

Expression of the matrix metalloproteinase 9 in Hodgkin's disease is independent of EBV status

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Abstract

Background—In vitro the Epstein-Barr virus (EBV) encoded latent membrane protein 1 (LMP-1) has been shown to upregulate expression of matrix metalloproteinase 9 (MMP-9), a member of a family of zinc dependent endopeptidases that is believed to facilitate tumour invasion and metastasis by degradation of the extracellular matrix.

Aim—To test whether the expression of MMP-9 in Hodgkin's disease correlates with EBV status and survival and to investigate whether LMP-1 expression affects MMP-9 concentrations in the Hodgkin's disease cell line, L428.

Methods—MMP-9 expression was measured by means of immunohistochemistry in a series of Hodgkin's disease tumours and this expression was correlated with EBV status and survival. The influence of LMP-1 on MMP-9 expression was also investigated in the Hodgkin's disease cell line, L428.

Results—MMP-9 expression was demonstrated in the malignant Hodgkin and Reed-Sternberg cells of all (n = 86) formalin fixed, paraffin wax embedded Hodgkin's disease tumours examined. Although the intensity of MMP-9 immunostaining varied between cases, there was no correlation between MMP-9 expression and EBV status or survival. MMP-9 expression was also detected in a variety of non-malignant cells, including fibroblasts. MMP-9 was detected by zymography in the L428 and KMH2 Hodgkin's disease cell lines, whereas low or undetectable amounts of MMP-9 were found in the L591 Hodgkin's disease cell line. Induction of LMP-1 expression in the Hodgkin's disease cell line L428 did not result in a detectable increase in the values of MMP-9 as measured by zymography.

Conclusions—These results demonstrate that MMP-9 is consistently expressed by the Hodgkin and Reed-Sternberg cells of Hodgkin's disease tumours and by the Hodgkin's disease cell lines, L428 and KMH2. However, this expression does not appear to be related either to LMP-1 values or to survival.

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In the Western world, Hodgkin's disease is the most common malignant lymphoma affecting individuals between the ages of 10 and 30 years.¹ One of its characteristic features is the presence of a small population of large mononucleated or multinucleated Hodgkin and Reed-Sternberg cells surrounded by a much larger mass of non-malignant cell types.²

The Epstein-Barr virus (EBV), a B cell human herpesvirus that infects most of the world's adult population, is associated with the development of several malignancies, including undifferentiated nasopharyngeal carcinoma, Burkitt's lymphoma, and post-transplant lymphoproliferative disease in immunosuppressed individuals. More recently, EBV has been linked to the development of Hodgkin's disease. Evidence to support a role for EBV in Hodgkin's disease is provided by the consistently high degree of expression of the EBV transforming protein, latent membrane protein 1 (LMP-1), by Hodgkin and Reed-Sternberg cells in EBV associated patients. Recently, LMP-1 has been shown to upregulate expression of the matrix metalloproteinase 9 (MMP-9) gene in the C33A cell line.³ MMP-9 is a member of a family of zinc dependent endopeptidases that are believed to facilitate tumour invasion and metastasis through degradation of the extracellular matrix.^{4,5} Previous studies suggest that MMP-9 is a key enzyme responsible for the biological aggressiveness of human non-Hodgkin's lymphomas.⁶ Raised MMP-9 expression has been reported in several other malignancies including breast, colorectal, and lung cancers.⁷ These data suggest that LMP-1 mediated upregulation of MMP-9 might contribute to the progression and spread of EBV associated Hodgkin's disease. Therefore, we wished to determine whether, in Hodgkin's disease, expression of MMP-9 correlated with EBV status and survival. We also examined the influence of LMP-1 expression on the production of MMP-9 in the Hodgkin's disease cell line, L428.

Materials and methods

PATIENT GROUPS

A total of 86 samples of paraffin wax embedded tissue from patients with Hodgkin's disease were examined. A histological diagnosis of Hodgkin's disease was confirmed by review and all cases were subtyped according to the REAL classification system.⁸

IMMUNOHISTOCHEMISTRY

Sections (4 µm thick) were cut from each formalin fixed, paraffin wax embedded tissue

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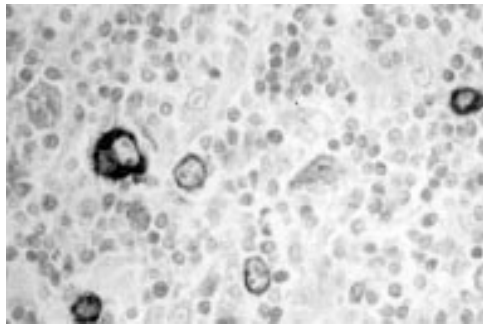


Figure 1 Immunohistochemistry for matrix metalloproteinase 9 (MMP-9). Paraffin wax embedded section of Hodgkin's disease showing strong positive staining in the malignant Hodgkin and Reed-Sternberg cells.

sample, adhered to VECTABOND treated slides (Vector Laboratories, Peterborough, UK), and incubated overnight at 37°C. After dewaxing and clearing, endogenous peroxidase activity was blocked with 0.3% hydrogen peroxide in methanol, and the sections were transferred to phosphate buffered saline (PBS) at pH 7.6. Sections were preincubated in normal rabbit serum (Dako, Cambridge, UK) for 10 minutes and then incubated at room temperature with a prediluted (1/50) goat polyclonal antibody directed against MMP-9 (C-20; Autogen Bioclear, Calne, Wiltshire, UK), for one hour. Biotinylated rabbit anti-goat immunoglobulin was used as the secondary antibody for 30 minutes, followed by streptavidin and biotinylated horseradish peroxidase complex for a further 30 minutes (both Dako). To confirm the specificity of the immunodetection, selected cases were also immunostained with a mouse monoclonal antibody to MMP-9 (clone VIIC2, Lab Vision Corp, Fremont, California, USA) diluted 1/100. Visualisation was carried out using the Sigma FAST™ DAB (3, 3'-diaminobenzidine) peroxidase substrate system (Sigma-Aldrich, Poole, Dorset, UK). Sections were counterstained in haematoxylin and mounted in DPX. One section known to give strong staining was included in each subsequent run as a positive control. Negative controls consisted of the substitution of the MMP-9 primary antibody with appropriate non-immune serum. For the evaluation of immunohistochemical staining, intensity of staining in the malignant cells was graded between + and +++ (where + was weak staining and +++ was the most intense staining).

STATISTICAL ANALYSIS

Kaplan-Meier plots were used to compare the potential differences in survival between the three Hodgkin's disease groups with differing intensities of MMP-9 expression. The Mann-Whitney test was also used to compare the distribution of MMP-9 categories within EBV negative and EBV positive specimens.

HODGKIN'S DISEASE CELL LINE

Hodgkin's disease cell lines, including KMH2, L591, parental L428, and L428 cells transfected with a cadmium inducible LMP-1 construct (F1L428) were cultured in RPMI-1640

(Gibco-BRL, Paisley, Scotland; UK) supplemented with 5% vol/vol fetal calf serum (FCS; Gibco-BRL), 2 mM L-glutamine, streptomycin (5 mg/100 ml), penicillin (5000 U/100 ml) and amphotericin (250 µg/100 ml) (all Sigma-Aldrich).⁹⁻¹² LMP-1 expression was induced in F1L428 cells by the addition of cadmium chloride (final concentration of 6 µM) to the medium (induction was carried out for six hours). L428 cells that had been transfected with vector DNA only (H2L428) were used as controls.

WESTERN BLOTTING

For western blot analysis, 10 ml aliquots (approximately 2×10^6 cells) of each of the L428 cell lines (H2, F1, and F1 induced) were pelleted by centrifugation at $200 \times g$ for five minutes. The resulting cell pellets were resuspended in 1000 µl of distilled water and sonicated at 4°C for 2.5 minutes (5×30 seconds). The protein concentration was determined using the Bio-Rad protein assay (Bio-Rad, Hemel Hempstead, Hertfordshire, UK). Cells were centrifuged and the pellets were resuspended in 200 µl of sodium dodecyl sulphate (SDS) buffer and equal amounts of

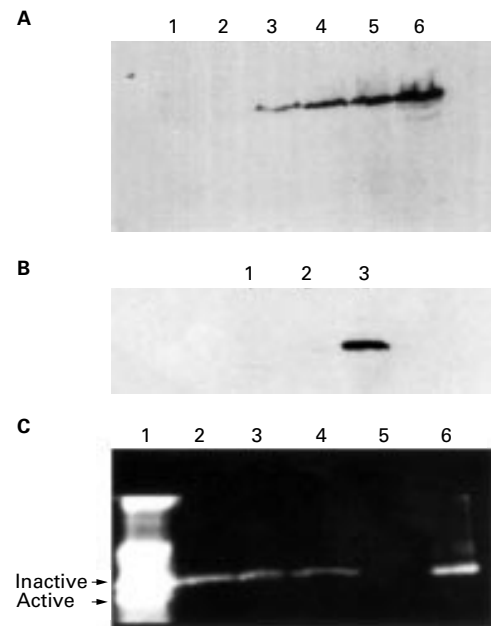


Figure 2 (A) Western blot analysis demonstrates that latent membrane protein 1 (LMP-1) expression can be induced in L428 cells transfected with the LMP-1 gene (F1L428 cells) by incubation with 6 µM cadmium chloride for time periods ranging from zero hours (lane 1) to 24 hours (lane 6). Protein was detected using the CS1-4 monoclonal antibody reagent. (B) Western blot analysis of the L428 Hodgkin's disease cell line using the monoclonal reagent CS1-4. Lane 1 represents protein from the parental L428 Hodgkin's disease cell line, lane 2 is from L428 cells transfected with vector only, and lane 3 the vector plus LMP-1 insert (F1L428 cells). LMP-1 expression was induced in the F1L428 cells by incubation with 6 µM cadmium chloride for six hours and shows a protein band corresponding to LMP-1. As expected, treatment of parental L428 cells and H2 cells with cadmium produced no such band. (C) Zymography analysis of matrix metalloproteinase 9 (MMP-9) expression. Conditioned media from: lane 1, control cells showing bands for active and inactive forms of MMP-9; lane 2, L428 cells with LMP-1 insert (F1L428) uninduced; lane 3, F1L428 cells after induction of LMP-1 expression; lane 4, H2L428 cells (vector only); lane 5, L591 cells; lane 6, KMH2 cells.

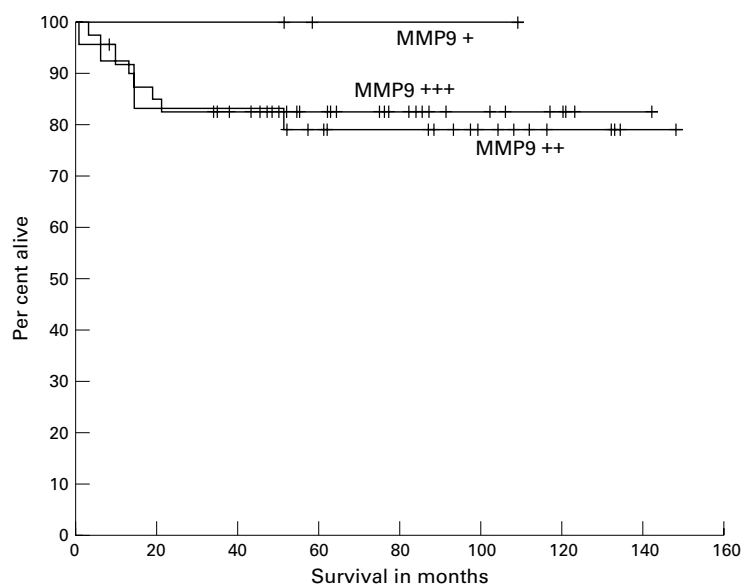


Figure 3 Kaplan-Meier plots showing the survival curves for the three groups of Hodgkin's disease with differing intensities of matrix metalloproteinase 9 (MMP-9) expression (+, ++, and +++). There were no significant differences between the groups.

protein (40 µg/well) were separated on 8% polyacrylamide/SDS Laemmli gels. Proteins were transferred (90 V for 90 minutes) on to a nitrocellulose membrane (Hybond-C; Amersham, Little Chalfont, Buckinghamshire, UK). The expression of LMP-1 was analysed using antiserum against LMP-1 (CS1-4 monoclonal mouse antiserum; Dako) at a 1/50 dilution. Horseradish peroxidase conjugated antimouse secondary antibodies (Dako) were used for detection with the enhanced chemiluminescence system (ECL; Amersham-Pharmacia Biotech). The standard immunodetection protocol as detailed in the ECL handbook was used. Exposure times were in the region of 15–60 seconds.

ZYMOGRAPHY

Zymography was performed on lysates prepared from pelleted Hodgkin's disease cells and also on the conditioned media in which the cells had been cultured. Lysates were prepared by incubation in lysis buffer (50 mM Tris (pH 7.8), 250 mM NaCl, 0.5% Triton X-100, 0.1% SDS). The sample of conditioned medium was prepared for analysis by ethanol precipitation (–70°C overnight), and after centrifugation the pellet was resuspended in protein sample buffer (0.2 M Tris (pH 6.8), 0.1% wt/vol SDS, 0.5% vol/vol Triton X-100). The protein concentrations of the samples were determined using the Bio-Rad DC protein assay. Samples were mixed with 2× SDS sample buffer (0.2 M Tris (pH 6.8), 2.25% wt/vol SDS, 20% glycerol, 0.05% wt/vol bromophenol blue) to yield an equivalent final protein concentration. Controls were conditioned cell culture media from Chinese hamster origin cell lines transfected with the human recombinant gene for MMP-2 or MMP-9. The MMP-2 and MMP-9 controls were diluted 1/5 and 1/10, respectively, with 2× SDS sample buffer. Samples and controls were incubated at 37°C for 30 minutes in sample buffer before

separation on 7.5% acrylamide gels containing 0.05% wt/vol gelatin. The gels were renatured at room temperature for 30 minutes in a 2.5% vol/vol Triton X-100 solution, before incubating at room temperature for 30 minutes in developing buffer (50 mM Tris (pH 7.6), 0.02 M NaCl, 5 mM CaCl₂, 0.02% Brij 35). Gels were incubated in fresh developing buffer overnight at 37°C. The gels were stained (2.5% Coomassie blue) and MMP-9 activity demonstrated by the presence of clear bands upon a blue background.

Results

IMMUNOHISTOCHEMISTRY

MMP-9 expression was detected in the malignant Hodgkin and Reed-Sternberg cells of all 86 paraffin wax embedded Hodgkin's disease specimens (fig 1). In most cases, strong cytoplasmic staining of Hodgkin and Reed-Sternberg cells was seen. In all cases, several non-malignant cell types were stained, including fibroblasts and macrophages. The specificity of the goat polyclonal antibody for MMP-9 was confirmed by the staining of selected cases with a monoclonal antibody to MMP-9 because both antibodies produced identical staining patterns.

ZYMOGRAPHY

Gelatin zymography of the cell lysates and conditioned media from the parental L428 Hodgkin's disease cell line showed the presence of a band at the molecular weight corresponding to inactive MMP-9. LMP-1 expression was successfully induced in the F1L428 line as demonstrated by western blotting using the CS1-4 reagent (fig 2A and B). However, when conditioned media or cell pellets from this cell line were subjected to zymography, MMP-9 values were identical to those seen in the parental L428 cells (fig 2C). MMP-9 was also detected by zymography in the KMH2 Hodgkin's disease cell line, whereas low or undetectable amounts of MMP-9 were found in the L591 Hodgkin's disease cell line. None of the samples contained detectable amounts of activated MMP-9.

STATISTICAL ANALYSIS

The intensity of MMP-9 immunostaining of individual Hodgkin's disease cases was variable and there was no correlation between the degree of MMP-9 expression and EBV status. In addition, MMP-9 expression was independent of histological subtype. For the Mann-Whitney test, in which the distribution of MMP-9 categories is compared between EBV negative and EBV positive status, the *Z* statistic was –0.16, and the *p* value was 0.88.

The Kaplan-Meier plots (fig 3) showed that there were no significant differences between the degree of MMP-9 expression and patient outcome. However, there were too few patients in the MMP-9 positive category for a meaningful analysis of this group.

Discussion

Although several MMPs have been implicated in the spread of tumours, MMP-9 may be particu-

larly relevant to the progression of lymphomas. MMP-9 has been shown to be important for the in vitro degradation of extracellular matrix components by non-Hodgkin's lymphoma cells.⁶ In vivo, MMP-9 is also overexpressed in a subset of high grade non-Hodgkin's lymphomas, and this correlates with a poor clinical outcome.¹³⁻¹⁵

Previous studies have shown that MMP-9 expression is increased in EBV latency type III B cell lymphoma cell lines compared with type I cells, where there is a restricted expression of virus genes.³ Furthermore, expression of MMP-9 in the C33A cell line was increased by transfection of the EBV encoded LMP-1.³ LMP-1 is transforming in Rat-1 cells and induces many of the phenotypic changes seen in EBV infected B cells, including expression of the B cell activation markers, CD23 and CD40; upregulation of cell adhesion molecules such as intercellular adhesion molecule 1 (ICAM-1), lymphocyte function associated antigen 1 (LFA-1), and LFA-3; and cytokine production.¹⁶⁻¹⁸ LMP-1 also protects B cells from cell death by the upregulation of several anti-apoptosis genes including Bcl-2, Mcl-1, and A20.¹⁹⁻²² Many of the phenotypic and growth transforming effects of LMP-1 are the result of the activation of a variety of signalling pathways, many of which converge on the NF- κ B (nuclear factor κ B) and AP-1 transcription factors.²³⁻²⁴

Given that LMP-1 is highly expressed in a proportion of Hodgkin's disease tumours,²⁵⁻²⁶ and also the potential importance of MMP-9 in the progression of lymphomas, we examined the expression of MMP-9 in a series of Hodgkin's disease tumours.

The results of our study show that MMP-9 is consistently expressed by the malignant Hodgkin and Reed-Sternberg cells of Hodgkin's disease. Furthermore, studies on the Hodgkin's disease cell lines showed that L428 and KMH2 cells secreted MMP-9. Although the intensity of MMP-9 immunostaining within individual Hodgkin's disease tumours varied, there was no correlation between the degree of MMP-9 expression and EBV status. This was confirmed by the induction of LMP-1 expression in L428 cells, which resulted in no detectable increase in MMP-9 values.

A recent study has shown that upregulation of MMP-9 by LMP-1 is dependent upon the two activating regions of LMP-1 (known as C terminus activating regions, CTAR-1 and CTAR-2) and that the NF- κ B and AP-1 sites in the MMP-9 promoter are also required.²⁷ Constitutive activation of NF- κ B (p50/p65) has been described as a common feature of Hodgkin and Reed-Sternberg cells.²⁸⁻²⁹ Thus, it may be that NF- κ B mediated upregulation of MMP-9 is likely in Hodgkin and Reed-Sternberg cells even in the absence of LMP-1.

Although the exact role of MMP-9 expression in the spread of Hodgkin's disease tumours has yet to be established, its consistent expression suggests the potential for therapeutic interventions using MMP-9 inhibitors.

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- Cossman J, Messineo C, Bagg A. Reed-Sternberg cell: survival in a hostile sea. *Lab Invest* 1998;78:229-35.
- Kuppers R, Rajewsky K. The origin of Hodgkin and Reed/Sternberg cells in Hodgkin's disease. *Annu Rev Immunol* 1998;16:471-93.
- Yoshizaki T, Sato H, Furukawa M, et al. The expression of matrix metalloproteinase 9 is enhanced by Epstein-Barr virus latent membrane protein 1. *Proc Natl Acad Sci U S A* 1998;95:3621-6.
- Trocme C, Gaudin P, Berthier S, et al. Human B lymphocytes synthesize the 92-kDa gelatinase, matrix metalloproteinase-9. *J Biol Chem* 1998;273:20677-84.
- Gokaslan ZL, Chintala SK, York JE, et al. Expression and role of matrix metalloproteinases MMP-2 and MMP-9 in human spinal column tumors. *Clin Exp Metastasis* 1998;16:721-8.
- Kossakowska AE, Hinek A, Edwards DR, et al. Proteolytic activity of human non-Hodgkin's lymphomas. *Am J Pathol* 1998;152:565-76.
- Kossakowska AE, Huchcroft SA, Urbanski SJ, et al. Comparative analysis of the expression patterns of metalloproteinases and their inhibitors in breast neoplasia, sporadic colorectal neoplasia, pulmonary carcinomas and malignant non-Hodgkin's lymphoma in humans. *Br J Cancer* 1996;73:1401-8.
- Harris NL, Jaffe ES, Stein H, et al. A revised European-American classification of lymphoid neoplasms: a proposal from the international lymphoma study group. *Blood* 1994;84:1361-92.
- Stein H, Gerdes J, Kirchner H, et al. Hodgkin and Sternberg-Reed cell antigen(s) detected by an antiserum to a cell line (L428) derived from Hodgkin's disease. *Int J Cancer* 1981;28:425-9.
- Burrlicher H, Schaadt M, Diehl V. Conclusions from Hodgkin-derived cell lines. *Cancer Treat Res* 1989;41:29-41.
- Drexler HG, Leber BF, Norton J, et al. Genotypes and immunophenotypes of Hodgkin's disease-derived cell lines. *Leukaemia* 1988;2:371-6.
- Diehl V, Pfreundschuh M, Fonatsch C, et al. Phenotypic and genotypic analysis of Hodgkin's disease derived cell lines: histopathological and clinical implications. *Cancer Surv* 1985;4:399-419.
- Kossakowska AE, Urbanski SJ, Edwards DR. Tissue inhibitor of metalloproteinases-1 (TIMP-1) RNA is expressed at elevated levels in malignant non-Hodgkin's lymphomas. *Blood* 1991;77:2475-81.
- Kossakowska AE, Urbanski SJ, Huchcroft SA, et al. Relationship between the clinical aggressiveness of large cell immunoblastic lymphomas and expression of 92 kDa gelatinase (type IV collagenase) and tissue inhibitor of metalloproteinases-1 (TIMP-1) RNAs. *Oncol Res* 1992;4:233-40.
- Kossakowska AE, Urbanski SJ, Watson A, et al. Patterns of expression of metalloproteinases and their inhibitors in human malignant lymphomas. *Oncol Res* 1993;5:19-28.
- Wang F, Gregory C, Sample C, et al. Epstein-Barr virus latent membrane protein (LMP1) and nuclear proteins 2 and 3C are effectors of phenotypic changes in B lymphocytes: EBNA-2 and LMP1 cooperatively induce CD23. *J Virol* 1990;64:2309-18.
- Nakagomi H, Dolcetti R, Bejarano MT, et al. The Epstein-Barr virus latent membrane protein-1 (LMP1) induces interleukin-10 production in Burkitt lymphoma lines. *Int J Cancer* 1994;57:240-4.
- Huen DS, Henderson SA, Croom-Carter D, et al. The Epstein-Barr virus latent membrane protein-1 (LMP1) mediates activation of NF- κ B and cell surface phenotype via two effector regions in its carboxy-terminal cytoplasmic domain. *Oncogene* 1995;10:549-60.
- Gregory CD, Dive C, Henderson S, et al. Activation of Epstein-Barr virus latent genes protects human B cells from death by apoptosis. *Nature* 1991;349:612-14.
- Henderson S, Rowe M, Gregory C, et al. Induction of bcl-2 expression by Epstein-Barr virus latent membrane protein 1 protects infected B cells from programmed cell death. *Cell* 1991;65:1107-15.
- Wang S, Rowe M, Lundgren E. Expression of the Epstein Barr virus transforming protein LMP1 causes a rapid and transient stimulation of the Bcl-2 homologue Mcl-1 levels in B-cell lines. *Cancer Res* 1996;56:4610-13.
- Laherty CD, Hu HM, Opiari AW, et al. The Epstein-Barr virus LMP1 gene product induces A20 zinc finger protein expression by activating nuclear factor kappa B. *J Biol Chem* 1992;267:24157-60.
- Devergne O, Hatzivassiliou E, Izumi KM, et al. Association of TRAF1, TRAF2, and TRAF3 with an Epstein-Barr virus LMP1 domain important for B-lymphocyte transformation: role in NF-(κ)B activation. *Mol Cell Biol* 1996;16:7098-108.
- Eliopoulos AG, Young LS. Activation of the cJun N-terminal kinase (JNK) pathway by the Epstein-Barr virus-encoded latent membrane protein 1 (LMP1). *Oncogene* 1998;16:1731-42.
- Pallesen G, Hamilton-Dutoit SJ, Rowe M, et al. Expression of Epstein-Barr virus latent gene products in tumour cells of Hodgkin's disease. *Lancet* 1991;337:320-2.

- 26 Murray PG, Young LS, Rowe M, *et al.* Immunohistochemical demonstration of the Epstein-Barr virus-encoded latent membrane protein in paraffin sections of Hodgkin's disease. *J Pathol* 1992;**166**:1-5.
- 27 Takeshita H, Yoshizaki T, Miller WE, *et al.* Matrix metalloproteinase 9 expression is induced by Epstein-Barr virus latent membrane protein 1 C-terminal activation regions 1 and 2. *J Virol* 1999;**73**:5548-55.
- 28 Bargou RC, Leng C, Krappmann D, *et al.* High-level nuclear NF-kappa B and Oct-2 is a common feature of cultured Hodgkin/Reed-Sternberg cells. *Blood* 1996;**87**:4340-7.
- 29 Krappmann D, Emmerich F, Kordes U, *et al.* Molecular mechanisms of constitutive NF-kappaB/Rel activation in Hodgkin/Reed-Sternberg cells. *Oncogene* 1999;**18**:943-53.

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