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Pathology and Pathogenesis of Ovine Pulmonary Adenocarcinoma

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Abstract

Ovine pulmonary adenocarcinoma (OPA), also known as jaagsiekte, is a transmissible lung cancer of sheep caused by jaagsiekte sheep retrovirus (JSRV). JSRV induces neoplastic transformation of alveolar and bronchiolar secretory epithelial cells and the resulting tumours can grow to occupy a significant portion of the lung. Tumour growth is frequently accompanied by the over-production of fluid in the lung, which further compromises normal respiration. The period between infection and the appearance of clinical signs may be several months or years, and many JSRV-infected sheep do not exhibit clinical signs at all during their lifespan. This allows the spread of OPA into new flocks through contact with infected but apparently normal animals. OPA was first described in the early 19th century. However, it has still not been possible to devise effective methods for controlling its spread and it remains an important problem in most countries where sheep are farmed. This is due in part to the absence of an immunological response to JSRV in infected animals which has hindered the development of serological diagnostic tests and vaccines.

In addition to its veterinary importance, OPA is regarded as a potential large animal model for human lung adenocarcinoma and this has stimulated research into the pathogenesis of the ovine disease. This work has produced some significant results, including the finding that one of the JSRV structural proteins is directly involved in oncogenesis. The recent advances in understanding JSRV and the pathogenesis of OPA should lead to novel strategies for diagnosis and control of this disease and for its exploitation as a comparative model for human lung cancer.

Keywords: jaagsiekte sheep retrovirus; ovine pulmonary adenocarcinoma; pathogenesis; lung; cancer; enzootic nasal tumour

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Introduction

Ovine pulmonary adenocarcinoma (OPA) is a contagious lung cancer of sheep previously known as sheep pulmonary adenomatosis and ovine pulmonary carcinoma (Fan, 2003). OPA was first described in South Africa in the 19th century where it was called Jaagsiekte (derived from the Afrikaans for ‘chasing sickness’), because affected sheep have the appearance of having been chased and the disease is most noticeable when sheep are being herded (Tustin, 1969; York and Querat, 2003). Since then, OPA has been identified in a wide variety of breeds in many countries around the world and it is an important economic and animal welfare problem. Notably, the earliest description of OPA in the UK was in the first volume of this journal (Dykes and McFadyean, 1888), although at that time the true nature of the disease was not understood (Taylor, 1938).

OPA is caused by jaagsiekte sheep retrovirus (JSRV), which induces oncogenic transformation of alveolar and bronchiolar secretory epithelial cells. The characterization of JSRV and OPA has advanced significantly in recent years due primarily to the cloning and sequencing of the viral genome (York *et al.*, 1992; Palmarini *et al.*, 1999; DeMartini *et al.*, 2001), which has permitted the development of improved reagents and techniques for studying the virus and its relationship with the disease. In addition to its importance as a veterinary problem, OPA has wider relevance for fundamental studies on cancer since it provides a new model for understanding the molecular events involved in the development of epithelial cell tumours (Palmarini and Fan, 2001). Moreover, OPA may have particular significance for some forms of human pulmonary adenocarcinoma, which share histological similarity with the ovine disease (Mornex *et al.*, 2003). A number of recent reviews have highlighted the mechanisms underlying viral oncogenesis in OPA (Fan *et al.*, 2003; Leroux *et al.*, 2007; Liu and Miller, 2007; Maeda *et al.*, 2008). Here, we describe the clinical and pathological features of OPA and some recent advances in research on JSRV and related retroviruses. We also discuss the prospects for controlling this important disease.

Pathology of OPA

Clinical signs

The first indicator of OPA in a flock is often an increased number of deaths in adult sheep from pneumonia that does not respond to antibiotic treatment. Affected animals struggle to breathe, especially when exercised, and they may become very thin despite having a normal appetite. A pathognomonic sign of OPA is the production of copious amounts of fluid in the lung that is frothy, clear, milky or at times pinkish and drains from the sheep’s nostrils when it lowers its head. Up to 400 ml per day may be collected from such animals by lifting the rear end (the ‘wheelbarrow’ test) (Figure 1), though 10 - 40 ml per day is more common (Cousens *et al.*, 2009). Once the clinical signs are seen the sheep usually lives for only a few more days and may die abruptly following exercise or exposure to cold. Despite the unique clinical signs in some affected animals, in many cases no lung fluid is seen. A definitive diagnosis of OPA in an individual animal therefore always requires the identification of the characteristic gross and histopathological findings by post-mortem examination. OPA occurs in domestic and wild sheep species and affects no other livestock except goats, in which natural cases have been described only in subclinically affected animals (De las Heras *et al.*, 2003a).



Figure 1. Collection of lung fluid from an OPA-affected sheep.

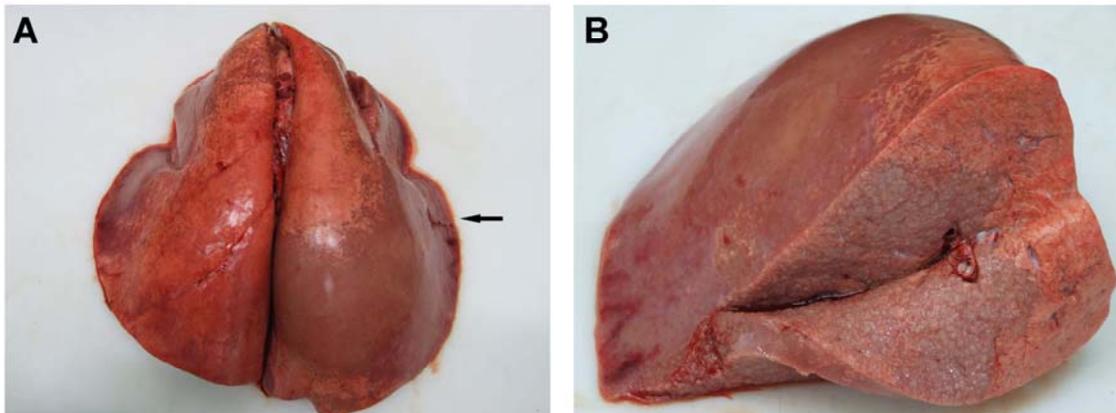


Figure 2. Gross appearance of OPA.

A natural case of OPA from an adult sheep is shown. Note the asymmetric enlargement of the right caudal lung lobe due to the presence of tumour (Panel A). In this lung, affected areas appear darker than normal tissue. Panel B shows a cut section of lung at the level of the arrow on Panel A. This shows the dense, greyish tumour with an uneven cobbled texture, adjacent to normal pink lung in the dorsal region of the lobe. Note that the precise hue, texture and distribution of tumour vary considerably between cases.

Gross pathology

There are numerous descriptions of OPA in the literature, and from these it can be seen that there has been little change in the pathological presentation in over 100 years (Dykes and McFadyean, 1888; Dungal *et al.*, 1938; Tustin, 1969; De las Heras *et al.*, 2003a). A typical post-mortem examination of a naturally infected animal in the advanced stages of disease will find a thin carcass with frothy fluid filling the trachea and exuding from the nares. On incision into the thoracic cavity, the lungs will fail to collapse and are enlarged, heavy and oedematous.

Careful palpation will reveal consolidated foci or diffuse areas within the bulk of some or all of the lung lobes. This represents tumour, which, when extensive, can grossly distort the normal architecture of the organ. Externally, affected areas appear darker (purple/greyish) than adjacent normal tissue but the smooth contour of the overlying pleura generally remains uninterrupted (Figure 2A). Sectioning through the lesion displays the solid, grey, granular surface of the tumour, which frequently exudes frothy fluid (Figure 2B). There is a clear boundary between this and adjacent normal aerated, pink, functioning lung tissue. In larger masses, the centre is more resistant to the knife and may feel gritty. In addition, firm foci of fibrosis or soft pockets of necrosis and abscessation are also frequently found. The bronchial and mediastinal lymph nodes are often enlarged and oedematous and up to 10% contain metastases (DeMartini *et al.*, 1988; Rosadio *et al.*, 1988). Distant metastases have also been reported in liver, kidney, heart and skeletal muscle but are generally rare (Mackay and Nisbet, 1966; Nobel *et al.*, 1969; Hunter and Munro, 1983). Animals that have died naturally often have a secondary bacterial pneumonia. In these cases, detailed dissection is important as extensive pleurisy and adhesions can mask the underlying pathology of OPA.

Histology and electron microscopy

Histological examination of OPA reveals non-encapsulated neoplastic foci that emanate from the alveolar and bronchiolar epithelia forming acinar and papillary proliferations that expand into adjacent structures (Figure 3A, 3B). These are supported by a fibrovascular connective tissue framework that can dominate the centre of large tumour nodules (Wootton *et al.*, 2006b). As defined by immunohistochemistry, type II alveolar epithelial cells are the principal neoplastic cell type (82%) with Clara cells (7%) and undifferentiated cells (11%) making up the remainder (Platt *et al.*, 2002). Tumour cells vary in shape and malignancy both within and between tumour nodules. Classically, they are cuboidal or columnar, with or without cytoplasmic vacuolation, and have a low mitotic index (0.002% in any tumour) (Platt *et al.*, 2002). In areas of increased malignancy, solid masses of pleomorphic cells with a high mitotic rate and scattered foci of necrosis are found. Occasionally, nodules of loose mesenchymal tissue appear admixed with tumour, as part of the fibrovascular core, or on their own, and these are also assumed to be neoplastic tissue (Wootton *et al.*, 2006b). In all tumours, the fibrovascular connective tissue acts as a scaffold for infiltrating inflammatory cells, which vary according to the size and age of tumour and the presence of secondary infectious agents. A further common feature of OPA is the large number of macrophages seen infiltrating alveolar spaces around neoplasms (Hunter and Munro, 1983; Payne and Verwoerd, 1984; Summers *et al.*, 2005). These are often accompanied by neutrophils if there is a secondary bacterial infection. Immunohistochemical labelling with antibodies raised against JSRV proteins confirms the presence of JSRV in the transformed cells (Figure 3C, 3D) (Palmarini *et al.*, 1995; Salvatori *et al.*, 2004; Wootton *et al.*, 2006b) and PCR with primers specific for JSRV is always positive when applied to samples of OPA lesions in lung tissue (Palmarini *et al.*, 1996).

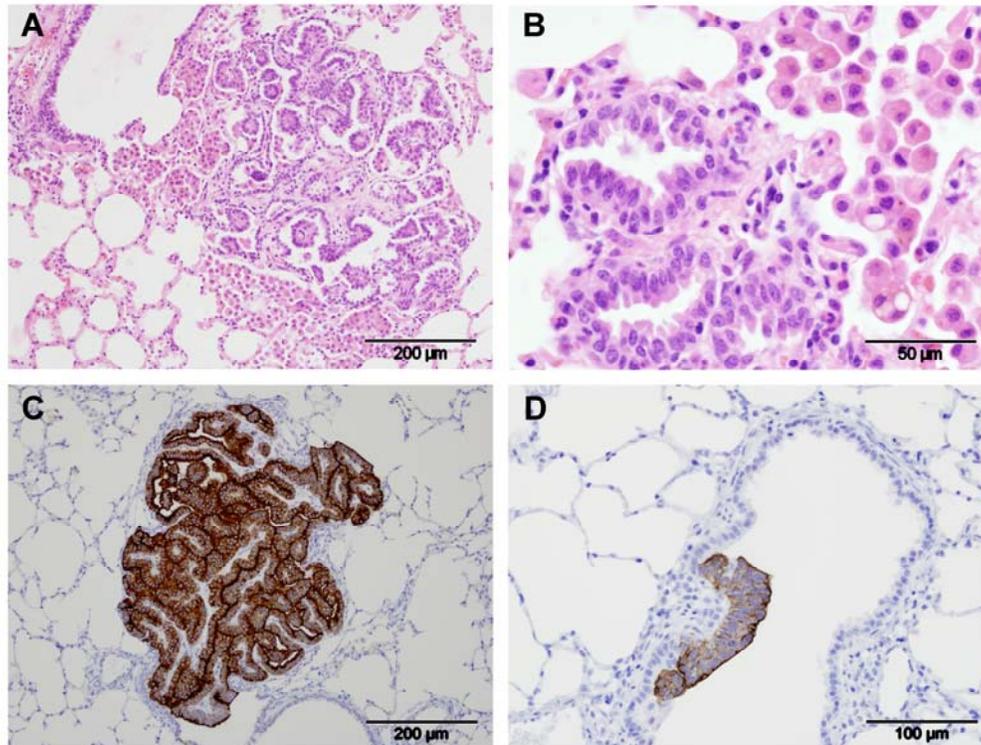


Figure 3. Histological presentation of OPA.

Panels A and B show an OPA-affected sheep lung stained with haematoxylin and eosin. A typical tumour nodule infiltrating alveoli immediately distal to a terminal bronchiole is shown. The tumour is a mixed adenocarcinoma with acinar and papillary growth patterns. Note the localized infiltrate of macrophages around a neoplastic focus, some of which have a vacuolated cytoplasm. The degree of macrophage infiltration varies between cases and between different tumour nodules from the same case. Panels C and D show immunohistochemical labelling of JSRV proteins in OPA lesions in the lung using an anti-Env (SU) monoclonal antibody. This antibody was a kind gift of Dusty Miller, Fred Hutchinson Cancer Research Center, Seattle and was used as described (Wootton *et al.*, 2006b). Note the strong labelling of JSRV SU that localizes to the plasma membrane (D).

Electron microscopy has been used to document the early growth of the tumour, to identify ultrastructural characteristics of neoplastic cells and to support the histological findings (reviewed in De las Heras *et al.*, 2003a). The tumours appear to originate from cells resembling fetal pneumocytes and type II pneumocytes (Cutlip and Young, 1982; Payne and Verwoerd, 1984). These cells proliferate to line alveoli with cuboidal or columnar cells, before forming papilliform or varicose clusters that can extend into bronchioles (Payne and Verwoerd, 1984). Tumour cells have microvilli, basal or centrally located nuclei and are connected by desmosomes (Perk *et al.*, 1971; Payne and Verwoerd, 1984). They contain variable numbers of secretory granules which can appear as electron-dense structures or are filled with myelinoid whorls, in comparison to the electron-lucent granules found in normal granular pneumocytes (Payne and Verwoerd, 1984). The cells have rough endoplasmic reticulum, large numbers of free polysomes, a well developed Golgi apparatus and hypertrophic mitochondria (Perk *et al.*, 1971; Payne and Verwoerd, 1984). Cytoplasmic glycogen granules have also been identified, sometimes in large quantities, and confirmed using periodic acid Schiff staining (Perk *et al.*, 1971; Cutlip and Young, 1982). The macrophages identified by histology are highly ‘ruffled’ and enlarged, confirming their activated

state (Payne and Verwoerd, 1984). They either attach to the surface of normal or neoplastic cells, or form separate clusters. In addition to lysosomes and phagolysosomes they contain ingested JSRV particles, bacteria and mycoplasma-like organisms (Payne and Verwoerd, 1984).

Atypical OPA

In addition to the 'classical' form of OPA described above, an 'atypical' form has been reported in which tumour nodules are hard, pearly-white and have a dry cut surface (García-Goti *et al.*, 2000; De las Heras *et al.*, 2003a). Usually located in the diaphragmatic lobe, such nodules are clearly demarcated from the surrounding parenchyma, either underneath the pleura or deep in the lung tissue, and fluid is absent in the bronchi. The histopathological features of atypical OPA are very similar to those of classical OPA but in the atypical form there is heavy infiltration of the stroma by mononuclear cells, mostly lymphocytes and plasma cells, and connective tissue fibres. Lymphoid proliferations are also consistently seen around the bronchioles of neoplastic areas. Immunohistochemical analysis reveals fewer JSRV-positive cells in atypical tumours than in the classical form of OPA (García-Goti *et al.*, 2000).

Due to the restricted tumour development in atypical OPA and the lack of overproduction of lung fluid, the atypical form of OPA occurs only as a subclinical finding in abattoir studies or when the animal is autopsied for unrelated reasons. Atypical OPA has been described in Spain and Peru (García-Goti *et al.*, 2000; De las Heras *et al.*, 2003a) and there are no reports of its occurrence in other countries where OPA is well recognized, such as the UK and South Africa. However, nodules resembling atypical lesions have been noted in the same lungs in which the classical form of the tumour is present (García-Goti *et al.*, 2000). Therefore, these two forms of OPA appear to represent two extremes of a spectrum of histological presentations in this disease.

Epidemiology of OPA

OPA has been recorded in sheep as young as two months old and as old as 11 years although most clinical cases are thought to occur in animals aged two to four years (Hunter and Munro, 1983). The disease has been reported in many sheep-rearing countries, with the notable exceptions of New Zealand, Australia and the Falkland islands (Figure 4). Iceland is also now free from OPA following a rigorous slaughter policy in the 1950s (Pálsson, 1985). Isolated case reports of lung tumours in New Zealand and Australia appear to be unrelated to JSRV infection (Dalefield and Alley, 1988; Hooper and Ellard, 1995).

The highest mortality rates from OPA are reported in the first few years after the initial diagnosis of OPA in a flock (Tustin, 1969; Hunter and Munro, 1983; Sharp and DeMartini, 2003). This finding also fits with the epizootic appearance of the disease in previously unaffected areas. For example, in the outbreak in Iceland in the 1930s, 30-50% of animals in each affected flock were lost within 18 months of the first clinical case of OPA (Dungal *et al.*, 1938). As the disease becomes endemic the mortality rate from OPA falls to around 1-5% (Tustin, 1969; Sharp and DeMartini, 2003). However, accurate information on the prevalence of OPA is difficult to obtain because OPA is not a notifiable disease and therefore very few countries collate data regarding the number of cases diagnosed.



Figure 4. Geographical distribution of OPA.

OPA is found in almost all sheep rearing countries of the world. Countries where OPA has been reported are shown in grey. Countries where OPA is absent are shown in black. The OPA status of countries shown as white is unknown.

Perhaps the best available indicator of the prevalence of OPA in a country where the disease is endemic is the UK Veterinary Investigation Diagnosis Analysis (VIDA) data. This is despite the recognized limitations that such data will underestimate disease prevalence (Veterinary Laboratories Agency, 2007). In the UK, OPA has been endemic for many years, where it was first described in 1888 (Dykes and McFadyean, 1888). Although at that time the disease was not recognized as OPA, the description and photomicrographs published later (McFadyean, 1920) clearly fit a diagnosis of OPA (Taylor, 1938). As shown in Figure 5, approx 0.2% to 0.65% of sheep per year submitted to UK Veterinary Investigation (VI) centres for post-mortem examination between 1975 and 2008 were diagnosed with OPA (personal communication, Sue Kidd and Helen Gartner, Veterinary Laboratories Agency). The proportion of sheep submitted that were diagnosed with OPA was highest throughout the 1980s and has been increasing again since 2000. Interestingly, the latest increase coincides with post-2001 foot and mouth disease (FMD) restocking of Welsh and English farms with sheep obtained from FMD-free areas such as the North of Scotland. Scotland has consistently had a higher proportion of OPA cases than England and Wales.

It is clear that clinical OPA is under-reported but also that many cases of OPA remain sub-clinical at the end of the sheep's commercial lifespan (Caporale *et al.*, 2005). An abattoir study in 1964 recorded visible OPA lesions in 52 of 280,000 sheep slaughtered in an abattoir in Edinburgh that year (Mackay and Nisbet, 1966). A countrywide slaughterhouse survey was advocated at that time in order to properly establish the overall prevalence and geographical distribution of OPA in the UK but this has never been undertaken. With the introduction of a compulsory tagging system and movement records it should now be possible to trace the source of every diseased animal and thereby obtain more robust data.

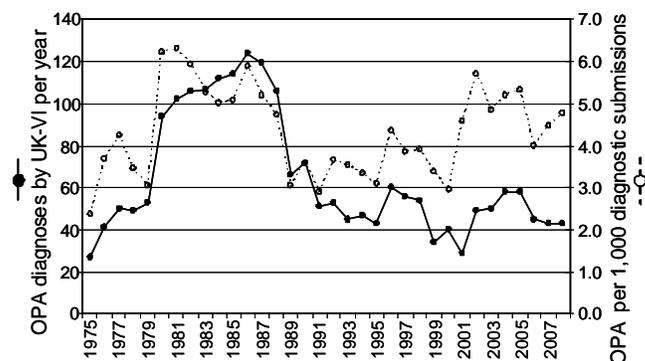


Figure 5. OPA diagnoses in the UK.

Diagnoses of OPA by the UK Veterinary Investigation service (UK-VI) presented as total per year (●) and as a proportion of the total number of sheep diagnostic submissions each year (○). This will be an underestimate of the disease incidence as only a small proportion of ill or dead sheep are submitted for VI diagnosis and on-farm diagnoses by veterinary surgeons or farmers are not recorded (personal communication, Sue Kidd and Helen Gartner, Veterinary Laboratories Agency).

In the UK, January and February are the peak months for deaths from OPA probably because out-wintered OPA-affected sheep are less able to cope with the adverse weather conditions and nutritional restrictions at that time of year. Early studies noted the apparent sensitivity of affected sheep to cold temperatures (Mackay *et al.*, 1971). In contrast, during the outbreak in Iceland in the 1930s there appeared to be fewest cases of OPA in January and February, but this was a period during which the sheep were housed (Dungal *et al.*, 1938). In Iceland, large numbers of sheep died when the animals were turned back out to pasture in the spring, especially during bad weather. Apart from mortality, there could be production losses associated with a flock carrying OPA such as reduced fertility, or decreased weight gain but this has not yet been systematically investigated.

Biology of JSRV

Identification of JSRV as the causative agent

The involvement of a retrovirus in OPA was first suggested by electron microscopy studies of tumour cells in which particles resembling retroviruses were observed (Perk *et al.*, 1971; Malmquist *et al.*, 1972; Payne *et al.*, 1983). Subsequent biochemical analysis identified reverse transcriptase activity, retroviral RNA and retroviral antigens in tumour extracts (Perk *et al.*, 1974; Verwoerd *et al.*, 1980; Sharp and Herring, 1983) and further work led to the cloning of the JSRV genome from a South African lung tumour specimen (York *et al.*, 1991; York *et al.*, 1992). JSRV was later confirmed to be both necessary and sufficient to induce OPA (Palmarini *et al.*, 1999; DeMartini *et al.*, 2001). It is worth noting that, in older studies, various other agents were associated with OPA including sheep lungworm (Dykes and McFadyean, 1888; McFadyean, 1920; Taylor, 1938), mycoplasma (Krauss and Wandera, 1970) and herpesviruses (Mackay, 1969; Tustin, 1969; De Villiers and Verwoerd, 1980). Although these agents are not directly involved in tumourigenesis they are commonly found in the lungs of OPA-affected (and unaffected) sheep and one cannot rule out the possibility that they act synergistically to enhance disease progression in natural infection.

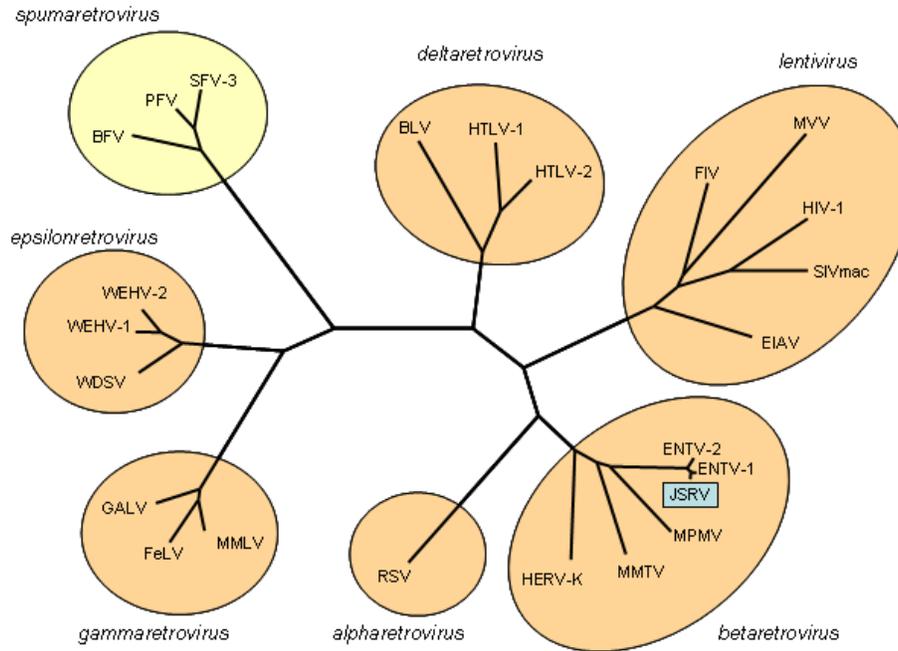


Figure 6. Classification of retroviruses

The relationship of selected retroviruses is shown based on an alignment of reverse transcriptase proteins. Retroviruses are classified into two subfamilies; *orthoretrovirinae* (orange shading) and *spumaretrovirinae* (yellow), and seven genera (italics) (Linial *et al.*, 2005). JSRV (blue box) is classified as a betaretrovirus. HIV-1, human immunodeficiency virus type 1; SIV, simian immunodeficiency virus; FIV, feline immunodeficiency virus; EIAV, equine infectious anaemia virus; MVV, maedi-visna virus; HTLV-1, -2, human T-lymphotropic virus type 1 and type 2; BLV, bovine leukaemia virus; RSV, Rous sarcoma virus; MMTV, mouse mammary tumour virus; MPMV, Mason-Pfizer monkey virus; HERV-K, human endogenous retrovirus-K; ENTV-1, enzootic nasal tumour virus type 1 (ovine strain); ENTV-2, enzootic nasal tumour virus type 2 (caprine strain); FeLV, feline leukaemia virus; GALV, gibbon ape leukaemia virus; MMLV, Moloney murine leukaemia virus; PFV, primate foamy virus; SFV-3, simian foamy virus type 3; BFV, bovine foamy virus; WDSV, walleye dermal sarcoma virus; WEHV-1, -2, walleye epithelial hyperplasia viruses type 1 and type 2. The tree was inferred using the neighbour-joining algorithm and MEGA 4.1 software (Tamura *et al.*, 2007).

Classification

Retroviruses are a diverse group of human and animal pathogens that cause a variety of diseases, in particular cancers and immunological conditions (Coffin *et al.*, 1997). Under the current taxonomy, retroviruses are classified into 2 subfamilies and 7 genera based on the genetic similarity of the reverse transcriptase (RT) protein (Linial *et al.*, 2005) (Figure 6). In contrast, previous classification systems categorized retroviruses by their associated diseases into oncoretroviruses, which cause cancer; lentiviruses, which cause complex chronic diseases with a long incubation period, and foamy viruses (spumaviruses), which are not associated with disease but are highly cytopathic in cultured cells. Although some early reports referred to JSRV as a lentivirus because of the long incubation period of OPA, under the current genetic taxonomy it is designated a betaretrovirus.

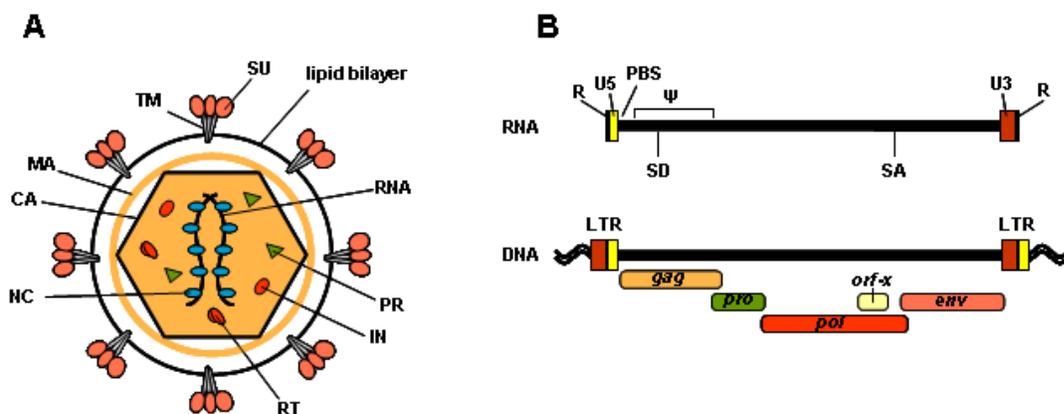


Figure 7. Structure of the JSRV virion and genome

A). Particle structure. JSRV virions contain 2 identical copies of the RNA genome associated with a highly basic nucleocapsid (NC) protein that are enclosed within the virus capsid (CA) and matrix (MA) proteins. The particles have a lipid bilayer envelope that is associated with transmembrane (TM) and surface (SU) envelope glycoproteins. The viral core also contains a number of enzymes involved in replication including an aspartic protease (PR), reverse transcriptase (RT) and integrase (IN).

B). Genome organization. The RNA and DNA forms of the JSRV genome are shown for comparison. The RNA genome of JSRV is approximately 7460 nucleotides in length. R, repeated element at both ends of the RNA genome. U5 and U3, unique elements close to the 5' and 3' termini of the genome respectively; Ψ, encapsidation signal; SD, splice donor; SA, splice acceptor; PBS, primer binding site for initiating reverse transcription. The long terminal repeat (LTR) is generated by recombination events that occur during reverse transcription. The LTR contains gene promoter and enhancer elements that regulate JSRV expression. The protein-coding region of JSRV is located in the central part of the genome and includes 5 genes. The structural proteins of the virus core (MA, CA, NC) are encoded by the *gag* gene, while the envelope glycoproteins (SU and TM) are encoded by the *env* gene. The *pro* and *pol* genes encode the viral enzymes (PR, RT, IN). The *orf-x* gene is unique to JSRV and is conserved across the various sequenced isolates although it is not known whether this gene encodes an authentic JSRV protein.

Structure of particles and genome

Retroviruses have enveloped virions that contain two identical copies of the single-stranded positive-sense RNA genome (Figure 7A). For JSRV, the genome is approximately 7,460 nucleotides in length and, in common with other retroviruses, it contains four genes that encode essential viral proteins (Figure 7B). These are *gag* encoding the structural internal virion proteins comprising matrix (MA), capsid (CA), and nucleocapsid (NC); *pro* which encodes an aspartic protease (PR); *pol* encoding the reverse transcriptase (RT) and integrase (IN) enzymes; and *env* which encodes the surface (SU) and transmembrane (TM) envelope glycoproteins. The viral proteins are synthesized initially as large precursors and are later processed into the mature proteins by proteolytic cleavage. The genome also contains several *cis*-acting elements that are required for expression of the viral proteins.

In addition to the four common retroviral genes, JSRV has a further open reading frame, denoted *orf-x*, which overlaps the *pol* gene and is of undefined function. *Orf-x* has a codon usage markedly different from that of other genes within JSRV, and the predicted amino-acid sequence is extremely hydrophobic. Expression of the putative Orf-x protein has not been demonstrated although there appears to be a specific *orf-x* mRNA transcript in JSRV-transfected cells and in lung tumour (Palmarini *et al.*, 2002). In addition, the sequence is highly conserved in JSRV isolates

collected from the UK, Italy, Spain, South Africa and the USA, which is consistent with the notion that *orf-x* encodes an authentic viral protein (Bai *et al.*, 1999; Rosati *et al.*, 2000). Sequence similarity with the adenosine 3A receptor has been reported and may give a clue to its function (Bai *et al.*, 1999), but as most of the ‘homologous’ regions are in hydrophobic domains the significance of this similarity is uncertain. Whatever its function, Orf-x is not essential for cellular transformation *in vitro* (Maeda *et al.*, 2001) or for oncogenesis *in vivo* (Wootton *et al.*, 2005; Caporale *et al.*, 2006; Cousens *et al.*, 2007).

Replication

A generalized scheme for JSRV replication is presented in Figure 8, based largely on established mechanisms used by other retroviruses (Coffin *et al.*, 1997). The two characteristic events of the retrovirus replication cycle are reverse transcription of the RNA genome to a DNA form and insertion (‘integration’) of the viral DNA into the host cell’s chromosomal DNA. The integrated form of the viral genome is known as a ‘provirus’. Recent cell culture studies have begun to examine the details of JSRV replication and some notable features that are relevant to pathogenesis are worth highlighting here.

(i) The receptor used by JSRV is hyaluronidase 2 (Hyal2), a glycosylphosphatidylinositol-anchored protein that is present on the surface of many cell types and is not restricted to lung epithelial cells (Rai *et al.*, 2001; Miller, 2008). JSRV therefore has the potential to enter and infect a range of different cells and tissues in addition to lung epithelial cells, including lymphocytes and macrophages (Holland *et al.*, 1999). Consequently, receptor distribution does not appear to determine the lung pathogenesis. Instead, the specificity of expression of JSRV to the lung appears to be determined by the viral long terminal repeat (LTR), which contains enhancer elements that bind lung-specific transcription factors. If JSRV were to integrate in a cell without the required factors, it would not be efficiently expressed. Several studies have identified factors that are required for expression from the JSRV LTR (Palmarini *et al.*, 2000a; McGee-Estrada *et al.*, 2002; McGee-Estrada *et al.*, 2005; McGee-Estrada and Fan, 2006; McGee-Estrada and Fan, 2007) but further work is necessary to gain a complete understanding of JSRV transcriptional control.

(ii) To become integrated in the chromosomal DNA of the target cell, the retroviral DNA must enter the nucleus. However, all retroviruses other than lentiviruses are unable to cross the nuclear envelope and so require the disassembly of this membrane during cell division to gain access to the chromosomal DNA. Efficient infection is therefore restricted to actively dividing cells. This is likely to determine the population of cells in the ovine lung that are susceptible to JSRV infection since, in adults, type II pneumocytes and Clara cells normally show a low degree of proliferation unless activated by lung injury (Wright and Alison, 1984). This perhaps explains why in experimental infections younger animals are more susceptible to OPA than older animals, as they will have a greater amount of actively growing lung tissue (see below).

(iii) Recent studies have revealed that the *env* gene of JSRV encodes an additional protein, designated Rej, which is required for efficient production of JSRV virions. Rej may be expressed either from a multiply spliced mRNA (Hofacre *et al.*, 2009) or by cleavage from the full-length Env protein (Caporale *et al.*, 2009). The mechanism of action has yet to be determined but Rej may be involved in several steps in JSRV replication including nuclear export of the viral genomic RNA, translation of Gag-Pro-Pol proteins, and the release of JSRV particles from the cell (Caporale *et al.*, 2009; Hofacre *et al.*, 2009). In this respect it appears to be functionally similar to the Rev protein of HIV-1 (Groom *et al.*, 2009). This recent finding indicates that JSRV replication is more complex than previously thought. This may be relevant to the cell and tissue tropism of the virus and therefore also to the pathogenesis of the subsequent disease.

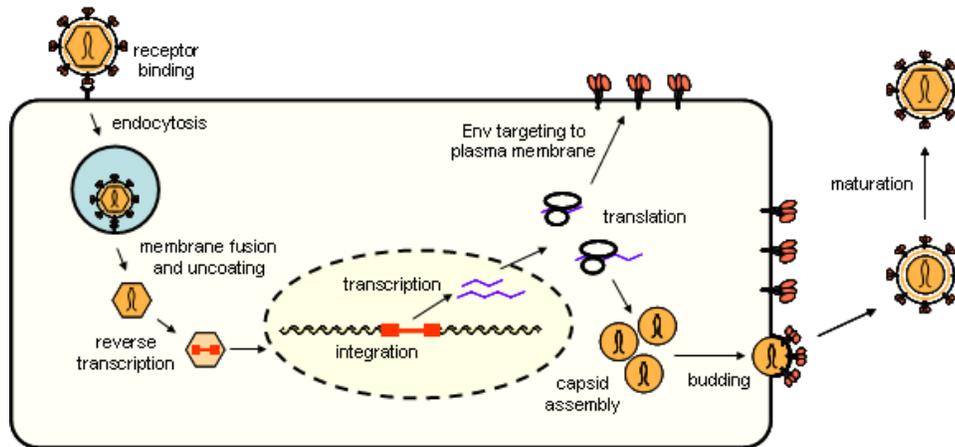


Figure 8. Replication cycle of JSRV

The initial attachment of JSRV to its target cell is mediated through the binding of the SU subunit of the Env glycoprotein to a specific cell surface receptor molecule, hyaluronidase 2 (Hyal2). Recent evidence suggests that this leads to endocytosis of the virus particle and that subsequent fusion of the viral and endosomal membranes releases the viral core into the cytoplasm (uncoating) (Bertrand *et al.*, 2008; Côté *et al.*, 2008b). The entry of the JSRV core into the cytoplasm activates reverse transcription, during which the single-stranded RNA genome is converted into a double-stranded DNA form. The viral DNA subsequently becomes inserted (integrated) into the chromosomal DNA of the cell to form the provirus, a step that is catalysed by the viral integrase enzyme. Following integration, expression of JSRV RNA from the viral promoter in the LTR is controlled by the host transcriptional machinery. The cellular specificity of JSRV expression is dependent on enhancer elements located in the U3 region of the LTR. Two major JSRV transcripts are produced by alternative splicing; (i) the full length viral RNA, used for expression of Gag, Pro and Pol proteins and also encapsidated into new virus particles, and (ii) a spliced sub-genomic RNA that is used for translation of Env proteins. Other transcripts may also be produced (Palmarini *et al.*, 2002; Caporale *et al.*, 2009; Hofacre *et al.*, 2009). Retroviral protein synthesis occurs on free ribosomes in the cytoplasm for Gag, Pro and Pol, whereas Env proteins are targeted to the endoplasmic reticulum and subsequently traffic to the plasma membrane. Assembly of new JSRV core particles and RNA encapsidation occur at a specific cytosolic location, closely associated with the microtubule trafficking and organization centre (Arnaud *et al.*, 2007b; Murcia *et al.*, 2007). The nascent capsids migrate from here to the cell surface where they are released by budding through the plasma membrane. As they bud they acquire a lipid envelope and their Env glycoproteins. Following release from the cell, the Gag-Pro-Pol polyproteins are cleaved into their mature forms by the viral protease. This step (maturation) is essential for the formation of infectious particles.

Genetic variation of JSRV

Three strains of JSRV have been completely sequenced including a South-African isolate, denoted JSRV-SA (York *et al.*, 1991; York *et al.*, 1992), and 2 strains from the UK, denoted JSRV₂₁ (Palmarini *et al.*, 1999) and JSRV-JS7 (DeMartini *et al.*, 2001). JSRV-SA was isolated from lung fluid, JSRV₂₁ was cloned from lung tumour tissue and JS7 was cloned from a cell line derived from an OPA tumour. JSRV₂₁ and JS7 are the infectious molecular clones that were first used to demonstrate the aetiological role of JSRV in OPA in experimentally inoculated lambs (Palmarini *et al.*, 1999; DeMartini *et al.*, 2001) and have been used in most of the recent studies on the mechanisms of viral oncogenesis (see below). In addition to these fully sequenced genomes, partial sequences of other JSRV isolates from various geographical locations have been obtained by PCR amplification from tumours and blood (Bai *et al.*, 1996; Palmarini *et al.*, 1996; Bai *et al.*, 1999; Rosati *et al.*, 2000). Phylogenetic analysis of Env and LTR sequences has differentiated two genotypes of JSRV, one from South Africa and Kenya (JSRV-I) and one from the UK and USA

(JSRV-II) (Bai *et al.*, 1996; Bai *et al.*, 1999; DeMartini *et al.*, 2003). These genotypes differ at a small number of sites but it is not yet known whether the sequence variation confers functional differences between the two subtypes.

Endogenous JSRV

On rare occasions retroviruses can infect sperm or egg cells and become integrated in the germ-line DNA of the host. Such proviruses are then inherited by any descendants along with other nuclear DNA in a Mendelian fashion. These elements are known as endogenous retroviruses (ERV) and have been found in all vertebrate species examined (Palmarini *et al.*, 2004; Weiss, 2006). Colonization of the genomes of vertebrates has been an ongoing process over millions of years of evolution and continues today (Tarlinton *et al.*, 2006). In some species ERVs have accumulated to form a sizable proportion of the genome; for example, around 8% of the human genome is derived from ERVs (Li *et al.*, 2001).

ERVs are important in the context of OPA because the sheep genome contains many proviruses that are closely related to JSRV. (York *et al.*, 1992; Palmarini *et al.*, 2000b; Palmarini *et al.*, 2004; Arnaud *et al.*, 2007a). Twenty-seven endogenous JSRV-related proviruses (denoted enJSRV) have so far been identified (Palmarini *et al.*, 2000b; Arnaud *et al.*, 2007a) and they have over 90% sequence similarity with exogenous JSRV in most parts of the genome (Bai *et al.*, 1996; Palmarini *et al.*, 1996; Bai *et al.*, 1999; Palmarini *et al.*, 2000b; Rosati *et al.*, 2000; Arnaud *et al.*, 2007a). The majority of enJSRVs are defective in at least one viral gene due to the acquisition of mutations since they integrated into the germ-line. However, at least five enJSRV proviruses have intact open reading frames for all genes and are potentially capable of encoding virus particles (Arnaud *et al.*, 2007a). In addition, a few enJSRV proviruses show ‘insertional polymorphism’, *i.e.*, they are present only in some breeds or in individual animals within a breed (Arnaud *et al.*, 2007a). This indicates that this subset of enJSRV proviruses was formed very recently in the evolution of sheep and, in the case of one provirus, possibly within the last 200 years (Arnaud *et al.*, 2007a). In contrast, some other enJSRV proviruses are very old and integrated before the evolutionary split of the sheep and goat lineages. The distribution of enJSRV proviruses between different sheep breeds has been used to infer the history of sheep domestication (Chessa *et al.*, 2009). The presence of some very ‘young’ enJSRV proviruses in the sheep genome raises the question of whether viruses related to enJSRV (*i.e.*, in addition to tumour-causing JSRV) are still circulating among sheep today.

It is important to note that enJSRVs are not oncogenic and most proviruses are defective in at least one gene. Nevertheless, their RNA and/or proteins are abundantly expressed, particularly in the female reproductive tract and in fetal tissues (Palmarini *et al.*, 2001a; Spencer *et al.*, 2003). Expression in the thymus during development may have relevance to the clinical outcome of subsequent infection by exogenous JSRV through the induction of immunological tolerance (discussed below). In addition, the ability of enJSRV proviruses to produce RNA and proteins has led to studies investigating their possible physiological and pathological functions.

One area of interest has been the potential for enJSRV proteins to interfere with the replication of exogenous JSRV. Two mechanisms have been described. Firstly, the enJSRV Env protein is able to bind to Hyal2 and thereby block infection by exogenous JSRV, a phenomenon known as receptor interference (Spencer *et al.*, 2003). Secondly, the Gag proteins of some enJSRV proviruses are able to block assembly and release of exogenous JSRV (Mura *et al.*, 2004; Murcia *et al.*, 2007). It has been suggested that the inhibitory effects of these enJSRV proteins on JSRV replication may have driven a change in tissue tropism for the exogenous virus from the genital tract (where enJSRV is expressed) to the respiratory tract (where enJSRV is not expressed) during the evolution of the exogenous virus (Palmarini *et al.*, 2004).

In addition to its ‘anti-viral’ activity, the enJSRV Env protein has a crucial role in placental morphogenesis and differentiation. The mechanism(s) involved are unclear, but it is possible that enJSRV Env binds to Hyal2 to mediate cell fusion events that drive the formation of trinucleate

cells and placental multinucleate syncytial plaques (Dunlap *et al.*, 2006a; Dunlap *et al.*, 2006b). An analogous function has been proposed for ERV proteins in the formation of syncytiotrophoblast cell layers in rodent and human placentas (Dupressoir *et al.*, 2009; Vargas *et al.*, 2009). Alternatively, the enJSRV Env protein could be involved in the regulation of cell differentiation events that occur during the formation of the trinucleate cells.

Pathogenesis of OPA

Transmission and incubation period

Several early studies determined that natural transmission of JSRV occurs most commonly via the aerosol route (Dungal *et al.*, 1938; Dungal, 1946; Tustin, 1969). Animals producing lung fluid, which contains 10^7 - 10^{10} JSRV particles per ml (Cousens *et al.*, 2009), are likely to be the most effective in spreading the disease. However, it is also apparent that the virus can spread before clinical signs appear (Dungal *et al.*, 1938; Tustin, 1969; Caporale *et al.*, 2005; Cousens *et al.*, 2008). Close contact between susceptible animals is likely to be a major risk factor for infection and the practice of indoor housing of sheep therefore provides an increased opportunity for the transmission of JSRV.

Infection of lambs can occur soon after birth from a JSRV-infected dam and it has been shown that the virus is present in milk and colostrum and that this may permit its transmission to the suckling lamb (Grego *et al.*, 2008). In an attempt to derive a JSRV-free flock from a severely affected flock, lambs were isolated immediately after birth so that they had no contact with their dams (Voigt *et al.*, 2007b). This strategy was successful in reducing JSRV transmission but it is unclear whether this was due to preventing access to the ewe's milk in particular or whether restricting other close contact with the ewe was also important. In another study, transmission was prevented by embryo transfer from OPA-affected donor ewes to unaffected recipients (Parker *et al.*, 1998). Unfortunately both these approaches are expensive and labour-intensive and could not easily be undertaken on most commercial farms. However, they could be useful control measures for high-value flocks. It should be noted that the success of these programmes in preventing transmission to lambs appears to be in conflict with PCR data that show that JSRV can be transmitted vertically to fetuses from OPA-affected ewes (Salvatori, 2004).

It is clear that the prevalence of JSRV is not necessarily a reflection of the prevalence of clinical OPA since a much larger number of animals is infected than ever develop the clinical signs (Salvatori, 2004; Caporale *et al.*, 2005). Additional factors such as genetic resistance or secondary infections may potentially influence the outcome of JSRV infection. There is some evidence for genetic resistance to OPA. In the Icelandic epizootic described above, up to 90% of Gottorp breed but only around 10% of Adalbol breed sheep died in OPA-affected flocks (Dungal *et al.*, 1938). Furthermore, as noted above where flocks have carried OPA for some time the number of affected animals reduces to a low level (less than 5% per year) after high losses (20% or more) in the first few years (Tustin, 1969; Sharp and DeMartini, 2003). Although this pattern would fit with the development of flock immunity to JSRV, infected animals do not develop a detectable immune response to the virus. Therefore, the reduction of cases may reflect the selection of more resistant animals after the most susceptible are killed off. This would lead to breeding stock in endemic areas being comprised mostly of OPA-resistant breedlines. Whether this resistance to OPA disease is indicative of resistance to JSRV infection or to the subsequent growth of tumour has yet to be investigated.

Experimental model for OPA

A reliable procedure for the experimental production of OPA has been developed based on the results of experiments carried out over many decades. The first demonstration of experimental transmission of OPA was by de Kock in 1929 (Tustin, 1969). Later, Dungal (1946) proved that the disease could be transmitted through the air in an elegant experiment in which three of eight lambs housed above an OPA-affected sheep contracted the disease after 4-6 months. Subsequent reports have demonstrated that OPA can be transmitted via tumour cells (Coetzee *et al.*, 1976), tumour homogenate (Wandera, 1970; Verwoerd *et al.*, 1980), lung fluid (Martin *et al.*, 1976; Sharp *et al.*, 1983; Salvatori *et al.*, 2004) or by JSRV produced in cultured cells (Palmarini *et al.*, 1999; DeMartini *et al.*, 2001; Cousens *et al.*, 2007) when these preparations are administered by the intratracheal route.

OPA can be reproduced most rapidly and efficiently in young lambs using JSRV concentrated from lung fluid or prepared *in vitro* by transient transfection (Sharp *et al.*, 1983; Palmarini *et al.*, 1999; DeMartini *et al.*, 2001; Salvatori *et al.*, 2004; Cousens *et al.*, 2007). Under these conditions clinical signs of OPA may be identified within a few weeks to months after inoculation. In one study, clinical signs were noted just 4 days after infection in one lamb (Sharp *et al.*, 1983). The incubation time increases with age at infection (Salvatori *et al.*, 2004). It has been hypothesized that younger lambs are most sensitive to infection because of the increased proportion of dividing type II pneumocytes and Clara cells, the likely target cells of JSRV, in their lungs (Salvatori *et al.*, 2004; Caporale *et al.*, 2005). The dose of infectious virus is also likely to affect the incubation time. For example, the incubation period between infection and appearance of clinical signs varies depending on the lung fluid used for inoculation (Sharp *et al.*, 1983), possibly because the concentration of JSRV in different lung fluids can vary by up to 1000-fold (Cousens *et al.*, 2009).

It should be noted that natural cases of OPA differ in several respects from cases that have been induced experimentally. Natural cases usually form one large tumour mass, possibly from coalescing smaller masses, whereas experimental cases show numerous small nodules widely disseminated throughout the lung. In addition, fluid production in experimental cases is not common but can occur as early as 7 weeks after inoculation. These differences possibly reflect the large infectious dose of JSRV in experimental animals compared to natural cases. In addition, for animal welfare reasons experimentally-infected lambs are closely monitored and culled as soon as any clinical signs appear and this may be much earlier in the course of tumour development than when clinical OPA is recognized in natural cases.

The reproduction of OPA using JSRV that had been generated *in vitro* by transient transfection with infectious molecular clones of JSRV finally demonstrated that JSRV alone was capable of causing OPA (Palmarini *et al.*, 1999; DeMartini *et al.*, 2001). This has opened the way for other experiments to elucidate the pathogenic role of individual viral proteins of JSRV *in vivo* (Palmarini *et al.*, 1999; Caporale *et al.*, 2006; Cousens *et al.*, 2007). The development of quantitative methods for standardising the dose of JSRV should now allow the experimental system to be robust and predictable (Cousens *et al.*, 2009).

Infection of target cells: origin of the tumour

JSRV is able to infect several cell types in addition to lung epithelial cells including lymphocytes (CD4+ and CD8+ T cells and B cells) and cells of the monocyte/macrophage lineage (Holland *et al.*, 1999). However, cells outside the lung do not express high levels of JSRV proteins or RNA (Holland *et al.*, 1999). As noted above, this appears to be due to a requirement for lung-specific transcription factors by enhancer elements in the viral LTR (Palmarini *et al.*, 2000a; McGee-Estrada *et al.*, 2002; McGee-Estrada *et al.*, 2005; McGee-Estrada and Fan, 2006; McGee-Estrada and Fan, 2007). An important question in understanding the pathogenesis of OPA is the identity of the target cell within the lung that is initially infected and transformed. The majority of tumour cells express markers of type II pneumocytes or Clara cells (Platt *et al.*, 2002). However,

the presence of undifferentiated cells (Wootton *et al.*, 2006b) and reports of those with mixed characteristics on EM suggest that a progenitor cell, possibly a stem cell, may be the actual target for transformation. This would also be consistent with the pattern of virus expression in infected lung, which is tightly restricted to tumour cells while adjacent normal tissue is negative (Wootton *et al.*, 2006b; and see Figure 3).

Several groups have described candidate stem cells in the lung. Studies in mice have identified cells at the bronchiolo-alveolar junction that express markers of Clara cells (Clara Cell Specific Protein) and type II pneumocytes (Surfactant Protein-C) as well as stem cell antigens (Kim *et al.*, 2005). These cells, denoted bronchioloalveolar stem cells (BASCs), have also been proposed as the cells of origin for lung adenocarcinoma (Kim *et al.*, 2005). Others have suggested that additional epithelial cell types in the bronchiolar epithelium can have stem cell-like properties (Stripp and Reynolds, 2008; Teisanu *et al.*, 2009).

To investigate the role of lung epithelial progenitor cells in OPA, transgenic mice have been developed that express a β -galactosidase reporter gene under the control of the JSRV promoter (Dakessian and Fan, 2008). These mice should therefore express β -galactosidase in cells and tissues where the JSRV LTR is active. Interestingly, the mice exhibited β -galactosidase expression only in type II pneumocytes but not in BASCs. This suggests that BASCs may not be a target for JSRV. However, further investigation is warranted in ovine tissues since a high degree of transcriptional repression was observed in the transgenic mice and this mechanism of inactivation may be more potent in some cell types than others; for example, specific inhibition of retrovirus expression has been observed in embryonic stem cells (Wolf and Goff, 2009). In addition, there might be species-specific differences in the susceptibility of BASCs to JSRV infection.

Mechanisms of oncogenesis in OPA

Once JSRV has infected its target cell in the lung, it is able to promote neoplastic transformation of that cell. The mechanism by which JSRV induces lung tumours is not completely understood (reviewed in detail by Liu and Miller, 2007; Maeda *et al.*, 2008). However, it appears that the classical mechanisms of retroviral oncogenesis, namely oncogene transduction and insertional mutagenesis, are not important in OPA. Instead, the JSRV Env glycoprotein functions as a viral oncoprotein to stimulate cellular transformation directly, in addition to its primary role in cellular entry. This was first shown *in vitro* in cultured rodent fibroblast cell lines in which transfection with an Env-expressing plasmid led to the development of foci of morphologically transformed cells (Maeda *et al.*, 2001; Rai *et al.*, 2001). Subsequently, similar results were obtained with chicken fibroblasts (Allen *et al.*, 2002; Zavala *et al.*, 2003), and with human and canine epithelial cells (Danilkovitch-Miagkova *et al.*, 2003; Liu and Miller, 2005). *In vitro* transformation of ovine type II pneumocytes has so far not been demonstrated due to the difficulty of maintaining these cells in culture. Nevertheless, studies in mice and sheep have confirmed that the Env glycoprotein alone is able to induce lung tumours *in vivo* (Wootton *et al.*, 2005; Caporale *et al.*, 2006; Chitra *et al.*, 2009).

Within the Env molecule, it appears that the cytoplasmic tail of the JSRV TM protein is critical for transformation because deletion of this region eliminates the transforming activity (Palmarini *et al.*, 2001b; Allen *et al.*, 2002; Liu *et al.*, 2003; Liu and Miller, 2005). Notably, this region is divergent between JSRV and the non-oncogenic enJSRV proviruses. Moreover, specific mutation of a single tyrosine residue in this protein is reported to abrogate the transforming activity (Cousens *et al.*, 2007; Liu and Miller, 2007). Other experiments have indicated that the SU subunit of Env might also participate in transformation, possibly by interaction with the receptor molecule Hyal2 (Danilkovitch-Miagkova *et al.*, 2003; Hofacre and Fan, 2004). The evidence for this is dependent on the species and type of cell line used but it appears that the SU and TM domains of JSRV Env may both be important for oncogenesis in OPA.

Although the oncogenic activity of JSRV Env is clearly established, the mechanisms underlying this process are not completely understood. This issue has attracted considerable attention because the identification of signalling pathways stimulated by JSRV Env could have broader relevance for understanding lung carcinogenesis in man (Maeda *et al.*, 2008). In cell culture assays JSRV Env appears to activate a number of protein kinase signalling cascades that promote cellular proliferation, with the PI3K-Akt and MEK-ERK pathways being of particular interest. Again, the relative importance of these 2 pathways appears to be dependent on the cell type and species of the cells used in the assays (Alberti *et al.*, 2002; Maeda *et al.*, 2005; Archer *et al.*, 2007; Liu and Miller, 2007). Phosphorylated (activated) forms of Akt and ERK can be identified in some OPA cases using immunohistochemistry, indicating that these pathways are also important *in vivo* in sheep (De las Heras *et al.*, 2006). In addition, transgenic mice expressing Env from the lung-specific surfactant protein-C promoter developed lung tumours similar to OPA that had significant induction of the MEK-ERK pathway (Chitra *et al.*, 2009). Identification of specific target molecules that bind the JSRV Env protein should clarify the role of the various pathways in transformation.

Following the initial activation of signalling pathways by JSRV Env, additional mutations or lesions are likely to be necessary for OPA to develop (Hanahan and Weinberg, 2000). This would be consistent with the long incubation period of natural OPA. Such events include the activation of telomerase, which has been reported in cultures of primary OPA tumour cells (Suau *et al.*, 2006). Indeed, multiple events are generally required for other retrovirus-induced tumours such as the activation of additional cellular oncogenes or inactivation of tumour-suppressors (Rosenberg and Jolicoeur, 1997). Despite the primary importance of Env-mediated activation of signalling pathways, a role for insertional mutagenesis in OPA in the natural disease cannot be entirely ruled out. In one study, 70 independent JSRV integration sites from sheep were isolated from OPA tumours and sequenced (Cousens *et al.*, 2004). The results indicated that in tumour DNA extracted from two sheep with OPA there is a common integration site for JSRV on chromosome 16 (Cousens *et al.*, 2004). While further evidence of integration sites in this region is required, this is the first suggestion that insertional mutagenesis might be relevant in OPA.

Immune and inflammatory responses to JSRV

An important feature of JSRV infection is the absence of specific cellular or humoral immune responses to viral proteins (Ortín *et al.*, 1998; Summers *et al.*, 2002; Sharp and DeMartini, 2003). This is the case both for the natural disease and for experimental infections. There is one report of the positive detection of anti-beta-retroviral Gag antibodies in infected sheep by immunoblotting (Kwang *et al.*, 1995), but this is now thought to be due to antibodies to the glutathione-S-transferase fusion partner of the recombinant Gag antigen used in the assays (Ortín *et al.*, 1998).

The mechanism underlying the lack of an adaptive immune response to JSRV is unknown but perhaps the most likely explanation is that sheep are immunologically tolerant of JSRV antigens due to the expression of closely related enJSRV proteins in the fetal thymus during T-lymphocyte development (discussed above) (Spencer *et al.*, 2003). Any JSRV-reactive T-cells should be recognized as 'anti-self' and selectively removed. Central tolerance due to ERV expression is known to be active in some other retroviral infections and in at least some circumstances may be beneficial to the host, since pathogenic effects may be immune-mediated (Crittenden *et al.*, 1982; Crittenden *et al.*, 1984). In natural cases of OPA, the lack of inflammation in the lung might explain why sheep with tumour survive for a prolonged period with no outward signs of disease.

An alternative mechanism that could contribute to the lack of an adaptive immune response to JSRV is local immunosuppression induced within the tumour microenvironment. Little is known about local events in the lung following JSRV infection, although several studies have demonstrated an influx of macrophages into the OPA lung (Hunter and Munro, 1983; Payne and Verwoerd, 1984; De las Heras *et al.*, 2003a; Summers *et al.*, 2005). In addition, one immunohistochemical study has suggested that these alveolar macrophages produce large amounts of interferon-gamma (Summers *et al.*, 2005). However, it is unclear why this initial host response is not followed by the activation of T-cells and a JSRV-specific immune response. Tumour cells appear to down-regulate expression of MHC-I (Summers *et al.*, 2005), and since these cells seem to be the predominant site of virus protein expression, this would prevent recognition by cognate CD8⁺ T-lymphocytes. In addition, the overproduction of surfactant proteins in OPA has been proposed to contribute to the absence of an effective immune response to JSRV, since these proteins are known to be immunomodulatory (Summers *et al.*, 2005). Regulatory T cells (Tregs) are also likely to be important in the host response to JSRV but their potential role in OPA has yet to be investigated (Rouse *et al.*, 2006). Further studies on the local response to JSRV infection are required to obtain a better understanding of the events involved. This information might then be valuable for the design of vaccines for JSRV.

Additional immunosuppressive mechanisms might be directly induced by JSRV itself. Although the infection of lymphoid and myeloid cells does not appear to result in significant levels of virus production, it remains possible that the infection of these cell types could have functional consequences for immune responses in JSRV-infected sheep. Indeed, peripheral blood mononuclear cells from natural and experimental cases of OPA exhibit reduced responsiveness to the T-cell mitogen concanavalin A, suggesting a virus-induced effect (Summers *et al.*, 2002). In addition, sheep with natural OPA have a reduced number of CD4⁺ T cells in the peripheral blood and an increased number of circulating neutrophils (Holland *et al.*, 1999; Summers *et al.*, 2002; Sharp and DeMartini, 2003). However, these changes are not found in experimentally-infected lambs, suggesting that they are not a direct consequence of JSRV infection. Instead, these effects are possibly due to the bacterial infections common in late stage natural OPA (Summers *et al.*, 2002; Sharp and DeMartini, 2003).

Several retroviruses are known to encode an immunosuppressive domain within their Env protein (Haraguchi *et al.*, 1995). This short peptide sequence is conserved among several otherwise divergent retroviruses, although it is not present in JSRV. Nevertheless, studies on the transformation activity of the JSRV Env clearly show that this protein can activate cellular signalling pathways and it is possible that this could have immunosuppressive effects in addition to stimulating proliferation. Here too, further investigation is required due to its importance for vaccine development.

Terminal stages – clinical disease

By the stage at which clinical OPA is apparent and the animal either dies or is euthanased a large proportion of the lungs is commonly taken over by tumour and multiple lobes of the lung may be affected. The tumour mass is frequently necrotic at the centre. Tumours may metastasize to thoracic lymph nodes and occasionally to other tissues. The reduction in functional lung parenchyma and the increased production of lung fluid cause respiratory difficulties and the resultant coughing or spilling out of fluid via the nose promotes transmission of JSRV through aerosol production and contamination of the environment. Frequently, a bacterial infection appears to be the cause of death in the OPA-affected animal. However, the heavy tumour burden and the increased volume of fluid can compromise lung function sufficiently to kill the animal in the absence of a secondary infection. An overview of the pathogenesis of OPA is presented in Figure 9.

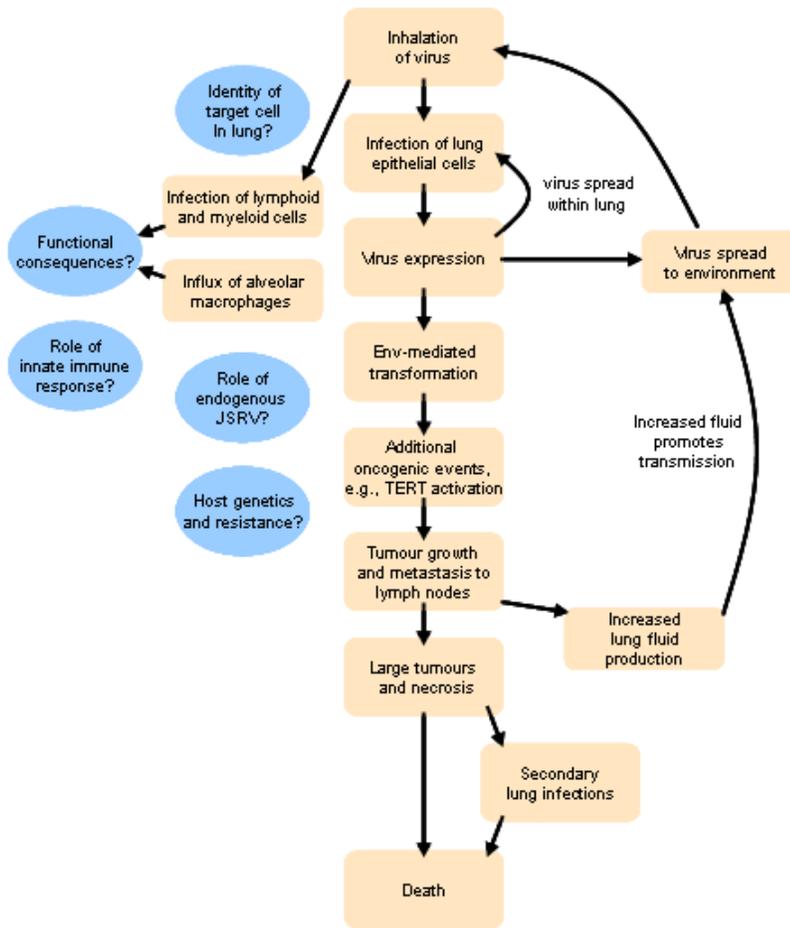


Figure 9. Overview of the pathogenesis of OPA

The important events in the pathogenesis of OPA are shown (orange rectangles). Some remaining questions are shown in blue ovals. JSRV is transmitted primarily by the respiratory route and once inhaled the virus may infect a variety of cells including lymphocytes and myeloid cells, in addition to the epithelial cells of the lung. The functional consequences of infection of non-epithelial cells are unclear as very little virus gene expression appears to occur there. Within type II pneumocytes and Clara cells (or their precursors), expression of the JSRV Env protein activates signalling cascades that promote cellular proliferation and drive malignant transformation of the cells. Initially, the tumour cells grow along the alveolar walls in a pattern reminiscent of human bronchiolar-alveolar adenocarcinoma but subsequently become more invasive and metastasize to the local lymph nodes. In rare cases, extra-thoracic metastases may occur. Larger tumours may be necrotic and fibromatous at their centre. For the weeks to months while the tumour grows there may be little outward indication and most infected animals show no clinical signs. As the tumour grows, fluid production in the lung increases and this is likely to promote virus spread to other sheep. Only when the tumour reaches a size large enough to compromise lung function, or when fluid production reaches a noticeable level do clinical signs appear. Critically, the majority of infected animals in endemic areas never show outward signs of infection but may be shedding virus, thus promoting inadvertent introduction of the disease into previously unaffected flocks and new geographical areas.

Prospects for control of OPA

Although OPA has been recognized in sheep for almost 200 years, we still do not have effective tools for controlling the disease and it continues to cause significant economic losses. In typical cases, infection with JSRV is clinically silent until the tumour is sufficiently advanced to compromise respiration or to cause the animal to lose condition and, as noted above, this incubation period may last from months to years (Dungal *et al.*, 1938; Tustin, 1969; Sharp and DeMartini, 2003). Even animals with small tumours are frequently not clinically identifiable (Dungal *et al.*, 1938; Cousens *et al.*, 2008) and in many cases animals may be infected but never develop OPA lesions within their normal or commercial lifespan (Salvatori, 2004; Caporale *et al.*, 2005). Such apparently healthy animals may nevertheless be infectious and this facilitates the inadvertent introduction of the disease into naïve flocks and to new countries by the importation of infected animals. In this way new outbreaks continue to be reported in previously unaffected flocks and in areas where the disease has not been seen before.

Diagnostic tests

Screening and culling strategies that have been useful in some other sheep diseases, notably maedi-visna (Synge *et al.*, 2008), might also be valuable in controlling OPA. However, this would require the development of sensitive and specific tests for identifying infected but pre-clinical animals. The absence of JSRV-specific antibodies in infected sheep has precluded the use of serological tests for infection and greatly hindered the development of diagnostic assays (Sharp *et al.*, 1983; Ortín *et al.*, 1998; Summers *et al.*, 2002). PCR on blood samples can be employed to detect JSRV-infected cells (González *et al.*, 2001; Salvatori *et al.*, 2004; Caporale *et al.*, 2005; De las Heras *et al.*, 2005) but the proportion of infected cells in blood is very low (Holland *et al.*, 1999) and such blood tests therefore fail to identify many infected animals (Lewis *et al.*, 2009). Despite the high false negative rate, PCR tests are informative for epidemiological studies and for identifying infected flocks, but would not be suitable for testing individual animals for eradication or accreditation purposes. The most successful method for identifying early OPA has been PCR testing of bronchoalveolar lavage samples collected from live animals (Voigt *et al.*, 2007a). However, the sampling technique is laborious and samples only a small region of the lung so could also miss early cases. PCR and RT-PCR on saliva and nasal secretions are negative for JSRV (C. Cousens, unpublished data).

Work to develop improved pre-clinical tests for OPA is continuing since there is clearly a demand from farmers and veterinarians. In particular there would be good uptake of the test to confirm the disease status of high-value breeding sheep, especially rams that are to be introduced into otherwise closed flocks. If a reliable and affordable diagnostic test were available then the development of OPA-accredited flocks could provide a significant economic advantage to flocks specialising in the supply of breeding rams.

Vaccines

An alternative to the screening and culling of infected sheep is vaccination, but this is also challenging since it is unclear which immune mechanisms might correlate with protection. Even if tolerance can be broken, the expression of related antigens from enJSRV in normal adult tissues, particularly the reproductive tract in the ewe, could result in autoimmune inflammatory reactions. In addition, an inflammatory response to infectious JSRV within the lung elicited by successful vaccination could itself do more harm than the tumour. A final consideration relates to the potential for retroviruses to evolve rapidly by mutation to escape immune responses (Overbaugh and Bangham, 2001). If sterilising immunity were not established following vaccination, the immune response to JSRV could potentially drive the selection of new strains of the virus, which might not only evade the immune response elicited by vaccination but could also acquire increased or altered

pathogenicity. A similar phenomenon has been observed previously for vaccines to Marek's disease herpesvirus in chickens (Nair, 2005). Despite these potential issues, vaccines for OPA may yet be attainable and antibodies to JSRV Gag proteins can be generated experimentally by inoculating sheep with recombinant protein in adjuvant (Summers *et al.*, 2006). Studies are underway to characterize and improve these responses and to determine whether they are protective against infection with JSRV and/or the development of OPA.

In the absence of appropriate diagnostics and vaccines the best strategy for controlling JSRV is quarantining of new stock for several months and isolation of affected animals and their lambs as soon as clinical signs are suspected. Contaminated areas and equipment where sheep are held should be disinfected or left without sheep for up to two months (Cousens *et al.*, 2009). However, these measures may not be practical for all farms. Eradication of JSRV from high value flocks or rare breeds can be attempted by removal of lambs from the ewe at birth (Voigt *et al.*, 2007b), or by embryo transfer (Parker *et al.*, 1998) but this is likely to be too expensive for most commercial flocks.

Enzootic nasal tumour

OPA is the most common neoplasm of sheep, but another tumour of the respiratory tract, known as enzootic nasal tumour (ENT; also enzootic nasal adenocarcinoma, ENA), has been described in sheep and goats (De las Heras *et al.*, 2003b). ENT is an adenocarcinoma of the secretory epithelial cells of the nasal glands and is associated with two different retroviruses: enzootic nasal tumour virus type 1 (ENTV-1, in sheep, also ovine nasal tumour virus) and type 2 (ENTV-2, in goats, also caprine nasal tumour virus) (Cousens *et al.*, 1996; Cousens *et al.*, 1999; Ortín *et al.*, 2003). Although ENTV-1 and ENTV-2 are distinct viruses, the disease they cause is essentially the same in the two host species.

As with OPA, tumours in ENT are often fluid-producing and they are also the main site of replication of the virus. In addition, there is no antibody response to ENTV in infected animals (Ortín *et al.*, 1998). Respiratory distress, bulging eyes and skull deformation can be seen as a result of the tumour growing in the nasal cavity. Cases may appear in a flock or herd at low levels (0.1-6.6% per annum) over many years (Charray *et al.*, 1985). ENT has been reported in many countries in Africa, Europe, Asia and North and South America (reviewed by De las Heras *et al.*, 2003b.), with notable exceptions being Australia, New Zealand and the UK. Diseases with similar pathology have also been seen in cattle in India (Rajan, 1987) and in deer in Sweden (Borg and Nilsson, 1985) but their aetiology has yet to be investigated. Co-infection of sheep with JSRV and ENTV-1 has been noted (Ortín *et al.*, 2004), but OPA and ENT are clearly independent diseases as ENT has never been reported in the UK where OPA is common.

The genomes of ENTV-1, ENTV-2 and JSRV share more than 92% sequence similarity with each other but there are distinct sequence differences between them, in particular in the LTR regions, and this has enabled the development of specific PCR tests for each virus (Cousens *et al.*, 1996; Cousens *et al.*, 1999; Ortín *et al.*, 2003). The majority of *in vitro* studies have concentrated on ENTV-1 (referred to as ENTV in those reports). Like JSRV, ENTV-1 uses Hyal2 as a receptor for entry into cells (Dirks *et al.*, 2002) but it appears to have a lower affinity for Hyal2 than does JSRV (Van Hoesen and Miller, 2005). The ENTV-1 Env protein is also less fusogenic than JSRV and requires a lower pH for fusion and cell entry (Côté *et al.*, 2008a). However, ENTV-1 Env has similar transforming activity to JSRV Env both *in vitro* and *in vivo* (Alberti *et al.*, 2002; Liu and Miller, 2005; Van Hoesen and Miller, 2005; Wootton *et al.*, 2006a) and appears to act via the same signal transduction pathways (De las Heras *et al.*, 2006; Maeda and Fan, 2008).

The most striking sequence difference between this group of betaretroviruses is in the U3 region of the LTR and although some of the enhancer elements are conserved there are also many differences (McGee-Estrada and Fan, 2007). Together with receptor affinity and pH entry

requirements, this most likely determines the cell type specificity of the different viruses and could explain the different tumours that result. The similarities between these viruses imply that control measures developed for JSRV will be adaptable for ENTV-1 and ENTV-2.

OPA as a model for human lung cancer

Some of the earliest descriptions of OPA noted the histological similarities between the ovine tumour and human lung adenocarcinoma (Bonné, 1939; Sims, 1943; Watson and Smith, 1951) and this led to the suggestion that OPA may be a valuable animal model for the human disease (Perk and Hod, 1982; Palmarini and Fan, 2001; Mornex *et al.*, 2003). Human lung adenocarcinomas are classified into several subtypes based on their histological appearance and this taxonomy is reviewed periodically (Travis *et al.*, 2006). OPA has particular similarities to bronchiolar-alveolar adenocarcinoma (BAC), a non-invasive neoplasm that grows along alveolar walls. However, since OPA exhibits some invasive growth it is currently better described as an adenocarcinoma of mixed subtype with a prominent BAC component (Mornex *et al.*, 2003).

Although the human and sheep tumours have a number of shared characteristics, there are interesting differences in precursor morphology. Analysis of mature human adenocarcinomas often finds that more than one pattern of tumour is present, typically with more malignant cells in the centre with BAC or atypical alveolar hyperplasia (AAH) towards the periphery (Raz *et al.*, 2006). This and data from a mouse lung cancer model indicate that AAH and BAC act as precursors to the development of invasive adenocarcinomas (Jackson *et al.*, 2001). There is no evidence of a similar progression in OPA, where epithelial hyperplasia is the only precursor lesion noted so far. However, further analysis of genetic alterations in progressing stages of disease might identify novel markers that would be useful for human disease treatment or early detection.

Several studies have examined gene expression patterns to better define the various subtypes of human lung cancers. In the case of adenocarcinoma, microarray analysis has identified a number of subtypes that extend the histological classification (Bhattacharjee *et al.*, 2001; Garber *et al.*, 2001; Hayes *et al.*, 2006; Takeuchi *et al.*, 2006). Although there are some inconsistencies between the findings of the different studies, the subtypes identified correlate with features such as morphology, tumour differentiation stage and clinical prognosis. Other studies have revealed that specific gene mutations can have important biological consequences, for example influencing survival rates and responsiveness to therapeutic drugs (Lynch *et al.*, 2004). A detailed transcriptional profiling of OPA would enable the similarities between the human and sheep diseases to be defined in more detail and the potential of the ovine disease as a model system to be exploited fully. In addition, the OPA model may also be valuable for testing anti-cancer therapies (Varela *et al.*, 2007).

A retrovirus in human lung adenocarcinoma?

Up to a quarter of human lung cancers have only a weak association with tobacco smoking, suggesting the involvement of other environmental aetiological factors, potentially including viruses (Sun *et al.*, 2007). JSRV itself is not thought to be infectious for humans, although two studies have described JSRV DNA sequences in human tissues by PCR (Morozov *et al.*, 2004; Rocca *et al.*, 2008). These reports have yet to be widely accepted and other workers have failed to detect JSRV in human tumours (Yousem *et al.*, 2001; Hiatt and Highsmith, 2002). Nevertheless, around 30-40% of human lung adenocarcinomas express an antigen that binds antibodies to JSRV and other betaretroviruses on immunohistochemistry (De las Heras *et al.*, 2000; De las Heras *et al.*, 2007). The identity of this antigen is unknown but it could possibly represent a novel betaretrovirus, an endogenous human retrovirus or another human protein that coincidentally binds to the antisera used. Attempts to identify this antigen have so far been unsuccessful. Nevertheless,

the identification of a virus in human lung cancer would have significant impact on the treatment of this disease and further work on this is warranted.

Conclusions and future work

OPA is an intriguing disease that presents some difficult challenges for control but that provides opportunities for development as a comparative model for human lung cancer. Work on improving early diagnosis and on vaccination must continue, although novel strategies for diagnostics are necessary given the absence of serological responses and the limitations of current PCR tests. For example, one potential alternative is ultrasonographic examination, which has been used to detect large tumours in subclinically affected animals (Scott and Gessert, 1998; Scott et al., 2009). In addition, the development of a cell culture system for the *in vitro* isolation of JSRV would also be valuable. With regard to vaccination, a better understanding of the mechanisms underlying the absence of an immune response to JSRV antigens in natural infection is required before rational vaccine design can be continued. These experiments are currently underway in our laboratory.

The finding that the JSRV Env protein is directly oncogenic has focussed attention on the potential relevance of OPA for fundamental studies on lung carcinogenesis. Two important targets for research include identification of the signalling molecules that bind JSRV Env to stimulate transformation and determination of the target cell or cells that are initially infected and transformed. Transcriptional profiling of OPA would also be valuable in placing this animal tumour in the context of the spectrum of phenotypes observed in human lung adenocarcinomas.

Historically, the study of oncogenic retroviruses has provided numerous insights into the role of oncogenes in cellular transformation and cancer and this information has led to important advances in cancer research and treatment. Research on novel mechanisms of retroviral oncogenesis such as that of JSRV should lead to further advances in our understanding of cancer biology with implications for the treatment of human tumours as well as the control of OPA.

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References

- Alberti, A., Murgia, C., Liu, S. L., Mura, M., Cousens, C., *et al.* (2002). Envelope-induced cell transformation by ovine betaretroviruses. *Journal of Virology*, **76**, 5387-5394.
- Allen, T. E., Sherrill, K. J., Crispell, S. M., Perrott, M. R., Carlson, J. O., *et al.* (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. *Journal of General Virology*, **83**, 2733-2742.
- Archer, F., Jacquier, E., Lyon, M., Chastang, J., Cottin, V., *et al.* (2007). Alveolar type II cells isolated from pulmonary adenocarcinoma: a model for JSRV expression in vitro. *American Journal of Respiratory Cell and Molecular Biology*, **36**, 534-540.
- Arnaud, F., Caporale, M., Varela, M., Biek, R., Chessa, B., *et al.* (2007a). A Paradigm for Virus-Host Coevolution: Sequential Counter-Adaptations between Endogenous and Exogenous Retroviruses. *PLoS Pathogens*, **3**, e170.
- Arnaud, F., Murcia, P.R., Palmarini, M. (2007b). Mechanisms of late restriction induced by an endogenous retrovirus. *Journal of Virology*, **81**, 11441-11451.
- Bai, J., Bishop, J. V., Carlson, J. O. DeMartini, J. C. (1999). Sequence comparison of JSRV with endogenous proviruses: envelope genotypes and a novel ORF with similarity to a G-protein-coupled receptor. *Virology*, **258**, 333-343.
- Bai, J., Zhu, R. Y., Stedman, K., Cousens, C., Carlson, J., *et al.* (1996). Unique long terminal repeat U3 sequences distinguish exogenous jaagsiekte sheep retroviruses associated with ovine pulmonary carcinoma from endogenous loci in the sheep genome. *Journal of Virology*, **70**, 3159-3168.
- Bertrand, P., Côté, M., Zheng, Y. M., Albritton, L. M. Liu, S. L. (2008). Jaagsiekte sheep retrovirus utilizes a pH-dependent endocytosis pathway for entry. *Journal of Virology*, **82**, 2555-2559.
- Bhattacharjee, A., Richards, W. G., Staunton, J., Li, C., Monti, S., *et al.* (2001). Classification of human lung carcinomas by mRNA expression profiling reveals distinct adenocarcinoma subclasses. *Proceedings of the National Academy of Sciences of the USA*, **98**, 13790-13795.
- Bonné, C. (1939). Morphological resemblance of pulmonary adenomatosis (jaagsiekte) in sheep and certain cases of cancer of the lung in man. *American Journal of Cancer*, **35**, 491-501.
- Borg, K. and Nilsson, P. O. (1985). Ethmoid tumors in moose and roe deer. *Nordisk Veterinaermedicin*, **37**, 145-160.
- Caporale, M., Arnaud, F., Mura, M., Golder, M., Murgia, C., *et al.* (2009). The signal peptide of a simple retrovirus envelope functions as a posttranscriptional regulator of viral gene expression. *Journal of Virology*, **83**, 4591-4604.
- Caporale, M., Centorame, P., Giovannini, A., Sacchini, F., Di Ventura, M., *et al.* (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., *et al.* (2006). Expression of the jaagsiekte sheep retrovirus envelope glycoprotein is sufficient to induce lung tumors in sheep. *Journal of Virology*, **80**, 8030-8037.
- Charray, J., Aman, N., Tanoh, K. G. (1985). Note sur une enzootie d'adenocarcinoma de la muqueuse pituitaire chez des brebis djallonké. *Revue Elevage Médecine Veterinaire Pays Tropicaux*, **38**, 406-410.
- Chessa, B., Pereira, F., Arnaud, F., Amorim, A., Goyache, F., *et al.* (2009). Revealing the history of sheep domestication using retrovirus integrations. *Science*, **324**, 532-536.
- Chitra, E., Yu, S. L., Hsiao, K. N., Shao, H. Y., Sia, C., *et al.* (2009). Generation and characterization of JSRV envelope transgenic mice in FVB background. *Virology*. Published ahead of print, 19 August 2009, doi:10.1016/j.virol.2009.07.023.
- Coetsee, S., Els, H. J., Verwoerd, D. W. (1976). Transmission of jaagsiekte (ovine pulmonary adenomatosis) by means of a permanent epithelial cell line established from affected lungs. *Onderstepoort Journal of Veterinary Research*, **43**, 133-141.
- Coffin, J. M., Hughes, S. H., Varmus, H. E. (1997). *Retroviruses*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.
- Côté, M., Kucharski, T. J., Liu, S. L. (2008a). Enzootic nasal tumor virus envelope requires a very acidic pH for fusion activation and infection. *Journal of Virology*, **82**, 9023-9034.
- Côté, M., Zheng, Y. M., Albritton, L. M., Liu, S. L. (2008b). Fusogenicity of Jaagsiekte sheep retrovirus envelope protein is dependent on low pH and is enhanced by cytoplasmic tail truncations. *Journal of Virology*, **82**, 2543-2554.
- Cousens, C., Bishop, J.V., Philbey, A.W., Gill, C.A., *et al.* (2004). Analysis of integration sites of Jaagsiekte sheep retrovirus in ovine pulmonary adenocarcinoma. *Journal of Virology* **78**, 8506-8512.
- Cousens, C., Graham, M., Sales, J., Dagleish, M. P. (2008). Evaluation of the efficacy of clinical diagnosis of ovine pulmonary adenocarcinoma. *Veterinary Record*, **162**, 88-90.

- Cousens, C., Maeda, N., Murgia, C., Dagleish, M. P., Palmarini, M., *et al.* (2007). In vivo tumorigenesis by Jaagsiekte sheep retrovirus (JSRV) requires Y590 in Env TM, but not full-length orfX open reading frame. *Virology*, **367**, 413-421.
- Cousens, C., Minguijón, E., Dalziel, R. G., Ortín, A., Garcia, M., *et al.* (1999). Complete sequence of enzootic nasal tumor virus, a retrovirus associated with transmissible intranasal tumors of sheep. *Journal of Virology*, **73**, 3986-3993.
- Cousens, C., Minguijón, E., Garcia, M., Ferrer, L. M., Dalziel, R. G., *et al.* (1996). PCR-based detection and partial characterization of a retrovirus associated with contagious intranasal tumors of sheep and goats. *Journal of Virology*, **70**, 7580-7583.
- Cousens, C., Thonur, L., Imlach, S., Crawford, J., Sales, J., *et al.* (2009). Jaagsiekte sheep retrovirus is present at high concentration in lung fluid produced by ovine pulmonary adenocarcinoma-affected sheep and can survive for several weeks at ambient temperatures. *Research in Veterinary Science*, **87**, 154-156.
- Crittenden, L. B., Fadly, A. M., Smith, E. J. (1982). Effect of endogenous leukosis virus genes on response to infection with avian leukosis and reticuloendotheliosis viruses. *Avian Disease*, **26**, 279-294.
- Crittenden, L. B., Smith, E. J. and Fadly, A. M. (1984). Influence of endogenous viral (ev) gene expression and strain of exogenous avian leukosis virus (ALV) on mortality and ALV infection and shedding in chickens. *Avian Disease*, **28**, 1037-1056.
- Cutlip, R. C. and Young, S. (1982). Sheep pulmonary adenomatosis (jaagsiekte) in the United States. *American Journal of Veterinary Research*, **43**, 2108-2113.
- Dakessian, R. M. and Fan, H. (2008). Specific in vivo expression in type II pneumocytes of the Jaagsiekte sheep retrovirus long terminal repeat in transgenic mice. *Virology*, **372**, 398-408.
- Dalefield, R. R. and Alley, M. R. (1988). An ovine pulmonary tumour of alveolar epithelial Type II cells. *New Zealand Veterinary Journal*, **36**, 25-27.
- Danilkovitch-Miagkova, A., Duh, F. M., Kuzmin, I., Angeloni, D., Liu, S. L., *et al.* (2003). Hyaluronidase 2 negatively regulates RON receptor tyrosine kinase and mediates transformation of epithelial cells by jaagsiekte sheep retrovirus. *Proceedings of the National Academy of Sciences of the USA*, **100**, 4580-4585.
- De las Heras, M., Barsky, S. H., Hasleton, P., Wagner, M., Larson, E., *et al.* (2000). Evidence for a protein related immunologically to the jaagsiekte sheep retrovirus in some human lung tumours. *European Respiratory Journal*, **16**, 330-332.
- De las Heras, M., González, L. and Sharp, J. M. (2003a). Pathology of ovine pulmonary adenocarcinoma. *Current Topics in Microbiology and Immunology*, **275**, 25-54.
- De las Heras, M., Murcia, P., Ortín, A., Azúa, J., Borderías, L., *et al.* (2007). Jaagsiekte sheep retrovirus is not detected in human lung adenocarcinomas expressing antigens related to the Gag polyprotein of betaretroviruses. *Cancer Letters*, **258**, 22-30.
- De las Heras, M., Ortín, A., Benito, A., Summers, C., Ferrer, L. M., *et al.* (2006). In-situ demonstration of mitogen-activated protein kinase Erk 1/2 signalling pathway in contagious respiratory tumours of sheep and goats. *Journal of Comparative Pathology*, **135**, 1-10.
- De las Heras, M., Ortín, A., Cousens, C., Minguijón, E. and Sharp, J. M. (2003b). Enzootic nasal adenocarcinoma of sheep and goats. *Current Topics in Microbiology and Immunology*, **275**, 201-223.
- De las Heras, M., Ortín, A., Salvatori, D., Pérez de Villareal, M., Cousens, C., *et al.* (2005). A PCR technique for the detection of Jaagsiekte sheep retrovirus in the blood suitable for the screening of ovine pulmonary adenocarcinoma in field conditions. *Research in Veterinary Science*, **79**, 259-264.
- De Villiers, E. M. and Verwoerd, D. W. (1980). Presence of Herpesvirus ovis DNA sequences in cellular DNA from sheep lungs affected with jaagsiekte (pulmonary adenomatosis). *Onderstepoort Journal of Veterinary Research*, **47**, 109-112.
- DeMartini, J. C., Bishop, J. V., Allen, T. E., Jassim, F. A., Sharp, J. M., *et al.* (2001). Jaagsiekte sheep retrovirus proviral clone JSRV(JS7), derived from the JS7 lung tumor cell line, induces ovine pulmonary carcinoma and is integrated into the surfactant protein A gene. *Journal of Virology*, **75**, 4239-4246.
- DeMartini, J. C., Carlson, J. O., Leroux, C., Spencer, T. and Palmarini, M. (2003). Endogenous retroviruses related to jaagsiekte sheep retrovirus. *Current Topics in Microbiology and Immunology*, **275**, 117-137.
- DeMartini, J. C., Rosadio, R. H. and Lairmore, M. D. (1988). The etiology and pathogenesis of ovine pulmonary carcinoma (sheep pulmonary adenomatosis). *Veterinary Microbiology*, **17**, 219-236.
- Dirks, C., Duh, F. M., Rai, S. K., Lerman, M. I. and Miller, A. D. (2002). Mechanism of cell entry and transformation by enzootic nasal tumor virus. *Journal of Virology*, **76**, 2141-2149.
- Dungal, N. (1946). Experiments with jaagsiekte. *American Journal of Pathology*, **22**, 737-759.

- Dungal, N., Gíslason, G. and Taylow, E. L. (1938). Epizootic adenomatosis in the lungs of sheep - comparisons with jaagsiekte, verminous pneumonia and progressive pneumonia. *Journal of Comparative Pathology and Therapeutics*, **51**, 46-66.
- Dunlap, K. A., Palmarini, M. and Spencer, T. E. (2006a). Ovine endogenous betaretroviruses (enJSRVs) and placental morphogenesis. *Placenta*, **27 Suppl A**, S135-140.
- Dunlap, K. A., Palmarini, M., Varela, M., Burghardt, R. C., Hayashi, K., *et al.* (2006b). Endogenous retroviruses regulate periimplantation placental growth and differentiation. *Proceedings of the National Academy of Sciences of the USA*, **103**, 14390-14395.
- Dupressoir, A., Vernochet, C., Bawa, O., Harper, F., Pierron, G., *et al.* (2009). Syncytin-A knockout mice demonstrate the critical role in placentation of a fusogenic, endogenous retrovirus-derived, envelope gene. *Proceedings of the National Academy of Sciences of the USA*, **106**, 12127-12132.
- Dykes, R. J. and McFadyean, J. (1888). Lung disease in sheep, caused by the *Strongylus Rufescens*. *Journal of Comparative Pathology and Therapeutics*, **1**, 139-146.
- Fan, H. (2003). Jaagsiekte sheep retrovirus and lung cancer. *Current Topics in Microbiology and Immunology*, **275**. Springer-Verlag, Berlin.
- Fan, H., Palmarini, M. and DeMartini, J. C. (2003). Transformation and oncogenesis by jaagsiekte sheep retrovirus. *Current Topics in Microbiology and Immunology*, **275**, 139-177.
- Garber, M. E., Troyanskaya, O. G., Schluens, K., Petersen, S., Thaessler, Z., *et al.* (2001). Diversity of gene expression in adenocarcinoma of the lung. *Proceedings of the National Academy of Sciences of the USA*, **98**, 13784-13789.
- García-Goti, M., González, L., Cousens, C., Cortabarría, N., Extramiana, A. B., *et al.* (2000). Sheep pulmonary adenomatosis: characterization of two pathological forms associated with jaagsiekte retrovirus. *Journal of Comparative Pathology*, **122**, 55-65.
- González, L., García-Goti, M., Cousens, C., Dewar, P., Cortabarría, N., *et al.* (2001). Jaagsiekte sheep retrovirus can be detected in the peripheral blood during the pre-clinical period of sheep pulmonary adenomatosis. *Journal of General Virology*, **82**, 1355-1358.
- Grego, E., De Meneghi, D., Alvarez, V., Benito, A. A., Minguijón, E., *et al.* (2008). Colostrum and milk can transmit jaagsiekte retrovirus to lambs. *Veterinary Microbiology*, **130**, 247-257.
- Groom, H. C., Anderson, E. C. and Lever, A. M. (2009). Rev: beyond nuclear export. *Journal of General Virology*, **90**, 1303-1318.
- Hanahan, D. and Weinberg, R. A. (2000). The hallmarks of cancer. *Cell*, **100**, 57-70.
- Haraguchi, S., Good, R. A. and Day, N. K. (1995). Immunosuppressive retroviral peptides: cAMP and cytokine patterns. *Immunology Today*, **16**, 595-603.
- Hayes, D. N., Monti, S., Parmigiani, G., Gilks, C. B., Naoki, K., *et al.* (2006). Gene expression profiling reveals reproducible human lung adenocarcinoma subtypes in multiple independent patient cohorts. *Journal of Clinical Oncology*, **24**, 5079-5090.
- Hiatt, K. M. and Highsmith, W. E. (2002). Lack of DNA evidence for jaagsiekte sheep retrovirus in human bronchioloalveolar carcinoma. *Human Pathology*, **33**, 680.
- Hofacre, A. and Fan, H. (2004). Multiple domains of the Jaagsiekte sheep retrovirus envelope protein are required for transformation of rodent fibroblasts. *Journal of Virology*, **78**, 10479-10489.
- Hofacre, A., Nitta, T. and Fan, H. (2009). Jaagsiekte sheep retrovirus encodes a regulatory factor Rej required for synthesis of Gag protein. *Journal of Virology*, **83**, 12483-12498.
- Holland, M. J., Palmarini, M., Garcia-Goti, M., Gonzalez, L., McKendrick, I., *et al.* (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. *Journal of Virology*, **73**, 4004-4008.
- Hooper, P. T. and Ellard, K. A. (1995). A pulmonary adenoma in an Australian sheep. *Australian Veterinary Journal*, **72**, 477.
- Hunter, A. R. and Munro, R. (1983). The diagnosis, occurrence and distribution of sheep pulmonary adenomatosis in Scotland 1975 to 1981. *British Veterinary Journal*, **139**, 153-164.
- Jackson, E. L., Willis, N., Mercer, K., Bronson, R. T., Crowley, D., *et al.* (2001). Analysis of lung tumor initiation and progression using conditional expression of oncogenic K-ras. *Genes and Development*, **15**, 3243-3248.
- Kim, C. F., Jackson, E. L., Woolfenden, A. E., Lawrence, S., Babar, I., *et al.* (2005). Identification of bronchioalveolar stem cells in normal lung and lung cancer. *Cell*, **121**, 823-835.
- Krauss, H. and Wandera, J. G. (1970). Isolation and properties of mycoplasma from respiratory tract of sheep with Jaagsiekte in Kenya. *Journal of Comparative Pathology*, **80**, 389-397.

- Kwang, J., Keen, J., Rosati, S. and Tolari, F. (1995). Development and application of an antibody Elisa for the marker protein of ovine pulmonary-carcinoma. *Veterinary Immunology and Immunopathology*, **47**, 323-331.
- Leroux, C., Girard, N., Cottin, V., Greenland, T., Mornex, J. F., *et al.* (2007). Jaagsiekte Sheep Retrovirus (JSRV): from virus to lung cancer in sheep. *Veterinary Research*, **38**, 211-228.
- Lewis, F.I., Brülisauer, F., Cousens, C., McKendrick, I.J., Gunn G.J. Diagnostic accuracy of PCR for Jaagsiekte sheep retrovirus using field data from 125 Scottish sheep flocks. *The Veterinary Journal*. Published ahead of print, 20 November 2009, doi:10.1016/j.tvjl.2009.10.024.
- Li, W. H., Gu, Z., Wang, H. and Nekrutenko, A. (2001). Evolutionary analyses of the human genome. *Nature*, **409**, 847-849.
- Linial, M., Fan, H., Hahn, B., Löwer, R., Neil, J., *et al.* (2005). Retroviridae. In: *Virus Taxonomy. Eighth Report of the International Committee on Taxonomy of Viruses.*, C. M. Fauquet, M. A. Mayo, J. Maniloff, U. Desselberger and L. A. Ball, Eds, Elsevier Academic Press, San Diego (CA), pp. 421-440.
- Liu, S. L., Lerman, M. I. and Miller, A. D. (2003). Putative phosphatidylinositol 3-kinase (PI3K) binding motifs in ovine betaretrovirus Env proteins are not essential for rodent fibroblast transformation and PI3K/Akt activation. *Journal of Virology*, **77**, 7924-7935.
- Liu, S. L. and Miller, A. D. (2005). Transformation of madin-darby canine kidney epithelial cells by sheep retrovirus envelope proteins. *Journal of Virology*, **79**, 927-933.
- Liu, S. L. and Miller, A. D. (2007). Oncogenic transformation by the jaagsiekte sheep retrovirus envelope protein. *Oncogene*, **26**, 789-801.
- Lynch, T. J., Bell, D. W., Sordella, R., Gurubhagavatula, S., Okimoto, R. A., *et al.* (2004). Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib. *New England Journal of Medicine*, **350**, 2129-2139.
- Mackay, J. M. (1969). Tissue culture studies of sheep pulmonary adenomatosis (jaagsiekte). II. Transmission of cytopathic effects to normal cultures. *Journal of Comparative Pathology*, **79**, 147-154.
- Mackay, J. M. and Nisbet, D. I. (1966). Jaagsiekte - a hazard of intensified sheep husbandry. *Veterinary Record*, **78**, 18-24.
- Mackay, J. M., Nisbet, D. I. and Slee, J. (1971). A high incidence of jaagsiekte following experimental cold exposures in housed sheep. *International Journal of Biometeorology*, **15**, 65-69.
- Maeda, N. and Fan, H. (2008). Signal transduction pathways utilized by enzootic nasal tumor virus (ENTV-1) envelope protein in transformation of rat epithelial cells resemble those used by jaagsiekte sheep retrovirus. *Virus Genes*, **36**, 147-155.
- Maeda, N., Fan, H. and Yoshikai, Y. (2008). Oncogenesis by retroviruses: old and new paradigms. *Reviews in Medical Virology*, **18**, 387-405.
- Maeda, N., Fu, W., Ortín, A., De las Heras, M. and 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. *Journal of Virology*, **79**, 4440-4450.
- Maeda, N., Palmarini, M., Murgia, C. and Fan, H. (2001). Direct transformation of rodent fibroblasts by jaagsiekte sheep retrovirus DNA. *Proceedings of the National Academy of Sciences of the USA*, **98**, 4449-4454.
- Malmquist, W. A., Krauss, H. H., Moulton, J. E. and Wandera, J. G. (1972). Morphologic study of virus-infected lung cell cultures from sheep pulmonary adenomatosis (Jaagsiekte). *Laboratory Investigation*, **26**, 528-533.
- Martin, W. B., Scott, F. M., Sharp, J. M., Angus, K. W. and Norval, M. (1976). Experimental production of sheep pulmonary adenomatosis (Jaagsiekte). *Nature*, **264**, 183-185.
- McFadyean, J. (1920). Transformation of the alveolar epithelium in verminous pneumonia in the sheep. *Journal of Comparative Pathology and Therapeutics*, **33**, 5-10.
- McGee-Estrada, K. and Fan, H. (2006). In vivo and in vitro analysis of factor binding sites in Jaagsiekte sheep retrovirus long terminal repeat enhancer sequences: roles of HNF-3, NF-I, and C/EBP for activity in lung epithelial cells. *Journal of Virology*, **80**, 332-341.
- McGee-Estrada, K. and Fan, H. (2007). Comparison of LTR enhancer elements in sheep beta retroviruses: insights into the basis for tissue-specific expression. *Virus Genes*, **35**, 303-312.
- McGee-Estrada, K., Palmarini, M. and 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. and 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. (2008). Hyaluronidase 2 and its intriguing role as a cell-entry receptor for oncogenic sheep retroviruses. *Seminars in Cancer Biology*, **18**, 296-301.

- Mornex, J. F., Thivolet, F., De las Heras, M. and Leroux, C. (2003). Pathology of human bronchioloalveolar carcinoma and its relationship to the ovine disease. *Current Topics in Microbiology and Immunology*, **275**, 225-248.
- Morozov, V. A., Lagaye, S., Löwer, J. and Löwer, 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., *et al.* (2004). Late viral interference induced by transdominant Gag of an endogenous retrovirus. *Proceedings of the National Academy of Sciences of the USA*, **101**, 11117-11122.
- Murcia, P. R., Arnaud, F. and Palmarini, M. (2007). The transdominant endogenous retrovirus enJS56A1 associates with and blocks intracellular trafficking of Jaagsiekte sheep retrovirus Gag. *Journal of Virology*, **81**, 1762-1772.
- Nair, V. (2005). Evolution of Marek's disease - a paradigm for incessant race between the pathogen and the host. *Veterinary Journal*, **170**, 175-183.
- Nobel, T. A., Neumann, F. and Klopfer, U. (1969). Histological patterns of the metastases in pulmonary adenomatosis of sheep (jaagsiekte). *Journal of Comparative Pathology*, **79**, 537-540.
- Ortín, A., Cousens, C., Minguijón, E., Pascual, Z., Pérez de Villarreal, M., *et al.* (2003). Characterization of enzootic nasal tumour virus of goats: complete sequence and tissue distribution. *Journal of General Virology*, **84**, 2245-2252.
- Ortín, A., Minguijón, E., Dewar, P., García, M., Ferrer, L. M., *et al.* (1998). Lack of a specific immune response against a recombinant capsid protein of Jaagsiekte sheep retrovirus in sheep and goats naturally affected by enzootic nasal tumour or sheep pulmonary adenomatosis. *Veterinary Immunology and Immunopathology*, **61**, 229-237.
- Ortín, A., Pérez de Villarreal, M., Minguijón, E., Cousens, C., Sharp, J. M., *et al.* (2004). Coexistence of enzootic nasal adenocarcinoma and jaagsiekte retrovirus infection in sheep. *Journal of Comparative Pathology*, **131**, 253-258.
- Overbaugh, J. and Bangham, C. R. (2001). Selection forces and constraints on retroviral sequence variation. *Science*, **292**, 1106-1109.
- Palmarini, M., Cousens, C., Dalziel, R. G., Bai, J., Stedman, K., *et al.* (1996). The exogenous form of Jaagsiekte retrovirus is specifically associated with a contagious lung cancer of sheep. *Journal of Virology*, **70**, 1618-1623.
- Palmarini, M., Datta, S., Omid, R., Murgia, C. and Fan, H. (2000a). The long terminal repeat of Jaagsiekte sheep retrovirus is preferentially active in differentiated epithelial cells of the lungs. *Journal of Virology*, **74**, 5776-5787.
- Palmarini, M., Dewar, P., De las Heras, M., Inglis, N. F., Dalziel, R. G., *et al.* (1995). Epithelial tumour cells in the lungs of sheep with pulmonary adenomatosis are major sites of replication for Jaagsiekte retrovirus. *Journal of General Virology*, **76**, 2731-2737.
- Palmarini, M. and Fan, H. (2001). Retrovirus-induced ovine pulmonary adenocarcinoma, an animal model for lung cancer. *Journal of the National Cancer Institutes*, **93**, 1603-1614.
- Palmarini, M., Gray, C. A., Carpenter, K., Fan, H., Bazer, F. W., *et al.* (2001a). Expression of endogenous betaretroviruses in the ovine uterus: effects of neonatal age, estrous cycle, pregnancy, and progesterone. *Journal of Virology*, **75**, 11319-11327.
- Palmarini, M., Hallwirth, C., York, D., Murgia, C., de Oliveira, T., *et al.* (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. *Journal of Virology*, **74**, 8065-8076.
- Palmarini, M., Maeda, N., Murgia, C., De-Fraja, C., Hofacre, A., *et al.* (2001b). 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. *Journal of Virology*, **75**, 11002-11009.
- Palmarini, M., Mura, M. and Spencer, T. E. (2004). Endogenous betaretroviruses of sheep: teaching new lessons in retroviral interference and adaptation. *Journal of General Virology*, **85**, 1-13.
- Palmarini, M., Murgia, C. and Fan, H. (2002). Spliced and prematurely polyadenylated Jaagsiekte sheep retrovirus-specific RNAs from infected or transfected cells. *Virology*, **294**, 180-188.
- Palmarini, M., Sharp, J. M., De las Heras, M. and Fan, H. (1999). Jaagsiekte sheep retrovirus is necessary and sufficient to induce a contagious lung cancer in sheep. *Journal of Virology*, **73**, 6964-6972.
- Pálsson, P. A. (1985). Maedi/visna of sheep in Iceland, introduction of the disease in Iceland, clinical features, control measures and eradication. In: *Slow viruses in sheep, goats, and cattle. The Commission of the European Communities publication EUR 8076 EN.*, J. M. Sharp and R. Hoff-Jørgensen, Eds, The Commission of the European Communities. Luxembourg, pp. 3-19.
- Parker, B. N., Wrathall, A. E., Saunders, R. W., Dawson, M., Done, S. H., *et al.* (1998). Prevention of transmission of sheep pulmonary adenomatosis by embryo transfer. *Veterinary Record*, **142**, 687-689.
- Payne, A. L. and Verwoerd, D. W. (1984). A scanning and transmission electron microscopy study of jaagsiekte lesions. *Onderstepoort Journal of Veterinary Research*, **51**, 1-13.

- Payne, A. L., Verwoerd, D. W. and Garnett, H. M. (1983). The morphology and morphogenesis of jaagsiekte retrovirus (JSRV). *Onderstepoort Journal of Veterinary Research*, **50**, 317-322.
- Perk, K. and Hod, I. (1982). Sheep lung carcinoma: an endemic analogue of a sporadic human neoplasm. *Journal of the National Cancer Institutes*, **69**, 747-749.
- Perk, K., Hod, I. and Nobel, T. A. (1971). Pulmonary adenomatosis of sheep (jaagsiekte). I. Ultrastructure of the tumor. *Journal of the National Cancer Institutes*, **46**, 525-537.
- Perk, K., Michalides, R., Spiegelman, S. and Schlom, J. (1974). Biochemical and morphologic evidence for the presence of an RNA tumor virus in pulmonary carcinoma of sheep (Jaagsiekte). *Journal of the National Cancer Institutes*, **53**, 131-135.
- Platt, J. A., Kraipowich, N., Villafane, F. and 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. *Veterinary Pathology*, **39**, 341-352.
- Rai, S. K., Duh, F. M., Vigdorovich, V., Danilkovitch-Miagkova, A., Lerman, M. I., *et al.* (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. *Proceedings of the National Academy of Sciences of the USA*, **98**, 4443-4448.
- Rajan, A. (1987). Carcinoma of the mucosa of the ethmoid in domestic animals. *Annales de recherches vétérinaires.*, **18**, 13-17.
- Raz, D. J., He, B., Rosell, R. and Jablons, D. M. (2006). Current concepts in bronchioloalveolar carcinoma biology. *Clinical Cancer Research*, **12**, 3698-3704.
- Rocca, S., Sanna, M. P., Leoni, A., Cossu, A., Lissia, A., *et al.* (2008). Presence of Jaagsiekte sheep retrovirus in tissue sections from human bronchioloalveolar carcinoma depends on patients' geographical origin. *Human Pathology*, **39**, 303-304.
- Rosadio, R. H., Lairmore, M. D., Russell, H. I. and DeMartini, J. C. (1988). Retrovirus-associated ovine pulmonary carcinoma (sheep pulmonary adenomatosis) and lymphoid interstitial pneumonia. I. Lesion development and age susceptibility. *Veterinary Pathology*, **25**, 475-483.
- Rosati, S., Pittau, M., Alberti, A., Pozzi, S., York, D. F., *et al.* (2000). An accessory open reading frame (orf-x) of jaagsiekte sheep retrovirus is conserved between different virus isolates. *Virus Research*, **66**, 109-116.
- Rosenberg, N. and Jolicoeur, P. (1997). Retroviral Pathogenesis. In: *Retroviruses*, J. M. Coffin, S. H. Hughes and H. E. Varmus, Eds, Cold Spring Harbor Laboratory Press, New York, pp. 475-585.
- Rouse, B. T., Sarangi, P. P. and Suvas, S. (2006). Regulatory T cells in virus infections. *Immunological Reviews*, **212**, 272-286.
- Salvatori, D. (2004). Studies of the pathogenesis and epidemiology of ovine pulmonary adenocarcinoma., PhD Thesis, University of Edinburgh.
- Salvatori, D., Gonzalez, L., Dewar, P., Cousens, C., De las Heras, M., *et al.* (2004). Successful induction of ovine pulmonary adenocarcinoma in lambs of different ages and detection of viraemia during the preclinical period. *Journal of General Virology*, **85**, 3319-3324.
- Scott, P. R. and Gessert, M. E. (1998). Ultrasonographic examination of the ovine thorax. *Veterinary Journal*, **155**, 305-310.
- Scott, P., D., Collie, D., McGorum, B., Sargison, N. (2009). Relationship between thoracic auscultation and lung pathology detected by ultrasonography in sheep. *The Veterinary Journal*. Published ahead of print, 2 September 2009, doi:10.1016/j.tvjl.2009.07.020.
- Sharp, J. M., Angus, K. W., Gray, E. W. and Scott, F. M. (1983). Rapid transmission of sheep pulmonary adenomatosis (jaagsiekte) in young lambs. *Archives of Virology*, **78**, 89-95.
- Sharp, J. M. and DeMartini, J. C. (2003). Natural history of JSRV in sheep. *Current Topics in Microbiology and Immunology*, **275**, 55-79.
- Sharp, J. M. and Herring, A. J. (1983). Sheep pulmonary adenomatosis: demonstration of a protein which cross-reacts with the major core proteins of Mason-Pfizer monkey virus and mouse mammary tumour virus. *Journal of General Virology*, **64**, 2323-2327.
- Sims, J. L. (1943). Multiple bilateral pulmonary adenomatosis in man. *Archives of Internal Medicine*, **71**, 403-409.
- Spencer, T. E., Mura, M., Gray, C. A., Griebel, P. J. and Palmarini, M. (2003). Receptor usage and fetal expression of ovine endogenous betaretroviruses: implications for coevolution of endogenous and exogenous retroviruses. *Journal of Virology*, **77**, 749-753.
- Stripp, B. R. and Reynolds, S. D. (2008). Maintenance and repair of the bronchiolar epithelium. *Proceedings of the American Thoracic Society*, **5**, 328-333.

- Suau, F., Cottin, V., Archer, F., Croze, S., Chastang, J., *et al.* (2006). Telomerase activation in a model of lung adenocarcinoma. *European Respiratory Journal*, **27**, 1175-1182.
- Summers, C., Dewar, P., van der Molen, R., Cousens, C., Salvatori, D., *et al.* (2006). Jaagsiekte sheep retrovirus-specific immune responses induced by vaccination: a comparison of immunisation strategies. *Vaccine*, **24**, 1821-1829.
- Summers, C., Neill, W., Dewar, P., Gonzalez, L., van der Molen, R., *et al.* (2002). Systemic immune responses following infection with Jaagsiekte sheep retrovirus and in the terminal stages of ovine pulmonary adenocarcinoma. *Journal of General Virology*, **83**, 1753-1757.
- Summers, C., Norval, M., De las Heras, M., Gonzalez, L., Sharp, J. M., *et al.* (2005). An influx of macrophages is the predominant local immune response in ovine pulmonary adenocarcinoma. *Veterinary Immunology and Immunopathology*, **106**, 285-294.
- Sun, S., Schiller, J. H. and Gazdar, A. F. (2007). Lung cancer in never smokers - a different disease. *Nature Reviews Cancer*, **7**, 778-790.
- Synge, B., Hosie, B. and Pritchard, I. (2008). Changes to the SAC's maedi-visna accreditation scheme. *Veterinary Record*, **162**, 796.
- Takeuchi, T., Tomida, S., Yatabe, Y., Kosaka, T., Osada, H., *et al.* (2006). Expression profile-defined classification of lung adenocarcinoma shows close relationship with underlying major genetic changes and clinicopathologic behaviors. *Journal of Clinical Oncology*, **24**, 1679-1688.
- Tamura, K., Dudley, J., Nei, M., and Kumar, S. (2007). MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. *Molecular Biology and Evolution* **24**, 1596-1599.
- Tarlinton, R. E., Meers, J. and Young, P. R. (2006). Retroviral invasion of the koala genome. *Nature*, **442**, 79-81.
- Taylor, E. L. (1938). The lungworm theory for the origin of epizootic adenomatosis and the question of the existence of adenomatosis in Great Britain. *Proceedings of the Royal Society of Medicine*, **31**, 505-508.
- Teisanu, R. M., Lagasse, E., Whitesides, J. F. and Stripp, B. R. (2009). Prospective isolation of bronchiolar stem cells based upon immunophenotypic and autofluorescence characteristics. *Stem Cells*, **27**, 612-622.
- Travis, W. D., Garg, K., Franklin, W. A., Wistuba, II, Sabloff, B., *et al.* (2006). Bronchioloalveolar carcinoma and lung adenocarcinoma: the clinical importance and research relevance of the 2004 World Health Organization pathologic criteria. *Journal of Thoracic Oncology*, **1**, S13-19.
- Tustin, R. C. (1969). Ovine Jaagsiekte. *Journal of the South African Veterinary Medicine Association*, **40**, 3-23.
- Van Hoesen, N. S. and Miller, A. D. (2005). Improved enzootic nasal tumor virus pseudotype packaging cell lines reveal virus entry requirements in addition to the primary receptor Hyal2. *Journal of Virology*, **79**, 87-94.
- Varela, M., Golder, M., Archer, F., De las Heras, M., Leroux, C., *et al.* (2007). A large animal model to evaluate the effects of Hsp90 inhibitors for the treatment of lung adenocarcinoma. *Virology*, **371**, 206-215.
- Vargas, A., Moreau, J., Landry, S., LeBellego, F., Toufaily, C., *et al.* (2009). Syncytin-2 plays an important role in the fusion of human trophoblast cells. *Journal of Molecular Biology*, **392**, 301-318.
- Verwoerd, D. W., Williamson, A. L. and De Villiers, E. M. (1980). Aetiology of jaagsiekte: transmission by means of subcellular fractions and evidence for the involvement of a retrovirus. *Onderstepoort Journal of Veterinary Research*, **47**, 275-280.
- Veterinary Laboratories Agency. (2007). Website report. http://www.defra.gov.uk/vla/reports/docs/rep_vida07_intro.pdf
- Voigt, K., Brugmann, M., Huber, K., Dewar, P., Cousens, C., *et al.* (2007a). PCR examination of bronchoalveolar lavage samples is a useful tool in pre-clinical diagnosis of ovine pulmonary adenocarcinoma (Jaagsiekte). *Research in Veterinary Science*, **83**, 419-427.
- Voigt, K., Kramer, U., Brugmann, M., Dewar, P., Sharp, J. M., *et al.* (2007b). Eradication of ovine pulmonary adenocarcinoma by motherless rearing of lambs. *Veterinary Record*, **161**, 129-132.
- Wandera, J. G. (1970). Clinical pulmonary adenomatosis of sheep produced experimentally. *British Veterinary Journal*, **126**, 185-193.
- Watson, W. L. and Smith, R. R. (1951). Terminal bronchiolar or "alveolar cell" cancer of the lung: Report of thirty-three cases. *Journal of the American Medical Association*, **147**, 7-13.
- Weiss, R. A. (2006). The discovery of endogenous retroviruses. *Retrovirology*, **3**, 67.
- Wolf, D. and Goff, S. P. (2009). Embryonic stem cells use ZFP809 to silence retroviral DNAs. *Nature*, **458**, 1201-1204.
- Wootton, S. K., Halbert, C. L. and Miller, A. D. (2005). Sheep retrovirus structural protein induces lung tumours. *Nature*, **434**, 904-907.

- Wootton, S. K., Halbert, C. L. and Miller, A. D. (2006a). Envelope proteins of Jaagsiekte sheep retrovirus and enzootic nasal tumor virus induce similar bronchioalveolar tumors in lungs of mice. *Journal of Virology*, **80**, 9322-9325.
- Wootton, S. K., Metzger, M. J., Hudkins, K. L., Alpers, C. E., York, D., *et al.* (2006b). Lung cancer induced in mice by the envelope protein of jaagsiekte sheep retrovirus (JSRV) closely resembles lung cancer in sheep infected with JSRV. *Retrovirology*, **3**, 94.
- Wright, N. A. and Alison, M. (1984). Cell proliferation in respiratory epithelia. In: *The Biology of Epithelial Cell Populations: Volume 2*, Clarendon Press, Oxford, pp. 1068-1078.
- York, D. F. and Querat, G. (2003). A history of ovine pulmonary adenocarcinoma (jaagsiekte) and experiments leading to the deduction of the JSRV nucleotide sequence. *Current Topics in Microbiology and Immunology*, **275**, 1-23.
- York, D. F., Vigne, R., Verwoerd, D. W. and Querat, G. (1991). Isolation, identification, and partial cDNA cloning of genomic RNA of jaagsiekte retrovirus, the etiological agent of sheep pulmonary adenomatosis. *Journal of Virology*, **65**, 5061-5067.
- York, D. F., Vigne, R., Verwoerd, D. W. and Querat, G. (1992). Nucleotide sequence of the jaagsiekte retrovirus, an exogenous and endogenous type D and B retrovirus of sheep and goats. *Journal of Virology*, **66**, 4930-4939.
- Yousem, S. A., Finkelstein, S. D., Swalsky, P. A., Bakker, A. and Ohori, N. P. (2001). Absence of jaagsiekte sheep retrovirus DNA and RNA in bronchioalveolar and conventional human pulmonary adenocarcinoma by PCR and RT-PCR analysis. *Human Pathology*, **32**, 1039-1042.
- Zavala, G., Pretto, C., Chow, Y. H., Jones, L., Alberti, A., *et al.* (2003). Relevance of Akt phosphorylation in cell transformation induced by Jaagsiekte sheep retrovirus. *Virology*, **312**, 95-105.