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Jaagsiekte Sheep Retrovirus (JSRV): from virus to lung cancer in sheep

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Abstract – Jaagsiekte Sheep Retrovirus (JSRV) is a betaretrovirus infecting sheep. This virus is responsible for a pulmonary adenocarcinoma, by transformation of epithelial cells from the bronchioli and alveoli. This animal cancer is similar to human bronchioloalveolar cancer (BAC), a specific form of human lung cancer for which a viral aetiology has not yet been identified. JSRV interacts with target cells through the membrane receptor Hyal2. The JSRV genome is simple and contains no recognised oncogene. It is now well established that the viral envelope protein is oncogenic by itself, via the cytoplasmic domain of the transmembrane glycoprotein and some domains of the surface glycoprotein. Activation of the PI3K/Akt and MAPK pathways participates in the envelope-induced transformation. Tumour development is associated with telomerase activation. This review will focus on the induction of cancer by JSRV.

JSRV / ovine pulmonary adenocarcinoma / bronchioloalveolar cancer / type II pneumocytes / lung

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1. INTRODUCTION

Ovine pulmonary adenocarcinoma (OPA) is a contagious tumour originating in the distal lung after infection by Jaagsiekte Sheep Retrovirus (JSRV). The disease was initially described in 1915 in South Africa and is present worldwide. JSRV belongs to the *Retroviridae* family and the *Betaretrovirus* genus. OPA is similar to a peculiar form of human cancer, bronchioloalveolar cancer (BAC), with which it shares clinical, radiological and histopathological features [51]. Although the molecular mechanisms of JSRV induced- tumorigenesis are still only partially understood, the significant recent efforts in the field of OPA research reinforce the relevance of this model for the study of both the virological and cancerous aspects of lung adenocarcinoma.

2. OPA: A VIRUS-INDUCED CANCER

2.1. Natural history

Transmission of OPA among sheep has been suspected for almost two centuries. The first report dates back to 1825, with a letter written by a farmer who complained about the loss of many of his sheep. The disease was called “Jaagsiekte”, after the Afrikaans words for “chase” (Jaag) and “sickness” (sieckte), to describe the respiratory distress observed in an animal out of breath from being chased [91]. The first evidence of a viral cause came from the observation of retrovirus particles in the lungs from sheep presenting clinical signs of cancer [66], and was further clearly confirmed by experimental induction of the disease by intratracheal inoculation of viral particles with a reverse transcriptase activity [47], cytoplasmic fractions of tumoral cells [82–84] or pulmonary secretions [76].

The disease can also be efficiently transmitted to goats by experimental inoculation [77, 81]. JSRV has been definitively demonstrated as the aetiological agent of OPA by experimental inoculation of particles produced from a JSRV-molecular clone [57].

OPA is present on all continents. Its incidence is difficult to evaluate in the absence of an appropriate screening tool. The virus is transmitted between animals by close contact, mainly through aerosolized particles. The breeding conditions are of major importance for the dissemination of the virus. The incubation period in naturally infected animals ranges between 2 and 4 years [78], but the cancer may be diagnosed as early as a few months after birth. The incubation period may vary according to the type of infection (experimental versus spontaneous infection), and the age of the animals [78]. Interestingly, injection of tumoral tissues into newborn lambs rapidly induces the disease in 3–6 weeks [57]. In natural conditions, the rapid development of tumoral lesions in young animals suggests a greater susceptibility of the developing lung to JSRV [8]. In utero transmission to the fetus has been suggested [8].

2.2. JSRV: a member of the *Betaretrovirus* genus

JSRV is the virus responsible for the induction of OPA. JSRV belongs to the family *Retroviridae*, to the subfamily *Orthoretrovirinae* and the genus *Betaretrovirus*. The *Betaretrovirus* genus also comprises Mouse Mammary Tumour Virus (MMTV) responsible for a mammary adenocarcinoma in the mouse, Mason-Pfizer Monkey Virus (MPMV) isolated from a Rhesus monkey, and Squirrel Monkey RetroVirus (SMRV). In 2003, Dolly the sheep, the first mammal cloned from an adult cell, died after being diagnosed with

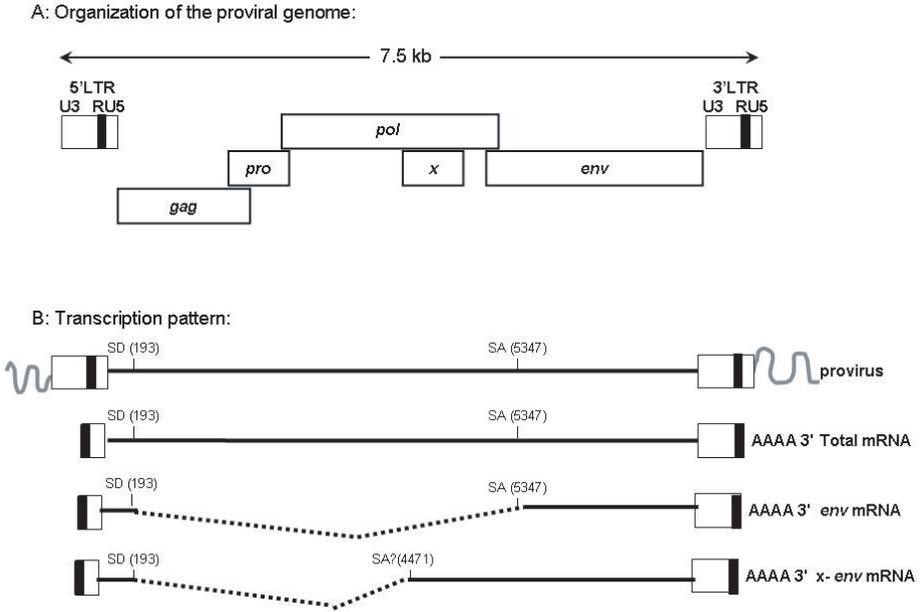


Figure 1. Organisation (A) and transcription pattern (B) of JSRV provirus (from [30]). The *gag*, *pro*, *pol* and *env* genes respectively encode the core proteins, the protease, the enzymatic activities and the envelope glycoproteins. “x” is an additional open reading frame encoding a putative protein of unknown function. LTR: Long Terminal Repeat. SA: splice acceptor; SD: splice donor (adapted from [80]).

an incurable pulmonary adenocarcinoma induced by JSRV.

The first attempts to characterise the virus were made in the early 1980's by purification of the virus from lung lavage [91]. In 1991, a cDNA library was obtained from purified 1.186 g/mL density fractions [89], thus allowing the first sequencing of the entire JSRV genome of a South African isolate [90].

2.3. Virus and genomic organisation

Retroviruses are RNA viruses infecting vertebrate species and many non-vertebrates. Virions (80–100 nm in diameter) are spherical and surrounded by an envelope, with spikes composed of virus-encoded glycoproteins. The envelope is composed of viral proteins and elements of

the host-cell membrane (lipid bilayer and proteins). The virions carry two copies of the genome, composed of linear, positive, single-stranded RNA. The 7.58 kb genome of the infectious virus comprises four main genes, organised as 5'-*gag-pro-pol-env*-3' (Fig. 1), which encode the virus proteins (reviewed in [64, 90]). Interestingly, while the nucleotide sequences of *gag*, *pro* and *pol* are homologous to their counterparts in MPMV, the *env* gene of JSRV is more related to that of MMTV and Human Endogenous Retrovirus -K (HERV-K). JSRV is organised as a simple retrovirus, with an additional open reading frame, named ORF-x, that overlaps the 3' end of the *pol* gene. ORF-x is unique among retroviruses and may encode a putative accessory protein of 166 amino acids. Although the existence of the protein is debated, ORF-x is strongly conserved in various isolates,

and sequence analyses suggest a selective pressure for its conservation [6, 63, 72, 91] (and our unpublished data). Interestingly, two sub-genomic mRNA with splice acceptor sites within or in the vicinity of ORF-x have been identified, suggesting that this putative gene may actually be transcribed [63]. We have also identified these two mRNA in tumoral lungs (unpublished data).

The JRSV genome contains non-coding regions at the ends of the genome, that are essential for the virus replication: R repeated at both ends; U5 unique to the 5' end and U3 unique to the 3' end. The integrated genome is flanked by the long terminal repeat (LTR) composed of the 3 regions U3-R-U5. The LTR serve as the sites of transcriptional initiation. The U3 region contains several elements important for viral transcription and tropism. The *gag* gene (*group-specific-antigen*) encodes a single polypeptide that is cleaved into at least three proteins: the matrix (MA), the major capsid protein (CA) and the nucleocapsid (NC). The *pro* gene encodes a protein compatible with already-described dUTPase in its 5' part and a protease (PRO) in its 3' end. Whereas the protease cleaves the precursor polypeptides, the dUTPase (reviewed in [11]) prevents the incorporation of deoxyuridine triphosphate (dUTP) by the reverse transcriptase [91]. The *pol* gene is predicted to encode the enzymatic activities: Reverse Transcriptase (RT) and integrase (IN), respectively implicated in the replication of the viral RNA and in the integration of the retrotranscribed DNA provirus into the host genome. The RT is a RNA-dependent DNA polymerase, essential for the conversion of viral RNA into DNA. The *env* gene encodes the surface (SU) and transmembrane (TM) glycoproteins. The SU glycoprotein interacts with the cellular receptor of JRSV in sheep, Hyal-2, a glycosylphosphatidylinositol anchored hyaluronidase-2 [70]. The TM glycoproteins presumably anchor SU into the

lipid bilayer and are composed of a hydrophobic stretch of amino acids followed by a short cytoplasmic tail. The JRSV envelope is a major determinant for the cellular transformation as discussed in detail below.

JRSV is phylogenetically related to the Enzootic Nasal Tumour Virus (ENTV), the agent responsible for nasal adenocarcinoma, a contagious tumour of the mucosal nasal glands affecting sheep and goats. In infected animals, epithelial cell proliferation is responsible for continuous nasal discharge, respiratory distress, exophthalmos and important skull deformations (for review see [19]). Co-infection of ENTV and JRSV has been reported [54].

A family of endogenous retroviruses, enJRSV (endogenous JRSV) closely related to JRSV, is present in domestic and wild sheep and goats [34]. JRSV and enJRSV genomes are highly related with 90–98% homology in deduced amino-acid sequences [59]. Endogenous retroviruses (ERV) are vertically transmitted as stable Mendelian genes in the germline of most eukaryotes. They derive from the integration of exogenous viruses in the host genomes, followed by genetic stabilisation through accumulation of mutations. Several families of HERV such as HERV-F, HERV-FRD, HERV-K, HERV-R, HERV-T and HERV-W (defined by the tRNA complementary to their putative primer binding site using the one letter code for the tRNA's corresponding amino acid) have been described in the human genome. Eight to twelve copies of enJRSV have been located on metaphase chromosome spreads of sheep and goats using fluorescent in situ hybridisation [10] (Fig. 2).

The biological significance of ERV is still largely debated. Expression of ERV has been described in the placenta and genital tract of mammals including humans with HERV-W. In the female genital tract, enJRSV expression is limited to the epithelia and is particularly abundant in the

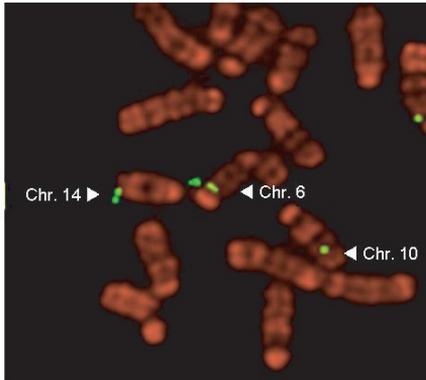


Figure 2. Chromosomal localisation of the en-JSRV copies. Integration sites were determined by in situ hybridisation with a full-length JSRV probe on ovine metaphase spreads. Chromosomes were identified by R-banding, and integration sites are indicated by arrows. Chr.: Chromosome. (A color version of this figure is available at www.edpsciences.org/vetres.)

endometrium [22,25,26,62]. Envelope proteins and *env* mRNA are present in the mononuclear trophoblasts and particularly abundant in the binucleate cells and the syncytiotrophoblast plaques of the ovine placenta [24,25,62]. Only low levels of expression have been described in the lung epithelium [62].

2.4. JSRV tropism

JSRV is unique among the retroviruses in that it induces transformation of differentiated lung epithelial cells, i.e. type II pneumocytes (also called alveolar type II cells) in the alveoli, and Clara cells in the bronchioli. Tumours exclusively occur in the sheep lung, as a result of selective replication of JSRV in these two cell types demonstrated by the detection of the viral protein only in the tumoural cells and their neighbouring epithelial cells [55, 68, 75]. However JSRV may infect different cell types in vitro, with a larger

cellular tropism. Hence, ovine cell lines issued from various tissues may be infected in vitro by JSRV, however viral production stays weak [58]. Presence of viral DNA can be demonstrated in naturally infected animals in lymphoid tissues, alveolar macrophages and in peripheral blood mononuclear cells such as monocytes or B and T lymphocytes [29,33,37,56,75].

Viral envelope and LTR regions are essential determinants for the tropism and the expression of retroviruses. Located at the surface of the viral particle, surface (SU) glycoproteins specifically interact with cellular receptors, allowing entry of the virus into the cell. It is now well established that Hyal2 (hyaluronidase 2) is the cellular receptor for JSRV (Fig. 3) in sheep [23,70]. Hyal2 is a member of the hyaluronoglucosaminidase family, enzymes that degrade hyaluronic acids of the vertebrates' extracellular matrix. Hyal2 only shows a weak hyaluronidase activity in comparison to other proteins of the same family. Hyal2 a glycosylphosphatidylinositol (GPI)-anchored cell-surface receptor, is ubiquitously expressed in human and mouse tissues [14]. Hyal2 also acts as a cellular receptor for ENTV [23], the related *Betaretrovirus* that induces nasal tumours. Ubiquitous expression of Hyal2 is expected in sheep, in accordance with the capacity of JSRV to infect different cell types. JSRV may thus be able to enter different cells via its ubiquitous receptor [56,58], however its active replication is restricted to bronchioloalveolar epithelial cells (tropism restriction). Indeed, the productive infection is strictly controlled at the cellular and molecular level. Retroviral LTR contain the viral promoter and enhancer elements that interact with cellular transcription factors; they are specifically activated in cells expressing transcription factors that bind to the enhancer regions. Restriction of JSRV expression to lung epithelial cells is largely due to its LTR transcriptional specificity

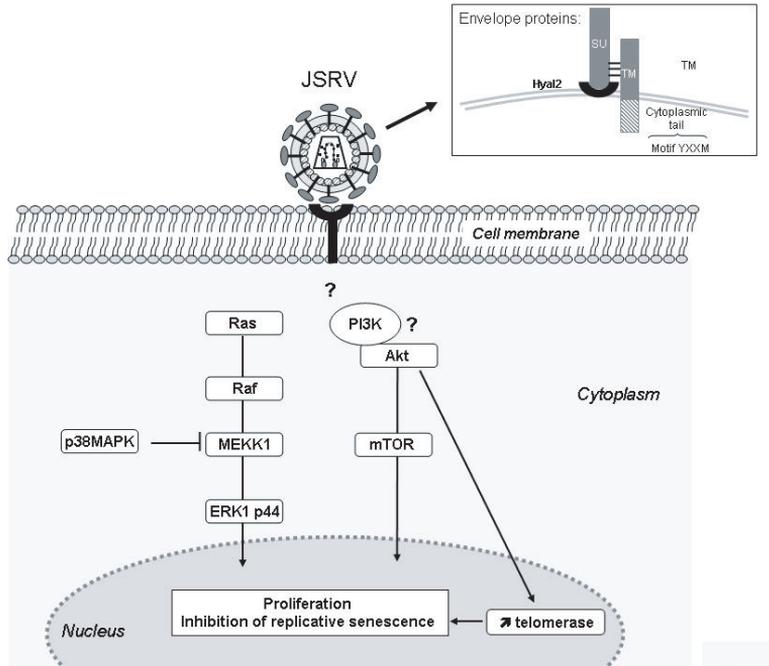


Figure 3. Pathways involved in JSRV-induced transformation and in the maintenance of the tumour (adapted from [80]). Two main pathways have been shown to be activated following *in vitro* expression of the JSRV envelope in different cell lines: the MAPKinase and PI3K/Akt pathways.

[60]. Promoters of specific genes from type II pneumocytes or Clara cells, such as surfactant proteins SP-A, SP-B, SP-C, SP-D and CCSP (Clara Cell Secretory Protein or CC10) share different regulatory elements. JSRV LTR have several binding sites for transcription factors. Among those, HNF-3 (Hepatocyte Nuclear Factor 3) a factor involved in regulating the expression of surfactant protein genes, and C/EBP (CCAAT/Enhancer Binding Protein), are essential for the transcriptional activity of JSRV LTR. Directed mutagenesis experiments have established that alterations of NF- κ B, HNF3 β and C/EBP binding sites reduce LTR activity of JSRV by 40 to 70% in MLE-15, a murine type II pneumocyte cell line [48, 60]. C/EBP is also an important activator of transcriptional activity of the LTR in MtCC1-2, a murine Clara cell line, while HNF-3 is not essential [48].

The endogenous virus enJSRV uses the same Hyal2-cellular receptor as JSRV. This could lead to a phenomenon of interference at the time of cell infection by the exogenous form of the virus [79]. Exogenous JSRV and enJSRV are differentially regulated, since LTR of enJSRV may respond to progesterone but not to the transcription factors that regulate expression of exogenous virus LTR [59].

3. THE JSRV INDUCED DISEASE

3.1. Clinical presentation and histopathology

OPA shows different symptoms such as progressive dyspnea, abundant bronchorrhea, cough, anorexia and cachexia. Death usually results from end-stage respiratory failure. A typical sign (known as the

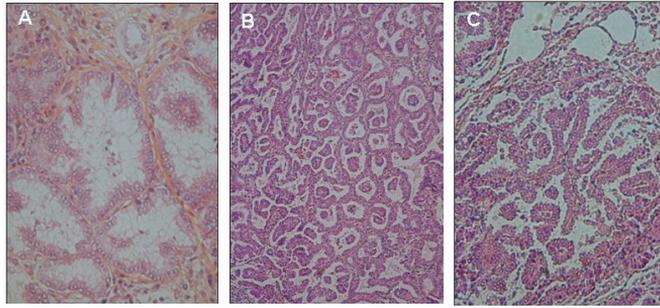


Figure 4. Subtypes of lesions observed in OPA: bronchioloalveolar (A), acinar (B) and papillary (C). (A color version of this figure is available at www.edpsciences.org/vetres.)

“wheelbarrow test”) is the flow of abundant mucoid fluid (up to 500 mL per day in advanced disease) from nostrils when the rear legs of the animal are raised. Extrathoracic metastases are rare. Macroscopic examination shows enlarged lungs infiltrated with tumoural areas, varying from small nodules (1–30 mm) to lobar consolidation [18, 51]. The disease is multifocal, disseminated in both lungs, pneumonic in appearance, with the airways filled with fluid produced by the tumoural cells.

According to the latest WHO classification for human lung cancers [7], OPA should be referred to as a mixed adenocarcinoma with associated bronchioloalveolar, papillary and/or acinar subtypes. The subtypes are defined by their cell growth and differentiation patterns [51] (Fig. 4). The bronchioloalveolar differentiation is characterised by the expansion of cells following the alveolar septa, also referred to as the lepidic spread, without destruction of the alveolar architecture. The papillary subtype is defined by the presence of papilla-like structures protruding above the epithelial layer and replacing the underlying alveolar architecture. Finally, the acinar subtype is composed of duct-like structures or acini and tubules composed of cells resembling bronchial glands. In JSRV-induced adenocarcinoma,

these three histopathological subtypes may be present within the same tumoural tissue.

The OPA tumoural cells derive from epithelial cells of the distal lung, namely alveolar type II cells (for ~80% of the cells) and Clara cells (for ~15% of the cells) [68] (Fig. 5). Type II pneumocytes produce different components of surfactant, a tensio-active agent that allows the maintenance of alveolar integrity.

Interestingly, tumoural cells over-express CD208/DC-LAMP (Dendritic Cell Lysosomal Associated Membrane Protein) [74], a protein belonging to the LAMP family of proteins (Lysosomal-Associated Membrane Protein), and initially described in activated dendritic cells. CD208/DC-LAMP is also constitutively expressed in human, murine and ovine type II pneumocytes, and is over-expressed in human bronchioloalveolar carcinoma and OPA [74]. In type II pneumocytes, CD208 is expressed in the constitutive membranes of the lamellar bodies, specific vesicles specialised in surfactant production. The role of CD208 over-expression in tumoural development is still unknown.

3.2. Mechanisms of oncogenesis

Oncogenic retroviruses have been historically divided into two groups

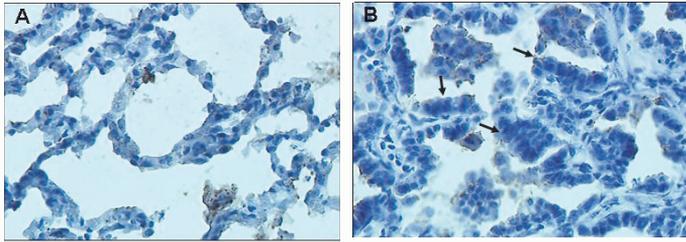


Figure 5. Microscopic pattern of lesions from OPA. Typical aspect of a non-tumoural (A) and tumoural ovine lung (B) labelled with an anti SP-A (Surfactant Protein A) antibody. Arrows show tumoural cells, i.e. type II pneumocytes labelled with an anti SP-A antibody. (A color version of this figure is available at www.edpsciences.org/vetres.)

depending on the speed of disease induction. Acute and non acute retroviruses cause tumours with a short or long latency period respectively. Acute retroviruses have been described in mice, birds, primates and are oncogene-bearing retroviruses. Viral oncogenes are derived from normal cellular genes or proto-oncogenes, involved in regulation of cell proliferation. Once captured by the virus, proto-oncogenes undergo mutations that lead to uncontrolled cell proliferation. Rous Sarcoma Virus (RSV) is an example of avian retrovirus bearing an oncogene, *v-src*, deriving from a mammalian cellular kinase.

Typical lesions of OPA may be observed in just a few weeks following experimental inoculation of concentrated JSRV particles [64, 83, 84]. This timeframe of disease induction and the multifocal pattern of OPA are compatible with the presence of an oncogene in the JSRV genome [18]. JSRV DNA transfection in murine NIH 3T3 fibroblasts induces foci of transformed cells, clearly indicating that the JSRV genome contains an oncogenic element [44, 70]. However, to date no sequence homologous to any cellular oncogene has been detected in the JSRV genome [72]. The open reading frame (orf-x) of JSRV might be a potential oncogene, although it does

not present any sequence homology with known oncogenes [6, 72], and its deletion does not prevent transformation *in vitro*.

It is now clearly established that JSRV induces tumours via the oncogenic properties of its envelope, which is both necessary and sufficient to induce transformation. The transforming property of the JSRV envelope has been demonstrated *in vitro* in various cell lines including murine NIH 3T3 fibroblasts [44], rat 208F fibroblasts [70], avian DF-1 fibroblasts [4, 93], bronchial human BEAS-2B epithelial cells [16], canine kidney MDCK epithelial cells [43], and rat kidney RK3E cells [46]. The oncogenic property of the JSRV envelope has also been shown *in vivo* in an immuno-deficient mouse model [87] and recently in sheep [9]. Expression of the JSRV envelope in mice using a replication-incompetent adeno-associated virus vector, AAV6, results in lung tumours similar to those observed in sheep [87]. The bronchioloalveolar localisation of the tumours and the expression of the surfactant protein SP-C show that the transformed cells are derived from type II pneumocytes [87]. Using a replication-defective virus carrying the *env* gene under the control of the JSRV LTR, it has been shown that the JSRV envelope is sufficient to induce lung tumours in sheep. The envelope of the related

ENTV virus displays the same oncogenic property both in vitro [2, 23] and in vivo [88].

Deletion experiments show that the TM (transmembrane) region of the envelope is the main determinant for cell transformation [12, 36, 38]. In addition to the TM region, deletions of the SU (surface) glycoprotein (going from the signal peptide to the junction between the SU and TM subunits) also abolish transformation induced by the envelope, suggesting that multiple domains of SU may be involved in cellular transformation. The cytoplasmic tail of TM, composed of 43 amino acids, is essential for the transformation process in MDCK and NIH-3T3 cells [43, 61]. This region contains a peptidic YXXM motif (Fig. 3), corresponding to a potential consensus site (phosphorylated on tyrosine Y) linked to the SH2 domain of the p85 subunit of PI3K (Phosphatidylinositol-3 Kinase), a kinase that activates Akt. The PI3K-Akt signalling pathway is determinant in cellular proliferation and survival (for review [15]) (Fig. 3). Following a membrane stimulus such as a growth factor binding to its receptor, PI3K is recruited to the cell membrane. Phosphorylated-PI3K phosphorylates the second messenger PIP2 (phosphatidylinositol (4,5) biphosphate) into active PIP3 (phosphatidylinositol (3,4,5) triphosphate). PIP3 can then recruit PDK1 (phosphatidylinositol-dependent kinase 1), which in turn phosphorylates Akt. The kinase Akt can phosphorylate diverse substrates involved in signalling cascades controlling cellular proliferation, survival and metabolism. Akt phosphorylation inhibits proteins such as GSK-3 (Glycogen Synthase Kinase 3), FOXO (forkhead box transcription factor), p24 or Kip1, and activates mTOR (mammalian target of rapamycin), an important regulator of cell growth.

Mutations of the YXXM motif of the JSRV-*env* gene abolish cell transformation of NIH-3T3 [43, 61] and rat 208F and

RK3E cells [38]. Akt activation was observed in different cell lines transformed by JSRV (Fig. 3), while it was absent from parental cells. OPA-derived type II pneumocytes do not respond to EGF (Epidermal Growth Factor) stimulation, an activator of the PI3K-Akt pathway [80], suggesting dysregulation of the Akt pathway by JSRV infection. We recently showed that Akt activation was observed in 37% of OPA tumours sampled at a late stage of disease [80]. These results support the involvement of the Akt signalling pathway in the development and/or the maintenance of OPA, and also suggest the involvement of an Akt-independent pathway [80].

The mechanisms leading to JSRV-induced cell transformation are in fact much more complex than previously considered. Several experiments rule out a direct role for the YXXM motif in Akt activation. In JSRV-transformed cells, phosphorylation of the Y590 residue of the intra-cytoplasmic TM tail, or interaction between the p85 subunit of PI3K and the envelope (a prerequisite for Akt activation), have never been shown [42, 43]. Mutations of the YXXM motif do not abolish Akt phosphorylation, so that the exact role of this motif remains to be determined [42, 43, 93]. Moreover, implication of the YXXM motif may differ between cell types in in vitro experiments [4, 36, 41, 42, 61, 93]; while it is essential for transformation of NIH 3T3 fibroblasts, it is not required for transformation of avian DF-1 fibroblasts. Nevertheless, the presence of the YXXM motif seems to affect the efficiency of envelope-mediated transformation [43]. Since the YXXM motif does not explain the oncogenic properties of JSRV, the mechanism of Akt activation in transformed cells still need to be determined. Interestingly, treatment of cells with LY294002, a PI3K-specific inhibitor, drastically reduces Akt phosphorylation in NIH 3T3 cells [42-45, 93], suggesting that PI3K-dependent Akt activation may occur.

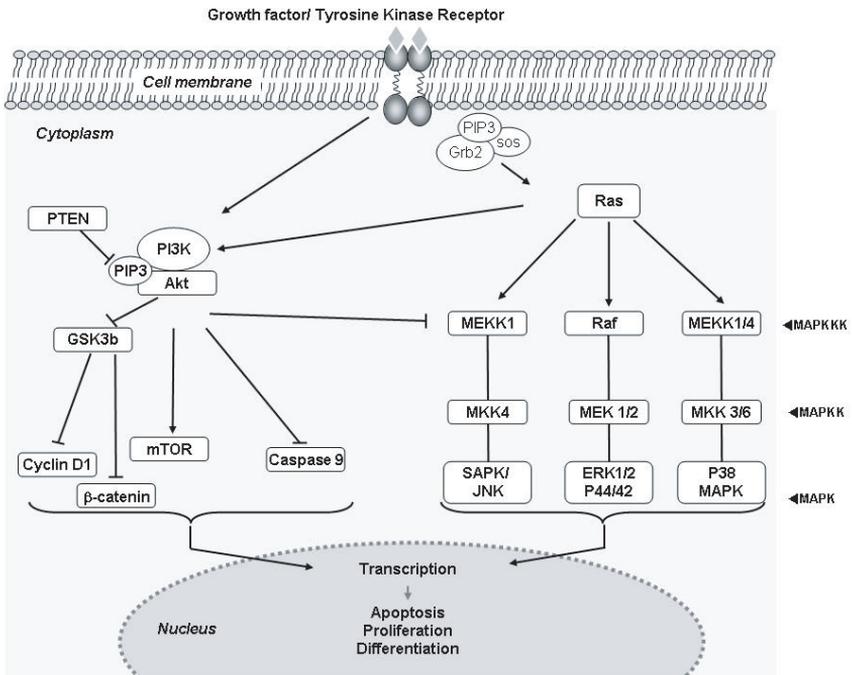


Figure 6. Activation of PI3K/Akt and MAPK pathways. Binding of growth factor, such as epidermal growth factor (EGF) to its tyrosine kinase receptor (such as EGF receptor) may induce activation of different pathways involved in the control of cell proliferation, apoptosis and differentiation (adapted from [80]).

In addition, PI3K inactivation does not prevent cell transformation [46], and Akt activation may be observed in NIH 3T3 cells deficient for the p85 subunit of PI3K [45], suggesting that PI3K may not be necessary for Akt activation [28, 73].

JSRV-mediated cell transformation is associated with the activation of the Ras-MEK-MAPK pathway. Mitogen-activated Protein Kinase (MAPK) are a group of kinases important in signal transduction, that may be activated by the small Ras protein (Fig. 6). The MAPK pathway includes 4 protein families: ERK1/2 (extracellular signal-regulated kinase 1 and 2) also named p44/42; SAPK/JNK (stress-activated protein kinase/c-Jun NH₂-terminal kinase); MAPK p38 and BMK (big mitogen-activated protein kinase 1).

MAPK are regulated and phosphorylated by the MAPK kinase (MAPKK) such as MEK1/2, themselves activated by the MAPKK kinase (MAPKKK) such as Raf (Fig. 6). Activation of this signalling cascade leads to translocation of MAPK into the nucleus, where they activate different transcription factors. The Ras-MEK-MAPK pathway is activated in JSRV-transformed NIH 3T3 and RK3E cells [38, 46] (Fig. 3). As we have reviewed above for Akt, the significance of this signalling pathway in cell transformation seems to vary according to the cell types used in the *in vitro* studies [46]. Inhibitors of MEK1 (MAPKK), can totally abolish transformation in fibroblasts and epithelial cells. On the contrary, although an inhibitor of MAPKKK totally abolishes the

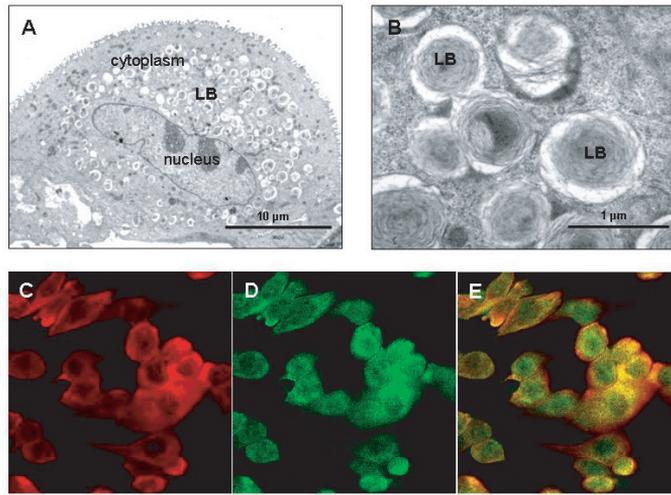


Figure 7. Tumoural type II pneumocytes in OPA-derived primary cultures. Cytoplasm is rich in lamellar bodies (LB) (A) as shown in an enlarged view (B). In vitro-derived cells express cytokeratin (C), the surfactant protein C (SP-C) (D) and co-express both cytokeratin and SP-C (E). (A color version of this figure is available at www.edpsciences.org/vetres.)

transformation of fibroblast NIH 3T3, its effect on epithelial RK3E cells is only partial. A recent study on JSRV and ENTV tumoural tissues gives more clues to understand the JSRV complex signalling cascades. In naturally and experimentally induced OPA, MAPK Erk1/2 seems to be the predominant activated pathway [20].

Since the first description of the oncogenic properties of the JSRV envelope, many studies performed in various cell types (fibroblasts, epithelial cells) and species (human, sheep, mouse, rat) have led to a better understanding of the mechanisms leading to cell transformation. Although a number of points remain unanswered, the transformation steps are believed to be dependent on the nature and origin of the cells. Hence, understanding the key events in the transformation of ovine type II pneumocytes (i.e. cells that represent the cells at the origin of the tumours) is now the challenge of ongoing work. Our group has developed primary cell cultures isolated from tumoural ovine

lungs [5, 80]; those tumour-derived cells are clearly type II pneumocytes as revealed by the presence of lamellar bodies in the cytoplasm, and the expression of surfactant protein C (SP-C) (Fig. 7). Analysis of transformation mechanisms in the ovine type II pneumocytes is in progress.

The activation of telomerase was recently evidenced in OPA-derived type II pneumocytes and in tumoural lung tissues, suggesting that replicative senescence may be negatively regulated in this tumour [80] (Fig. 3). Replicative senescence, leading to the natural death of the cells after several divisions, is essential for the maintenance of homeostasis and strict regulation of the cell life span. Replicative senescence is mainly regulated by the telomerase, a ribonucleoprotein enzyme complex able to maintain telomere length during cell division, by de novo synthesis of telomeres and elongation of existing telomeres. Telomerase activation has been implicated as a crucial factor in oncogenesis through inhibition of replicative senescence and

down-regulation of cell death. Telomerase activity is itself regulated by a variety of factors, including the Akt kinase pathway. We reported telomerase activation and Akt activation in OPA tumours [80], suggesting that Akt activation may contribute to telomerase activation and inhibition of senescence and cell death in JSRV infected cells, thereby contributing to the accumulation of tumoural cells in the ovine lung.

In most of the cell lines studied, JSRV envelope-induced transformation is independent of its interaction with the cellular receptor Hyal2. Hyal2 is weakly active as a JSRV receptor in mice and the deletion of the receptor binding domain (RBD), predicted by sequence homology with other retroviruses, from the JSRV envelope does not abolish the transformation [41, 49, 50, 70].

Although incompletely understood, the original mechanism of JSRV-cell transformation may not be unique among retroviruses. Envelope-mediated activation of cellular proliferation has also been shown for MMTV [39], Spleen Focus Forming Virus (SFFV) [1, 86] and Avian Hemangioma Virus (AHV) [3]. Expression of the MMTV envelope in the human and murine mammary epithelial cell line induces morphological changes compatible with cell transformation [39]; cell transformation is dependent on an Immunoreceptor Tyrosine-based Activation Motif (ITAM motif), a potential anchor site for signalling proteins carrying a SH2 motif, and encoded by the MMTV *env* gene [39]. ITAM (Yxx(L/I)_{x6-8}Yxx(L/I)) motifs are associated with cell survival, activation and differentiation; tyrosine (Y) residues are necessary and sufficient for signalling function. The envelope-mediated transformation has been well established in the case of SFFV, a murine retrovirus causing erythroleukemia. The SFFV genome encodes a truncated envelope protein that interacts with and activates the erythropoietin receptor, and a truncated form of the

receptor tyrosine kinase Stk/Ron [40, 53] that induces the proliferation of the target cell. Despite the direct effect of the envelope on cell signalling, other molecular events are necessary for cell transformation, such as integration of the virus genome in the vicinity of protooncogenes [71].

Tumorigenesis is a multistep process and the JSRV envelope might not be sufficient to induce transformation of cells *in vivo*. Insertional mutagenesis cannot be ruled out. Except in neonates, OPA is characterised by a slow progression from infection to disease, suggesting a non-acute mechanism of transformation [78]. A multi step gene walking technique was recently used to clone and sequence JSRV integration sites from sheep with OPA [13] and identified multiple integration sites in most tumours, suggesting a random distribution. Interestingly, a common integration site has been described in sheep chromosome 16 [13] and in or near the receptor protein tyrosine phosphatase γ (RPT Pase γ) gene [67]. In this context, insertional mutagenesis may participate to the development of the tumour in sheep.

4. JSRV AND HUMAN LUNG CANCER

Fifteen to 20% of all human cancers are thought to be associated with infectious agents (reviewed in [31]). OPA has been considered as a model for human adenocarcinomas, and especially pneumonic-type bronchioloalveolar carcinoma (BAC), since the two tumours share common clinical, radiological and histopathological features [51]. Similarly to OPA, BAC is a slow-growing tumour with rare metastatic spread. It is clinically associated with highly productive cough and progressive restrictive respiratory failure [51, 85]. The epidemiology of BAC differs from that of other non-small cell lung cancers, with a

Table I. JSRV and human lung cancer.

For	Against
Analogy to the ovine pulmonary adenocarcinoma	
Specific epidemiology of the bronchioloalveolar cancer	No epidemiologic evidence of JSRV transmission from sheep to humans
Presence of JSRV-receptor Hyal2 in human cells	Lack of JSRV DNA or RNA in human adenocarcinoma
Possible neoplastic transformation of human cells with JSRV envelope protein	
Evidence for a protein related immunologically to JSRV in some human lung tumours	
Detection of JSRV-related DNA sequences in Africans	

less important epidemiologic link to tobacco smoking, an increased frequency in women and younger patients, and a better outcome than other non-small cell lung cancers (with a 5-year survival of about 60%). Given the similarities of OPA and human BAC, a viral cause to human BAC has long been hypothesised; several recent clinical and molecular observations have been reported studying the link between JSRV and human lung carcinoma (Tab. I).

Clinically, an interesting argument in favour of an underlying infectious condition in human lung cancer came from the pattern of recurrence of BAC after lung transplantation. Although usually contraindicated in cancer, lung transplantation has been reported as a feasible treatment of unresectable BAC because of the lack of metastasis in this tumour type. Since our first report of lung transplantation in a patient with BAC [27], about thirty cases have been described, showing recurrence rates of about 50%, within a median time of 45 months [21]. Microsatellite analysis of the lung specimens of the donor and the recipient have showed that recurrent tumours originated from the transplant recipient [30, 65]. Since BAC is considered as a localised disease (and excluding possible tumoural contamination during the transplantation procedure), these ob-

servations suggest the existence of either extra-pulmonary tumoural stem cells that would remain dormant for several years [32], or of infectious agents with an extra-pulmonary preclinical reservoir.

Hyal2, the cellular receptor of JSRV, has been shown to be present at the surface of a wide range of human cells, including alveolar cells, and moreover to bind the JSRV envelope protein [70]. Furthermore, the gene coding for Hyal2 is located in the chromosomal region 3p21 which is frequently deleted in human lung cancer, making this gene a potential tumour suppressor in lung carcinogenesis [69, 70].

Interestingly, de las Heras et al. [17] showed the presence of an antigen cross-reacting with JSRV-Gag antiserum in about 30% of human BAC, and 26% of lung adenocarcinomas. However, these results have not been confirmed by further molecular studies and 2 PCR-based analyses failed to detect any exogenous or endogenous JSRV-related genome in human BAC and adenocarcinoma [35, 92].

More recently, Morozov et al. [52] reported the presence of JSRV-related sequences in healthy and HIV positive Africans, but not in the few lung cancer patients tested. Their significance remains to be evaluated.

Up to date, no conclusive molecular evidence of a link between JSRV and human BAC has been provided (Tab. I). As an alternative approach, a case control epidemiologic study is currently underway in France, conducted by our group. The objective is to determine if ovine or caprine exposure is a risk factor for BAC in humans.

5. CONCLUSION

Over the last few years, research on JSRV and the resulting OPA has focussed on the molecular mechanisms of cell transformation. Interesting results came from the discovery that the virus envelope acts as a potent oncogene. Several cellular pathways seem to occur in the cell transformation.

To conclude, research into the biology of JSRV and mechanisms leading to the development of OPA is of great interest both for the naturally induced cancer in sheep and for BAC, the related human cancer. Even though a viral agent remains uncertain in BAC patients, understanding the steps leading to the transformation of lung epithelia may be of interest in the context of therapeutic approaches in human lung cancers in general and BAC in particular.

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