Acta Otorrinolaringol Esp. 2011;**62(1):**1–9

Acta Otorrinolaringológica Española

www.elsevier.es/otorrino

ORIGINAL ARTICLE

Usefulness of antimitochondrial antibody 113-1 in diagnosis and classification of salivary gland tumours with oncocytic differentiation

Francisco Vera-Sempere,[a,*] Beatriz Vera-Sirera[b]

[a] *Departamento de Patología, Universidad de Valencia, Servicio de Anatomía Patológica, Hospital Universitario La Fe, Valencia, Spain*
[b] *Master en Endodoncia, Facultad de Medicina y Odontología, Universidad de Valencia, Spain*

Received March 9, 2010; accepted August 5, 2010

KEYWORDS
Salivary gland;
Tumours;
Antimitochondrial;
Classification;
Oncocytic;
Differentiation;
Immunohistochemistry

Abstract
Introduction and objectives: Salivary gland tumours usually show great variability both in their morphopathology as well as their clinical behaviour. In the present study, the usefulness of antimitochondrial monoclonal antibody 113-1 in the diagnosis and categorization of salivary tumours was studied.
Material and methods: A series of 22 benign and malignant salivary tumours and 5 non-tumoral salivary gland specimens were immunohistochemically analysed using an antimitochondrial monoclonal antibody (Ab Mo 113-1), which recognises a non-glycosylated mitochondrial protein of 60 Kd.
Results: The use of this antibody allowed us to recognize all salivary tumours with oncocytic differentiation. Two salivary tumours (1 papillary cystadenoma and 1 epithelial-myoepithelial carcinoma) (2/22; 10%) were also reclassified as oncocytic tumoral subtypes, in principle unidentified.
Conclusion: Our study highlights the usefulness of this antibody to facilitate the classification of salivary tumours, an aspect that may sometimes have not only diagnostic implications, but also prognostic.

PALABRAS CLAVE
Glándula salival;
Tumores;
Antimitocondrial;
Clasificación;
Oncocítico;

Utilidad del anticuerpo antimitocondrial 113-1 en el diagnóstico y categorización de los tumores de glándula salival con diferenciación oncocítica

Resumen
Introducción y objetivos: Los tumores de glándulas salivales se caracterizan por presentar una gran variabilidad y heterogeneidad, tanto en su morfopatología como en su evolutividad clínica.

*Corresponding author.
E-mail address: fco.jose.vera@uv.es (F. Vera-Sempere).

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Diferenciación;
Inmunohistoquimia

En el presente estudio se analiza la utilidad del anticuerpo antimitocondrial (Ac.113-1) en el diagnóstico y categorización de los tumores salivales.
Material y métodos: Se estudian inmunohistoquímicamente una serie de 22 tumores benignos y malignos salivales, así como 5 especímenes correspondientes a glándulas salivales no tumorales aplicando un anticuerpo monoclonal antimitocondrial (Ac Mo 113-1), que reconoce una proteína no glicosilada de 60 Kd mitocondrial.
Resultados: La utilización del anticuerpo 113-1 permitió reconocer todos los tumores con diferenciación oncocítica y además dos tumores salivales (un cistoadenoma papilar y un carcinoma epimiopitelial) (2/22; 10%) fueron reclasificados como variedades tumorales oncocíticas en principio no identificadas.
Conclusión: El estudio realizado resalta la utilidad de este anticuerpo al facilitar la categorización de los tumores salivales, aspecto este que puede tener en ocasiones implicaciones no sólo diagnósticas sino también pronósticas.

Introduction

Within the organism, the salivary glands are one of the structures that present the greatest diversity and complexity in their tumoral growth. Their tumours show great variability and heterogeneity, both in morphology-pathology and in clinical evolution. Within this broad diversity of salivary tumours, there is a group of tumours characterised by a morphological feature known as oncocytic differentiation. This differentiation is likewise characterised by the appearance of eosinophilic cellular elements with wide cytoplasm, known as oncocytes or oxyphilic cells, which are full of mitochondrial structures when observed with electron microscopy.[1] These cells do not appear in only the salivary glands, as oncocytic lesions with a metaplastic or neoplastic tendency (usually called oncocytomas) have been described in other organ sites (kidneys, thyroid, parathyroid, pituitary, stomach, etc.), with all of them presenting a similar morphology.[2-5]

Tumours with oncocytic differentiation at the salivary level are usually benign tumour lesions (Warthin tumour, oncocytoma, oncocytic cystadenoma), although there are also some rare forms of malignant tumour in which subvarieties or types have been described,[6,7] which present oncocytic differentiation. Interestingly, some of these are less aggressive than their non-oncocytic counterparts[6] and have a higher age of onset.[7] This suggests that, as is the case with other oncocytic lesions, tissue senescence may play a role in the pathogenesis of these tumour variants.[8,9]

In general, oncocytic cells are easily recognisable in observations using the usual histological stains (H&E), although sometimes there are other cell types that can simulate an oncocytic appearance at an optical level; this makes it necessary to employ histoenzymological techniques or an ultrastructural observation[10,11] for an accurate recognition of oncocytes. In the last decade, immunohistochemical techniques, with the use of antimitochondrial antibodies (AMA) such as 113-1 or MAB-127, have emerged as an effective method with immediate recognition of this oncocytic differentiation.[2,12,13]

In this sense, in the present study we have attempted to immunohistochemically evaluate a number of benign and malignant salivary glands, comparing them with non-tumoral salivary structures, using the monoclonal antibody 113-1. This antibody recognises a non-glycosylated protein present in mitochondrial structures, allowing optical microscope identification of the presence of mitochondrial structures and thus oncocytic cells. The goal is assessing the usefulness of this antibody in the diagnosis and classification of salivary tumour oncocytic lesions.

Material and methods

We retrospectively studied a series of 22 salivary gland tumours (Table 1) corresponding to extirpated surgical pieces of the following tumoral forms: pleomorphic adenoma (4 cases), Warthin tumour (8 cases), oncocytoma (one case), cystadenoma (two cases), epithelial/myoepithelial carcinoma (two cases) and mucoepidermoid carcinoma (5 cases). These tumours affected 12 females and 10 males with a mean age at onset of 56.3 years (52.5 years and 62 years respectively for malignant and benign tumours). Only one phenomenon of local recurrence of pleomorphic adenoma was found in the 15 benign tumours after a mean clinical follow-up of 5 years. Three of the 7 malignant tumours (corresponding to the 3 mucoepidermoid carcinomas) showed the presence of locoregional lymph node metastases, detected at the time of diagnosis in two cases and at 5 months after the initial surgery in the third observation. After a mean clinical follow-up of 5 years, no metastasis was found in any of the malignant tumours analysed. Macroscopic photographic images taken at the time of surgical excision were available retrospectively for all tumour lesions studied.

The tumour lesions studied affected the major salivary glands: parotid (12 cases), submandibular (5 cases) and sublingual (2 cases) glands, with the remaining 3 cases being minor salivary gland tumours (soft palate, lip, buccal mucosa). All surgical tumour specimens were obtained from the same hospital, processed after being enclosed in paraffin, following a uniform protocol in all cases.

We also analysed 5 major salivary specimens in parallel (Table 2) (3 parotid and 2 submaxillary glands) from 5 patients aged between 26 and 75 years (3 men and 2 women), who presented no salivary tumour pathology

Table 1 Salivary gland tumours studied

	Location	Gender	Age	Diagnosis	Surgery
1	Parotid	Female	46	Pleomorphic adenoma	Superficial parotidectomy
2	Parotid	Male	53	Pleomorphic adenoma	Superficial parotidectomy
3	Submaxillary	Female	44	Pleomorphic adenoma	Sub-maxillectomy
4	Parotid	Female	49	Recurrence of pleomorphic adenoma	Tumorectomy
5	Parotid	Male	45	Warthin tumour	Superficial parotidectomy
6	Parotid	Male	60	Warthin tumour	Superficial parotidectomy
7	Parotid	Male	68	Warthin tumour	Superficial parotidectomy
8	Parotid	Female	55	Warthin tumour	Superficial parotidectomy
9	Parotid	Male	61	Bilateral Warthin tumour	Bilateral superficial parotidectomy
10	Parotid	Male	64	Warthin tumour	Superficial parotidectomy
11	Parotid	Male	73	Warthin tumour	Parotidectomy
12	Parotid	Female	58	Warthin tumour	Tumorectomy
13	Submaxillary	Male	73	Oncocytoma	Sub-maxillectomy
14	Lower lip	Female	57	Cystadenoma	Tumorectomy
15	Submaxillary	Female	65	Cystadenoma	Sub-maxillectomy
16	Parotid	Male	48	Mucoepidermoid carcinoma	Radical parotidectomy
17	Sublingual	Male	55	Mucoepidermoid carcinoma	Extended tumorectomy
18	Submaxillary	Female	46	Mucoepidermoid carcinoma	Radical sub-maxillectomy
19	Mucosa buccal	Female	44	Mucoepidermoid carcinoma	Extended tumorectomy
20	Soft palate	Female	51	Mucoepidermoid carcinoma	Extended tumorectomy
21	Sublingual	Female	60	Epimyoepithelial carcinoma	Extended tumorectomy
22	Submaxillary	Female	64	Epimyoepithelial carcinoma	Radical sub-maxillectomy

morphologically. These specimens came from radical surgical excision, performed at the same hospital, due to malignant neoplasms (extra-salivary) in the head and neck region.

We selected a representative paraffin block from each tumour specimen, as well as the parts corresponding to the non-tumoral salivary gland. Three serial histological sections were made from each specimen, which were then stained with H&E, phosphotungstic acid haematoxylin (PTAH) and monoclonal antibody 131-1.

Immunostaining was performed with Ab Mo concentrate MU213-UC, clone 113-1 (BioGenex) (Ab No. 213M), at 1/80 dilution, using the polymer-based detection system Envision-Plus™from Dako®.[14] This system is more efficient and has a lower background noise than the usual method of avidin-biotin complex, commonly used for monoclonal antibodies.[15] The monoclonal antibody employed, MU213-UC, recognises all 60Kd non-glycosylated proteins, present in all mitochondria of human cells specifically and sensitively.[2] Antigen retrieval was performed by heat treatment, using the EnVision Flex Target Retrieval solution, High pH (cat. No. K8000/K8004) (Dako).

As a positive immunostaining control, we used sections of renal parenchyma, where the 113-1 antibody marked mitochondrial structures in the basal area, called basal labyrinth, of the proximal convoluted kidney tubules. As a negative control, we used histological sections from both tumour specimens and non-tumour salivary samples, in which we omitted the primary antibody, replacing it with distilled water throughout the immunohistochemical process.

A tumour lesion was considered to possess oncocytic differentiation when the presence of oncocytic elements was found at the optical microscopy level (analysed with haematoxylin, eosin and PTAH staining). In the immunohistochemical evaluation, a cellular element

Table 2 Non-tumour salivary glands

	Location	Gender	Age	Surgery
1	Parotid	Male	58	Cervical emptying + parotidectomy
2	Parotid	Male	63	Cervical emptying + parotidectomy
3	Parotid	Female	60	Left hemi-maxillectomy
4	Submaxillary	Male	51	Right radical cervical emptying
5	Submaxillary	Female	48	Right radical cervical emptying

was considered oncocytic when positive cytoplasmic immunoreactivity was expressed (+++) with monoclonal antibody 113-1. A tumour lesion was considered to present oncocytic differentiation when at least 50% of the proliferating cells were positive (+++) towards the monoclonal antibody.

The immuno-morphological results obtained were evaluated by two observers using a double observation binocular microscope Nikon Eclipse-50i, evaluating both the intensity of immunostaining (classified from + to +++) and the percentage (%) of labelled epithelial cells; micrographs were obtained with a photomicroscope Olympus Vanox AHBT-3 equipped with a DS-5Mc cooled digital camera and a DS-L2 (Nikon) camera control unit. The macroscopic images corresponding to the surgical specimens were obtained at the time of tumour resection using a MAKAM® macro system equipped with a CCD camera (752x582 pixels) with a motorized 18x optical zoom.

Results

The study of the 5 samples corresponding to non-tumoral salivary tissue (4 samples of parotid gland and 1 of submaxillary gland) (Figure 1, Figure 2) with the monoclonal antibody 131-1 showed normal salivary tissue with intense immunostaining in columnar cells lining the striated excretory ducts. This positivity was more marked in the basal portion of the cytoplasm of these cells, and immunostaining–often elongated–was arranged linearly and perpendicularly to the basal surface of these cells.

In the remaining epithelial cells of the glandular excretory tree (intercalated ducts), there was a form of positivity consisting of fine cytoplasmic granules arranged diffusely, but with a much lower intensity than in the striated ducts.

The differences between the positivity found in the striated ducts as opposed to the intercalated ducts were highlighted when comparing the parotid gland tissue (Figure 2) and the submandibular tissue (Figure 1). The greater length of the striated ducts and the lower representation of the intercalated ducts in the submandibular gland made the distinction with 113-1 immunostaining more apparent in the case of the submandibular gland (Figure 1A).

In parotid and submaxillary epithelial acinar cells (Figure 2B) (in the latter, in the so-called crescent or serous half-moons), as in the mucosal cells of the submaxillary gland (Figure 1B), the positivity with 113-1 was much weaker and in the form of small, very thin cytoplasmic granules. Similarly, fibroblastic and adipose stromal cells as well as vascular endothelial cells present in normal salivary tissue showed a very weak granular positivity rate, dispersed on a cytoplasmic level. In the field of intraparotid nodes and follicular centre areas, some large lymphoid cells, from the germinal centre, expressed a very thin, scattered granular positivity.

In all cases, the results obtained with 113-1 immunostaining were more precise, specific and recognisable for the purpose of identifying the presence of oncocytic cells than the results obtained with PTAH. The PTAH staining had to be prolonged 24 hours to achieve a mitochondrial marking, which was always less precise and less constant.

In the study of the whole series of salivary tumours (22 tumours), immunohistochemical analysis accurately and specifically marked all morphologically oncocytic cellular elements, which were present in different representations in the tumour lesions studied. Table 3 shows the overall results obtained in relation to immunostaining with 113-1 monoclonal antibody for each of the tumours studied and the new diagnostic categorisation adopted after the immunohistochemical study.

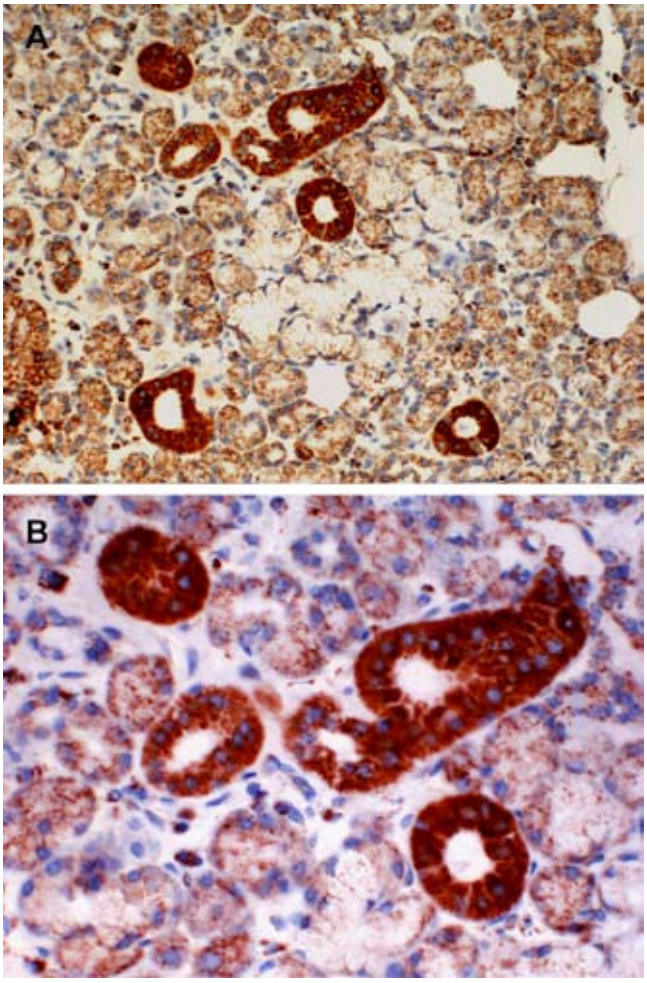

Figure 1 Non-tumoral submandibular gland. (A) stained with Ab 113-1, the intense immunostaining of striated ducts is in contrast with the weak staining of acinar cells. Mucous cells are negative. (B) shows 4 striated ducts stained intensely in detail, in the vicinity of acinar cells with low reactivity (113-1 300 and 400x).

In pleomorphic adenomas (4 cases), the presence of oncocytic elements was generally very scarce, weak (rated as + or +++) and scattered (not over 2%4% of the epithelial component). In the structure of these tumours, 113-1 positivity was not a predominant component, with no apparent diagnostic value, at least in the 4 pleomorphic adenomas studied. In none of the 4 cases did oncocytic cellularity account for more than 4% of the epithelial component of the tumour.

However, in the 8 cases of Warthin tumour, the presence of immunoreactivity for 113-1 was always very strong (Figure 3), defining the layers of oncocytic cells very

Table 3 Salivary gland tumours studied

Observation	Location	Diagnosis	Reactivity 113-1*		New categorisation**
1	Parotid	Pleomorphic adenoma	+	4%	
2	Parotid	Pleomorphic adenoma	+	2%	-
3	Submaxillary	Pleomorphic adenoma	—	0%	-
4	Parotid	Recurrence of pleomorphic adenoma	—	0%	-
5	Parotid	Warthin tumour	+++	95%	-
6	Parotid	Warthin tumour	+++	95%	-
7	Parotid	Warthin tumour	+++	95%	-
8	Parotid	Warthin tumour	+++	100%	-
9	Parotid	Bilateral Warthin tumour	+++	95%	-
10	Parotid	Warthin tumour	+++	100%	-
11	Parotid	Warthin tumour	+++	100%	-
12	Parotid	Warthin tumour	+++	100%	-
13	Submaxillary	Oncocytoma	+++	100%	-
14	Lower lip	Cystadenoma	+	<10%	-
15	Submaxillary	Papillary cystadenoma	+++	90%	Oncocytic papillary cystadenoma
16	Parotid	Mucoepidermoid carcinoma	—	0%	-
17	Sublingual	Mucoepidermoid carcinoma	—	0%	-
18	Submaxillary	Mucoepidermoid carcinoma	+	5%	-
19	Buccal mucosa	Mucoepidermoid carcinoma	—	0%	-
20	Soft palate	Mucoepidermoid carcinoma	—	0%	-
21	Sublingual	Carcinoma epimyoepithelial	+	<10%	-
22	Submaxillary	Carcinoma epimyoepithelial	+++	60-70%	Oncocytic epimyoepithelial cystadenoma

*113-1 reactivity: expressed in intensity of immunostaining (rated from + to +++) and in %of epithelial cells labelled.
**New ranking: new diagnostic categorisation of the tumour lesion after the Ab Mo 113 study.

specifically and precisely, especially in the luminal layers of the tumour (Figure 3B), where 95%or 100%of epithelial cells appeared intensely marked (+++). There was no variability observed in 113-1 immunostaining in the 8 cases studied, nor between the different areas of each of the tumours analysed. Immunohistochemical reactivity in the luminal epithelial layer was very intense and comparable in intensity to that found in the striated ducts of salivary non-tumoral glands. The layer of basal cells available in this tumour showed slightly less positivity, and the presence of negative or weakly positive round core basal elements for 113-1 was detected. In two of the Warthin tumours studied, we found pockets of mucoid metaplasia, which were negative for antimitochondrial antibody. In addition, the areas of squamous metaplasia (present in one case) were only weakly positive, without reaching an intensity comparable to that of oncocytic cells.

Immunostaining with 113-1 marked the presence of salivary duct inclusions within intraglandular lymphadenopathies equally for all Warthin tumours, especially at the intraparotid level. This antibody also accurately marked the presence of foci of cystic tumoral growth–of Warthin tumour type–within the intraglandular lymphadenopathies. This was especially marked in one of the observations that included multiple tumour growth foci, occupying part of the surface of intraparotid lymphadenopathies, showing the histogenesis of this benign tumour from ductal intranodal inclusions.

In the case of the submaxillary gland oncocytoma studied (Figure 4), all tumour cells (100% of tumoral cellularity) presented intense (+++), diffuse cytoplasmic positivity (Figure 4B). The reactivity was granular in character and of very strong intensity, comparable to that found in the cytoplasm of the ductal cells of striated ducts of normal glands (Figure 4B). Only a few cells with clear cytoplasm interspersed with oncocytic cells showed no reactivity with the antimitochondrial antibody. The tumour architecture was of a solid pattern; the glandular tissue attached presented small nests of oncocytic cells, immunoreactive to 113-I, forming images of nodular oncocytosis or oncocytic intraglandular nodular hyperplasia, which had gone unnoticed during H&E observation.

In both cystadenoma observations, one in the submaxillary gland and the other in the minor salivary glands of the lip, we detected the presence of oncocytic cell changes with a varied representation. In one of the two cases studied (corresponding to the submaxillary gland) (Figure 5), the epithelial lining of the cysts was very predominantly oncocytic (90% of the epithelial component) (Figure 5B), with strong reactivity (+++) for 113-1, and with a very isolated presence of mucosal cells. In addition, some focal areas of papillary growth could be identified (Figure 5A),

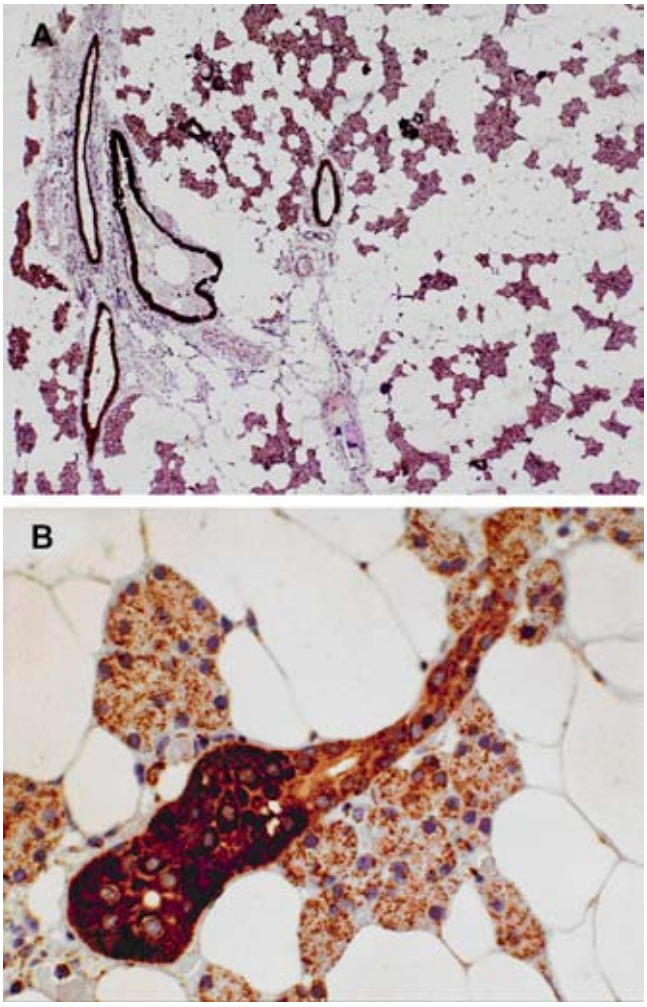

Figure 2 Non-tumoral parotid gland stained with Ab 113-1, showing immunostaining of the striated ducts (A). (B) shows finely granular immunostaining in the acinar cells. The positivity is increased in the intercalated ducts and especially in the striated ducts, with the entire surface of the cytoplasm being immunoreactive (113-1, 200 and 300x).

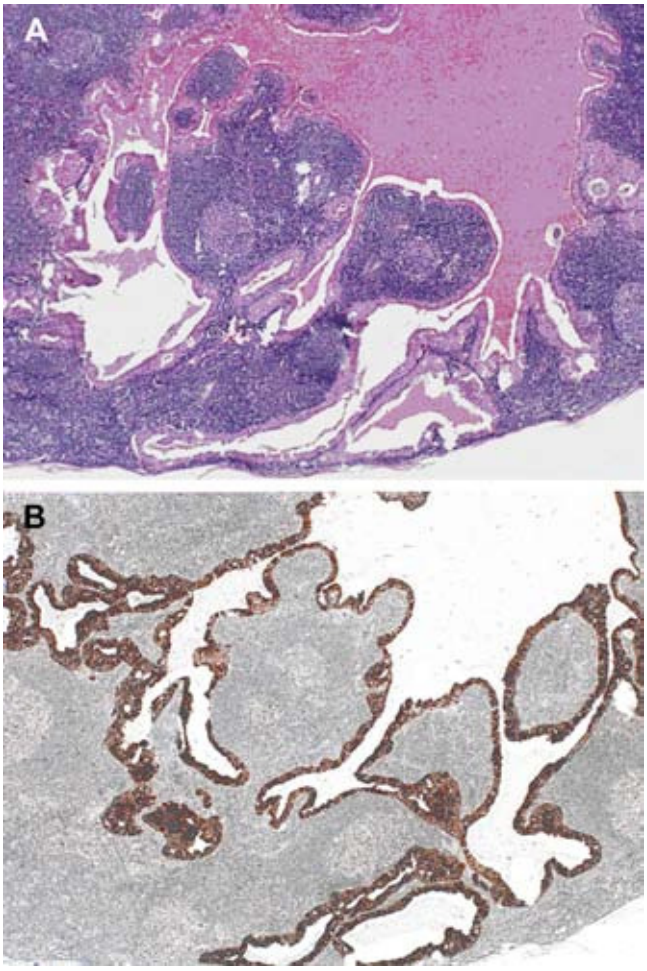

Figure 3 Serial sections of a Warthin tumour stained with H&E and 113-1, showing the cystic architecture of the tumour (A), with papillae lined with oncocytic cells, reactive to antimitochondrial Ab (B), and showing a lymphoid stroma with germinal centres (H&E and 113-1, 200x).

which also showed features of an oncocytic character (Figure 5B). After verifying this information, the lesion was finally considered as an oncocytic papillary cystadenoma. In contrast, the remaining forms of cystadenoma (from minor labial salivary glands), presented a low proportion (less than 10%of the epithelial component) of cells weakly marked (+) by the 113-1 antibody, with very few identifiable as real oncocytic cells.

The two epimyoepithelial carcinomas (Figure 6) showed the characteristic biphasic morphology of this tumour type, confirmed by epithelial markers (cytokeratin cocktail AE1-3 +) and myoepithelial markers (CD10–neuropeptidase–, vimentin, muscle-specific actin, PS-100 +) (Figure 6A). It should be noted that in one of them (corresponding to an epimyoepithelial submaxillary gland carcinoma), immunoreactivity for 113-1 was much stronger, showing that approximately 60%70%of the cells from the epithelial component presented 113-1 immunoreactive cytoplasmic granules (Figure 6B) with a strong positivity (+++). This last observation was therefore reclassified as an oncocytic variety of epimyoepithelial carcinoma.

With regard to mucoepidermoid carcinomas (5 cases), 2 of them corresponded to carcinomas of high nuclear grade, with marked polymorphism and nuclear atypia, showing no cystic tumour areas; the remaining 3 were low-grade lesions, often showing cystic epithelial areas with presence of mucus secreting cells and scarce squamous component. The 113-1 antibody reactivity in these mucoepidermoid carcinomas was nil or very low, being restricted to some isolated cellular elements (less than 10% of epithelial cellularity), with a weak positivity.

Discussion

Within the broad framework of salivary tumours, there is a variety of primary tumours that predominantly present cells having eosinophilic cytoplasm. Such tumours include tumoral lesions of oncocytic, epidermoid and/or myoepithelial

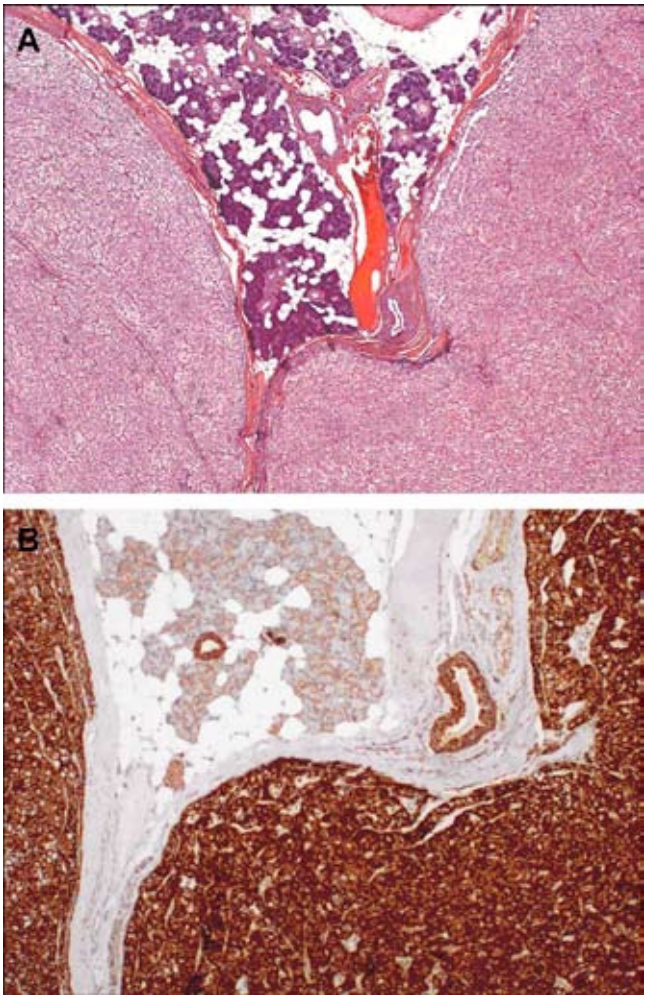

Figure 4 Serial sections of a submandibular oncocytoma (A) stained with H&E and 113-1, showing intense, diffuse immunoreactivity of the tumour cells compared to antimitochondrial Ab (B), similar to that found in neighbouring striated glandular tissue (H&E and 113-1, 200 and 350x).

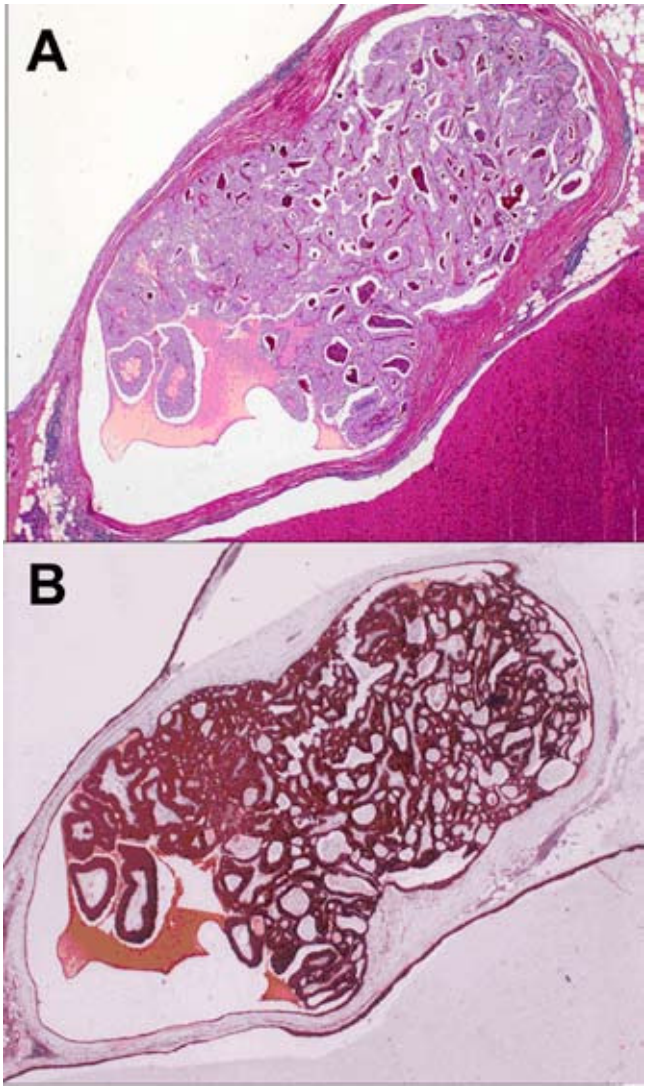

Figure 5 Submaxillary papillary cystadenoma with gland cavities filled by secretory material and complex tubulo-papillary papillary projections (A). Ab 131-1 (B) intensely marks the lining of intra-cystic papillary projections (PAS and 113-1, 200x).

type.[12] Within purely oncocytic tumour lesions, Warthin tumours are the most common among the benign and oncocytic carcinoma among malignant lesions.

The term "oncocytic" or "oncocytic change" is used to describe the presence of cell changes consisting of an enlargement of the cytoplasmic surface or volume, accompanied by a marked eosinophilia, with slight granularity, usually showing hyperchromatic nuclei. All these factors result in large volume cells with well-defined cell boundaries,[2-5] with cytoplasmic changes being the result of a massive presence of mitochondrial structures in the cytoplasm, identified in the ultrastructural review.[1]

The term "oncocytic" comes from the Greek , which etymologically means swollen or bloated. It was introduced into the medical literature by the German pathologist Hamperl in 1931[20] to describe cells that constitute salivary oncocytomas, and in which a marked eosinophilia appears, along with a cell swelling or enlargement. They are consequently also referred to as oxyphilic cells.

Oncocytic changes are not unique to the salivary glands. They are also verifiable in endocrine epithelial organs such as the thyroid gland (where oncocytic cells are known as Askanazy cells or Hürthle cells)[16] and in the pituitary gland, adrenal gland, parathyroid gland, kidney, etc.[2-5] The existence of high concentrations of oxidative enzymes[17] with high metabolic activity has been reported in oncocytic cells. This high metabolic activity would make it easier for them to suffer ischemic lesions and infarction after needle aspiration cytology.[18]

Oncocytic cellular forms or oxyphilic cells are usually easily recognisable on an optical level and do not generally pose diagnostic difficulties. However, they can be difficult to recognise when analysing small tumour samples, making it necessary to apply immunohistochemical techniques[2,12,19] for their precise classification as oncocytic cells.

The exact significance of an increase in the number of mitochondria present in oncocytic cells is not well known. It has been suggested that this mitochondrial

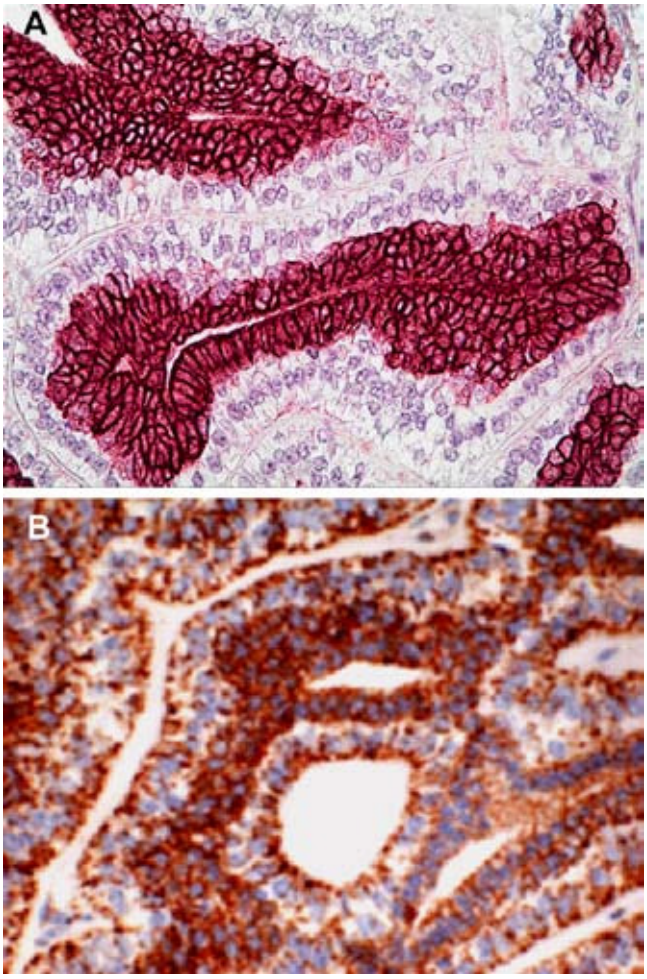

Figure 6 Epimyoepithelial carcinoma of the submaxillary gland, showing the biphasic tumoral character with a double layer of epithelial and myoepithelial cells (positive PS-100) (A). Staining with Ab 113-1 1 (B) shows the oncocytic nature of the epithelial tumour component (P-S-100 and 113-1, 400 and 250x).

hyperplasia would be a compensatory mechanism for an intrinsic mitochondrial defect that would result in a deficit in energy production and an increase in free radicals and reactive oxygen species, including H_2O_2. This would explain the immunohistochemical reactivity of oncocytes towards peroxiredoxin-I,[21] an H_2O_2 compaction protein. The accumulation of reactive oxygen species would predispose these cells towards genomic instability and neoplastic cell transformation phenomena.

It has also been suggested that oncocytic cells are the result of a degenerative cell change linked to aging,[8,9] as they may appear sporadically between normal ductal and acinar cells in relation to advanced age, or forming small oncocytic cell micronodules in the so-called salivary oncocytosis or nodular oncocytic hyperplasia.[22] This lesion is sometimes associated with the development of a truly encapsulated oncocytic tumour nodule; this was the case in the submandibular gland oncocytoma studied in our series (case No. 13), where the existence of an oncocytoma was associated with a multiple intraglandular oncocytosis. In our observation, antibody 113-1 perfectly delineated the oncocytoma structure and highlighted the associated presence of nodular oncocytosis or nodular oncocytic hyperplasia. The latter aspect went unnoticed in observation with H&E, which had been reported by other authors[23] previously and could explain the possible multiplicity and/or bilateral nature of this benign salivary tumour, as well as the occasional relapses described after simple tumorectomy.

Despite the relatively simple optical morphological recognition of oncocytic cells, it should be noted that a variety of intracytoplasmic organelle disorders may produce a certain morphological similarity to oncocytic cells or leads to confusion.[2] These changes include the accumulation of intermediate filaments as in the so-called rhabdoid tumours, the accumulation of secretion products in acinar cells or cellular filling by lysosomal structures. An example of this last is the case of so-called granular cell tumours, which can sometimes mimic oncocytic cells, although these cells are immunoreactive to CD68, a feature which does not occur with oncocytic cells.[2]

Classically, PTAH staining, and in particular the prolonged variation (48 hours) of this stain,[8] also used in our study, has been the methodology used to recognise oncocytes, although it is clear that PTAH staining is not entirely specific towards mitochondrial structures. Therefore, the immunohistochemistry methods are the fastest and most useful for oncocyte recognition. In this respect, Barnes and Bedetti (1984)[19] proposed immunohistochemical staining using an antibody against cytochrome C oxidase, an oxidative enzyme confined to the inner mitochondrial membrane ridge. Honda and Shintaku[2] proposed, as in our study, the use of 113-l monoclonal antibody that recognises a 60-kDa non-glycosylated mitochondrial protein as a more specific and reproducible methodology.

In our study, the immunohistochemical method using 113-1 antibody showed a marked specificity for the detection of oncocytic cells, which were easily identified in lesions such as Warthin tumour and oncocytoma. It also helped to identify the presence of oncocytic changes that normally go unnoticed in conventional H&E staining in cases such as one of the epimyoepithelial carcinomas studied or a cystadenoma of the submaxillary gland, where exclusive H&E observation did not allow us to establish the existence of oncocytic differentiation. The results obtained led to the reclassification of 2 of the 22 tumoral cases studied.

Several subtypes of epimyoepithelial carcinoma have been described in recent years,[7,24] including the so-called oncocytic variety observed in our study, in case No. 22. This affected the submaxillary gland, with a broad reactivity against 113-1 antibody. However, no specific prognostic significance has been attached to this tumoral subvariety.

In the case of salivary mucoepidermoid carcinoma, there have recently been reports of an oncocytic variety[6,25] that appears in older patients. These are generally low-grade tumours with a better prognosis. We did not detect any cases of this oncocytic variety in a total of 5 cases of mucoepidermoid carcinoma. Nevertheless, it is clear that applying the antimitochondrial antibody method makes it possible to identify this tumoral subvariety of mucoepidermoid carcinoma[6,25] if it exists, thus giving this categorisation a diagnostic and prognostic value.

Conclusions

As a conclusion, in our study we found a series of data and results that support the usefulness of the 113-1 antibody when applied to the study of tumour pathology of the salivary glands. These may be summarised in three aspects:

1) the studied antibody, 113-1, has a higher efficiency than classical, prolonged staining with PTAH for the recognition of oncocytic changes in salivary pathology;
2) the systematic use of the 113-1 monoclonal antibody makes it possible to recognise some unsuspected tumours during conventional studies, as was proven in our study when we detected an oncocytic hyperplasia associated to an oncocytoma. We also detected two very rare tumoral subtypes, an oncocytic variant of epimyoepithelial carcinoma and an oncocytic papillary cystadenoma. The technique led to the consequent reclassification of 10% (2/22) of the tumour lesions we studied;
3) immunostaining with 113-1 also enables the histogenesis of some tumour forms such as Warthin tumour and oncocytoma to be established, by demonstrating a similarity in their immunoreactivity and the immunophenotypic profile of the striated ducts. This proves how the origin of these tumours is established from a proliferation of striated ducts of the salivary tree glands.

Conflict of interest

The authors declare no conflict of interest.

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