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ORIGINAL ARTICLE

The *HTN3-MSANTD3* Fusion Gene Defines a Subset of Acinic Cell Carcinoma of the Salivary Gland

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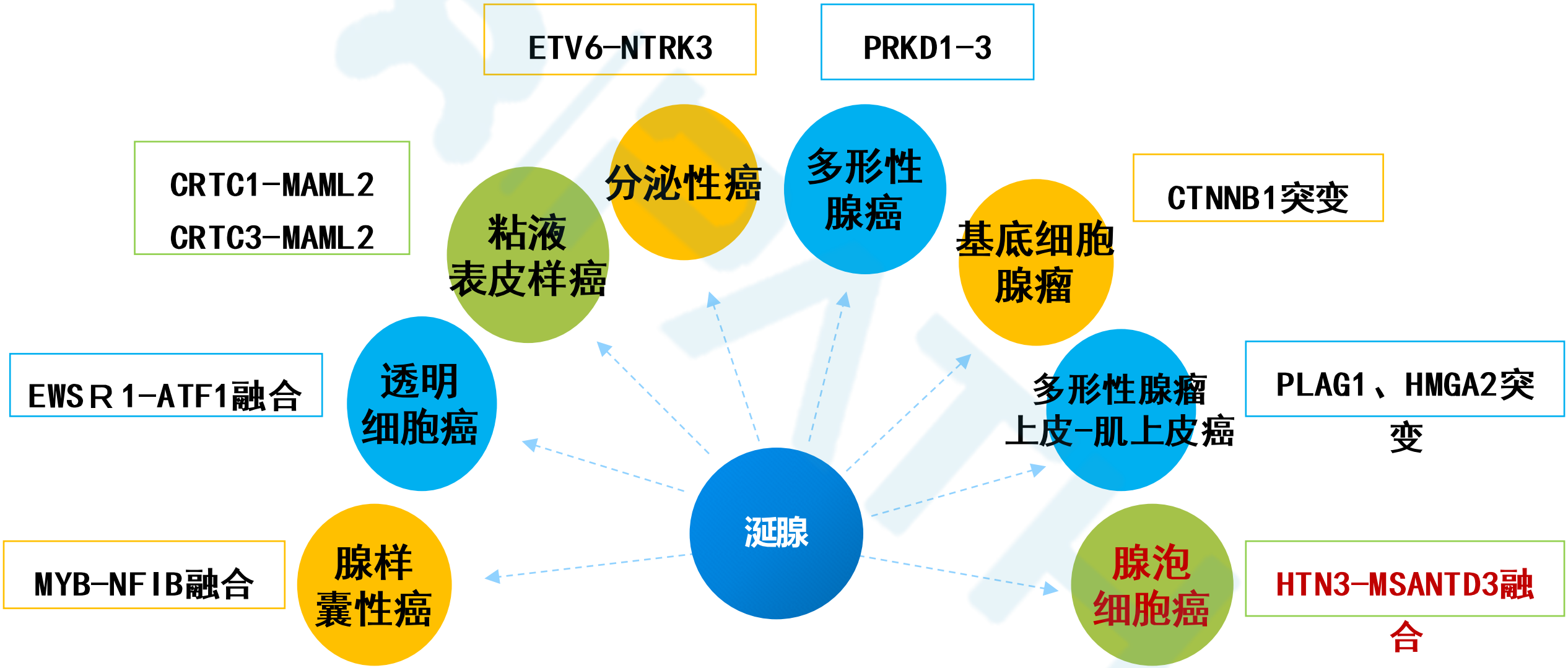
ETV6-NTRK3
PRKD1-3
CRTC1-MAML2
CRTC3-MAML2
分泌性癌
多形性腺癌
粘液表皮样癌
基底细胞腺瘤
CTNNB1突变
EWSR1-ATF1融合
透明细胞癌
多形性腺瘤
上皮-肌上皮癌
PLAG1、HMGA2突变
涎腺
MYB-NFIB融合
腺样囊性癌
腺泡细胞癌
HTN3-MSANTD3融合

涎腺腺泡细胞癌

- 占涎腺恶性肿瘤的10%左右
- 女性较男性稍常见
- 发病年龄10-70岁
- 绝大多数（90%以上）位于腮腺
- 临床表现为缓慢增大、实性、活动性肿瘤，部分伴有疼痛
- 大体表现为直径1-3cm、界限清楚的实性结节。切面分叶状，实性至囊性。
- 低度恶性，惰性的生物学行为，可转移至颈部淋巴结和肺，平均复发率约35%，转移率和因病死亡率约16%

- 病理学特征：

➢ 以腺泡样细胞和闰管样细胞为主，排列紧密，呈片状、结节状；还可见不等量的腺泡样、空泡样、透明样和非特异腺样细胞，可呈实性/小叶状、微囊性、乳头状-囊性、滤泡样结构。

➢ 腺泡细胞为大的、多角形细胞，有微嗜碱性、颗粒状胞质和圆形、偏中心的细胞核；胞质酶原样颗粒呈PAS阳性，抗淀粉酶消化；

➢ 闰管样细胞较小，嗜酸性或双嗜性，立方状，核居中；细胞围绕成大小不一的腔隙。

➢ 可见明显的间质淋巴样浸润

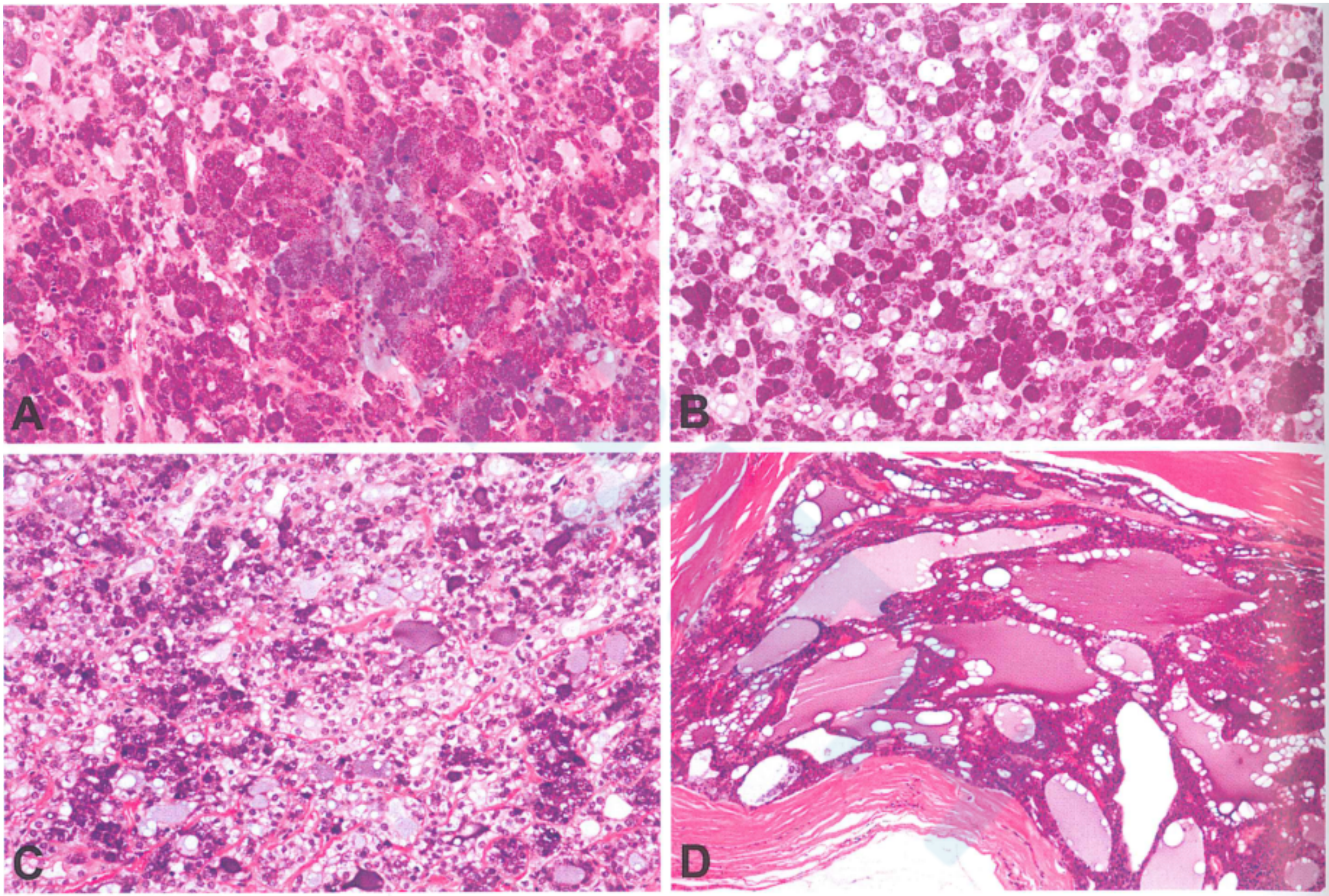

Fig. 7.07 Acinic cell carcinoma. **A** Serous acinar cell type. **B** Microcystic type: vacuolated/microcyst formation along with serous acinar cell type. **C** Clear plus serous acinar cell type. **D** Follicular growth pattern.

- 免疫表型无特异性，腺泡细胞表达DOG1，闰管样细胞表达角蛋白等
- 分子机制尚不明确

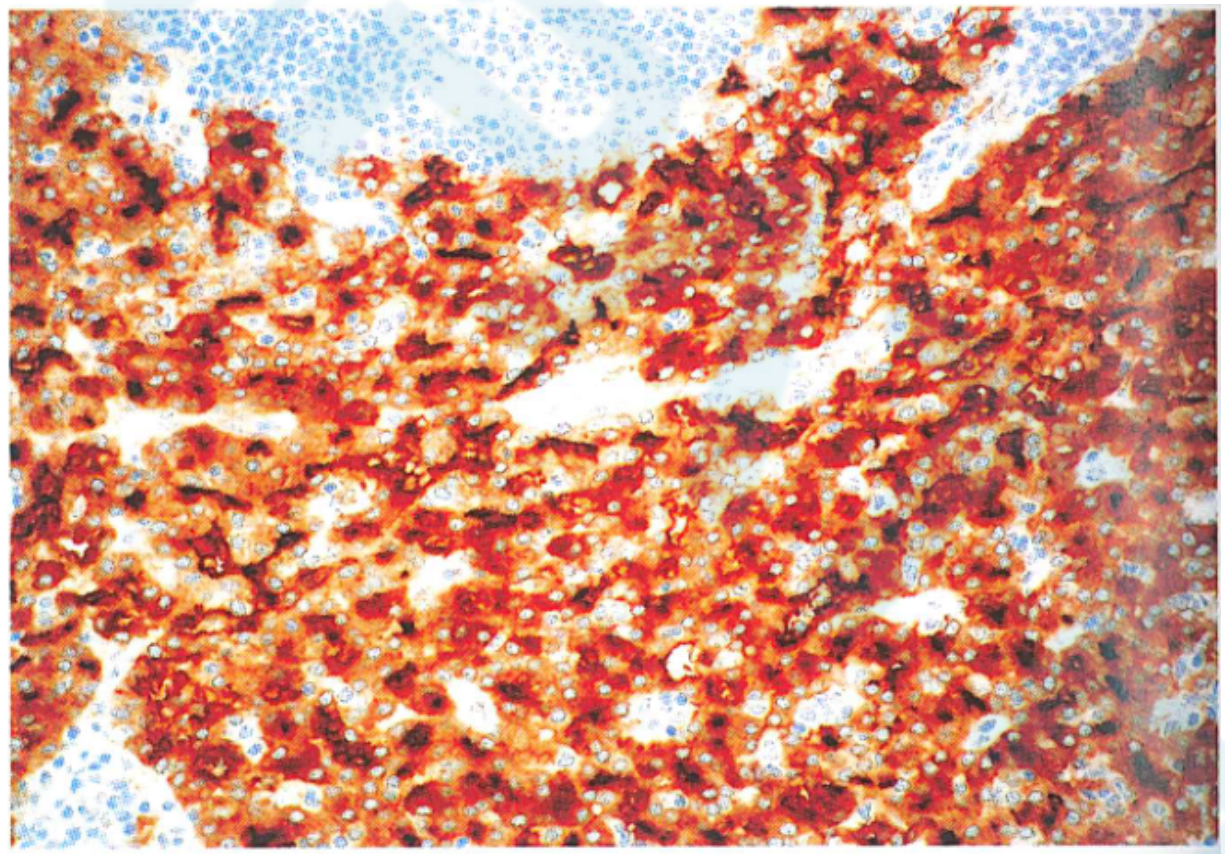

Fig. 7.08 Acinic cell carcinoma. DOG1 stains most acinic cell carcinomas.

PLoS One. 2017 Feb 17;12(2):e0171265. doi: 10.1371/journal.pone.0171265. eCollection 2017.

Recurrent rearrangements of the Myb/SANT-like DNA-binding domain containing 3 gene (MSANTD3) in salivary gland acinic cell carcinoma.

Barasch N[1], Gong X[1], Kwei KA[2], Varma S[1], Biscocho J[1], Qu K[2], Xiao N[3], Lipsick JS[1], Pelham RJ[2], West RB[1], Pollack JR[1].

- 对3例AciCC病例进行了全基因组测序，其中一例发现了一种新的HTN3-MSANTD3基因融合
- 在20例AciCC中，检测到3例(15%)MSANTD3重排
- MSANTD3免疫组化染色显示27例AciCC中，有8例(30%)呈弥漫性核表达，包含MSANTD3重排的3例。
- 新确定了MSANTD3重排是涎腺AciCC的一个重复性事件

- MSANTD3编码一种先前未被鉴定的Myb/SANT结构域蛋白。
- MSANTD3在一系列正常和肿瘤组织标本中均有不同的表达，在正常的唾液腺导管上皮细胞和其他组织学类型的唾液腺癌中均有异质表达
- HTN3基因编码富组蛋白3，富组蛋白是人类腮腺和颌下腺分泌的一组富含组氨酸的多肽，具有广谱抗菌及抗真菌作用，对维持口腔卫生有重要作
- 在先前的报道中，这2种基因都未曾重复性的出现于任何类型的恶性肿瘤中

材料和方法

- 273例AciCCs，包含Barasch et al之前报道的3例
- HE染色
- 特染（PAS、PAS+D、普鲁士蓝染色、磷钨酸苏木精染色）

✓ 磷钨酸苏木精(PTAH)染色是一种常用的结缔组织染色法，主要用于显示心肌、骨骼肌的肌纤维主要结构及病变

- 正常心肌、骨骼肌的肌纤维和横纹呈清晰的蓝色；

  变性的肌纤维颗粒、团块和收缩带呈深蓝色；

  神经胶质纤维、纤维素、线粒体、粘液等呈深蓝色；

  间质结缔组织呈浅红色或不着色，胶原、网状纤维、骨基质呈粉红色或棕红色。

Mammaglobin	304-1A5	Ready-to-use	Dako
S-100	Polyclonal	1:4,000	Dako

- FISH

  MSANTD3分离探针

  标准：每个病例计数60-100个细胞核，≥10%细胞核出现≥1个分离信号认为重排阳性，≥10%细胞核中一个或两个信号出现2个以上时认为有增多

- RNA测序

- Rt-PCR及核酸序列分析

结果

- 9例(3.3%)检测出17%--48%的肿瘤细胞存在MSANTD3分离信号
- 1个病例显示额外的1-2个绿色和红色信号
- 共11例（4%）检测出MSANTD3异常
- 除先前报道的一例HTN3-MSANTD3融合病例外，6例检测出HTN3外显子1和MSANTD3外显子2融合

TABLE 2. Demographics, Presentation, Treatment, and Outcome of Patients With *MSANTD3* Aberrated Acinic Cell Carcinoma

Case#	*MSANTD3* FISH	RT-PCR	Age (y), Sex	Site	Size (mm)	Presentation	Stage	Treatment	Outcome, follow-up (mo)
1	Break	*HTN3-MSANTD3*	59, M	Parotid	30×35×30	Parotid mass	T2N0M0	Surgery	DOC 98
2	Break	*HTN3-MSANTD3*	52, F	Parotid	80×110	Parotid mass	T3N0M0	Surgery+RT	NED 269
3	Break	*HTN3-MSANTD3*	51, F	Parotid	NA	NA	NA	NA	NA
4	Break	*HTN3-MSANTD3*	75, F	Parotid	25	Parotid mass	T2cN0M0	Surgery+RT	NED 120
5	Break	*HTN3-MSANTD3*	65, F	Parotid, accesssory	18	Buccal mass	T1cN0M0	Surgery	NED 36
6	Break	*HTN3-MSANTD3*	28, F	Parotid	32	Incidental finding on MRI	T2cN0M0	Surgery	NED 24
7	Break	—*	59, M	Submandibular	25×20×25	Submandibular mass	T2N0M0	Surgery+RT	NED 58
8†	Break	*HTN3-MSANTD3*‡	76, F	Parotid	25	NA	NA	Surgery	NA
9†	Break	NA	49, F	Parotid	12	NA	NA	Surgery	NA
10†	Break	NA	30, M	Parotid	12 (recurrence)	NA	NA	Surgery	NA
11	Break	NA	NA	Parotid	NA	NA	NA	NA	NA

*Absence of *HTN3-MSANTD3* fusion transcript by RT-PCR and uninformative RNA sequencing.
†Cases previously reported by Barasch et al[13] "Complex pattern, 1 to 2 extra green signals." "Complex pattern, 1 to 2 extra red signals."
‡Fusion identified by RNA sequencing.
DOC indicates died of other causes; MRI, magnetic resonance imaging; NA, not available; NED, no evidence of disease; RT, radiotherapy.

- 年龄28—76岁，平均年龄54岁
- 女性7人，男性3人，1例未知
- 腮腺10例，下颌下腺1例
- 最大径12—110mm，平均33mm
- 6例获知分期，均无淋巴结和远处转移
- 9例获知治疗方式，原发病灶手术切除，3例辅助放疗
- 6例获知随访情况，随访时间24—269个月，随访期间均无复发
- MSANTD3基因增多者，出现颈部淋巴结转移和肺转移，肺切除3个月后失随访

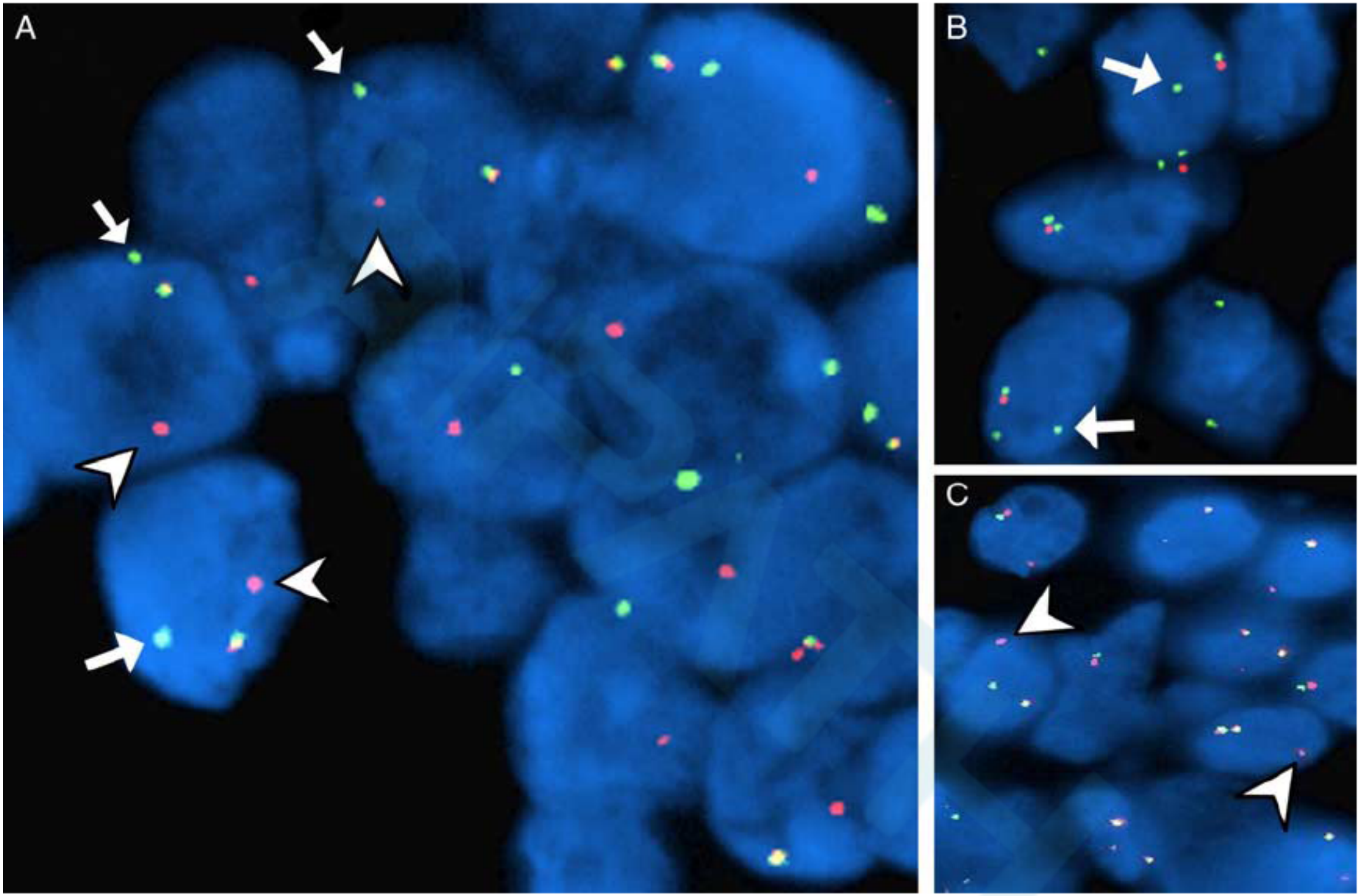

FIGURE 1. Patterns of MSANTD3 aberrations in acinic cell carcinoma of the salivary gland. A, FISH demonstrating separate green(arrows) and red (arrowheads) signals, consistent with fusion gene formation. The gain of one green signal (arrows) (B) and onered signal (arrowheads) (C) was present in a subset of cases.

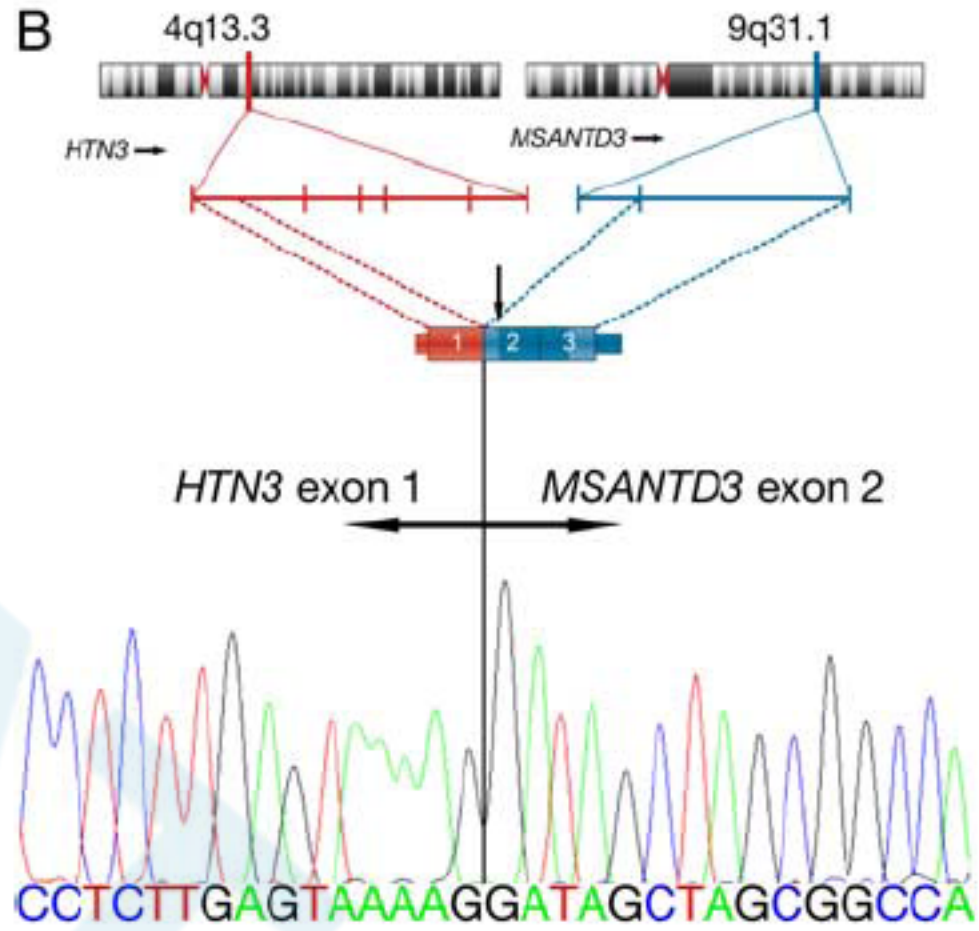

FIGURE 2. HTN3-MSANTD3 fusion transcripts in acinic cell carcinoma of the salivary gland. A, RT-PCR revealed a 160 bp fragment in cases 1 to 6 but not in case 7. RT-PCR did not yield an amplicon in any of the cases with complex MSANTD3 FISH patterns (not shown). All cases had PGK1 products, ensuring sufficient RNA quality (not shown). B, The breakpoints within the genomic location of the HTN3 gene on chromosome 4 and MSANTD3 gene on chromosome 9 with exons denoted by vertical bars schematically presented. An illustrative part of the fusion transcript demonstrating the fusion of exon 1 of HTN3 and exon 2 of MSANTD3, which was shown to be the same in all 6 cases with amplified PCR fragments by nucleotide sequencing.Note that the start codon (vertical arrow) lies within exon 2 of MSANTD3, causing for full-length MSANTD3 transcripts as marked by dark blue. Bp indicates base pair; M,marker; NC, negative control.

- 病变表现为AciCC的典型形态，分叶状，肿瘤细胞主要为浆液性腺泡细胞，呈实性生长，胞浆内可见显著的颗粒；

- 闰管样细胞数量多少不等，大小、形状不一致，胞浆嗜酸性或苍白，排列成囊状、微囊状、筛状，内充均质嗜酸性物质

- 无乳头状或滤泡样结构、透明细胞或嗜酸性细胞、淋巴细胞性间质、高级别转化区域

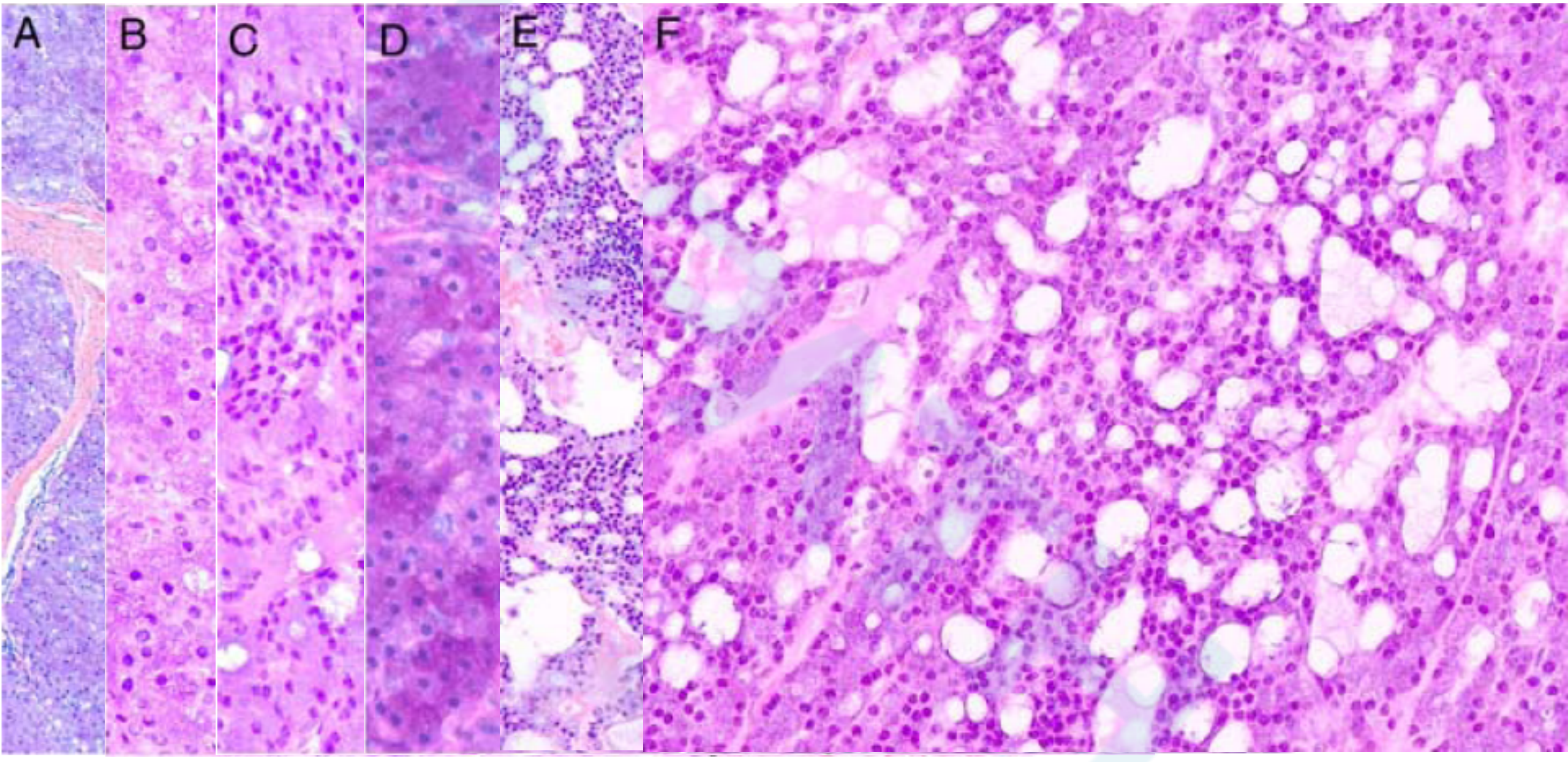
A
B
C
D
E
F

TABLE 3. Immunohistochemical Profile of Acinic Cell Carcinoma With and Without *MSANTD3* Aberrations

Case#	Alpha-amylase	CK7	DOG-1	Ki-67	Mammaglobin	S-100
1	+	+	+	2%	−	+
2	+	+	+	1%	−	+
3	NA	NA	+	NA	NA	NA
4	+	+	+	2%	−	−
5	−	+	+	1%	−	−
6	−	+	+	2%	−	−
7	−	+	+	4%	−	+
8	+	+	+	1%	−	+
9	−	+	+	2%	−	+
10	+	+	+	5%	−	+
Total	6/9	9/9	10/10	Mean 2.2	0/9	6/9
Non-rearranged AciCC ($n=20$)	11/20	20/20	20/20	Mean 2	0/20	20/20

No material from case 11 was available for immunohistochemistry.
NA indicates not available.

- 免疫组化同MSANTD3野生型
- 闰管样成分CK7和S-100强阳性
- 浆液性细胞DOG-1腔面细胞膜强阳性
- 胞浆内颗粒α- 淀粉酶阳性
- Mammaglobin阴性
- Ki-67：1%--5%

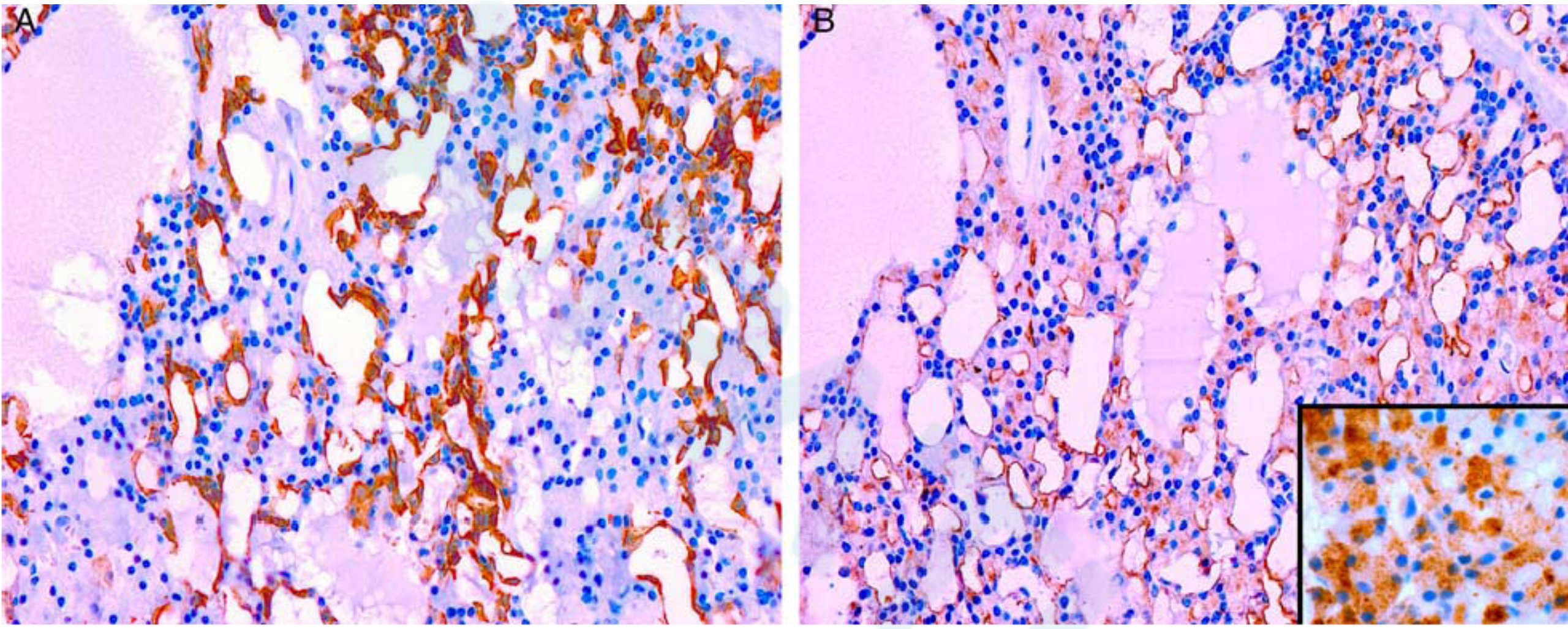

FIGURE 5. Immunohistochemical profile of salivary gland acinic cell carcinoma with MSANTD3 aberrations. A, S-100 highlights the cytoplasm of intercalated duct-like cells with serous cells being negative. B, DOG-1 highlight the luminal membrane of serous tumor cells with only a few, scattered intercalated duct-like cells showing faint cytoplasmic staining. Alpha-amylase highlights the cytoplasmic granules of the serous tumor cells (inset).

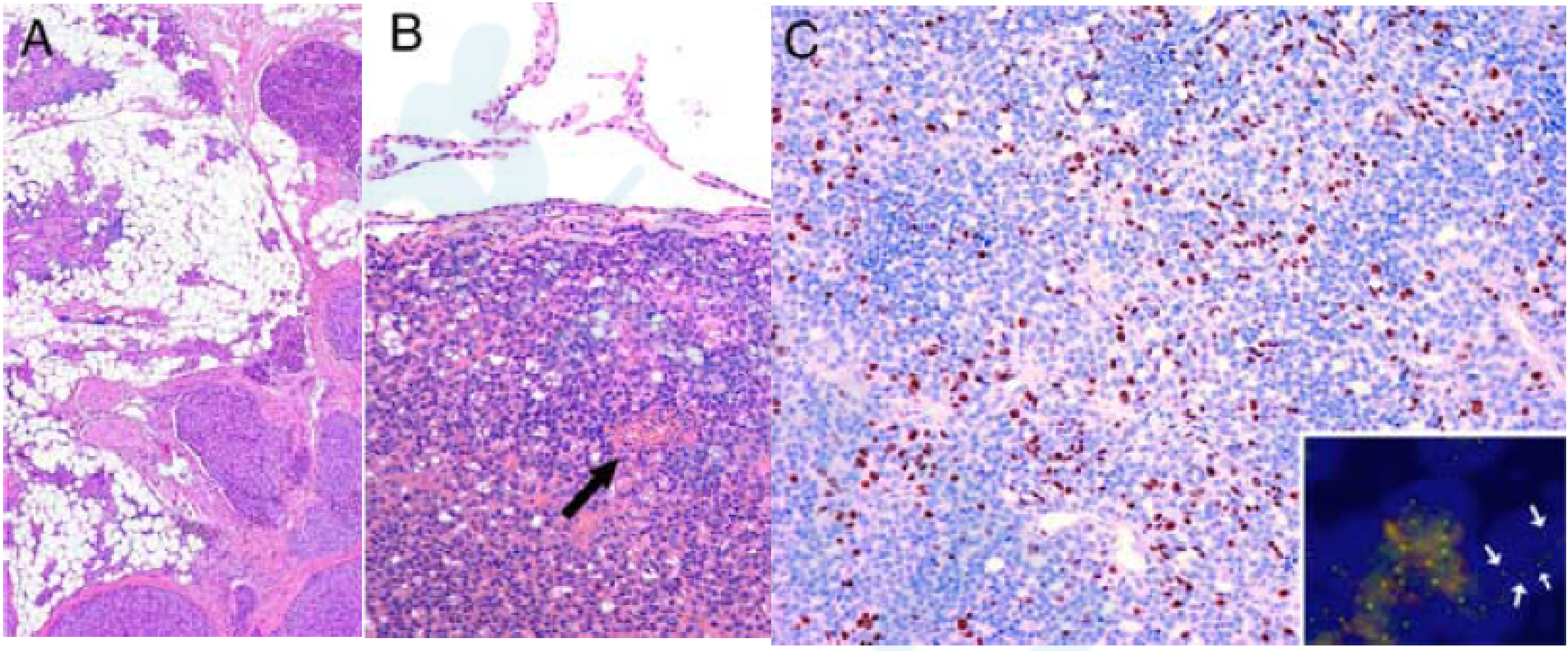

FIGURE 3. Invasive growth of salivary gland acinic cell carcinoma with MSANTD3 gain. A, A locally invasive tumor growing along interlobular fibrous septae in an atrophic parotid gland (H&E). Note the preservation of cytoplasmic PAS+D-positive granulation(inset). B, A microcystic pulmonary metastasis from the tumor shown in (A) surrounded by a pseudocapsule with a small necrotic area (arrow, H&E). C, The proliferative activity as ascertained with Ki-67 was 10%, up to 25% in hotspots (Ki-67, IHC). The gain of the green signal in MSANTD3 was found in the primary tumor as well as the metastasis (inset, arrows).

讨论

1，MSANTD3致瘤性目前尚不明确，本文中MSANTD3基因增多的病例发生淋巴结和肺转移，显示出不同寻常的侵袭性的临床进展，一定程度证实了MSANTD3基因的致瘤性

- MSANTD3增多尚未报道于任何类型的恶性肿瘤中

2，本研究显示在涎腺AciCC中，HTN3基因是MSANTD3变异的伴侣基因

- 由于HTN3唯一性的表达于浆液性腺泡细胞，因此，浆液性细胞在HTN3-MSANTD3融合性肿瘤中占主要成分

3，由于所有病变中均含有不同数量的闰管样细胞，提示浆液性细胞可能有分化为闰管样细胞的活性

4，经典的涎腺AciCC组织形态表现为数量不等的浆液性细胞与闰管样细胞构成一种或多种结构，如实性、微囊状、乳头状-囊状、滤泡样等，伴不等量的腺泡样、空泡样、透明样和非特异腺样细胞，多数可见明显的间质淋巴样浸润；而MSANTD3突变的AciCCs表现为以浆液性细胞为主，呈实性生长，不含淋巴性间质，表现为惰性的生物学行为

5，MSANTD3突变的AciCCs免疫表型与MSANTD3野生型类似

6，由于MSANTD3突变的AciCCs形态并非特异性表现，临床预后也没有显著差异，因此，不足以将其作为独立的诊断实体，而称之为AciCC亚型的一种遗传特征更为合适

结论

- HTN3-MSANTD3融合是涎腺AciCC的重复性事件，但比例很低，仅见于<5%的病例

- HTN3-MSANTD3融合性涎腺AciCC以浆液性细胞为主，呈实性生长，不伴淋巴细胞性间质，其临床病程与传统AciCC并无差异


Thank
You!