Expression and regulation of the endogenous retrovirus 3 in Hodgkin’s lymphoma cells

Stefanie Kewitz and Martin Sebastian Staeger*
Department of Pediatrics, Martin-Luther-University Halle-Wittenberg, Halle, Germany

INTRODUCTION

The exact etiology of Hodgkin's lymphoma (HL) is unknown, but immunological and molecular properties suggest that the majority of HL are derived from B cells (1, 2). HL cells have a characteristic gene-expression profile that discriminates these cells from other normal and transformed cells (3, 4). Especially for pediatric HL patients the prognosis is relatively good, and with the combination of radio- and chemo-therapy the majority of patients with HL can be cured. However, the established therapy is associated with a plethora of late adverse side effects and some patients with chemoresistant disease cannot be cured (5, 6). Therefore, it is important to search for new targets for treatment of patients with HL.

Recently, reactivation of endogenous retrovirus (ERV) activity has been observed in HL (7). This reactivation leads to expression of the receptor for macrophage colony-stimulating factor (CSF1R), a known oncogene, in HL cells. ERV are an integral part of the genome of virtually all eukaryotes, and ERV loci have been extensively analyzed in plants, insects, and vertebrates (8–13). In the human genome, ERV derived sequences constitute at least 8% of the complete DNA. Usually, ERV are silenced epigenetically and are not transcribed into RNA. Reactivation of ERV has been observed under pathological conditions, e.g., in cancer cells. Such reactivation can result even in the formation of virus particles (14, 15). A small number of ERV-encoded proteins can be found under normal physiological conditions. Such proteins can exert variable biological functions (16, 17). One notable example is ERVW-1 (also known as syncytin 1) which is required for proper formation of the syncytial layer of the placenta (16). In this ERV only the envelope protein is functional. Other open reading frames (ORF) have been inactivated by deleterious mutations. Such mutations destroy the ORF of the majority of ERV. Some ERV with intact ORF encode superantigens (18, 19). Such superantigens can activate a high percentage of all T cells. The activation of these T cells can lead to hyper-reactivity of the immune system but can also lead to the final deletion of the activated T cells.

In addition to the potential involvement in the pathogenesis of human diseases, ERV might also represent interesting target structures for the development of future treatment strategies. Immune responses against ERV-encoded antigens have been described in cancer patients (20, 21). In melanoma patients, antibodies against ERV are associated with shorter disease free survival (21). On the other hand, cytotoxic T cells with specificity for ERV-encoded antigens can kill melanoma cells (22), colorectal cancer cells (23), and renal cancer cells (24). In addition, antibodies against ERV products can inhibit growth of breast cancer cells in vitro and in an animal model (25). Successful immunization of rhesus macaques against simian ERV suggests that ERV derived antigens can be used as safe vaccines without development of auto-immunity (26). Interestingly, ERV-specific T cells have been detected in patients after allogeneic hematopoietic stem cell transplantation (alloHSCT) (24). These T cells can kill the tumor cells and might be responsible for graft-versus-tumor effects after alloHSCT (24). Graft-versus-tumor effects have also been described in HL patients.
after alloHSCT (27). It remains unclear whether ERV reactivation in HL cells (7) has an impact on such graft-versus-tumor effects. We asked whether ERV reactivation in HL is a phenomenon affecting multiple (or all) ERV loci or whether this reactivation is specific for single ERV loci. Therefore, we used DNA microarray data for the analysis of multiple ERV loci in HL cells. DNA microarrays can be used for the characterization of complete gene-expression profiles from normal and malignant cells in a single experiment (28). Modern DNA exon microarrays contain several probe sets with specificity for ERV and, therefore, can be used for analysis of expression of multiple ERV loci at once.

MATERIALS AND METHODS

CELL LINES AND CELL CULTURE

Hodgkin’s lymphoma-cell lines HDLM-2, KM-H2, L-1236, L-428, and L-540 (29–33) were obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ), Braunschweig, Germany. P439-6 cells were kindly provided by G. Bornkamm and G. Laux, Munich, Germany. P493-6 cells carry an EBV nuclear antigen 2 (EBNA2)-estrogen receptor fusion gene and MYC under control of a promoter which can be regulated by tetracycline (34–36). All cells were cultured in RPMI-1640 (Invitrogen, Karlsruhe, Germany) supplemented with 10% fetal calf serum, 100 U/mL penicillin, and 100 µg/mL streptomycin (PAA, Pasching, Germany) at 37°C in a humidified atmosphere at 5% CO2. Treatment of HL cells with 1 µM vorinostat was carried out as described (37) at a cell density of 1 × 106 cells/mL for 24 h. Dimethyl sulfoxide (DMSO) was used as control. For simulation of hypoxia, HL cells were treated for 2 days at a cell density of 1 × 106 cells/mL with 200 µM cobalt(II) chloride. P439-6 cells were cultured for 4 days in medium with or without 2 µM estradiol and/or 1 µg/mL tetracycline.

GENE-EXPRESSIOANALYSIS

RNA from cell lines were isolated using TriFast reagent (peqlab, Erlangen, Germany) following manufacturer’s protocol. Two micrograms of the RNA were transcribed into cDNA and used as template for polymerase chain reaction (PCR). The following primer combinations were used for real-time quantitative reverse transcription-PCR (qRT-PCR): actin beta (ACTB): 5′-CTC CAA GGG ATG AGA ACC AA-3′, 5′-GGG AGT ATG CGG AAA GTT CA-3′; ERV3: 5′-GGG AGT ATG CGG AAA GTT CA-3′, 5′-GGG AGT ATG CGG AAA GTT CA-3′; ERVFH21-1: 5′-GGT ATC GTG GAC GCC GAA 3′; ERV18-1: 5′-GGG AGT ATG CGG AAA GTT CA-3′, 5′-CCC CCA GGG AGG TGG ACA GCC A-3′; ERV3: 5′-GGG AGT ATG CGG AAA GTT CA-3′, 5′-GGG AGT ATG CGG AAA GTT CA-3′. Quantitative RT-PCR was performed using the Go Taq qPCR Master Mix (Promega, Mannheim, Germany). The reaction was performed with 10 µL Go Taq qPCR Master Mix, 6 µL water, 1 µL primer combination, and 2 µL cDNA using the following conditions: 94°C, 30 s; 60°C, 30 s; 72°C, 45 s (40 cycles). Determination of gene expression was performed using the 2−ΔΔCt method (38). Global gene expression in HL cell lines was analyzed by using Affymetrix Human Exon 1.0ST arrays (Affymetrix, Santa Clara, USA). In addition to microarray data from HL cell lines L-540, HDLM-2 and L-428 (39), microarray data from normal peripheral blood cells (40), P493-6 cells (41), and normal B cells (42, 43) were used for comparative analysis. These cell files were down-loaded from the gene-expression omnibus (GEO) data base. All cell files were processed together using the robust microarray analysis (RMA) algorithm with Expression Console 1.1 (Affymetrix). Cell files from DNA microarrays from HL cell lines have been submitted to the GEO data base (accession number GSE47686). Signal intensities from ERV-specific probe sets were visualized with the Genesis software (44).

RESULTS

ANALYSIS OF ERV EXPRESSION IN DNA MICROARRAY DATA

We analyzed expression of human ERV in DNA microarray data from HL cell lines HDLM-2, L-428, and L-540 in comparison to normal blood cells, normal B cells, and the conditionally immortalized B cell line P493-6. A total of 169 probe sets with specificity for ERV sequences were analyzed (Figure 1). Signal intensities for single ERV loci. Therefore, we used DNA microarray data for the analysis of multiple ERV loci in HL cells. DNA microarrays can be used for the characterization of complete gene-expression profiles from normal and malignant cells in a single experiment (28). Modern DNA exon microarrays contain several probe sets with specificity for ERV and, therefore, can be used for analysis of expression of multiple ERV loci at once.

MATERIALS AND METHODS

CELL LINES AND CELL CULTURE

Hodgkin’s lymphoma-cell lines HDLM-2, KM-H2, L-1236, L-428, and L-540 (29–33) were obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ), Braunschweig, Germany. P439-6 cells were kindly provided by G. Bornkamm and G. Laux, Munich, Germany. P493-6 cells carry an EBV nuclear antigen 2 (EBNA2)-estrogen receptor fusion gene and MYC under control of a promoter which can be regulated by tetracycline (34–36). All cells were cultured in RPMI-1640 (Invitrogen, Karlsruhe, Germany) supplemented with 10% fetal calf serum, 100 U/mL penicillin, and 100 µg/mL streptomycin (PAA, Pasching, Germany) at 37°C in a humidified atmosphere at 5% CO2. Treatment of HL cells with 1 µM vorinostat was carried out as described (37) at a cell density of 1 × 106 cells/mL for 24 h. Dimethyl sulfoxide (DMSO) was used as control. For simulation of hypoxia, HL cells were treated for 2 days at a cell density of 1 × 106 cells/mL with 200 µM cobalt(II) chloride. P439-6 cells were cultured for 4 days in medium with or without 2 µM estradiol and/or 1 µg/mL tetracycline.

GENE-EXPRESSIOANALYSIS

RNA from cell lines were isolated using TriFast reagent (peqlab, Erlangen, Germany) following manufacturer’s protocol. Two micrograms of the RNA were transcribed into cDNA and used as template for polymerase chain reaction (PCR). The following primer combinations were used for real-time quantitative reverse transcription-PCR (qRT-PCR): actin beta (ACTB): 5′-GGG AGT ATG CGG AAA GTT CA-3′, 5′-GGG AGT ATG CGG AAA GTT CA-3′; ERV3: 5′-GGG AGT ATG CGG AAA GTT CA-3′, 5′-GGG AGT ATG CGG AAA GTT CA-3′; ERVFH21-1: 5′-GGT ATC GTG GAC GCC GAA 3′; ERV18-1: 5′-GGG AGT ATG CGG AAA GTT CA-3′, 5′-CCC CCA GGG AGG TGG ACA GCC A-3′; ERV3: 5′-GGG AGT ATG CGG AAA GTT CA-3′, 5′-GGG AGT ATG CGG AAA GTT CA-3′. Quantitative RT-PCR was performed using the Go Taq qPCR Master Mix (Promega, Mannheim, Germany). The reaction was performed with 10 µL Go Taq qPCR Master Mix, 6 µL water, 1 µL primer combination, and 2 µL cDNA using the following conditions: 94°C, 30 s; 60°C, 30 s; 72°C, 45 s (40 cycles). Determination of gene expression was performed using the 2−ΔΔCt method (38). Global gene expression in HL cell lines was analyzed by using Affymetrix Human Exon 1.0ST arrays (Affymetrix, Santa Clara, USA). In addition to microarray data from HL cell lines L-540, HDLM-2 and L-428 (39), microarray data from normal peripheral blood cells (40), P493-6 cells (41), and normal B cells (42, 43) were used for comparative analysis. These cell files were down-loaded from the gene-expression omnibus (GEO) data base. All cell files were processed together using the robust microarray analysis (RMA) algorithm with Expression Console 1.1 (Affymetrix). Cell files from DNA microarrays from HL cell lines have been submitted to the GEO data base (accession number GSE47686). Signal intensities from ERV-specific probe sets were visualized with the Genesis software (44).

RESULTS

ANALYSIS OF ERV EXPRESSION IN DNA MICROARRAY DATA

We analyzed expression of human ERV in DNA microarray data from HL cell lines HDLM-2, L-428, and L-540 in comparison to normal blood cells, normal B cells, and the conditionally immortalized B cell line P493-6. A total of 169 probe sets with specificity for ERV sequences were analyzed (Figure 1). Signal intensities...
(RMA normalized, linear values) above 100 were considered to be expressed in the corresponding samples. According to this threshold, HDLM-2 cells expressed 13 different ERV (represented by 31 probe sets), L-428 cells expressed 10 ERV (36 probe sets), and L-540 cells expressed 11 ERV (28 probe sets). In normal blood cells, 13 ERV were expressed (43 probe sets with mean signal intensities above the threshold). Interestingly, the eight ERV that were expressed in all HL cell lines (ERVFC1-1, ERVH-1, ERVH-4, ERVH48-1, ERVK3-1, ERVK-7, ERVK-9/-4/-19, ERVK13-1), were also expressed in normal blood cells. In addition, normal blood cells expressed ERV3-1, ERVK-6, ERVW-6, and ERVMER34-1. Isolated B cells expressed 15 ERV (45 probe sets with mean signal intensities above the threshold). In addition to all ERV that were found in normal blood, isolated B cells had high signal intensities for ERV9-1 and ERVK11-1. Additional ERV were found only in single HL cell lines: L-428 cells expressed ERVH-6; L-540 cells expressed ERVW-1 and ERVK3-2; HDLM-2 cells expressed ERVFRD-1 and ERVFRD-2. Taken all together, the number of expressed ERV in HL cell lines did not exceed the number of ERV expressed in normal blood or isolated B cells. Probe sets for ERV3-1 and ERVK13-1 showed significantly lower (p < 0.01) signal intensities in HL cells than in normal blood cells (Figure 2A). We found no ERV that were significantly up-regulated in HL cells. Mean signal intensities for ERV3-1 and ERVK13-1 in isolated B cells were lower than the signals in whole blood. It is known from the literature that ERV3 is up-regulated in cell cycle arrested differentiating cells (45). We tested whether ERV3 is also up-regulated in cell cycle arrested B cells. For this end, we analyzed DNA microarray data (41) from P493-6 cells that have been treated with tetracycline. Tetracycline switches off expression of MYC in this Burkitt lymphoma model cell line leading to cell cycle arrest (34–36). As shown in Figure 2B, we detected up-regulation of all probe sets from Figure 2A in arrested P493-6 cells.

VALIDATION OF ERV3 EXPRESSION IN CELL CYCLE ARRESTED B CELLS

To validate the observation of cell cycle dependent regulation of ERV3 we performed quantitative RT-PCR with P439-6 cells that had been cultured under different conditions. Cells were grown in medium (cycling cells) or after addition of tetracycline (arrested cells). In addition, the same cells were cultured in the presence of estrogen. Estrogen switches on functional EBNA2 in these cells and allows the proliferation in the presence of tetracycline. As shown in Figure 3, incubation of P493-6 cells with tetracycline resulted in marked up-regulation of ERV3. Switching on the EBV transformation program by addition of estrogen to tetracycline-treated P493-6 cells inhibited this up-regulation. Similar results were obtained with the conditional EBV-immortalized cell line EREB2-5 (46). This cell line is the parental cell line of P493-6 cells (without exogenous MYC) and proliferates only in the presence of estrogen in the culture medium. In this cell line we observed up-regulation of ERV3 after withdrawal of estrogen (data not shown).

REGULATION OF ERV3 EXPRESSION IN HL CELL LINES

In our previous work we observed that treatment of HL cells with the histone deacetylase inhibitor vorinostat induces cell cycle arrest (37). Therefore, we asked whether this cell cycle arrest is also accompanied by up-regulation of ERV3 in HL cells. Figure 4 shows the results of this analysis. Incubation of all tested HL cell lines with vorinostat resulted in an increased expression of ERV3. We observed that treatment of HL cells with the hypoxia-mimetic
CoCl₂ led to a pronounced inhibition of proliferation (Figure 5). As shown in Figure 6, the expression of ERV3 again increased when cells were cultured under conditions of inhibited proliferation.

**DISCUSSION**

Increased expression of ERV derived sequences has been observed in cancer cells (14, 15, 47–51) and in patients with autoimmune diseases or neurodegenerative diseases (52–54). ERV can act as alternative promoters for adjacent genes (7, 55–58). The resulting fusion transcripts can result in new protein isoforms, or the ERV component of these fusion transcripts can inhibit translation (58). In addition, ERV expression can interfere with the expression of adjacent genes at the level of transcription (59). In cancer cells such interference may lead to the inactivation of tumor suppressor genes. Some ERV-encoded proteins can directly bind and inactivate tumor suppressor genes (60). In our present study we provide preliminary evidence for a differential expression of ERV3 in HL cells under conditions of growth arrest. The method used for analysis of ERV expression in HL has several limitations. Not all human ERV loci are represented on the arrays and it might be that other ERV are differentially expressed in the investigated cells. In addition, the comparability of our data sets from HL cells and published data sets from normal blood and B cells might also be sub-optimal. However, our results gave no evidence for a general up-regulation of the investigated ERV loci in HL cells. The ERV3 signals in normal B cells are relatively low. Therefore and based on the limitations of the study, we cannot conclude that the low ERV3 expression is a specific feature of HL cells. However, up-regulation of ERV3 in HL cells under conditions of growth arrest suggests that ERV3 might be an interesting gene for further studies. ERV3 is unique among ERV as it is considered to be a tumor suppressor (61). ERV3 is abundantly expressed in the placenta and it is expressed in most other tissues at lower levels (62). Absence of expression in choriocarcinoma was observed (62), and transgenic expression of ERV3 in choriocarcinoma cells inhibits cell proliferation (63). This growth inhibition is associated
with down-regulation of cyclin B and up-regulation of the cyclin dependent kinase inhibitor p21 (63). Expression of ERV3 is up-regulated during terminal differentiation of leukemia cells and highest in cell cycle arrested cells (45, 64). ERV3 is a member of an ERV family with more than 40 members, but only ERV3 has intact ORF for viral proteins (65). The chromosomal location of ERV3 is characterized by a high number of pseudogenes (data not shown), and the complete ERV3 locus is present only in Old World primates with the exception of gorillas (66). Surprisingly, approximately 1% of Caucasians with normal phenotype have mutations in ERV3 which interrupt the ORF of the ERV3 envelope (67). This observation suggests that the ERV3 encoded envelope protein is not critically involved in the physiological function of this gene. Interestingly, read-through transcript between ERV3 and the neighboring zinc finger protein 117 (ZNF117) have been described (68). Lower expression of these transcripts has been observed in patients with multiple sclerosis (69). The human zinc finger proteins ZNF107, ZNF138, and ZNF92 have high homology with ZNF117. Together with other zinc finger proteins these genes form a cluster on human chromosome 7. The physiological function of ZNF117 has not been clarified, but it seems possible that this gene contributes to the biological effects of ERV3.

Endogenous retrovirus reactivation might occur only in transcriptionally active regions of the genome. In such cases, ERV reactivation might be only an epiphenomenon of chromatin opening and depends on the presence of adequate competence factors allowing transcription of the ERV. In such a model one would expect that several ERV loci are activated at the same time point. Our results show no evidence for such a general activation of ERV loci in HL. However, our analysis includes only well characterized ERV loci which are detectable by the used microarrays. Reactivation of ERV associated alternative promoter in the CSF1R gene seems to be involved in the pathophysiology of HL (7). The identification of such alternative transcription start sites by means of DNA exon microarray analysis requires new bioinformatics tools which are currently being developed in our lab.

The up-regulation of ERV3 under conditions of cell cycle inhibition and/or terminal differentiation is not specific for HL. Whether such up-regulation occurs only in transformed hematopoietic cells or also in other cell types has to be determined. Up-regulation of ERV3 in HL cells occurred under conditions which are characterized by increased apoptosis. CoCl2 can induce apoptosis in hematopoietic and non-hematopoietic tumor cells (70–73). Similarly, vonostat and other histone deacetylase inhibitors induce apoptosis in HL cells (74–76). If ERV3 is a tumor suppressor gene (as suggested by the choriocarcinoma data discussed above), expression of ERV3 in HL cells and other hematopoietic cells under pro-apoptotic and anti-proliferative conditions might indicate a tumor suppressing activity of ERV3 also in these cell types. The elucidation of ERV3 activities in the context of growth inhibition and apoptosis might help the identification of new targets for the treatment of HL and other malignant diseases.

ACKNOWLEDGMENTS

We thank I. Volkmer for grateful technical assistance. We thank Vera Marks (communication skills) for critically reading and copy editing of the manuscript. This work was supported by a fellowship from the Konrad-Adenauer-Stiftung (Stefanie Kewitz) and the Wilhelm-Roux-Program of the Martin-Luther-University Halle-Wittenberg (FKZ 25/22).

REFERENCES

1. Tzankov A, Dirnhofer S. Pathobiology of classical Hodgkin lymphoma. Pathobiology (2006) 73:107–25. doi:10.1159/000095558

2. Drexler HG, Minowada J. Pathobiology of classical Hodgkin lymphoma. Pathobiology (2006) 73:107–25. doi:10.1159/000095558

3. Schwering I, Bäzninger A, Klein U, Jungnickel B, Tinguely M, Drieb V, et al. Loss of the B-lineage-specific gene expression program in Hodgkin and Reed-Sternberg cells of Hodgkin lymphoma. Blood (2003) 101:1503–12. doi:10.1182/blood-2002-03-0839

4. Staeger MS, Banning-Eichenseer U, Weissflog G, Volkmer I, Burdach S, Richter G, et al. Gene expression profiles of Hodgkin’s lymphoma cell lines with different sensitivity to cytotoxic drugs. Exp Hematol (2008) 36:886–96. doi:10.1016/j.exphem.2008.02.014

5. Korholz D, Clavier A, Hasenclever D, Klinge R, Hirsch W, Kamprad F, et al. The concept of the GPOHD-HD 2003 therapy study for pediatric Hodgkin’s disease: evolution in the tradition of the DALLGPOH studies. Klin Padiatr (2004) 216:150–6. doi:10.1585/issn-2004-822627

6. Maat-Korholz C, Hasenclever D, Dorfel W, Ruschke K, Pefz T, Voigt A, et al. Procarbamazine-free OEPA-COP-DM chemotherapy in boys and standard OPPA-COP in girls have comparable effectiveness in pediatric Hodgkin’s lymphoma: the GPOHD-HD-2002 study. J Clin Oncol (2010) 28:3680–6. doi:10.1200/JCO.2009.26.9381

7. Lamprecht B, Walter K, Kreher S, Kumar R, Hummel M, Lenze D, et al. Derepression of an endogenous long terminal repeat activates the CSFIR proto-oncogene in human lymphoma. Nat Med (2010) 16:571–9. doi:10.1038/nm.2129

8. Weiss RA. The discovery of endogenous retroviruses. Retrovirology (2006) 3:67. doi:10.1186/1742-4690-3-51-S67

9. Burzdon A. Human-specific endogenous retroviruses. ScientificWorldJournal (2007) 7:1848–68. doi:10.1108/issn-2007-270

10. Basta HA, Cleveland SB, Clinton RA, Dimitrov AG, McClure MA. Evolution of teleost fish retroviruses: characterization of new retroviruses with cellular genes. J Virol (2009) 83:10152–62. doi:10.1128/JVI.02546-08

11. Du J, Tian Z, Hans CS, Laten HM, Cannon SB, Jackson SA, et al. Evolutionary conservation, diversity and specificity of LTR-retrotransposons in flowering plants: insights from genome-wide analysis and multi-specific comparison. Plant J (2010) 63:584–98. doi:10.1111/j.1365-313X.2010.04263.x

12. Cui J, Holmes EC. Endogenous RNA viruses of plants in insect genomes. Virology (2012) 427: 77–9. doi:10.1016/j.virol.2012.02.014

13. Hanrzmann R, Lower J, Lower R, Bichler KH, Kurth R. Synthesis of retrovirus-like particles in testicular teratocarcinomas. J Urol (1982) 128:1055–9.

14. Stefanov Y, Salenko Y, Glukhov I. Drosophila endogenous retroviruses. Mob Genet Elements (2012) 2:36–45. doi:10.4161/mge.19234

15. Bieda K, Hoffmann A, Boller K. Phenotypic heterogeneity of human endogenous retrovirus particles produced by teratocarcinoma cell lines. J Gen Virol (2001) 82:591–6.

16. Dupressoir A, Lavielle C, Heidmann H. From ancestral insectous retroviruses to bona fide cellular genes: role of the captured syncytins in placenta. Placenta (2012) 33:663–71. doi:10.1016/j.placenta.2012.05.005

17. Beyer U, Moll-Rocek J, Moll UM, Dobbelstein M. Endogeneous retrovirus drives hitherto unknown proapoptotic p63 isoforms in the mouse germ line of humans and great apes. Proc Natl Acad Sci U S A (2011) 108: 9624–9. doi:10.1073/pnas.1016201108

www.frontiersin.org

July 2013 | Volume 3 | Article 179 | 5

ERV3 in Hodgkin’s lymphoma
23. Mullins CS, Linnebacher M. Frontiers in Oncology | Pediatric Oncology 19. Sutkowski N, Conrad B, Kewitz and Staege ERV3 in Hodgkin’s lymphoma CTL epitopes. HERV-H env encoding strong Endogenous retrovirus sequences 27. Peggs KS, Hunter A, Chopra R, Parker A, Mahendra P, Milligan D, et al. Clinical evidence of a graft-versus-Hodgkin’s lymphoma effect after reduced-intensity allogeneic transplantation. Lancet (2005) 365:1934–41, doi:10.1016/S0140-6736(05)66659-7

28. Staeger MS, Hattenhorst UE, Neu- manne UE, Hutter C, Foja S, Burdach S. DNA-microarrays as tools for the identification of tumor specific gene expression profiles: applications in tumor biology, diagnosis and therapy. Klin Padiatr (2003) 215:135–9, doi:10.1055/s-2003-3971

29. Drexler HG, Gaedicke G, Lok MS, Diehl V, Minowada J. J Natl Cancer Inst (2012) 104:189–210. doi:10.1093/jnci/djr540

24. Wang-Johanning F, Radvanyi L, Royaj K, Plummer JB, Yan P, Satriy KJ, et al. Human endogenous retrovirus K triggers an antigen-specific immune response in breast cancer patients. Cancer Res (2008) 68:5869–77, doi:10.1158/0008-5472.CAN-07-6838

25. Mullins CS, Linnebacher M. Endogenous retrovirus sequences as a novel class of tumor-specific antigens: an example of HERV-H env encoding strong CTL epitopes. Cancer Immunol Immunother (2012) 61:1093–100, doi:10.1007/s00262-011-1183-3

26. Sachs JB, Kim II, Chen L, Ullah JH, Goodwin DA, Simmons HA, et al. Vaccination with cancer- and HIV infection-associated endoge nous retrotransposable elements is safe and immunogenic. J Immunol (2012) 189:1467–79, doi:10.4049/jimmunol.1200797

27. Kapil R, Alwani A, Al-Hassani J, Barakat EE, Salinas RE, Dave SS, Luftig MV, Korbmacher C, Müller-Lantzsch H, Stein H, Fonatsch C, Gerdes J, et al. Characteristics of Hodgkin’s disease-derived cell lines. Cancer Treat Rev (1982) 66:135–32.

30. Schuhmacher M, Staege MS, Pajic A, Polack A, Weidle UH, Bornkamm GW, et al. Control of cell growth by c-Myc in the absence of cell division. Curr Biol (1999) 9:1255–8, doi:10.1016/S0960-9822(99)80507-7

31. Wolf J, Kapp U, Bohlen H, Korbmacher M, Schoch C, Stahl B, et al. Peripheral blood mononuclear cells of a patient with advanced Hodgkin’s lymphoma give rise to permanently growing Hodgkin-Reed Sternberg cells. Blood (1996) 88:285–92.

32. Takahashi Y, Harashima N, Kaji- gaya S, Yokoyama H, Cherkasova E, McCoy JP, et al. Regression of human kidney cancer following allogeneic stem cell transplantation is associated with recognition of an HERV-E antigen by T cells. J Clin Invest (2008) 118:1099–109, doi:10.1177/00220364083124409

33. Wang-Johanning F, Royaj K, Plummer JB, Li M, Yin B, Frerich K, et al. Immunotherapeutic potential of anti-human endoge nous retrovirus-K envelope protein antibodies in targeting breast tumors. J Natl Canc er Inst (2012) 104:189–210, doi:10.1093/jnci/djr540

34. Salminen A, Staege MS, Pajic A, Polack A, Weidle UH, Bornkamm GW, et al. Control of cell growth by c-Myc in the absence of cell division. Curr Biol (1999) 9:1255–8, doi:10.1016/S0960-9822(99)80507-7

35. Pajic A, Spitkovsky D, Christoph M, Kempkes B, Schuhmacher M, Staeger MS, et al. Cell cycle activation by c-myc in a Burkitt lymphoma model cell line. Int J Cancer (2000) 87:787–93, doi:10.1002/1097-0215(20000915)87:6<787::AID-AD IC>3.0.CO;2-6

36. Pajic A, Staeger MS, Dudziak D, Schuhmacher M, Spitkovsky D, Eissner G, et al. Antagonistic effects of c-myc and Epstein-Barr virus latent genes on the phenotype of human B cells. Int J Cancer (2001) 93:810–6, doi:10.1002/ijc.1404

37. Kewitz S, Bernig T, Staeger MS. Histone deacetylase inhibition restores cisplatin sensitivity of Hodgkin’s lymphoma cells. Leuk Res (2012) 36:773–8, doi:10.1016/j.leukres.2012.02.021

38. Liu J, Schaad MT, Schrieber LD, Koprowski H, Stein H, et al. Identification of an HERV-E antigen by T cells. J Clin Invest (2000) 105:9104, doi:10.1289/ji.2000.9104

39. Saigusa Y, Fujii K, Nonomura M, Takakura K, Suzuki H, et al. Restriction site modifications by DNA polymerase lambda in the mammary carcinoma cell line. J Biol Chem (1993) 268:3148–28.

40. Grigorov Y, Kurian SM, Nakocrevisky AA, Burke JP, Campbell DL, Head SR, et al. Genome-wide analysis of immune activation in human T and B cells reveals distinct classes of alternatively spliced genes. PLoS ONE (2010) 5:e9104, doi:10.1371/journal.pone.0009104

41. Li H, Wu G, Zhan X, Nolan A, Koh C, De Marzo A, et al. Cell-type independent MYC target genes reveal a primordial signature involved in biomass accumulation. PLoS ONE (2011) 6:e26057, doi:10.1371/journal.pone.0026057

42. Balada E, Vilardell-Tarrés M, Balas M, et al. Expression of human endogenous retroviral envelope transcripts in human breast cancer. Cancer Res (2012) 62:5510–6.

Protein 1-mediated NF-κB activa tion. J Virol (2012) 86:11096–106, doi:10.1128/JVI.01069-12

43. Sturm A, Quackenbush J, Jano zanski Z. Genetic cluster analysis of microarray data. Bioinfor matics (2002) 18:207–8, doi:10.1093/bioinformatics/18.1.207

44. Abdulkarim M, Larson E, Hell man L. Methylation of ERV3, an endogenous retrovirus regulat ing the Kruppel-related zinc finger gene H-plk, in several human cell lines arrested during early monocyte development. DNA Cell Biol (1996) 15:727–37, doi:10.1089/dna.1996.15.727

45. McClain K, Wilkowski C. Activation of endogenous retro virus sequences in human leukemia. Biochem Bio phys Res Commun (1985) 133:945–50, doi:10.1016/0006-291X(85)91227-6

46. Tomita N, Hori R, Guo W, Yok ouchi H, Ogawa M, Mori T, et al. Transcription of human endogenous retroviral long terminal repeat (LTR) sequence in a lung cancer cell line. Biochem Biophys Res Commun (1996) 166:1–10, doi:10.1006/bbrc.1996.10961, doi:10.1016/0006-291X(90)91404-7

47. Lowe R, Boller K, Hasenmaier B, Korbmacher M, Müller-Lantzsch N, Lower J, et al. Identification of human endogenous retroviruses with complex mRNA expression and particle formation. Proc Natl Acad Sci U S A (1993) 90:4480–4, doi:10.1073/pnas.90.10.4480

48. Patience C, Simpson GR, Colletta AA, Welch HM, Weiss RA, Boyd MT. Human endogenous retrovirus expression and reverse transcriptase activity in the T47D mammary carcinoma cell line. J Virol (1996) 70:2654–7.

49. Wang-Johanning F, Frost AR, Johanning GL, Khazaeli MB, LoBuglio AF, Shaw DR, et al. Expression of human endogenous retrovirus K envelope transcripts in human breast cancer. Clin Cancer Res (2001) 7:1535–40.

50. Balada E, Vilardell-Tarrés M, Ordi-Ros J. Implication of human endogenous retroviruses in the development of autoimmune diseases. Int Rev Immunol (2010) 29:351–70.
ERV3 in Hodgkin’s lymphoma

Kewitz and Staege

DOI: 10.3109/08830185.2010.48533

53. Antony JM, Deslauriers AM, Blat RK, Ellestad KK, Power C. Human endogenous retroviruses and multiple sclerosis: innocent bystanders or disease determinants? Biochim Biophys Acta (2011) 812:162–76. doi:10.1016/j.bbadis.2010.07.016

54. Douville R, Liu J, Rothstein J, Nath A. Identification of active loci of a human endogenous retrovirus in neurons of patients with amytrophic lateral sclerosis. Ann Neurol (2011) 69:141–51. doi:10.1002/anna.22149

55. Liu AY, Abraham BA. Subtraction of an associated fusion transcript, PLA2L. Transcription of an associated fusion transcript, PLA2L. Transcription of an associated fusion transcript, PLA2L.

56. Gosenca D, Gabriel U, Steidler K, Mager DL. The Opitz syndrome gene MID1 is transcribed from a human endogenous retroviral promoter. Mol Biol Evol (2002) 19:1934–42. doi:10.1093/oxfordjournals.molbev.a004017

57. Landry JR, Rouhi A, Medstrand P, Mager DL. The Opitz syndrome gene MID1 is transcribed from a human endogenous retroviral promoter. Mol Biol Evol (2002) 19:1934–42. doi:10.1093/oxfordjournals.molbev.a004017

58. Kovalski PE, Mager DL. A human endogenous retrovirus suppresses translation of an associated fusion transcript, PLA2L. J Virol (1998) 72:6164–8.

59. Gosenca D, Gabriel U, Steidler K, Mager DL. The Opitz syndrome gene MID1 is transcribed from a human endogenous retroviral promoter. Mol Biol Evol (2002) 19:1934–42. doi:10.1093/oxfordjournals.molbev.a004017

60. Denne M, Sauter M, Armbruester V, Licht JD, Roemer K, Mueller-Lantzsch N. Physical and functional interactions of human endogenous retrovirus proteins Np9 and rec with the promyelocytic leukemia zinc finger protein. J Virol (2007) 81:5607–16. doi:10.1128/JVI.02771-06

61. Matsuda T, Sasaki M, Kato H, Yamada H, Cohen M, Barrett JC, et al. Human chromosome 7 carries a putative tumor suppressor gene(s) involved in choriocarcinoma. Oncogene (1997) 15:2773–81. doi:10.1038/ onc.1201461

62. Cohen M, Kato N, Larsson E. ERV3 human endogenous provirus mRNAs are expressed in normal and malignant tissues and cells, but not in choriocarcinoma tumor cells. J Cell Biochem (1988) 36:121–8. doi:10.1002/jcb.240360203

63. Lin L, Xu B, Rote NS. The cellular mechanism by which the human endogenous retrovirus ERV-3 env gene affects proliferation and differentiation in a human placental trophoblast model, BeWo. Placenta (2000) 21:73–8. doi:10.1053/plac.1999.0443

64. Larsson E, Venables P, Andersson AC, Fan W, Rigby S, Botling J, et al. Tissue and differentiation specific expression on the endogenous retrovirus ERV3 (HERV-R) in normal human tissues and during induced monocytic differentiation in the U-937 cell line. Leukemia (1997) 11(Suppl 3):142–4.

65. Andersson AC, Yun Z, Sperber GO, Larsson E, Blomborg I. ERV3 and related sequences in humans: structure and RNA expression. J Virol (2005) 79:9270–84. doi:10.1128/JVI.79.14.9270–9284.2005

66. Hervé CA, Forrest G, Lörwer R, Griffiths DJ, Venables PJ. Conservation and loss of the ERV3 open reading frame in pri-mates. Genomics (2004) 83:940–3. doi:10.1016/j.ygeno.2003.10.003

67. de Parseval N, Heidmann T. Physiological knockout of the envelope gene of the single-copy ERV-3 human endogenous retrovirus in a fraction of the Caucasian population. J Virol (1998) 72:3442–5. doi:10.1128/JVI.72.8.3442–3445.2000

68. Kato N, Shimotohno K, Van Leeuwen D, Cohen M. Human proviral mRNAs down regulated in choriocarcinoma encode a zinc finger protein related to Kruppel. Mol Cell Biol (1990) 10:4401–5.

69. Rasmussen HB, Ghey G, Dejorge L, Perron H, Touré et al. Expression of endogenous retrovirus in blood mononuclear cells and brain tissue from multiple sclerosis patients. Mult Scler (1995) 1:82–7.

70. Bae S, Jeong HJ, Cha HJ, Kim K, Choi YM, An IS, et al. The hypoxia-mimetic agent cobalt chloride induces cell cycle arrest and alters gene expression in U266 multiple myeloma cells. Int J Mol Med (2012) 30:1180–6. doi:10.3892/immm.2012.1115

71. Guo M, Song LP, Jiang Y, Liu W, Yu Y, Chen HQ. Hypoxia-mimetic agents deresoramine and cobalt chloride induce leukemic cell apoptosis through different hypoxia-inducible factor-Lipha independent mechanisms. Apoptosis (2006) 11:673–7. doi:10.1007/s10495-005-3085-3

72. Zeno S, Zaalst J, Leshner S, Veenman L, Vogt G, Mammel R, et al. The pan-deacetylase inhibitor panobinostat induces cell death and synergizes with everolimus in Hodgkin lymphoma cell lines. Blood (2012) 119:4017–25. doi:10.1182/blood-2011-03-331421

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 27 March 2013; accepted: 25 June 2013; published online: 10 July 2013.

Citation: Kewitz S and Staege MS (2013) Expression and regulation of the endogenous retrovirus 3 in Hodgkin’s lymphoma cells. Front. Oncol. 3:179. doi: 10.3389/fonc.2013.00179

This article was submitted to Frontiers in Pediatric Oncology, a specialty of Frontiers in Oncology.

Copyright © 2013 Kewitz and Staege. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in other forums, provided the original authors and source are credited and subject to any copyright notices concerning any third-party graphics etc.