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Detection of occult nasopharyngeal primary tumours by means of *in situ* hybridization

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Abstract

Detection of nasopharyngeal carcinoma primaries in patients presenting with neck node metastases may sometimes demand considerable efforts. By using the '*in situ* hybridization' technique, we manage to identify the Epstein-Barr virus in neck metastases secondary to nasopharyngeal carcinomas. We propose that such identification in neck node metastases where the primary lesion is unknown indicates a nasopharyngeal primary.

Introduction

The most prevalent malignant nasopharyngeal tumour is the WHO type 3 nasopharyngeal carcinoma. This is an undifferentiated type of tumour also known as lymphoepithelioma or Shminke's tumour (Tarr and Glaser, 1989). The tumour usually infiltrates submucosally and therefore the mucosal lining may appear to be normal to the examining clinician. Patients can present with a neck mass or serous otitis with no clinically identifiable topological abnormality in the nasopharynx. Furthermore random biopsies taken from the nasopharynx, if not sufficiently deep, may demonstrate no pathology. The cases presented in this study suggest that the '*in-situ* hybridization' technique, by detecting Epstein-Barr viral fingerprints may help in determining a nasopharyngeal primary in patients presenting with neck metastases of unknown origin.

Material and patients

This study investigates paraffin blocks from four metastatic neck nodes where the primary tumour was unknown for presence of Epstein-Barr virus fingerprints with the '*in-situ* hybridization' technique (Chan *et al.*, 1989b). Case studies of two of the four are presented here.

The first biopsy examined was a metastatic lymph node taken from a 50-year-old male with no other findings or symptoms but that of a 2.0 × 2.0 cm jugulodigastric neck node.

A complete head and neck investigation including random biopsies from the nasopharynx were all negative. Excision biopsy of the neck node was performed. Histology showed this to be an undifferentiated carcinoma and as no primary was revealed the patient was given the label of a 'metastatic carcinoma of unknown primary'. A section from the neck node was submitted for '*in situ* hybridization', in search of EBV fingerprints. A few weeks later, the patient developed overt nasopharyngeal pathology, and was referred for radiotherapy.

The second patient was a 55-year-old female presenting with unilateral serous otitis. Since the condition did not resolve over a period of a few weeks she was suspected of having a nasopharyngeal tumour and evaluated as such. Random biopsies from the nasopharyngeal area were taken, but all turned out to be normal. A month later she returned with a 1.5 × 1.5 cm neck node, in the upper jugular chain. Excision biopsy was performed. Histology revealed this to be an undifferentiated squamous cell carcinoma. The patient was then referred for radiation therapy. A section of the excised gland was examined retrospectively for EBV fingerprints.

Two 5 micron sections from each of the specimens were deparafinized with xylene and then rehydrated with decreasing concentrations of alcohol. The tissue was digested in proteinase K for 15 mins at 37°C. Denaturation by heating to 100°C for 5 mins was followed by hybridization. A probe consistent of a combination of EBV DNA fragments 'enzo biochemistry' and labelled with biotin, in hybridization solution was added onto the slide and incubated at 37°C for 2 h. Positively hybridized spots were detected by avidin biotin reaction (Hawkins *et al.*, 1990).

Results

Two out of the four metastatic lymph node biopsies, examined in this study showed positive hybridization with the EBV probe. A representative picture of a positive hybridization between a section taken from the metastatic lymph node belonging to patient No. 2 is presented (Fig. 1).

A B-95 cell lines which is EBV positive was the positive control in this study. A representative picture of the hybridization is presented (Fig. 2).

Normal peripheral lymphocytes were used as a negative control (Fig. 3).

Discussion

Association between the Epstein-Barr virus and naso-

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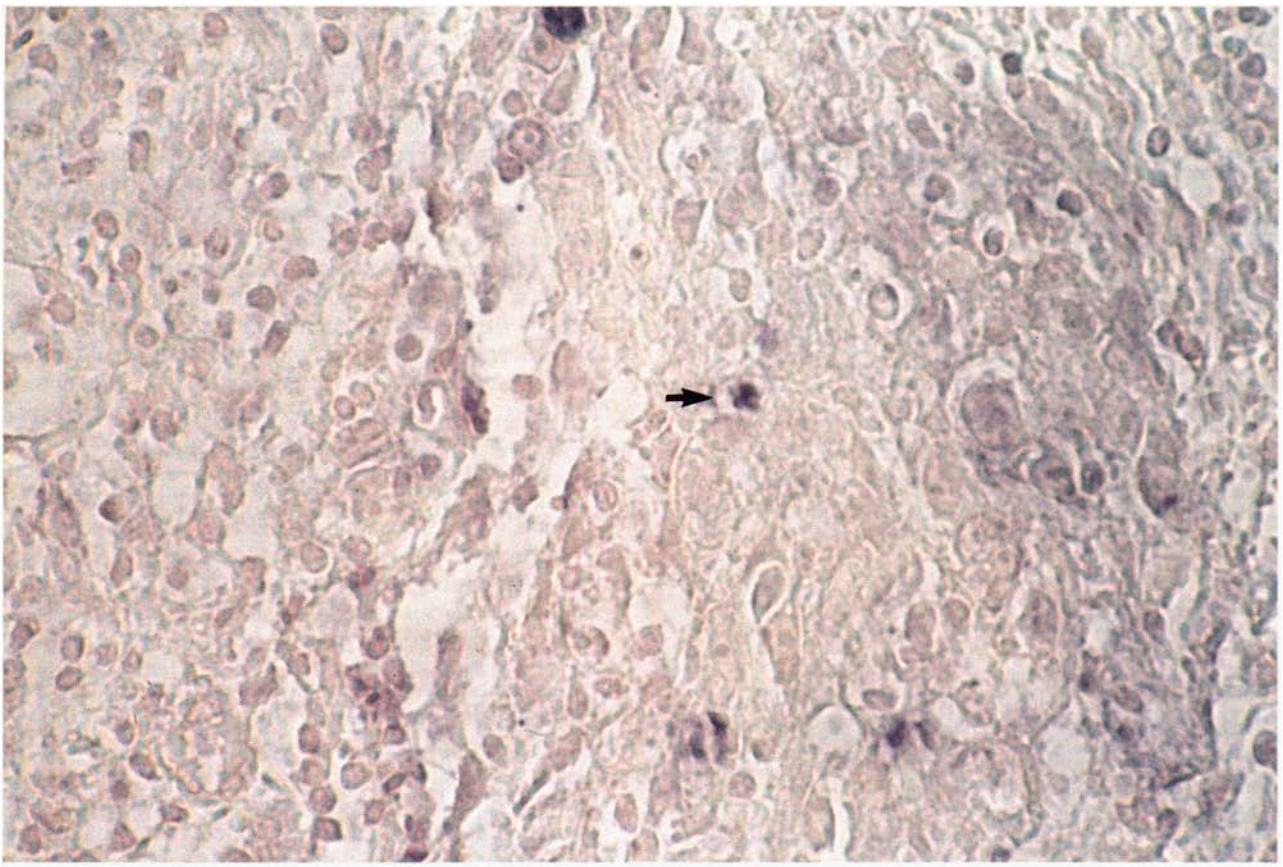

FIG. 1

A section of a lymph node taken from the first patient described, whose primary was not identified at the time of biopsy.
The arrow points to a cell stained positively as evidence for hybridization between the probe that was added and the viral genome in the section.

pharyngeal carcinoma was first described by Henle *et al.* (1973). This was initially done by the demonstration of high titres of antibodies against viral proteins in the serum of patients with nasopharyngeal carcinoma (Henle *et al.*, 1977). The finding was confirmed by Wolf and Zur-Hausen (1973) who by means of *in situ* hybridization were able not only to confirm the association between tumour and virus, but also localize the virus to the epithelial cells of the tumour. In the late 1980s studies, in which DNA extracted from NPC and hybridized with an EBV probe, reported positive hybridization in 70 per cent of patients (Zhang *et al.*, 1989). Lately neck metastases secondary to a nasopharyngeal primary were also found to have the EBV genome (Chan *et al.*, 1989a; Chan *et al.*, 1989b).

Although the role of the virus in carcinogenesis is unclear, it seems that its fingerprints can serve as a marker for a nasopharyngeal primary. Attempts to use this association between virus and tumour in order to decipher the riddle of the 'unknown primary' have been made before. Neel *et al.* (1981) suggested that elevated anti-EBV antibodies in patients with neck metastases but with no other pathology indicating a primary, may suggest a nasopharyngeal primary.

This, however, was not accepted as conclusive evidence. The present study indicates that positive EBV DNA fingerprints traced in a metastastic neck node by means of *in situ* hybridization may support the presence of a nasopharyngeal primary tumour. In patients presenting with a neck metastasis and a clinically normal nasopharynx, this may be an important contribution.

Although B-cells are known to carry the receptor for the EBV, it is not quite clear whether epithelial cells carry the receptor as well. It is speculated that the basilar layer of the mucosa lining the nasopharynx, which is the least mature, may carry the receptor. As the epithelium matures towards the surface, this characteristic is lost. It is the basilar layer that is infected and later transformed. This may offer an explanation as to why nasopharyngeal tumours may be deeply situated and infiltrative, and why the surface in some of these patients may be normal. The two cases presented here are two such cases. The positive hybridization between metastatic deposits and viral probe suggest that the primary lesion is a nasopharyngeal carcinoma as this is the only head and neck tumour that is closely associated with the Epstein-Barr virus. This, in addition to high levels of serum anti-viral antibodies, increases the probability of a nasopharyngeal primary.

Conclusions

The results obtained in this study suggest that ' *in situ* hybridization' may be an important tool in detecting Epstein-Barr viral fingerprints. Together with high levels of anti Epstein-Barr viral antibodies positive hybridization may indicate a nasopharyngeal primary lesion in patients presenting with a neck metastasis where the primary lesion cannot yet be detected.

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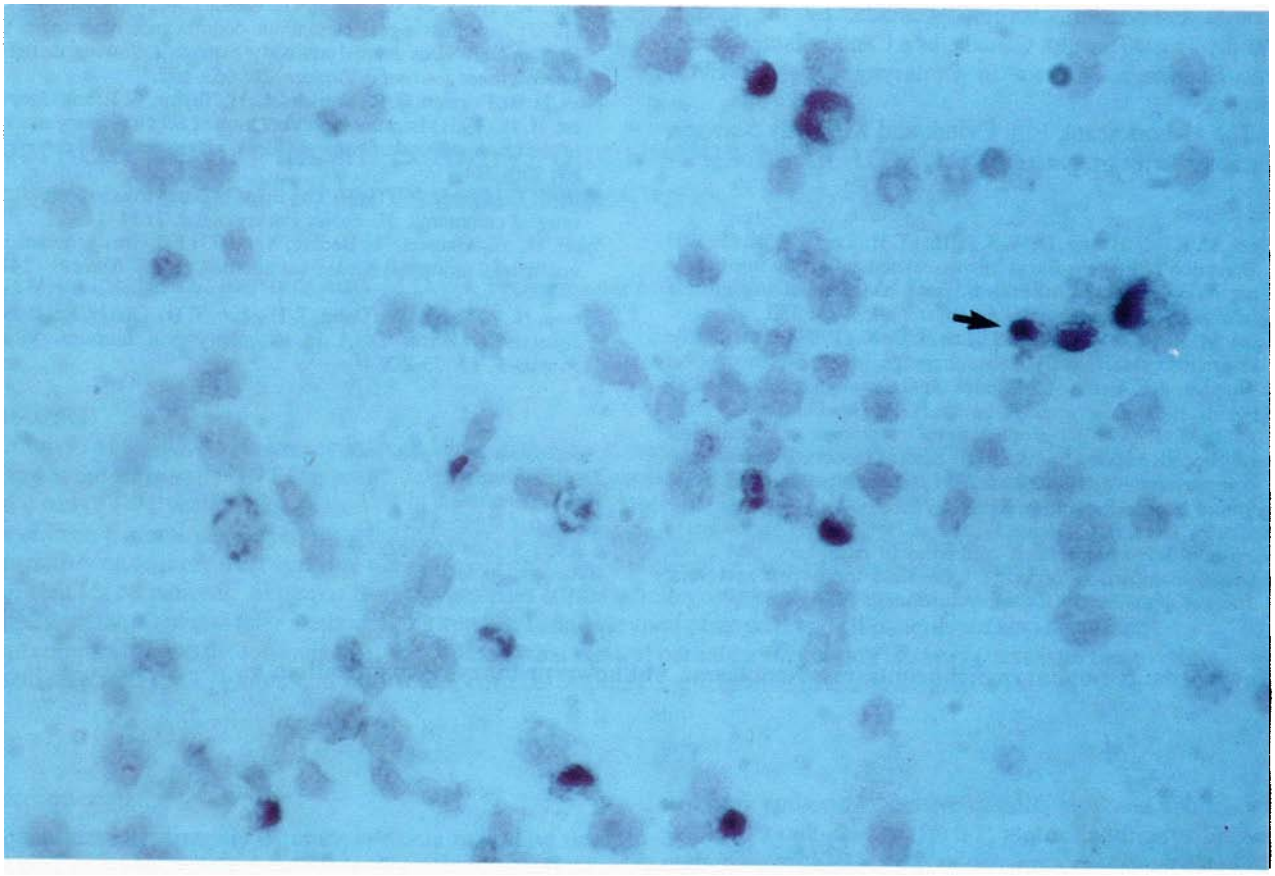

FIG. 2
A B-95 Burkitt's lymphoma cell line which is EBV positive, was the positive control in this study (arrow points towards positive hybridization).

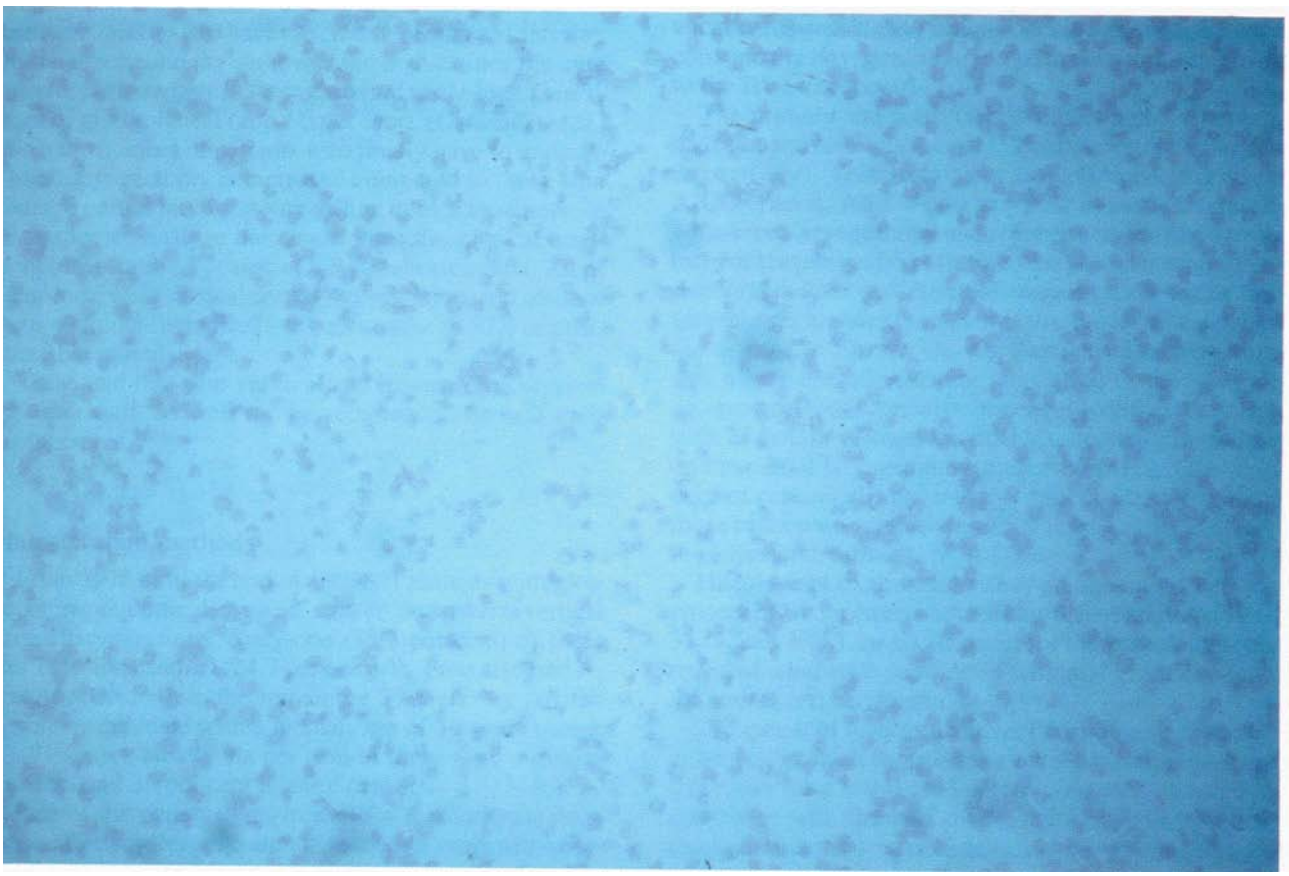

FIG. 3
Normal peripheral lymphocytes were the negative control in this study. No hybridization is detected.

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