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Phenotypic heterogeneity of human endogenous retrovirus particles produced by teratocarcinoma cell lines

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Human endogenous retrovirus (HERV) sequences represent about 0·5% of the human genome. The only HERV known to express virus particles is human teratocarcinoma-derived virus (HTDV), which is now termed HTDV/HERV-K. Between 25 and 50 different copies of HERV-K are present in the human genome, three of which contain full-length genes for viral structural proteins. To determine whether genes of different HERV-K proviruses can be expressed, the morphologies and protein expression patterns of HTDV/HERV-K produced by various human teratocarcinoma cell lines were compared. Three different types of retrovirus-like particles were observed, showing differences in the presence of viral surface proteins and the existence of free mature virions. These distinct morphological features between virion types were in accordance with the results of immunoblotting analyses that revealed differences in the cleavage of a viral Gag protein precursor and the presence of a putative Env protein. These data suggest that different HERV-K proviruses are transcribed in human teratocarcinoma cell lines.

Human teratocarcinoma-derived virus (HTDV) particles are retrovirus-like structures that were first detected by electron microscopy in cell lines established from human teratocarcinomas (Bronson *et al.*, 1979; Kurth *et al.*, 1980). As they have been shown to be encoded by the human endogenous retrovirus (HERV)-K sequence (Boller *et al.*, 1993; Löwer *et al.*, 1993), HTDV is now termed HTDV/HERV-K. HTDV/HERV-K particles exhibit no immunological cross-reactivity to other known human or animal retroviruses and are morphologically distinct as they lack an electron-lucent space between the viral core and envelope. HTDV/HERV-K particles also appear to be defective, as surface spikes and free mature virus particles with condensed cores are virtually never observed (Boller *et al.*, 1983). This is supported by the fact that all efforts to demonstrate infectivity of HTDV/HERV-K have failed (Löwer *et al.*, 1984). HERV-K is clearly the most active HERV sequence and to date is the only one shown to be expressed in the form of virus particles (Tönjes *et al.*, 1996).

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Virus particles with a morphology similar to HTDV have been observed budding from the syncytial layer of full-term human placentas (Kalter *et al.*, 1973; Vernon *et al.*, 1974), although they have not yet been unequivocally identified as HTDV/HERV-K. Viral proteins have been detected in biopsies of seminomas (Sauter *et al.*, 1995), and functional viral proteases (Schommer *et al.*, 1996) as well as reverse transcriptase (Berkhout *et al.*, 1999) have been demonstrated *in vitro* after the expression of cloned sequences. The frequent presence of antibodies specific for HTDV/HERV-K proteins in patients suffering from testicular tumours (Sauter *et al.*, 1995; Boller *et al.*, 1997) provides indirect evidence of expression *in vivo*. However, apart from the expression of HTDV/HERV-K proteins in teratocarcinoma cell lines, testicular tumours and most probably in the placenta, their presence in other neoplastic or normal tissues has not been demonstrated. As with endogenous animal retroviruses, the possible involvement of HERV in tumour diseases or autoimmune disorders has been suggested (Urnovitz & Murphy, 1996; Löwer, 1999). Furthermore, the involvement of an endogenous retrovirus sequence, IDDMK$_{1,2}$22, with a sequence similarity to HERV-K in type I diabetes has been claimed recently (Conrad *et al.*, 1997), but could not be confirmed (Löwer *et al.*, 1998). A beneficial role of HERV in humans has also been discussed (Venables *et al.*, 1995; Harris, 1998). However, direct evidence for a biological function of HTDV/HERV-K has yet to be produced.

Between 25 and 50 different HERV-K sequences are present in the human genome (Tönjes *et al.*, 1996) and open reading frames for all three structural protein genes have been cloned from human genomic libraries or from expression libraries of human teratocarcinoma cells. It is not clear whether they originate from a unique provirus because no completely intact replication-competent virus genome has yet been characterized (Tönjes *et al.*, 1999). This raises the question of whether the virus particles observed in teratocarcinoma cell lines are encoded by one or several HERV-K proviruses and whether all cell lines producing HTDV have identical active sequences. To investigate the possibility of differential HERV-K provirus

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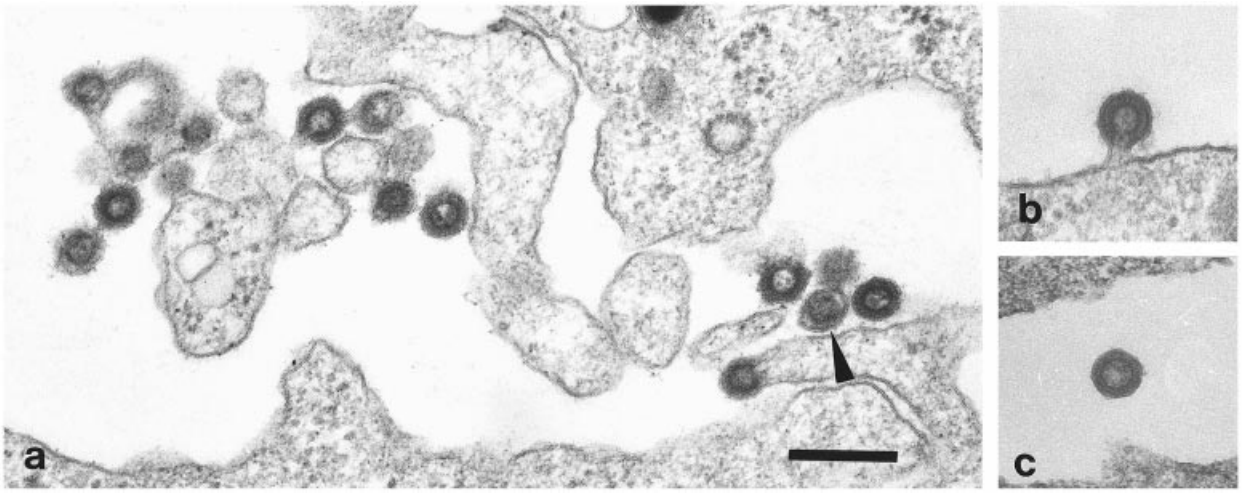

Fig. 1. Electron micrographs of HTDV particles budding from GH (*a*) and Tera-1 (*b*, *c*) cells. Most of the virus particles adhere to the producing cell, spikes on the virus surface are not visible, and mature virus particles, as shown by the arrowhead (*a*), are extremely rare. While virus particles are abundant in GH cells, virions in Tera-1 cells are rare and usually occur as single virions. Bar, 250 nm.

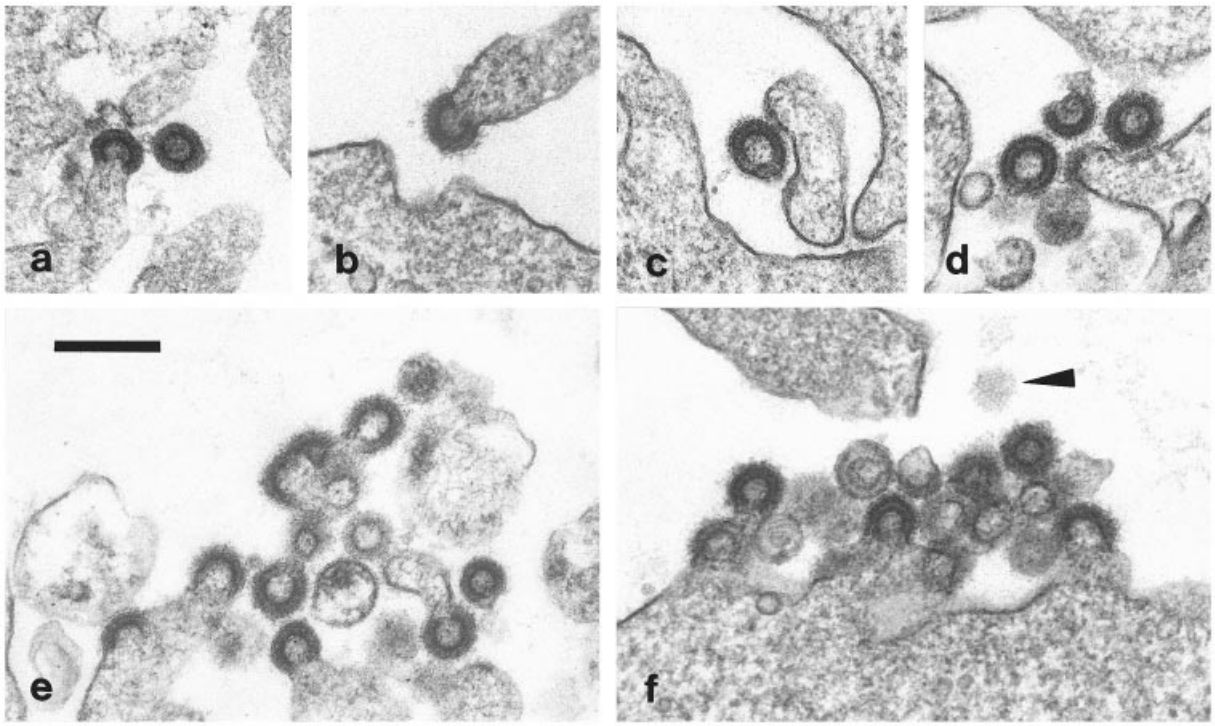

Fig. 2. Electron micrographs of HTDV particles synthesized by 2102Ep cells. Virions appear mainly at the budding stage and mature virus particles are not found. Apart from the naked virus particles without surface spikes (*a*), virions with prominent spikes are usually abundant (*b–f*). Note the spike pattern of the virion (arrowhead) cut tangentially in (*f*). Bar, 250 nm.

expression, we studied the morphology and protein banding pattern of HTDV/HERV-K produced by different human germ cell tumour cell lines.

For these studies, six human teratocarcinoma cell lines were examined: GH (Löwer *et al.*, 1984) and Tera-1 cells (Fogh & Trempe, 1975), which are both known to produce HTDV/HERV-K particles (Boller *et al.*, 1997); NCCIT (Teshima *et al.*, 1988) and 2102Ep cells (Andrews *et al.*, 1980); and Tera-2 (Fogh & Trempe, 1975) and PA-1 cells (Zeuthen *et al.*, 1980) as virus-negative controls. GH cell lines were established in our institute (Löwer *et al.*, 1984). NCCIT and 2102Ep cell lines were kindly provided by H.-J. Schmoll (Halle, Germany) and

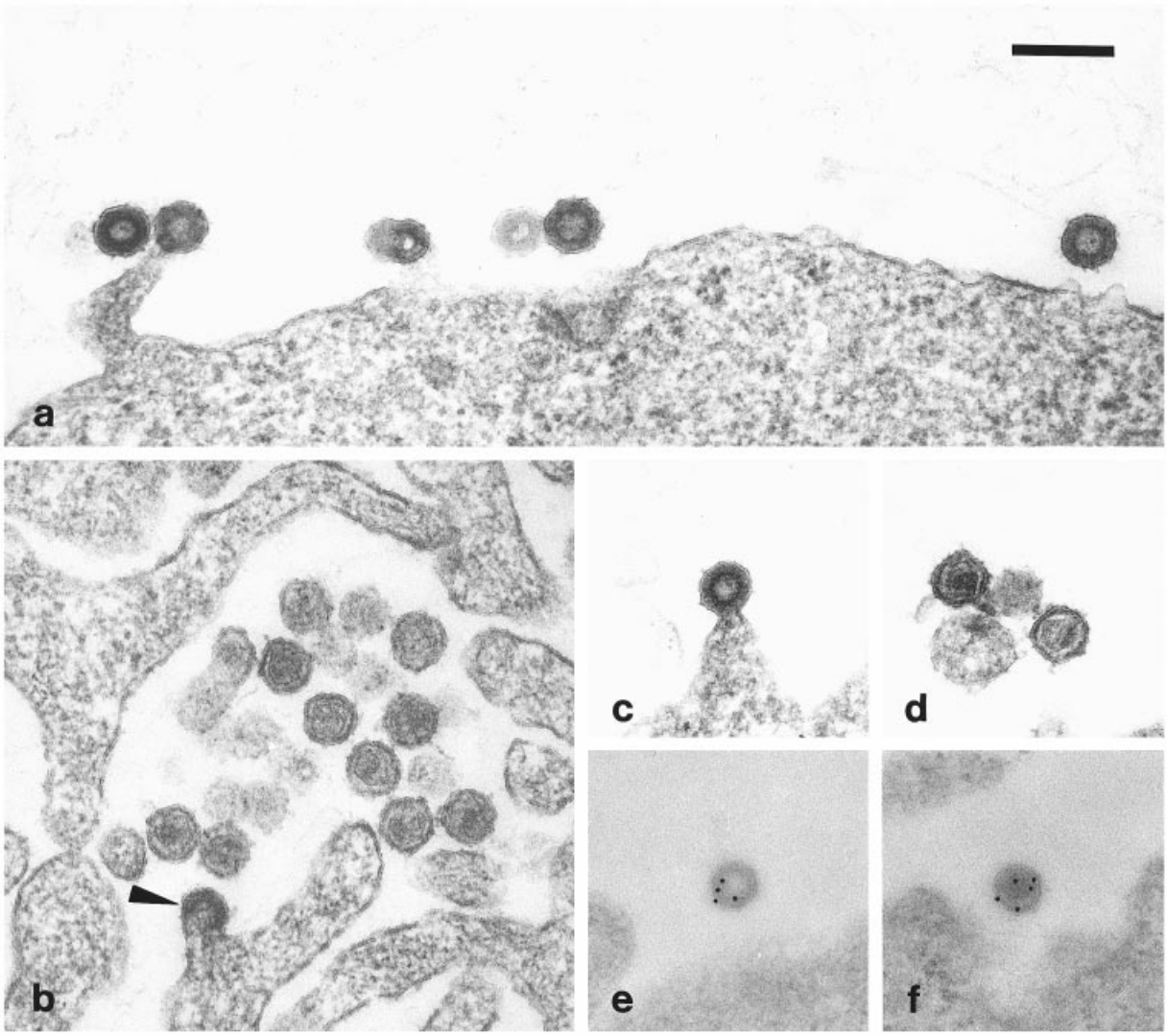

Fig. 3. Electron micrographs of HTDV particles synthesized by NCCIT cells. Apart from the immature virions, arrowhead in (*b*), adhering to the producing cell (*a*, *c*), mature virus particles with condensed cores (*b*, *d*) are often observed. In immunoelectron microscopy on ultra-thin sections, both types of virions show similar patterns of labelling with colloidal gold-coupled antibody HERMA-1. The immature (*e*) and mature (*f*) virions can be seen. Bar, 250 nm.

the others were purchased from the ATCC. To detect virus particles and proteins, we used various antibodies specific for HTDV: (i) the HTDV/HERV-K/Gag-specific monoclonal antibody HERMA-1, which was produced by immunizing BALB/c mice with the membrane fraction of GH cells; (ii) a goat serum that was raised against recombinant HERV-K/Gag and kindly provided by R. Tönjes (Boller *et al.*, 1997); and (iii) a serum from a teratocarcinoma patient that was previously shown to react with HTDV. For immunofluorescence studies, this last antibody was preabsorbed with recombinant HERV-K/Gag protein in order to leave it specific for the putative viral Env protein (Boller *et al.*, 1997). All three antibodies reacted with the cell lines GH, Tera-1 and 2102Ep in immunofluorescence assays, whereas no reaction was seen with the HERV-K-negative teratocarcinoma cell lines, Tera-2 and PA-1 (data not shown). Surprisingly, only the Gag-specific antibodies and not the Env-specific human serum reacted with the human teratocarcinoma cell line NCCIT. The particulate staining pattern of all three antibodies was located at the cell surface of positive cell lines, as would be expected for C-type virus staining. Immunofluorescence double-labelling experiments with Gag- and Env-specific antibodies on GH, Tera-1 and 2102Ep cells clearly showed a colocalization, indicating that both proteins are incorporated into virus particles (results not shown).

In the three cell lines that were reactive with anti-HTDV/HERV-K antibodies in immunofluorescence analyses, retrovirus-like particles of the HTDV type described earlier could be detected by electron microscopy on ultra-thin sections performed according to standard methods. Virus particles were

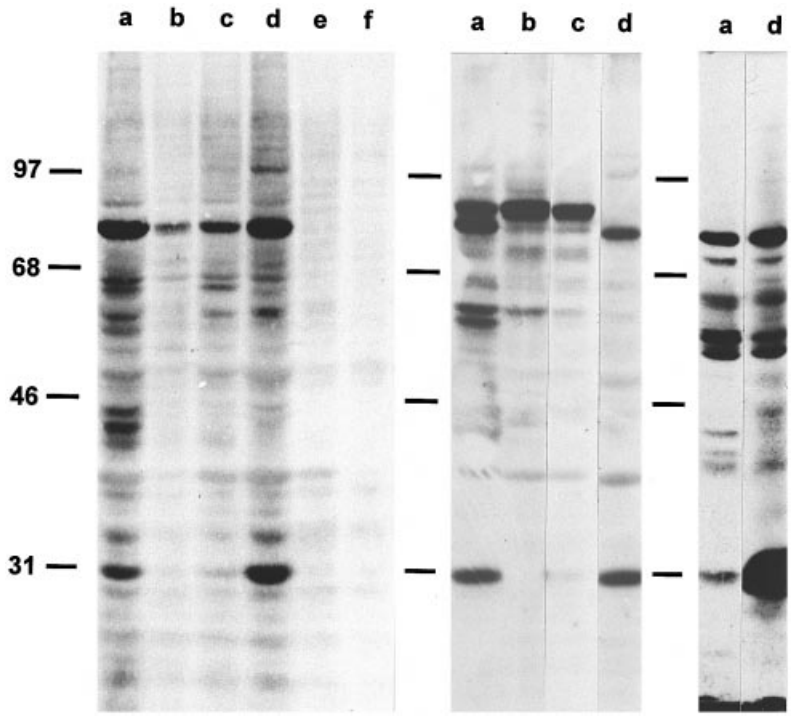

Fig. 4. Immunoblot analysis of various cell lines with the anti-HTDV/Gag-specific monoclonal antibody HERMA-1 (left) or serum from a teratocarcinoma patient (middle). Immunoblot analysis of cell culture supernatant with a goat anti-HERV-K/Gag antiserum (right) is also shown. Lanes a–f: GH, Tera-1, 2102Ep, NCCIT, Tera-2 and PA-1 cell lines, respectively. While Tera-1 (lanes b) and 2102Ep (lanes c) cell lysates exclusively exhibit the 80 kDa Gag precursor protein, GH (lanes a) and NCCIT (lanes d) cell lysates also reveal the cleaved 30 kDa core protein for all antibodies. The HERV-negative Tera-2 (lane e) and PA-1 (lane f) cell lines show no specific bands. The human serum also recognizes the 90 kDa putative Env protein in GH, Tera-1 and 2102Ep cells (lanes a–c) but not in NCCIT cells (lane d). In the supernatant of GH cells (lanes a), a weak band of processed Gag protein of 30 kDa is observed, whereas in the NCCIT cell supernatant (lanes d), a large broad band appears, indicating that most free virus particles exist as mature virions with cleaved Gag protein. Note that the protein quantities loaded on the gel were not identical for all cell lines but standardized for equal banding intensity of the main band. Molecular mass markers (kDa) are shown on the left.

most easily found in GH cells, where groups of more than 10 virions were frequent (Fig. 1*a*). The cell line producing the smallest number of virus particles was the Tera-1 cell line, where single virus particles could only occasionally be observed (Fig. 1*b*, *c*). Virus production in NCCIT and 2102Ep cells was moderate, with single as well as small groups of virus particles being found within some minutes of examination. When comparing virus particle morphology, particular attention was paid to the presence of surface spikes (representing the envelope glycoprotein) and free mature virus particles with condensed cores, which are typical for infectious retroviruses in the virus maturation stage (Frank *et al.*, 1978). During earlier studies using GH cells, virus particles with spikes were not found and free mature virus particles were only rarely observed. This was confirmed for both GH and Tera-1 cells (Fig. 1). In contrast, many virus particles with spikes were clearly observed budding from 2102Ep cells (Fig. 2), with about 10–20% of virions showing prominent surface projections of equal length and structure. While free mature virus particles with condensed cores were hardly seen in GH, Tera-1 and 2102Ep cells, a significant proportion of these mature virions was found in the NCCIT cell line. These virus particles usually occurred in groups and were often associated with budding virus particles showing the classical HTDV morphology, i.e. exhibiting the typical structure of a mature mammalian C-type retrovirus with a centrally located polygonal core (Fig. 3*b*, *d*). To confirm that the free mature virus particles in NCCIT cells did not represent a retrovirus of different origin (endogenous or exogenous), we performed immunoelectron microscopy on freeze-substituted Lowicryl-embedded cells using goat antiserum specific for recombinant HERV-K/Gag, where bound antibodies were detected with anti-goat antibodies labelled with 10 nm colloidal gold. Virions with condensed cores were found to be labelled as efficiently as the immature virus particles or virus particles budding at the cytoplasmic membrane (Fig. 3*e*, *f*).

In summary, three different types of virions were observed: (i) the classical HTDV type, i.e. immature virus particles without spikes, which were present in all cell lines examined; (ii) immature virus particles with prominent spikes found in 2102Ep cells; and (iii) free mature virus particles without surface spikes found in NCCIT cells.

To investigate whether these observations could be confirmed at the protein level, immunoblotting analyses (Boller *et al.*, 1997) were performed using the different antibodies available. The monoclonal antibody HERMA-1 (specific for the HTDV/HERV-K/Gag protein) showed only minor differences between whole cell lysates of the HTDV-positive cell lines (Fig. 4). In Tera-1 and 2102Ep cells, however, only one major band with a molecular mass of 80 kDa, corresponding to the Gag precursor protein, was seen. In GH and NCCIT cells, an additional prominent band with a molecular mass of 30 kDa was observed, representing the cleaved Gag of HTDV/HERV-K. Using the teratocarcinoma patient serum, the same bands

were seen together with bands representing the putative 90 kDa HERV-K Env protein, which was observed in GH, Tera-1 and 2102Ep cells but not in NCCIT cells. Interestingly, in Tera-1 and 2102Ep cells, the band representing the Gag protein was consistently less prominent than the band thought to be the Env protein. In GH cells, however, both viral proteins were detectable in approximately equal amounts.

Mature virus particles were seen by electron microscopy in the NCCIT cell line and an apparently cleaved Gag protein was found by immunoblot analysis in NCCIT and GH cell lysates. The cell supernatant of these lines was therefore examined for the presence of free virus particles. Cell culture supernatants of GH and NCCIT cells were centrifuged at 20000 r.p.m. in an ultracentrifuge, lysates were prepared from the pellet (Boller *et al*., 1997) and immunoblots were probed with goat anti-HERV-K/Gag antibody. In the supernatant of GH cells, a prominent band of 80 kDa, corresponding to the Gag precursor, and only a weak band at 30 kDa, representing the cleaved core protein, were seen. In contrast, the overwhelming majority of protein detected in NCCIT cell supernatant corresponded to the 30 kDa band and significantly less 80 kDa protein was observed. We therefore conclude that only a minor proportion of virus particles are released from GH cells to become mature virions, whereas most viruses produced by NCCIT cells are released into the cell culture supernatant.

The production of morphologically distinct retrovirus-like particles from different human teratocarcinoma cell lines has been observed previously (Bronson *et al*., 1984). According to our results, these viruses were probably just different forms of HTDV/HERV-K virions. This study is the first to report the existence of free and mature HTDV particles, although this type of HTDV/HERV-K (as observed in the NCCIT cell line) lacks the Env protein and therefore cannot be infectious, given the role played by Env in cellular binding and uptake. There would therefore appear to be a selection pressure for the production of non-infectious viruses.

In conclusion, the levels of transcription (Medstrand *et al*., 1992; Li *et al*., 1995), translation and virus particle formation (this work) clearly indicate that various HERV-K proviruses are expressed. As a completely intact replication-competent sequence probably does not exist, it has been speculated that HTDV/HERV-K virus particles might originate by complementation of transcripts from different proviruses (Löwer *et al*., 1996); Li *et al*. (1995) observed that different HERV-K sequences can be expressed in a single teratocarcinoma cell line and the results reported here would support this hypothesis.

In two of the cell lines studied here, two types of particle were observed. Although this does not demonstrate unequivocally that different proviruses are active (the same sequence could be responsible for different morphotypes as immature and mature virus particles), there seems to be a heterogeneity in virus expression. However, if several proviruses are transcribed in one cell and can participate in virus particle formation, phenotypic mixing could occur and eventually lead to infectious virions. It would therefore be worthwhile to look further for signs of infection by HTDV/HERV-K.

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References

Andrews, P. W., Bronson, D. L., Benham, F., Strickland, S. & Knowles, B. B. (1980). A comparative study of eight cell lines derived from human testicular teratocarcinoma. *International Journal of Cancer* **26**, 269–280.

Berkhout, B., Jebbink, M. & Zsiros, J. (1999). Identification of an active reverse transcriptase enzyme encoded by a human endogenous HERV-K retrovirus. *Journal of Virology* **73**, 2365–2375.

Boller, K., Frank, H., Löwer, J., Löwer, R. & Kurth, R. (1983). Structural organization of unique retrovirus-like particles budding from human teratocarcinoma cell lines. *Journal of General Virology* **64**, 2549–2559.

Boller, K., König, H., Sauter, M., Mueller-Lantzsch, N., Löwer, R., Löwer, J. & Kurth, R. (1993). Evidence that HERV-K is the endogenous retrovirus sequence that codes for the human teratocarcinoma-derived retrovirus HTDV. *Virology* **196**, 349–353.

Boller, K., Janssen, O., Schuldes, H., Tönjes, R. R. & Kurth, R. (1997). Characterization of the antibody response specific for the human endogenous retrovirus HTDV/HERV-K. *Journal of Virology* **71**, 4581–4588.

Bronson, D. L., Fraley, E. E., Fogh, J. & Kalter, S. S. (1979). Induction of retrovirus particles in human testicular tumor (Tera-1) cell cultures: an electron microscopic study. *Journal of the National Cancer Institute* **63**, 337–339.

Bronson, D. L., Saxinger, W. C., Ritzi, D. M. & Fraley, E. E. (1984). Production of virions with retrovirus morphology by human embryonal carcinoma cells *in vitro*. *Journal of General Virology* **65**, 1043–1051.

Conrad, B., Weissmahr, R. N., Boni, J., Arcari, R., Schupbach, J. & Mach, B. (1997). A human endogenous retroviral superantigen as candidate autoimmune gene in type I diabetes. *AIDS Research and Human Retroviruses* **90**, 303–313.

Fogh, J. & Trempe, G. (1975). In *Human Tumor Cells In Vitro*, pp. 115–138. Edited by J. Fogh. New York: Plenum Press.

Frank, H., Schwarz, H., Graf, T. & Schafer, W. (1978). Properties of mouse leukemia viruses. XV. Electron microscopic studies on the organization of Friend leukemia virus and other mammalian C-type viruses. *Zeitschrift für Naturforschung Section C Biosciences* **33**, 124–138.

Harris, J. R. (1998). Placental endogenous retrovirus (ERV): structural, functional, and evolutionary significance. *Bioessays* **20**, 307–316.

Kalter, S. S., Helmke, R. J., Heberling, R. L., Panigel, M., Fowler, A. K., Strickland, J. E. & Hellman, A. (1973). Brief communication: C-type particles in normal human placentas. *Journal of the National Cancer Institute* **50**, 1081–1084.

Kurth, R., Löwer, R., Löwer, J., Harzmann, R., Pfeiffer, R., Schmidt, C. G., Fogh, J. & Frank, H. (1980). In *Cold Spring Harbor Conferences On Cell Proliferation*, pp. 835–846. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.

Li, M. D., Bronson, D. L., Lemke, T. D. & Faras, A. J. (1995). Restricted expression of new HERV-K members in human teratocarcinoma cells. *Virology* **208**, 733–741.

Löwer, R. (1999). The pathogenic potential of endogenous retroviruses: facts and fantasies. *Trends in Microbiology* **7**, 350–356.

Löwer, R., Löwer, J., Frank, H., Harzmann, R. & Kurth, R. (1984). Human teratocarcinomas cultured *in vitro* produce unique retrovirus-like viruses. *Journal of General Virology* **65**, 887–898.

Löwer, R., Boller, K., Hasenmaier, B., Korbmacher, C., Mueller-Lantzsch, N., Löwer, J. & Kurth, R. (1993). Identification of human endogenous retroviruses with complex mRNA expression and particle formation. *Proceedings of the National Academy of Sciences, USA* **90**, 4480–4484.

Löwer, R., Löwer, J. & Kurth, R. (1996). The viruses in all of us: characteristics and biological significance of human endogenous retrovirus sequences. *Proceedings of the National Academy of Sciences, USA* **93**, 5177–5184.

Löwer, R., Tönjes, R. R., Boller, K., Denner, J., Kaiser, B., Phelps, R. C., Löwer, J., Kurth, R., Badenhoop, K., Donner, H., Usadel, K. H., Miethke, T., Lapatschek, M. & Wagner, H. (1998). Development of insulin-dependent diabetes mellitus does not depend on specific expression of the human endogenous retrovirus HERV-K. *AIDS Research and Human Retroviruses* **95**, 11–14.

Medstrand, P., Lindeskog, M. & Blomberg, J. (1992). Expression of human endogenous retroviral sequences in peripheral blood mononuclear cells of healthy individuals. *Journal of General Virology* **73**, 2463–2466.

Sauter, M., Schommer, S., Kremmer, E., Remberger, K., Dolken, G., Lemm, I., Buck, M., Best, B., Neumann-Haefelin, D. & Mueller-Lantzsch, N. (1995). Human endogenous retrovirus K10: expression of Gag protein and detection of antibodies in patients with seminomas. *Journal of Virology* **69**, 414–421.

Schommer, S., Sauter, M., Kräusslich, H.-G., Best, B. & Mueller-Lantzsch, N. (1996). Characterization of the human endogenous retrovirus K proteinase. *Journal of General Virology* **77**, 375–379.

Teshima, S., Shimosato, Y., Hirohashi, S., Tome, Y., Hayashi, I., Kanazawa, H. & Kakizoe, T. (1988). Four new human germ cell tumor cell lines. *Laboratory Investigation* **59**, 328–336.

Tönjes, R. R., Löwer, R., Boller, K., Denner, J., Hasenmaier, B., Kirsch, H., König, H., Korbmacher, C., Limbach, C., Lugert, R., Phelps, R. C., Scherer, J., Thelen, K., Löwer, J. & Kurth, R. (1996). HERV-K: the biologically most active human endogenous retrovirus family. *Journal of Acquired Immune Deficiency Syndromes and Human Retrovirology* **13** (Suppl. 1), S261–267.

Tönjes, R. R., Czauderna, F. & Kurth, R. (1999). Genome-wide screening, cloning, chromosomal assignment, and expression of full-length human endogenous retrovirus type K. *Journal of Virology* **73**, 9187–9195.

Urnovitz, H. B. & Murphy, W. H. (1996). Human endogenous retroviruses: nature, occurrence, and clinical implications in human disease. *Clinical Microbiology Reviews* **9**, 72–99.

Venables, P. J., Brookes, S. M., Griffiths, D., Weiss, R. A. & Boyd, M. T. (1995). Abundance of an endogenous retroviral envelope protein in placental trophoblasts suggests a biological function. *Virology* **211**, 589–592.

Vernon, M. L., McMahon, J. M. & Hackett, J. J. (1974). Additional evidence of type-C particles in human placentas. *Journal of the National Cancer Institute* **52**, 987–989.

Zeuthen, J., Norgaard, J. O., Avner, P., Fellous, M., Wartiovaara, J., Vaheri, A., Rosen, A. & Giovanella, B. C. (1980). Characterization of a human ovarian teratocarcinoma-derived cell line. *International Journal of Cancer* **25**, 19–32.

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