Thyroid transcription factor-1 (TTF-1/Nkx2.1/TITF1) gene regulation in the lung

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ABSTRACT

TTF-1 [thyroid transcription factor-1; also known as Nkx2.1, T/EBP (thyroid-specific-enhancer-binding protein) or TITF1] is a homeodomain-containing transcription factor essential for the morphogenesis and differentiation of the thyroid, lung and ventral forebrain. TTF-1 controls the expression of select genes in the thyroid, lung and the central nervous system. In the lung, TTF-1 controls the expression of surfactant proteins that are essential for lung stability and lung host defence. Human TTF-1 is encoded by a single gene located on chromosome 14 and is organized into two/three exons and one/two introns. Multiple transcription start sites and alternative splicing produce mRNAs with heterogeneity at the 5′ end. The 3′ end of the TTF-1 mRNA is characterized by a rather long untranslated region. The amino acid sequences of TTF-1 from human, rat, mouse and other species are very similar, indicating a high degree of sequence conservation. TTF-1 promoter activity is maintained by the combinatorial or cooperative actions of HNF-3 [hepatocyte nuclear factor-3; also known as FOXA (forkhead box A)], Sp (specificity protein) 1, Sp3, GATA-6 and HOXB3 (homeobox B3) transcription factors. There is limited information on the regulation of TTF-1 gene expression by hormones, cytokines and other biological agents. Glucocorticoids, cAMP and TGF-β (transforming growth factor-β) have stimulatory effects on TTF-1 expression, whereas TNF-α (tumour necrosis factor-α) and ceramide have inhibitory effects on TTF-1 DNA-binding activity in lung cells. Haplo-insufficiency of TTF-1 in humans causes hypothyroidism, respiratory dysfunction and recurring pulmonary infections, underlining the importance of optimal TTF-1 levels for the maintenance of thyroid and lung function. Recent studies have implicated TTF-1 as a lineage-specific proto-oncogene for lung cancer.

INTRODUCTION

TTF-1 [thyroid transcription factor-1; also known as Nkx2.1, T/EBP (thyroid-specific-enhancer-binding protein) or TITF1], a member of the homeodomain-containing transcription factor family, activates the expression of select genes in the thyroid, lung and restricted regions of the brain[1]. Homeodomain-containing transcriptional factors play key roles in the control of embryonic development and differentiation [2,3]. A homeobox is a 180 bp DNA sequence motif encoding a protein domain that can bind the DNA in a sequence-specific manner. Homeodomain-containing proteins control the transcriptional activation of target genes by binding to specific

Key words: inflammation, lung cancer, morphogenesis, promoter regulation, surfactant, thyroid transcription factor (TTF).

Abbreviations: CCSP, Clara cell secretory protein; DEE, diesel engine emissions; EMT, epithelial–mesenchymal transition; FOX, forkhead box; HNF, hepatocyte nuclear factor; HOX, homeobox; IPF, idiopathic pulmonary fibrosis; MITF, microphthalmia-associated transcription factor; NSCLC, non-SCLC; PKA, protein kinase A; RNAi, RNA interference; SCC, squamous cell carcinoma; SCLC, small-cell lung carcinoma; SP, surfactant protein; T/EBP, thyroid-specific-enhancer-binding protein; TGF-β, transforming growth factor-β; TNF-α, tumour necrosis factor-α; TSH, thyroid-stimulating hormone; TTF-1, thyroid transcription factor-1.

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DNA sequences via the homeodomain. The homeobox was first identified in the HOM and HOX (homeobox) genes that control body axis patterning in the fruit fly *Drosophila melanogaster*. TTF-1 was first purified from rat thyroid FRTL-5 cells as a transcription factor activating the rat thyroglobulin promoter in a thyroid-cell-specific manner [4]. cDNA cloning experiments determined that the TTF-1 mRNA encodes a protein of approx. 38 kDa, and that the TTF-1 DNA-binding domain is a novel mammalian homeodomain that displays significant homology with the *Drosophila* NK-2 homeodomain [5]. These studies also demonstrated the presence of TTF-1 mRNA in the lung and TTF-1 DNA-binding activity in lung nuclear extracts. Soon after the first report on the cloning of TTF-1, independent studies reported the cloning of T/EBP from FRTL-5 cells that had sequence identity with TTF-1 [6].

TTF-1 controls the expression of several important thyroid-specific and lung-specific genes. In the thyroid, TTF-1 controls the expression of the thyroglobulin [4], thyroperoxidase [6–9], thyrotropin receptor [10,11] and sodium iodide symporter [12] genes and, in the lung, TTF-1 is essential for the expression of SP (surfactant protein)-A [13,14], SP-B [15,16], SP-C [17,18], CCSP (Clara cell secretory protein) [19,20] and ABCA3 (ATP-binding-cassette transporter A3) [21] genes (Figure 1). Recent studies have determined that, in lung type II cells, TTF-1 is required for the induction of a subset of genes that include LAMP3 (lysosomal-associated membrane protein 3) and CEACAM6 (carcinoembryonic antigen-related cell adhesion molecule 6) [22]. Ectopic expression of TTF-1 in NIH 3T3 cells activates the expression of nestin, an intermediate filament protein expressed in neuroepithelial stem cells, identifying nestin as a TTF-1-regulated gene in the central nervous system [23]. TTF-1 appears to be important for the expression of the T1α promoter [24]. The expression of T1α is restricted to alveolar type I, but not type II, epithelial cells. It is not known whether TTF-1 is expressed in alveolar type I cells, but the occurrence of a TTF-1 DNA-binding site in the T1α promoter and activation of the T1α promoter by TTF-1 suggest that TTF-1 might be expressed in type I cells.

TTF-1 is expressed at the earliest stages of thyroid, lung and brain development and well before the expression of its target genes [25–27], suggesting that TTF-1 may have important roles in the development and differentiation of these organs. Consistent with a role for TTF-1 in the organogenesis of the thyroid, lung and brain, blocking TTF-1 expression in fetal lung explants in culture resulted in an inhibition of branching morphogenesis [28]. Deletion of T/EBP (TTF-1) in mice resulted in pups that are born dead, due to the complete lack of lung parenchyma and thyroid, and to extensive defects in the ventral region of the forebrain [29]. In the adult mouse lung, TTF-1 is expressed predominantly in alveolar type II epithelial cells, epithelial cells lining

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**Figure 1** Expression of TTF-1 and TTF-1-responsive genes in the lung

A schematic diagram of an alveolus showing the locations of alveolar type I and type II cells and Clara epithelial cells. TTF-1 is expressed by alveolar type II and Clara epithelial cells and regulates the expression of SP and CCSP by these cells.
bronchi and bronchioles, and with a weaker expression in the epithelial cells lining the trachea in a pattern similar to the expression of SPs and CCSP [27]. TTF-1 expression remained constant during fetal human lung development and in the adult lung, indicating that TTF-1 levels may not be subject to developmental regulation [30].

**Figure 2**  **TTF-1 protein organization**

A schematic diagram of the functional domains of TTF-1 protein. The N- and C-terminal activation domains (AD), the homeodomain (HD) and the inhibitor domain (ID) are shown. The locations of redox-sensitive cysteine residues (C) and the serine-phosphorylation sites (S) in the TTF-1 protein are indicated. The domain organization of TTF-1 protein shown in this diagram is based on the work described by De Felice et al. [35]. The identification of redox-sensitive cysteine residues and serine-phosphorylation sites was described by Arnone et al. [37] and Zannini et al. [40] respectively.

**TTF-1 PROTEIN AND GENE STRUCTURE**

TTF-1 from calf [5], rat [6], mouse [31] and human [32] comprises a single polypeptide chain of 371–378 amino acids with a molecular mass in the range of 38–42 kDa (Figure 2). The amino acid sequences of human [32], rat [6] and mouse [31] TTF-1 have 98 % sequence similarity, with the complete conservation of the 60-amino-acid homeodomain. A minor variant form of the human TTF-1 transcript encoding a 30-amino-acid extension with the complete conservation of the 60-amino-acid homeodomain and the serine-phosphorylation sites (S) in the TTF-1 protein are indicated. The domain organization of TTF-1 protein shown in this diagram is based on the work described by De Felice et al. [35]. The identification of redox-sensitive cysteine residues and serine-phosphorylation sites was described by Arnone et al. [37] and Zannini et al. [40] respectively.

The TTF-1 homeodomain shares 82 % identity with the Drosophila NK-2 homeodomain and only 27–40 % identity with other homeodomains, identifying it as a member of the NK2 family of homeodomain proteins [5]. Deletion mapping studies have shown that most of the DNA-binding activity of TTF-1 is contributed by the homeodomain [5]. Mapping studies have identified two activation domains located at the N- and C-termini of the protein that are involved in the transcriptional activation of TTF-1 [35]. Either of the two activation domains was found to activate a target promoter, indicating probable functional redundancy [35]. The TTF-1 transcriptional activation domains do not have any sequence similarity with the activation domains of transcription factors characterized previously [36]. Despite this lack of similarity with the activation domains of other transcription factors, the N-terminal TTF-1 activation domain activates transcription by mechanisms similar to those used by acidic activation domains [36]. Interestingly a glutamine-rich region located C-terminal to the homeodomain functions as an inhibitory domain [35].

In vitro experiments have demonstrated that treatment of TTF-1 with oxidizing agents, such as oxidized glutathione and diamide, inactivated TTF-1 DNA-binding activity, which was reversed by dithiothreitol [37]. The decrease in the TTF-1 DNA-binding activity was found to be due to the oxidation of two specific cysteine residues located outside the TTF-1 homeodomain that lead to the formation of higher-order oligomers [37]. Specifically, oxidation of Cys87 that resides in the N-terminal activation domain was implicated in the reduced DNA-binding activity of the TTF-1 homeodomain [38]. It was suggested that oxidation of Cys87 interferes with the correct folding of the homeodomain, resulting in its decreased DNA-binding activity. Ref-1 (redox effector factor-1) was shown to mediate the redox effects on the TTF-1 homeodomain, thereby modulating TTF-1 transcriptional activity [38]. Redox regulation of TTF-1 DNA-binding activity could play important roles in the control of target gene expression. In thyroid FRTL-5 cells, TSH (thyroid-stimulating hormone) up-regulates thyroglobulin gene expression via increased binding of Pax-8 and TTF-1 transcription factors to the thyroglobulin promoter [39]. Redox regulation by thioredoxin was suggested to be responsible for the increased binding of Pax-8 and TTF-1 to the thyroglobulin promoter in TSH-treated FRTL-5 cells. TTF-1 DNA-binding activity appears to be highly sensitive to oxidation, as shown by its loss during the preparation of nuclear extracts in the absence of reducing agents and the restoration of binding activity by reducing agents ([37], and V. Boggaram, unpublished work). TTF-1 is phosphorylated on seven serine residues in thyroid FRTL-5 cells and in heterologous cells such as HeLa cells [40]. PKA (protein kinase A) [14,41], PKC (protein kinase C) [40] and MST2 (mammalian Ste20 (sterile 20)-like protein kinase 2)}
kinase [42] are able to phosphorylate TTF-1. Increased TTF-1 phosphorylation by PKA activated gene transcription of SP-B [41] and SP-A [14] in H441 lung epithelial cells and primary alveolar type II cells respectively, suggesting that alterations in TTF-1 phosphorylation status can modulate target gene expression. Abnormalities in lung morphogenesis and decreased expression of SPs in homozygous mice expressing a TTF-1 mutant in which all of the seven serine phosphorylation sites had been mutated underlines the importance of TTF-1 phosphorylation in the control of target gene expression [45].

TTF-1 is encoded by a single-copy gene that is located on chromosome 14 in humans and chromosome 12 in the mouse [5]. Initial studies concluded that rat [44,45], mouse [31] and human [32,33] TTF-1 genes were organized into two exons and one intron. Other studies have determined that the rat TTF-1 gene is organized into three exons and two introns [46] (Figure 3). Previous studies on the cloning and sequencing of a large number of TTF-1 cDNAs from a fetal human lung cDNA library have concluded that the human TTF-1 gene, similar to the rat TTF-1 gene, is also organized into three exons and two introns [33]. The newly identified exon I contains an ATG codon that falls in-frame with the initiator ATG identified previously. In MLE-15 and H441 human lung epithelial cells, the start site of transcription for the TTF-1 gene was mapped to −196 bp relative to the initiator ATG codon [32]. In rat FRTL-5 thyroid cells and human lung H441 cells, multiple T/EBP (TTF-1) transcripts having variable lengths of 5′ untranslated regions were identified by cDNA cloning and sequencing, indicating the use of multiple promoters and the occurrence of alternative splicing [33,45,46]. Determination of transcription start sites for TTF-1 transcription in thyroid and lung showed multiple start sites of transcription in agreement with the existence of multiple TTF-1 mRNAs [45,46].

**TTF-1 PROMOTER REGULATION IN THE LUNG**

The 5′ flanking region of the TTF-1 gene supports high level reporter gene expression in H441 and MLE-15 lung cells compared with non-lung cells, such as NIH 3T3 cells, and to A549 lung cells that do not display differentiated characteristics of type II cells, indicating the presence of cis-DNA elements necessary for lung-specific expression [32,33,47]. The identification of a new exon I in the TTF-1 gene and analysis of genomic regions indicated the presence of two promoter regions which support reporter gene expression in lung cells in vitro [33]. One lies within the first intron (proximal promoter) and the other upstream of the first exon (distal promoter). A TTF-1 genomic fragment that included both the upstream and the intronic promoter regions expressed the reporter gene at a significantly higher level than a fragment containing the intronic promoter alone [32]. These findings suggest interactions between the two promoters. The proximal promoter does not contain a traditional TATA sequence, but instead contains a sequence TAAA that has some degree of similarity to the TATA element, whereas the distal promoter lacks a TATA-like sequence altogether. The TTF-1 proximal promoter contains two closely located DNA elements that bind HNF-3α [hepatocyte nuclear factor-3α; also known as FOXA1 (forkhead box A1)] and HNF-3β (also known as FOXA2) factors in MLE-15 lung cells and activate promoter activity [32]. The distal promoter contains a GC-rich sequence that binds Sp1 and Sp3 (specificity protein 1 and 3 respectively) transcription factors that regulate TTF-1 promoter activity [48]. The TTF-1 promoter is activated by co-expression of TTF-1 in FRTL-5 thyroid cells and HepG2 cells, indicating that the TTF-1 promoter is subject to positive autoregulation [45,49]. The TTF-1-binding site(s) that controls positive autoregulation of the TTF-1 promoter has not been identified or characterized. A DNA element that binds the zinc finger protein GATA-6 was identified in the mouse proximal TTF-1 promoter and found to be important for promoter activity in MLE-15 lung epithelial cells [50]. However, mice deficient in GATA-6 expressed TTF-1 in bronchiolar epithelial cells, suggesting that GATA-6 may not be required for TTF-1 expression in bronchiolar epithelial cells [51]. The homeobox protein HOX3B binds to a highly conserved DNA element in the TTF-1 proximal promoter and activates promoter activity in cotransfection experiments in HeLa cells [52]. The significance of HOX3B regulation of TTF-1 promoter activity in lung epithelial cells is not clear because HOX3B expression appears to be restricted to the mesenchyme in the developing lung [53]. The occurrence of multiple functionally important binding sites in the TTF-1 promoter suggests that the promoter activity is controlled via combinatorial or co-operative interactions between

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**Figure 3** TTF-1 gene organization

A schematic diagram of the structure of human TTF-1 gene. Exons are represented by rectangles and introns and 5′ and 3′ flanking regions are represented by lines. Filled and open areas represent untranslated and translated regions of the exons respectively. The arrows indicate the locations of transcription start sites. The locations of translation initiation (ATG) and translation termination (TGA) codons and of functionally important transcription-factor-binding sites are also shown. The organization of the TTF-1 gene shown is based on the structure of the TTF-1 gene described by Hamdan et al. [33].
the transcription factors. Although TTF-1 5’ flanking genomic DNA fragments from the baboon that included both the distal and proximal promoters or only the distal promoter failed to direct LacZ reporter gene expression in the lung or thyroid, they expressed the LacZ gene in the trachea and subsets of cells localized in the hypothalamus in transgenic mice [34]. These intriguing findings suggest that additional TTF-1 regulatory sequences may be required for lung- and thyroid-specific expression.

**TTF-1 EXPRESSION IN LUNG DISEASES**

TTF-1 is expressed at the onset of lung morphogenesis in epithelial cells and throughout fetal lung development. In the postnatal lung, TTF-1 expression is restricted primarily to alveolar type II cells and subsets of non-ciliated bronchiolar epithelial cells. There is limited information on the regulation of TTF-1 levels in lung diseases. TTF-1 immunostaining was reduced or absent in type II cells in regions of acute inflammation, oedema, haemorrhage and atelectasis in infants with HMD (hyaline membrane disease) and BPD (bronchopulmonary dysplasia) [25]. In contrast, TTF-1 immunostaining was prominent in cells in regions of the lung that were undergoing regeneration [25]. Partial deficiency or haplo-insufficiency of TTF-1 in humans heterozygous for TTF-1 mutations is associated with hypothyroidism, choreoathetosis, muscular hypotonia, respiratory dysfunction and recurrent pulmonary infections [55–59]. TTF-1 mutations in these patients were characterized as complete gene deletions, missense mutations or nonsense mutations. Respiratory dysfunction and recurrent pulmonary infections in these patients could be the result of reduced expression of SPs as a consequence of TTF-1 deficiency. TTF-1 expression was significantly reduced in nitrogen-induced lung hypoplasia in rats, and glucocorticoid treatment prevented the reduction in TTF-1 levels [60]. Similarly, nitrogen decreased TTF-1 promoter activity in H441 lung epithelial cells, indicating that the decreased TTF-1 expression is due to reduced gene transcription [60]. Other studies have found that TTF-1 expression is unaltered [61] or increased [62,63] in hypoplastic lungs of nitrogen-treated rats. In contrast with the alterations in TTF-1 levels in hypoplastic lungs of nitrogen-treated rats, no changes in lung TTF-1 expression were observed in an ovine model of surgically created congenital diaphragmatic hernia [64] or in human fetuses with diaphragmatic hernia [65]. These findings suggest that the alterations in TTF-1 levels in nitrogen-induced diaphragmatic hernia may be the result of the effects of nitrogen on TTF-1 expression and not due to diaphragmatic hernia itself.

**TTF-1 AS A LINEAGE-SPECIFIC ONCOGENE IN LUNG CANCER**

Owing to its cell/tissue-restricted expression, TTF-1 has been used widely as a marker for the diagnosis of primary and metastatic lung cancer, in particular for the identification of the lung as the primary site of metastatic adenocarcinoma [66–68]. TTF-1 is also useful in distinguishing malignant pleural mesotheliomas from adenocarcinomas [69]. SCLCs (small-cell lung carcinomas) also have TTF-1 immunoreactivity. Although SCLCs display a positive reaction for TTF-1, TTF-1 may not be a specific marker for SCLCs, as small cell carcinomas from tissues other than lung have positive reactions [70]. It has been suggested that TTF-1 in combination with cytokeratin 20 would be very useful as a marker for distinguishing SCLCs from small cell carcinomas of other primary sites [70]. In primary NSCLC (non-SCLC), TTF-1 immunoreactivity was significantly correlated with adenocarcinomas, rather than SCCs (squamous cell carcinomas) [71,72]. There appears to be considerable variation in TTF-1 immunoreactivity in NSCLC. Although TTF-1 immunoreactivity is not frequently encountered in SCCs, reverse transcriptase–PCR analysis of lung SCC cell lines detected TTF-1 expression in three out of four cell lines [73], indicating that the lack of or low TTF-1 immunoreactivity in SCCs is due to low TTF-1 expression.

Several studies have investigated whether TTF-1 expression can serve as a prognostic indicator for survival in lung cancer patients [71,72,74–76]. There appears to be a strong correlation between TTF-1 expression and median survival rates in patients with lung cancer. Patients with tumours having strong TTF-1 expression had statistically significant better median survival rates than those with tumours having no or weak expression (> 57.3 compared with 39.4 ± 5.2 months respectively; P = 0.006 according to a long-rank test) [71]. Indeed a meta-analysis concluded that TTF-1 expression is a good prognostic factor for survival in NSCLC [77]. The reasons for the close association between TTF-1 expression and survival rates in patients with NSCLC are not well understood. Although one study found no correlation between TTF-1 expression levels and tumour differentiation [75], another study found that the TTF-1 expression level was positively associated with tumour differentiation in NSCLCs [71].

There exists an intimate association between cell lineage and tumorigenesis, indicating common mechanisms for cell lineage and tumour progression and survival. Indeed results from several studies have supported a lineage-dependency model for tumorigenesis ([78], but see [78a]). It has been suggested that aberrant functioning of lineage-survival pathways might underlie carcinogenesis and tumour progression. For example, lineage-dependency in tumorigenesis might rely on the deregulated expression of a master gene(s) that controls developmental and essential functions. It has been suggested that transcription factors and signalling proteins might fill the role of lineage-survival oncogenes. The master transcriptional regulator MITF (microphthalmia-associated transcription factor)
that is involved in the differentiation and survival of melanocytes is a case in point [79]. Deregulation of MITF has been linked to metastatic melanoma.

The characteristics of TTF-1, namely its role in lung development, cell-restricted expression in the lung and high-level expression in lung adenocarcinomas, strongly point to TTF-1 as a potential lineage-survival oncogene in lung cancer [80]. Analysis of large numbers of lung tumours and lung cancer cell lines has revealed amplification of the TTF-1 gene in lung adenocarcinoma. Although one study reported amplification of the TTF-1 gene in 2% of the tumours analysed [82], other studies have reported amplification rates of 12% [81] and 11% [82]. Interestingly, the occurrence of higher TTF-1 gene amplifications at metastatic sites than primary sites was observed [82]. Amplification of the TTF-1 gene in lung adenocarcinoma cell lines is associated with increased expression of TTF-1 [80,82]. Sequencing of the TTF-1 open reading frame from four NSCLC cell lines exhibiting amplification did not reveal any DNA mutations, indicating that TTF-1 levels would be of importance [82]. Reduction in endogenous TTF-1 levels by RNAi (RNA interference)-mediated knockdown in a variety of lung cancer cell lines with TTF-1 amplification decreased cell proliferation, indicating the importance of elevated TTF-1 levels for the growth of cells [80–82]. Lung cancer cell lines without amplification of the TTF-1 gene but with detectable expression of TTF-1 (H1155) [82] and those without detectable expression of TTF-1 (NCI-H23, A549) [80] were not affected by RNAi-mediated knockdown of TTF-1, underlining further the functional importance of elevated TTF-1 levels for cell survival and proliferation. A reduction in endogenous TTF-1 levels by RNAi-mediated knockdown resulted in decreased cell-cycle progression and increased apoptosis [80–82]. These findings have demonstrated that sustained expression of TTF-1 is necessary for the growth and survival of a subset of lung adenocarcinoma and implicate TTF-1 as lineage-specific proto-oncogene for lung cancer. Apart from gene amplification, TTF-1 levels in lung adenocarcinoma cell lines and tumours can be up-regulated by transcriptional and post-transcriptional mechanisms, and by the control of protein stability. It remains to be determined whether any of these mechanisms operate along with TTF-1 gene amplification to elevate TTF-1 levels in lung adenocarcinomas. It is not known whether TTF-1 directly controls the survival of cancer cells or via another downstream effector molecule(s), whose identity remains to be determined.

REGULATION OF TTF-1 GENE EXPRESSION IN THE LUNG

There is limited information on the regulation of TTF-1 expression in the lung. Intratracheal instillation of silicon carbide whiskers, an asbestos substitute, into rats produced fibrotic changes and decreased the expression of SP-A and TTF-1 [83]. Changes in SP-A and TTF-1 levels were apparent as early as 3 days after the exposure and reduced expression was maintained for up to 6 months following the single exposure. In studies to assess the impact of inhaled DEE (diesel engine emissions) on the clearance of \textit{Pseudomonas aeruginosa} infection in mice, it was found that prior exposure of mice to DEE significantly reduced the expression of TTF-1 as well as of CCSP [84]. Exposure to DEE decreased lung clearance of \textit{P. aeruginosa} and exacerbated lung inflammation and injury. Pro-inflammatory agents such as TNF-\(\alpha\) (tumour necrosis factor-\(\alpha\)) [85] and ceramide [86] inhibited SP-B promoter activity in H441 lung cells by reducing TTF-1 DNA-binding activity. The molecular mechanisms responsible for the TNF-\(\alpha\) and ceramide inhibition of TTF-1 DNA-binding activity are not known. Glucocorticoid- and cAMP-dependent differentiation of alveolar type II epithelial cells \textit{in vitro} is associated with the up-regulation of TTF-1 and SP expression [87]. It is not known whether the inductive effects of glucocorticoid and cAMP on TTF-1 levels are due to their direct effects on TTF-1 gene expression or secondary to their effects on the differentiation of alveolar type II cells. Chronic exposure of primary rat alveolar epithelial cells to a combination of TGF-\(\beta\) (transforming growth factor-\(\beta\)) and TNF-\(\alpha\) resulted in decreased TTF-1 immunoreactivity with a concomitant increase in immunoreactivity for \(\alpha\)-SMA (\(\alpha\)-smooth muscle actin) and other mesenchymal markers, indicating induction of an EMT (epithelial–mesenchymal transition) [88]. It was suggested that EMT could be responsible for the accumulation of fibroblasts and myofibroblasts in IPF (idiopathic pulmonary fibrosis). Although TGF-\(\beta\) increases TTF-1 expression in non-tumorigenic E10 mouse lung epithelial cells, it had no effect on TTF-1 expression in tumorigenic E9 mouse lung epithelial cells [89]. In E10 cells, TGF-\(\beta 1\) induction of TTF-1 expression occurs via the TGF-\(\beta\)II receptor and Smad signalling with the involvement of Sp1 and Sp3 transcription factors. Sp1 and Sp3 exert opposing effects on the TTF-1 promoter; although Sp1 increases TTF-1 promoter activity, Sp3 negated the inductive effects of Sp1.

CONCLUSIONS

TTF-1 plays key roles in the control of cell/tissue-specific gene expression and in the morphogenesis and differentiation of the thyroid, lung and brain. Recent studies have suggested that TTF-1 plays the role of a proto-oncogene in lung cancer. Considering the important roles that TTF-1 plays, abnormal expression of TTF-1 probably contributes to the pathogenesis of lung, thyroid and other diseases. Molecular mechanisms that control cell/tissue-specific expression of TTF-1 and TTF-1 transcriptional
activity are not fully understood. Likewise cellular pathways and agents that regulate TTF-1 expression remain to be explored. Because TTF-1 is essential for the expression of SP and other genes important for lung function, abnormal expression and/or activity of TTF-1 may underlie the pathogenesis of a variety of lung inflammatory diseases. The recent discovery of TTF-1 as a proto-oncogene for lung cancer has opened new avenues of research that might lead to novel therapies for lung cancer. A better understanding of agents and pathways and molecular mechanisms that control TTF-1 expression in the lung will be useful for the development of novel treatment strategies for lung diseases such as ARDS (acute respiratory distress syndrome), IPF, lung cancer and others.

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