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RESEARCH

MT1 Melatonin Receptor Expression in Warthin's Tumor

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Abstract We contribute the first immunohistochemical study of MT1 melatonin receptor in Warthin's tumor and normal parotid gland. All 14 Warthin's tumors studied showed intense cytoplasmic positivity for MT1 receptor in all cylindrical epithelial cells lining spaces and a less intense positivity in basal cells. The lymphoid component accompanying the tumor was always negative for MT1 receptor. The parotid structure surrounding the tumor showed intense cytoplasmic positivity in all cells lining excretory ducts (lobar and lobulillar), with a lesser and focal positivity in cells of the acinar component. The biological activity of MT1 receptor in epithelial cells lining parotid excretory ducts may resemble its activity in Warthin's tumor cells. Hence, we propose Warthin's tumor as a useful positive control in immunohistochemical studies of MT1 melatonin receptor.

Keywords Immunohistochemistry · Melatonin · MT1 receptor · Normal parotid gland

All authors approve the submission

All authors have participated sufficiently to take public responsibility for appropriate portions of the work

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Introduction

Two types of melatonin receptor, Mel1a (MT1) and Mel2a (MT2), have been reported in the cytoplasmic membrane of human cells [1]. The MT1 gene is located at chromosome 4 and MT2 gene at chromosome 11 [2, 3] These receptors, which are coupled to heterotrimeric guanine nucleotide binding proteins (G proteins), are found in high concentrations in the pituitary, the suprachiasmatic nucleus of the hypothalamus, retina, and ependymal cells of the choroid plexus [4, 5]. MT1 receptor has been implicated in the inhibition of melatonin in the suprachiasmatic nucleus of the hypothalamus and in the effect of melatonin on MCF-7 breast cancer cells [6]. Experimental findings in rats suggest that the MT2 receptor mediates the action of melatonin in the retina and induces phase shift of the circadian rhythm in the suprachiasmatic nucleus of the hypothalamus [7].

The MT1 receptor has been described in multiple sites of the human body [8] and in some benign and malignant conditions [9–16]. To our best knowledge, this is the first report on the distribution and expression of MT1 receptor in Warthin's tumor and normal parotid gland.

Material and Methods

Patient Samples

The study included 14 patients (8 females and 6 males) with Warthin's tumor aged 32 to 57 years; normal parotid gland

was also examined in 8 of these patients. Written informed consent was obtained from all subjects.

Immunohistochemical Analysis

Samples were fixed in 10 % buffered formalin for 24 hrs and embedded in paraffin. Paraffin-embedded 4-μm sections were dewaxed, hydrated, and heat-treated at 95 °C for 20 min in 1 mM EDTA buffer pH 8 for antigenic unmasking. Sections were incubated for 30 min at room temperature with goat polyclonal antibody raised against a peptide mapping at the N-terminus of MEL-1A R of human origin; it was applied at a dilution of 1:500 (Santa Cruz). The immunohistochemical study was done on an automatic immunostainer (Autostainer 480, LabVision Fremont, CA) by indirect polymer-peroxidase-based method followed by development with diaminobenzidine (Masvision, Master Diagnostica, Granada, Spain).

The MT1 receptor cytoplasmic staining pattern was graded as weakly positive (+), moderately positive (++), or strongly positive (+++); samples of brain and retina tissue were used as positive controls.

Results

Histopathological Study

Epithelial Component This is formed by clefts, spaces and tubular structures lined by a cylindrical epithelium with monomorphic nuclei and abundant eosinophilic cytoplasm; the clefts and spaces contain secretion (Fig. 1a and c). Small, discontinuous basal cells were also observed.

Lymphoid Component This revealed abundant reactive lymphoid cellularity comprising mature lymphocytes that formed lymphoid follicles; some lymphocytes were observed among epithelial cells (Fig. 1c).

Immunohistochemical Study

Epithelial Component This presented intense (+++) positivity for MT1 receptor in the cytoplasm and a lesser positivity (+) in the cell membrane; positivity (++) was also observed in the cytoplasm of basal cells (Fig. 1b and d).

Lymphoid component: this was negative for the MT1 receptor, although a slight positivity (+) was detected in some histiocytary cells in lymphoid follicles; lymphocytes located among epithelial cells were negative for MT1 receptor.

The normal parotid structure (Fig. 1e), examined in eight of these patients, showed moderate cytoplasmic positivity (++) for MT1 receptor in the epithelium lining excretory ducts and a lesser positivity (+) in acinar ducts, which exhibited a granular cytoplasmic pattern (Fig. 1f).

Discussion

The present study has shown the expression of the MT1 melatonin receptor in the epithelial cells of the Warthin's tumor and in the excretory ductal epithelium and its absence in the secretory acinic cells.

Melatonin (*N*-acetyl-5-methoxytryptamine) is a lipophilic hormone that is primarily synthesized and secreted by the pineal gland [17] and is widely distributed throughout the human body [18]. It exerts chronobiotic, immunomodulatory, oncostatic and antioxidant actions, among others, via direct and indirect mechanisms [19–22]. Melatonin is also synthesized in the digestive system by enterochromaffin cells of the intestinal mucosa in response to food intake [23], sometime reaching concentrations up to 400-fold higher than those produced by the pineal gland [24].

After its release into the blood, melatonin enters the oral cavity by passive diffusion in the saliva. Melatonin concentrations in saliva are 15–33 % of those in plasma, since 70 % of plasma melatonin is bound to albumin and does not enter the saliva to any appreciable extent. Hence, salivary melatonin represents the percentage of circulating melatonin that is not albumin-bound, *i.e.,* free melatonin. Evaluating the levels of salivary melatonin is a reliable technique for monitoring circadian rhythms [18].

The presence of MT1 and MT2 receptors was recently reported in rat parotid gland in an immunoblotting study [25] but immunohistochemical distribution and expression of MT1 receptor has not previously investigated in normal or pathologic human glands. The MT1 receptor has been found in multiple sites in the human body [8], in cancers of the prostate, breast, bone, and gallbladder, and in melanomas [9–16]. To our best knowledge, this is the first report of MT1 expression in Warthin's tumor and in normal neighboring parotid gland.

Besides its circadian and sleep promoting effects, the functional role of the action of melatonin on its receptors (MT1 and MT2) remains unclear [8], and the biological role and clinical relevance of the MT1 receptor in normal and tumor tissues are poorly understood. The MT1 receptor was found to modulate the proliferation of malignant cells [26] and was reported to mediate the effects of melatonin on growth suppression and gene modulation in

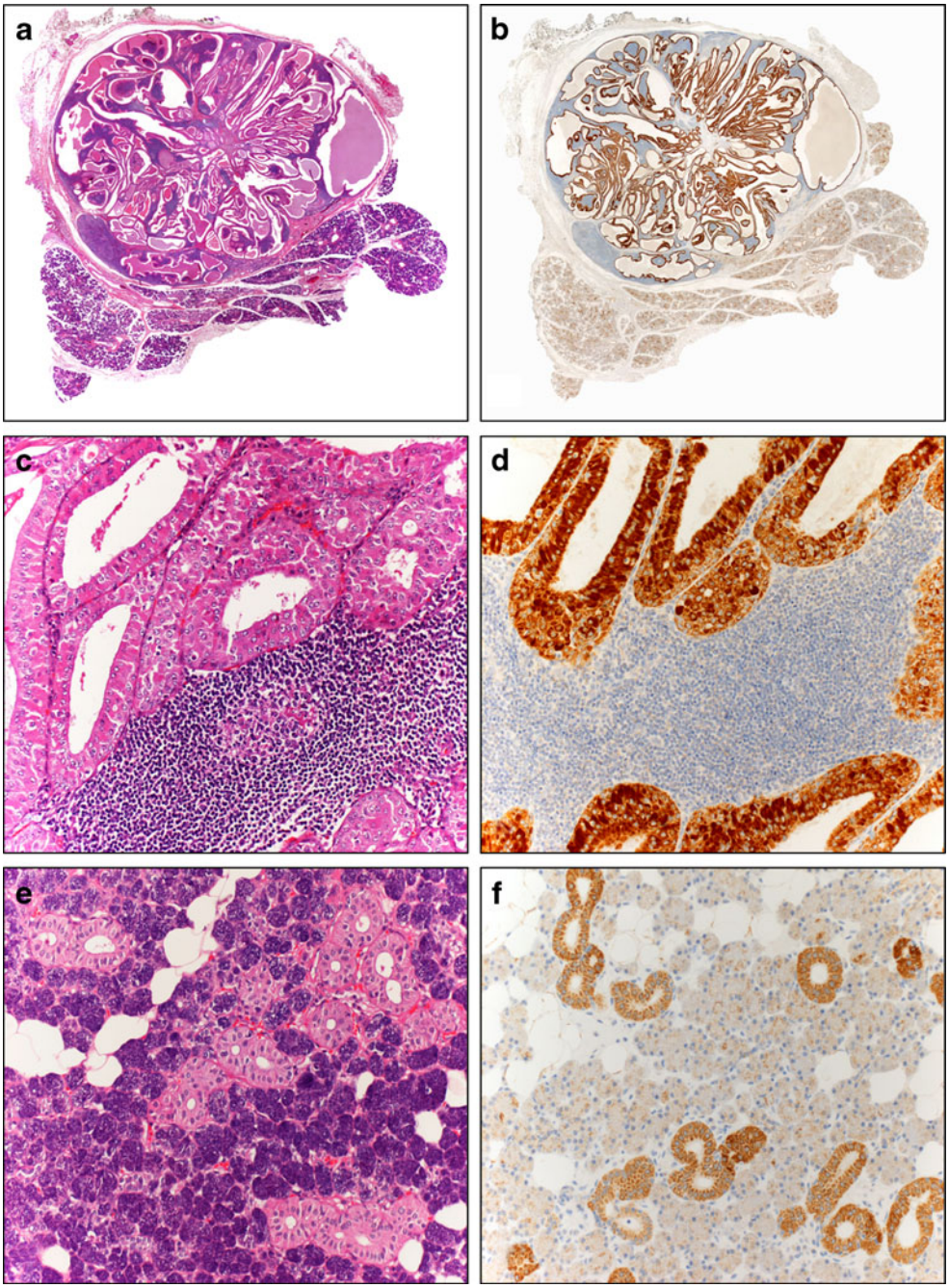

Fig. 1 **a**. Well-defined tumor comprised of clefts and cystic spaces containing secretion, which are lined by epithelial cells with lymphoid stroma (Warthin's Tumor). The tumor is accompanied by a normal parotid gland (hematoxylin & eosin, Panoramica). **b**. Intense positivity for MT1 in cytoplasm of epithelial cells of the Warthin's tumor and in ducts of the normal parotid gland (panoramic). **c**. Tubular structures lined by epithelium with abundant eosinophilous cytoplasm with monomorphic nuclei; a few basal cells can be observed. The stroma shows an abundant lymphoid component with lymphoid follicles. **d**. Intense positivity for MT1 can be observed in epithelial cells of Warthin's tumor, with no MT1 expression in stroma. **e**. Acinar and ductal components of the normal parotid gland. **f** Moderate cytoplasmic positivity in the cells that form the ducts (intercalated and striated) and slight granular cytoplasmic positivity (+) for MT1 in acinar cells

breast cancer cells [15]. However, no data have been published on MT1 receptor expression in benign tumors. In the present study, the cylindrical tumor cells showed intense cytoplasmic granular positivity for this receptor, consistent with the location of the targeted antigen (peptide 536), i.e., the 19 amino acid sequence of the c-terminal region of the receptor. Given that Warthin's tumor cells have abundant mitochondria [27], we consider that the target antigen would be preferentially localized in the mitochondrial membranes.

Finally the intense positivity for the MT1 receptor in excretory ducts of the normal parotid gland may indicate that this receptor has a comparable biological function in the epithelium of these ducts as it has in Warthin's tumor. We also propose Warthin's tumor as a useful positive control in immunohistochemical studies of MT1 melatonin receptor.

Conflict of interest The authors have no conflict of interest to declare.

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