Original Paper

Are there *EWSR1* rearranged cutaneous hidradenomas and mucoepidermoid carcinomas of salivary glands? A FISH study and review of the literature

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MAML2 rearrangements have been previously described for hidradenomas and mucoepidermoid carcinomas (MEC). However, one report showed *EWSR1* rearrangement in both tumours. In this study, *EWSR1* and *MAML2* translocation were investigated in hidradenomas and MECs.

Specimens from thirteen MECs of the salivary glands and twenty hidradenomas of the skin were evaluated. Fluorescence *in situ* hybridisation (FISH) studies with *EWSR1* and *MAML2* break-apart probes were used.

Forty percent of hidradenomas and 84.6% of MECs showed a positive *MAML2* break signal. *EWSR1* break signal was absent in hidradenomas. Only two MECs showed positive EWSR1 signal and were, thus, reclassified as clear cell carcinoma (CCC). A statistically significant relationship was also observed between clear cells containing hidradenomas and the FISH status.

Despite the previous study, *EWSR1* translocations could not be established in hidradenomas and MECs. The study further suggests that evaluation of *EWSR1* might be obligatory for the correct diagnosis of *MAML2*-negative MECs to exclude the chance of CCCs. The present study also supports the notion that *MAML2* can be used as a marker for hidradenomas and MECs.

Key words: *MAML2*, *EWSR1*, FISH, hidradenomas, mucoepidermoid carcinomas.

Introduction

Hidradenomas refer to benign tumours of the sweat glands. In general, hidradenomas are characterised by a complex morphology and the presence of a variety of cells. These tumours are composed of solid, cystic and glandular areas [1]. Besides these morphological patterns, hidradenomas also harbour t(11;19) translocation, resulting in *CRTC1-MAML2* fusion, which has been reported also in cases of mucoepidermoid carcinoma (MEC) of salivary glands [2].

In addition to *CRTC1-MAML2* fusion, in 2008, Moller *et al*. reported the fusion of *EWSR1* and *POU5F1* genes in hidradenomas of the skin and MECs of the salivary glands [3]. This rearrangement has also been described in a subgroup of soft tissue myoepitheliomas with distinctive clear cell morphology that occurs in children and young adults [4]. Despite Moller's study, the presence of *EWSR1* rearrangement in hidradenomas and MECs is a controversial issue [3, 4, 5]. Furthermore, the presence of an *EWSR1* translocation in clear cell carcinoma (CCC) of salivary glands, closely mimics MECs in routine practice, which increases its complexity [6].

This study aimed to clarify the presence of *EWSR1* rearrangement in cutaneous hidradenomas and MECs of minor and major salivary glands with the fluorescence *in situ* hybridisation (FISH) technique and to

evaluate the *MAML2* status in these groups of tumours.

Material and methods

Study group

Specimens from thirteen MECs of the major and the minor salivary glands and twenty hidradenomas were included in this study. All hidradenoma cases were re-evaluated and confirmed based on the new WHO classification of skin tumours. Poroid hidradenomas which are now classified under the poroma group were excluded from the study [7]. The morphological features of MECs were also evaluated and graded using the Armed Force Institute of Pathology grading (AFIP) scheme [8]. The clinical data for the tumour groups were obtained from hospital information systems. The details of the study groups are summarised in Table I.

Table I. Study groups

Hidradenoma
n = 20
Gender:
14 F/6 M
Age:
Mean ±SD: 52.5 ±14.5
Range: 24-74 year
Localisation:
Head and neck: 6
Body: 6
Upper extremities: 3
Lower extremities: 5
Diameter:
Mean ±SD: 1 ±0.6
Range: 0.2-3.2 cm
Mucoepidermoid carcinoma
n = 13
Gender:
8 F/5 M
Age:
Mean ±SD: 47.2 ±20.8
Range: 9-83 years
Localisation:
Parotis: 10
Oral (Minor SG): 3
Diameter:
Mean ±SD: 1.5 ±0.7
Range: 0.7-3 cm
Grade:
AFIP:
High: 1
Intermediate: 1
Low: 11

Ethics statement

This study was financially supported by the Ankara Numune Research and Education Hospital Medical Speciality Education Board (TUEK, Project no. 217-0033). All the experiments were conducted according to the institutional ethical guidelines (Ankara Numune Research and Education hospital Local Ethics Committee, 26.04.2017, 1383/2017).

Fluorescence *in situ* hybridisation (FISH) study

FISH studies were performed by using a ZytoLight SPEC *EWSR1* Dual Colour Break Apart Probe (Zytovision, Bremerhaven, Germany) and a ZytoLight SPEC *MAML2* Dual Colour Break Apart Probe. All analyses and evaluations were carried out using a Nikon Eclipse 80i fluorescence microscope (Nikon Europe, Amsterdam, Netherlands). A total of 100 non-overlapping intact nuclei were scored using Nikon NIS Elements 3.0 software (Nikon Europe, Amsterdam, Netherlands), and a cut-off value of > 10% of neoplastic cells showing the break-apart signal was used for analysis.

Statistics

The χ^2 test using the PASW 17 statistic programme (Chicago, USA) was used to analyse the relationship between morphological details of the hidradenomas and FISH status. Because the minimum expected count was less than 5 for all comparisons, Fisher's Exact Test was used. The comparison between FISH-positive and FISH-negative hidradenomas for age, diameter and mitotic count was established with the help of the Mann-Whitney U test.

Results

The FISH analysis of hidradenoma specimens revealed the presence of *MAML2* break-apart signal in eight hidradenomas (40%), but the *EWSR1* break-apart signal was completely absent.

For *MAML2*, the expected signal pattern of one orange-green fusion (F), one orange (O) and one separate green signal (G) (1F1O1G) was observed in 14-83% of the cells of hidradenomas. Furthermore, unusual expression patterns were also observed which could be attributed to polysomy of the tumour cells.

Table II. Morphological and clinical features of FISH(+) and FISH(–) hidradenomas*

	FISH (+)	FISH (–)	Total
Gender	5 F/3 M	9 F/3 M	14 F/6 M
Age (mean ±SD)	43.7 ±12.8 years	58.4 ±12.9 years	52.5 ±14.5 years
	24-65 years	35-74 years	24-74 years
Localisation	Head and neck: 2	Head and neck: 4	Head and neck: 6
	Body: 4	Body: 2	Body: 6
	Upper ext.: 1	Upper ext.: 2	Upper ext.: 3
	Lower ext.: 1	Lower ext.: 4	Lower ext.: 5
Diameter (mean ±SD)	1 ±0.4 cm	0.9 ±0.7 cm	1 ±0.6 cm
	0.5-2 cm	0.2-3.2 cm	0.2-3.2 cm
Dermal (D)/subcutaneous (SC)	D: 4	D: 8	D: 12
	SC: 0	SC: 0	SC: 0
	D/SC: 4	D/SC: 4	D/SC: 8
Tubule	5/8	5/12	10/20
Cysts	6/8	10/12	16/20
Polygonal cells	8/8	12/12	20/20
Clear cells	8/8	3/12	11/20
Squamoid cells	1/8	5/12	6/20
Sebaceous cells	0/8	1/12	1/20
Mucinous cells	0/8	1/12	1/20
Poroid areas/cells	0/8	4/12	4/20
Calcification	0/8	0/12	0/20
Decap. secret	1/8	2/12	3/20
Nuclear Groove	6/8	6/12	12/20
Necrosis	0/8	1/12	1/20
Epidermal connection**	0/6	1/9	1/15
Stroma	Hyalinized: 6	Hyalinized: 9	Hyalinized: 15
	Other:2	Other:3	Other:5
Mitosis (mean ±SD) 10 HPF	0.8 ±1.1	2.2 ±2.4	1.7 ±2.1

** The morphological data for hidradenomas was evaluated on the basis of the morphologic features previously summarised by Nandeesh and Rajalakshmi* [1]

*** Epidermis cannot be detected in all of the cases due to surgical procedure*

A statistically significant relationship was observed between clear cells containing hidradenomas and the FISH status (p = 0. 001). However, no significant relationship was observed between other observed morphological parameters, including cysts, tubule formation, nuclear grooves, decapitation secretions, dominant stroma type, mucinous, poroid and squamous cells (Table II). Clinically, FISH-positive hidradenomas were present in younger patients compared with FISH-negative ones (Mean: 43.7 vs. 58.4; p = 0. 031)

In the MEC group, 11 cases showed a *MAML2* break signal (84.6%), whereas the remaining two cases showed only an *EWSR1* break signal. These 2 specimens were obtained from minor salivary glands.

As observed in the hidradenoma group, the expected signal pattern of 1F1O1G for *MAML2* was seen in 13-80% of the cells of MEC. Unusual expression patterns similar to hidradenomas were also detected. *EWSR1* break signals of 72% and 83% of the cells were detected in the two FISH-positive MEC cases.

Discussion

In recent years, similar to salivary gland tumours, several molecular markers for skin adnexal tumours such as MAML2, have been identified with advances in molecular pathology [9, 10, 11, 12, 13]. This

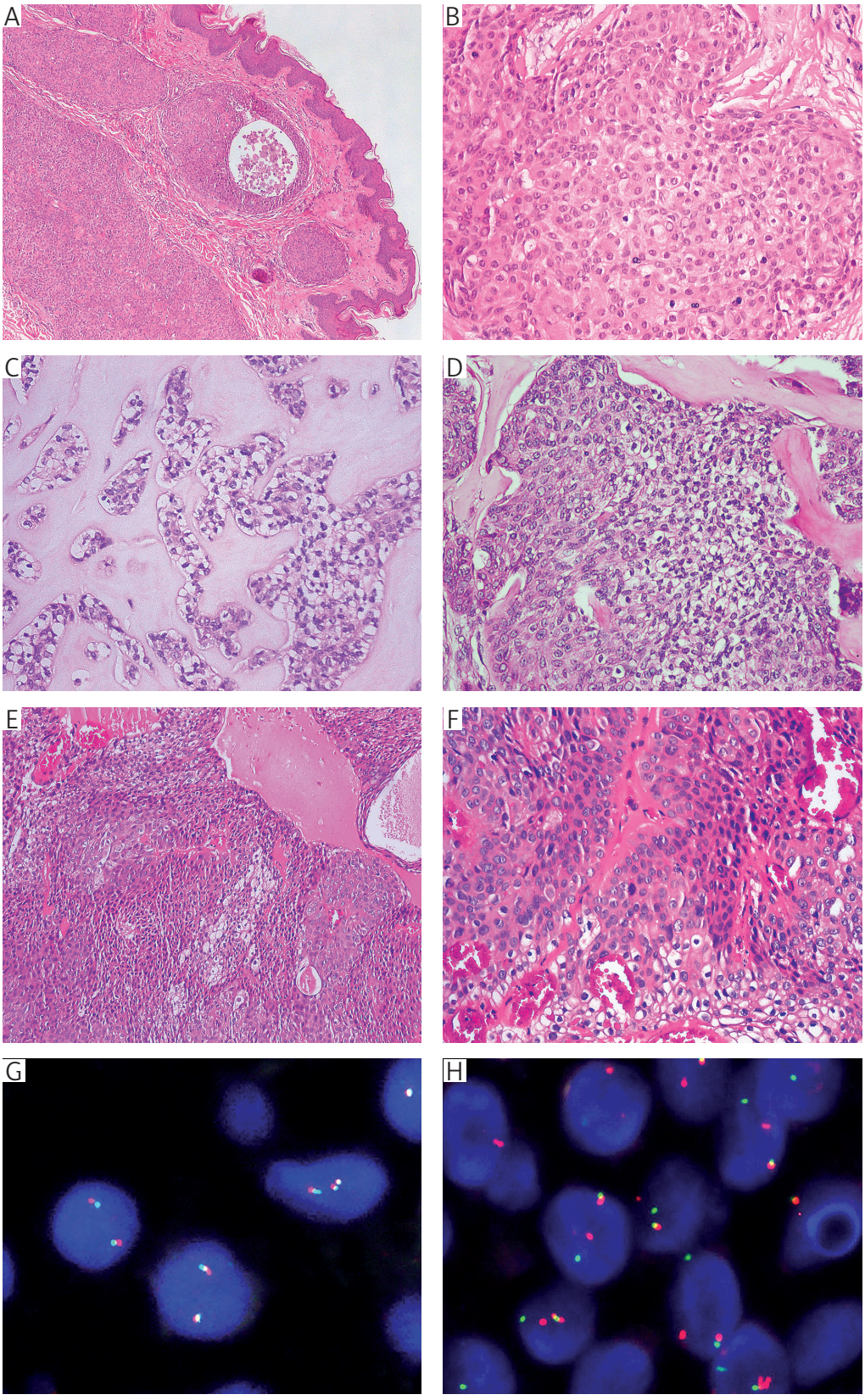

Fig. 1. Three hidradenoma cases positive for *MAML2* FISH analysis. First case shows the presence of eosinophilic round cells, with focal areas indicative of hardly detectable clear cells (A; B). In the second case, both eosinophilic and clear cell islands can be seen embedded in the hyaline stroma (C; D). The third case includes cystic areas, and eosinophilic cell areas, and clear cells can also be easily detected (E, F). FISH studies of the first case clearly demonstrate a *EWSR1*-negative (G) but *MAML2*-positive (H) signal

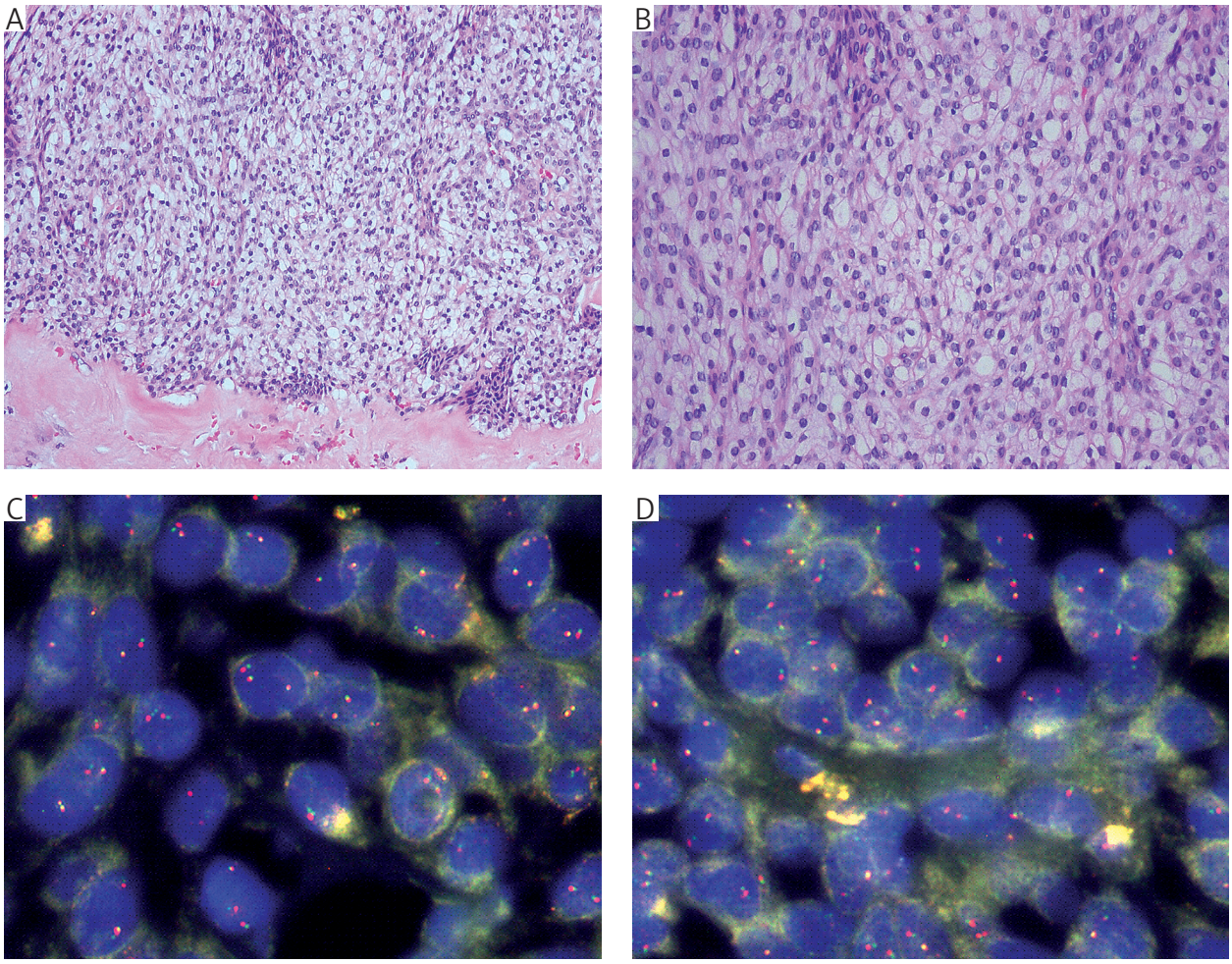

Fig. 2. Clear cell dominant hidradenoma (A; B) with negative *EWSR1* (C) and *MAML2* (D) FISH studies

indicates a close similarity between skin adnexal tumours and salivary gland neoplasms.

The *EWSR1* gene region is a common translocation partner in various soft tissue tumours [14]. However, its occurrence in epithelial tumours is rare. CCCs of salivary glands, clear cell odontogenic carcinomas and soft tissue myoepithelial carcinomas are well known examples of epithelial tumours with EWSR1 translocations [15, 16]. *EWSR1* translocation in MEC has remained a source of controversy in head and neck pathology. Moller *et al.* showed the presence *EWSR1* rearrangements (*EWSR1–POU5F1*) in an 85-year-old man [3]. However, it is not clear whether these rearrangements were an *EWSR1* translocation-positive MEC or not. Shah *et al*. and Antonescu *et al.* reported that none of the mucoepidermoid carcinomas in their studies demonstrated *EWSR1* rearrangements [4, 17]. In a recent study involving *MAML2* translocation negative MECs, 3 out of 17 *MAML2* fusion-negative cases were reported to be positive for *EWSR1* translocation. However, these cases were reclassified as hyalinising CCC despite morphological similarity and MEC diagnosis [5]. Similar to this study, our two cases originally diagnosed as MEC were *EWSR1* translocation-positive. Both tumours were excised from the oral cavity. Immunohistochemically, both cases showed p63 positivity but no S100 expression. Cytokeratin 7 positivity was seen in one patient. These cases were morphologically re-evaluated by an experienced head and neck pathologist (see Acknowledgments). Therefore, these two cases were finally diagnosed as CCC (Fig. 3) with consensus. This is suggestive of a false diagnosis of CCC as MEC in the absence of FISH studies, particularly in cases of minor salivary glands.

The presence of *EWSR1* rearrangement has been shown in cutaneous myoepithelial tumours including mixed tumour, myoepithelioma and myoepithelial carcinoma [4, 18]. However, Moller *et al.* also reported *EWSR1* translocation in 5 hidradenomas [3]. In our study, no *EWSR1* rearrangement was detected in the hidradenomas of the skin. In another study, which focused on *EWSR1* rearrangement in soft tissue myoepithelial tumours, Antonescu *et al.* reported that five cutaneous eccrine hidradenomas were used

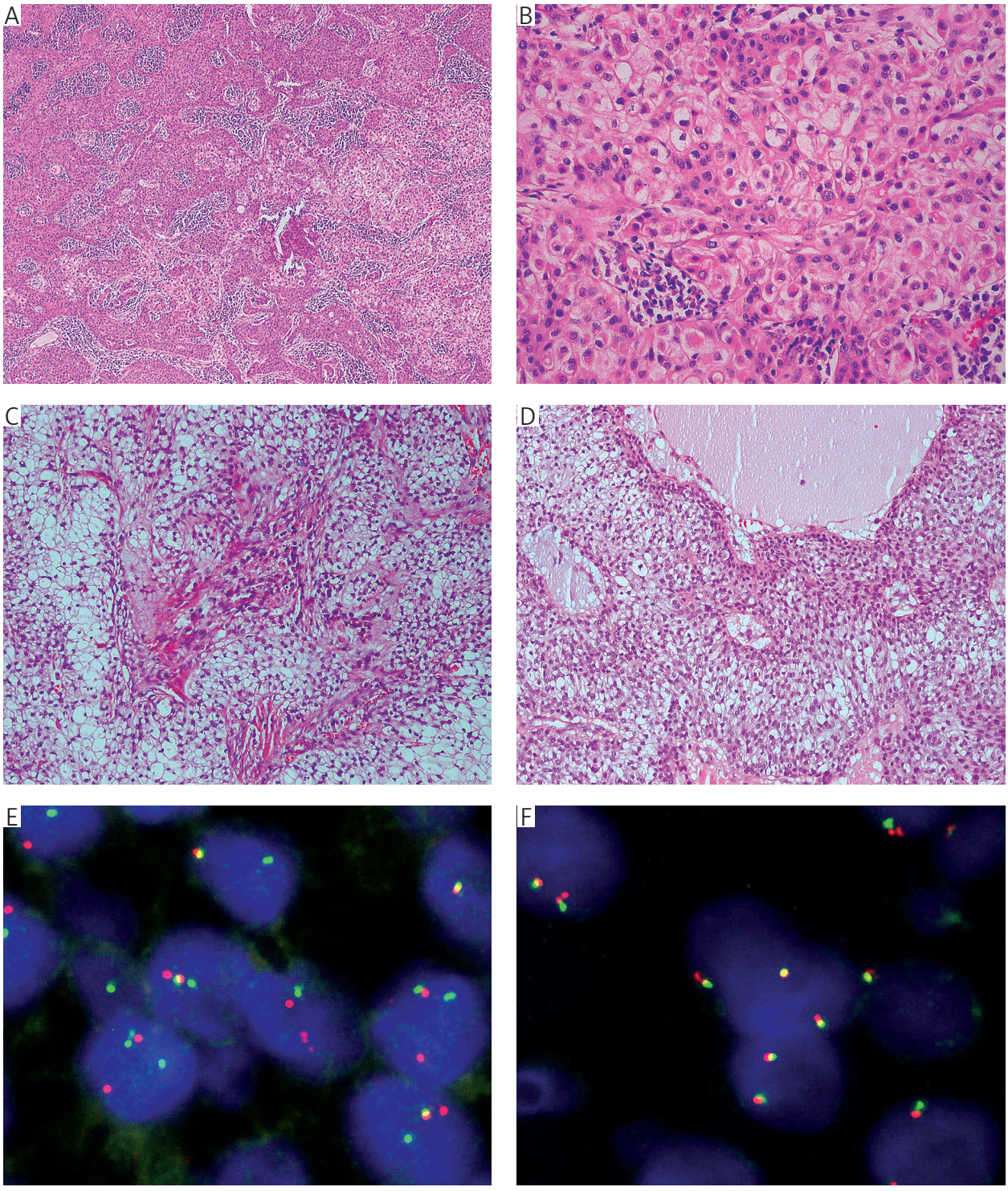

Fig. 3. Two salivary gland cancers positive for *EWSR1* fusion. The first case shows solid and trabecular areas of eosinophilic cells with focal clear cell areas (A; B). The second case contains clear cells with some cystic areas (C; D). EWSR1 FISH study of the second case clearly demonstrates a break signal in significant number of cells (E). However, no *MAML2* break signal was detected (F)

as a control group and that none of them showed an abnormal *EWSR1* gene. [4] Therefore, *EWSR1* rearrangement is a very rare event in hidradenomas – if it exists. Future studies involving larger groups might be helpful to explore this theory in detail.

Translocations involving *MAML2 locus* have been previously described for both MECs and hidradenomas [19, 20]. The translocation of *CRTC1-MAML2* has been found to be commonly associated with hidradenomas. Besides this, there are several reports on

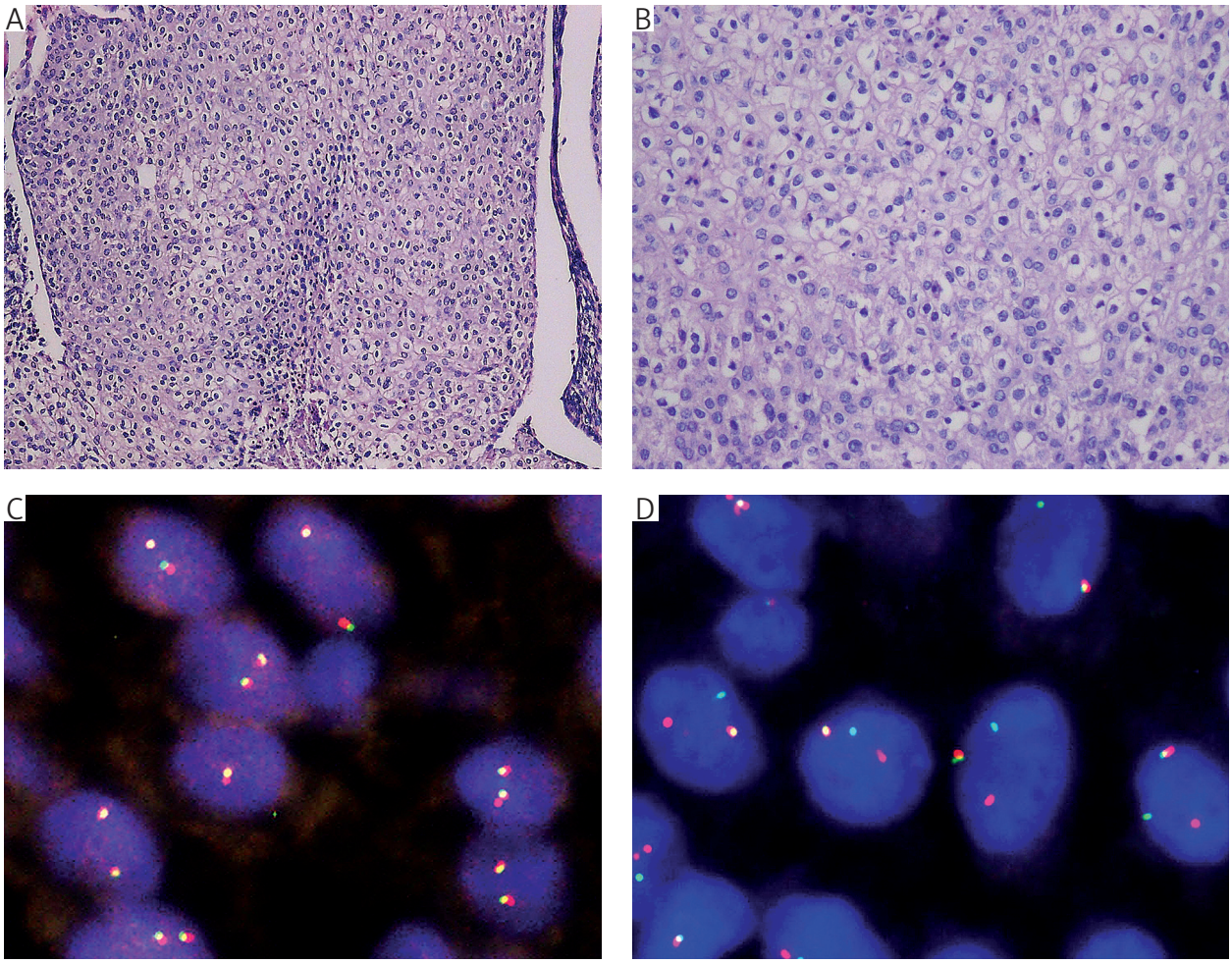

Fig. 4. Clear cell dominant MEC of parotid gland. Despite the presence of clear morphological similarities with *EWSR1*-positive cases, FISH studies show a *EWSR1*-negative (C) but *MAML2*-positive signal (D)

the occurrence of *CRTC3-MAML2* fusion in a small group of tumours [2, 19]. In 1994, Gorunova *et al*. reported genetic alteration for this *locus* in hidradenomas for the first time. The study further pointed out t(11;19) (q21;p13) translocation in one of the cases, in addition to other karyotypic changes [21]. In 2005, Behboudi *et al.* demonstrated t(11;19) translocation in one hidradenoma case with the help of the RT-PCR technique [22]. Following this, several studies were conducted focussing on this issue [2, 12, 19, 23]. In our study, *MAML2* translocations were detected in 40% of the hidradenomas (Fig. 1). Similarly, several reports clearly highlight that approximately half of the hidradenomas are characterised by *MAML2* translocations [2, 12, 19, 23].

It is important questions to evaluate whether any morphological and/or clinical differences exist between fusion-positive and fusion-negative hidradenomas. In this study, a statistically significant correlation was observed between clear cells of hidradenoma and *MAML2* translocation status ($p = 0.001$). Similar results were reported by Winnes *et al*., whose studies showed the presence of more or less abundant areas of clear cells in all fusion-positive tumours, whereas all fusion-negative tumours were completely devoid of clear cells. This indicated an association between *MAML2* gene rearrangement and clear cell variants of this tumour [12]. In comparison to this study, investigations by Kyrpychova *et al*. showed no correlations between translocation-positive hidradenomas and morphological details [19]. In another study, Kuma *et al.* reported no *MAML2* rearrangement in prominent cystic tumours and poroid hidradenomas [2]. In our study, more or less clear cell component was clearly correlated with FISH status; however, this was not true for all cases (Fig. 2). In one of our cases, a significant clear cell component with FISH-negative status was observed. As mentioned above, fusion-positive tumours were seen in younger patients as compared to negative ones, but the clinical significance remains unclear.

The practical use of *MAML2* and *EWSR1* rearrangements in skin tumours has not been well established. Requena and Sangüeza revealed the presence

of *MAML2* translocation in apocrine hidradenomas [24]. This translocation may be helpful in the differential diagnosis of poromatous lesions including poroid (eccrine) hidradenoma. Because *EWSR1* rearrangements have been described in mixed tumours, myoepithelioma and myoepithelial carcinoma, it can be used for the differential diagnosis of these lesions with hidradenomas [4]. In salivary gland tumours, showing *MAML2* and *EWSR1* rearrangements is very helpful for distinguishing between MEC and CCC in daily practice [13, 17, 20].

Despite Moller's article, we did not observe any *EWSR1* FISH positivity in our study group that included *MAML2* negative ones, similarly to many other studies [4]. The results of our study support the theory that *MAML2* is a marker for hidradenomas particularly the clear cell variant. Furthermore, *EWSR1* study seems to be obligatory for correct diagnosis of *MAML2*-negative MECs, especially those originating from minor salivary glands, to exclude the possibility of a closed mimic CCCs.

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The authors declare no conflict of interest.

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