Lacrimal Gland Pleomorphic Adenoma and Carcinoma ex Pleomorphic Adenoma

Genomic Profiles, Gene Fusions, and Clinical Characteristics

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Purpose: To study genetic alterations in lacrimal gland pleomorphic adenoma (PA) and carcinoma ex pleomorphic adenoma (Ca-ex-PA) with focus on copy number changes and expression patterns of the translocation target genes *PLAG1*, *HMGA2,* and *CRTC1-MAML2* in relation to clinical data.

Design: Experimental study.

Participants: A total of 36 tumors from 32 patients with lacrimal gland PA or Ca-ex-PA were included in the study.

Methods: Genome wide, high-resolution array-based comparative genomic hybridization (arrayCGH) and immunohistochemistry were used to study the genomic profiles and expression patterns of the translocation targets PLAG1, HMGA2, and CRTC1-MAML2.

Main Outcome Measures: Copy number alterations (gains/losses) and protein expression of PLAG1, HMGA2, and CRTC1-MAML2.

Results: Genome-wide arrayCGH analysis revealed normal genomic profiles in 10 of 17 PA samples. The average number of genomic imbalances per tumor was 3.25 (range, 1−7) in primary and recurrent PAs with alterations compared with 7.7 (range, 4−12) in Ca-ex-PAs. Five recurrent copy number alterations were identified in PAs, including losses of 1pter-p31.3, 6q22.1-q24.3, 8q24.22-q24.3, and 13q21.31-q21.33, and gain of 9p23-p22.3. Gain of 9p23-p22.3 also was seen in a Ca-ex-PA. In Ca-ex-PA, gain of 22q12.3-qter was the only recurrent alteration. Detailed analysis of the array data identified *NFIB* and *PDGFB* as the 2 major candidate target oncogenes that may be activated as a result of copy number gains involving 9p and 22q. Both genes have been implicated in the pathogenesis of PA and other types of salivary gland tumors. Immunohistochemical analysis revealed frequent overexpression of the translocation target gene *PLAG1* in PAs and in 1 Ca-ex-PA. In contrast, overexpression of *HMGA2* was observed in only a small subset of PAs. The CRTC1-MAML2 fusion oncoprotein was overexpressed in 2 mucoepidermoid Ca-ex-PAs.

Conclusions: Lacrimal and salivary gland PAs and Ca-ex-PAs have similar genomic profiles and frequently overexpress the PLAG1 oncoprotein. Copy number gains involving 9p23-p22.3 (*NFIB*) and 22q12-qter (*PDGFB*) may be of importance for disease progression in a subset of lacrimal gland PAs. *Ophthalmology 2014;121:1125-1133 © 2014 by the American Academy of Ophthalmology.*

Pleomorphic adenoma (PA) is the most common epithelial tumor of the lacrimal gland.[1−3] It is a benign, slow-growing tumor that is clinically and histopathologically virtually identical to its salivary gland counterpart. The mean age at presentation is 40 years, but the tumors may occur in all age groups.[4] Histopathologically, PAs show a remarkable degree of morphologic diversity, including epithelial and myoepithelial cells forming a variety of growth patterns in an often mucoid/myxoid or chondroid matrix. Treatment consists of surgical excision, and if the tumor is completely removed, the prognosis is excellent. However, PAs have a tendency for local recurrence and may undergo malignant transformation to carcinoma ex pleomorphic adenoma (Ca-ex-PA). The latter tumors often have a poor prognosis.[5−8]

Ca-ex-PAs constitute approximately 10% of all malignant lacrimal gland tumors.[9,10] The carcinomatous component of Ca-ex-PA may show a variety of morphologies, for example, mucoepidermoid carcinoma, adenoid cystic carcinoma, salivary duct carcinoma, and adenocarcinoma not otherwise specified (NOS). The malignant potential of Ca-ex-PA may vary considerably. In a recent survey, up to 50% of the patients developed recurrence and up to 70% developed local or distant metastases.[11] There are currently no biomarker(s) available that may help to predict the risk of malignant transformation of benign PAs.

Extensive cytogenetic studies of approximately 500 salivary gland PAs have revealed that they are characterized by a highly specific pattern of chromosome rearrangements

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Table 1. Clinical Data and Copy Number Alterations in Lacrimal Gland Pleomorphic Adenoma and Carcinoma ex Pleomorphic Adenoma

Case	Age/ Sex	Diagnosis	PLAG1 IHC	HMGA2 IHC	Array Format	arrayCGH Results		Follow-up (Years after Diagnosis)
						Gains	*Losses*	
1	NDA	PA	NDA	NDA	400K			NDA
2	NDA	PA	NDA	NDA	400K			NDA
3	57/M	PA	+	−	180K			NED (10)
4	31/M	PA	−	++	180K			NED (5)
5	57/F	PA	+	−	180K			DOC (1)
6	14/F	PA	−	−	180K			NED (30)
7	57/F	PA	−	−	180K			NED (17)
8	81/F	PA	+	−	180K			LR (2), NED (5)
9	37/M	PA	+	−	180K			LR (17), NED (28)
10	28/M	PA	++	−	400K		1pter-p31.3, 6q14.1-qter	NED (3)
11	39/M	PA	++	−	400K		7p14.1, 8q13.2-q13.3, 8q13.3, 8q21.2-q22.1, 13q21.33-q31.3	NED (3)
12	50/F	PA	++	−	400K	1p12-q41, 8p22-qter	1pter-p12, 8pter-p22, 13q21.31-q21.33	NED (3)
13	53/M	PA	−	−	180K	9p23-p22.3, 9q33.2, 11q12.2-q13.2, 12p13.2-p13.1, 12q13.13, 14q24.1, 20q13.13		NED (24)
14	64/F	PA	++	−	180K		8q11.23, 8q24.22-qter, 14q32.2-qter	NED (12)
15	71/M	PA	−	−	180K		8q24.22-q24.3	DOC (12)
16	62/F	PA	++	−	180K	7q31.2, 9p23-p22.3		LR (15), DOC (20)
17a	13/F	PA	−	−	244K			LR x 5 (2,4,17,21,24), Ca-ex-PA (32), DM (42), NED (51)
17b	30/F	PA (3rd LR)	++	−	244K			−
17c	37/F	PA (5th LR)	−	−	244K		6q22.1-q24.3	−
17d	55/F	Ca-ex-PA (DM)	−	−	244K	1q21.1-qter, 5, 11q21-qter, 21q11.2-q21.1, 22q12.3-qter	11pter-p15.2, 17q25.1	−
18	65/F	Ca-ex-PA	−	−	244K	7pter-q22.2, 10q22.3-q23.1, 10q25.1-qter, 17q12-q21.33, 19p13.13, 20p11.21-qter, 22	5q14.2-q23.3, 9p24.1-p22.1, 9p21.3-p21.2, 14q12, 14q12-q13.1	NED (5)
19a	31/F	Ca-ex-PA	+	−	NDA	NDA	NDA	LR (2), NED (20)
19b	33/F	Ca-ex-PA (LR)	−	−	244K	9p23-p22.3	3p22.1-p21.1, 9p22.3-p21.2, 9p21.2-p21.1	−
20	57/M	PA	+	++	NDA	NDA	NDA	NED (5)
21	46/F	PA	−	−	NDA	NDA	NDA	NED (7)
22	42/M	PA	+	−	NDA	NDA	NDA	NED (30)
23	44/F	PA	+	−	NDA	NDA	NDA	NED (15)
24	63/F	PA	NDA	−	NDA	NDA	NDA	NED (19)
25	37/M	PA	−	−	NDA	NDA	NDA	NED (30)
26	20/M	PA	−	−	NDA	NDA	NDA	LR x 3 (3,6,13), NED (23)
27	32/M	PA	NDA	NDA	NDA	NDA	NDA	NED (31)
28	40/M	PA	+	−	NDA	NDA	NDA	NED (16)
29	37/F	PA	NDA	−	NDA	NDA	NDA	NED (27)
30	76/F	PA	−	−	NDA	NDA	NDA	NED (4)
31	35/F	PA	+	−	NDA	NDA	NDA	LR (19), NED (28)
32	39/M	Ca-ex-PA	−	−	NDA	NDA	NDA	PA,* TRD (4)

arrayCGH = array-based comparative genomic hybridization; Ca-ex-PA = carcinoma ex pleomorphic adenoma; DM = distant metastases; DOC = dead of other causes; F = female; IHC = immunohistochemistry; LR = local recurrence; M = male; NED = no evidence of disease; NDA = no data available; PA = pleomorphic adenoma; TRD = tumor-related death.
*The patient was diagnosed with PA (sample not available for analysis) 1 year before the diagnosis of Ca-ex-PA.

including at least 4 major cytogenetic subgroups: (1) tumors with rearrangements of 8q12; (2) tumors with rearrangements of 12q14-15; (3) tumors with nonrecurrent clonal changes; and (4) tumors with an apparently normal karyotype.[12,13] We have previously shown that the target genes of the 8q12 and 12q14-15 rearrangements are the transcription factor genes *PLAG1* and *HMGA2*.[12–14] PLAG1 is a DNA-binding zinc finger protein that belongs