

Nasal natural killer/T-cell lymphoma and its association with type "i"/*XhoI* loss strain Epstein–Barr virus in Chile

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Nasal T/natural killer (NK)-cell lymphoma is a disease entity that has been recognised since the 1990s, but was defined as a distinct clinicopathological entity highly associated with Epstein–Barr virus (EBV) only after a workshop held in Hong Kong in 1996.¹ The World Health Organization (WHO) Classification listed this neoplasia in the category of mature T-cell and NK-cell neoplasms and defined it as an extranodal NK/T-cell lymphoma, nasal type.² Typically, the immunophenotype is CD2 and CD56 positive and surface CD3 is usually negative; cytoplasmic CD3 can be detected in paraffin wax sections, and clonal T-cell receptor gene rearrangement is not found, indicating an NK cell origin.¹ Histological features of this lymphoma are angiocentric infiltration by lymphoma cells and invasion of blood vessels, which results in notable ischaemic necrosis of normal and neoplastic tissues. Interestingly, tumour cells usually show evidence of clonal EBV, suggesting its aetiological role rather than a "silent passenger" in the pathogenesis of this lymphoma.^{3–4} Patients commonly present with nasal symptoms, such as nasal obstruction, facial mass and bleeding. The response of this lymphoma to therapy is inadequate even when radiotherapy (RT) and chemotherapy (CH) are combined, and therefore this lymphoma has a distinctly poor prognosis.^{5–6}

Although nasal NK/T-cell lymphoma is relatively uncommon worldwide, its incidence shows striking geographical variations. This disease is unusual in Western countries, accounting for <1% of lymphomas in Europe and North America.^{7–10} By contrast, it is relatively common in Asia, making up 6–8% of all lymphomas in China and Japan.^{3–11–13} Among Latin American countries, previous studies show an incidence in between that in Western and in Asian countries.^{14–18}

Among the two major types of EBV, type 1 EBV is the predominant strain all over the world, with the exception of

Background: Nasal T/natural killer (NK)-cell lymphoma is an aggressive type of non-Hodgkin's lymphoma associated with Epstein–Barr virus (EBV) and striking geographical variations worldwide.

Aim: To characterise nasal NK/T-cell lymphoma associated with genotypes of EBV in Chile, a Latin American country, where multiple strains of EBV, including two new recombinant strains, in healthy individuals were recently found.

Methods: Cases with diagnosis of primary nasal lymphoma were selected for histological and immunohistochemical analysis (CD3, CD3e, CD4, CD8, CD79a, CD56, CD57 and TIA-1) and in-situ hybridisation, serology and genotyping analysis for EBV.

Results: Out of 22 cases, 9 (41%) cases fulfilled the World Health Organization criteria for nasal NK/T-cell lymphoma; of these 7 (78%) cases were positive for EBV. Genotyping analysis revealed 6 cases of type 1 EBV and wildtype F at the *Bam*HI-F region, 4 cases type "i" EBV at the *Bam*HI-W1/I1 region; *XhoI* wild type was found in 2 and *XhoI* loss in 4 cases, respectively. Cosegregation analysis of the *Bam*HI-W1/I1 region and *XhoI* restriction site showed the new recombinant strain type "i"/*XhoI* loss in 3 cases and type "i"/*XhoI* wild-type strain in 1 case. Most patients were treated with combined anthracycline-containing regimens. Half of the cases attained complete remission.

Conclusion: Although nasal NK/T-cell lymphomas from Chile share similar clinicopathological features, high association with EBV and unfavourable prognosis with those described elsewhere, genotype analysis shows that the new recombinant type "i"/*XhoI* loss strain might contribute to explain the intermediate incidence of nasal NK/T-cell lymphomas in Latin America.

Africa, whereas type 2 EBV prevails.^{19–22} Regarding the *Bam*HI-F region, the prototype F has a worldwide distribution, but variant "f", featured by the presence of an extra *Bam*HI site, is found only in China, where it is associated with nasopharyngeal carcinoma.²³ The presence of an extra *Bam*HI site at the *Bam*HI-W1/I1 region (type "i" variant) and the presence of an *XhoI* restriction site at exon 1 of the LMP1 gene (*XhoI* wild-type variant) define genotypes for healthy people and EBV-associated diseases in Western countries.^{24–29} Conversely, the lack of this extra *Bam*HI site at the *Bam*HI-W1/I1 region and the loss of *XhoI* restriction site at LMP1 gene define type I and *XhoI* loss, respectively. These genotypes prevail in healthy donors and EBV-associated disease in Japan and China.^{27–28} These observations raise the possibility that EBV genotypes or variants might contribute to explain geographical variations of nasal NK/T-cell lymphoma around the world. The aim of this study was to characterise the nasal NK/T-cell lymphoma associated with genotypes and variants of EBV in Chile, a Latin American country, where we have recently found multiple EBV infections including two novel recombinant strains (type "i"/*XhoI* loss and type I/*XhoI* wild type) in healthy individuals.²⁰

MATERIALS AND METHODS

Patients and clinical data

From 1989 to 2001, 22 patients were diagnosed with and treated for primary nasal lymphoma by the National Adult Program for Antineoplastic Drugs. After immunophenotypic analysis, only nine cases were found fulfilling the criteria of the

Abbreviations: CH, chemotherapy; EBER-1, Epstein–Barr virus-encoded small RNA type-1; EBV, Epstein–Barr virus; HTLV-1, human T-lymphotropic leukaemia virus type-1; NK, natural killer; RT, radiotherapy; WHO, World Health Organization

WHO Classification for extranodal NK/T-cell lymphoma, nasal type, which is the subject of this study.

Histology and immunohistochemistry

Paraffin-wax-embedded sections were stained with H&E, and all cases were classified according to the new WHO Classification.² Immunohistochemical analysis was conducted using the following monoclonal antibodies: CD3 (Novocastra Laboratories, Newcastle upon Tyne, UK, dilution 1:100); CD3e (Dako, Glostrup, Denmark, dilution 1:50); CD4 (Novocastra Laboratories, dilution 1:40); CD8 (Dako, Denmark, dilution 1:25); CD79a (Dako, Copenhagen, Denmark, dilution 1:25); CD56 (Novocastra Laboratories, dilution 1:100); CD57 (Immunotech, Marseille, France, dilution 1:1); and TIA-1 (Coulter Immunology, Hialeah, Florida, USA, dilution 1:2). The avidin-biotin-peroxidase complex method was used (VECTASTAIN ABC KIT, Vector Laboratories, Burlingame, California, USA). The expression of each molecule was graded as follows: (1) - (0%), (2) + (1–49%) and (3) ++ (>50%), according to the proportion of positive cells. Replicate slides devoid of monoclonal antibodies were included as a negative control.

In-situ hybridisation for EBV-encoded small RNA type 1

EBV-encoded small RNA type-1 (EBER-1) expression was detected with a complementary digoxigenin-labelled 30-base oligomer, using the procedure described previously.³⁰ A case was considered to be EBER-1 positive based on a positive signal under microscopy in at least 10% of tumour cells. Lymph node section from a patient with infectious mononucleosis was used as positive control, and a sense probe for EBER-1 was used as negative control.

Genotype-specific primer sets and probes for EBV

Genotypes of EBV were examined by PCR, restriction enzyme and Southern blot analysis as described previously.²⁰ For distinguishing between type 1 and 2 EBV, we used primers and probes described by Sample *et al*³¹ to produce 153 and 246 bp fragments for type 1 and 2, respectively. The *Bam*HI-F region was amplified with primers described by Lung *et al*^{24, 32} that yield a 198-bp fragment for the prototype F and 127 and 71 bp fragments for “f” variant. The *Bam*HI-W1/I1 region was amplified by primer pair.^{24, 32} Type I, 205 bp fragment, and type “i”, 130 and 75 bp fragments, were determined by *Bam*HI restriction enzyme digestion. *Xho*I restriction site polymorphisms were performed with a set of primers described,³³ and digestion with *Xho*I restriction enzyme resulted in 67 and 46 bp fragments for *Xho*I wildtype and undigested 113 bp PCR product for *Xho*I loss type. Cell line B95-8 served as positive control for type 1, prototype F, type I and *Xho*I wildtype virus. Cell lines AG786 and Akata served as positive controls for type 2 and *Xho*I loss virus, respectively. Cloned *Bam*HI-“f” and

*Bam*HI-“i” DNA fragments served as positive controls for variant “f” and type “i”, respectively.

Serology for human T-lymphotropic leukaemia virus type-1 and HIV

We conducted serological analyses of human T-lymphotropic leukaemia virus type-1 (HTLV-1) and HIV by Enzyme Immunoassay (Abbott, Abbott Park, Illinois, USA).

Treatment

The treatment for the cases was anthracycline-contained CH, such as cyclophosphamide, doxorubicin hydrochloride, oncovin and prednisolone or cyclophosphamide, doxorubicin hydrochloride, oncovin, prednisolone and bleomycin, or a combination therapy with RT at a dose of 40–50 Gy.

RESULTS

Patients' characteristics

Table 1 summarises clinical data obtained from nine patients with extranodal nasal type lymphoma. There were five women and four men. Their median age was 43 years (range 24–62). Family names suggested that all nine patients were of Hispanic origin. The nasal cavity was the main site involved in all patients, but one, which presented with a middle-line mass of the hard palate and invasion to adjoining tissues such as the nasopharynx, ethmoid and orbit. In all, 8 (88%) patients had a localised disease (stage I–II), except one (stage IV) with stomach involved.

Histology and immunohistochemistry

There was a broad cytologic spectrum of tumour cells, from atypical small cells, to a mixture of small and large cells to large cells, with frequent mitotic figures, admixing with a variable number of inflammatory cells, such as granulocytes, macrophages, plasma cells and small lymphocytes. Angiocentricity and angioinvasive pattern were reported in all cases. Necrosis was also found in all cases, but was most prominent in tissues with large cells. Table 2 summarises the immunophenotypic findings. Six patients met the criteria of the WHO Classification for nasal lymphoma (CD3e positive, CD56 positive, TIA-1 positive and CD79a negative). Three patients were CD3e positive, TIA-1 positive, CD79a negative but CD56 negative.

Serology to HTLV-1 and HIV

Antibodies against HTLV-1 and HIV were negative in four patients studied.

EBV in-situ hybridisation and genotyping analysis

In total, 7 (78%) cases were considered EBV positive according to the expression of EBER-1 by in-situ hybridisation in ≥10% of the tumour cells (table 2). Two cases were negative for EBER-1 expression. Figure 1 shows the representative examples of EBV

Table 1 Clinical features of nasal natural killer/T-cell lymphomas

Case	Age/sex	Tumour involvement	Clinical stage	Treatment	Response	Survival period, months	Outcome
1	62/F	Nose	I	CT	Failure	5	Died
2	24/F	Nose	I	CT and RT	Failure	4	Died
3	52/F	Nose	I	CT	Failure	22	Alive
4	40/M	Nose	I	CT	CR	12	Alive
5	28/M	Nasopharynx, supraglottis, stomach	IV	CT	Failure	1	Died
6	58/F	Nose	I	NA	NA	NA	NA
7	53/F	Nose (at 46 months)	I	CT and RT	CR	52	Relapsed
8	43/M	Hard palate, orbit, ethmoid	II	CT	CR	14	Died
9	26/M	Nose	I	CT	CR	7	Died

CR, complete remission; CT, chemotherapy; F, female; M, male; NA, not available; RT, radiotherapy.

Table 2 Summary of immunohistochemical and Epstein–Barr virus RNA in-situ hybridisation results in nasal natural killer/T-cell lymphomas

Case	CD3	CD4	CD8	CD79a	CD56	CD57	CD3e	TIA-1	EBER (%)*	Type	F/"F"	I/"i"	XhoI
1	++	+/-	++	-†	-	-	++	++	80	1	F	"i"	Loss
2	++‡	-	-	-†	++	-	++	++	90<	1	F	NA	Wild type
3	-†	-	-§	-†	++	-	-§	++	90<	1	F	NA	Loss
4	++‡	-†	-†	-†	++	-§	++	++	80	NA	"F"	NA	NA
5	++	-§	++	-†	-	-†	+	++	70	1	F	"i"	Loss
6	++‡	-	-†	-	++	-§	++	++	0	NT	NT	NT	NT
7	-§	-	-§	-†	++	NT	++	++	0	NT	NT	NT	NT
8	-§	-	+	-	+	NT	++	++	90<	1	F	"i"	Loss
9	++‡	-	++	-†	-	NT	++	++	70	1	F	"i"	Wild type

EBER, Epstein–Barr virus-encoded small RNA; NA, not amplified; NT, not tested.

*In-situ hybridisation results were expressed as number of positive cells per medium-powered field ($\times 200$). Cases were considered positive when a positive signal was observed in $\geq 10\%$ of the tumour cells.

†Background was also reacted.

‡Cytoplasmic expression was observed.

§Positive for reactive cells but not for neoplastic cells.

positive nasal lymphoma cases. Genotyping analysis revealed that six out of seven positive cases were type 1 EBV and wild-type F at the *Bam*HI-F region. The remaining case (case 3) did not amplify for type 1 or 2 EBV, and was variant "F" at the *Bam*HI-F region. All four amplified cases of the *Bam*HI-W1/I1 region were type "i" EBV. In the remaining three cases, no amplification of this region could be obtained after several attempts. Amplification of *Xho*I restriction site was successful in six cases, two were *Xho*I wild type and four showed *Xho*I loss. Figure 2 shows the representative examples. As it is known that polymorphisms at the *Bam*HI-W1/I1 region cosegregate with *Xho*I restriction site polymorphisms,²⁷ we analysed these two variants. Among four type "i" nasal lymphomas, three harboured a novel recombinant strain type "i"/*Xho*I loss (cases 4, 7 and 8) and one harboured type "i"/*Xho*I wildtype strain (case 9).

Treatment and outcome

The initial treatment was CH in eight cases, combined with RT in two cases and unknown for the remaining one. Complete remission was achieved in 4 (50%) of 8 cases whose treatment data were available. The median survival period of these four cases with complete remission was 13 months. All of the four patients who were unresponsive to the treatment died of progressive disease, except one patient (median survival period 4.5 months). Survival analysis could not be conducted because of the small number of patients.

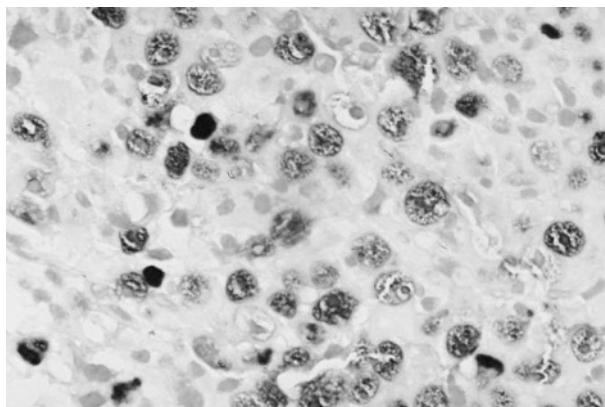


Figure 1 Identification of Epstein–Barr virus (EBV) in a case of nasal natural killer/T-cell lymphoma (case 1) by EBV-encoded small RNA type-1 in situ hybridisation ($\times 400$).

DISCUSSION

The present study shows that the clinical and phenotypic characteristics of nasal NK/T-cell lymphoma in Chile are similar to those described in other Latin American as well as Asian and Western countries.^{3 7–15 17 18} Here, we also confirm that most nasal NK/T-cell lymphomas were EBV positive,^{3 4} although we failed to show this association in two cases. Possible explanations may be the low quality of preservation of paraffin-wax-embedded blocks, or the lack of EBER-1 expression, as has been shown in other tumours.³⁴ Nevertheless, these negative cases strictly met the criteria for classification as nasal NK/T-cell lymphomas, as they were CD56 positive, CD3e positive and TIA-1 positive.² Genotype analysis showed the presence of a new recombinant strain characterised by the presence of an extra

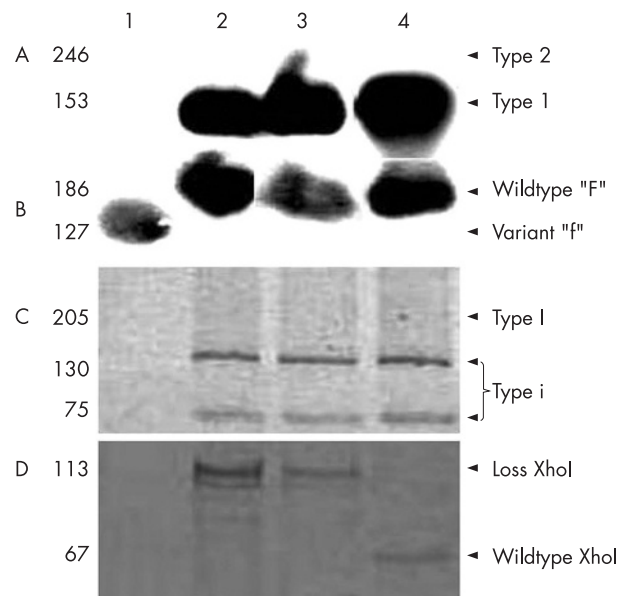


Figure 2 Genotypes of Epstein–Barr virus in cases of nasal natural killer/T-cell lymphomas. (A) Southern blot analysis after PCR amplification and hybridisation with specific probes for type 1 and 2 strains. (B) Southern blot analysis after PCR amplification, digestion with *Bam*HI restriction enzyme and hybridisation with specific probes for polymorphisms at the *Bam*HI-F region. (C) Polyacrylamide gel after PCR amplification and digestion with *Bam*HI restriction enzyme at the *Bam*HI-W1/I1 region. (D) Polyacrylamide gel after PCR amplification and digestion with *Xho*I restriction enzyme at *Xho*I site polymorphisms. The second fragment after *Xho*I digestion (46 bp fragment) for the wild type is not seen in the gel.

Take-home messages

- Six out of seven Epstein–Barr virus (EBV)-positive nasal natural killer (NK)/T-cell lymphoma cases were type 1 EBV and wildtype F at the *Bam*HI-F region.
- Among six EBV-positive nasal NK/T-cell lymphoma cases, four were type “i” EBV at the *Bam*HI-W1/I1 region.
- Among six EBV-positive nasal NK/T-cell lymphoma cases, four showed *Xho*I loss and two had *Xho*I wild-type restriction site.
- Cosegregation analysis of the *Bam*HI W1/I1 region and *Xho*I restriction site showed a novel recombinant strain type “i”/*Xho*I loss in three cases and type “i”/*Xho*I wildtype strain in one case.
- Genotype analysis revealed that the novel recombinant strain type “i”/*Xho*I loss might contribute to explain the intermediate incidence of nasal NK/T-cell lymphomas in Latin America.

*Bam*HI site at the *Bam*HI-W1/I1 region, together with the loss of the *Xho*I restriction site at the LMP1 gene (type “i”/*Xho*I loss strain). Type “i”/*Xho*I loss strain has been recently identified in a proportion of 329 healthy adults in two Latin American countries, including Chile.²⁰ In that study, type “i”/*Xho*I loss strain was found admixed with another recombinant strain, type I/*Xho*I wild-type strain, and strains described previously in Western countries (type “i”/*Xho*I wild type) and Asian countries (type I/*Xho*I loss).^{20–24–29} Therefore, these results provide the first evidence that type “i”/*Xho*I loss strain can be found in nasal NK/T-cell lymphomas. Furthermore, the presence of type “i”/*Xho*I loss might contribute to explain the intermediate incidence of nasal NK/T-cell lymphoma in Latin America, in between that in Western and in Asian regions.^{14–18} The frequency of other genotypes, type 1 and wild-type F at the *Bam*HI-F region in our patients were similar to that of patients with nasal NK/T-cell lymphoma described in Japan.³⁵

Even early stages have an overall survival rate lower than that in other extranodal or nodal lymphomas. Besides being a resistant disease, relapses are also frequent.³⁶ To date, there is no agreement on the best treatment. RT is effective in limited disease, whereas combined therapy seems to be more effective for advanced disease but does not ensure prolonged survival.^{5–6} Recent reports suggested that high dose CH with stem-cell transplantation may be an effective treatment modality for refractory NK lymphomas.³⁷ The survival benefit of this modality still needs to be shown.

In conclusion, we describe nasal NK/T-cell lymphomas from Chile, with similar clinicopathological features as those described elsewhere, strong association with EBV and unfavourable prognosis. Genotype analysis revealed the presence of a novel recombinant strain (type “i”/*Xho*I loss) in most of the analysed cases, providing evidence that, although previously described in healthy donors, this strain can also be found in nasal NK/T-cell lymphomas. Further research is necessary to clarify whether this finding is specific of Latin America or can be found in Western and Asian countries as well.

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Competing interests: None declared.

REFERENCES

- 1 Nava VE, Jaffe ES. The pathology of NK-cell lymphomas and leukemias. *Adv Anat Pathol* 2005;**12**:27–34.
- 2 Jaffe E, Harris N, Stein H, et al. *Pathology and genetics of tumors of haematopoietic and lymphoid tissues*. Lyon: IARC Press, 2001.
- 3 Harabuchi Y, Imai S, Wakashima J, et al. Nasal T-cell lymphoma causally associated with Epstein-Barr virus: clinicopathologic, phenotypic, and genotypic studies. *Cancer* 1996;**77**:2137–49.
- 4 Kanavaros P, Briere J, Emile JF, et al. Epstein-Barr virus in T and natural killer (NK) cell non-Hodgkin's lymphomas. *Leukemia* 1996;**10**(Suppl 2):s84–7.
- 5 Egger G, Liang G, Aparicio A, et al. Epigenetics in human disease and prospects for epigenetic therapy. *Nature* 2004;**429**:457–63.
- 6 Oshimi K. NK cell lymphoma. *Int J Hematol* 2002;**76**(Suppl 2):118–21.
- 7 Kanavaros P, Lescs MC, Briere J, et al. Nasal T-cell lymphoma: a clinicopathologic entity associated with peculiar phenotype and with Epstein-Barr virus. *Blood* 1993;**81**:2688–95.
- 8 Garcia-Cosio M, Santon A, Mendez MC, et al. Nasopharyngeal/nasal type T/NK lymphomas: analysis of 14 cases and review of the literature. *Tumori* 2003;**89**:278–84.
- 9 Rodriguez J, Romaguera JE, Manning J, et al. Nasal-type T/NK lymphomas: a clinicopathologic study of 13 cases. *Leuk Lymphoma* 2000;**39**:139–44.
- 10 Gaal K, Sun NC, Hernandez AM, et al. Sinonasal NK/T-cell lymphomas in the United States. *Am J Surg Pathol* 2000;**24**:1511–17.
- 11 Ho FC, Todd D, Loke SL, et al. Clinico-pathological features of malignant lymphomas in 294 Hong Kong Chinese patients, retrospective study covering an eight-year period. *Int J Cancer* 1984;**34**:143–8.
- 12 Ng CS, Chan JK, Lo ST, et al. Immunophenotypic analysis of non-Hodgkin's lymphomas in Chinese. A study of 75 cases in Hong Kong. *Pathology* 1986;**18**:419–25.
- 13 Aozasa K, Ohsawa M, Tajima K, et al. Nation-wide study of lethal mid-line granuloma in Japan: frequencies of Wegener's granulomatosis, polymorphic reticulosis, malignant lymphoma and other related conditions. *Int J Cancer* 1989;**44**:63–6.
- 14 Arber DA, Weiss LM, Albuja PF, et al. Nasal lymphomas in Peru. High incidence of T-cell immunophenotype and Epstein-Barr virus infection. *Am J Surg Pathol* 1993;**17**:392–9.
- 15 Aviles A, Diaz NR, Neri N, et al. Angiocentric nasal T/natural killer cell lymphoma: a single centre study of prognostic factors in 108 patients. *Clin Lab Haematol* 2000;**22**:215–20.
- 16 Calderon-Garciduenas L, Delgado R, Calderon-Garciduenas A, et al. Malignant neoplasms of the nasal cavity and paranasal sinuses: a series of 256 patients in Mexico City and Monterrey. Is air pollution the missing link? *Otolaryngol Head Neck Surg* 2000;**122**:499–508.
- 17 Altmani A, Barbosa AC, Kulka M, et al. Characteristics of nasal T/NK-cell lymphoma among Brazilians. *Neoplasia* 2002;**49**:55–60.
- 18 Elenitoba-Johnson KS, Zarate-Osorno A, Meneses A, et al. Cytotoxic granular protein expression, Epstein-Barr virus strain type, and latent membrane protein-1 oncogene deletions in nasal T-lymphocyte/natural killer cell lymphomas from Mexico. *Mod Pathol* 1998;**11**:754–61.
- 19 Zimmer U, Addlinger HK, Lenoir GM, et al. Geographical prevalence of two types of Epstein-Barr virus. *Virology* 1986;**154**:56–66.
- 20 Corvalan A, Ding S, Koriyama C, et al. Association of a distinctive strain of Epstein-Barr virus with gastric cancer. *Int J Cancer* 2006;**118**:1736–42.
- 21 Young LS, Murray PG. Epstein-Barr virus and oncogenesis: from latent genes to tumours. *Oncogene* 2003;**22**:5108–21.
- 22 Young LS, Yao QY, Rooney CM, et al. New type B isolates of Epstein-Barr virus from Burkitt's lymphoma and from normal individuals in endemic areas. *J Gen Virol* 1987;**68**(Pt 11):2853–62.
- 23 Lung ML, Lam WP, Sham J, et al. Detection and prevalence of the “F” variant of Epstein-Barr virus in southern China. *Virology* 1991;**185**:67–71.
- 24 Lung ML, Chang GC. Detection of distinct Epstein-Barr virus genotypes in NPC biopsies from southern Chinese and Caucasians. *Int J Cancer* 1992;**52**:34–7.
- 25 Lung ML, Chang RS, Huang ML, et al. Epstein-Barr virus genotypes associated with nasopharyngeal carcinoma in southern China. *Virology* 1990;**177**:44–53.

- 26 **Lung ML**, Chang RS, Jones JH. Genetic polymorphism of natural Epstein-Barr virus isolates from infectious mononucleosis patients and healthy carriers. *J Virol* 1988;**62**:3862-6.
- 27 **Abdel-Hamid M**, Chen JJ, Constantine N, et al. EBV strain variation: geographical distribution and relation to disease state. *Virology* 1992;**190**:168-75.
- 28 **Khanim F**, Yao QY, Niedobitek G, et al. Analysis of Epstein-Barr virus gene polymorphisms in normal donors and in virus-associated tumors from different geographic locations. *Blood* 1996;**88**:3491-501.
- 29 **Young LS**, Murray PG. Epstein-Barr virus and oncogenesis: from latent genes to tumors. *Oncogene* 2003;**22**:5108-21.
- 30 **Wu MS**, Huang SP, Chang YT, et al. Association of the -160 C --> a promoter polymorphism of E-cadherin gene with gastric carcinoma risk. *Cancer* 2002;**94**:1443-8.
- 31 **Sample J**, Young L, Martin B, et al. Epstein-Barr virus types 1 and 2 differ in their EBNA-3A, EBNA-3B, and EBNA-3C genes. *J Virol* 1990;**64**:4084-92.
- 32 **Lung ML**, Chang GC, Miller TR, et al. Genotypic analysis of Epstein-Barr virus isolates associated with nasopharyngeal carcinoma in Chinese immigrants to the United States. *Int J Cancer* 1994;**59**:743-6.
- 33 **Sandvej K**, Gratama JW, Munch M, et al. Sequence analysis of the Epstein-Barr virus (EBV) latent membrane protein-1 gene and promoter region: identification of four variants among wild-type EBV isolates. *Blood* 1997;**90**:323-30.
- 34 **Jaffe ES**, Chan JK, Su JJ, et al. Report of the workshop on nasal and related extranodal angiocentric T/natural killer cell lymphomas. Definitions, differential diagnosis, and epidemiology. *Am J Surg Pathol* 1996;**20**:103-11.
- 35 **Sidagis J**, Ueno K, Tokunaga M, et al. Molecular epidemiology of Epstein-Barr virus (EBV) in EBV-related malignancies. *Int J Cancer* 1997;**72**:72-6.
- 36 **Lee HK**, Wilder RB, Jones D, et al. Outcomes using doxorubicin-based chemotherapy with or without radiotherapy for early-stage peripheral T-cell lymphomas. *Leuk Lymphoma* 2002;**43**:1769-75.
- 37 **Au WY**, Lie AK, Liang R, et al. Autologous stem cell transplantation for nasal NK/T-cell lymphoma: a progress report on its value. *Ann Oncol* 2003;**14**:1673-6.

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