Veterinary Microbiology 181 (2015) 170–177

Contents lists available at ScienceDirect

Veterinary Microbiology

journal homepage: www.elsevier.com/locate/vetmic

Advances in the study of transmissible respiratory tumours in small ruminants

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ARTICLE INFO

Keywords:
Cancer
ENTV
Goat
JSRV
Lepidic
Respiratory infection
Retrovirus
Sheep

ABSTRACT

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

1. Introduction

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

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

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

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http://dx.doi.org/10.1016/j.vetmic.2015.08.008
0378-1135/

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

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

2. Retroviruses and cancer in small ruminants

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

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

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

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

3. Cell tropism and tissue specificity

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

Table 1
Retroviruses inducing tumours in animals or humans.

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