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BR22, a 26 kDa thyroid transcription factor-1 associated protein (TAP26), is expressed in human lung cells

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BR22, a 26 kDa thyroid transcription factor-1 associated protein (TAP26), is expressed in human lung cells. M-C.W. Yang, B. Wang, J.C. Weissler, L.R. Margraf, Y-S. Yang. ©ERS Journals Ltd 2003.

ABSTRACT: The current authors have previously identified BR22, a thyroid transcription factor (TTF)-1 associated protein 26 (TAP26), which interacts with TTF-1 to enhance human surfactant protein (SP)-B promoter activity in transfected 293 cells. However, the expression of TAP26 in the lung cells and its biological relevance to the SP-B production under physiological conditions were not examined.

In this study, endogenous co-immunoprecipitation and *in situ* immunohistochemical staining techniques were employed to explore the presence of TAP26 and TTF-1 complex in the lung epithelial cells. The correlation of TAP26, TTF-1 and SP-B expression was inspected in H441 cells in the presence of dexamethasone, a known positive effector of the SP-B promoter.

Monoclonal antibody (mAb) against TAP26 can co-immunoprecipitate both TAP26 and TTF-1 from H441 cells. Using this antibody in *in situ* staining of human lung sections, the data show that TAP26 is present in the lung alveolar epithelial cells. Reverse transcriptase-polymerase chain reaction and Western blot analyses of type-II cells as well as dexamethasone-treated H441 cells suggest that TAP26 expression is modulated coordinately with SP-B and TTF-1 in these cells.

In summary, the current study demonstrates that thyroid transcription factor-1 associated protein 26 is an associated protein of thyroid transcription factor-1 in the lung alveolar epithelial cells where surfactant protein gene expressions take place *in vivo*.

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Thyroid transcription factor (TTF)-1, a member of the NK2 family of homeodomain-containing deoxyribonucleic acid (DNA)-binding protein, is required for normal development of the lung, thyroid, pituitary and forebrain [1]. In a study of mice with disrupted TTF-1, the mice were dead at birth and lacked lung parenchyma [1]. This result suggested an essential role of TTF-1 during lung development. Proper expression of TTF-1 is also necessary to initiate and maintain lung morphogenesis and to sustain the differentiation of lung epithelial cell lineages during embryogenesis [2–5]. TTF-1 expression decreases in the proximal bronchial and bronchiolar epithelia as the embryo matures. After birth, and in the adult lung, TTF-1 expression remains in Clara cells located at the distal airway epithelium and in alveolar epithelium type-II cells [6], where it regulates the expression of many lung-specific genes, namely surfactant protein (SP) genes and Clara cell-specific protein (CCSP) [7]. In addition to TTF-1, many other transcription factors, such as the Winged helix DNA-binding domain containing forkhead box proteins (hepatocyte nuclear factor (HNF)-3 (FoxA1 and FoxA2), HNF-3/forkhead homolog (Foxj1a and Foxf1)) and zinc finger proteins Gli and GATA, also participate in the regulation of lung development or lung-specific gene expression [4].

TTF-1 is a known key factor that controls the gene expression of SPs and CCSP [7]. The activity of TTF-1 is subject to modification by a variety of potential regulators. For example, altering phosphorylation of TTF-1 [8–12], as well as redox/oligomerisation of TTF-1 [13], can amend its DNA-binding activity. Other reports demonstrate that forming protein complexes through interactions with various proteins could also modulate TTF-1 DNA-binding, as well as its transactivation activities [14–17]. The cellular location of TTF-1 can be another factor to modulate its affected gene expression. Under a condition of cells treated with phorbol ester or transforming growth factor-β, TTF-1 was retained in the cytoplasm instead of becoming nuclear bound, leading to the blockage of TTF-1 binding to its DNA targets. The lack of TTF-1 binding to its target consequently prevents the activation of these TTF-1 dependent genes [18, 19].

In a previous study, using the proximal TTF-1 binding sites located in the human SP-B promoter as a target in yeast one-hybrid analysis, the current authors identified a novel transfactor, BR22, which interacts with the SP-B promoter fragment and transactivates reporter genes in yeast. BR22, a 26 kDa protein, now designated as TTF-1-associated protein 26 (TAP26), forms a protein complex with TTF-1 *in vitro* and is capable of enhancing human SP-B promoter activity in transfected 293 cells [14]. However, their *in vivo* interactions and the biological relevance under physiological conditions have not been addressed. Here, by using the TAP26-specific mAb42, the expression of TAP26 and its protein complex with TTF-1 can be identified *in vivo* in the lung alveolar

For editorial comments see page 6.

epithelial cells. Furthermore, by comparing the expression pattern of TAP26 and TTF-1 with SP-B in dexamethasone-treated lung adenocarcinoma cells, the correlation of TAP26 and TTF-1 complex with the SP-B expression can be examined *in vivo*.

Materials and methods

Reverse transcriptase-polymerase chain reaction analysis

One microgram of total ribonucleic acid (RNA) from adult lung tissue, foetal lung tissue, isolated type-II cells treated with dibutyryl cyclic adenosine monophosphate (Bt_2cAMP) for 5 days (D5) (a gift from C.R.M. Mendelson at UT Southwestern Medical Center, Dallas, Texas) and H441 cells (NCI-H441, a human lung, papillary adenocarcinoma cells) was used to generate complimentary DNA (cDNA) by using the SMART (BD Biosciences, Palo Alto, CA, USA) protocol. One nanogram of synthesised cDNA was used in the polymerase chain reaction (PCR) analysis of TAP26 message. Primers used in the PCR analysis are as indicated in figure 1. The PCR amplification of TAP26 message with these primers span a region containing exon II and III. These primers were chosen to complement the TAP26 message only, not the pseudogene. Aliquots of amplified sample were collected every three cycles and then loaded onto 1.5% agarose gel for analysis. Controls that used glyceraldehyde-3-phosphate dehydrogenase (GADPH) or SP-B specific primers were also amplified separately. Aliquots were collected from each sample at the indicated cycles of amplification.

Monoclonal antibody

Purified recombinant protein glutathione-S-transferase (GST)-TAP26 was used as the antigen to immunise mice. Two bipartite primers, 5′ primer TCTGACGGATCC**ATGGCGCCGGTGAGGCGGT** and 3′ primer ATGGTGGGATCC-**ACATTTTTCTTGTATTTTTTGAAGAA** (TAP26 sequences are in bold) were used for reverse transcriptase (RT)-PCR amplification of the full-length TAP26 cDNA using human lung polyA RNA (BD Biosciences) as the template. Utilising the bipartite primers in T4 DNA polymerase exonuclease recession method [20], the PCR-amplified TAP26 fragment can be directly in-frame fused to GST in vector pGEX-VH [21] without the introduction of extra unnecessary amino acid residues. The pGEX-VH vector contains a six-histidine stretch that is designed at the end of fusion protein, which can be purified to homogeneity as a full-length protein (GST-TAP26) by performing sequential GST- and Ni-beads affinity chromatography. Each mouse (female Balb/c, 6 weeks of age, n=10) received 0.1 mL of GST-TAP26 (250 $\mu g \cdot mL^{-1}$ in saline) in RIBI adjuvant system (MPL+ TDM Emulsion, R-700; Corixa, Hamilton, MT, USA) at each of two subcutaneous sites on day 0 and day 21. The mice sera were tested for TAP26 antibodies on enzyme-linked immunosorbent assay (ELISA) coated with MAL-TAP26, which was constructed by inserting TAP26 into the vector pMAL^{TM}-p2X (New England Biolab, Beverly, MA, USA). The sera were also tested for positive endogenous TAP26 on Western blots using nuclear extracts from 293 (adenovirus 5 DNA transformed human embryonic kidney cells) or Hela cells. Only those mice that showed strong immune responses with a band corresponding to the endogenous TAP26 on the blots then received the final immunisation intraperitoneally. Three days later, splenectomy was performed on these mice and the splenocytes were fused with mouse myeloma cell line (P3X63Ag8.653, ATCC CRL-1580) by PEG-1500. The successfully fused hybridomas were selected in Roswell Park Memorial Institute (RPMI)-1640 medium supplemented with HAT (10 mM hypoxanthine, 40 μM aminopterin, 1.6 mM thymidine; GIBCOBRL, Carlsbad, CA, USA), 10% foetal bovine serum, 24 μM β-mercaptoethanol and Nutridoma-CS (Roche, Indianapolis, IN, USA). The culture supernatants were collected, screened by ELISA, and the reactions were confirmed with Western blot analysis. Positive clones were clonally selected twice by limiting dilution. The secreted antibody from culture was purified using either Protein-A Sepharose CL-4B column or MABTrap Kit (Amersham, Piscataway, NJ, USA).

Fig. 1. – The structure of human thyroid transcription factor-1 associated protein (TAP26) gene and a similarity comparison of deoxyribonucleic acid (DNA) sequences between TAP26 and a pseudogene. a) The genomic gene structure of human TAP26 gene (sequence information obtained from AC083811). Open boxes denote the exon regions and their sequence ranges that correspond to the complimentary DNA (cDNA) sequence in panel b are indicated. b) Sequence homology alignment of TAP26 cDNA (AF213377) and the pseudogene (sequence information extracted from AL031133). Polymerase chain reaction primer sequences, which are specific for the TAP26 gene not the pseudogene, are highlighted.

Endogenous co-immunoprecipitation

A total of 200 μg H441 or 293 cell nuclear extracts were incubated with 16 μg mAb42 (immunoglobulin (Ig)G_{2b} subtype) in buffer C (20 mM *N*-2-hydroxyethylpiperazine-*N*-2-ethanesulphonic acid (HEPES), pH 7.5; 150 mM NaCl, 1 μg

antipain, 1 μg leupeptin, 1 μg pepstatin A and 1 mM phenylmethylsulphonyl fluoride) overnight at 4°C. The antibody and protein complexes were bound to protein A beads, washed with binding buffer, eluted and fractionated in 10% sodium dodecylsulphate-polyacrylamide gel electrophoresis (SDS-PAGE) for Western blot analysis. Anti-TTF-1 mAb (IgG_1 subtype; Zymed, South San Francisco, CA, USA) was applied to detect the presence of TTF-1 in the complex. Goat antimouse IgG_1 labelled with biotin was used as the secondary antibody, and streptavidin-conjugated horseradish peroxidase (HRP) was applied to develop the immunocomplex. For the IgG_{2b} isotype control, antihaemaglutinin (HA) mAb 12C5 was used. Nuclear extracts from 293 were also used to substitute the H441 extracts as a negative control.

Immunohistochemistry staining

Formaldehyde-fixed, paraffin-embedded, human adult lung sections were employed to explore the expression of TAP26 *in vivo*. Tissue sections were blocked with normal serum and then incubated with antibodies against TAP26 (mAb42), TTF-1 (Zymed), and/or SP-B (Chemicon, Temecula, CA, USA) in phosphate-buffered saline with 0.5% bovine serum albumin at 4°C overnight. For immunohistochemical staining, an avidin and biotinylated macromolecular complex (ABC) system (Vector Labs, Burlingame, CA, USA) was applied to detect the antibody-antigen complex by following the manufacturer's procedure. To detect antigen-bound antibodies, a horseradish peroxidase (HRP)-conjugated ABC complex was used. The substrate of peroxidase-diaminobenzidine tetrahydrochloride (DAB) was used to develop a brown colour at the antigen-present locations. After the colour development, cells were counterstained with Mayer's Haematoxylin for nuclei. The counterstained nuclei were shown in blue. For the SP-B detection, the counterstaining procedure was omitted.

Results

Genomic gene and pseudogene

Using yeast genetic studies, TAP26 was identified to be a factor that interacts with TTF-1 *in vitro*, through a direct protein-protein interaction and activates the SP-B promoter in cotransfected 293 cells [14]. The gene, which encodes for TAP26 message, is located at human chromosome 12q (Accession #AC083811). This gene is composed of four exons with traditional intron-exon splicing sites (fig. 1a). In addition, a pseudogene, with >95% of sequence identity to the TAP26 cDNA (fig. 1b), was identified in the human genome at chromosome 6q (Accession #AL031133). It is a processed pseudogene by RT. This pseudogene contains several mutations, which render amino acid substitutions, deletions and a premature termination codon at position 212 (C→A). Prior to the current authors' analysis, both genomic genes had already been sequenced and deposited in the Genebank, however, neither were identified as a gene for transcripts.

Endogenous co-immunoprecipitation

On Western blot analysis, mAb42 identified a 34 kDa polypeptide in H441 and 293 cell lysates (fig. 2a). Based on the cDNA sequence, the expected protein size is 26 kDa which is smaller than the detected polypeptide in the gel. The present authors believe that the 34 kDa polypeptide is endogenous TAP26 since overexpressed protein has a similar size as the endogenous TAP26 (fig. 2a, lane 3); likewise, mouse

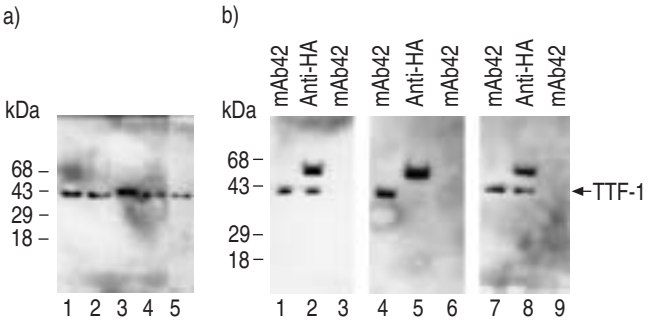

Fig. 2.–a) Western blot analysis of thyroid transcription factor (TTF)-1 associated protein (TAP26). Totals of 30 μg protein from different samples were fractionated in 12% sodium dodecylsulphate-polyacrylamide gel electrophoresis (SDS-PAGE), transferred to a polyvinylidene fluoride membrane and probed with monoclonal antibody (mAb)42. Lane 1: 293 nuclear extracts; Lane 2: 293 whole cell lysates; Lane 3: nuclear extracts of haemaglutinin (HA)-TAP26; Lane 4: nuclear extracts of Flag-TTF-1-transfected 293 cells; Lane 5: H441 nuclear extracts. b) Endogenous TTF-1 is co-immunoprecipitated with TAP26 from the nuclear extracts of H441 cells. A total of 16 μg mAb42 or antiHA monoclonal antibody was incubated with 200 μg of nuclear extracts from H441 (lanes 1, 2, 4, 5, 7, 8) or 293 cells (lanes 3, 6 and 9). Lanes 1–3 are a mixture of antibody and nuclear extracts before binding to protein A beads. Lanes 4–6 are proteins eluted off the protein A beads. Lanes 7–9 are proteins left in supernatant after incubating with the protein A beads. Reaction mixtures containing antibodies are as labelled. Protein samples were fractionated on 10% SDS-PAGE gel and then subjected to Western blot analysis for TTF-1 by using anti-TTF-1 mAb as described in the Materials and methods section.

polyclonal antibody to TAP26 also detects a 34 kDa polypeptide on the SDS-PAGE gel (data not shown). Controls using anti-HA mAb (12C5), which has the same isotype as mAb42, did not develop a signal at the size of TAP26. This result suggests that the signal detected by mAb42 is specific to TAP26. Only one band appeared on the blot when probed with mAb42 and longer exposure yielded no extra signals.

In an earlier study, the current authors had used recombinant proteins to demonstrate the protein-protein interactions between TTF-1 and TAP26 *in vitro* and *in vivo*. The present report shows that the endogenous protein complex can be identified in H441 cells by co-immunoprecipitating the complex. As shown in figure 2b, anti-TTF-1 antibody detects a band from H441 nuclear extracts retained on the beads co-immunoprecipitated with TAP26 by mAb42 (lane 4). The isotype control antibody, 12C5, did not precipitate TTF-1 (lane 5), and the other control, of using 293 cell nuclear extracts in the experiment, also did not show the same size band identified by anti-TTF-1 antibody (lane 6). This endogenous co-immunoprecipitation of TAP26 and TTF-1 strongly suggests that they are a physically associated stable compound *in vivo*. Obviously, the binding of mAb42 to TAP26 does not interfere with the interaction of TAP26 with TTF-1. When 293 nuclear extracts were applied in the experiment as a cellular protein control, no signal was detected. This suggests that the identified co-immunoprecipitating band is a specific signal of TTF-1. There were no irrelevant proteins aggregated nonspecifically with TAP26 or to the antibody column. Thus, the present authors are confident in using mAb42 to detect the endogenous TAP26 and TTF-1 compound in the lung cells (fig. 2b).

TAP26 is expressed in the lung alveolar epithelial cells

In order to examine the expression pattern of TAP26 in the lung and to test the capability of mAb42 on tissue staining,

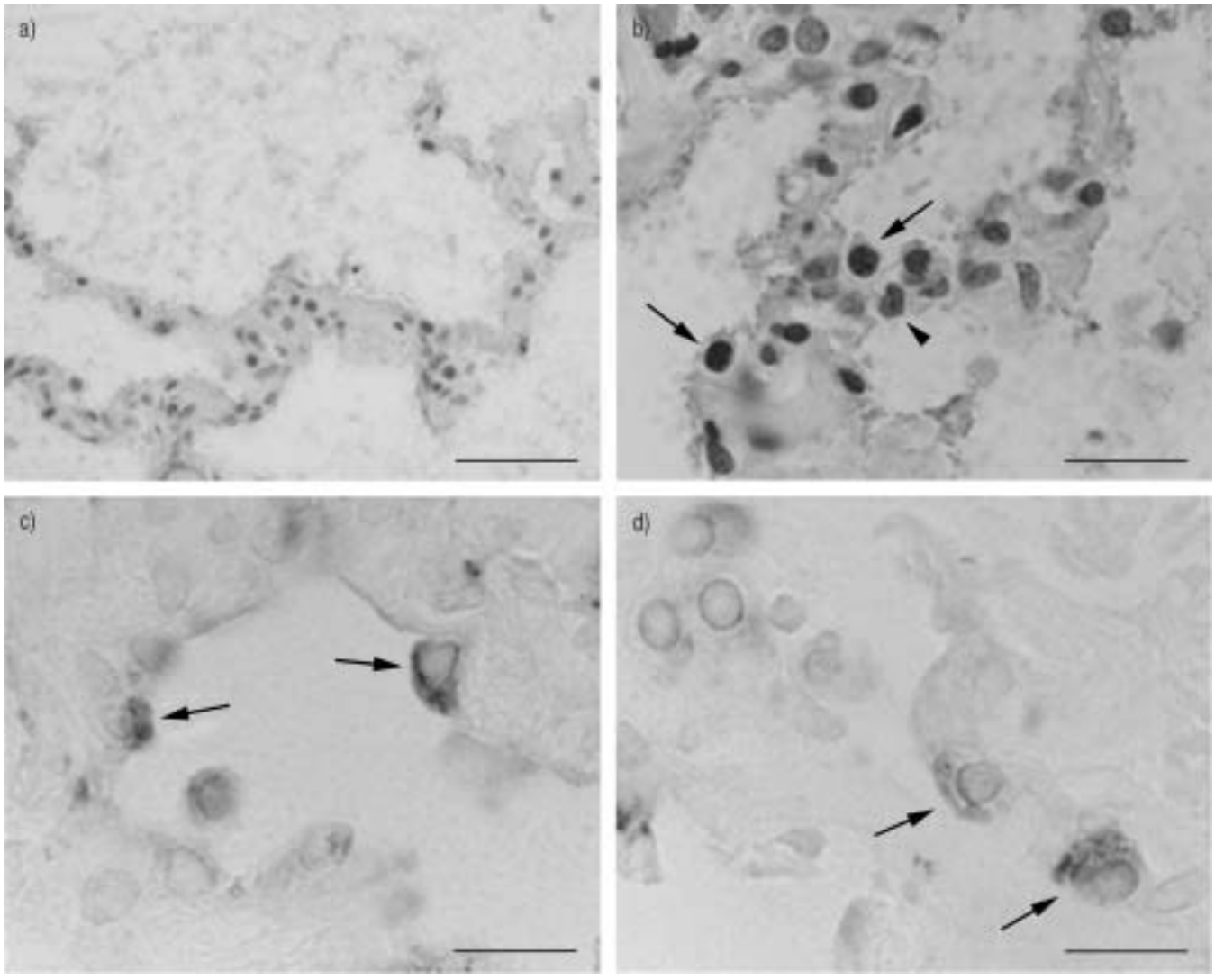

Fig. 3. – *In situ* staining of thyroid transcription factor-1 associated protein (TAP26) in the human lung alveolar epithelial cells. Formalin-fixed and paraffin-embedded human lung sections were probed with monoclonal antibody (mAb)42 or polyclonal antisurfactant protein (SP)-B antibodies. Cellular antigen interacting with the antibodies was developed with diaminobenzidine tetrahydrochloride (DAB) as described in the Materials and methods section. a) and b) were probed with mAb42 (the mAb against TAP26). c) and d) were continuous lung sections from the same tissue block probed with anti-SP-B antibody. Arrows mark the cells stained by the antibodies and the arrowhead shows the staining by DAB. Scale bars: a) 75 μm; b) 30 μm; c) 15 μm; d) 15 μm.

in situ immunohistochemical staining of lung sections was conducted. As shown in figure 3, mAb42 selectively stained some nuclei of lung alveolar epithelial cells. Not all the cells in the section were stained. mAb42 staining was absent from cells in the mesenchyme region as well as in the distal airway epithelium (a and b). These stained cells have a round shape and bulge from the alveolar epithelial surface. When these cell locations in the lung are compared with the distribution pattern of SP-B expressing cells (c and d), they are quite similar (fig. 3). This result implies that TAP26 could be expressed in the SP-B producing cells. Controls without mAb42 showed no staining signal, which eliminates the possibility of secondary antibody contribution to the stained signal. Occasionally, some cells located in the interstitial tissue or in the blood vessel were also stained by DAB (fig. 3b, arrowhead). These cells could be macrophages or leukocytes which also express TAP26 *in vivo*, or may represent non-specific staining caused by the presence of endogenous peroxidase (see Discussion Section).

TAP26 is coordinately expressed with SP-B and TTF-1 in vivo

In order to determine whether the expression of TAP26 is physiologically relevant to SP-B production, the patterns of TAP26, TTF-1, and SP-B expression were examined *in vivo*. To prevent detecting the transcripts from TAP26 pseudogene or the gene itself, primers selected for TAP26 transcripts were used in RT-PCR analysis. As shown in figure 1b, the selected TAP26 primers can detect TAP26 message in the adult lung, foetal lung, isolated type-II cells, and H441 cells (fig. 4). In the adult lung and in Bt_2cAMP-induced type-II cells (D5), the amounts of TAP26 transcripts are significantly higher than that in the foetal lung tissue and the uninduced type-II cells (D0) (normalised with a GAPDH control). Likewise, the amounts of SP-B transcripts in these samples also appear abundant. Such coordinated expression suggests that the level of TAP26 may be able to modulate the expression of SP-B *in vivo*. The possibility of amplifying the genomic gene with

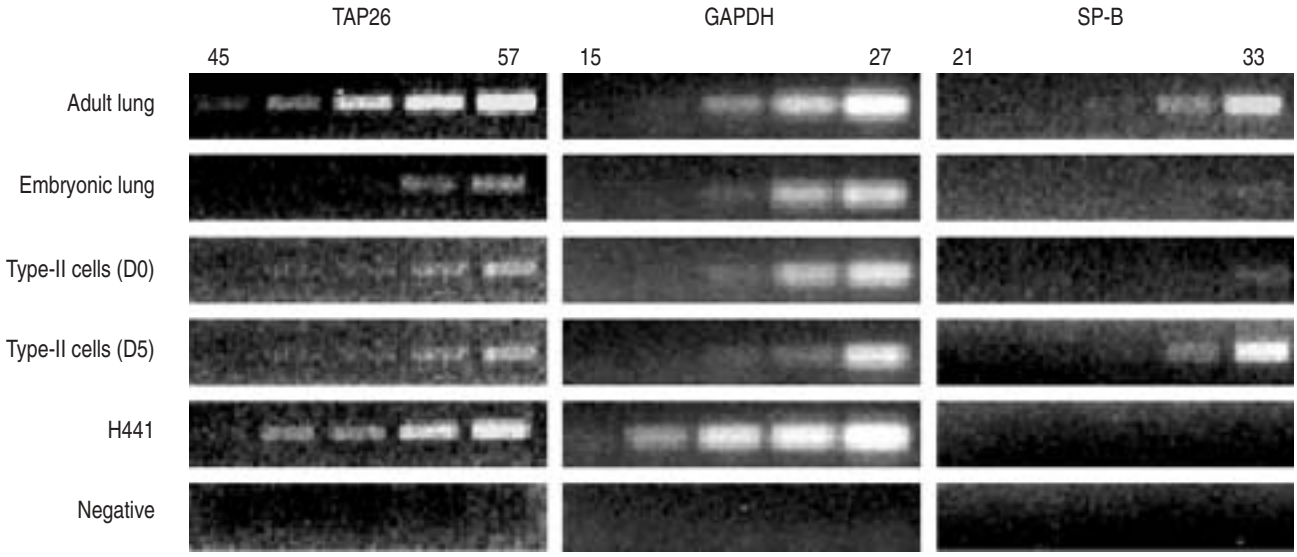

Fig. 4. – Thyroid transcription factor-1 associated protein (TAP26) message is expressed in lung cells. Total ribonucleic acid from human adult lung, embryonic lung tissue, type II cells isolated from foetal lung explants at day 0 (D0) and cultured in dibutyryl cyclic adenosine monophosphate for 5 days (D5), and H441 cells were utilised to generate complimentary deoxyribonucleic acid (cDNA) by the SMART protocol (Clontech, Palo Alto, CA, USA). One nanogram of each cDNA was applied for polymerase chain reaction analysis using specific primer sets for TAP26, glyceraldehydes-3-phosphate dehydrogenase (GAPDH), and surfactant protein B (SP-B) messages. Aliquots from various amplification cycles were collected and analysed in agarose gel. The numbers of cycles amplified for each sample are labelled at the top.

these primers is unlikely since the primers used for this analysis are located on different exons (II and III; fig. 1). The amplification of the genomic gene with these primers will yield a DNA fragment >2 kb size that is distinct from the RT-PCR products (186 base pairs). Thus, the signals detected in the RT-PCR analysis should represent the relative level of TAP26 expressions in these cells.

It is known that the expression of SP-B and TTF-1 in type-II or H441 cells can be induced by dexamethasone and/or cyclic adenosine monophosphate (cAMP) [22, 23]. In order to examine whether the upregulation of SP-B could be contributed by the increase of TAP26 expression *in vivo*, the protein level of TAP26 under the treatment of dexamethasone induction was inspected. As shown in figure 5, dexamethasone can induce both TAP26 and TTF-1 expression in H441 cells in a parallel and dose-dependent manner. The other effector, cAMP, also shows a mild induction of both proteins at 25 μM. Given the fact that increased TAP26 expression in type-II or H441 cells is accompanied by elevated SP-B and/or TTF-1 expression, the present data suggest a role of TAP26 as

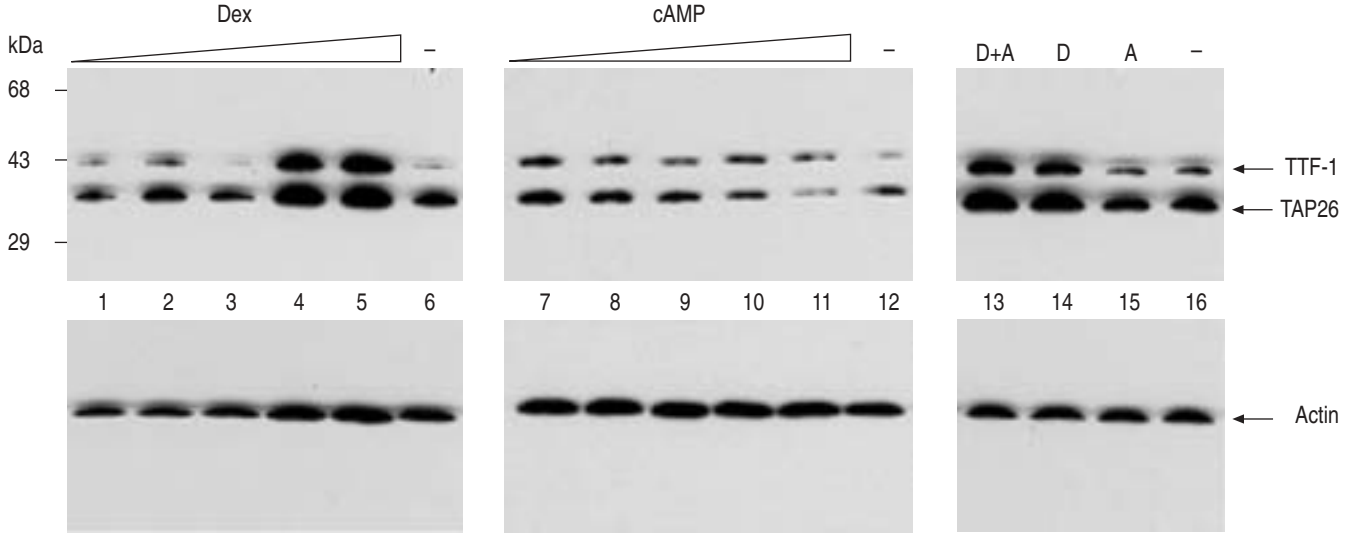

Fig. 5. – Thyroid transcription factor (TTF)-1 associated protein (TAP26) can be induced in parallel with TTF-1 by dexamethasone (Dex) and cyclic adenosine monophosphate (cAMP) in H441 cells. Exponentially growing H441 cells were treated with 1, 10, 25, 50 and 100 nM of Dex (lanes 1–5), or with 25, 50, 100, 200 and 500 μM of cAMP (lanes 7–11) for 24 h in culture before harvested for nuclear extracts preparation. Samples in the right panel (lanes 13–16) were collected from cells treated with 50 nM Dex and 100 μM cAMP (D+A), 50 nM Dex (D) or 100 μM cAMP (A) alone for 48 h before beng harvested for analysis. Total of 20 μg nuclear extracts protein were loaded in each lane and fractioned in 10% sodium dodecylsulphate-polyacrylamide gel electrophoresis, Western transferred, and then probed with TTF-1 and TAP26 (mAb42) antibodies. Both TTF-1 and TAP26 signals are indicated. Lower panels are the same protein blots from above that have been stripped and reprobed with actin antibody to show the amounts of protein loaded in each lane.

a co-activator of TTF-1 in regulating the surfactant gene expression in the lung alveolar cells.

Discussion

There are two genes in the human genome that have DNA sequence homology to TAP26. One is localised to chromosome 12q and the other to chromosome 6q25. Given the fact that the gene on chromosome 6q25 has multiple mutations, deletions, a premature termination codon, a stretch of polyA tail at the end of the gene, and no intron; the gene is suspected to be a processed pseudogene. The 5′ end of this gene contains a 270 nucleotide Alu element. The sequence of this Alu element is identical to the human endogenous retrovirus family (HERV) Alu element [24, 25], which is located toward the 5′ end of HERV-H/env60 *pol* gene [24] and toward the leader region of the HERV-K family [25]. Thus, it is likely that the 5′ Alu segment is a functional element that can drive the pseudogene expression in adult tissues or cultured cells as shown in other reports [26]. However, when the pseudogene transcribes, a mature polypeptide will not be produced, since the translation will be stopped at a premature termination codon.

Western blot analysis showed that endogenous TAP26 has a larger size than that expected from the deduced polypeptide. It is unlikely that the incomplete cDNA sequence accounts for the size variations because of the following reasons: 1) two different cDNA sequences recorded in the Genebank have the same length of encoded polypeptide and identical amino acid sequence as the TAP26 cDNA; 2) recombinant TAP26 migrated at the same size range as the endogenous protein (fig. 2a); and 3) polyclonal antibodies from rabbit or mouse also identify the same polypeptide. Thus, the current authors suspect that an unusual protein structure or post-translational modification of the protein could contribute to the altered TAP26 migration in the SDS-PAGE gel.

A previous report by the current authors, using *in vitro* biochemical assays and yeast two-hybrid analysis, documented a protein-protein interaction between TAP26 and TTF-1 [14]. Here, the present authors would like to identify this complex *in vivo* under a physiological condition. To this end, the existence of endogenous TAP26 and TTF-1 complex in H441 cells is characterised by co-immunoprecipitation studies. The result from co-immunoprecipitation indicates that the complex is stable at the physiological concentration. By using Northern blot analyses the present data indicates that TAP26 is widely expressed in adult tissues. However, the result of immunohistochemical staining shows a staining pattern restricted to the alveolar epithelial cells. The stained cells have round-shaped nuclei and are positioned at the alveolar epithelial surface, not the mesenchyme region. In addition, the distribution pattern of these cells largely overlaps with anti-SP-B antibody stained cells (fig. 3). It has been reported that in the adult lung alveolar epithelium, TTF-1 is only expressed in type-II cells. Neither differentiated type I cells nor other interstitial cells express TTF-1 [27]. Thus, the present RT-PCR and co-immunoprecipitation results strongly suggest the presence of TAP26/TTF-1 complex in type-II cells. The fact that not all of the alveolar epithelial cells were probed by mAb42 may be caused by the restriction of TAP26 expression in the distal lung region, or by a low expression in these unstained cells. In addition to these alveolar epithelial cells, the other cells that can be stained by mAb42 are frequently located within the blood vessels or the interstitial mesenchymal regions. This could represent either a true expression of TAP26 in alveolar macrophages or leucocytes, or endogenous peroxidase that was not fully depleted during the staining procedure. Especially in macrophages, dense granules stained in the cytoplasm can often be found. It is clear that such types of staining are caused by the incomplete depletion of endogenous peroxidase *in vivo*. However, the majority of interstitial cells, as well as the epithelial cells in the distal airway, were not detected by mAb42.

TTF-1 is mainly expressed in the thyroid, lung and certain brain tissues in adults. In thyroid, TAP26 is also detected in the follicular cells (data not shown) suggesting that it may also play a role in modulating the TTF-1-affected thyroid gene expression as well. As in the alveolar epithelium, the endothelial cells, blood cells and other interstitial cells in thyroid are all negative for TAP26 expression. Therefore, the co-expression of TAP26 along with TTF-1 in various tissues could suggest a general role of TAP26 as a cofactor of TTF-1 to modulate its affected gene expression *in vivo*.

Changes in TAP26 expression were coordinated with the variations of TTF-1 *in vivo* in dexamethasone-treated H441 cells (fig. 5). The observation of parallel induction of both TAP26 and TTF-1 in H441 cells strengthens the authors′ option that TAP26 is functionally associated with TTF-1 *in vivo*. Evidently, the interaction promotes TTF-1 activity to modulate the affected gene expression. Another line of evidence that suggests the relationship of this protein complex and the surfactant gene expression is the higher level expression of TAP26 in adult lung and isolated type II cells induced by Bt_2cAMP, where the SP-B expression is also largely increased *in vivo* (fig. 4). Such a coordinative regulation indicates that the complex plays an important role in mediating the gene expression under physiological conditions. Indeed, when TAP26 was cotransfected with TTF-1 and SP-B or SP-C promoter-driven reporters into 293 cells, TTF-1-modulated promoter activities were synergistically stimulated by TAP26 in a dose-dependent manner [14] (and data not shown). Collectively, it is clear that TAP26 is an associated co-activator of TTF-1 in the lung. Indeed, its function on TTF-1 effected gene expression in other tissues requires further examination.

Many proteins have been reported to associate with thyroid transcription factor-1 and modulate its affected gene expression. Calreticulin and retinoic acid receptor-α are two of the proteins that individually increase the thyroid transcription factor-1 deoxyribonucleic acid-binding activity through protein-protein interactions, and subsequently promote its transcription activity [15, 17]. On the other hand, proteins such as thyroid transcription factor-2 and T:G mismatch-specific thymine deoxyribonucleic acid glycosylase can repress thyroid transcription factor-1 activity either through a direct interaction with thyroid transcription factor-1 [28], or by interfering with the co-factor which is required for thyroid transcription factor-1 activity [16]. Although the current authors′ studies strongly indicate that thyroid transcription factor-1 associated protein 26 has a direct protein contact with thyroid transcription factor-1 in lung cells, the mechanism by which this interaction modulates the activity of thyroid transcription factor-1, such as by changing its deoxyribonucleic acid-binding activity or by enhancing its transactivation activity, requires further examination.

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