ORIGINAL ARTICLE

SDHB Expression in Warthin’s Tumour

Beatriz Vera-Sirera, a,∗ Judith Pérez-Rojas, b Cecilia López-Valdivia, b
Enrique Jiménez, b Diego Collado-Martín, c Francisco Vera-Sempere b, d

a Departamento de Estomatología, Universidad de Valencia, Valencia, Spain
b Servicio de Anatomía Patológica, Hospital Universitario La Fe, Valencia, Spain
c Servicio de Otorrinolaringología, Hospital Universitario La Fe, Valencia, Spain
d Departamento de Patología, Universidad de Valencia, Valencia, Spain

Received 9 February 2011; accepted 27 March 2011

KEYWORDS
SDHB; Warthin’s tumour; Oncocytes; Immunohistochemistry

Abstract
Introduction: Succinic dehydrogenase subunit B (SDHB) is an enzyme belonging to the mitochondrial complex II. The aim of this study is to analyse SDHB expression in a series of Warthin’s tumours, studying its relationship with oncocytic changes, constantly present in this form of tumour.

Material and methods: In resection tumour specimens from a series of ten Warthin’s tumours (all from the parotid gland), immunohistochemical expression of SDHB was analysed using a commercially available monoclonal antibody.

Results: The Warthin’s tumours studied affected 10 men (mean age: 64.2 yrs, range 40–80), all with smoking habits, and 2 with metachronous bilateral involvement. Two patients presented associated urothelial carcinoma. Our SDHB study showed marked reactivity (++/+++) in all cases in the oncocytic epithelial component and also in striated duct cytoplasm (+) from non-tumorous parotid tissue. Expression was not influenced by age, smoking intensity or bilateral character.

Discussion and conclusions: Due to the constant and intense SDHB reactivity in oncocytic cells in our observations, oncocytic changes are not considered to be associated with defective enzyme activity in the mitochondrial complex II. Strong SDHB reactivity is an additional marker of oncocytic changes in Warthin’s tumour. Neither of these facts has been described previously.© 2011 Elsevier España, S.L. All rights reserved.

PALABRAS CLAVE
SDHB; Tumor de Warthin; Oncocitos; Inmunohistoquímica

Expresión de SDHB en el tumor de Warthin

Resumen
Introducción: El objetivo del presente estudio es analizar la expresión de la succinodeshidrogenasa B (SDHB), enzima perteneciente al complejo mitocondrial II, en el tumor de Warthin

∗ Corresponding author.
E-mail address: vera_fra@gva.es (B. Vera-Sirera).
Introduction

Warthin’s tumour is a frequent benign tumour of the salivary glands, accounting in some series for up to 30% of all primary epithelial tumours of the parotid gland, which is its most frequent location. The morphology of the tumour is very characteristic, presenting an epithelial component with papillary projections and cystic transformation, as well as a lymphoid stromal component, with germinal centers, with a constant presence of oncocytic luminal epithelial cells.

The term “oncocytic” describes a cellular change consisting of an enlargement of the cytoplasmic surface, accompanied by marked eosinophilia, with granular character, presenting hyperchromatic nuclei, and appearing as large cells, with well-defined cell boundaries. This oncocytic change is the result of a massive presence of mitochondria, which are detectable ultrastructurally or by immunohistochemical analysis.

Succinate dehydrogenase (SDH) is a mitochondrial enzyme belonging to enzyme complex II. It has a crucial role in cellular energy metabolism, being involved in the aerobic respiratory chain and tricarboxylic acid cycle. There are several subunits (A, B, C, D) of this enzyme, and it is known that mutations of the SDH gene determine the genetic basis of paragangliomas/pheochromocytomas. Mutations in the SDH gene produce a loss of enzymatic activity with functional instability in the mitochondrial respiratory chain, accompanied by alterations in mitochondrial morphology with numerous giant mitochondria that are functionally inefficient and which explain the occasional oncocytic appearance of these paragangliomas/pheochromocytomas.

It has recently been proven that the study of expression of the B subunit of SDH by itself is sufficient to establish the existence of mutational changes, susceptible to genetic screening, in any of the subunits of SDH.

In the present article, we intend to explore the immunohistochemical expression of SDHB in Warthin’s tumour, analyzing whether the presence of oncocytic changes in this tumoural form is related to alterations in the expression of mitochondrial enzyme complex II.

Materials and Methods

We studied specimens from surgical excision in a series of 10 Warthin’s tumours, all affecting the parotid gland, analysed over the last year in the Pathology Department at Hospital Universitario La Fe in Valencia. In all cases, we obtained the following clinical–pathological data: age, gender, location, smoking history referred to number of cigarettes/day (stratifying according to the scheme followed in the Fagerström nicotine dependence test) and number of years of habit, bilateral character, type of surgery performed and associated diseases at the time of surgery. All these data were obtained through our medical records software application MIZAR 2.0, using the Luna browser available in the Intranet of Hospital Universitario La Fe. The original histological preparations on which the diagnoses were based were available in all cases, as were the corresponding paraffin blocks. In each case, we selected a paraffin block containing tumoral representation and non-tumoral salivary parotid tissue.

We performed a histochemical PTAH stain (Mallory’s phosphotungstic acid-haematoxylin), for the identification of oncocytic changes in the samples studied, using the DiaPath kit (Martinegno, Italy), with prolonged incubation for 16–24 h according to the recommendations by other authors. In addition, 2 immunohistochemical stains against anti-mitochondrial antibody (Ab 113-1) (Ab Mo MU213-UC, clone 113-1-BioGenex®, San Ramon, CA, USA), at a 1/80 dilution, and against anti-peroxiredoxin antibody (Prx-1)
(clone Poly Prx-1, Alexis Co-Enzo Life Science, Lausen, Switzerland), at a 1/500 dilution, were used.

The 113-1 anti-mitochondrial antibody used recognises a 60 kDa non-glycosylated protein present in mitochondria, which enables identification of oncocytic cells in different tumours,\(^\text{15}\) including those with a salivary location.\(^\text{6}\) The Prx-1 antibody recognises an isoform of proteins involved in peroxide detoxification, as a protective mechanism against oxidative stress, and which is overexpressed in oncocytic lesions of the salivary gland.\(^\text{16}\)

Subsequently, as per the aim of the study, we carried out an immunohistochemical technique against a 30 kDa subunit of mitochondrial enzyme complex II using a mouse monoclonal antibody anti-SDHB (clone 21A 11AE7) (Molecular Probes®-Invitrogen Co, CA, USA), used at a 1/100 dilution and pH 9, with an incubation time of 20 min. Immunostaining was assessed in a semiquantitative form as negative (−) or positive (+), and the latter was scored into +/++/+++ depending on the intensity of the marking.

All immunostains were performed using a Dako EnVision™ Flex+ visualisation system with the Dako Autostainer Plus Link automatic immunostainer (Dako® Denmark). As positive controls, we used sections of liver and myocardium (given their mitochondrial abundance) and, as the negative control, we omitted the primary antibody, replacing it with phosphate saline buffer.

## Results

### Clinical—Pathological Data

Table 1 outlines the clinical–pathological data of 10 patients included in the series studied, all of them diagnosed with Warthin’s tumour affecting the parotid gland. All 10 patients were males, aged between 45 and 80 years with a mean age of 64.2 years, with only 3 aged ≤50 years. All patients had a history of smoking, with 6 of them indicating that they consumed >20 cigarettes/day. Of the remaining 4 patients, 2 reported consumption of between 11 and 20 cigarettes/day and another 2 of ≤10 cigarettes/day. However, they all indicated duration of the habit over 20 years. A total of 4 patients were still smokers at the time of diagnosis. Nevertheless, none of the patients presented a history of irradiation of the parotid area or of the head and neck region. In all cases, the surgery performed was a superficial parotidectomy, with the exception of 3 patients who underwent a partial lateral parotidectomy, given the small diameter of their lesions and good delineation.

In 6 patients, the tumour was located on the left parotid gland and in 4 on the right; the most common location (7 cases) was the lower pole of the parotid superficial lobe. In 2 patients, there was evidence of bilateral tumour involvement of metachronous nature (4 and 8 years before the current surgery). Among the associated diseases present at the time of diagnosis, it is noteworthy that 3 patients (66, 70, and 80 years old, respectively) presented concomitant urinary tract disorders, with transitional cell tumours of the bladder. Four patients were hypertensive and in clinical treatment, and another 3 suffered type 2 diabetes, one of them with an associated dyslipidemia metabolic syndrome.

The SDHB immunostaining showed a granular marking (+) in the cytoplasm of striated duct cells (Fig. 4) of the non-tumoral parotid gland. In Warthin’s tumour, there was a specific granular marking (++/+++) of the entire epithelial component (Figs. 5 and 7). Fine granular staining marked all epithelial cells with broad cytoplasm and an oncocytic tendency (Fig. 6). Only in one observation, in which there
<table>
<thead>
<tr>
<th>Case</th>
<th>Gender</th>
<th>Age</th>
<th>Smoking Habit (^a)</th>
<th>Location</th>
<th>Bilaterality</th>
<th>Surgery</th>
<th>Associated Disease (^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Male</td>
<td>79</td>
<td>Yes (15 c/d)</td>
<td>Left parotid gland Inferolateral segment</td>
<td>No</td>
<td>Superficial parotidectomy</td>
<td>AHT, prostate syndrome DM type 2</td>
</tr>
<tr>
<td>2</td>
<td>Male</td>
<td>63</td>
<td>Yes (20–30 c/d)</td>
<td>Right parotid gland Tail of the parotid</td>
<td>Yes (MT)</td>
<td>Partial lateral parotidectomy</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>Male</td>
<td>77</td>
<td>Yes (20 c/d)</td>
<td>Right parotid gland Superficial lobe</td>
<td>Yes (MT)</td>
<td>Superficial parotidectomy</td>
<td>AHT, DM type 2</td>
</tr>
<tr>
<td>4</td>
<td>Male</td>
<td>80</td>
<td>Yes (20 c/d)</td>
<td>Left parotid gland Inferolateral segment</td>
<td>No</td>
<td>Superficial parotidectomy</td>
<td>Vesical carcinoma, transitional cells</td>
</tr>
<tr>
<td>5</td>
<td>Male</td>
<td>47</td>
<td>Yes (20 c/d)</td>
<td>Left parotid gland Superficial lobe</td>
<td>No</td>
<td>Superficial parotidectomy</td>
<td>AHT</td>
</tr>
<tr>
<td>6</td>
<td>Male</td>
<td>65</td>
<td>Yes (10 c/d)</td>
<td>Right parotid gland Inferolateral segment</td>
<td>No</td>
<td>Partial lateral parotidectomy</td>
<td>COPD</td>
</tr>
<tr>
<td>7</td>
<td>Male</td>
<td>45</td>
<td>Yes (20 c/d)</td>
<td>Right parotid gland Superficial lobe</td>
<td>No</td>
<td>Superficial parotidectomy</td>
<td>DM-2, dyslipidemia, metabolic syndrome</td>
</tr>
<tr>
<td>8</td>
<td>Male</td>
<td>70</td>
<td>Yes (20 c/d)</td>
<td>Left parotid gland Inferolateral segment</td>
<td>No</td>
<td>Superficial parotidectomy</td>
<td>Vesical carcinoma, thrombotic stroke</td>
</tr>
<tr>
<td>9</td>
<td>Male</td>
<td>66</td>
<td>Yes (20 c/d)</td>
<td>Left parotid gland Superficial lobe</td>
<td>No</td>
<td>Superficial parotidectomy</td>
<td>Vesical carcinoma, transitional cells</td>
</tr>
<tr>
<td>10</td>
<td>Male</td>
<td>50</td>
<td>Yes (5 c/d)</td>
<td>Left parotid gland Inferolateral segment</td>
<td>No</td>
<td>Partial lateral parotidectomy</td>
<td>AHT</td>
</tr>
</tbody>
</table>

AHT, arterial hypertension; COPD, chronic obstructive pulmonary disease; DM-2, diabetes mellitus type 2.

\(^a\) Smoking habit as referred to no. of cigarettes/day.

\(^b\) Associated disease at the time of surgery.
SDHB Expression in Warthin’s Tumour

Figure 2 Immunohistochemical staining with anti-mitochondrial antibody 113-1, showing specific staining of oncocytic changes present in Warthin’s tumour (Ab 113-1, 150×).

were foci of squamous metaplasia in the epithelial component of the tumour, was there absence of reactivity (Fig. 7). According to the intensity of immunostaining in the tumour component, 4 cases were classified as intensity ++ and 6 cases as +++. None of the 10 cases studied presented epithelial areas of oncocytic tendency that were defective or had a mute expression of SDHB, except for the areas of squamous metaplasia (Fig. 7). By comparing the intensity of immunostaining with patient age (based on 2 age groups: patients <50 years and ≥50 years) with the intensity of smoking habit identified in all patients for more than 10 years (relative to <10, 10–20, and >20 cigarettes/day) and the bilateral character (Table 2), we found no apparent differences in the reactivity of SDHB found in these different groups of patients with parotid Warthin’s tumour.

Discussion

Warthin’s tumour is the second most frequent benign tumour of the salivary glands. It affects mainly the parotid gland, especially in male patients in their sixth decade of life. Its incidence is higher in patients with smoking habits, with the age at start, duration and intensity of the habit (dose–response association) all having an influence on the incidence of this benign salivary tumour. Its predominance in males has been explained as due to the fact that they are smokers more frequently; however, an increase in tumour incidence in women has also been noted over the past 50 years, in relation to the increase of female smokers.

In our series, all patients were male, with a mean age of 64.2 years. All of them had been smokers for more than 20 years, with a daily intake of 20 cigarettes/day in 6 patients. At the time of surgery, 2 patients presented concomitant urinary tract transitional tumours, a tumoral disease also associated with smoking habit. The most frequent tumour location was the lower pole of the parotid superficial lobe, an aspect that has been linked to the greater abundance of intraparotid nodes at this level. The surgical interventions performed were limited in all cases, in line with current trends in the surgical treatment of benign tumours, especially those of small size. Two patients (20%) presented bilateral tumour lesions of a metachronous nature, and this bilaterality was also reported in association to patients with strong chronic tobacco consumption. However, none of our patients reported a history of irradiation of the parotid gland or the anatomical region of the head and neck, another aetiological factor identified in oncocytic tumours of different locations, including those of the salivary gland.
Figure 5  (A and B) Serial sections of a Warthin’s tumour stained with H&E and with SDHB. It is possible to note the marking of tumoral oncocytic epithelium and the clear differentiation from the lymphoid component (HE and SDHB, 100x).

Figure 6  (A and B) Detail of SDHB expression in Warthin’s tumour, with the cytoplasm of the tumoral oncocytic epithelium being marked in specific and granular manner (HE and SDHB, 250x).

The aetiological and epidemiological factors mentioned above (tobacco consumption, irradiation and involvement of patients in advanced ages) appear in possible relation to the presence of genetic damage (genomic deletion of 4977 bp), observed in mitochondrial DNA (mtDNA) of oncocytic Warthin’s tumour cells in the last decade. Thus, cellular aging or senescence would produce a cumulative oxidative damage to mtDNA. Likewise, the consumption of tobacco causes an increase in reactive oxygen species (ROS) which cause cumulative damage in mtDNA, accompanied by a deficiency in oxidative phosphorylation (OXPHOS) phenomena. All these are associated to morphological mitochondrial changes characterised by the appearance of giant mitochondria with a hypertrophic character and attached layout, responsible for the so-called oncocytic changes, which are detectable ultrastructurally or through immunohistochemical techniques.

In our study, we first used 3 different staining methods (PTAH, 113-1 and Prx-1) to highlight oncocytic changes present in Warthin’s tumour. Comparatively, the most

Figure 7  (A and B) SDHB expression delimits the cytoplasm of oncocytic cells of Warthin’s tumour, which line a papillary tumour (A). The foci of squamous metaplasia (B) which can optionally be found inside the tumour, unlike oncocytic epithelium, are negative for SDHB (SDHB 400, 250x).
accurate and specific methodology was that obtained with 113-1 monoclonal antibody, as already noted previously.6,6 The PTAH staining used in our study, with prolonged incubation from 16 to 48 h, previously recommended by other authors,6,14 is the classic methodology used to identify cells with oncocytic changes, producing a blue, granular, cytoplasmic staining, although it is clear that PTAH staining is not entirely specific to mitochondrial structures. The coarse granular immunostaining obtained in oncocytic cells with Prx-1, a H2O2 compaction protein, can be explained by the accumulation of free radicals and ROS that occurs in these cells,6,14 although the immunostaining obtained is less specific than that of antibody 113-1, probably due to the polyclonal nature of the Prx-1 antibody.

After identification of oncocytic cells, the main objective of our study was to explore the expression of SDHB (B subunit of succinate dehydrogenase) in the oncocytic component of Warthin's tumour. Succinate dehydrogenase (SDH), also known as succinate-ubiquinone oxidoreductase, is a mitochondrial enzyme complex that catalyses the oxidation of succinate to fumarate in the citric acid cycle, also participating in the electron transport chain.

Located in the inner mitochondrial membrane, SDH consists of several subunits (SDHA, SDHB, SDHC and SDHD) and has been shown that mutations in the SDH gene underlie the pathogenesis of paragangliomas/pheochromocytomas7,8 and associated tumour syndromes (Carney–Stratakis syndrome and Carney triad).14 In these processes, there is often an absence of immunohistochemical expression of SDHB2,26 and the immunohistochemical study of SDHB has consequently become a powerful diagnostic tool, since the absence of expression of SDHB is indirectly indicative of probable presence of mutations in one of the subunits of SDH. In this regard, mutations in the SDHB and SDHD genes are one of the components of the appearance of paragangliomas, contributing to the majority of familial cases and perhaps to as much as 8% of the apparently sporadic cases. However, the frequency of SDHC mutations is variable and generally very low, as is also the case with SDH5, through the flavination of SDHA.8 Therefore, the tumour genesis of paragangliomas implies mutational changes in the SDH gene,7,12 probably related to alterations of the mechanisms involved in cell proliferation, apoptosis and the role of oxygen sensing.

Mutational changes of SDH are accompanied by inefficient mitochondrial enzyme activity, with deficient ATP production that induces the appearance of oncocytic phenotypic changes, due to mitochondrial hyperplasia.10 Interestingly, ultrastructural studies7 have shown mitochondrial changes (appearance of giant mitochondria, with paracrystalline inclusions) of an oncocytic type in paragangliomas, in which the pathogenetic role of SDH mutations is already well known.7,8,12,26 This would explain the presence of oncocytic variants or subtypes described in paragangliomas/pheochromocytomas.7,11

However, in our study of a series limited to 10 observations of Warthin's tumour, we found an increased expression of SDHB, both at the level of the cytoplasm and of the striated ducts of the non-tumoral parotid gland, and in a more intense manner in oncocytic cells of the epithelial component of Warthin's tumour. These data, not previously described in the medical literature, lead us to consider that it is likely that the genetic damage reported21 in the mtDNA of Warthin's tumour does not involve a defective alteration in the enzyme activity of SDHB (mitochondrial enzyme complex II). In addition, in line with what has been described in other forms of tumoral disease,7,8,12,26 it is likely that mutations in the SDH gene do not play a pathogenetic role in Warthin's tumour. It remains to be confirmed if our hypothesis holds true in larger series of Warthin's tumours, thus clarifying whether the genetic damage existing in Warthin's tumour is in mtDNA mutations affecting enzyme complex I genes, as was recently demonstrated in renal28 and thyroid17 tumours of oncocytic phenotype.

Conflict of Interests

The authors have no conflicts of interest to declare.

References


