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Detection of Epstein-Barr Virus DNA in a Japanese Case of Lymphoepithelioma-like Thymic Carcinoma

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Epstein-Barr virus DNA was detected in a case of lymphoepithelioma-like thymic carcinoma. A homogeneous terminal structure of the viral DNA was demonstrated in this case, indicating the presence of the viral genome in clonally expanded tumor cells. Since all of 26 other thymic epithelial tumors (eight non-invasive, 13 invasive thymomas and four non-lymphoepithelioma-like thymic carcinomas) in Japanese were negative by polymerase chain reaction, it is suggested that lymphoepithelioma-like thymic carcinoma may represent a unique pathological entity distinct from Epstein-Barr virus-negative thymic epithelial tumors, which are in the majority in Japan.

Key words: Epstein-Barr virus — Thymus — Lymphoepithelioma — Polymerase chain reaction — Viral oncogenesis

The pathoepidemiologic or etiologic profiles of thymic epithelial tumors (TETs) have not yet been fully characterized. Since Leyvraz proved the presence of Epstein-Barr virus (EBV) genomic DNA in thymic carcinoma tissue,[1] it has been suggested that EBV may play an important role in the histogenesis of TETs. Recent reports have shown that thymic tumors in southern China occasionally contain EBV-DNA,[2] while European and American cases do not.[3] In order to evaluate the involvement of EBV in Japanese TETs, tumor tissues from 26 Japanese patients were examined for the presence of EBV-DNA.

The pathological diagnosis of each case is listed in Table I. Genomic DNA was extracted from 21 thymoma and four thymic carcinoma tissues, which had been freshly obtained by surgical resection or at autopsy. DNA extracted from formalin-fixed paraffin-embedded tissue, principally following a described method,[4] of another thymic carcinoma case was also included in the study. All tissue samples were obtained from the National Cancer Center Hospital, Tokyo, during the period between 1980 and 1989.

Extracted DNA was subjected to polymerase chain reaction (PCR).[5] TETs often contain numerous infiltrating lymphocytes but few tumor cells, and therefore PCR is an effective method of detecting the EBV-DNA sequence with low copy number in the material. In addition, PCR can be successfully performed using DNA from paraffin-embedded tissue samples.[6,7] Oligonucleotide primer construction and PCR conditions including specific hybridization were the same as described in detail previously.[8] DNA extracted from Raji cells (carrying EBV genome) served as a positive control, while DNA from Ramos cells (EBV-negative) was used as a negative control. Both cell lines were obtained from the Japanese Cancer Research Resources Bank, Tokyo.

After specific hybridization, no positive signal was detected in any of 21 thymomas examined (Table I). Among five thymic carcinoma cases, only one case was positive for 129-bp PCR product, which proved the presence of EBV-DNA in the tumor tissue (Fig. 1). This was the only case of lymphoepithelioma-like undifferentiated carcinoma examined in this study, showing special histologic features mimicking so-called lymphoepithelioma of the nasopharynx (Fig. 2).[9] This patient, a 10-year-old boy, had elevated serum antibody titers against EBV-related antigens, such as IgG against viral capsid antigen and against early antigen, with titers of 1:2560 and 1:640, respectively. He died one year and two months after diagnosis in spite of intensive chemo- and radiotherapy. Tumor tissues taken from the metastatic tumors in lung and liver at autopsy also gave positive results by PCR (data not shown). None of the thymic carcinomas of other histologic types, squamous cell carcinoma or adenosquamous carcinoma, gave a positive signal for EBV-DNA (Table I). Using DNA from formalin-fixed, paraffin-embedded tissues, undifferentiated carcinoma, which is dissimilar to lymphoepithelioma, gave negative PCR results (Table I). However, a DNA sample from formalin-fixed, paraffin-embedded tissue of the above-mentioned lymphoepithelioma-like undifferentiated carcinoma case was positive (data not shown).

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Table I. Cases Examined and EBV-DNA Detected by PCR

Diagnosis	No. of EBV-DNA positive cases/ No. of cases examined
Non-invasive thymoma	0/8
mixed	0/8
Invasive thymoma	0/13
lymphocytic predominant	0/3
mixed	0/7
epithelial predominant	0/3
Thymic carcinoma	1/5
squamous cell carcinoma	0/1
adenosquamous carcinoma	0/2
"lymphoepithelioma-like" undifferentiated carcinoma	1/1[a]
non-"lymphoepithelioma-like" undifferentiated carcinoma	0/1[b]
Total	1/26

a) Positive in both fresh and formalin-fixed, paraffin-embedded tumor tissues.
b) Examined only in formalin-fixed, paraffin-embedded tissue.

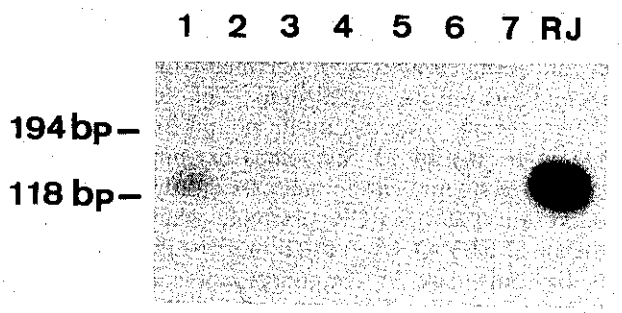

Fig. 1. Autoradiogram demonstrating the detection of 129-bp PCR product in TETs. Lane 1, "lymphoepithelioma-like" undifferentiated carcinoma; 2, undifferentiated carcinoma, not "lymphoepithelioma-like"; 3 and 4, adenosquamous carcinoma; 5, squamous cell carcinoma; 6, non-invasive thymoma, mixed type; 7, invasive thymoma, mixed type; RJ, Raji cell.

In Hong Kong, which is an EBV-endemic area, EBV genome has been detected not only in lymphoepithelioma-like undifferentiated carcinoma, but also in TET of other histologic types and in thymic lymphoid hyperplasia cases.[2] In non-EBV-endemic areas, however, it has been found only in lymphoepithelioma-like undifferentiated carcinoma (two reported cases).[1,10] The present results suggest that in Japanese cases of TET, EBV is involved only in lymphoepithelioma-like undifferentiated carcinoma, but not in thymoma or thymic carcinoma of other histologic types.

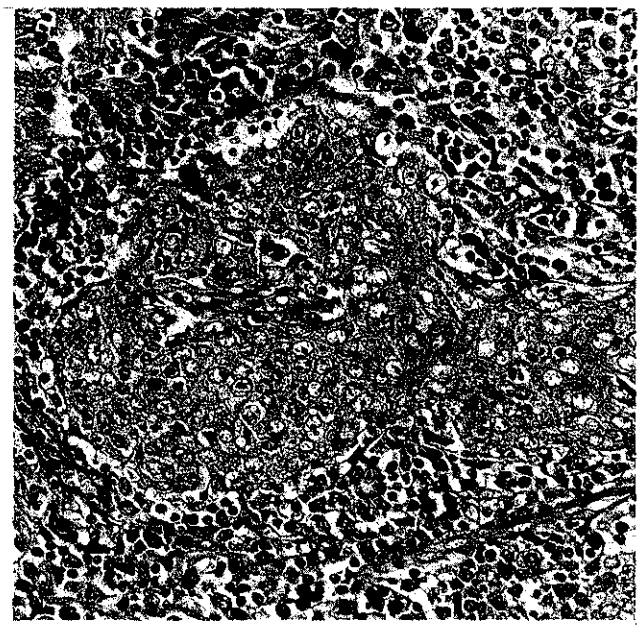

Fig. 2. "Lymphoepithelioma-like" undifferentiated carcinoma consists of nests of large polygonal tumor cells and lymphoid stroma. Hematoxylin and eosin, ×200.

It is well known that so-called lymphoepithelioma of the nasopharynx[11] and lymphoepithelial lesions of the parotid gland[12] are closely associated with EBV infection. Embryologically, the nasopharynx, parotid gland and thymus derive from the common anlage named the primary pharynx. Therefore, it is conceivable that EBV-DNA is frequently detected in lymphoepithelioma or lymphoepithelioma-like carcinoma of the organs derived from the primary pharynx, though EBV genome has also been detected in lymphoepithelioma-like undifferentiated carcinoma of the lung.[13]

In order to determine whether EBV-DNA is present in the tumor cells or in the non-tumor cells in lymphoepithelioma-like undifferentiated carcinoma tissue, the terminal structure of EBV-DNA was analyzed. The genomic DNA extracted from frozen tissues was subjected to Southern blot analysis after *Bam*HI digestion. *Bam*HI-W fragment, which detects internal repetitive sequence (IR1), and *Xho*I 1.9 kb fragment, detecting the terminal repeat of EBV genomic sequence,[14] were used as probes after ^{32}P labeling by the random priming method. Specific hybridization using *Bam*HI-W revealed that EBV genome (3.3 kb band) was present in tumor tissue (liver metastasis) but not in non-tumorous liver or spleen tissue obtained from the same patient (Fig. 3a). In the latter two tissues, EBV-DNA was also not detected

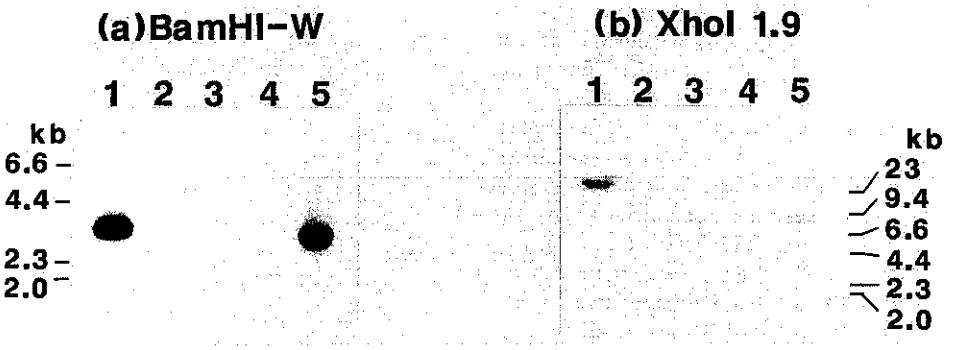

Fig. 3. Southern blot analysis demonstrating homogeneous terminal structure of EBV-DNA in tumor tissue. DNA extracted from cultured cell lines, or fresh autopsy material of "lymphoepithelioma-like" undifferentiated carcinoma case was subjected to Southern blot hybridization after *Bam*HI digestion. As probes, ^{32}P-labeled *Bam*HI-W (detecting an internal repetitive sequence) and *Xho*I 1.9 (detecting a terminal repetitive sequence) fragments were used in (a) and (b), respectively. 1, Raji cell; 2, Ramos cell; 3, 4, and 5; spleen, liver and liver metastatic tissues from the positive case, respectively.

by PCR (data not shown). *Xho*I 1.9 probe detected a single band in metastatic liver tumor tissue, indicating a homogeneous terminal structure of EBV (Fig. 3b). This result demonstrates a clonal origin of the EBV-infected cells in the tumor tissue.[14] Because immunohistochemical study revealed that infiltrating small lymphocytes consisted of mixed B and T lymphocytes (data not shown), it is unlikely that the lymphocytes expanded clonally. It is concluded, therefore, that the majority of EBV was integrated in tumor cells. Supporting evidence would be obtained if EBV genome were detected in the tumor cells by *in situ* hybridization, but we have not succeeded in this. Although the presence of viral DNA in tumor cells does not necessarily imply that the virus is the causative agent of the tumor, our findings support that possibility.

Our results strongly suggest that it is unlikely that Japanese thymomas are associated with antecedent EBV infection. Thus, the present data confirm that thymomas in Hong Kong are exceptional in terms of association with EBV.[3] This may be explained in part by the endemicity of EBV in that area, or by the genetic predisposition of the population to EBV infection. However, it is possible that EBV-DNA detected in TETs and thymic hyperplasia in EBV-endemic areas was present in infiltrating lymphocytes, but not in epithelial tumor cells. In order to exclude this possibility, clonality of EBV-infected cell populations requires demonstration. Also, lymphoepithelioma-like undifferentiated thymic carcinoma must be closely associated with EBV infection, probably irrespective of EBV-endemicity of the areas. Although we cannot come to a definite conclusion at present because of the rarity of such cases, lymphoepithelioma-like undifferentiated carcinoma seems to represent a distinct entity in TETs, from both morphological and etiological standpoints.

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