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Advances in the study of transmissible respiratory tumours in small ruminants

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ABSTRACT

Sheep and goats are widely infected by oncogenic retroviruses, namely *Jaagsiekte Sheep RetroVirus* (JSRV) and *Enzootic Nasal Tumour Virus* (ENTV). Under field conditions, these viruses induce transformation of differentiated epithelial cells in the lungs for *Jaagsiekte Sheep RetroVirus* or the nasal cavities for *Enzootic Nasal Tumour Virus*. As in other vertebrates, a family of endogenous retroviruses named endogenous *Jaagsiekte Sheep RetroVirus (enJSRV)* and closely related to exogenous *Jaagsiekte Sheep RetroVirus* is present in domestic and wild small ruminants. Interestingly, *Jaagsiekte Sheep RetroVirus* and *Enzootic Nasal Tumour Virus* are able to promote cell transformation, leading to cancer through their envelope glycoproteins. *In vitro*, it has been demonstrated that the envelope is able to deregulate some of the important signaling pathways that control cell proliferation. The role of the retroviral envelope in cell transformation has attracted considerable attention in the past years, but it appears to be highly dependent of the nature and origin of the cells used. Aside from its health impact in animals, it has been reported for many years that the *Jaagsiekte Sheep RetroVirus*-induced lung cancer is analogous to a rare, peculiar form of lung adenocarcinoma in humans, namely lepidic pulmonary adenocarcinoma. The implication of a retrovirus related to *Jaagsiekte Sheep RetroVirus* is still controversial and under investigation, but the identification of an infectious agent associated with the development of lepidic pulmonary adenocarcinomas might help us to understand cancer development. This review explores the mechanisms of induction of respiratory cancers in small ruminants and the possible link between retrovirus and lepidic pulmonary adenocarcinomas in humans.

1. Introduction

Cancers are complex and multi-causal diseases and constitute a major threat for humans. Environmental factors, as well as genetic events, have been associated to tumour induction. Worldwide, up to 20% of human cancers might be attributed to infectious agents (Gatza et al., 2005). Among them, several viruses have been associated with cancer induction, *e.g.*,*Hepatitis B Virus*, *Hepatitis C Virus*, *Human Papilloma Virus*, *Epstein Barr Virus*, *Kaposi's Sarcoma Herpesvirus* or *Human T-Lymphotropic Virus type 1*.

The association between viruses and cancers has been reported as early as 1894, although the term 'virus' did not exist at the time, with the description of a contagious pulmonary disease affecting sheep in South Africa. The disease was at the time named Jaagsiekte, a term associating two Afrikaans words ('Jaag' for chase and 'siekte' for disease), describing the respiratory condition of the sick animals that appeared as if they had been chased, as a result of the induced dyspnoea (York and Querat, 2003). Since the seminal description, the disease has been reported worldwide from Europe to China. The link between *Jaagsiekte Sheep RetroVirus* infection and cancer has become evident in the late 1970s, by imaging of retrovirus particles in the lung of affected sheep (Perk et al., 1974) and experimental induction of tumours by inoculation of viral particles (Martin et al., 1976), cytoplasmic fractions of tumoural cells (Verwoerd et al., 1980a,b) or pulmonary secretions (Sharp et al., 1983). Reproduction of the cancer upon inoculation of particles produced from a *Jaagsiekte Sheep RetroVirus* molecular clone definitively linked virus to cancer (Palmarini et al., 1999).

In 1909, Peyton Rous evidenced the association between viruses and cancer when he successfully transmitted a tumour from hen to chicken by the injection of cell-free extracts (Rous, 1910, 1911). The retrovirology field initiated by Rous and other pioneers at the beginning of the 20th century set up the foundations for important discoveries in the following decades and marked a major step in

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virology. The studies on retroviruses have been essential to decipher the molecular events during carcinogenesis and they remain relevant to the understanding of tumour development.

This review explores the mechanisms of induction of respiratory cancers in small ruminants and the possible link between retrovirus and lepidic pulmonary adenocarcinomas in humans.

2. Retroviruses and cancer in small ruminants

The Retroviridae family is classified into two subfamilies, Spumaretrovirinae and Orthoretrovirinae; the latter is divided into six genera: alpha-retrovirus, beta-retrovirus, gamma-retrovirus, delta-retrovirus, epsilon-retrovirus and lentivirus. They are enveloped RNA viruses, dependent for their replication of the reverse transcriptase, a RNA dependent DNA-polymerase. Importantly, the viral DNA or provirus definitively integrates into the cellular genome during the early steps of the virus cycle and will remain for the rest of the life into the host DNA. Among the large Retroviridae family, oncogenic retroviruses are present in various species from humans to fish and responsible for the induction of various types of tumours, such as lymphomas or leukaemia in humans, cattle, cats or captive koalas as recently demonstrated (Xu et al., 2013), or solid tumours in the lung in sheep or in the mammary gland in mouse (Table 1).

Sheep and goats are widely infected by the non-oncogenic *Small Ruminant LentiViruses* (SRLV), related to *Human Immunodeficiency Virus-1* and responsible for slowly evolving inflammatory and/or degenerative diseases, and by oncogenic retroviruses, namely *Jaagsiekte Sheep RetroVirus* (JSRV) and *Enzootic Nasal Tumour Virus (ENTV)*, respectively inducing lung or nasal adenocarcinomas (Table 1) (Leroux et al., 1995; Leroux and Mornex, 2008; Leroux et al., 2010). Under field conditions, it has been widely observed that the transmission of cancer occurs among flocks by trade of clinically unaffected animals (Sharp and DeMartini, 2003). The respiratory route of transmission has been reported as early as 1934 during the Icelandic epidemic (Dungal, 1938); the evidence that adult sheep can be infected when naive and infected animals were kept together stresses out the importance of this route (Caporale et al., 2005). Besides inhalation of aerosolised particles in adults, *Jaagsiekte Sheep RetroVirus* can infect animals very early in life, with virus detectable even at birth, suggesting *in utero* transmission to the foetus (Caporale et al., 2005). *Jaagsiekte Sheep RetroVirus* has been detected in colostrum, which supports the evidence that the virus can spread to newborns by colostrum and milk (Grego et al., 2008). Interestingly, eradication of ovine pulmonary adenocarcinoma has been accomplished by motherless-rearing of lambs from flocks incurring a high prevalence of animals with gross and histological lesions (Voigt et al., 2007), as had been done in the past for *Small Ruminant Lentiviruses*. For the anecdote, *Jaagsiekte Sheep RetroVirus*-induced pulmonary adenocarcinoma has been responsible for the demise of the ewe Dolly, the first mammal cloned by nuclear transfer from an adult cell; post mortem examination confirmed the presence of lung tumours.

The incubation period in naturally infected animals ranges from few months to two to four years. This may vary according to the type of infection, with shorter incubation period after experimental inoculation than spontaneous infection, and in young animals (Sharp and DeMartini, 2003). In our experience, clinical signs confirmed by macroscopic lesions and presence of the virus may be diagnosed as early as at the age of three to six months; moreover, we have recently diagnosed the disease in lambs younger than six months. Injection of tumoural tissues to newborn lambs rapidly induces the disease in three to six weeks (Palmarini et al., 1999). Under clinical conditions, the rapid development of tumoural lesions in young animals suggests an increased susceptibility of the developing lung to the virus (Caporale et al., 2005).

Sheep can be co-infected by *Jaagsiekte Sheep RetroVirus* and *Enzootic Nasal Tumour Virus* and coexistence of enzootic nasal tumour and *Jaagsiekte Sheep RetroVirus* infection has been reported in naturally infected sheep (Ortin et al., 2004). While *Enzootic Nasal Tumour Virus* induces transformation of epithelial cells of the ethmoid turbinates in sheep and goats, *Jaagsiekte Sheep RetroVirus* transforms epithelial cells of the distal lung, namely alveolar epithelial type II cells in the alveoli and Club cells (formerly named Clara cells [Winkelmann and Noack, 2010]) in the bronchioles. Interestingly, the comparison of the complete sequences of *Enzootic Nasal Tumour Virus-1* isolated from sheep and of *Enzootic Nasal Tumour Virus-2* isolated from goats (both able to induce transformation of nasal epithelium), revealed that they were closely-related but distinct viruses (Cousens et al., 1999; Ortin et al., 2003; Walsh et al., 2010). While *Enzootic Nasal Tumour Virus-2* establishes a disseminated lymphoid infection, *Enzootic Nasal Tumour Virus -1* is mainly restricted to the tumour (Ortin et al., 2003). The causative relationship between *Enzootic Nasal Tumour Virus-1* and nasal tumour has only been recently demonstrated after reproduction of lesions in the nasal cavity associated with retrovirus particles (Walsh et al., 2013).

3. Cell tropism and tissue specificity

In naturally infected animals, viral DNA can be detected in lymphoid tissues, blood mononuclear cells, *e.g.*, monocytes or B or T lymphocytes and alveolar macrophages (Palmarini et al., 1996; Holland et al., 1999; Garcia-Goti et al., 2000; Gonzalez et al., 2001; Salvatori et al., 2004). The virus burden is higher in adherent mononuclear cells than in non-adherent lymphocytes (Holland et al., 1999). The role of the infection of blood cells remains to be established, but the infection of lymphoid cells precedes the tumour development (Holland et al., 1999; Archer et al., 2012). In infected animals, tumours exclusively occur in the deep lung and affect epithelial cells, *i.e.* alveolar epithelial type II cells in the alveoli and Club cells in the bronchiole (Palmarini et al., 1995;

Table 1
Retroviruses inducing tumours in animals or humans.

Host	Virus name	Genus	Induced tumours
Sheep, goats	*Jaagsiekte Sheep Retrovirus*	beta	Pulmonary adenocarcinoma
	Enzootic Nasal Tumour Virus	beta	Nasal adenocarcinoma
Cattle	*Bovine Leukaemia Virus*	delta	Lymphomas
Cats	*Feline Leukaemia Virus*	gamma	Leukaemia
Chicken	*Avian Leukosis Virus*	alpha	B cell, erythroid or myeloid leucosis
	Rous Sarcoma Virus	alpha	Sarcoma, fibrosarcoma
Fish	*Walleye Dermal Sarcoma Virus*	epsilon	Cutaneous mesenchymal neoplasm
Mice	*Mouse Mammary Tumour Virus*	beta	Mammary adenocarcinomas
	Murine Leukaemia Virus	gamma	B/T cell lymphoma
Captive koalas	*Koala Retrovirus B*	gamma	Leukaemia, lymphoma
Humans	*Human T-lymphotropic virus*	delta	Adult T-cell Leukaemia

Platt et al., 2002; Salvatori et al., 2004). Under *ex vivo* conditions, tumour-derived alveolar epithelial type II cells contain the viral genome and, when polarised in 3D matrix, produced virus particles (Archer et al., 2007).

Viral envelope and long terminal repeat regions are essential determinants for the tropism and the expression of retroviruses. The very first step of the virus replication cycle is the specific interaction between its surface glycoproteins and the cellular receptor present at the cell membrane which is a determining factor for the host range and potentially the nature of induced lesions. Both *Jaagsiekte Sheep RetroVirus* and *Enzootic Nasal Tumour Virus* interact with the mammalian cells through HYAL-2 (hyaluronoglucosaminidase 2) (Rai et al., 2001; Dirks et al., 2002; Miller, 2003), a ubiquitous membrane surface protein that belongs to the hyaluronidases. Hyaluronidases degrade hyaluronan, a major polysaccharide component of the extracellular matrix (Lepperdinger et al., 1998) and are involved in various biological processes, among them development and tumourigenesis. As an example, HYAL-2 is over expressed in colorectal cancers, mainly in advanced stages (Bouga et al., 2010). The hyaluronidase activity of HYAL-2 is not required for its retrovirus receptor function, as demonstrated by inactivating mutations of the catalytic residues, which do not affect virus-receptor interaction (Vigdorovich et al., 2007). The *Jaagsiekte Sheep RetroVirus*-envelope mediated fusion requires a low pH and the cytoplasmic tail of the envelope negatively regulates the fusion activity (Cote et al., 2011). While sharing the same cellular receptor for virus attachment and entry, *Jaagsiekte Sheep RetroVirus* and *Enzootic Nasal Tumour Virus* differ in their entry process. The fusogenicity of *Enzootic Nasal Tumour Virus* is much lower than that of *Jaagsiekte Sheep RetroVirus* and its envelope requires a very acidic pH for fusion activation and cell entry (Cote et al., 2008).

Due to the ubiquitous nature of the HYAL-2 receptor, *Jaagsiekte Sheep RetroVirus* and *Enzootic Nasal Tumour Virus* may thus be able to enter different cell types. However, their active replication is restricted to epithelial cells of nasal cavity and lung parenchyma. Their tropism restriction is then not only controlled by their interaction with the receptor. The expression of *Jaagsiekte Sheep RetroVirus* or *Enzootic Nasal Tumour Virus* envelopes in mouse airway epithelial cells using an adeno-associated virus vector induces tumours in the distal airway, similar to those observed in *Jaagsiekte Sheep RetroVirus*-infected sheep (Wootton et al., 2005, 2006). This strongly suggests that the tissue-specificity of the induced tumours is not mediated by the envelope. The retroviral Long Terminal Repeats (LTRs) contain the viral promoter and enhancer elements interacting with cellular transcription factors; they are specifically activated in cells expressing transcription factors that bind to their enhancer regions. The *Jaagsiekte Sheep RetroVirus* LTRs are active in all airway epithelial cells, while the *Enzootic Nasal Tumour Virus* LTRs are active in the nasal epithelium and alveolar epithelial type II cells, but poorly in tracheal and bronchial epithelial cells (Yu et al., 2011). Several *cis*-regulatory elements have been identified in the long terminal repeats of *Jaagsiekte Sheep RetroVirus* and are important for transcription activity in pulmonary epithelial cells. They include docking sites for Hepatocyte Nuclear Factor 3β (HNF3β), a factor involved in specific regulation of gene expression in lung epithelial cells, C/EBP (CCAAT/Enhancer Binding Protein) and NF-I (Palmarini et al., 2000a; McGee-Estrada et al., 2002; McGee-Estrada et al., 2005; McGee-Estrada and Fan, 2007). In sharp contrast, the critical Hepatocyte Nuclear Factor 3β binding sites are absent in the *Enzootic Nasal Tumour Virus-1* and *Enzootic Nasal Tumour Virus-2* LTRs; consistently, *Enzootic Nasal Tumour Virus-1* LTR has a low activity in lung epithelial cells (McGee-Estrada and Fan, 2007). All the above elements may play a role in localisation of the induced tumours, nasal *versus* pulmonary epithelia.

4. Exogenous *versus* endogenous *Jaagsiekte Sheep RetroVirus* in cancer

As a consequence of the mandatory integration of the retroviral genome (provirus) into the host genome during their replicative cycle, retroviruses exist as exogenous transmissible and related endogenous retroviruses (ERV). These are part of the host heritage and are germinally transmitted to the next generation as Mendelian genes. Endogenous retroviruses derive from rare events of ancestral integration of exogenous viruses into germ line cells, followed by genetic stabilisation through point mutations and/or insertion–deletion. As a consequence of multiple events of retrovirus integration into the germline, endogenous retroviruses represent 18% of the cattle genome and ~8% of the human genome with >500,000 elements (Adelson et al., 2009; Kurth and Bannert, 2010). As in other vertebrates, a family of endogenous retroviruses named endogenous *Jaagsiekte Sheep RetroVirus* (or *enJSRV*s), closely related to the exogenous forms, is present in domestic and wild small ruminants (Hecht et al., 1996; Carlson et al., 2003; DeMartini et al., 2003; Sistiaga-Poveda and Jugo, 2014), but absent from cattle. This suggests that they integrated into the small ruminant genomes before the split between sheep and goats, ~5–7 million years ago, and continued after domestication (Arnaud et al., 2007a; Chessa et al., 2009). Interestingly, the exogenous and endogenous forms of *Jaagsiekte Sheep RetroVirus* are highly related with 90–98% identity at the amino acid level (Palmarini et al., 2000b) and the endogenous *Jaagsiekte Sheep RetroVirus* retained their full genomes integrated into the host genome. While most endogenous retroviruses lost their ability to produce viral proteins or infectious particles, some of them retained functional retroviral proteins. It has been hypothesised that maintenance of the encoding ability of endogenous retroviruses may have important roles, *e.g.*, maintenance of physiological functions during reproduction, pathophysiological negative effects in cancer (Prudhomme et al., 2005; Jern and Coffin, 2008) or protection against exogenous retroviruses at the entry level by inducing cellular resistance to exogenous viruses through receptor interference (Ikeda and Sugimura, 1989; Ponferrada et al., 2003) or during retroviral replication (Pryciak and Varmus, 1992). The endogenous *Jaagsiekte Sheep RetroViruses* are essential for placental development in sheep as shown for independently acquired endogenous retroviruses in humans or rodents (Taruscio and Mantovani, 2004; Prudhomme et al., 2005; Dunlap et al., 2006; Dupressoir et al., 2012; Lavialle et al., 2013). A role, if any, of them in cancer development is not clearly identified. But, they can interfere with infection by the exogenous *Jaagsiekte Sheep RetroViruses* responsible for the disease, both at early and late stages of infection. During the initial step of virus-cell interaction, the envelope of endogenous *Jaagsiekte Sheep RetroVirus* can block the entry of the exogenous retroviruses into cells by receptor interference (Spencer et al., 2003), characterised by competition between the two envelopes for the HYAL-2 receptor (Miller, 2003). Among the endogenous *Jaagsiekte Sheep RetroVirus*, two trans-dominant loci, namely enJSRV56A1 and enJSRV20, have a protective effect at the late stage of infection by a mechanism referred to '*Jaagsiekte Sheep RetroVirus* late restriction' (Mura et al., 2004). These trans-dominant endogenous *Jaagsiekte Sheep RetroVirus* carry a defective substitution at amino-acid position 21 in the Gag precursor protein, in which the arginine (R21) of the replication competent *Jaagsiekte Sheep RetroVirus* is replaced by a tryptophan (W21) and prevent the Gag proteins of the exogenous JSRV to interact with the trafficking cellular machinery, resulting in blockage of the virus release at the plasma membrane (Arnaud et al., 2007b; Murcia et al., 2007). By preventing the release of

exogenous *Jaagsiekte Sheep RetroVirus*, the interfering W21 endogenous viruses may play a role in cancer induction. We have used a fast mutation detection assay, based on the oligo ligation assay, and reported that the expression of mRNA of the W21 *gag* was null or lower in tumoural alveolar epithelial type II cells or lung tissues as compared to non-tumoural samples (Viginier et al., 2012). From our study, it is not possible to conclude that absence of interfering W21 Gag allowed development of cancer in absence of protection against exogenous *Jaagsiekte Sheep RetroVirus*; but the fact that the expression of mRNA of W21 was low or absent in the tumours questioned its role *in vivo*.

5. Molecular mechanisms leading to transformation of the epithelia

Acute and non-acute transforming retroviruses can be identified based on the way they induce tumours (Maeda et al., 2008). Acute transforming retroviruses are replication defective and rapidly induce tumours; they carry viral oncogenes corresponding to captured forms of normal cellular genes or proto-oncogenes acting as positive regulator of cell proliferation. Non-acute retroviruses are replication competent and they promote tumours by their integration in the vicinity of proto-oncogenes, inducing their abnormal activation. A third group of retroviruses induces cell transformation through the properties of their viral proteins, *e.g.*, *Human T-Lymphotropic Virus type 1* with its regulatory tax protein, or through their envelopes, as for *Jaagsiekte Sheep RetroVirus*, *Enzootic Nasal Tumour Virus*, *Friend Spleen Focus-Forming Virus*, *Avian Haemangioma Virus* or *Mouse Mammary Tumour Virus*. While the acute and non-acute retroviruses have been described for decades, the transforming properties of the retroviral envelopes have been reported more recently.

Initially expressed as a 615 amino acid polyprotein anchored to the membrane, the *Jaagsiekte Sheep RetroVirus* envelope is cleaved by cellular furin proteases into surface (SU) and transmembrane (TM) domains linked by disulfide bonds (Hull and Fan, 2006). The surface glycoprotein is expressed at the virus surface and interacts with the cellular receptor, whilst the TM ensures the SU anchoring in the lipid bilayer and the fusion of virus and cell membranes during *Jaagsiekte Sheep RetroVirus* infection. The transmembrane comprises a 44 amino acid intracellular domain or cytoplasmic tail (CT) that is required for *Jaagsiekte Sheep RetroVirus*-induced transformation, as shown by abrogation of its transformation capacity when deleted (Palmarini et al., 2001; Allen et al., 2002; Liu et al., 2003b; Hofacre and Fan, 2004; Hull and Fan, 2006). Oncogenic property of *Jaagsiekte Sheep RetroVirus* envelope has been demonstrated in various cell lines *in vitro*, *e.g.*, human bronchial epithelial cells BEAS-2B (Danilkovitch-Miagkova et al., 2003), rat fibroblasts 208F (Hofacre and Fan, 2004), canine kidney epithelial cells MDCK (Liu and Miller, 2005), rat kidney epithelial cells RK3E (Maeda et al., 2005) or mouse fibroblasts NIH3T3 (Maeda et al., 2001; Palmarini et al., 2001). *In vivo*, oncogenic properties of the envelope have been reported in sheep and mice, using viral vectors bearing *Jaagsiekte Sheep RetroVirus*-envelope (Wootton et al., 2005; Caporale et al., 2006). Similarly, *Enzootic Nasal Tumour Virus*-1 is able to transform fibroblastic and epithelial cell lines (Alberti et al., 2002; Dirks et al., 2002; Liu et al., 2003a) and to induce pulmonary tumours in mice (Wootton et al., 2006). The surface subunit may also play a role in cell transformation, presumably through its interaction with HYAL-2 (Danilkovitch-Miagkova et al., 2003; Hofacre and Fan, 2004). The importance of HYAL-2 remains unclear and might be cell dependent; it plays no role in the transformation of murine cells, but the human HYAL-2 suppresses envelope-mediated transformation by increasing its degradation (Liu et al., 2003a).

Interestingly, the transmembrane glycoprotein of *Jaagsiekte Sheep RetroVirus* contains an YXXM sequence motif that may potentially bind phosphatidylinositol 3-kinase (PI3K) (Palmarini et al., 2001), suggesting that the envelope of *Jaagsiekte Sheep RetroVirus* could transform cells by docking PI3K to the plasma membrane with production of 3′-phosphorylated phosphatidylinositols followed by recruitment and phosphorylation of downstream signaling molecules. The PI3K/Akt/mTOR (phosphatidylinositol 3 Kinase/v-akt murine thymoma viral oncogene homolog/mammalian Target of Rapamycin) pathway is involved in *Jaagsiekte Sheep RetroVirus* - or *Enzootic Nasal Tumour Virus* -induced transformation of various cell lines. This signaling pathway is crucial for cell growth and survival in physiological and pathological situations and is activated by multiple factors, *e.g.*, hormones, growth factors or mutations of components of the tyrosine kinase pathways (Datta et al., 1999; Porta et al., 2014). Activation of the PI3K/Akt pathway disturbs the control of cell growth and survival leading to a growth advantage of deregulated cells. Consistently, activation by phosphorylation of Akt/protein kinase B, a downstream kinase of PI3K, has been reported in *Jaagsiekte Sheep RetroVirus*-transformed cell lines (Palmarini et al., 2001; Liu et al., 2003b; Zavala et al., 2003; Monot et al., 2015). *In vitro*, cell transformation by *Jaagsiekte Sheep RetroVirus* envelope is dependent on Akt phosphorylation through PI3K, as shown by Pi3K inhibitors (Alberti et al., 2002; Zavala et al., 2003; Liu and Miller, 2005). Murine NIH3T3 cells deficient for the PI3K subunit activator of Akt can be transformed by *Jaagsiekte Sheep RetroVirus*; Akt is still activated in these cells, questioning the role of Pi3K in Akt activation (Liu et al., 2003b; Maeda et al., 2003). Overall, the role of PI3K/Akt pathway seems to be cell line dependent, with murine cells depending on Akt activation, while chicken cell lines can be transformed by mutants in the YXXM motifs that fail to activate Akt (Zavala et al., 2003). Interestingly, phosphorylated Akt is not detectable (Zavala et al., 2003) or only in one third of the tested ovine lung adenocarcinomas (Suau et al., 2006). Akt activation is low in alveolar epithelial type II cells derived from tumours, meanwhile these primary cells are insensitive to stimulation by Epidermal Growth Factor, an activator of the PI3K/Akt/mTOR pathway, suggesting the deregulation of this pathway in primary cells infected with *Jaagsiekte Sheep RetroVirus* (Suau et al., 2006; Monot et al., 2015).

Cellular senescence is mainly regulated by telomerase reverse transcriptase, stabilising telomere length. Activation of telomerase reverse transcriptase has been well established in human cancer cell lines and tumours, including lung cancer. The complex regulation of telomerase activity involves several pathways, including the phosphorylation and activation of telomerase reverse transcriptase by Akt. As in most cancers, high level of telomerase activity has been demonstrated in ovine lung tumours, as well as in alveolar epithelial type II cells derived from tumours, suggesting that inhibition of cell senescence participates to cancer progression in small ruminants (Suau et al., 2006). All together, these studies strongly suggest that Akt phosphorylation may participate to cell transformation of some, but not all, cell lines *in vitro* and is not the sole deregulation leading to development and maintenance of tumour cells. Various Hsp90 (Heat shock protein) inhibitors efficiently blocked transformation of *Jaagsiekte Sheep RetroVirus*, partly due to Akt degradation and Hsp90 inhibitors specifically inhibited proliferation of immortalised and primary cells derived from ovine lung tumours (Varela et al., 2007). Many viruses, among them *Epstein-Barr Virus*, papilloma viruses, polyoma viruses, *Human Herpesvirus 8*, *Hepatitis B Virus*, *Hepatitis C Virus*, *Human Immunodeficiency Viruses*, cytomegaloviruses, *Respiratory Syncytial Virus* or *Rubella Virus*, require up-regulation of the Pi3K/Akt pathway to sustain long-standing infections and to create a favorable environment for cell transformation or virus replication (Cooray, 2004).

The Ras/MEK/MAPK pathway (Ras/Mitogen Extracellular regulated kinase/Mitogen activated protein kinase) is involved in transformation of cell lines by *Jaagsiekte Sheep RetroVirus* (Maeda et al., 2005). Ras activation results in phosphorylation of MAPKKK (as Raf), which in turn phosphorylates MAPKK (as MEK 1/2), which, ultimately, phosphorylates MAPK (as ERK 1/2). The activated MAPKs are translocated into the nucleus, where they phosphorylate and activate transcription factors leading to the activation of genes involved in proliferation, cell differentiation and apoptosis. This pathway has been shown to be important for transformation by *Jaagsiekte Sheep RetroVirus* envelope in some cell lines (Maeda et al., 2005; Hull and Fan, 2006). Proteins of the Erk1/2 pathway (Raf-1, Mek1/2, p44/42MAPK) are activated in natural cases of lung or nasal adenocarcinomas in small ruminants and may be important for *in vivo* transformation (De Las Heras et al., 2006).

The role of *Jaagsiekte Sheep RetroVirus* envelope in cell transformation has attracted considerable attention in the past years, but still more needs to be done to decipher the early steps of envelope-mediated cell transformation and to identify cellular proteins able to interact with the envelope during the very early steps in the natural cell targets. In cell culture assays, *Jaagsiekte Sheep RetroVirus* envelope clearly activates a number of proteins involved in signaling cascades controlling cell growth and fate. Of importance, the proteins involved appear to be dependent of the type and origin of the cell system used *in vitro*, emphasing the need to control the candidates in the natural targets of the virus, such as alveolar epithelial type II cells (Suau et al., 2006). Even if they are not easy to manipulate, these cells, as well as bronchioloalveolar progenitors, can be generated from ovine lungs tissues (Archer et al., 2007, 2013) and are valuable tools to understand the pathological situation in the animal. The identification of cellular partners of the JSRV envelope remains crucial for deciphering mechanisms leading to cell transformation. We recently reported RALBP1 (RalA binding protein 1; also known as RLIP76 or RIP), a cellular protein implicated in the *ras* pathway, as a partner of JSRV Env and confirmed formation of RALBP1/Env complexes in mammalian cells (Monot et al., 2015). Expression of the RALBP1 protein was repressed in tumoral lungs and in tumor-derived alveolar type II cells. Through its inhibition using specific small interfering RNA (siRNA), we demonstrated that RALBP1 was involved in envelope-induced cell transformation and in modulation of the mTOR (mammalian target of rapamycin)/p70S6K pathway (Monot et al., 2015). Finally, other mechanisms leading to cell transformation, such as targeted integration of the exogenous *Jaagsiekte Sheep RetroVirus* cannot be totally ruled out (Cousens et al., 2004).

6. Ovine pulmonary adenocarcinoma and related human lung cancer

Worldwide, lung cancer is the leading cause of cancer death in humans, with around 1.5 million deaths annually, *i.e.* ~20% of the total number of cancer deaths. Only few animal models are available for its study, mainly developed in rodents. Despite development of new target-specific drugs, less than 15% of patients with lung cancer achieve a 5-year survival. Of the cases of lung cancers, 80% are 'non-small cell lung cancer'; among them adenocarcinomas are the most frequent type, accounting for 40% of all cases of lung cancers. The human lepidic pulmonary adenocarcinoma (formerly referred to as bronchioloalveolar cancer) is a rare form of lung adenocarcinoma redefined on the latest World Health Organisation classification of lung adenocarcinoma (Travis et al., 2011; Van Schil et al., 2013). By its peculiar presentation and its epidemiology, it has always intrigued chest physicians and pathologists. The lepidic spread used to define these tumours refers to the bronchioloalveolar growth pattern with tumour cells lining the alveolar septa without evidence of stromal, vascular or pleural invasion. Lepidic adenocarcinomas are clinically associated with highly productive cough and progressive restrictive respiratory failure (Mornex et al., 2003; Wislez et al., 2005). The tumour is slow-growing with rare metastatic spread. Lepidic adenocarcinomas share striking clinical, radiological and histopathological similarities with ovine pulmonary adenocarcinomas induced by *Jaagsiekte Sheep RetroVirus* (Mornex et al., 2003). The role of a virus in the induction of this human cancer has been hypothesised for many years; as early as in 1954, chest physicians questioned the potential implication of a virus (Dufourt et al., 1954). There is growing evidence about the implication of infectious agents in induction of cancer in humans (Gatza et al., 2005). Could a virus, possibly related to *Jaagsiekte Sheep RetroVirus*, be at the origin of human lepidic pulmonary adenocarcinomas? As for other hypotheses regarding the implication of retroviruses in human cancers, *e.g. Mouse Mammary Tumour Virus* in breast cancers in women, the question is still open and quite controversial, nevertheless we should carefully considered the following reports.

Despite some evolution in the treatment, such as administration of tyrosine kinase inhibitor of the of Epidermal Growth Factor Receptor, the lepidic pulmonary adenocarcinoma still has a poor prognosis, with a 5 year survival less than 10%. Cancer is generally considered to be a contra-indication for transplantation, but the local extension of lepidic pulmonary adenocarcinoma makes the affected patients eligible for lung transplantation. In 1991, the first double-lung transplantation has been reported and the patient survived for 5.5 years with no cancer relapse (Etienne et al., 1997). In 2014, among the fifteen reported cases by the 'International Society for Heart and Lung Transplantation', recurrence limited to the transplanted lung occurred in over 50% of cases with a median occurrence of 30.1 months (Garver et al., 1999; Paloyan et al., 2000; de Perrot et al., 2004; Avrillon et al., 2012). Microsatellite analyses of lung specimens of donors and recipients have shown that recurring tumours originated from the transplant recipient (Garver et al., 1999; Paloyan et al., 2000). Several hypotheses have been proposed to explain these relapses: peri-surgical contamination with aerogenous diffusion of tumoural cells, latent tumoural cells in extra pulmonary sites, deregulated bronchiolalveolar stem cells able to relocate and differentiate into the transplanted lungs or infection-reinfection with an infectious agent with an extra-pulmonary preclinical reservoir.

We have conducted a case-control study on 44 cancer patients and 132 healthy individuals from 11 French university hospitals to determine whether chronic exposure to domestic small ruminants may increase the risk factor of lepidic pulmonary adenocarcinomas compared to other lung cancers (Lutringer-Magnin et al., 2012). Data were collected using a detailed 356-item questionnaire, addressing frequency, type (professional or leisure) and age-period (0–12, 13–20, 21–40, >40 year-old) of contacts, as well as type of domestic animals the patient was exposed to. By using multivariate analysis, lepidic adenocarcinoma was associated significantly more frequently with female gender, 'never-smoker' status, personal history of extra-thoracic cancer and professional exposure to goats with a 5.09 odds ratio, as compared to other subtypes of lung cancer (Lutringer-Magnin et al., 2012). Our epidemiological study did not address viral infection, but the observed risk with professional exposure to goats might, possibly, be linked to presence of oncogenic retroviruses in goats.

Several reports have pointed to a possible interaction between the *Jaagsiekte Sheep RetroVirus* or *Enzootic Nasal Tumour Virus* and the human cells. As shown by immunostaining on lung slices using specific antibodies against *Jaagsiekte Sheep RetroVirus*, viral antigens might be expressed in one third of human lepidic adenocarcinomas, as well as in other types of lung adenocarcinomas (De las Heras et al., 2000; Linnerth-Petrik et al., 2014). JSRV genome has been detected in tissues from lepidic

adenocarcinomas from Sardinian patients (Rocca et al., 2008), as well as in the blood of African individuals and cancer patients (Morozov et al., 2004; Linnerth-Petrik et al., 2014). Nevertheless, other studies using specific PCR-based analyses failed to detect any exogenous or endogenous *Jaagsiekte Sheep RetroVirus* or beta retrovirus related sequences in human adenocarcinoma (Yousem et al., 2001). The HYAL-2 ubiquitous receptor for envelopes of both *Jaagsiekte Sheep RetroVirus* and *Enzootic Nasal Tumour Virus* is expressed at the surface of human cells and is able to mediate infection by retroviral vectors pseudotyped with the *Jaagsiekte Sheep RetroVirus* or *Enzootic Nasal Tumour Virus*-envelope (Rai et al., 2000).

From these studies, it is not possible to conclude to a zoonotic agent related to *Jaagsiekte Sheep RetroVirus*, responsible for the induction of this rare human cancer. If *Jaagsiekte Sheep RetroVirus* is not implicated, a related known or unknown retrovirus might be involved. In a study conducted in Japan, *Human T-Lymphotropic Virus type 1* has been proposed as a risk factor for lepidic adenocarcinoma in humans; prevalence of lepidic adenocarcinoma in individuals infected with the *Human T-Lymphotropic Virus type 1* was significantly higher than in negative patients (Nomori et al., 2011). To note that this virus is almost exclusively present in Japan and the Caribbean and that the observed link may be associated to its frequency among the Japanese population (Watanabe, 2011)

Up to date, there is no conclusive answer to the possible link between *Jaagsiekte Sheep RetroVirus* or *Enzootic Nasal Tumour Virus* or a related retrovirus and the development of lepidic adenocarcinomas in humans. The reported results should be considered as hypothesis-generating and prompt us for further epidemiological studies in a more selected population, living in the countryside, possibly in *Jaagsiekte Sheep RetroVirus* or *Enzootic Nasal Tumour Virus* endemic regions and working with domestic small ruminants with a precise quantification of animal exposure.

7. Concluding remarks

In conclusion, *Jaagsiekte Sheep RetroVirus* and *Enzootic Nasal Tumour Virus* induce cancers in animals and are of importance in animal health. Even if the role of the oncogenic envelope has been largely studied in cell lines, much needs to be done to elucidate the initial steps leading to the tumour induction *in vivo*. Development of *ex vivo* models as close as possible to the lung of infected animals are of great importance and tools for future discoveries.

Besides the economic impact of these infections in the field, their potential link with human cancers should be fully explored. The identification of an infectious agent associated with the development of lepidic pulmonary adenocarcinomas may open new therapeutic approaches and have an impact for patient care. As in the past in the history of retrovirology, *Jaagsiekte Sheep RetroVirus* and *Enzootic Nasal Tumour Virus* could help us to decipher new and original routes in cancer biology.

Conflict of interest

The authors declare no conflict of interest.

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References

Adelson, D.L., Raison, J.M., Edgar, R.C., 2009. Characterization and distribution of retrotransposons and simple sequence repeats in the bovine genome. Proc. Natl. Acad. Sci. U. S. A. 106, 12855–12860.

Alberti, A., Murgia, C., Liu, S.L., Mura, M., Cousens, C., Sharp, M., Miller, A.D., Palmarini, M., 2002. Envelope-induced cell transformation by ovine betaretroviruses. J. Virol. 76, 5387–5394.

Allen, T.E., Sherrill, K.J., Crispell, S.M., Perrott, M.R., Carlson, J.O., DeMartini, J.C., 2002. The jaagsiekte sheep retrovirus envelope gene induces transformation of the avian fibroblast cell line DF-1 but does not require a conserved SH2 binding domain. J. Gen. Virol. 83, 2733–2742.

Archer, F., Abi-Rizk, A., Desloire, S., Dolmazon, C., Gineys, B., Guiguen, F., Cottin, V., Mornex, J.F., Leroux, C., 2013. Lung progenitors from lambs can differentiate into specialized alveolar or bronchiolar epithelial cells. BMC Vet. Res. 9, 224.

Archer, F., Jacquier, E., Lyon, M., Chastang, J., Cottin, V., Mornex, J.F., Leroux, C., 2007. Alveolar type II cells isolated from pulmonary adenocarcinoma: a model for JSRV expression in vitro. Am. J. Respir. Cell Mol. Biol. 36, 534–540.

Archer, F., Mornex, J.F., Leroux, C., 2012. Interplay between JSRV, an oncogenic retrovirus, and the pulmonary epithelium. Curr. Immunol. Rev. 7, 209–215.

Arnaud, F., Caporale, M., Varela, M., Biek, R., Chessa, B., Alberti, A., Golder, M., Mura, M., Zhang, Y.P., Yu, L., Pereira, F., Demartini, J.C., Leymaster, K., Spencer, T.E., Palmarini, M., 2007a. A paradigm for virus-host coevolution: sequential counter-adaptations between endogenous and exogenous retroviruses. PLoS Pathog. 3, e170.

Arnaud, F., Murcia, P.R., Palmarini, M., 2007b. Mechanisms of late restriction induced by an endogenous retrovirus. J. Virol. 81, 11441–11451.

Avrillon, V., Philit, F., Chalabreysse, L., Chumbi-Flores, R.W., Tronc, F., Thivolet-Bejui, F., Mornex, J.F., 2012. Bronchiolo-alveolar carcinoma and lung transplantation. Rev. Mal. Respir. 29, 84–88.

Bouga, H., Tsouros, I., Bounias, D., Kyriakopoulou, D., Stavropoulos, M.S., Papageorgakopoulou, N., Theocharis, D.A., Vynios, D.H., 2010. Involvement of hyaluronidases in colorectal cancer. BMC Cancer 10, 499.

Caporale, M., Centorame, P., Giovannini, A., Sacchini, F., Di Ventura, M., De las Heras, M., Palmarini, M., 2005. Infection of lung epithelial cells and induction of pulmonary adenocarcinoma is not the most common outcome of naturally occurring JSRV infection during the commercial lifespan of sheep. Virology 338, 144–153.

Caporale, M., Cousens, C., Centorame, P., Pinoni, C., De las Heras, M., Palmarini, M., 2006. Expression of the jaagsiekte sheep retrovirus envelope glycoprotein is sufficient to induce lung tumors in sheep. J. Virol. 80, 8030–8037.

Carlson, J., Lyon, M., Bishop, J., Vaiman, A., Cribiu, E., Mornex, J.F., Brown, S., Knudson, D., DeMartini, J., Leroux, C., 2003. Chromosomal distribution of endogenous Jaagsiekte sheep retrovirus proviral sequences in the sheep genome. J. Virol. 77, 9662–9668.

Chessa, B., Pereira, F., Arnaud, F., Amorim, A., Goyache, F., Mainland, I., Kao, R.R., Pemberton, J.M., Beraldi, D., Stear, M.J., Alberti, A., Pittau, M., Iannuzzi, L., Banabazi, M.H., Kazwala, R.R., Zhang, Y.P., Arranz, J.J., Ali, B.A., Wang, Z., Uzun, M., Dione, M.M., Olsaker, I., Holm, L.E., Saarma, U., Ahmad, S., Marzanov, N., Eythorsdottir, E., Holland, M.J., Ajmone-Marsan, P., Bruford, M.W., Kantanen, J., Spencer, T.E., Palmarini, M., 2009. Revealing the history of sheep domestication using retrovirus integrations. Science 324, 532–536.

Cooray, S., 2004. The pivotal role of phosphatidylinositol 3-kinase-Akt signal transduction in virus survival. J. Gen. Virol. 85, 1065–1076.

Cote, M., Kucharski, T.J., Liu, S.L., 2008. Enzootic nasal tumor virus envelope requires a very acidic pH for fusion activation and infection. J. Virol. 82, 9023–9034.

Cote, M., Zheng, Y.M., Albritton, L.M., Liu, S.L., 2011. Single residues in the surface subunits of oncogenic sheep retrovirus envelopes distinguish receptor-mediated triggering for fusion at low pH and infection. Virology 421, 173–183.

Cousens, C., Bishop, J.V., Philbey, A.W., Gill, C.A., Palmarini, M., Carlson, J.O., DeMartini, J.C., Sharp, J.M., 2004. Analysis of integration sites of Jaagsiekte sheep retrovirus in ovine pulmonary adenocarcinoma. J. Virol. 78, 8506–8512.

Cousens, C., Minguijon, E., Dalziel, R.G., Ortin, A., Garcia, M., Park, J., Gonzalez, L., Sharp, J.M., de las Heras, M., 1999. Complete sequence of enzootic nasal tumor virus, a retrovirus associated with transmissible intranasal tumors of sheep. J. Virol. 73, 3986–3993.

Danilkovitch-Miagkova, A., Duh, F.M., Kuzmin, I., Angeloni, D., Liu, S.L., Miller, A.D., Lerman, M.I., 2003. Hyaluronidase 2 negatively regulates RON receptor tyrosine kinase and mediates transformation of epithelial cells by jaagsiekte sheep retrovirus. Proc. Natl. Acad. Sci. U. S. A. 100, 4580–4585.

Datta, S.R., Brunet, A., Greenberg, M.E., 1999. Cellular survival: a play in three Akts. Genes Dev. 13, 2905–2927.

De las Heras, M., Barsky, S.H., Hasleton, P., Wagner, M., Larson, E., Egan, J., Ortin, A., Gimenez-Mas, J.A., Palmarini, M., Sharp, J.M., 2000. Evidence for a protein related immunologically to the jaagsiekte sheep retrovirus in some human lung tumours. Eur. Respir. J. 16, 330–332.

De Las Heras, M., Ortin, A., Benito, A., Summers, C., Ferrer, L.M., Sharp, J.M., 2006. In-situ demonstration of mitogen-activated protein kinase Erk 1/2 signalling pathway in contagious respiratory tumours of sheep and goats. J. Comp. Pathol. 135, 1–10.

de Perrot, M., Chernenko, S., Waddell, T.K., Shargall, Y., Pierre, A.F., Hutcheon, M., Keshavjee, S., 2004. Role of lung transplantation in the treatment of bronchogenic carcinomas for patients with end-stage pulmonary disease. J. Clin. Oncol. 22, 4351–4356.

DeMartini, J.C., Carlson, J.O., Leroux, C., Spencer, T., Palmarini, M., 2003. Endogenous retroviruses related to jaagsiekte sheep retrovirus. Curr. Top. Microbiol. Immunol. 275, 117–137.

Dirks, C., Duh, F.M., Rai, S.K., Lerman, M.I., Miller, A.D., 2002. Mechanism of cell entry and transformation by enzootic nasal tumor virus. J. Virol. 76, 2141–2149.

Dufourt, A., Santy, P., Galy, P., Touraine, R.G.R., 1954. Adenomatosis et soi-disant cancer alvéolaire. Les proliférations épithéliales intra-alvéolaires de type bronchique. Sem. Hôp. (Paris) 30, 1209.
Dungal, N., 1938. Epizootic adenomatosis of the lungs of sheep: its relation to verminous pneumonia and Jaagsiekte. Proc. R. Soc. Med. 31, 497–505.
Dunlap, K.A., Palmarini, M., Spencer, T.E., 2006. Ovine endogenous betaretroviruses (enJSRVs) and placental morphogenesis. Placenta 27 (Suppl. A), S135–S140.
Dupressoir, A., Lavialle, C., Heidmann, T., 2012. From ancestral infectious retroviruses to bona fide cellular genes: role of the captured syncytins in placentation. Placenta 33, 663–671.
Etienne, B., Bertocchi, M., Gamondes, J.P., Wiesendanger, T., Brune, J., Mornex, J.F., 1997. Successful double-lung transplantation for bronchioalveolar carcinoma. Chest 112, 1423–1424.
Garcia-Goti, M., Gonzalez, L., Cousens, C., Cortabarria, N., Extramiana, A.B., Minguijon, E., Ortin, A., De las Heras, M., Sharp, J.M., 2000. Sheep pulmonary adenomatosis: characterization of two pathological forms associated with jaagsiekte retrovirus. J. Comp. Pathol. 122, 55–65.
Garver Jr., R.I., Zorn, G.L., Wu, X., McGiffin, D.C., Young Jr., K.R., Pinkard, N.B., 1999. Recurrence of bronchioloalveolar carcinoma in transplanted lungs. N. Engl. J. Med. 340, 1071–1074.
Gatza, M.L., Chandhasin, C., Ducu, R.I., Marriott, S.J., 2005. Impact of transforming viruses on cellular mutagenesis, genome stability, and cellular transformation. Environ. Mol. Mutagen. 45, 304–325.
Gonzalez, L., Garcia-Goti, M., Cousens, C., Dewar, P., Cortabarria, N., Extramiana, A.B., Ortin, A., De Las Heras, M., Sharp, J.M., 2001. Jaagsiekte sheep retrovirus can be detected in the peripheral blood during the pre-clinical period of sheep pulmonary adenomatosis. J. Gen. Virol. 82, 1355–1358.
Grego, E., De Meneghi, D., Alvarez, V., Benito, A.A., Minguijon, E., Ortin, A., Mattoni, M., Moreno, B., Perez de Villarreal, M., Alberti, A., Capucchio, M.T., Caporale, M., Juste, R., Rosati, S., De las Heras, M., 2008. Colostrum and milk can transmit jaagsiekte retrovirus to lambs. Vet. Microbiol. 130, 247–257.
Hecht, S.J., Stedman, K.E., Carlson, J.O., DeMartini, J.C., 1996. Distribution of endogenous type B and type D sheep retrovirus sequences in ungulates and other mammals. Proc. Natl. Acad. Sci. U. S. A. 93, 3297–3302.
Hofacre, A., Fan, H., 2004. Multiple domains of the Jaagsiekte sheep retrovirus envelope protein are required for transformation of rodent fibroblasts. J. Virol. 78, 10479–10489.
Holland, M.J., Palmarini, M., Garcia-Goti, M., Gonzalez, L., McKendrick, I., de las Heras, M., Sharp, J.M., 1999. Jaagsiekte retrovirus is widely distributed both in T and B lymphocytes and in mononuclear phagocytes of sheep with naturally and experimentally acquired pulmonary adenomatosis. J. Virol. 73, 4004–4008.
Hull, S., Fan, H., 2006. Mutational analysis of the cytoplasmic tail of jaagsiekte sheep retrovirus envelope protein. J. Virol. 80, 8069–8080.
Ikeda, H., Sugimura, H., 1989. Fv-4 resistance gene: a truncated endogenous murine leukemia virus with ecotropic interference properties. J. Virol. 63, 5405–5412.
Jern, P., Coffin, J.M., 2008. Effects of retroviruses on host genome function. Annu. Rev. Genet. 42, 709–732.
Kurth, R., Bannert, N., 2010. Beneficial and detrimental effects of human endogenous retroviruses. Int. J. Cancer 126, 306–314.
Lavialle, C., Cornelis, G., Dupressoir, A., Esnault, C., Heidmann, O., Vernochet, C., Heidmann, T., 2013. Paleovirology of 'syncytins', retroviral env genes exapted for a role in placentation. Philos. Trans. R. Soc. Lond. B Biol. Sci. 368 20120507.
Lepperdinger, G., Strobl, B., Kreil, G., 1998. HYAL2, a human gene expressed in many cells, encodes a lysosomal hyaluronidase with a novel type of specificity. J. Biol. Chem. 273, 22466–22470.
Leroux, C., Cruz, J.C., Mornex, J.F., 2010. SRLVs: a genetic continuum of lentiviral species in sheep and goats with cumulative evidence of cross species transmission. Curr. HIV Res. 8, 94–100.
Leroux, C., Mornex, J.F., 2008. Retroviral infections in sheep and the associated diseases. Small Rumin. Res. 76, 68–76.
Leroux, C., Vuillermoz, S., Mornex, J.F., Greenland, T., 1995. Genomic heterogeneity in the pol region of ovine lentiviruses obtained from bronchoalveolar cells of infected sheep from France. J. Gen. Virol. 76, 1533–1537.
Linnerth-Petrik, N.M., Walsh, S.R., Bogner, P.N., Morrison, C., Wootton, S.K., 2014. Jaagsiekte sheep retrovirus detected in human lung cancer tissue arrays. BMC Res. Notes 7, 160.
Liu, S.L., Duh, F.M., Lerman, M.I., Miller, A.D., 2003a. Role of virus receptor Hyal2 in oncogenic transformation of rodent fibroblasts by sheep betaretrovirus env proteins. J. Virol. 77, 2850–2858.
Liu, S.L., Lerman, M.I., Miller, A.D., 2003b. Putative phosphatidylinositol 3-kinase (PI3K) binding motifs in ovine betaretrovirus Env proteins are not essential for rodent fibroblast transformation and PI3K/Akt activation. J. Virol. 77, 7924–7935.
Liu, S.L., Miller, A.D., 2005. Transformation of madin-darby canine kidney epithelial cells by sheep retrovirus envelope proteins. J. Virol. 79, 927–933.
Lutringer-Magnin, D., Girard, N., Cadranel, J., Leroux, C., Quoix, E., Cottin, V., Signore, C.D., Lebitasy, M.P., Cordier, G., Vanhems, P., Mornex, J.F., 2012. Professional exposure to goats increases the risk of pneumonic-type lung adenocarcinoma: results of the IFCT-0504-epidemio study. PLoS One 7, e37889.
Maeda, N., Fan, H., Yoshikai, Y., 2008. Oncogenesis by retroviruses: old and new paradigms. Rev. Med. Virol. 18, 387–405.
Maeda, N., Fu, W., Ortin, A., de las Heras, M., Fan, H., 2005. Roles of the Ras-MEK-mitogen-activated protein kinase and phosphatidylinositol 3-kinase-Akt-mTOR pathways in Jaagsiekte sheep retrovirus-induced transformation of rodent fibroblast and epithelial cell lines. J. Virol. 79, 4440–4450.
Maeda, N., Inoshima, Y., Fruman, D.A., Brachmann, S.M., Fan, H., 2003. Transformation of mouse fibroblasts by Jaagsiekte sheep retrovirus envelope does not require phosphatidylinositol 3-kinase. J. Virol. 77, 9951–9959.
Maeda, N., Palmarini, M., Murgia, C., Fan, H., 2001. Direct transformation of rodent fibroblasts by jaagsiekte sheep retrovirus DNA. Proc. Natl. Acad. Sci. U. S. A. 98, 4449–4454.
Martin, W.B., Scott, F.M., Sharp, J.M., Angus, K.W., Norval, M., 1976. Experimental production of sheep pulmonary adenomatosis (Jaagsiekte). Nature 264, 183–185.
McGee-Estrada, K., Fan, H., 2007. Comparison of LTR enhancer elements in sheep betaretroviruses: insights into the basis for tissue-specific expression. Virus Genes 35, 303–312.
McGee-Estrada, K., Palmarini, M., Fan, H., 2002. HNF-3beta is a critical factor for the expression of the jaagsiekte sheep retrovirus long terminal repeat in type II pneumocytes but not in clara cells. Virology 292, 87–97.
McGee-Estrada, K., Palmarini, M., Hallwirth, C., Fan, H., 2005. A Moloney murine leukemia virus driven by the Jaagsiekte sheep retrovirus enhancers shows enhanced specificity for infectivity in lung epithelial cells. Virus Genes 31, 257–263.
Miller, A.D., 2003. Identification of Hyal2 as the cell-surface receptor for jaagsiekte sheep retrovirus and ovine nasal adenocarcinoma virus. Curr. Top. Microbiol. Immunol. 275, 179–199.
Monot, M., Erny, A., Gineys, B., Desloire, S., Dolmazon, C., Aublin-Gex, A., Lotteau, V., Archer, F., Leroux, C., 2015. Early steps of Jaagsiekte sheep retrovirus-mediated cell transformation involve the interaction between env and the RALBP1cellular protein. J. Virol. 89, 8462–8473.
Mornex, J.F., Thivolet, F., De las Heras, M., Leroux, C., 2003. Pathology of human bronchioloalveolar carcinoma and its relationship to the ovine disease. Curr. Top. Microbiol. Immunol. 275, 225–248.
Morozov, V.A., Lagaye, S., Lower, J., Lower, R., 2004. Detection and characterization of betaretroviral sequences, related to sheep Jaagsiekte virus, in Africans from Nigeria and Cameroon. Virology 327, 162–168.
Mura, M., Murcia, P., Caporale, M., Spencer, T.E., Nagashima, K., Rein, A., Palmarini, M., 2004. Late viral interference induced by transdominant Gag of an endogenous retrovirus. Proc. Natl. Acad. Sci. U. S. A. 101, 11117–11122.
Murcia, P.R., Arnaud, F., Palmarini, M., 2007. The transdominant endogenous retrovirus enJS56A1 associates with and blocks intracellular trafficking of Jaagsiekte sheep retrovirus Gag. J. Virol. 81, 1762–1772.
Nomori, H., Mori, T., Iyama, K., Okamoto, T., Kamakura, M., 2011. Risk of bronchioloalveolar carcinoma in patients with human T-cell lymphotropic virus type 1 (HTLV-I): case-control study results. Ann. Thorac. Cardiovasc. Surg. 17, 19–23.
Ortin, A., Cousens, C., Minguijon, E., Pascual, Z., Villarreal, M.P., Sharp, J.M., de las Heras, M., 2003. Characterization of enzootic nasal tumour virus of goats: complete sequence and tissue distribution. J. Gen. Virol. 84, 2245–2252.
Ortin, A., Perez de Villarreal, M., Minguijon, E., Cousens, C., Sharp, J.M., De las Heras, M., 2004. Coexistence of enzootic nasal adenocarcinoma and jaagsiekte retrovirus infection in sheep. J. Comp. Pathol. 131, 253–258.
Palmarini, M., Datta, S., Omid, R., Murgia, C., Fan, H., 2000a. The long terminal repeat of jaagsiekte sheep retrovirus is preferentially active in differentiated epithelial cells of the lungs. J. Virol. 74, 5776–5787.
Palmarini, M., Dewar, P., De las Heras, M., Inglis, N.F., Dalziel, R.G., Sharp, J.M., 1995. Epithelial tumour cells in the lungs of sheep with pulmonary adenomatosis are major sites of replication for Jaagsiekte retrovirus. J. Gen. Virol. 76, 2731–2737.
Palmarini, M., Hallwirth, C., York, D., Murgia, C., de Oliveira, T., Spencer, T., Fan, H., 2000b. Molecular cloning and functional analysis of three type D endogenous retroviruses of sheep reveal a different cell tropism from that of the highly related exogenous jaagsiekte sheep retrovirus. J. Virol. 74, 8065–8076.
Palmarini, M., Holland, M.J., Cousens, C., Dalziel, R.G., Sharp, J.M., 1996. Jaagsiekte retrovirus establishes a disseminated infection of the lymphoid tissues of sheep affected by pulmonary adenomatosis. J. Gen. Virol. 77, 2991–2998.
Palmarini, M., Maeda, N., Murgia, C., De-Fraja, C., Hofacre, A., Fan, H., 2001. A phosphatidylinositol 3-kinase docking site in the cytoplasmic tail of the jaagsiekte sheep retrovirus transmembrane protein is essential for envelope-induced transformation of nih 3t3 cells. J. Virol. 75, 11002–11009.
Palmarini, M., Sharp, J.M., de las Heras, M., Fan, H., 1999. Jaagsiekte sheep retrovirus is necessary and sufficient to induce a contagious lung cancer in sheep. J. Virol. 73, 6964–6972.
Paloyan, E.B., Swinnen, L.J., Montoya, A., Lonchyna, V., Sullivan, H.J., Garrity, E., 2000. Lung transplantation for advanced bronchioloalveolar carcinoma confined to the lungs. Transplantation 69, 2446–2448.
Perk, K., Michalides, R., Spiegelman, S., Schlom, J., 1974. Biochemical and morphologic evidence for the presence of an RNA tumor virus in pulmonary carcinoma of sheep (Jaagsiekte). J. Natl. Cancer Inst. 53, 131–135.
Platt, J.A., Kraipowich, N., Villafane, F., DeMartini, J.C., 2002. Alveolar type II cells expressing jaagsiekte sheep retrovirus capsid protein and surfactant proteins are the predominant neoplastic cell type in ovine pulmonary adenocarcinoma. Vet. Pathol. 39, 341–352.
Ponferrada, V.G., Mauck, B.S., Wooley, D.P., 2003. The envelope glycoprotein of human endogenous retrovirus HERV-W induces cellular resistance to spleen necrosis virus. Arch. Virol. 148, 659–675.
Porta, C., Paglino, C., Mosca, A., 2014. Targeting PI3K/Akt/mTOR signaling in cancer. Front. Oncol. 4, 64.
Prudhomme, S., Bonnaud, B., Mallet, F., 2005. Endogenous retroviruses and animal reproduction. Cytogenet. Genome Res. 110, 353–364.

Pryciak, P.M., Varmus, H.E., 1992. Fv-1 restriction and its effects on murine leukemia virus integration *in vivo* and *in vitro*. J. Virol. 66, 5959–5966.
Rai, S.K., DeMartini, J.C., Miller, A.D., 2000. Retrovirus vectors bearing jaagsiekte sheep retrovirus Env transduce human cells by using a new receptor localized to chromosome 3p21.3. J. Virol. 74, 4698–4704.
Rai, S.K., Duh, F.M., Vigdorovich, V., Danilkovitch-Miagkova, A., Lerman, M.I., Miller, A.D., 2001. Candidate tumor suppressor HYAL2 is a glycosylphosphatidylinositol (GPI)-anchored cell-surface receptor for jaagsiekte sheep retrovirus, the envelope protein of which mediates oncogenic transformation. Proc. Natl. Acad. Sci. U. S. A. 98, 4443–4448.
Rocca, S., Sanna, M.P., Leoni, A., Cossu, A., Lissia, A., Tanda, F., Satta, M.P., Palmieri, G., 2008. Presence of Jaagsiekte sheep retrovirus in tissue sections from human bronchioloalveolar carcinoma depends on patients' geographical origin. Hum. Pathol. 39, 303–304.
Rous, P., 1910. A transmissible avian neoplasm. (Sarcoma of the common fowl.). J. Exp. Med. 12, 696–705.
Rous, P., 1911. A sarcoma of the fowl transmissible by and agents separable from the tumor cells. J. Exp. Med. 13, 397.
Salvatori, D., Gonzalez, L., Dewar, P., Cousens, C., de las Heras, M., Dalziel, R.G., Sharp, J.M., 2004. Successful induction of ovine pulmonary adenocarcinoma in lambs of different ages and detection of viraemia during the preclinical period. J. Gen. Virol. 85, 3319–3324.
Sharp, J.M., Angus, K.W., Gray, E.W., Scott, F.M., 1983. Rapid transmission of sheep pulmonary adenomatosis (jaagsiekte) in young lambs. Brief report. Arch. Virol. 78, 89–95.
Sharp, J.M., DeMartini, J.C., 2003. Natural history of JSRV in sheep. Curr. Top. Microbiol. Immunol. 275, 55–79.
Sistiaga-Poveda, M., Jugo, B.M., 2014. Evolutionary dynamics of endogenous Jaagsiekte sheep retroviruses proliferation in the domestic sheep, mouflon and *Pyrenean chamois*. Heredity 112, 571–578.
Spencer, T.E., Mura, M., Gray, C.A., Griebel, P.J., Palmarini, M., 2003. Receptor usage and fetal expression of ovine endogenous betaretroviruses: implications for coevolution of endogenous and exogenous retroviruses. J. Virol. 77, 749–753.
Suau, F., Cottin, V., Archer, F., Croze, S., Chastang, J., Cordier, G., Thivolet-Bejui, F., Mornex, J.F., Leroux, C., 2006. Telomerase activation in a model of lung adenocarcinoma. Eur. Respir. J. 27, 1175–1182.
Taruscio, D., Mantovani, A., 2004. Factors regulating endogenous retroviral sequences in human and mouse. Cytogenet. Genome Res. 105, 351–362.
Travis, W.D., Brambilla, E., Noguchi, M., Nicholson, A.G., Geisinger, K.R., Yatabe, Y., Beer, D.G., Powell, C.A., Riely, G.J., Van Schil, P.E., Garg, K., Austin, J.H., Asamura, H., Rusch, V.W., Hirsch, F.R., Scagliotti, G., Mitsudomi, T., Huber, R.M., Ishikawa, Y., Jett, J., Sanchez-Cespedes, M., Sculier, J.P., Takahashi, T., Tsuboi, M., Vansteenkiste, J., Wistuba, I., Yang, P.C., Aberle, D., Brambilla, C., Flieder, D., Franklin, W., Gazdar, A., Gould, M., Hasleton, P., Henderson, D., Johnson, B., Johnson, D., Kerr, K., Kuriyama, K., Lee, J.S., Miller, V.A., Petersen, I., Roggli, V., Rosell, R., Saijo, N., Thunnissen, E., Tsao, M., Yankelewitz, D., 2011. International association for the study of lung cancer/american thoracic society/european respiratory society international multidisciplinary classification of lung adenocarcinoma. J. Thorac. Oncol. 6, 244–285.
Van Schil, P.E., Sihoe, A.D., Travis, W.D., 2013. Pathologic classification of adenocarcinoma of lung. J. Surg. Oncol. 108, 320–326.
Varela, M., Golder, M., Archer, F., de Las Heras, M., Leroux, C., Palmarini, M., 2007. A large animal model to evaluate the effects of Hsp90 inhibitors for the treatment of lung adenocarcinoma. Virology 371, 206–215.
Verwoerd, D.W., De Villiers, E.M., Tustin, R.C., 1980a. Aetiology of jaagsiekte: experimental transmission to lambs by means of cultured cells and cell homogenates. Onderstepoort J. Vet. Res. 47, 13–18.
Verwoerd, D.W., Williamson, A.L., De Villiers, E.M., 1980b. Aetiology of jaagsiekte: transmission by means of subcellular fractions and evidence for the involvement of a retrovirus. Onderstepoort J. Vet. Res. 47, 275–280.
Vigdorovich, V., Miller, A.D., Strong, R.K., 2007. Ability of hyaluronidase 2 to degrade extracellular hyaluronan is not required for its function as a receptor for jaagsiekte sheep retrovirus. J. Virol. 81, 3124–3129.
Viginier, B., Dolmazon, C., Lantier, I., Lantier, F., Archer, F., Leroux, C., Terzian, C., 2012. Copy number variation and differential expression of a protective endogenous retrovirus in sheep. PLoS One 7, e41965.
Voigt, K., Kramer, U., Brugmann, M., Dewar, P., Sharp, J.M., Ganter, M., 2007. Eradication of ovine pulmonary adenocarcinoma by motherless rearing of lambs. Vet. Rec. 161, 129–132.
Walsh, S.R., Linnerth-Petrik, N.M., Laporte, A.N., Menzies, P.I., Foster, R.A., Wootton, S.K., 2010. Full-length genome sequence analysis of enzootic nasal tumor virus reveals an unusually high degree of genetic stability. Virus Res. 151, 74–87.
Walsh, S.R., Linnerth-Petrik, N.M., Yu, D.L., Foster, R.A., Menzies, P.I., Diaz-Mendez, A., Chalmers, H.J., Wootton, S.K., 2013. Experimental transmission of enzootic nasal adenocarcinoma in sheep. Vet. Res. 44, 66.
Watanabe, T., 2011. Current status of HTLV-1 infection. Int. J. Hematol. 94, 430–434.
Winkelmann, A., Noack, T., 2010. The clara cell: a third reich eponym? Eur. Respir. J. 36, 722–727.
Wislez, M., Gounant, V., Cadranel, J., 2005. Le carcinome bronchioloalvéolaire. Rev. Mal. Respir. 22, 8S70–78S75.
Wootton, S.K., Halbert, C.L., Miller, A.D., 2005. Sheep retrovirus structural protein induces lung tumours. Nature 434, 904–907.
Wootton, S.K., Halbert, C.L., Miller, A.D., 2006. Envelope proteins of jaagsiekte sheep retrovirus and enzootic nasal tumor virus induce similar bronchioalveolar tumors in lungs of mice. J. Virol. 80, 9322–9325.
Xu, W., Stadler, C.K., Gorman, K., Jensen, N., Kim, D., Zheng, H., Tang, S., Switzer, W. M., Pye, G.W., Eiden, M.V., 2013. An exogenous retrovirus isolated from koalas with malignant neoplasias in a US zoo. Proc. Natl. Acad. Sci. U. S. A. 110, 11547–11552.
York, D.F., Querat, G., 2003. A history of ovine pulmonary adenocarcinoma (jaagsiekte) and experiments leading to the deduction of the JSRV nucleotide sequence. Curr. Top. Microbiol. Immunol. 275, 1–23.
Yousem, S.A., Finkelstein, S.D., Swalsky, P.A., Bakker, A., Ohori, N.P., 2001. Absence of jaagsiekte sheep retrovirus DNA and RNA in bronchioloalveolar and conventional human pulmonary adenocarcinoma by PCR and RT-PCR analysis. Hum. Pathol. 32, 1039–1042.
Yu, D.L., Linnerth-Petrik, N.M., Halbert, C.L., Walsh, S.R., Miller, A.D., Wootton, S.K., 2011. Jaagsiekte sheep retrovirus and enzootic nasal tumor virus promoters drive gene expression in all airway epithelial cells of mice but only induce tumors in the alveolar region of the lungs. J. Virol. 85, 7535–7545.
Zavala, G., Pretto, C., Chow, Y.H., Jones, L., Alberti, A., Grego, E., De las Heras, M., Palmarini, M., 2003. Relevance of Akt phosphorylation in cell transformation induced by Jaagsiekte sheep retrovirus. Virology 312, 95–105.