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Jaagsiekte sheep retrovirus is not detected in human lung adenocarcinomas expressing antigens related to the Gag polyprotein of betaretroviruses

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Abstract

A proportion of human lung adenocarcinomas (hLACs) express an antigen related to the major capsid protein (CA) of Jaagsiekte sheep retrovirus (JSRV), a *Betaretrovirus* that causes a transmissible lung cancer in sheep. In this study, we have investigated whether JSRV or related betaretroviruses are expressed in hLACs. Results obtained indicate that JSRV is not associated with human lung adenocarcinomas. However, a proportion of hLACs reacted positively in immunohistochemistry with antibodies specific towards different domains of the JSRV Gag suggesting that a *bona fide* retrovirus antigen could be expressed in these tumours. Further studies will be necessary to ascertain whether the detection of antigens cross-reacting with betaretrovirus Gag antisera in some hLACs is due to expression of a human endogenous retrovirus or, more unlikely, of an uncharacterized exogenous retrovirus.

Keywords: Retroviruses; Betaretroviruses; Jaagsiekte sheep retrovirus; JSRV; HERV-K; Lung adenocarcinoma

1. Introduction

Jaagsiekte sheep retrovirus (JSRV) is the etiological agent of a transmissible lung cancer of sheep known as ovine pulmonary adenocarcinoma (OPA). JSRV induces the transformation of secretory epithelial cells of the distal respiratory tract, type II pneumocytes and Clara cells (or a common precursor) [1–3]. OPA is one of the major infectious diseases of sheep [4] and displays several common features with some forms of human lung

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adenocarcinomas (hLACs) that have been reported for several decades [2,5–7].

The cellular receptor for JSRV is Hyaluronidase-2 (Hyal2), a putative tumour suppressor gene which, in humans, is localized in the chromosomal region 3p21.3. Notably, loss of heterozygosity at 3p21.3 is frequently observed in some human lung cancers [8]. JSRV can potentially infect human cells as human HYAL-2 mediates JSRV entry very efficiently *in vitro* [9]. In addition, the JSRV envelope glycoprotein functions as an oncoprotein *in vitro* [9–11], and *in vivo* [12,13]. In a previous study, we observed that approximately 30% of hLACs express a protein that cross-reacts with a polyclonal rabbit antiserum raised against the major capsid protein (CA) of JSRV [14]. However, there is no epidemiological association between human lung cancer and humans exposed to sheep in areas where OPA is endemic. JSRV is a betaretrovirus, phylogenetically and antigenically related to viruses such as mouse mammary tumour virus (MMTV), Mason-Pfizer monkey virus (MPMV) and the human endogenous retrovirus HERV-K. MMTV induces mammary carcinoma in mice but recent reports have suggested that MMTV can also infect human cells and be detected in a proportion of human breast cancers [15–18], although these data are controversial [19,20]. Expression of another betaretrovirus, the human endogenous retrovirus HERV-K has also been found in some human neoplasms, such as seminomas, teratomas and melanomas [21–24].

We decided to further investigate the presence of JSRV and JSRV-related betaretroviruses in hLACs because: (i) betaretroviruses have been associated with cancer in animals and humans [15–18,21–24]; (ii) approximately 30% of hLACs express antigens that cross-react with a JSRV-CA antiserum [14]; (iii) JSRV is the causative agent of an infectious lung adenocarcinoma in sheep [3]; (iv) JSRV can potentially infect human cells [9] and (v) JSRV-related DNA sequences were purposely detected in individuals from Nigeria and Cameroon [25].

Results obtained in this study indicate that JSRV *per se* is not associated with human lung adenocarcinomas. However, a proportion of hLACs reacted with antibodies specific for different domains of JSRV Gag. Further studies will be necessary to ascertain whether the presence of a betaretroviral Gag in some hLACs is due to expression of a human endogenous retrovirus or of an uncharacterized exogenous retrovirus.

2. Materials and methods

2.1. Antisera

Rabbit polyclonal antisera against the JSRV major capsid protein (CA) and surface domain (SU) of the envelope protein were used as previously described [26,27] and were kindly provided by the Moredun Research Institute (Edinburgh, Scotland). A polyclonal antiserum against the JSRV matrix (MA) was obtained by immunizing rabbits with a recombinant protein (expressed in bacteria) expressing the N-terminal domain of the JSRV Gag (Proteintech). Pre-immune serum was collected before immunization. Goat polyclonal antisera against the MPMV and MMTV CA proteins were kindly provided by Alan Rein. A rabbit polyclonal antiserum against the HERV-K Gag was kindly provided by Marlies Sauter [23].

2.2. Plasmids

Plasmid pCMV2JS21 expressing the JSRV molecular clone $JSRV_{21}$ has been described previously [3]. Plasmid pSARM4 expressing a MPMV infectious molecular clone has been kindly provided by Eric Hunter [28]. In order to express MMTV and HERV-K pseudoviruses we modified plasmid pCMV2JS21 by replacing the JSRV *gag* with the MMTV or HERV-K *gag*; the resulting chimeric plasmids were named, respectively, pCRU5-MMTVGag-HA and pCRU5-HERV-KGag and will be described elsewhere.

2.3. Virus preparations

JSRV, MPMV, chimeric MMTV-JSRV and HERV-K/JSRV particles were produced by transiently transfecting 293T cells with the appropriate plasmid using CalPhos (Clontech). Viral particles were concentrated by ultracentrifugation from cell supernatants 48 h after transfection as described previously [3].

2.4. SDS–PAGE/western blotting

SDS–PAGE and western blotting were performed as already published [3] using the appropriate antibodies described above.

2.5. Tumour and control tissue samples

Paraffin-embedded blocks of primary human lung adenocarcinomas (hLACs) were obtained from the tissue archive of the Hospital Miguel Servet in Zaragoza, Spain ($n = 43$) and from the Center of Excellence of Aging in Chieti, Italy ($n = 80$). Information on patient gender, smoking habit, epidermal growth factor (EGFR) mutation and histological tumour subtype were available from the latter sample subset.