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Immunohistochemical Detection of c-Myc in Lungs of Sheep with Ovine Pulmonary Adenocarcinoma

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Abstract

Ovine pulmonary adenocarcinoma, a naturally occurring lung cancer of sheep was diagnosed in seventeen cases at slaughter houses. Lung tumor tissues and associated lymph nodes were collected for histopathological and immunohistochemical studies. Histologically, the lung sections from OPA cases revealed areas of non encapsulated proliferation of alveolar epithelial cells in papillary or acinar pattern. Polyploid ingrowths were also noticed into the bronchioles. On immunostaining of lung sections, JSRV-MA protein was detected in the neoplastic cells confirming the JSRV infection. There was a strong nuclear labelling for c-Myc in most of the neoplastic cells indicating dysregulation of this particular oncogene in OPA.

Keywords*: Sheep, OPA, histopathology, immunohistochemistry, c-Myc*

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INTRODUCTION

Ovine Pulmonary adenocarcinoma, a contagious lung cancer of sheep previously known as Jaagsiekte, sheep pulmonary adenomatosis or ovine pulmonary carcinoma [1–3] is prevalent worldwide and is caused by jaagsiekte sheep retrovirus (JSRV), a beta retrovirus. OPA has a similar morphological features to those of human bronchioloalveolar carcinoma (BAC) and the etiology of BAC is yet to be established. OPA accounts for almost 70% of all sheep tumors [4]. *Myc* is a potent oncogene that can promote tumorigenesis in a wide range of tissues and the elevated expression of its gene product, the transcription factor c-Myc correlates with tumor aggression and poor clinical outcome. *Myc* expression is deregulated and often markedly elevated in human neoplasms [5]. The present study was carried out to know the expression of c-Myc in lung sections of OPA in sheep by immunohistochemistry.

MATERIALS AND METHODS

The materials for the present study were collected from local slaughter houses. Suspected lung tissues were collected into 10% neutral buffered formalin for histopathological and immunohistochemical studies. Paraffin sections of lung tissues were stained by H&E and immunohistochemical staining was performed on suspected OPA lung sections with antibodies to JSRV matrix (MA) protein (courtesy: Prof. Massimo Palmarini) and c-Myc (AM318-5M, Bio Genex) using Super-sensitive polymer-HRP detection kit (Bio Genex) as per the instructions of manufacturer with slight modifications. Expression of the markers used in the present study was recorded by a semi quantitative grading based upon the percentage of neoplastic cells that was labelled in three representative fields examined with the x40 objective of the microscope : high (+++) = > 60%, moderate (++) = 11–59%, low (+) = 1–10%, absent (–) = 0%.

RESULTS AND DISCUSSION

Grossly, the OPA lungs revealed diffuse areas of consolidation or presence of tumor nodules involving different lobes (Figure 1). On cut section, the surface was granular, greyish white and moist with exudation of fluid. Histologically, the lung sections revealed areas of non encapsulated proliferation of alveolar epithelial cells in papillary or acinar pattern

(Figure 2). Polyploid ingrowths were also noticed into the bronchioles and the gross and histopathological lesions in OPA lungs were similar to that of previous reports [6–9]. On immunostaining of lung sections, JSRV-MA protein was detected in the cytoplasm of neoplastic cells confirming the JSRV infection (Figure 3) as noticed previously in 10 OPA lung samples [10]. There was a strong nuclear immunolabelling for c-Myc in the neoplastic cells of papillary projections of alveolar and bronchiolar epithelium in OPA lung sections (Figures 4 and 5). Out of 17 lung samples, the percentage of tumor cells that showed nuclear staining for c-Myc was high in 8 (47.1%) samples, moderate in 6 (35.3%) samples and low in 3 (17.6%) samples.

Fig. 1: *OPA-Lung:Showing Diffuse Areas of Consolidation.*

Pawaiya and Ram Kumar [11] also noticed strong nuclear immunolabelling for c-Myc in lung sections of OPA. *Myc* is a potent oncogene that can promote tumorigenesis in a wide range of tissues and the elevated expression of its gene product, the transcription factor c-Myc correlates with tumor aggression and poor clinical outcome. *Myc* expression is deregulated and often markedly elevated in human neoplasms [5]. Elevated expression of c-Myc occurs through multiple mechanisms in tumor cells, including gene amplification, chromosomal translocation, single nucleotide polymorphism in regulatory regions, mutation of upstream signalling pathways, and mutations that enhance the stability of the protein [12–15]. c-Myc accumulates in the promoter regions of active genes across the cancer cell genome and causes transcriptional amplification, producing increased levels of transcripts within the cell's gene expression program. This transcriptional amplification could provide the sweeping changes in cellular physiology necessary for aggressive cellular growth and proliferation [16].

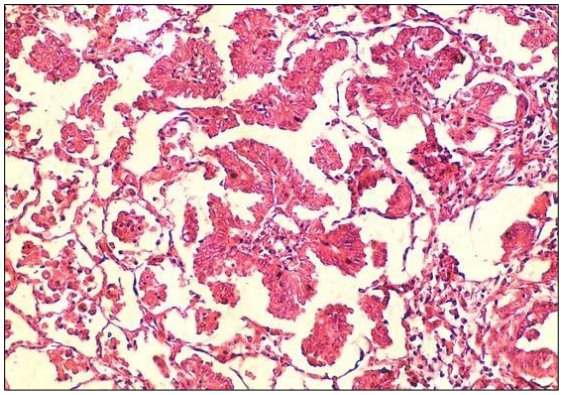

Fig. 2: *OPA-Lung: Note Papillary Projections of Alveolar Epithelium H&E x100.*

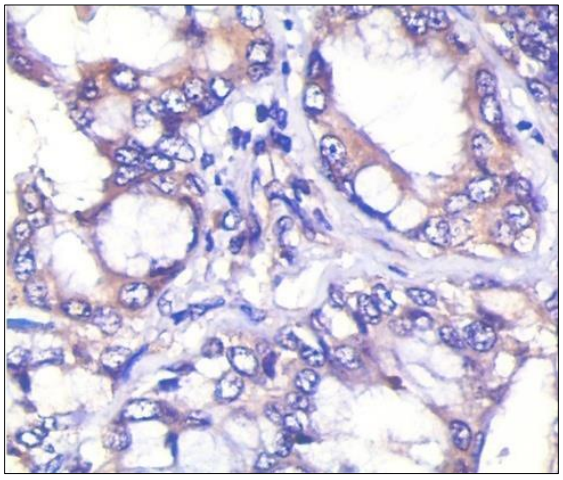

Fig. 3: *OPA-Lung: Immunostaining for JSRV-MA-Note Intracytoplasmic Brownish Granular Staining in the Neoplastic Epithelial Cells of Alveoli. X400.*

Retroviruses have contributed to our understanding of the role of c-Myc in tumors like leukemias and lymphomas [17]. Retroviruses can activate c-*Myc* oncogene expression in at least three ways; by use of virally encoded proteins to activate c-Myc transcription, by transduction and modification

of the c-Myc gene to make a virally encoded form of the gene and by cis-activation of c-Myc expression after proviral insertion [18–22].

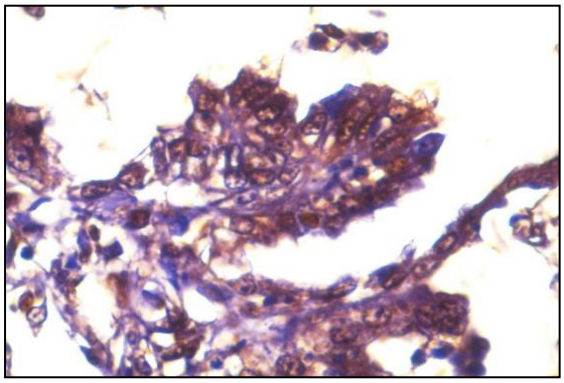

Fig. 4: *OPA-Lung: Immunostaining for c-Myc-Note Strong Nuclear Staining in the Neoplastic Epithelial Cells of Alveolar Projection. X400.*

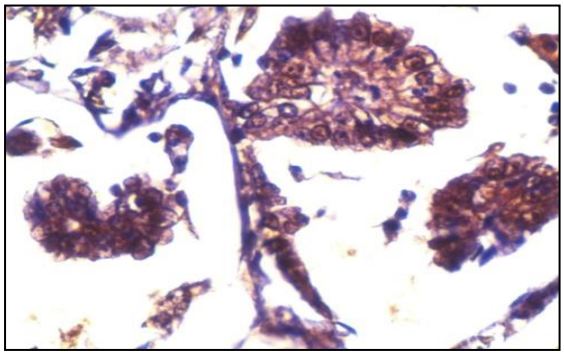

Fig. 5: *OPA-Lung: Immunostaining for c-Myc-Note Strong Nuclear Staining in the Neoplastic Epithelial Cells of Alveolar Projection X400.*

The mechanism of oncogenesis in retroviruses induced tumors is quite interesting. Transforming retroviruses carry oncogenes derived from cellular genes that are involved in mitogenic signalling and growth control. Non-transforming retro viruses activate cellular proto oncogenes by integrating a provirus near normal cellular proto oncogenes and activating their expression, by a mechanism termed 'proviral insertional mutagenesis' [23]. Lung cancers induced by JSRV infection in sheep and by JSRV Env expression in mice have similar histologic features and are primarily characterized by adenomatous proliferation of peripheral lung epithelial cells [24]. It was further stated that it is unnecessary to invoke a role for insertional mutagenesis, gene activation, viral replication, or expression of other viral gene products in sheep lung tumorigenesis, although these processes may play a role in other clinically less important sequelae of JSRV infection such as metastasis observed with variable frequency in sheep [24]. Unlike most acute transforming retroviruses, JSRV does not carry a host cell-derived oncogene, but rather its Env protein has transformation potential [25]. The role of oncogenes like *myc* in JSRV induced ovine pulmonary adenocarcinoma needs further studies to unravel the molecular mechanisms of oncogenesis in OPA.

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