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Cyclooxygenase-2 expression in Warthin's tumour

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Abstract

Objectives: To determine whether cyclooxygenase-2 (COX-2) is overexpressed in Warthin's tumours, and to characterize its pattern of expression.

Methods: Twenty-one paraffin-embedded Warthin's tumour specimens were analysed by immunohistochemical staining for expression of human COX-2. Semi-quantitative analysis of the staining was performed.

Results: In all of the specimens, we found that there was overexpression of COX-2 within the epithelial component of the tumours, with no expression in the lymphoid components. There was also overexpression of COX-2 in the salivary duct system of normal parotid tissue.

Conclusions: Our results suggest that COX-2 is up-regulated in the epithelial component of Warthin's tumours. Our findings support the hypothesis that Warthin's tumours originate from heterotopic ductal epithelial cells of the parotid gland. The role of COX-2 expression in the pathogenesis of Warthin's tumours remains to be determined.

Key words: Cyclooxygenase-2; Immunohistochemistry; Warthin's Tumour

Introduction

Warthin's tumour was initially described by Hildebrand,[1] and first reported in English by A S Warthin in 1929.[2] Over the years, this tumour has been known by many different names, with the three most popular being cystadenolymphoma, papillary cystadenoma lymphomatosum, and Warthin's tumour. It consists of two components: an oncocytic epithelial component, arranged in two layers, which develops cysts and papillary projections, and a variable lymphoid component containing follicles with germinal centres. The tumour is unique among salivary gland tumours in that it occurs almost exclusively in the parotid gland,[3] has its characteristic lymphoid component, and usually occurs in men at a peak age between the fourth and seventh decades of life.[4] This entity is a completely benign tumour, with the risk of malignant transformation being exceedingly rare, and only a few reports of such transformation have previously been published.

Cyclooxygenase (COX) catalyzes the synthesis of prostaglandins (PG) from arachidonic acid. Two enzyme isoforms have been identified: COX-1 which is constitutively expressed as a housekeeping gene in most cells, and COX-2 which is expressed as an early-response gene, activated by various stimuli including inflammatory cytokines, growth factors, and oncogenes.[5] Elevated COX-2 expression has been shown to occur in several malignancies, including colorectal,[6] breast,[7] lung,[8] pancreatic,[9] gastric,[10] hepatocellular,[11] and skin carcinomas.[12] In the head and neck, COX-2 has been reported to be up-regulated in squamous cell carcinomas,[13] and differentiated thyroid carcinomas.[14] To our knowledge, there have been no studies which have examined the expression of COX-2 in salivary tumours. In this study, our aim was to determine if there was COX-2 immunohistochemical expression in a series of Warthin's tumours, and to further characterize the pattern of distribution of this enzyme.

Materials and methods

Tissue specimens of Warthin's tumour diagnosed at the National University Hospital, Singapore between 1997 and 2001 were studied. The specimens were taken from a total of 21 consecutive patients who underwent surgical resection, all of whom were male. The mean age at the time of diagnosis was 57 years (age range from 38 to 82 years) and the specimens were all unilateral tumours.

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The paraffin-embedded tissues were cut serially 4–5 μm thick and mounted on the pre-coated slides. For each case, haematoxylin and eosin (H&E) staining for one tissue section was performed for histological review and the other sections were subjected to immunoperoxidase staining using COX-2 monoclonal antibody (catalogue no. 160112, Cayman Chemicals). Negative test controls in each staining series included sections in which the primary antibody was replaced by buffer solution. Normal parotid tissue specimens were used as negative tissue controls, and histological samples of COX-2-positive nasopharyngeal carcinoma specimens were used as positive external controls.

- **Cyclooxygenase-2 (COX-2) is overexpressed in many malignancies, but has not previously been shown to be expressed in salivary gland tumours**
- **This study shows that COX-2 is overexpressed in the epithelial component of Warthin's tumour and in the salivary ductal epithelium of the surrounding normal parotid tissue**
- **This supports the hypothesis that Warthin's tumours originate from heterotopic salivary ductal epithelium**

COX-2 immunohistochemistry

The slides were microwaved for 20 minutes in citrate buffer at pH 6.0, followed by endogenous peroxidase block by 0.6 per cent H_2O_2 in methanol for 30 minutes. The slides were then incubated overnight with COX-2 primary antibody (1:500) at room temperature. This was followed by DAB incubation and counterstaining with Mayer's haematoxylin.

Immunohistochemical assessment

After confirming the validity of the test batch, COX-2 staining intensity and immunoreactivity were evaluated by the same observer (T C P). A validated method of semiquantitative evaluation of the immunohistochemical stains was performed, and this involved comparison of the staining intensity and the estimated proportion of the positively stained cells with the total number of tumour cells.[15] In brief, the staining intensity was graded and subdivided into four categories (0 = negative, 1 = weakly positive, 2 = moderately positive, 3 = strongly positive), and the proportion of positively stained cells were also subdivided into four categories (0 = negative, 1 = <10% positive cells, 2 = 10–50% positive cells, 3 = >50% positive). The overall score for each test specimen was then obtained by multiplying the intensity score with the immunoreactivity score. An overall score of >4 would suggest an overexpression of COX-2 whereas a score of 1–4 suggested weak expression, and 0 was negative.

Results

All 21 analysed specimens of Warthin's tumour showed overexpression of COX-2 (scores > 4) within the epithelial component of the tumour entities. The staining pattern was localized to both the basal and luminal layers of the oncocytic epithelial component (Figure 1), with the cells showing strong cytoplasmic activity and little nuclear staining. There was no staining detected in the lymphoid component of the tumours. Strong COX-2 immunoreactivity was also present in the secretory ductal system of the surrounding normal parotid gland in all of the analysed specimens (Figure 1(d)). In stark contrast, there was no COX-2 expression in the rest of the normal parotid tissue.

Discussion

While COX-2 expression has been closely associated with carcinogenesis and has been shown to be up-regulated in many solid malignancies, we believe our study has shown for the first time the overexpression of COX-2 in a salivary gland tumour. Up-regulation of COX-2 has been postulated to promote carcinogenesis by inhibiting apoptosis,[16] promoting angiogenesis,[17] and enhancing invasiveness.[18] However, the exact mechanism by which COX-2 overexpression results in neoplasia is still debated.[19] Nevertheless, there have been suggestions of using selective COX-2 inhibitors to prevent and treat human cancers, although epidemiologic and randomized studies are lacking. There appears to be a definite positive relationship between Warthin's tumour and cigarette smoking, with smokers having an eight to nine times risk of developing Warthin's tumour when compared to non-smokers.[20] The increasing frequency of this tumour in women is also thought to be related to the increased tobacco use among them.[21] There is now increasing evidence that cigarette smoke can induce the expression of COX-2.[22,23] Further investigation is clearly needed to establish whether cigarette smoking influences the expression of COX-2 in salivary tissue and its role in the subsequent development of Warthin's tumours.

While the exact aetiology and pathogenesis of Warthin's tumour remain to be determined, several hypotheses have been proposed to try to explain the origin of this unusual tumour. The most common hypothesis suggests that the tumour originates from salivary ducts trapped in intra- and periparotid lymph nodes during embryological development.[24] The detection of normal nodal structures such as subcapsular sinuses, and the occurrence of these tumours in periparotid lymph nodes lends support to this hypothesis. This concept is further supported by the findings that the tumour has a specific predilection for the parotid gland and is not found in the submandibular or sublingual glands. A second hypothesis which is less commonly subscribed proposes that the tumour begins as an epithelial neoplasm, and that the lymphoid element represents a secondary inflammatory or immunological response.[25] Teymoortash *et al*. have recently investigated the status of sex hormone receptors in

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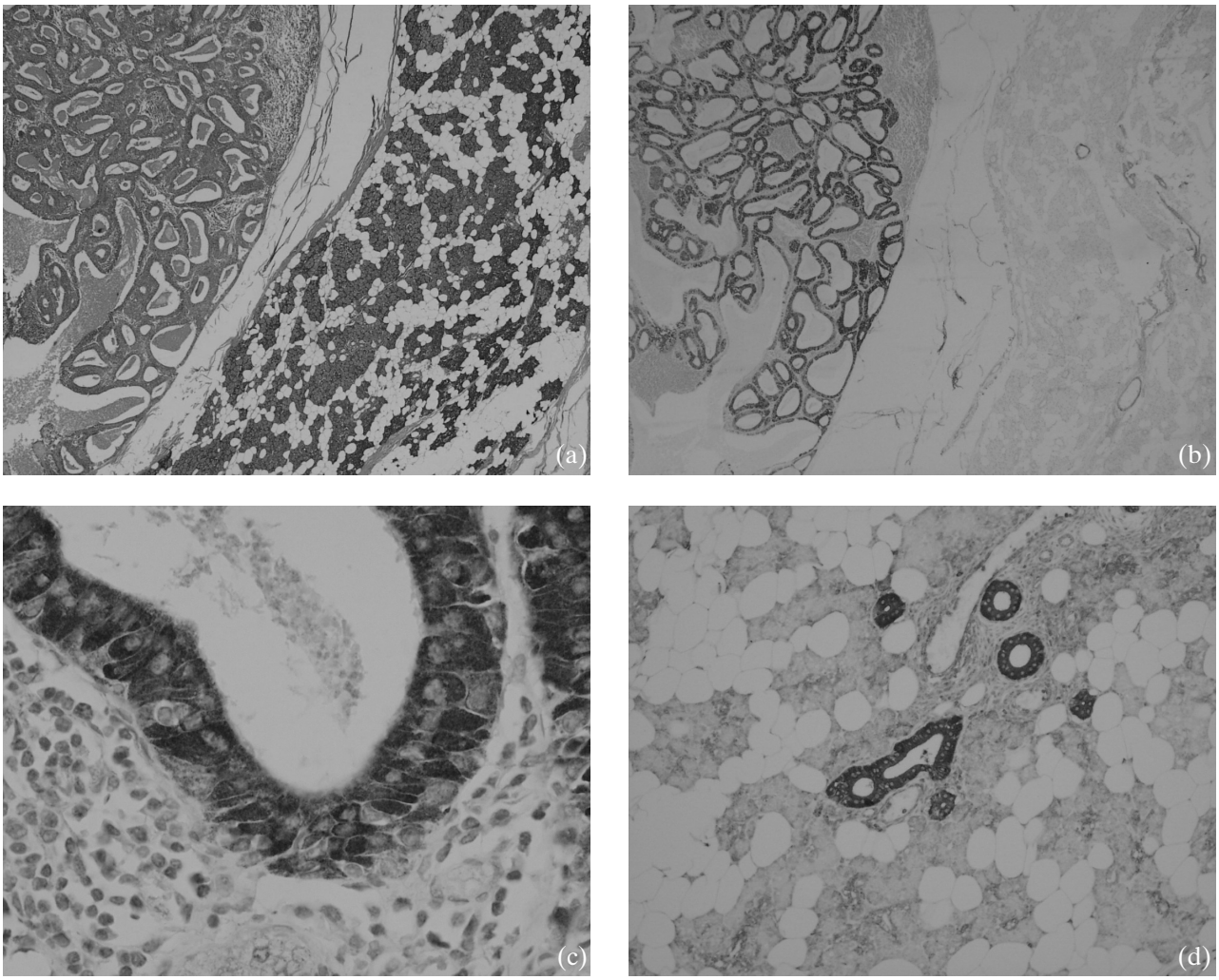

FIG. 1

COX-2 immunohistochemisty and H&E staining of Warthin's tumour and parotid tissue. (a) and (b) show Warthin's tumour stained with H&E and anti-COX-2 antibody, respectively (magnification ×40). (c) COX-2 expression was localized only to the epithelial component of Warthin's tumour (×100). (d) The salivary ductal system of normal parotid tissue also stained positively for COX-2 (×100).

Warthin's tumour and demonstrated that there is a similar pattern of expression of progesterone receptors in their series of Warthin's tumours, with expression being limited to the salivary duct system and epithelial components of the tumour only.[26] Brennan *et al.* also investigated the expression of the enzyme type 2 nitric oxide synthase (NOS2) in Warthin's tumours by immunohistochemistry. NOS2 is associated with the increased production of nitric oxide which has been implicated in the pathogenesis of many solid tumours.[27] They found widespread NOS2 expression in the epithelial cells of all 23 cases of Warthin's tumour studied, and additional expression in the salivary duct epithelium of normal parotid tissue. We have found in our study a very similar pattern of overexpression of COX-2 in the epithelial cells and in the normal surrounding salivary duct epithelium. Taking these findings in consideration, we favour the explanation that the proliferation of heterotopic salivary ductal epithelium results in the formation of this unique tumour.

Conclusion

We have demonstrated that COX-2 is up-regulated in the epithelial component of Warthin's tumours and in the salivary duct system of the normal parotid gland. These findings support the hypothesis that the oncocytic cell aggregates in Warthin's tumours are derived from normal salivary ductal epithelial cells. Further work is needed to establish the role of COX-2 in the pathogenesis of this unique and unusual salivary gland tumour.

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