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Article

Ovine Pulmonary Adenocarcinoma: A Large Animal Model for Human Lung Cancer

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Abstract

Lung cancer is the leading cause of cancer deaths worldwide. Recent progress in understanding the molecular pathogenesis of this disease has resulted in novel therapeutic strategies targeting specific groups of patients. Further studies are required to provide additional advances in diagnosis and treatment. Animal models are valuable tools for studying oncogenesis in lung cancer, particularly during the early stages of disease where tissues are rarely available from human cases. Mice have traditionally been used for studying lung cancer in vivo, and a variety of spontaneous and transgenic models are available. However, it is recognized that other species may also be informative for studies of cancer. Ovine pulmonary adenocarcinoma (OPA) is a naturally occurring lung cancer of sheep caused by retrovirus infection and has several features in common with adenocarcinoma of humans, including a similar histological appearance and activation of common cell signaling pathways. Additionally, the size and organization of human lungs are much closer to those of sheep lungs than to those of mice, which facilitates experimental approaches in sheep that are not available in mice. Thus OPA presents opportunities for studying lung tumor development that can complement conventional murine models. Here we describe the potential applications of OPA as a model for human lung adenocarcinoma with an emphasis on the various in vivo and in vitro experimental systems available.

Key words: animal models; cancer model; jaagsiekte; JSRV; lung cancer; NSCLC; OPA; ovine pulmonary adenocarcinoma

Introduction

Lung cancer is the most common cancer worldwide with an estimated 1.8 million new cases per year, of which over 85% are fatal within 5 years of diagnosis (Ferlay et al. 2015). The high mortality rate of lung cancer is due predominantly to the advanced stage at which diagnosis is made, which restricts the range and effectiveness of treatments that can be used. In addition, the disease is highly heterogeneous, with several clinical subtypes currently recognized. While tobacco smoking is the most significant environmental factor associated with lung cancer, the incidence of the disease among those who have never smoked is increasing and represents around 10%–15% of cases (Sun et al. 2007; Torok et al. 2011). Importantly, lung tumors of smokers and never-smokers have distinct histological and clinical features and exhibit divergent mutational profiles (Krishnan et al. 2014).

The significant mortality associated with lung cancer demonstrates the need for continued research to improve understanding of the causes of this disease and to define the molecular mechanisms involved in driving oncogenesis. Understanding the pathological changes that occur in early pre-metastatic tumors could help to identify novel biomarkers for the early detection of lesions, as well as prognostic markers to predict survival outcome.

Experimental animal models are valuable tools for studying pathogenic mechanisms in lung cancer, particularly in the early stages of disease when human tissue samples are rarely available. In vitro studies using cell lines have been important in characterizing genetic mutations in cancer, and animal models provide a tool for studying these pathways in the complex environment of a whole organism. Murine models have been widely used to study cancer pathogenesis, but it is recognized that there are a number of limitations in using mice for studying human disease, and alternative species may have some advantages (Flisikowska et al. 2013; Harding et al. 2013). In the case of lung cancer, an alternative to mice is the sheep, which has been exploited as a model species for several other pulmonary diseases due to the anatomical similarity of ovine and human lungs (Scheerlinck et al. 2008). Here we discuss the potential value of ovine pulmonary adenocarcinoma (OPA), a naturally occurring neoplastic disease of sheep, as a model for understanding lung cancer (Palmarini and Fan 2001).

Mouse Models for Lung Cancer

A variety of murine models for lung cancer have been described (reviewed in Dutt and Wong 2006; Vikis et al. 2013). The earliest studies used inbred mouse strains that exhibit a high frequency of spontaneous lung neoplasms or that reproducibly develop tumors following exposure to tobacco smoke (Hoenerhoff et al. 2009). In addition, transgenic murine models have been generated for various driver mutations common to lung cancer, and multiple mutations can be introduced to better phenocopy the human disease (Kwon and Berns 2013; Meuwissen and Berns 2005). Moreover, fluorescent reporters under the control of cell-specific promoters can be introduced to perform lineage tracing. This approach has been valuable in dissecting the involvement of individual oncogenes in tumorigenesis (Kim et al. 2005) and in defining specific cell types as the origin of lung cancer (Desai et al. 2014; Mainardi et al. 2014; Sutherland et al. 2014). Patient-derived xenografts can also be grafted onto nude athymic mice, and this has been particularly useful in preclinical drug testing (Kelland 2004; Moro et al. 2012). However, despite their advantages, murine models are a poor predictor of clinical outcome and do not always mimic the advanced stages of the disease very well.

Sheep as a Model Species for Human Lung Disease

Sheep have attracted attention as a model for studies of pulmonary function and disease for several reasons. First, the anatomy of the sheep lung is closer to that of humans than is the mouse lung. The pattern of airway branching and the transition between bronchioles and alveoli is more similar, as is the distribution of important differentiated respiratory epithelial cell types. This may be particularly relevant for studies in neonates, because human and sheep lungs are closer developmentally at birth than are those of humans and mice (Ackermann 2014). Second, the similar size of ovine and human lungs provides several opportunities not available in mouse models, for example, in tumor imaging (Viard et al. 2008) and transplantation models (Radu et al. 2010). In addition, the larger size of the sheep provides opportunities for repeated blood and tissue sampling in longitudinal studies and for collection of larger samples (e.g., for identification of circulating tumor markers). Moreover, the ability to use bronchoscopic instillation in the sheep permits the experimental design to include repeated treatment and sampling from the same site within the lung in a way that would not be possible with mice (Collie et al. 2013). These factors have led to sheep being adopted as a model species for a number of diseases, including asthma (Meeusen et al. 2009), cystic fibrosis (McLachlan et al. 2011) and respiratory syncytial virus infection (Ackermann 2014), as well as for stem cell therapy (Harding et al. 2013). For cancer studies, it may be possible to initiate multiple tumors in different lobes of the lung, potentially allowing complex interactions with components of the immune system to be examined. In addition, the introduction of multiple distinct mutations in the ovine lung may more faithfully reflect lung cancer heterogeneity.

There are, however, some disadvantages in using sheep. For example, containment facilities capable of handling large animals are not widely available and are expensive to maintain, and the number of animals that can be handled at any one time is more restrictive compared with small rodents. In addition, the range of commercially available reagents such as antibodies and off-the-shelf assays that can be used with sheep is more limited than for mice. However, the recent publication and improved annotation of the sheep genome sequence (Jiang et al. 2014) should lead to improved availability of such reagents and facilitate future studies. Similarly, the production of transgenic sheep is now an established technology, and the use of genome editing techniques has been described in this species (Proudfoot et al. 2014). Thus the practical disadvantages of sheep as an experimental model are not insurmountable and, depending on the research question, the potential benefits may outweigh the technical difficulties.

Ovine Pulmonary Adenocarcinoma

OPA is a naturally occurring neoplastic lung disease of sheep caused by jaagsiekte sheep retrovirus (JSRV) (Griffiths et al. 2010; Leroux et al. 2007). The disease was first recorded in South Africa in the early 19th century, where it was given the name *jaagsiekte*, derived from the Afrikaans for “chasing sickness” (*jaag-siekte*) (York and Querat 2003). Today, OPA is a significant economic and animal welfare problem for the sheep industry in many countries. Importantly, OPA appears to be highly specific for sheep, occasionally also affecting other small ruminants such as goats and mouflon but not other livestock species or humans (De las Heras et al. 2003; Sanna et al. 2001).

Natural cases of OPA typically appear as a progressive debilitating respiratory disease in which affected sheep struggle to recover after exercise, for example, during herding. The disease may occur in sheep of all ages, but is most commonly described in adult sheep of 2 to 4 years of age (Sharp and DeMartini 2003). The lungs of sheep clinically affected with OPA commonly have one or more large tumor masses that occupy a significant proportion of the total lung volume (Griffiths et al. 2010; Sharp and DeMartini 2003). A striking clinical feature of OPA is the excessive production of fluid in the lung, which may be discharged from the nose when the head is lowered. The appearance of lung fluid has been used as a crude method for diagnosing OPA in sheep for many decades and remains a useful, but inconsistent, antemortem diagnostic test. Lung fluid contains a high concentration of JSRV (Cousens et al. 2009) and therefore provides an efficient means to shed the virus into the environment and promote its transmission to other sheep. Similar to human lung cancer, OPA tumors may take many months or years to become clinically apparent. Many affected sheep do not develop clinical signs of respiratory disease during their commercial lifespan; however, such animals are still able to transmit JSRV to other sheep.

OPA is spread predominantly through the respiratory route via the inhalation of JSRV. JSRV DNA has also been detected in milk and colostrum of ewes, suggesting that lambs may also become infected when suckling (Grego et al. 2008). The virus can infect various cell types within the sheep, including B and T lymphocytes and myeloid cells (Holland et al. 1999). However, in these cell types, the expression of viral RNA and protein occurs at a very low level. In contrast, infection of respiratory epithelial cells in the distal lung results in a high level of virus production. OPA tumors are thought to arise predominantly from type II alveolar pneumocytes and possibly also from bronchiolar club cells (formerly called Clara cells) (De las Heras et al. 2014; Martineau et al. 2011; Murgia et al. 2011; Platt et al. 2002). Transformation of these cells results in the growth of tumors that spread along alveolar septa and within bronchioles. The infected cells release newly assembled JSRV virions, which spread within the lung to form new foci of infection and transformation, resulting in a highly polyclonal tumor (Cousens et al. 2004). These multiple tumor foci expand and coalesce to form the large OPA tumors observed in clinical cases. The respiratory distress caused by tumor growth and excess lung fluid is further exacerbated by secondary bacterial lung infections, which are a common finding in advanced cases of OPA. Once the advanced clinical signs of fluid production are evident, the disease typically results in death within a few weeks.

Jaagsiekte Sheep Retrovirus

JSRV has a structure and genetic organization similar to that of other retroviruses (Figure 1). The RNA genome is approximately 7460 nucleotides long. The central part of the genome contains the canonical retroviral genes *gag*, which encodes the structural proteins of the virus capsid (MA, CA, NC); *pro* and *pol*, which encode the viral enzymes, including an aspartic protease (PR), reverse transcriptase (RT), and integrase (IN); and *env*, which encodes the envelope glycoproteins (SU and TM) present on the surface of the virus particle. The JSRV proteins are expressed initially as large polyproteins but are subsequently cleaved to their mature forms by the viral protease (Gag and Pol) or by cellular furin (Env) (Griffiths et al. 2010). An additional open reading frame overlapping *pol*, named *orf-x*, is unique to JSRV, and its function is currently unknown. A small regulatory protein denoted Rej is also produced from the *env* gene by alternative splicing or following protease cleavage of the Env protein and has a role in promoting virus production (Caporale et al. 2009; Hofacre et al. 2009). Viral protein expression is driven by promoter and enhancer elements present at the 5′ and 3′ ends of the genome within a region known as the long terminal repeat (LTR).

The replication strategy of JSRV is characterized by reverse transcription of the viral RNA genome into a double-stranded DNA form that is subsequently integrated into the chromosomal DNA of the target cell (described in Figure 2). Several aspects of replication are relevant to the pathogenesis of OPA. Cellular entry of retroviruses requires binding of the viral Env protein to a specific cell surface receptor. The receptor for JSRV is hyaluronidase-2 (HYAL2), a glycosylphosphatidylinositol-linked protein that is expressed in many tissues and by many cell types (Rai et al. 2000, 2001). JSRV can therefore enter and integrate its genome in a broad range of cell types in vivo and in vitro (Holland et al. 1999; Palmarini et al. 1996, Palmarini, Sharp, Lee, et al. 1999). However, as noted above, in most cell types, infection does not result in efficient replication of the virus. This is primarily because transcription of JSRV RNA is active at high levels only in secretory epithelial cells of the lung, namely type 2 pneumocytes and club cells (McGee-Estrada and Fan 2007; Palmarini et al. 2000). This specificity is determined by binding sites for cell-specific transcription factors present in the viral LTR. Therefore, the LTR is the major determinant of JSRV cell tropism, which in turn results in the primary pathology of lung tumors. Notably, in naturally infected sheep, the expression of JSRV proteins is almost entirely restricted to OPA tumor cells (Platt et al. 2002).

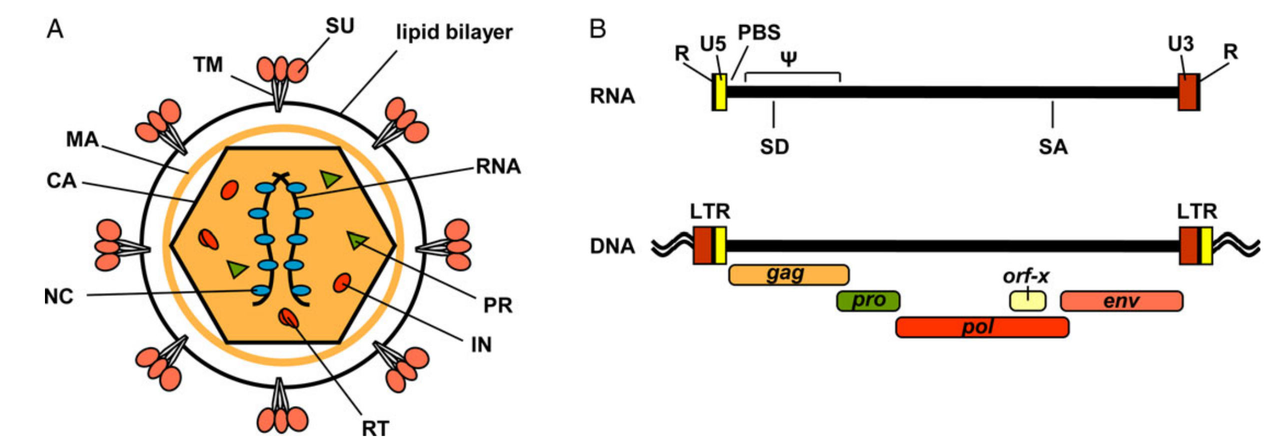

Figure 1. Structure of the JSRV virion and genome. (A) Particle structure. JSRV virions contain two identical copies of the RNA genome associated with a highly basic nucleocapsid (NC) protein and enclosed within the virus capsid (CA) and matrix (MA) proteins. The particles have a lipid bilayer envelope that is associated with transmembrane (TM) and surface (SU) envelope glycoproteins. The viral capsid 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. 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) contains gene promoter and enhancer elements that regulate JSRV expression. The LTRs are generated by recombination events that occur during reverse transcription and are present only in the DNA form of the genome. Protein-coding regions are located in the central part of the genome (*gag*, *pro*, *pol*, *env*, and *orf-x*). (Reprinted from Griffiths et al. 2010, with permission.)

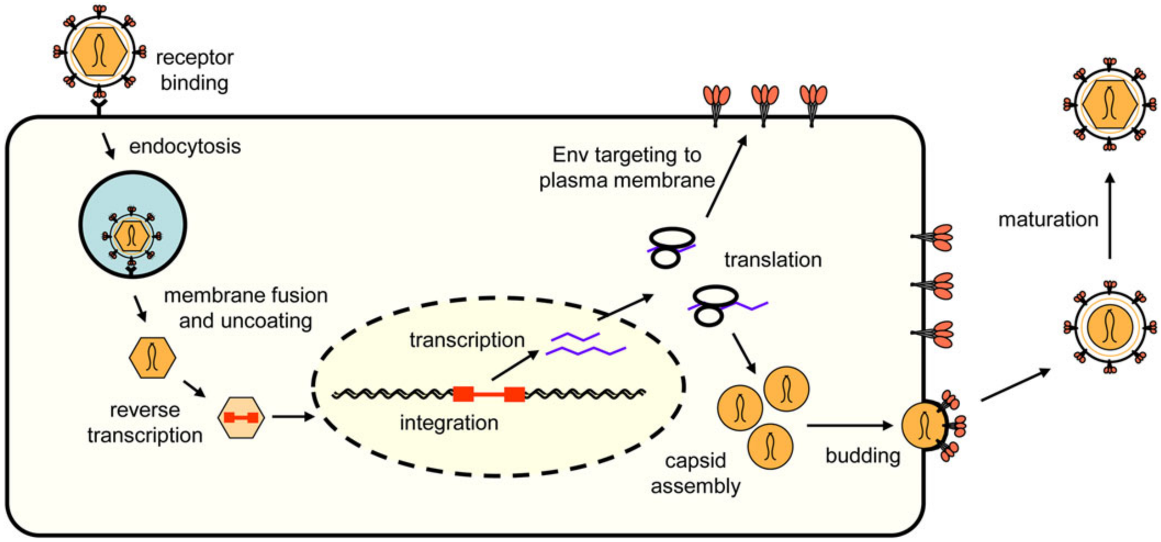

Figure 2. Replication cycle of JSRV. Attachment of JSRV to its target cell is mediated through the binding of the SU subunit of the Env glycoprotein to a specific cell surface receptor molecule, hyaluronidase 2 (HYAL2). Receptor binding leads to endocytosis of the virus particle. Subsequent fusion of the viral and endosomal membranes releases the viral core into the cytoplasm (uncoating). 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 in the cell nucleus to form the provirus. Following integration, expression of JSRV RNA from the viral promoter in the LTR is controlled by the host cell's transcriptional machinery. 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 (Caporale et al. 2009; Hofacre et al. 2009; Palmarini et al. 2002). Synthesis of Gag, Pro, and Pol occurs on cytoplasmic ribosomes, whereas Env proteins are synthesized on endoplasmic reticulum-bound ribosomes and traffic to the plasma membrane. Viral capsids assemble in the cytoplasm and migrate to the cell surface where they are released by budding through the plasma membrane. As they bud, they acquire a lipid envelope and the 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. (Reprinted from Griffiths et al. 2010, with permission).

Histological Similarity of Adenocarcinoma in Humans and Sheep

Lung cancer in humans is characterized by a high degree of heterogeneity, but distinct forms can be recognized by the architectural and cytomorphological appearance of the tumor cells (Travis et al. 2004). Around 15% are small-cell lung carcinoma (SCLC), an aggressive and highly metastatic tumor that is often widely disseminated at the time of diagnosis (Ettinger 2004). The remaining tumors can broadly be regarded as "non small-cell lung carcinomas" (NSCLC) and can be divided into several subtypes, the commonest of which are adenocarcinoma, squamous cell carcinoma, and large-cell carcinoma, with each of these having a variety of further subtypes (Travis et al. 2004).

The incidence of adenocarcinoma has, in recent years, become more common relative to squamous cell carcinoma particularly in North America and parts of Asia, although this change is less apparent in parts of Europe and the United Kingdom (Devesa et al. 2005). Adenocarcinomas can be subdivided into several pathological types depending on the growth pattern of the tumor (Travis et al. 2004), and their classification has been subject to periodic revision, with the most recent modifications being recommended by the International Association for the Study of Lung Cancer (IASLC) and European Respiratory Society (ERS) in 2011 (Travis et al. 2011).

The revisions in classification follow an increase in understanding of the biology and pathogenesis of pulmonary adenocarcinomas, which arise in the periphery of the lung rather than in relation to major conducting airways (Figure 3A–C). Premalignant lesions termed "atypical adenomatous hyperplasias" (AAHs) are thought to be the earliest morphological abnormality, and these may be identified in the histologically normal background lung of patients undergoing resection for pulmonary adenocarcinoma (Carey et al. 1992). As these lesions develop an increasing number of genetic abnormalities, the cells become more pleomorphic, developing into the lesions that were previously classified as nonmucinous bronchioloalveolar carcinoma (BAC) (Kerr 2001). The 2004 World Health Organization (WHO) classification defined these as being "non-invasive," with the tumor cells showing a lepidic growth pattern along alveolar walls, often associated with some fibrous thickening (Carey et al. 1992; Travis et al. 2004). Clinical data, particularly from studies of Asian populations, have shown that for lesions such as these, measuring less than 30 mm in diameter, surgical resection is associated with a 100% cure rate, and this led to the IASLC/ERS recommendation that these now be regarded as "adenocarcinoma-in-situ" (AIS) (Travis et al. 2011). As these lesions enlarge, it is thought that the central part collapses, resulting in the development of a fibrous area, and it is in relation to this that invasive adenocarcinoma develops. Depending on how far the lesion has extended down this pathway, the invasive disease may be at an early stage and have little, if any, impact on prognosis. This has again been recognized in the IASLC/ERS classification with the term "minimally invasive adenocarcinoma" (MIA) being suggested for lesions measuring less than 30 mm where the invasive component measures less than 5 mm (Travis et al. 2011). For tumors greater than 30 mm, these terms are not used, and they are classified as adenocarcinoma with an additional observation on the predominant pattern present (which may be lepidic, acinar, papillary, or solid) and a description of other minor patterns that are admixed (Travis et al. 2011). It should be noted that the invasive form is by far the most common clinical and pathological pattern, and early noninvasive disease is rare except in parts of Asia.

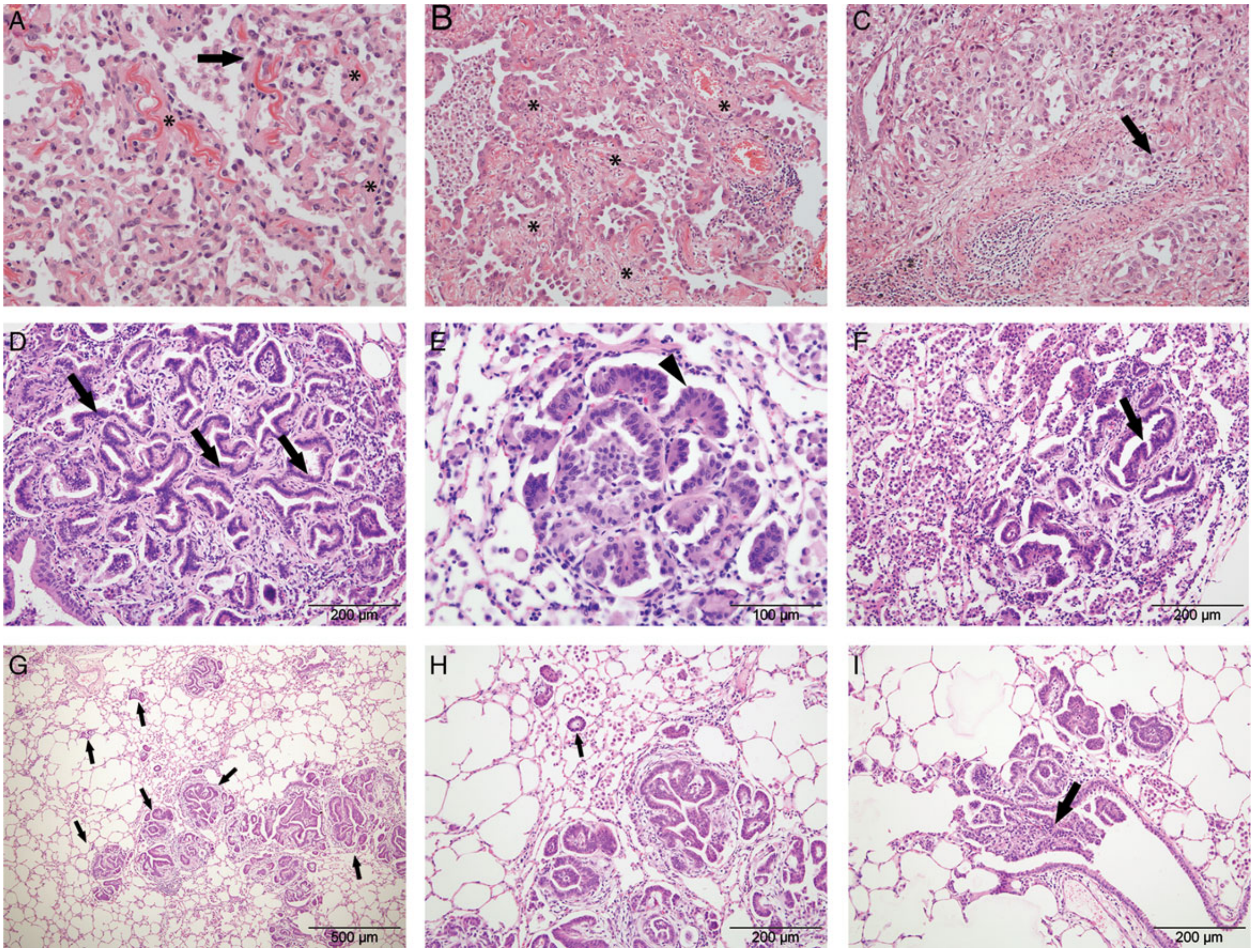

Figure 3. Histological appearance of human lung adenocarcinoma and OPA. Photomicrographs of human (A–C) and sheep (D–I) lung. (A) An area of atypical adenomatous hyperplasia (AAH) identified as an incidental finding in the background of a lung resected for adenocarcinoma. The alveolar walls are mildly thickened (marked by *) and are lined by a population of cells with a rather "hob-nail" appearance (arrow) showing mild cytological atypia with some nuclear enlargement. (B) An area of noninvasive "lepidic" pattern of adenocarcinoma. The cells are more atypical than that seen in AAH and are often associated with more significant alveolar wall thickening (*). No stromal invasion is seen. This pattern is frequently observed at the periphery of resected lung adenocarcinoma. (C) Invasive adenocarcinoma with an acinar pattern. Tumor is seen invading into a vessel (arrow). Original magnification in panels A-C was ×200. (D–F) Natural OPA displaying typical lepidic (arrows) and papillary (arrowhead) growth pattern of tumor cells within alveoli. Note the infiltration by macrophages and other myeloid cells into adjacent alveolar air spaces (left hand side of panel F). (G–I) Experimentally induced OPA. Note the alveolar (smaller arrows) and bronchiolar location of tumor cells (single large arrow, panel I). This pattern of growth is also seen on the outer edge of tumors in natural cases of OPA.

In addition, a "mucinous" pattern of lepidic adenocarcinoma may be encountered, which is often multifocal and bilateral and associated with extensive mucus consolidation of the lung (Travis et al. 2011). These patients often present with cough and extensive mucus production. While these multifocal lesions are often "noninvasive," minimally invasive and invasive lesions can be identified. Moreover, some of these patients may die due to excess mucous secretions resulting in fatal hypoxia, with no evidence of invasive disease (Nguyen et al. 2014).

OPA was first suggested as a potential model for human lung adenocarcinoma due to histological similarities between the human and ovine tumors (Bonne 1939; Perk and Hod 1982). The typical histological appearance of OPA is that of multiple foci of columnar or cuboidal neoplastic cells growing along the alveolar septa (i.e., lepidic growth) or from the wall of small bronchioles (Figure 3D–I). Larger foci typically have a papillary appearance, and advanced cases frequently have regions of necrosis at the center. Some early descriptions of OPA suggested that the disease is not invasive, and the term *sheep pulmonary adenomatosis* was used previously. However, intrathoracic and extrathoracic metastases are present in perhaps 10% of affected sheep, confirming that OPA is a malignant disease, at least in some animals or specific breeds (Demartini et al. 1988; Holland et al. 1999; Minguijón et al. 2013; Nobel et al. 1969).

It is important to recognize the difficulties in classifying human lung adenocarcinoma when comparing older literature on OPA with more recent studies and to specify exactly which features the ovine and human diseases have in common. In some early reports, OPA was said to have a particular similarity to human BAC but, as this term has not been applied consistently, we have avoided its use here. Instead, under the currently suggested classification for human adenocarcinomas, OPA is possibly better classified as a minimally invasive adenocarcinoma or lepidic-predominant adenocarcinoma in its early stages, for example, in experimentally induced disease (see below); whereas in more advanced cases, such as natural disease, it would fit the description of adenocarcinoma with a papillary or acinar-predominant growth pattern.

In this context, OPA more closely resembles the rare, multifocal, noninvasive form of lung cancer, including the mucinous forms, and is less similar to the aggressive, high-grade, metastatic tumors that are the more common presentation of the disease. Moreover, OPA shares few of the clinical and radiological features of the aggressive human tumors. As we describe below, the great advantage of OPA as an animal model is as a pathological model for studies of early disease. In addition, the comparative analysis of sheep and human diseases is of great interest in recognizing and defining interspecies differences and similarities.

Oncogenic Signaling in Human Adenocarcinoma and OPA

The histological similarity of OPA with some forms of human lung adenocarcinoma is strongly suggestive of common or shared mechanisms underlying the neoplastic changes. Understanding of the molecular basis of human cancer has advanced greatly in recent years and in particular has identified “driver” mutations that cause dysregulated signaling in pathways regulating the cell cycle (Greenman et al. 2007; Pao and Girard 2011; Sakashita et al. 2014). Importantly, several of these pathways are also perturbed in OPA, indicating that research into the sheep disease may reveal novel insights into the molecular pathogenesis of human cancer. For example, the existence of several common signaling pathways in OPA and NSCLC supports OPA as a model for studying the cross-talk between these pathways. This could potentially direct research toward unknown driver mutations in human cancer or allow the inference of regulatory relationships between the oncogenic pathways so that more targeted therapies that address tumor resistance can be realized.

Common Genetic Lesions in Human Lung Cancer

In a typical lung tumor, a patient can harbor hundreds of mutations, a minority of which will be driver mutations upon which the tumors are absolutely dependent (Gazdar et al. 2004; Pao and Girard 2011). For example, the oncogene Kirsten rat sarcoma viral oncogene homolog (KRAS), a member of the Ras family of small GTPases, is thought to be the target for underlying driver mutations in 25%–30% of patients with adenocarcinoma, whereas epidermal growth factor receptor (EGFR) hypermutations and anaplastic lymphoma receptor tyrosine kinase (ALK) gene fusions account for 15% and 5%, respectively (Korpanty et al. 2014). Other common driver mutations are found in the genes for BRAF (B-Raf serine/threonine kinase), PIK3CA (phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K), catalytic subunit alpha), PTEN (phosphatase and tensin homolog), and TP53 (tumor protein p53) (Sakashita et al. 2014; Shames and Wistuba 2014). One of the key challenges in developing effective therapeutic strategies for cancer is to distinguish the driver mutations from the background of hundreds of other mutations that may be present. This is important because it may guide decisions in the clinic regarding the treatment of individual patients. For example, KRAS and EGFR mutations are almost mutually exclusive in lung cancer, with KRAS mutations present more frequently in smokers and less commonly in never-smokers, whereas the opposite is found for EGFR mutations. Thus, genetic testing of tumors can guide physicians on the use of appropriate drugs such as EGFR tyrosine kinase inhibitors (Chen et al. 2013). Notably, the success of these selective inhibitors in the clinic has been limited because tumors often develop resistance due to compensatory mechanisms or the presence of additional driver mutations (Camidge et al. 2014; Lin et al. 2014).

Oncogenic Signaling in OPA

A notable aspect of JSRV biology is that the viral Env protein directly activates a number of cell signaling pathways that drive proliferation and and transformation in addition to its function in mediating entry to target cells (Hofacre and Fan 2010; Liu and Miller 2007). Therefore, Env is directly oncogenic, and this distinguishes JSRV from most other retroviruses, which cause cancer through insertional mutagenesis or oncogene capture (Maeda et al. 2008).

The transforming activity of Env was first demonstrated in vitro using cell lines (Maeda et al. 2001; Rai et al. 2001) and subsequently in vivo in mice (Chitra et al. 2009; Wootton et al. 2005) and sheep (Caporale et al. 2006). The precise mechanism(s) by which this protein is able to activate cellular signaling has not been fully determined but may involve several regions within the SU and TM domains of Env. The cytoplasmic tail of TM is thought to be particularly important as deletion or mutation of this region abrogates the transforming activity (Hull and Fan 2006; Liu and Miller 2007; Palmarini et al. 2001). However, the specific cellular factors that Env binds to initiate transformation have not been identified, and this remains a key question in OPA research. It appears likely that the cellular localization of Env at the plasma membrane places it in an appropriate context to interact with many protein kinases, adapter proteins, and other signaling factors that might trigger downstream signaling through a number of pathways.

The main pathways commonly activated in OPA and human lung adenocarcinoma are described below and summarized in Figure 4. The evidence for their activation by JSRV has been reviewed in detail previously by others (Hofacre and Fan 2010; Liu and Miller 2007).

Ras-MEK-ERK1/2 and PI3K-AKT-mTOR

As noted above, KRAS mutations are the leading driver mutations in NSCLC (Korpanty et al. 2014). Mutant KRAS can constitutively activate protein kinase signaling pathways that drive cellular proliferation and transformation. Two important pathways that are activated by KRAS in NSCLC are PI3K-AKT-mTOR and RAF-MEK-ERK1/2 (Castellano and Downward 2010; Scrima et al. 2012; Sheridan and Downward 2013; Stinchcombe and Johnson 2014). Together, these pathways promote cell survival and proliferation through a number of mechanisms including inhibiting apoptosis and activating telomerase and other proteins involved in cell growth and division. Several studies have shown that these two pathways are also activated by JSRV Env. For example, various fibroblast and epithelial cell lines phosphorylate (activate) AKT and/or ERK1/2 following transfection with plasmids encoding Env (Johnson et al. 2010; Liu et al. 2003; Liu and Miller 2005; Maeda et al. 2001, 2005; Palmarini and Fan 2001; Rai et al. 2001; Zavala et al. 2003). In addition, activated AKT and ERK1/2 are found in OPA tumor cells in natural and experimentally induced disease in sheep (Archer et al. 2007; Cousens et al. 2015; De las Heras et al. 2006; Maeda et al. 2005; Suau et al. 2006) (Figure 5) and in Env-transformed cells in mouse models (Linnerth-Petrik et al. 2012, Linnerth-Petrik, Santry et al. 2014). Importantly, studies in cell lines have shown that inhibitors of KRAS, MEK, PI3K, and mTOR can reduce or block the transformed cell phenotype, confirming the importance of these pathways in transformation.

Although the importance of these protein kinase signaling pathways is well established, few studies have examined the downstream effectors that drive oncogenesis in OPA. One mechanism by which AKT is known to promote cellular transformation is by phosphorylating (activating) the catalytic subunit of

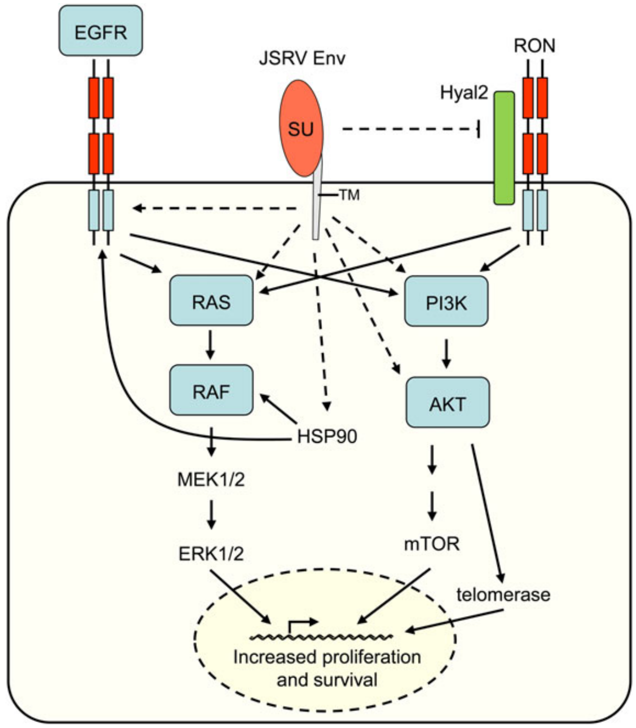

Figure 4. Signaling pathways activated in Env-mediated transformation. The major signaling pathways activated by the JSRV Env protein are shown, summarized from in vitro and in vivo studies (Hofacre and Fan 2010; Liu and Miller 2007). Env is thought to interact with one or more factors to activate these pathways and drive cellular proliferation, although the identities of the proteins that bind Env are currently unclear. This uncertainty is indicated by dashed lines. Molecules that are known targets for driver mutations in human NSCLC are boxed.

telomerase, thereby increasing the replicative potential of tumor cells (Hanahan and Weinberg 2011). Notably, increased telomerase activity has been described in human lung adenocarcinoma (Lantuéjoul et al. 2007; Lee et al. 1998) and has also been demonstrated in OPA tumor lysates and in primary tumor cells isolated from OPA (Suau et al. 2006).

Epidermal Growth Factor Receptor (EGFR)

EGFR is overexpressed in more than 60% of lung cancers (Stella et al. 2012) and is a common target for driver mutations in NSCLC (Rosell et al. 2009), in particular in tumors of never-smokers. The development of EGFR kinase inhibitors has had significant impact on treatment strategies for these patients. Phosphorylation (activation) of EGFR results in downstream activation of several pathways, including the PI3K-AKT-mTOR and RAF-MEK-ERK1/2 signaling cascades, among others (Stella et al. 2012). Despite the importance of these pathways in OPA, the role of EGFR in JSRV Env-mediated transformation is unclear. In vitro studies have shown that JSRV Env does not bind EGFR in cultured cell lines (Varela et al. 2006), and EGFR inhibitors do not affect transformation of rodent fibroblasts by Env in vitro (Varela et al. 2008). In contrast, analysis of tumors induced by Env in a murine model of OPA (see below) found activation of EGFR signaling in vivo (Linnerth-Petrik et al. 2012). Thus, these results suggest that EGFR may be involved in JSRV-mediated transformation but that this depends on the experimental system used. Further studies are required to determine its relevance to oncogenesis in the sheep lung.

RON-HYAL2

A mechanism for activation of AKT and ERK1/2 pathways has been proposed in which binding of HYAL2 by JSRV Env results in activation of signaling by the RON receptor tyrosine kinase (Danilkovitch-Miagkova et al. 2003). This model predicts that, in the absence of Env, HYAL2 binds to RON and suppresses its kinase activity (Danilkovitch-Miagkova et al. 2003). In contrast, when Env is present it binds HYAL2, thereby releasing RON, which results in dimerization and autophosphorylation of RON and activation of downstream protein kinase pathways (Danilkovitch-Miagkova et al. 2003; Miller et al. 2004; Varela et al. 2006). This is an attractive model because it presents HYAL2 as a tumor suppressor, which would be consistent with its genomic location at human chromosome 3p21, a known loss of heterozygosity locus in human lung cancer (Hesson et al. 2007; Ji et al. 2005). However, this mechanism has so far been demonstrated only in BEAS-2B cells (a human bronchial epithelial cell line) and does not appear to be active in other cell lines. For example, Env can induce transformation independently of HYAL2 (Varela et al. 2006; Wootton et al. 2005), and RON activation was not observed in rat IEC-18 cells transformed by Env (Varela et al. 2006). Therefore, the involvement of RON in JSRV Env-mediated transformation depends on the cell line used, and it will be interesting to examine the activity of this pathway in vivo in sheep tumors. Interestingly, overexpression of RON and its ligand, MSP (macrophage-stimulating protein), is strongly mitogenic and is associated with reduced patient survival in NSCLC and other tumors (reviewed in Yao et al. 2013).

Heat Shock Protein 90

Heat Shock Protein 90 (HSP90) is a molecular chaperone that, in the event of cellular stress, allows proteins to escape the fate of degradation by the ubiquitin-proteasome machinery (Patki and Pawar 2013). HSP90 inhibitors are currently in clinical trials for ALK fusion and EGFR mutation subtypes of NSCLC (Garcia-

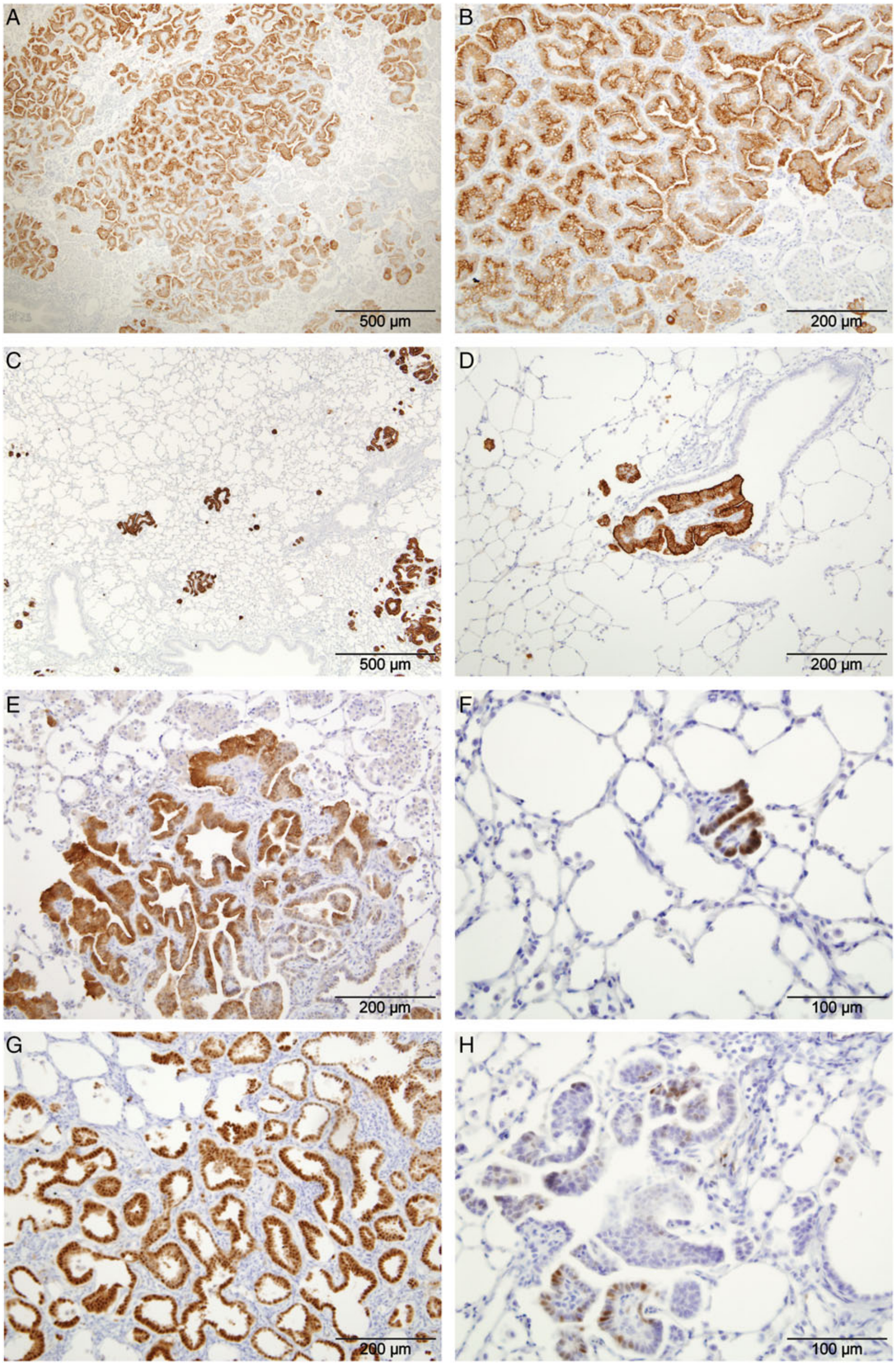

Figure 5. Immunohistochemical labeling of OPA tumor cells. (A and B) Natural OPA and (C and D) experimentally induced OPA lesions labeled with a monoclonal antibody to the JSRV Env (SU) protein (brown pigment in all panels). Note intense labeling of lepidic and papillary forms of OPA, which arise consistently from the respiratory epithelium, and the more extensive coalesced appearance of the natural OPA (A, B) compared with the more multifocal experimentally induced OPA (C, D) which represents an earlier point in the disease process. (E) Natural and (F) experimental OPA labeled with an antibody to phosphorylated AKT. (G) Natural and (H) experimental OPA labeled with an antibody to phosphorylated ERK1/2. Note phosphorylated AKT and phosphorylated ERK1/2 labeling is within OPA tumor cells.

Carbonero et al. 2013; Neckers and Workman 2012). Few studies have examined the role of HSP90 in the context of JSRV transformation, but one report showed that it is expressed in OPA tumors and in primary cell lines derived from these tumors (Varela et al. 2008). Interestingly, HSP90 inhibitors are able to reduce proliferation in these cells. A similar effect was described in an Env-transformed rat fibroblast cell line and was linked to AKT degradation (Varela et al. 2008). These findings suggest that the OPA model might have value in preclinical testing of drugs targeting HSP90.

Tumor-Associated Macrophages in Lung Cancer

OPA may also provide a model for investigating the role of tumor-associated macrophages (TAMs) in lung adenocarcinoma. Studies in a variety of cancers have shown that macrophages are intimately linked to tumor survival and metastasis, and, therefore, they represent a potential target for treatment (Lievense et al. 2013; Noy and Pollard 2014). TAMs may exhibit a broad spectrum of phenotypes depending on the specific activation signals they encounter, such as cytokines, growth factors, and microbial products, but they are commonly described as displaying polarization toward one of two different subpopulations, M1 and M2. M1 TAMs have cytotoxic and phagocytic activity and can inhibit cancer growth by expressing pro-inflammatory cytokines and promoting tumor cell killing. In contrast, M2 TAMs promote tumor survival through the production of angiogenic and anti-apoptotic signals and immunomodulatory cytokines (Lievense et al. 2013; Noy and Pollard 2014). It should be noted that the representation of phenotypic diversity of macrophages as a linear polarization from M1 to M2 is recognized to be an oversimplification (Murray et al. 2014); however, this concept is still useful when considering the role of macrophages in their interactions with tumor cells. For example, studies in several human cancers, including NSCLC, have shown that a high density of M2 macrophages in tumors is associated with a worse prognosis (Becker et al. 2014; Zhang et al. 2012). There is therefore significant interest in understanding the mechanisms involved in macrophage recruitment to tumors and the signaling events within the tumor microenvironment that favor the M2 phenotype over the M1 phenotype. This process may be dependent on other cell types within the surrounding tissue in addition to the tumor cells.

Association of macrophages with tumors is also a common histological feature of OPA. These cells tend to accumulate in alveolar spaces around the periphery of tumor foci or infiltrate the tumor stroma (see Figure 3F). Few studies have examined the phenotype of these cells, but Summers and colleagues reported that OPA-associated macrophages are IFNγ-positive, which is suggestive of an M1-like phenotype (Summers et al. 2005). However, further studies are required to profile these cells in more detail because it is possible that they have immunomodulatory activity. TAMs have also been described in a number of mouse models of cancer, although several of the markers used to phenotype murine TAMs differ from those in humans due to differences in the human and murine immune markers (Mestas and Hughes 2004). OPA therefore offers an alternative model to understand the function of these cells, particularly the signaling events involved in driving the generation of the M2 phenotype. In addition, OPA may have value in studies examining the potential for therapeutic intervention that targets their pro-tumorigenic activity.

Finally, it should be noted that later stages of natural OPA are also associated with significant neutrophil infiltration. These cells may be responding to concurrent bacterial infection in OPA-affected tissues, rather than the tumor itself; however, they are likely to further modulate cytokine signaling in tumors. Functional polarization of tumor-associated neutrophils has also been described in NSCLC, but to date these cells have not been characterized in as much detail as TAMs (Eruslanov et al. 2014; Fridlender et al. 2009).

Experimental Tools for Studying Cancer Pathogenesis in OPA

A major strength of OPA as a model for human lung cancer is the availability of several in vivo and in vitro experimental systems for studying tumorigenesis driven by JSRV Env. Together, these methods provide a broad platform for investigating disease mechanisms in lung cancer.

In Vivo Lamb Disease Model

The first experimental system to be developed for OPA was an in vivo disease model in neonatal lambs (Martin et al. 1976; Sharp et al. 1983). Intratracheal inoculation of concentrated JSRV results in successful infection of the lung and the appearance of tumors and clinical signs of respiratory disease within several weeks or months. In initial reports, the virus preparations used for in vivo infections were purified from lung tumor homogenates or lung fluid collected from natural field cases of OPA (Martin et al. 1976; Sharp et al. 1983). Those experiments were important in establishing the link between JSRV infection and development of OPA. Subsequent studies showed that the development of tumors and clinical disease are reproduced much more consistently and with shorter incubation periods in young lambs compared with older lambs or adult sheep (Murgia et al. 2011; Salvatori et al. 2004). In the majority of recent studies exploiting the in vivo OPA lamb model, animals were infected prior to 7 days of age. The age dependence of this disease model is most likely due to the availability of susceptible target cells for JSRV within the lung. In common with most other retroviruses, JSRV can only infect actively dividing cells (Lewis and Emerman 1994; Murgia et al. 2011), and such cells are much more abundant in the growing lung of younger animals than in adults. Consistent with this, adult sheep can be rendered susceptible to JSRV infection and tumor development if the lung is first subjected to mild injury, which stimulates cell division during tissue repair (Murgia et al. 2011).

A milestone in OPA research came with the cloning and sequencing of the JSRV genome (York et al. 1991, 1992) and the subsequent isolation of an infectious and oncogenic molecular clone (Palmarini, Sharp, de las Heras, et al. 1999). The availability of this clone (denoted $JSRV_{21}$) facilitated production of the virus through transient transfection of 293T cells (a human fetal kidney cell line), and this source of virus could then be used instead of lung fluid in the disease model (Palmarini, Sharp, de las Heras, et al. 1999). Because the concentration of JSRV in lung fluid from different cases of natural OPA can vary up to 1000-fold (Cousens et al. 2009), in vitro production of JSRV has provided greater consistency between preparations of inocula and resulted in a more reproducible model than was possible previously.

A further recent refinement of the lamb OPA model has been the use of a flexible bronchoscope to deliver JSRV to a specific lung lobe, which localizes the infection within a known volume of tissue, thereby facilitating accurate sampling in the early stages of infection (Caporale et al. 2013; Murgia et al. 2011). In principle, bronchoscopic instillation could be used to deliver a small volume of virus to multiple specific sites within the lung allowing different treatments to be compared in the same animal.

An important benefit of using the JSRV molecular clone is the ability to introduce targeted mutations into the virus that can be studied in lambs in vivo. The application of such mutants in the OPA disease model has provided several important insights into JSRV biology. For example, the essential role of Env in oncogenesis was demonstrated by infection of lambs with nonreplicating JSRV vectors expressing only Env (Caporale et al. 2006). Other studies have shown that the product of the JSRV Orf-X gene does not have an essential role in oncogenesis (Cousens et al. 2007) and that respiratory epithelial cells of goats are not fully permissive to JSRV infection in vivo (Caporale et al. 2013). In addition, the OPA lamb disease model has been used to study early events in disease by identifying virus-infected single cells in infected lung tissue just 10 days after intratracheal challenge with JSRV (Martineau et al. 2011; Murgia et al. 2011). Collectively, these studies demonstrate that the lamb disease model can be used to study OPA tumors throughout the course of disease, from the initial infected single cell through to the development of small tumors associated with early respiratory disease. When combined with studies of advanced natural cases (see below), in vivo studies of OPA permit analysis of the entire course of tumor growth.

The dose of infectious JSRV delivered in the lamb model has not yet been standardized. This is because there is currently no permissive cell line for JSRV that can be used to determine the infectious titer of viral preparations. Instead, the amount of virus used has been somewhat empirically determined. In general, JSRV has been concentrated between 10- and 200-fold from lung fluid or culture supernatant of transfected 293T cells and delivered in a volume of 2–5 ml using a transtracheal hypodermic needle or bronchoscope (Caporale et al. 2013; Cousens et al. 2007; Martin et al. 1976; Martineau et al. 2011; Murgia et al. 2011). This procedure causes only short-term moderate discomfort and is tolerated well by the lambs. In our laboratory, we use RT-qPCR to quantify JSRV produced by transient transfection and deliver a dose of approximately 10^{10} viral RNA copies per lamb (Martineau et al. 2011). This amount of virus has yielded clinical signs in 8 to 12 weeks in lambs challenged with JSRV within the first week postpartum (Martineau et al. 2011). Although the infectious dose of JSRV delivered in the lamb model is not known, it is likely to be much greater than that received during natural infection.

It is important to note that for welfare reasons it is not acceptable to prolong the experimental infection to the advanced clinical stage that occurs in natural disease. Therefore, in our laboratory, lambs are euthanized as soon as the early clinical signs of respiratory distress are apparent. Determining this point is somewhat subjective and relies on regular observation and assessment of the animal's breathing. Those that repeatedly exhibit signs of increased respiratory effort and recovery time after mild exercise can be predicted with reasonable confidence as having early OPA. Our standard protocol includes a maximum duration of 6 months post-infection, at which time all lambs are euthanized even if clinical signs of disease are not present. Computed tomography has been used to image subclinical tumors in OPA (Hudachek et al. 2010) but has not been broadly adopted in other studies to date. Ultrasonography has also been reported to detect large tumors in naturally infected sheep (Scott et al. 2013) but has not been used in an experimental setting.

The larger infectious dose and shorter clinical course of experimental OPA compared with natural cases results in a distinct histological pattern compared with natural disease. Experimentally induced OPA typically results in multiple neoplastic foci of varying sizes that presumably represent polyclonal tumor foci of different ages, many of which are at an earlier stage than in natural cases. The large tumor masses typical of natural field cases of OPA are not seen in the experimental model due to lambs being euthanized at a maximum of 6 months post-challenge for welfare reasons, but it is not unreasonable to assume they would develop if given time. Additionally, overt production of lung fluid is observed only rarely.

Natural OPA as a Model of Lung Cancer

While the lamb model facilitates analysis of early events in tumorigenesis in the lung, more advanced disease can be studied by examination of natural cases of OPA. As OPA is a relatively common disease of sheep in many countries, it is possible to obtain natural field cases of the disease from cooperative farmers. For example, in our laboratory in Edinburgh, we receive frequent donations of naturally affected animals from farms in southern and central Scotland for studies on the development of control strategies for OPA. Because the respiratory signs of clinical OPA do not became apparent until there is considerable lung deficit, natural cases typically exhibit very advanced disease in which approximately 20%–70% of the lung is composed of tumor tissue (De las Heras et al. 2003; Griffiths et al. 2010). Samples from such animals allow comparison of findings from in vitro studies and from experimentally infected lambs. Moreover, they offer the potential to study other events associated with advanced disease, such as metastasis (in some sheep [Demartini et al. 1988; Holland et al. 1999]) and systemic immune responses (Summers et al. 2002, 2006).

As noted above, in many cases of OPA, there is also evidence of a secondary bacterial infection. Concurrent bacterial lung infections also occur in human lung cancers and may present in the form of abscesses or as infection within an area of tumor necrosis (Sutton 2014). In both humans and sheep, bacterial infection is likely to be facilitated by the loss of integrity of the epithelial barrier, aberrant mucus production and mucociliary transport, or local airway obstruction leading to collapse. In addition, local immune suppression associated with the tumor may hamper immune clearance. Thus, while little is known about the infections secondary to OPA, it is possible that studies in this area might yield results that are relevant for human lung cancer.

In Vivo Mouse Tumor Model

In addition to the lamb disease model, an in vivo model of OPA has been described in mice (Wootton et al. 2005). Mouse cells are not naturally permissive to JSRV replication because the mouse ortholog of the JSRV receptor, HYAL2, does not support binding and cell entry by the virus (Dirks et al. 2002). However, intranasal administration of adeno-associated virus vectors encoding JSRV Env causes adenocarcinoma in mice that is histologically very similar to that in sheep (Linnerth-Petrik et al. 2012; Wootton et al. 2005). Importantly, the signaling molecules activated in this model are similar to those identified in human lung cancer and in sheep, including EGFR, KRAS, AKT, and ERK1/2 (Linnerth-Petrik et al. 2012; Wootton et al. 2005). This model provides an alternative in vivo system for studying JSRV Env transformation that does not require large animal facilities and that offers the potential for researchers to capitalize on the wide availability of transgenic mouse strains. However, an important difference from the lamb model is that the viruses used are not replicating forms of JSRV.

In Vitro Cell Culture Models

In vitro studies on JSRV have been somewhat restricted by the lack of a cell line that can support efficient replication of the virus (Palmarini, Sharp, Lee et al. 1999). Despite this, much has been learned about JSRV transformation by transfection of cell lines using the $JSRV_{21}$ molecular clone or expression plasmids encoding Env (reviewed in Hofacre and Fan 2010; Liu and Miller 2007). These experiments were crucial to identifying the oncogenic activity of Env and guided subsequent studies to characterize the specific pathways involved. The cell lines used include fibroblast and epithelial cells from a variety of species, including mice, rats, dogs, chickens, and humans. An important caveat is that JSRV Env may activate diverse pathways depending on the cell line used, and these may not necessarily reflect the events occurring in the sheep lung in vivo. Nevertheless, transfected cell lines are a valuable tool for studying JSRV pathogenesis, particularly when used in conjunction with in vivo disease models.

Some in vitro studies of JSRV transformation have focused on the characterization of primary OPA tumor cells obtained from sheep (Archer et al. 2007; Jassim et al. 1987; Suau et al. 2006). However, the cells described in these reports typically cease to produce JSRV after several passages in standard culture conditions. The loss of virus expression over repeated passage in culture is accompanied by a gradual reduction in expression of markers of differentiated epithelial cells, including surfactant protein-C (a marker of type II pneumocytes) during extended culture (Archer et al. 2007; Dobbs et al. 1997; Jassim et al. 1987; Suau et al. 2006). Interestingly, the changes in cellular differentiation state, including JSRV expression, can be delayed or reversed using three-dimensional culture systems, suggesting that cell polarization may be important for maintaining the phenotype of these cells (Archer et al. 2007; Johnson and Fan 2011; Johnson et al. 2010). This suggests that JSRV expression may be dependent upon the differentiated state of the infected cell. This knowledge should inform future work on the in vitro culture of JSRV. In particular 3-D culture systems may more faithfully reproduce the oncogenic events in sheep lung than monolayer cell cultures.

In Vitro Lung Slice Model of OPA

In light of the limitations of cell lines for studying JSRV replication in vitro, recent work from our laboratory has examined the potential for lung tissue explants to study early events in transformation (Cousens et al. 2015). This approach uses precision-cut lung slices (PCLS), which are discs of tissue approximately 8 mm in diameter and 300 μm thick that are prepared using an automated microtome and cultured in specialized medium (Liberati et al. 2010; Sanderson 2011). Lung slices offer an intermediate system between the in vitro cell culture and in vivo animal models and, because they have intact tissue morphology with multiple cell types present, they may offer a more faithful representation of JSRV-mediated transformation than cell lines. Moreover, the involvement of paracrine and autocrine signals from multiple cell types in modulating tumor growth can be analyzed, at least over a short time-frame. PCLS have been used in toxicological studies, for example, to study the effects of carcinogens found in tobacco smoke (Lin et al. 2012), and there have been several studies demonstrating that they can sustain virus replication (Booth et al. 2004; Kirchhoff et al. 2014; Nguyen et al. 2013). However, PCLS have not been used previously to study the activity of viral-driven oncogenesis nor to investigate dysregulated cell signaling.

Our recent data suggest that PCLS prepared from lamb lung tissue can be infected with JSRV and maintained in culture for 3 to 4 weeks (Cousens et al. 2015), during which time the infected cells express JSRV proteins and activate AKT and ERK1/2 pathways (Figure 6). These results indicate that this system can be used to study JSRV replication and to model very early events in transformation in lung tissue in vitro. Infected cells also express markers of type II pneumocytes, suggesting that this is an authentic model that mimics the behavior of infected cells in vivo. Studies are ongoing using this model to study the cross-

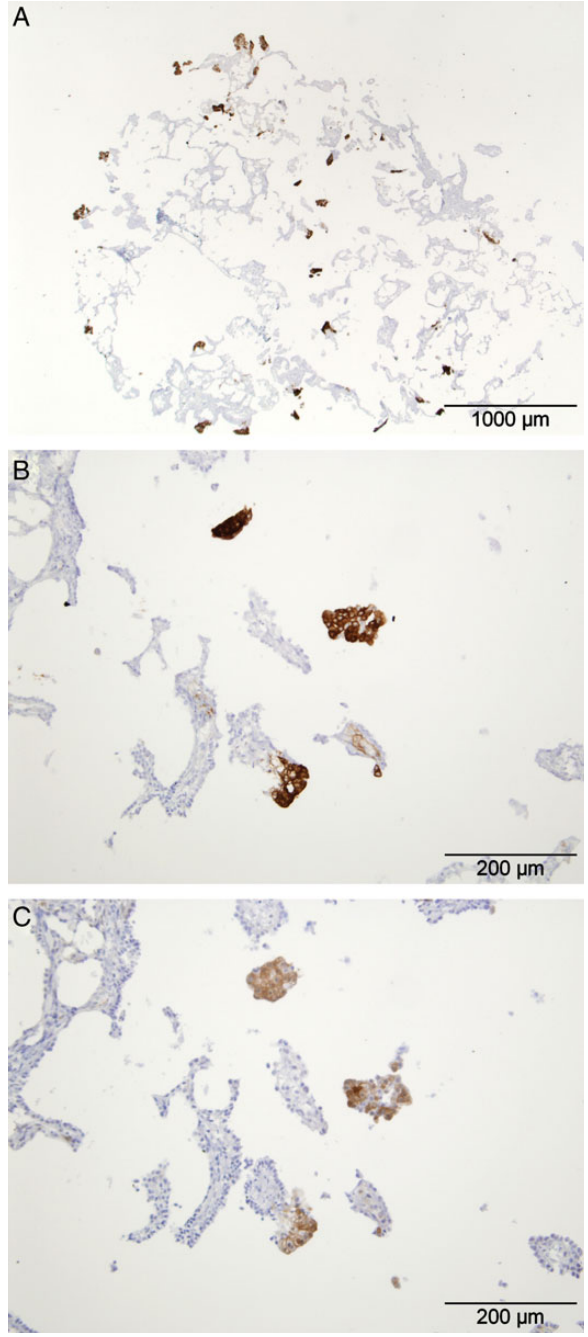

Figure 6. Ovine lung slices infected with JSRV. Immunohistochemical labeling (brown pigment) of precision-cut ovine lung slices that have been infected by JSRV. (A and B) Anti-JSRV Env (SU) at low and high power respectively, showing multiple foci of infected cells. (C) Semiserial section of (B) labeled with antiphosphorylated AKT. Note cells positive for phosphorylated AKT are also SU positive.

talk of signaling pathways and the interactions of multiple cell types in initiating and propagating tumor growth.

Is There a JSRV-Related Virus in Human Lung Cancer?

The increase in incidence of lung cancer in never-smokers has prompted a search for environmental etiological factors other than tobacco-related carcinogens (reviewed by Torok et al. 2011). The similarity between OPA and some forms of human lung adenocarcinoma has raised the question of whether JSRV or a related virus is able to contribute to lung cancer in humans. This is despite the lack of strong epidemiological evidence to support zoonotic transmission of JSRV to farmers or abattoir workers, who may be repeatedly exposed to high concentrations of the virus in lung fluid and tissue from affected sheep (De las Heras et al. 2007). Isolated epidemiological studies have shown an increased risk of lung cancer among farmers (as well as several other types of cancer) (Bardin-Mikolajczak et al. 2007; Bruske-Hohlfeld et al. 2000), but this might also be attributed to increased exposure to other carcinogens (e.g., pesticides). One recent study reported that occupational exposure to goats is associated with an increased risk of lung cancer although that association does not extend to sheep (Lutringer-Magnin et al. 2012).

The suggestion that JSRV might be a trigger for human lung adenocarcinoma may have some biological plausibility, because human HYAL2 is able to function as a cellular receptor for the virus, permitting its entry into human cells in culture (Rai et al. 2000, 2001). In addition, JSRV Env is able to transform the human pulmonary epithelial cell line BEAS-2B and to enhance the proliferative rate of A549 cells in culture (Chitra et al. 2010; Danilkovitch-Miagkova et al. 2003). However, receptor usage and/or transformation have also been described in cell lines from several other species, including dog, rabbit, and cattle (Dirks et al. 2002), which are not susceptible to JSRV in vivo. Even in goats, which are closely related to sheep, JSRV infection rarely results in clinical disease, and experimental infection of young goats results in very small, well circumscribed, encapsulated tumor foci and restricted intrapulmonary spread of the virus (Caporale et al. 2013).

Nevertheless, there is some evidence to support the presence of a JSRV-related virus in humans. Several studies have described positive immunohistochemical labeling in approximately a third of human lung tumors using antibodies raised to Gag proteins of JSRV or related retroviruses (De las Heras et al. 2000, 2007; Hopwood et al. 2010). More recently, the presence of a protein reacting with an anti-SU (Env) antibody was reported in human lung cancer tissue arrays (Linnerth-Petrik, Walsh et al. 2014). These studies are intriguing, but the reactive antigens have not been identified and so their significance is currently unclear. While these studies might be detecting a JSRV-related virus, it is also possible that they represent cross-reactive binding of the antibodies to cellular antigens, for example, endogenous retrovirus proteins, which may be upregulated in a proportion of human lung tumors. PCR studies have been inconsistent in identifying JSRV sequences in lung cancer patients (De las Heras et al. 2007; Hiatt and Highsmith 2002; Hopwood et al. 2010; Linnerth-Petrik, Walsh et al. 2014; Morozov et al. 2004; Rocca et al. 2008; Yousem et al. 2001). In light of the controversial status of JSRV as an infectious agent in humans, analysis of human lung adenocarcinomas using high-throughput sequencing is warranted to provide a definitive answer. Confirmation of a causative role for a virus in human lung cancer would radically change the options for diagnosis and treatment of this disease. Clearly, if that virus was related to JSRV, that would further strengthen the relevance of the OPA model to the human disease.

Conclusions and Future Perspectives

No animal model can entirely replicate a human disease. However, the close similarity of OPA with some forms of human lung adenocarcinoma, in particular, subtypes that exhibit a predominantly lepidic growth pattern, indicates that this naturally occurring and experimentally inducible tumor of sheep can be informative for understanding cancer in humans. The various experimental platforms that can be used to study JSRV Env-mediated oncogenesis, including in vivo models and in vitro cell and lung culture systems, provide a range of opportunities for uncovering novel molecular pathways involved in lung cancer and for studying potential therapeutics. With over 40% of human lung adenocarcinomas having no identified driver mutation (Korpanty et al. 2014), there remains much to learn about these tumors. Retroviruses have a long history of providing important insights into cancer biology (Vogt 2012), and the novel mechanisms of lung carcinogenesis triggered by JSRV Env may lead to further significant discoveries. In particular, the experimental lamb OPA model allows investigation of early premetastatic events in vivo, an area where there is a real gap in knowledge due to the lack of opportunity to obtain human samples at this stage of disease.

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