Eur Respir J 2006; 27: 1079–1081
DOI: 10.1183/09031936.06.00046606

EDITORIAL

Telomerase activation in adenocarcinoma–bronchioloalveolar carcinoma

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At present, human bronchioloalveolar carcinoma (BAC) is a disease with an evolving definition. The clinical concept of BAC needs to be re-evaluated with careful attention to the 2004 World Health Organization (WHO) criteria [1] due to major clinical implications. Series published in the literature before 2000, *i.e.* before the WHO definition of BAC, had not discerned a specific survival advantage and clinical behaviour for BAC as compared with other nonsmall cell lung cancers (NSCLC). Instead, existing data indicate that patients with solitary small peripheral BAC, characterised by pure lepidic growth pattern with no evidence of stromal vascular or pleural invasion, represent only 2–6% of NSCLC diseases and have 100% 5-yr survival. This survival advantage may be extended to the micro-invasive BAC according to Japanese observations [2].

Indeed, most lung adenocarcinomas, including those with a predominant BAC component, are invasive and consist of a mixture of BAC (noninvasive) with invasive histological patterns such as acinar, papillary or solid growth. The high predominance of these mixed subtype adenocarcinoma justified the moving of this category to the top of the list of subtypes in the 2004 WHO classification [1]. There are several pathological and radiological growth presentations of mixed subtype adenocarcinoma with a BAC component, including solitary peripheral nodules, multiple nodules and lobar consolidation, which can all be unilateral or bilateral. When BAC or mixed adenocarcinoma present with a diffuse parenchymal infiltration pattern (multicentric nodules and lobar consolidation), their macroscopic and radiological presentations are that of pneumonic-type adenocarcinoma. Although previously called pseudopneumonic BAC, most of these tumours are adenocarcinoma mixed subtype with the whole spectrum of the BAC acinar, papillary and solid pattern [3]. There are clear differences in the pathological, radiological and clinical implications of solitary small peripheral lung adenocarcinoma and these pseudopneumonic multicentric and diffuse consolidation patterns. The study of the latter tumours is more problematic because they are often unresectable, and a diagnosis is established only by partial biopsies or even cytological specimens. They also represent a staging problem since adenocarcinoma–BAC enter in T4 lesions (stage IIIB) if diffusion involves one lobe, and in M1 disease (classified as stage IV disease) when the tumour involves separate lobes. Recent studies indicate that although overstaged by the current tumour/node/metastasis classification, these tumours may be amenable to surgical resection and prolonged survival, and lack distant extrathoracic metastasis [4].

To increase the confusion about the old and current definitions of BAC, the term bronchioloalveolar was frequently used in experimental pathology and animal models, and it has been recognised that most of these animal adenocarcinoma models that histologically mimic pure human BACs are actually extremely rare. The human pseudopneumonic BAC is strikingly morphologically similar to a contagious form of pulmonary cancer in sheep infected by the β-retrovirus Jaagsiekte sheep retrovirus (JSRV) [5, 6] as discussed in the article by SUAU *et al.* [7] in this issue of the *European Respiratory Journal*.

Ovine pulmonary adenocarcinoma (OPA) arises from type II alveolar pneumocytes expressing JSRV capsid protein and thyroid transcription factor-1, producing surfactant A and C secretion with multilamellar bodies in the cell cytoplasm at electron microscopy. An obvious question, which is still unanswered, is whether viral agents are present in the BAC and mixed-type adenocarcinoma with BAC in humans. This does not rule out the crucial interest in OPA as representing an excellent outbred animal model of BAC, where JSRV host cell interactions and molecular mechanisms of oncogenesis are investigated with the highest priority objectives to: 1) study the sequence of events in the pathogenesis of adenocarcinoma; 2) determine the signalling pathways of oncogene and tumour suppressor genes involved in OPA tumorigenesis; and 3) establish the alternative to viral aetiology in human adenocarcinoma. In the present issue, SUAU *et al.* [7] pursue this objective by studying telomerase activity as a method for achieving immortalisation and continuous growth of the tumour in this model. Telomeres are nucleoprotein complexes composed of TTAGGG repeat array and associated proteins that function to protect and maintain the telomeric DNA, thus protecting chromosome ends from exonucleolitic degradation, end-to-end fusion, chromosomal rearrangements and telomere shortening. Since telomere shortening is the biological clock of cellular replicative senescence, telomerase activation is considered necessary for tumour cells to escape cell senescence and gain continuous proliferative capacities. Telomere maintenance proteins include TRF1, TRF2, RAP-1 and POT-1, which are critical for the ability of catalytic component human telomerase reverse transcriptase (hTERT) to be active in

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telomere elongation. Other mechanisms of telomerase regulation include post-translational modifications such as hTERT phosphorylation by protein kinase C and Akt protein kinase B. Independently, the roles of the Ras-mitogen extracellular kinase-mitogen-activated protein (MAP) kinase and phosphatidylinositol 3-kinase-AkT-mTOR pathways have been fully involved in JSRV-induced transformation of rodent fibroblast and epithelial cell lines [8]. Akt is a kinase involved in an important oncogenic process, providing an ability to increase cell survival, cell proliferation and tissue invasion. Evidence implicates involvement of PI3K-Akt in the signalling pathways induced by viral envelope protein (capsid peptides of JSRV). A phosphatidylinositol 3-kinase docking site is present in the cytoplasmic tail of the JSRV transmembrane protein, inducing Akt phosphorylation, which has been shown to be essential for envelope-induced cell transformation of NIH_3T_3 by JSRV.

SUAU *et al.* [7] demonstrate that cell immortalisation occurs in OPA lung tumours *via* a high level of telomerase activity, which was detected in all OPA tumours but also in two-thirds of cell cultures derived from telomerase-positive tumours, whereas no telomerase activity was found in control lung and normal lung type II pneumonocytes. SUAU *et al.* [7] explain the lack of telomerase activity in one third of cell cultures derived from telomerase-positive tumours by possible alternative pathways of telomerase activation, and their presence in tumours by contaminating activated lymphocytes or other inflammatory cells infiltrating the tumour. This indicates a limitation of the present study, which would have been avoided by an *in situ* demonstration of telomerase hTERT expression.

Previous results of hTERT expression and activity in adenocarcinoma using immunohistochemistry (an *in situ* approach) telomerase activity measurement by telomerase repeat amplification protocol (TRAP) assay (an enzymatic measurement of the reverse transcriptase hTERT activity), and *in situ* hybridisation (to evaluate the level of mRNA hTERT expression) showed that telomerase expression varies significantly according to the histological type of lung tumour [9], and adenocarcinoma was found to display the lowest level of telomerase activity (particularly in the bronchioloalveolar component) [10–12]. In contrast, basaloid carcinoma and aggressive forms of NSCLC and small cell lung carcinoma (SCLC) showed consistently high levels of telomerase expression. It was also reported that hTERT can preferentially locate on to nuclear structures in 45% of squamous cell carcinoma and 42% of adenocarcinoma, in contrast with its diffuse nuclear localisation in other tumour types, including SCLC and basaloid carcinoma. This nucleolar sublocalisation was associated with a shorter survival in stage I NSCLC [9]. This seems to correspond to a sequestration of hTERT away from its telomeric targets during the S-phase of replication and repair of DNA breaks [13, 14]. Conversely, in SV40 transfected cells, hTERT is released in the nucleoplasmic compartment where the telomeric sequence synthesis takes place [14]. *In situ* subcellular localisation of hTERT expression would have been important in the OPA model in comparison with other viral models of carcinogenesis to evaluate whether hTERT expression is purely nucleoplasmic or if it presents a docking site in the nucleoli in order to protect cells from high genetic instability and aberrant DNA repair. Indeed, telomere elongation should remain disconnected from the DNA repair process in order to prevent high genetic instability and inopportune mortality crisis.

Evidence whether transformation due to JSRV is caused by telomerase activation remains to be provided. The focus of the study by SUAU *et al.* [7] is an *in situ* hybridisation technique for telomere length assessment in order to postulate on the telomeric DNA maintenance in this model as compared with human adenocarcinoma and the chance of hTERT-targeted treatment to prevent immortalisation and continuous growth. The relationship between Akt activation as reflected by Akt phosphorylation and hTERT phosphorylation remains elusive. SUAU *et al.* [7] measured the level of hTERT activity (submitted to contamination by inflammatory cells) with a TRAP assay, but did not demonstrate phosphorylation of hTERT in this context.

In summary, the main strength of this animal model is that the histopathology is strongly similar to the human model, but the model is limited as it fails to show the pre-invasive lesion typical of bronchioloalveolar carcinoma in the form of atypical alveolar hyperplasia of type II Clara cells. It was also questioned whether sheep infected by JSRV develop this before adenocarcinoma, atypical alveolar hyperplasia, which could be the case since the tumour is of a pneumonocyte II Clara cell type and the mouse model of Ras conditional mutant develops in this way. Another strength of this model in determining an oncogenic tumour-signalling pathway involved in OPA and adenocarcinoma tumorigenesis resides in the activation of the MAP kinase/Akt pathway, which seems to be mandatory in the carcinogenesis pathway for adenocarcinoma in general: Ras mutation occurs in 20% of human adenocarcinoma; epidermal growth factor receptor mutation in 25% and especially in those with a BAC component; and Her2 mutation in 3%.

A large series of concomitant sequencing that searched for these three mutations upstream of the mitogen-activated protein kinase pathway [15] has demonstrated that 50% of adenocarcinomas were lacking any of these mutations, and only one per case could be present, *i.e.* the mutations are mutually exclusive. Jaagsiekte sheep retrovirus in an animal model is another way to activate Akt by phosphorylation and predicts that epidermal growth factor receptor and Ras mutations are absent in this model, although Akt constitutive activation and phosphorylation were not demonstrated in the study by SUAU *et al.* [7]. Actually, Akt phosphorylation was at the same baseline level as that of normal cells in culture under these culture conditions. Akt phosphorylation level was shown to be independent from and unresponsive to epidermal growth factor stimulation, which was indeed typical of impairment of mitogen-activated protein kinase pathway in this model of adenocarcinoma.

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