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Absence of Epstein-Barr virus encoded RNA and latent membrane protein (LMP1) in salivary gland neoplasms

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Abstract

A series of 55 (42 benign and 13 malignant) salivary gland tumours were investigated by immunohistochemistry, to detect Epstein-Barr virus (EBV) latent membrane protein (LMP1) and by *in situ* hybridization for EBV-encoded RNA. Non-neoplastic gland from all the patients with tumours and 15 control glands were also examined. All cases, both neoplastic and non-neoplastic were negative for LMP1 and failed to show any positive signal by *in situ* hybridization for EBV RNA. One undifferentiated carcinoma from a European patient was included in the group. These results confirm previous reports of an ethnic association between EBV and undifferentiated carcinomas of the salivary gland. They do not support an aetiological role for EBV in other salivary gland tumours.

Key words: Herpesvirus 4, human; Hybridization; Salivary glands

Introduction

Epstein-Barr virus (EBV) is a ubiquitous human herpesvirus which can establish latent infection with a lifelong carrier state. It has been closely associated with a variety of tumour types, such as Burkitt's lymphoma (Epstein *et al.*, 1964; Prevot *et al.*, 1992) nasopharyngeal undifferentiated carcinoma (Lopategui *et al.*, 1994), Hodgkin's disease (Weiss *et al.*, 1989), and lymphoepithelial carcinoma of the salivary gland (Leung *et al.*, 1995). It is detectable in the saliva of apparently healthy persons (Gerber *et al.*, 1972). The reservoir appears to be recirculating B lymphocytes rather than oropharyngeal epithelial cells as previously thought (Karajannis *et al.*, 1997). It has been suggested that it may be associated with Warthin's tumours (Santucci *et al.*, 1993). With this background we wished to investigate the presence or absence of EBV in salivary gland tumours in a predominately European population.

Materials and methods

The study was a retrospective analysis of all salivary gland neoplasms received in St James's hospital during a five-year period. The control group consisted of parotid and submandibular gland tissue taken either from patients who had had a neck dissection for oropharyngeal carcinoma, or from autopsy cases. Patients with a history of previous radiation to the head and neck region were excluded. All of the tissue had been formalin fixed and paraffin embedded.

In the study group the tumours were reviewed and one block of neoplastic and non-neoplastic gland was selected. In the control group one block of salivary tissue was evaluated. Immunohistochemical identification of EBV latent membrane protein (LMP1) was performed using a cocktail of antibodies (CS1–4, Dako), using a standard avidin-biotin peroxidase (ABC) Strepavidin technique. Sections were pretreated with 0.05 per cent pronane (Dako) for eight minutes, prior to immunostaining. A nodal post-transplant lymphoproliferative disorder, known to be positive by PCR was used as the positive control. *In situ* hybridization for EBV-encoded RNA was also performed, using NCL-EBV-K (Novocastra) with provided control.

Results

There were 50 European patients and five Asian (all Sri Lankan) patients in the study group, age range nine to 83 years (mean age 43 years). Forty-nine tumours were in the parotid, four submandibular and one was palatal. The tumour types are listed in Table I. A total of 42 were benign and 13 malignant. All original tumour diagnoses were confirmed. In the control group, there were 15

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TABLE I
TUMOUR TYPES

Benign (Total 42)	
Pleomorphic adenoma (PSA)*	35
Warthin's tumour	4
Basal cell adenoma	3
Malignant (Total 13)	
Mucoepidermoid carcinoma	2
Salivary duct carcinoma	2
Non-Hodgkin's lymphoma	2
Acinic cell carcinoma	2
Adenocarcinoma arising in PSA	2
Polymorphous low grade adenocarcinoma	1
Undifferentiated carcinoma	1
Epithelial-myoepithelial carcinoma	1

*Five Asian patients in this group.

patients, all European, with an age range 49–73 (mean age 55 years). This group was made up of 10 parotids and five submandibular glands.

Weak immunohistochemical staining was identified in the nuclei of ductal epithelium and myoepithelium in 15 of the tumours and also in 15 non-neoplastic glands (both study and control). Weak diffuse staining of the cytoplasm was seen in the serous glands, concentrated at the bases of the acini and there was patchy weak epithelial cytoplasmic positivity in 12 tumours. There was no convincing positive staining and no detectable pattern to the weak staining was observed.

Cytoplasmic membrane staining was not present. All the staining was interpreted as non-specific. There were no positive signals detected by *in situ* hybridization for EBV RNA.

Discussion

Salivary gland tumours are a group of neoplasms known for their histological complexity but, generally little is known about their aetiology. Previous exposure to radiation both therapeutic (Spitz *et al.*, 1984) and following the atomic bomb (Takeiche *et al.*, 1983) has been established as a risk factor. An association with primary carcinoma at another site has been noted, although the relative risk reported is variable, with the strongest associated reported with skin cancer in males (Spitz *et al.*, 1984); other sites include breast, bronchus and ovary (Prior and Waterhouse, 1977; Biggar *et al.*, 1983). Overexpression of c-*erb*B 2 oncogene has been detected in both salivary duct carcinoma and carcinoma ex pleomorphic adenoma (Hellquist *et al.*, 1994; Muller *et al.*, 1994).

Several viruses are known to replicate and latently infect the salivary glands (Weiland, 1988; Deacon *et al.*, 1991) including CMV and EBV, and there is an established link between EBV and undifferentiated carcinomas in Eskimos (Hamilton-Dutoit *et al.*, 1991), and in southern Chinese (Huang *et al.*, 1988; Chan *et al.*, 1994; Leung *et al.*, 1995). Chan *et al.* (1994) in addition examined 49 other various tumours in a largely Chinese population but found EBV restricted to undifferentiated carcinoma. EBV has not been reported in the few undifferentiated carcinomas of the salivary gland in Caucasians (Hamilton-Dutoit *et al.*, 1991). This is similar to sinonasal undifferentiated carcinoma (Lopategui *et al.*, 1994) and contrasts with EBV-related undifferentiated carcinomas occurring in the nasopharynx where EBV is identified irrespective of ethnic origin.

EBV DNA has also been demonstrated by *in situ* hybridization in multifocal or bilateral Warthin's tumours, while solitary tumours were negative (Santucci *et al.*, 1993). The signal was cytoplasmic and probably represents a false positive reaction as suggested by Chan *et al.* (1994). The four cases in our study were negative for EBV, and others have reported similar findings reporting 10 (Chan *et al.*, 1994) and 17 (Kärjä *et al.*, 1997) cases as negative. Taira *et al.* (1992) found positivity in various tumours by PCR but this may represent contamination by normal salivary gland or B lymphocytes (Kärjä *et al.*, 1997). Fox *et al.* (1990) did not detect EBV by immunohistochemistry and Southern blot in five pleomorphic adenomas and five malignant tumours, and very recently, Kärjä *et al.* (1997) in a study of 219 cases by *in situ* hybridization failed to identify EBV and CMV. The study is from Finland but no details of ethnic origin were provided and none of the cases were undifferentiated carcinomas.

In this population we failed to demonstrate EBV in a variety of benign and malignant salivary gland tumours and the adjacent non-neoplastic gland, using both *in situ* hybridization for EBV-encoded RNA and immunohistochemistry for EBV latent membrane protein (LMP1). This included one case of an undifferentiated carcinoma occurring in a European patient and five tumours occurring in an Asian population. These results confirm previous reports (Hamilton-Dutoit *et al.*, 1991; Chan *et al.*, 1994) of an ethnic association between EBV and undifferentiated salivary carcinomas. The immunohistochemical staining observed was interpreted as negative on the basis of pattern and weakness of staining, and is similar to that observed with LMP by Deacon *et al.* (1991).

To conclude, none of the 55 salivary gland neoplasms studied contained EBV latent membrane protein or encoded RNA by *in situ* hybridization. While it is possible that undetected parts of the viral genomes are present, the findings do not support an aetiological role for EBV in salivary gland tumours. However, they strengthen the argument that there is an ethnic association between EBV and undifferentiated salivary gland carcinomas.

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