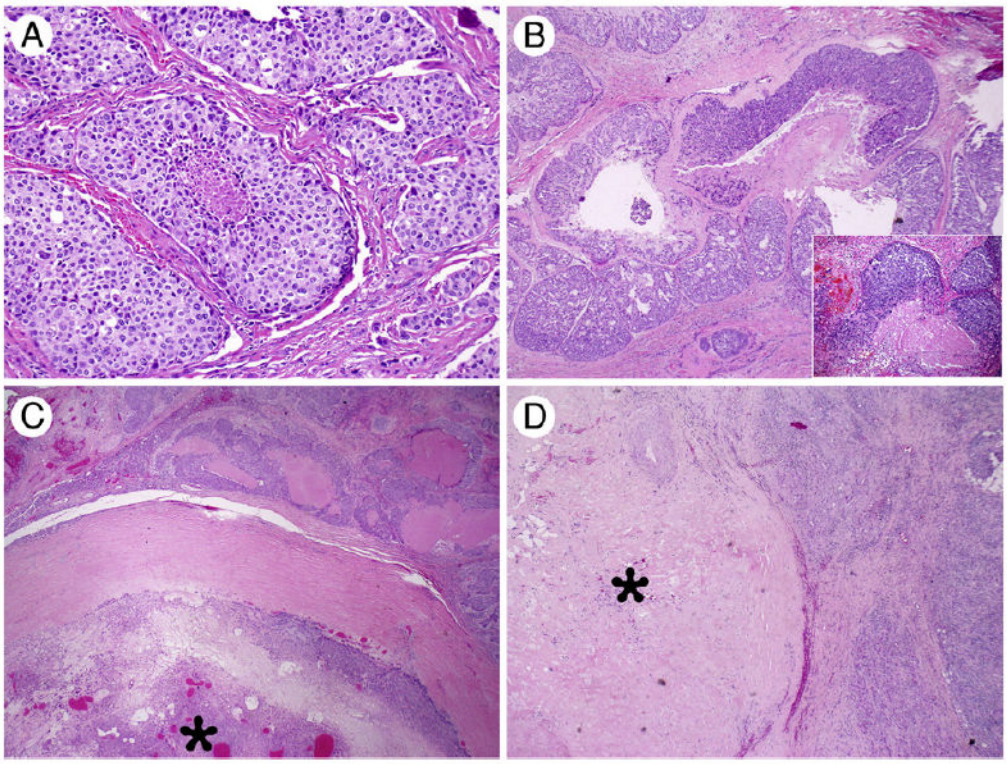

Fig. 1.

Morphologic spectrum of CA ex-PA. A, High-grade SDC type: the tumor cells show abundant eosinophilic cytoplasm and prominent nuclei, arranging in a solid growth pattern with central necrosis (×200). B, MECA with multinodular growth pattern and tumor necrosis (×40) with an inset (×400) showing high-power view of the tumor with hyalinized necrotic stroma and pseudoglandular spaces. C, CA ex-PA associated with a well-demarcated PA component (star; ×20). D, CA ex-PA in which only a discrete hyalinized stromal nodule (star) is noted, most likely representing a burned-out PA (×20).

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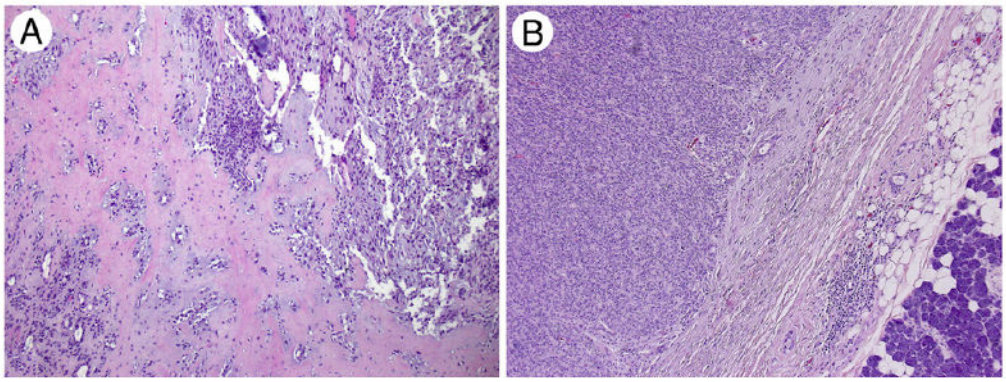

Fig. 2.

PA phenotype. A, Typical PA showing hypocellular proliferation of ductal and myoepithelial cells with a hyalinized/myxoid stroma (×10). B, Cellular PA with no stromal matrix (×40).

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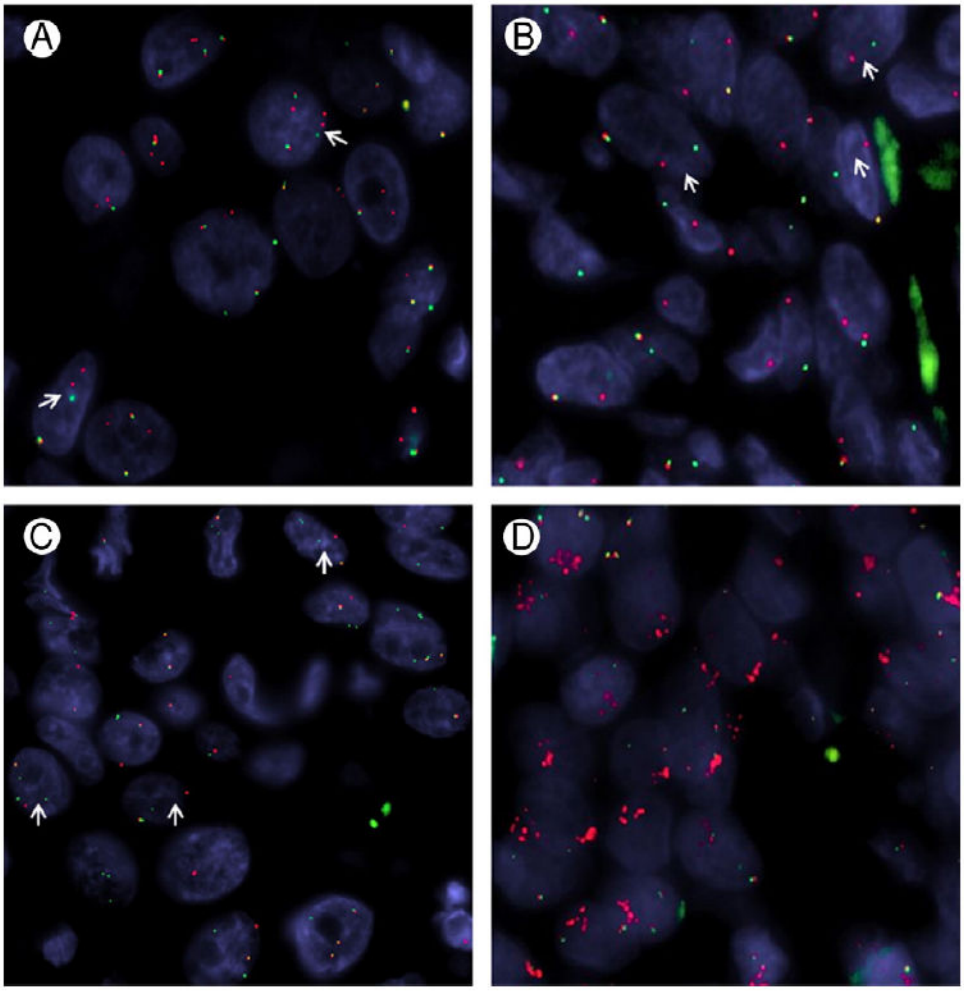

Fig. 3.

PLAG1, *HMGA2*, *CTNNB1*, and *FGFR1* gene abnormalities by FISH. A, *PLAG1* gene rearrangement (arrows) in an SDC CA ex-PA (CA ex-PA #12). B, *HMGA2* break-apart (arrows) in a typical PA with hypocellular matrix (PA #5). C, SDC-type CA ex-PA showing a *CTNNB1* gene rearrangement (arrows; CA ex-PA #12). D, Unbalanced *FGFR1* rearrangement pattern associated with telomeric deletion (green signal) and subsequent centromeric amplification (red signal), MECA ex-PA (CA ex-PA #2).

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Table 1

Clinicopathological and demographic characteristics of CA ex-PAs

No.	Age (y)/Sex	Location	Size (cm)	Subtype	Mitoses/10 HPFs	Necrosis	Nuclear pleomorphism	INV	PA%
1	47/F	Parotid	1.3	MECA	2	No	No	WI	15
2	41/M	Parotid	9.5	MECA	1	Yes	No	WI	<5
3	67/F	Parotid	1.1	MECA	12	No	Yes	WI	10
4	77/M	BOT	2.1	MECA	22	Yes	No	WI	15
5	67/F	Parotid	6	MECA	5	Yes	Yes	WI	<5
6r	63/F	Parotid	NA	MECA	NA	NA	NA	NA	NA [a]
7	64/M	Parotid	3	MECA	2	Yes	Yes	WI	NA [a]
8	F	Parotid	NA	MECA	6	Yes	No	WI	20
9	76/M	Parotid	3.5	MECA	7	Yes	Yes	WI	30
10	NA	NA	NA	MECA	NA	NA	NA	NA	NA [a]
11	67/F	Parotid	4.2	SDC	16	Yes	Yes	WI	25
12	63/M	Parotid	1.4	SDC	8	Yes	Yes	WI	10 [b]
13	75/M	Parotid	2	SDC	8	Yes	No	WI	60
14	67/F	Parotid	4.4	SDC	3	Yes	Yes	WI	10
15	70/M	Parotid	1.2	SDC	6	Yes	No	WI	15
16	51/M	Parotid	3.4	SDC	20	Yes	Yes	WI	30
17	87/F	Parotid	2.4	SDC	9	Yes	Yes	WI	5 [b]
18	43/M	Parotid	1.5	SDC	4	Yes	Yes	WI	30
19	68/F	Parotid	2	SDC	3	Yes	Yes	WI	70
20	98/M	Parotid	2.7	SDC	10	Yes	Yes	WI	5 [b]
21	74/F	Lacrimal gland	4.2	CA-SG	16	Yes	Yes	WI	25
22	35/M	Parotid	5.8	MECA and ACA NOS	6	Yes	Yes	NI	40

Abbreviations: INV, invasion; BOT, base of tongue; NA, not available; PA, pleomorphic adenoma; MECA, myoepithelial carcinoma; SDC, salivary duct carcinoma; F, female; M, male; WI, widely invasive; BOT, base of tongue; NA: not applicable; CA-SG, carcinoma with squamoglandular features; ACA NOS, adenocarcinoma not otherwise specified; WI, widely invasive; NI, noninvasive.

[a] The prior PA at the same site was not available for review.

[b] Hyalinized nodule.

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Table 2

Molecular characteristics of CA ex-PAs

No.	Subtype	*PLAG1*	*HMGA2*	*FGFR1*	*LIFR*	*CTNNB1*	*EWSR1*
1	MECA	Rearranged	Neg	Neg	Neg	Neg	Neg
2	MECA	Rearranged and C′ amp	NP	Rearranged and C′ amp	NP	NP	Neg
3	MECA	Rearranged and C′ amp	NP	Rearranged and C′ amp and T′ del	NP	NP	NP
4	MECA	Neg	Rearranged and T′ amp	NP	NP	NP	Neg
5	MECA	Neg [a]	Rearranged and C′T′ amp [b]	NP	NP	NP	Neg
6	MECA	Rearranged and C′ amp	Neg	Rearranged and C′ amp	Neg	Neg	Neg
7	MECA	Rearranged and C′/T′ amp	NP	Rearranged and C′ amp	Neg	Neg	Neg
8	MECA	Rearranged and T′ del and C′ amp NP	Rearranged and C′ amp	NP	NP	NP	
9	MECA	Rearranged [a]	NP	NP	NP	NP	NP
10	MECA	Neg	Rearranged and C′ amp	NP	NP	NP	Neg
11	SDC	Rearranged and T′ del	NP	Neg	NP	Rearranged	NP
12	SDC	Rearranged and T′ del	NP	Neg	NP	Rearranged	NP
13	SDC	Rearranged	NP	Neg	Neg	Neg	NP
14	SDC	Neg	Neg	NP	NP	NP	NP
15	SDC	Neg	Neg	NP	NP	NP	NP
16	SDC	Rearranged and T′ del	NP	NP	NP	Neg	NP
17	SDC	Rearranged and C′ del	NP	NP	NP	NP	NP
18	SDC	Rearranged and T′ del	NP	NP	NP	Neg	NP
19	SDC	Neg	Neg	NP	NP	NP	NP
20	SDC	Rearranged	NP	NP	NP	Neg	NP
21	CA-SG	Rearranged and C′ del	NP	NP	NP	Neg	NP
22	MECA and ACA	Rearranged and T′ del	NP	NP	NP	Neg	NP

Abbreviations: Neg, negative; C′, centromeric; amp, amplified; NP, not performed; T’, telomeric; del, deletion; MECA, myoepithelial carcinoma; SDC, salivary duct carcinoma; CA-SG, carcinoma with squamoglandular features; ACA, adenocarcinoma.

[a] Present in both carcinoma and PA.

[b] Present in carcinoma but not in PA component.

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Table 3

Molecular characteristics of de novo MECA and SDC

No.	Histology	*PLAG1*	*HMGA2*	*EWSR1*
1	MECA	Neg	Neg	Neg
2	MECA	Neg	Neg	Neg
3	MECA	Neg	Neg	Neg
4	MECA	Neg	Neg	Neg
5	MECA	Neg	Neg	NP
6	MECA	Neg	Neg	NP
7	MECA	Neg	Neg	NP
8	MECA	Neg	Neg	NP
9	MECA	Neg	Neg	NP
10	MECA	Neg	Rearranged	NP
11	MECA	NA	Neg	NP
12	SDC	Neg	Neg	NP
13	SDC	Neg	Neg	NP
14	SDC	Rearranged and C′ del	NP	NP
15	SDC	Neg	Neg	NP
16	SDC	Neg	Neg	NP
17	SDC	Neg	Neg	NP
18	SDC	Neg	Neg	NP
19	SDC	Neg	Neg	NP
20	SDC	Neg	Neg	NP

Abbreviations: MECA, myoepithelial carcinoma; SDC, salivary duct carcinoma; Neg, negative; NP, not performed; NA, not applicable; C′ del, deletion of centromeric part.

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Table 4

Histologic and molecular characteristics of PAs

No.	Cellular [a]	*PLGA1*	*HMGA2*	*CTNNB1*	*FGFR1*
1	No	Rearranged	NP	Rearranged	Neg
2	No	Neg	Neg	NP	NP
3	No	Neg	Neg	NP	NP
4	No	Neg	Rearranged	NP	NP
5	No	Neg	Rearranged	NP	NP
6	No	Neg	Neg	NP	NP
7	No	Rearranged	NP	NP	NP
8	No	Rearranged	NP	Neg	NP
9	No	Rearranged	NP	Rearranged	NP
10	Yes	Rearranged	NP	NP	NP
11	Yes	Neg	Neg	NP	NP
12	Yes	Rearranged/C′ amp	NP	NP	Rearranged/T del
13	Yes	Neg	Neg	NP	NP
14	Yes	Neg	Neg	NP	NP
15	Yes	Neg	Neg	NP	NP
16	Yes	Neg	Neg	NP	NP

Abbreviations: NP, not performed; Neg, negative; C′, centromeric; amp: amplified; T del, deletion of telomeric part.

[a] Cellular PAs are predominantly monomorphic with a predominant 1 cell type with focal stromal matrix.

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Table 5

Molecular features of control group

DX	n	*PLAG1*	*HMGA2*	*MYB*
EMC	2	Neg	Neg	NP
PLGA	7	Neg	Neg	Neg
BACA	2	Neg	Neg	NP

Abbreviations: DX, diagnosis; EMC, epithelial-myoepithelial carcinoma; PLGA, polymorphous low-grade adenocarcinoma; BACA, basal cell adenocarcinoma; Neg, negative; NP, not performed; BACA, basal cell adenocarcinoma.