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The potential of retroviral vectors to cotransfer human endogenous retroviruses (HERVs) from human packaging cell lines

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Abstract

Using a versatile and highly sensitive retroviral microarray, we have investigated particle preparations from three different human packaging cell lines harboring retroviral vector systems based on human immunodeficiency virus (HIV) and murine leukemia virus (MLV). 293Rev/Gag/Pol$_i$ cells inducibly express high titers of HIV-derived particles for packaging of HIV vectors. The Phoenix-GP and the Anjou 65 cell lines constitutively express MLV vector particles. We compared the transcription profiles of human endogenous retroviruses (HERVs) in all cell lines with the HERV sequences present in the particles. In addition, the influence of the transfected vector plasmid on the copackaging of HERVs was investigated. All particle preparations showed a defined pattern of endogenous retroviral sequences that differed from the cellular HERV expression pattern. HERV transcripts were observed in the particle preparations independent of whether a vector construct was coexpressed or not.

Furthermore, our results suggest that particle preparations are frequently contaminated by cellular vesicles (exosomes) containing cellular RNAs including HERV transcripts.

Keywords: Human endogenous retroviruses; HERV; Retroviral vector; Packaging cell line; Copackaging; DNA chip; Microarray; Gene therapy

1. Introduction

Development of safe and effective gene transfer systems is critical for the success of gene therapy protocols in clinical application. Retroviral vectors are promising tools for delivering therapeutic genes. However, there are particular safety concerns. First, the particle producing packaging cell line must not release replication-competent retroviruses (RCR). Second, expression of endogenous retroviral (ERV) sequences in the packaging cell line itself presents a potential danger. Copackaging of ERVs may result in the unwanted transfer of these sequences to target cells and lead to recombination with retroviral vectors or the cellular genome. Moreover, vesicles with non-specific packaged ERV genomes may detach from the plasma membrane of producer cells and become an undesired contaminant in retroviral vector preparations.

ERVs and non-viral reverse transcriptase (RT)-related sequences are normal components of all vertebrate genomes. Human endogenous retroviruses (HERVs) are considered to be remnants of ancient germ line infections by exogenous

Abbreviations: CMV, cytomegalovirus; DEPC, diethylpyrocarbonate; ELISA, enzyme-linked immunosorbent assay; ERV, endogenous retrovirus; GFP, green fluorescent protein; HERV, human endogenous retrovirus; HIV, human immunodeficiency virus; IRES, internal ribosome entry sequence; MLV, murine leukemia virus; MOP, mixed oligonucleotide primers; MMTV, mouse mammary tumor virus; PCR, polymerase chain reaction; PGK, phosphoglycerate kinase; RCR, replication-competent retrovirus; RT, reverse transcriptase; RRE, Rev responsive element; RSV, Rous sarcoma virus; SV40, simian virus 40; VSV-G, vesicular stomatitis virus glycoprotein.

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retroviruses that have become genetically fixed and in consequence inherited like Mendelian genes (for review see: Sverdlov, 2005). During evolution these elements have moved and spread around the host genome by retrotransposition. According to the human genome database, 8 to 9% of the human genome is of retroviral origin comprising about 200 distinct families (Lander et al., 2001; Jurka et al., 2005).

Activation and expression of HERV elements may result in unpredictable and undesired mobilization of genetic material of retroviral origin. This has been demonstrated, for example, for retrovirus-like particles released by the human breast cancer-derived cell line T47D. Characterization of transcripts isolated from T47D cells and from released particles revealed that complementation between several expressed HERVs may generate pseudotypes packaging retroviral RNA of different origin (Seifarth et al., 1995, 1998). Likewise, copackaging of ERV and vector sequences could lead to generation of recombinant proviruses in the target cells harboring genetic information from both parental RNAs (Mikkelsen and Pedersen, 2000).

We have recently developed a retrovirus-specific DNA chip for the detection of reverse transcriptase (RT)-related nucleic acids in samples of human and mammalian origin (Seifarth et al., 2003). The microarray contains synthetic capture probes representing 54 HERV *pol* sequences derived from 20 major HERV families and 12 mammalian ERVs (Frank et al., 2005; Seifarth et al., 2005). In this study, the microarray was used to screen several vector systems based on human packaging cell lines for copackaging of HERVs, packaging transcripts and cellular mRNAs.

2. Materials and methods

2.1. Vector constructs and packaging cell lines

Constitutive MLV-derived packaging cell lines Phoenix-GP and Anjou 65 (Pear et al., 1993) were obtained from G. Nolan, University of Stanford, USA. Both cell lines are derived from 293T cells. Phoenix-GP cells contain a MLV *gag-pol* expression plasmid under the control of the Rous sarcoma virus (RSV) promoter, Anjou 65 cells the MLV-derived pCRIPgag-2 packaging construct (Danos and Mulligan, 1988). A β-galactosidase expressing MLV vector has additionally been stably introduced into the Anjou 65 cells. The MLV-based vector construct pMX-GFP (Onishi et al., 1996) was used for transient transfection of both cell lines. The inducible HIV-1-derived packaging cell line 293Rev/Gag/Pol_i was described previously (Sparacio et al., 2001). The packaging construct pIND(Sp1)-ΔΨgag/pol-RRE and the helper construct pIND-Rev-IRES-Rev are both under the control of an ecdysone-inducible promoter. As a vector construct the HIV-based, tat-independent, GFP containing plasmid pRRL-CMV-GFP-Sin-18 was used. This construct was derived from plasmid pRRLhPGK.GFP-Sin-18 (Dull et al., 1998) by replacing the PGK promoter with the CMV promoter. The construct pEx-VSV-G encodes the vesicular stomatitis virus glycoprotein.

2.2. Purification of vector particles

Vector plasmids and pEx-VSV-G were cotransfected into the respective packaging cell lines using standard calcium phosphate procedures. 48 h post-transfection, culture supernatants potentially containing transducing vector particles were filtered through a 0.45 μm filter and concentrated by centrifugation through a cushion of sucrose (Sparacio et al., 2001). In the case of the HIV-1-based cell line 293Rev/Gag/Pol_i, particles were quantitated by ELISA for HIV-1-CA (Innogenetics, Belgium) before and after pelletation. Where applicable, plasmid expression was induced with ponasterone A 4 h post-transfection. All experiments were repeated at least four times.

2.3. Preparation of DNA-free RNA

Total RNA was extracted from cultured cells according to a guanidinium isothiocyanate/cesium chloride (GIT/CsCl) ultracentrifugation protocol (Sambrook et al., 1989) and dissolved in diethylpyrocarbonate (DEPC)-treated distilled water. RNA from retroviral particles was prepared as described previously (Seifarth et al., 1995). mRNA was purified using Dynabeads™ paramagnetic particles according to the manufacturer's instructions (Dynal, Hamburg, Germany). To exclude genomic DNA contamination, mRNA preparations were treated with DNase and tested by PCR using mixed oligo primers (MOP) omitting the reverse transcription step (Seifarth et al., 2003).

2.4. Reverse transcription PCR (RT-PCR) and microarray experiments

Reverse transcription of mRNA, and direct labeling by MOP-PCR using primer mixtures including Cy3-labeled reverse primers, as well as preparation, hybridization, processing and qualitative evaluation of retrovirus-specific microarrays were performed as described previously (Seifarth et al., 2003, 2005). For amplification of HERV *pol* sequences two separate multiplex PCRs were carried out using mixed oligonucleotide primers (MOP) for either class II HERVs or class I/III HERVs including human exogenous and animal retroviruses (Seifarth et al., 2003). Primers specific for housekeeping genes and green fluorescent protein (GFP) were added to both primer mixtures. Microarray hybridizations were performed at least in triplicate.

3. Results and discussion

3.1. HERV expression profiles in human packaging cell lines derived from 293 and 293T cells

To examine vector preparations for copackaging of HERV sequences, a recently established, retrovirus-specific microarray (HERV chip) that allows simultaneous detection and identification of a wide variety of human exogenous and endogenous retroviruses was employed (Seifarth et al., 2003, 2005). The microarray consists of 54 representative HERV reverse transcriptase (RT)-derived sequences from 20 major HERV