Journal of Zhejiang University-SCIENCE B (Biomedicine & Biotechnology)
ISSN 1673-1581 (Print); ISSN 1862-1783 (Online)
www.zju.edu.cn/jzus; www.springerlink.com
E-mail: jzus@zju.edu.cn

Review:

Nkx2-1: a novel tumor biomarker of lung cancer*

Li YANG[†1,3], Min LIN[2], Wen-jing RUAN[3], Liang-liang DONG[3],
En-guo CHEN[3], Xiao-hong WU[3], Ke-jing YING[†‡3]

([1]*Department of Respiratory Medicine, the First Affiliated Hospital of Zhejiang Chinese Medical University, Hangzhou 310006, China*)
([2]*Department of Radiology, the Third Affiliated Hospital of Zhejiang Chinese Medical University, Hangzhou 310005, China*)
([3]*Key Laboratory of Biotherapy of Zhejiang Province, Department of Respiratory Medicine, Sir Run Run Shaw Hospital, School of Medicine, Zhejiang University, Hangzhou 310016, China*)
[†]E-mail: hzforest_yang@163.com; ykjsrrsh@163.com

Received Dec. 22, 2011; Revision accepted May 30, 2012; Crosschecked Sept. 7, 2012

Abstract: *Nkx2-1* (Nkx homeobox-1 gene), also known as *TTF-1* (thyroid transcription factor-1), is a tissue-specific transcription factor of the thyroid, lung, and ventral forebrain. While it has been shown to play a critical role in lung development and lung cancer differentiation and morphogenesis, molecular mechanisms mediating *Nkx2-1* cell- and tissue-specific expression in normal and cancerous lungs have yet to be fully elucidated. The recent identification of prognostic biomarkers in lung cancer, particularly in lung adenocarcinoma (ADC), and the different reactivity of patients to chemotherapeutic drugs have opened new avenues for evaluating patient survival and the development of novel effective therapeutic strategies. The function of *Nkx2-1* as a proto-oncogene was recently characterized and the gene is implicated as a contributory factor in lung cancer development. In this review, we summarize the role of this transcription factor in the development, diagnosis, and prognosis of lung cancer in the hope of providing insights into the utility of Nkx2-1 as a novel biomarker of lung cancer.

Key words: *Nkx2-1*, *TTF-1*, Lung cancer, Biomarker, Diagnosis, Prognosis, Therapy
doi:10.1631/jzus.B1100382 **Document code:** A **CLC number:** R563.9

1 Introduction

During organogenesis, the lung buds are derived from the lateral-esophageal sulcus that evaginates into the thyroid and the stomach along with the foregut endoderm. This process is mediated by a dynamic profile of organ-specific gene expression. *Nkx2-1* (Nkx homeobox-1 gene), a member of the *Nkx2* family of homeodomain-containing transcription factors, is one of the key genes expressed. Also known as *TTF-1* (thyroid transcription factor-1), *T/EBP* (thyroid-specific enhancer-binding protein), or *TITF* (thyroid transcription factor-1 gene), *Nkx2-1* was identified in 1989 in the rat thyroid cell line FRTL-5, and shown to be a transcriptional regulator of the rat thyroglobulin promoter (Civitareale *et al*., 1989). Subsequent studies identified the high sequence homology of the homeodomain region with the *Drosophila Nkx2* gene family. Currently, *Nkx2-1* is known for its transcriptional activity in thyroid, lung, and ventral forebrain (Bingle, 1997). In the lung, *Nkx2-1* regulates normal development and morphogenesis, especially of lung epithelial cell differentiation and perinatal respiratory development. A more recent study (Weir *et al*., 2007; Kwei *et al*., 2008) showed that *Nkx2-1* can also function as a proto-oncogene and contribute to the pathogenesis of lung cancer. Since then, *Nkx2-1* has emerged as a useful molecular marker for lung cancer diagnosis and prognosis.

‡ Corresponding author
* Project supported by the Zhejiang Provincial Natural Science Foundation of China (No. LQ12H01001), and the Zhejiang Provincial Medicine & Health Scientific Foundation of China (No. 2012KYA143)

2 Structure of the *Nkx2-1* gene and protein, and its biological functions in the lung

The *Nkx2-1* gene is comprised of three exons and two introns, and is located on chromosome 14q13.3. The messenger RNA (mRNA) and protein expressions of Nkx2-1 are different in normal lung tissues and lung carcinomas (Bai and Shen, 2008). Missense and synonymous mutations of the *Nkx2-1* gene have been found in 16% of lung cancers. The NH_2-terminal harbors binding sites for co-activators, while the transactivation domain of the *Nkx2-1* gene is located in the COOH-terminal, and the DNA-binding homeodomain is located centrally. This structure facilitates *Nkx2-1* interactions with multiple transcription factors, by which the protein is able to regulate gene expression driving lung formation and biological functions (Fig. 1). In particular, the NH_2-terminus has been shown to bind to the following transcriptional coactivators: cyclic-adenosine monophosphate (AMP) response element binding protein (CBP) (Naltner *et al*., 2000a; 2000b; Yi *et al*., 2002), steroid receptor coactivator-1 (SRC-1) (Naltner *et al*., 2000a; 2000b; Yi *et al*., 2002; Yang *et al*., 2004), and transcriptional coactivator with PDZ-binding motif (TAZ) (Park *et al*., 2004). The centralized homeodomain is known to bind to other transcription factors, including retinoic acid receptors (RARs) (Yan *et al*., 2001), zinc finger GATA transcription-6 (GATA-6) (Liu *et al*., 2002; Weidenfeld *et al*., 2002), and nuclear factor of activated T cells (NFAT) (Dave *et al*., 2004). Finally, the COOH-terminus functionally interacts with the DNA repair protein thymine DNA glycosylase (TDG) (Missero *et al*., 2001), other transcriptional coactivators such as p300 (Bachurski *et al*., 2003; Grasberger *et al*., 2005), SRC-3, SRC-2, BR22 (amino acid 6–206) (Yang *et al*., 2001; 2003), poly(adenosine diphosphate-ribose) polymerase-1 (PARP-1), PARP-2 (Maeda *et al*., 2006), and other transcription factors such as nuclear factor κB (NF-κB) (Islam and Mendelson, 2006), signal transducers and activators of transcription 3 (STAT3) (Yan *et al*., 2002), *Drosophila* mothers against decapentaplegic 3 (SMAD3) (Li *et al*., 2002), nuclear factor I (NFI) (Missero *et al*., 2001), and ezrin-radixin-moesin (ERM) (Lin *et al*., 2006).

In human, calf, rat, and mouse, Nkx2-1 protein has been characterized as a single polypeptide chain of between 371 and 378 amino acids (aa) and with a molecular mass ranging from 38–42 kDa. The orthologues between the species have up to 98% sequence similarity. An alternative splice isoform, composed of three exons and encoding a 30-aa extension at the N-terminus, has been found in various mammalian species and has a molecular mass of 44 kDa (Hamdan *et al*., 1998). The two Nkx2-1 transcripts appear to have different functions in vivo (Li *et al*., 2000). It is well known that the 42-kDa human Nkx2-1 is a critical regulator of lung type II cell differentiation (Kolla *et al*., 2006); however, whether the 44-kDa variant is expressed or functions in the human lung remains unknown.

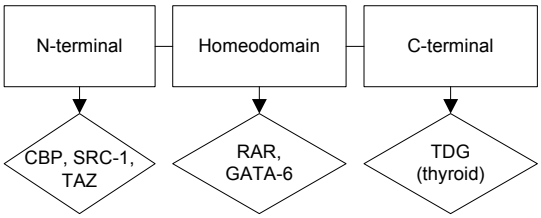

Fig. 1 Structure of the Nkx2-1 protein

Nkx2-1 has three domains: N-terminal, coactivator binding domain; center, DNA-binding homeodomain; C-terminal, transactivation domain. Different regions interact with different transcription factors to regulate the formation and biological functions of the lungs. CBP: cyclic-AMP response element binding protein; SRC-1: steroid receptor coactivator; TAZ: transcriptional coactivator with PDZ-binding motif; RAR: retinoic acid receptor; GATA-6: zinc finger GATA transcription-6; TDG: thymine DNA glycosylase

Expression of *Nkx2-1* in cells is dependent upon fibroblast growth factor (FGF) signaling, a key component of lung formation (Serls *et al*., 2005). *Nkx2-1*, as a pivotal gene itself in lung development and morphogenesis, directly binds to and activates the expression of several lung-specific genes, including surfactant protein B (SPB) (Bohinski *et al*., 1994), SPC (Kelly *et al*., 1996), SPA (Bruno *et al*., 1995), Clara cell secretory protein (CCSP) (Toonen *et al*., 1996), and adenosine triphosphate (ATP)-binding-cassette transporter 3 (ABCA3) (Besnard *et al*., 2007). More importantly, *Nkx2-1* is also strongly associated with expression of cancer-related genes in the lung, such as *LAMP3* (lysosomal-associated membrane protein 3) and *CEACAM6* (carcinoembryonic antigen-related cell adhesion molecule 6) (Kolla *et al*., 2006), and has been shown to be influenced by lung cancer growth.

3 Clinical application of *Nkx2-1* in lung cancer

3.1 Normal tissue expression

Nkx2-1 has been detected in mammalian samples of fetal lung, thyroid, and diencephalon. After birth, Nkx2-1 is expressed in subsets of epithelial cells, including alveolar type II cells, Clara cells, and bronchial basal cells. Deletion of Nkx2-1 in the mouse resulted in malformations of the forebrain, thyroid, and lung, consistent with its function as a transcriptional regulator of several important thyroid-specific and lung-specific genes. In normally developing epithelial cells of the lung, Nkx2-1 is synthesized in the cytoplasm and transported into the nucleus. In human lung cancer cells, however, Nkx2-1 accumulates in the cytoplasm, as evidenced by low nuclear and high cytoplasmic immunostaining with Nkx2-1 antibody (Fujita *et al*., 2003). However, the function and significance of cytoplasmic-restricted Nkx2-1 remain unknown (Table 1).

3.2 Lung cancer expression

Nkx2-1 has different expression profiles in different types of lung cancer. For example, *Nkx2-1* expression is high in lung adenocarcinomas (ADCs) and small cell lung cancers (SCLCs), but low or completely absent in large cell lung cancers (LCLCs) and lung squamous cell cancers (SCCs) (Table 1).

Tan *et al*. (2003) reported distinctive Nkx2-1 expression levels in different histological subtypes of 126 stages I–III non-small cell lung cancer (NSCLC) patients. Among those, 68% of ADCs (51 of 75) were positive but only 21% of squamous cell carcinomas (9 of 43) were positive. In a previous study, the positive expression of Nkx2-1 was found in 25%–76% of ADC subtypes (Bejarano *et al*., 1995; Fabbro *et al*., 1996; Holzinger *et al*., 1996; di Loreto *et al*., 1997; Bohinski *et al*., 1998; di Loreto *et al*., 1998; Harlamert *et al*., 1998; Kaufmann and Dietel, 2000) and 0%–38% of SCCs (Kaufmann and Dietel, 2000; Ordonez, 2000c; Pelosi *et al*., 2001; Haque *et al*., 2002). Several studies have evaluated the expression of Nkx2-1 in SCLCs; however, whether this can be used as a specific marker for SCLC remains unclear (Ordonez, 2000c). Nkx2-1-positive ADC was strongly associated with female sex, nonsmoking status, negative p53 staining, and preserved expression of p27 in NSCLC patients (Yatabe *et al*., 2002). Pelosi *et al*. (2001) found that Nkx2-1 immunoreactivity in ADC was associated with tumor sizes of <3 cm (P=0.08).

Recently, a clinical analysis of 371 tumors to determine single nucleotide polymorphisms associated with lung cancer found that over-expression of Nkx2-1 occurred in 12% of lung ADCs

Table 1 *Nkx2-1* expression in normal and cancerous lung tissues

Tissue and Disease	Disease status	Nkx2-1 expression profile	Reference
Normal tissue	Fetal lung	Epithelial cells	Stahlman *et al*., 1996
	Adult lung	Alveolar type II cells, Clara cells, and bronchial basal cells	Ikeda *et al*., 1995; Holzinger *et al*., 1996
	Thyroid		Kimura *et al*., 1996
	Diencephalon	Restricted regions of the brain	Stahlman *et al*., 1996; Kimura *et al*., 1996
Lung disease	Acute inflammation, oedema, haemorrhage, atelectasis	Reduced or absent	Stahlman *et al*., 1996
Respiratory dysfunction and recurrent infection		Perturbed expression due to gene deletions, missense mutations or nonsense mutations	Devriendt *et al*., 1998; Iwatani *et al*., 2000; Krude *et al*., 2002; Pohlenz *et al*., 2002; Doyle *et al*., 2004
Lung cancer	Adenocarcinoma	High expression	Myong *et al*., 2003; Tan *et al*., 2003
	Squamous carcinoma	Low expression or absent	Myong *et al*., 2003; Tan *et al*., 2003
	Large cell lung cancer	Low expression or absent	Nakamura *et al*., 2002
	Small cell lung cancer	High expression	Kwei *et al*., 2008; Tanaka *et al*., 2007

(Weir *et al*., 2007). Furthermore, amplification of the *Nkx2-1* gene was the single most common focal genetic event detected by high-resolution copy-number analysis of lung ADC. Another study (Zhang *et al*., 2009) of 404 NSCLCs and 28 benign pulmonary diseases (BPDs), which detected Nkx2-1 expression by the immunohistochemical EnVision two-step method, revealed that Nkx2-1 expression was negative in BPDs, mild in SCCs and tumor-adjacent normal alveolar epithelial cells (AECs) and bronchus tissue, and high in female, non-smoking, and asymptomatic patients. The sensitivity of Nkx2-1 for well or moderately differentiated ADC was reported as 84.0% and specificity as 89.8%.

3.3 Role of *Nkx2-1* in diagnosis of lung cancer

Nkx2-1 acts as a lung development gene, is a proto-oncogene, and has different efficacy in different biological sites. The histopathologic significance of defining the primary source of metastatic tumors is well recognized. Nkx2-1 was found to be expressed in about 60%–70% of lung ADCs and showed high sensitivity and specificity for diagnosis of primary lung cancer. Nkx2-1 was also characterized as a tissue specific marker, capable of distinguishing morphologically similar metastatic ADCs of lung origin from other unknown primary sites (except thyroid tumor metastases).

Stenhouse *et al*. (2004) confirmed that positive Nkx2-1 was a useful lineage marker for tumors arising from the peripheral airway of the alveolar epithelium. In this study, none of the 106 non-lung ADCs was found to express Nkx2-1, and only three out of 37 lung non-ADCs expressed Nkx2-1. However, 75% of 128 lung ADCs strongly expressed Nkx2-1. Mucinous and poorly differentiated lung ADCs showed reduced or no expression of Nkx2-1. Nkx2-1 has also been confirmed in clinical samples of atypical adenomatous hyperplasia, small cell carcinomas, and other neuroendocrine tumors (di Loreto *et al*., 1997; Agoff *et al*., 2000; Ordonez, 2000c; Cheuk *et al*., 2001; Oliveira *et al*., 2001; Stenhouse *et al*., 2004). On the contrary, Oliveira *et al*. (2001) and Ordonez (2000b) found that Nkx2-1 was mostly confined to cells arising from the lung and was absent from the neuroendocrine tissues.

Nkx2-1 is considered a highly specific marker for lung ADC. Gomez-Fernandez *et al*. (2002) examined body cavity fluids from 113 patients with lung ADC on the basis of cytology and found that Nkx2-1 was expressed in 54% of lung ADC, but was completely absent in other types. In another study using immunohistochemical staining to detect Nkx2-1, Nkx2-1, along with cytokeratin 20 (CK20) and neurofilaments (NF), was found to be effective for distinguishing Merkel cell carcinoma (MCC) from SCLC (Bobos *et al*., 2006). In addition, none of 138 MCC samples expressed Nkx2-1, while about 53%–100% of SCLC samples expressed Nkx2-1.

Lotan *et al*. (2009) determined an optimized molecular panel for distinguishing the most likely primary site of invasive micropapillary carcinoma (IMC), which included uroplakin, CK20, Nkx2-1, estrogen receptor (ER), Wilms tumor protein-1 (WT-1) and/or paired box protein 8 (PAX8), and mammaglobin. Use of Nkx2-1 was based on its uniformly positive expression in lung IMC cases, as detected by immunostaining. *Nkx2-1* mRNA (Jiang *et al*., 2008) had the best diagnostic performance in pleural effusions of primary pulmonary ADC (PPA): the sensitivity 93.0%, the specificity 100.0%, and the accuracy 96.6%. The mRNA expression rate of pleural effusions was higher in patients with PPA (93.0%) than that in either metastatic pulmonary ADC (0%) or primary pulmonary squamous cell carcinoma (12.5%). Moreover, Nkx2-1 proved to be a useful marker for distinguishing primary lung ADC from pleural mesothelioma (Ordonez, 2000a). Cytology specimens are known to be particularly challenging for distinguishing ADC of the lung from non-pulmonary ADC or malignant mesothelioma. However, Khoor *et al*. (2011) showed that, in 26 pulmonary ADCs, 26 non-pulmonary ADCs (13 breast, 5 ovarian, 2 gastric, 2 prostatic, 1 esophageal, 1 colonic, 1 pancreatic, and 1 renal) and 4 malignant mesothelioma patients, Nkx2-1 had high sensitivity (73%) and specificity (100%) in differentiating lung ADC.

Caudal-related homeobox 2 (CDX-2) and Nkx2-1 were also capable of differentiating gastrointestinal from pulmonary carcinoids (Saqi *et al*., 2005), and Nkx2-1 was proposed as a potentially useful marker to distinguish pulmonary from gastrointestinal or pancreatic well-differentiated neuroendocrine tumors. CDX-2 and Nkx2-1 (Lin *et al*., 2007; Strickland-Marmol *et al*., 2007) were characterized as

highly specific for identifying the original site of intestinal and lung tumors from metastatic neuroendocrine neoplasms. An immunohistochemical staining panel for distinguishing the primary site of metastatic well-differentiated neuroendocrine tumors (WDNETs) from the gastrointestinal tract, pancreas and lung included CDX-2, pancreatic duodenal homeobox-1 (PDX-1), neuroendocrine secretory protein-55 (NESP-55) and Nkx2-1 (Srivastava and Hornick, 2009).

Nkx2-1, *Nkx2-8* (Nkx homeobox-8 gene), and *PAX9* (paired box gene 9), the three transcription factors known to mediate lung development and maturation, were recently characterized as cooperating oncogenes located together on chromosome 14q13 (Hsu *et al*., 2009). NSCLC patients with early stage tumors that expressed both *Nkx2-1* and *Nkx2-8* had poor survival. In addition, *KRAS* and epidermal growth factor receptor (*EGFR*) mutations were correlated with tumors having coactivated *PAX9* and *Nkx2-1*, or *Nkx2-8* and *Nkx2-1*. This study also suggested that coactivation of *Nkx2-1* and *Nkx2-8* was associated with a potentially aggressive phenotype of NSCLC, and that the underlying molecular mechanism was independent of the rat sarcoma (RAS) pathway.

3.4 Role of *Nkx2-1* in progression of lung cancer

In lung ADC, *Nkx2-1* was shown to promote carcinogenesis by increasing the formation of new vessels (Pelosi *et al*., 2001) and enhancing the growth of cell proliferation (Puglisi *et al*., 1999). Kwei *et al*. (2008) showed that small interfering RNA (siRNA)-mediated knockdown of *Nkx2-1* reduced cell proliferation and increased apoptosis in lung cancer. *Nkx2-1*, *Nkx2-8*, and *PAX9* (Kendall *et al*., 2007) synergistically promote proliferation of immortalized human lung epithelial cells, as evidenced by over-expression of any combination of the three genes. In another study, *Nkx2-1* over-expression was shown to be independent of *Nkx2-8* (Harris *et al*., 2011). We have found that over-expressing *Nkx2-1* leads to inhibition of lung ADC cell proliferation and prompts necrosis (unpublished data). Epithelial-to-mesenchymal transition (EMT), which can be elicited by transforming growth factor-β (*TGF-β*), facilitates cancer cell invasion and metastasis. Satio *et al*. (2009) determined that *Nkx2-1* could inhibit the EMT process through down-regulation of TGF-β, thereby preventing metastasis of lung cancer. Meanwhile, in a recent study in our lab, we found that high expression of Nkx2-1 can diminish the migration ability of lung ADC cells, suggesting that lung cancer cells with low expression of Nkx2-1 have strong metastasis and invasion capabilities. Both of these properties involve down-regulation of TGF-β. A study by Winslow *et al*. (2011) suggested that Nkx2-1 might play a dual role of oncogenic and suppressive functions in a single tumor type. Thus, the true roles of Nkx2-1 in metastasis and migration of lung cancer cells are not yet fully understood and require further study.

3.5 Therapeutic role of *Nkx2-1* in lung cancer

To date, very few studies have investigated the relation between *Nkx2-1* and patient sensitivity to the epidermal growth factor receptor-tyrosine kinase inhibitors (EGFR-TKIs) or to antineoplastic agents. Wislez *et al*. (2010) studied bronchioloalveolar carcinoma (BAC) patients and found that 95% of non-mucinous tumors expressed Nkx2-1, compared to only 27% of mucinous tumors. Moreover, the non-mucinous tumors appeared to be more sensitive to EGFR-TKIs. However, whether over-expression of Nkx2-1 truly reflects an increased sensitivity to EGFR-TKIs in lung cancer patients remains to be determined. The mitotic inhibitor chemotherapeutic agent paclitaxel was more effective at treating mucinous BAC tumors than at treating non-mucinous tumors (West *et al*., 2005). Coactivation of Nkx2-1 and Nkx2-8 in human lung squamous carcinoma cells (NCI-H2170) was shown to elicit resistance to the alkylating chemotherapeutic agent cisplatin (Hsu *et al*., 2009). In a study performed in our lab, we found that high expression of Nkx2-1 in the lung adenocarinoma cell line NCI-H1299 produced increased sensitivity specifically to the nucleoside analogue gemcitabine, but did not affect sensitivity to cisplatin or the antimitotic docetaxel. We believe that new and improved therapeutic strategies for NSCLC can be designed based on the relationship between Nkx2-1 and subtypes of NSCLC and/or the status of EGFR, and that expression of Nkx2-1 might play a role in evaluating sensitivity to various anticancer drugs.

3.6 Prognostic role of *Nkx2-1* in lung cancer

While the 5-year survival rate of all lung cancer patients has increased by 3%–5% in recent years (Coleman *et al*., 2011), those patients with NSCLC have not experienced this benefit. Thus, it is very important to specifically evaluate the survival of NSCLC patients in response to established and new therapeutic approaches. This type of focused clinical study will likely provide information that will help in the design of optimal therapeutic schedules based on currently available treatments. Furthermore, by continuing experimental studies to determine the molecular mechanisms underlying NSCLC and disease progression and severity, manipulable biological factors, such as transcription factors like *Nkx2-1*, may be identified and targeted for molecular therapeutic intervention.

A multitude of studies have aimed to determine the role of *Nkx2-1* in lung cancer prognosis (Table 2). Yet, the results are largely conflicting. While nine studies have reported a positive correlation between Nkx2-1 over-expression and survival, two studies found a negative correlation and four found that Nkx2-1 was not significantly (NS) associated with lung cancer survival. Puglisi *et al*. (1999) published the first study on the potential of Nkx2-1 as a prognostic indicator of NSCLC, and used a multivariate analysis approach. A total of 89 patients with stages I–IIIB NSCLCs were categorized into three groups according to the extent of tumor immunoreactivity for Nkx2-1. Positive Nkx2-1 patients were associated with a poor prognosis (P=0.009). We should point out that this study has some limitations; for example, the performance status was low and the study relied solely on a polyclonal antibody, instead of a monoclonal antibody. Results from another study (Pelosi *et al*., 2001) found no relationship between Nkx2-1 immunoreactivity and patient survival. Two other studies (Haque *et al*., 2002; Tan *et al*., 2003), evaluated stages I–IIIA NSCLCs with long-term follow-up and found that patients with strong Nkx2-1 expression had significantly better survival than patients with no Nkx2-1 (P=0.0067). A meta-analysis (Berghmans *et al*., 2006) using ten related studies published in the lung cancer literature showed that Nkx2-1 positivity was indeed significantly associated with better survival in NSCLC patients, and especially with early and locally advanced stages and in ADC.

Still another study of 89 patients with lung ADC (Barletta *et al*., 2009) showed that patients with high Nkx2-1 expression but with no *Nkx2-1* gene amplification had improved overall survival (P<0.001). Patients with high gene expression of *Nkx2-1* had a higher risk of death, almost three times higher than patients with low *Nkx2-1* gene expression. Thus, the authors suggested that prognosis was related to *Nkx2-1* gene activity and not Nkx2-1 protein levels, but this hypothesis has not yet been confirmed.

Table 2 Studies aimed at assessing the prognostic role of Nkx2-1 on survival of patients with lung cancer

Reference	Specimen source	Diagnosis	Stage	*n*	Correlation with survival
Yoon *et al*., 2011	Peripheral blood	NSCLC	I–III	79	Negative
Martins *et al*., 2009	Histopathology	NSCLC	IIIB–IV	51	Positive
Barletta *et al*., 2009	Histopathology	ADC	I–IV	89	Positive
Wang *et al*., 2007	Histopathology	BAC	III	81	Positive
Berghmans *et al*., 2006	Ten-published articles	Meta-analysis	I–IV	10	Positive
Barlesi *et al*., 2005	Histopathology	ADC	I–IV	106	Positive
Stenhouse *et al*., 2004	Histopathology	Lung tumor	Unknown	165	NS
Au *et al*., 2004	Histopathology	NSCLC	Unknown	284	NS
Shah *et al*., 2004	Histopathology	NSCLC	I–III	63	NS
Saad *et al*., 2004	Histopathology	ADC	I	100	Positive
Myong, 2003	Histopathology	NSCLC	I–III	65	Positive
Tan *et al*., 2003	Histopathology	NSCLC	I–III	126	Positive
Haque *et al*., 2002	Histopathology	NSCLC	I–III	57	Positive
Pelosi *et al*., 2001	Histopathology	NSCLC	I	222	NS
Puglisi *et al*., 1999	Histopathology	NSCLC	I–III	88	Negative

NS: not significant; *n*: number of patients

Martins *et al*. (2009) assessed the prognostic ability of *Nkx2-1* and matrix metalloproteinases-9 (*MMP-9*). *MMP-9* is a protease that is commonly expressed in NSCLC and has recognized angiogenic and metastatic potential. They found that the expression of these factors was associated with the outcomes of stage IIIB or IV lung ADC patients treated with platinum regimens. High Nkx2-1 combined with low MMP-9 was associated with good survival (median survival: 127.6 weeks) and was designated as a low risk prognostic indicator. Either low Nkx2-1 or high MMP-9 alone was associated with a median survival of 39.0 weeks and was designated as an intermediate risk prognostic indicator. Low Nkx2-1 combined with high MMP-9 was associated with poor survival (median survival: 16.4 weeks) and was designated as a high risk prognostic indicator. Likewise, Wang *et al*. (2007) reported that the survival time of patients with positive Nkx2-1 was longer than those without Nkx2-1 in non-mucinous type and stage III BAC patients.

In another study (Yoon *et al*., 2011), the mRNA levels of *Nkx2-1* and *CK19* in circulating tumor cells (CTCs) were investigated in 79 surgically resected NSCLC patients by using nested real-time reverse transcription polymerase chain reaction (RT-PCR) assay. *Nkx2-1* mRNA was more highly expressed in NSCLC patients' CTCs than was *CK19*. Comparing expression in patient-matched CTCs prior to and after resection revealed that 36.1% of patients were *Nkx2-1*-positive before surgery and 37.5% after surgery. Post-surgery *Nkx2-1*-positive patients experienced shorter progression-free survival (P=0.004) compared to those with *Nkx2-1*-negative status. Moreover, patients who were pre-surgery *Nkx2-1*-negative and post-surgery *Nkx2-1*-positive also had shorter progression-free survival (P<0.001). This study suggested that *Nkx2-1* mRNA-expressing CTC could be used as a surrogate predictor prior to clinical manifestations of observable symptoms. Monitoring *Nkx2-1*(+) CTCs status after surgery was also proposed as useful for identifying high-risk patients among the surgically resected NSCLC population. In the advanced tumor stage, negative Nkx2-1 expression was an independent and significant marker for poor prognosis (Hiramatsu *et al*., 2010). Several other studies also evaluated the prognostic role of Nkx2-1 expression in NSCLC (Haque *et al*., 2002; Au *et al*., 2004; Berghmans *et al*., 2006), especially in lung ADC (Stenhouse *et al*., 2004; Barlési *et al*., 2005).

Thus, a definitive conclusion on the efficacy of *Nkx2-1* as a prognostic indicator of lung cancer outcomes, or that of NSCLC in particular, has not been reached. We recently found that over-expression of Nkx2-1 not only inhibited the migration and proliferation of lung cancer cells but also promoted necrosis, indicating that strong expression of Nkx2-1 would have anticancer protective effects. Thus, Nkx2-1 may yet become established as a good prognostic marker for survival in NSCLC, especially of the ADC subtype.

4 Conclusions

Nkx2-1 interacts with multiple transcription factors in a cell/tissue-specific manner to regulate a multitude of differentiation and developmental functions (Fig. 2). In the lung, *Nkx2-1* plays a pivotal role in normal lung development and also in that of tumors, especially contributing to tumor cell differentiation and morphogenesis processes. Nkx2-1 over-expression has been clinically detected in patient samples of NSCLC ADC subtype and SCLC, while low or no expression has been found in LCLCs or lung SCCs. Nkx2-1 has been successfully used to distinguish the metastatic and morphologically similar ADCs of pulmonary origin from those of other unknown primary sites. Furthermore, Nkx2-1 expression is strongly associated with the prognosis and efficacy of several commonly used therapeutic agents. Understanding the prognostic factors underlying different types of lung cancer, particularly of lung ADC, and their influence on tumor response to chemotherapeutic drugs will facilitate more in-depth evaluation of the survival of lung cancer patients and help to develop novel effective therapeutic strategies. Furthermore, the recently recognized inhibitory property of Nkx2-1 on mucous cell metaplasia and T helper 2 (Th2) inflammation (Maeda *et al*., 2011), suggests that a better understanding of Nkx2-1 molecular interactions will provide insights into new therapeutic targets to treat cancer-related and inflammation-related lung diseases.

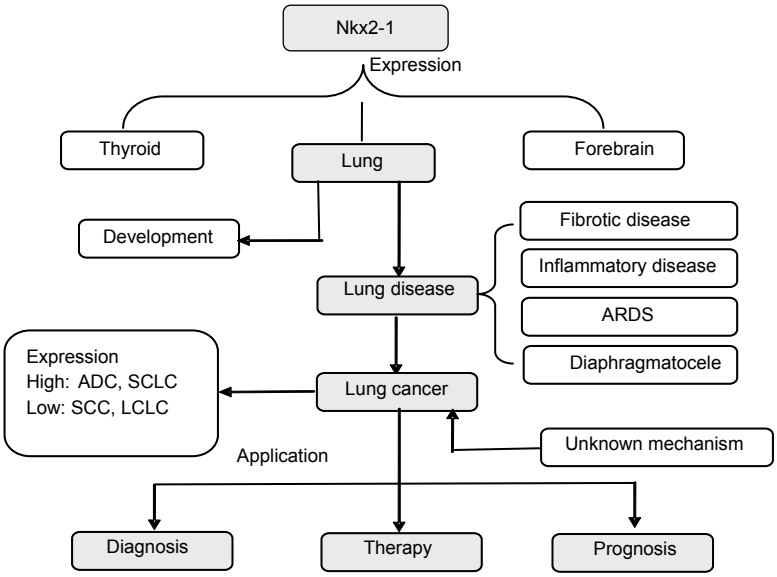

Fig. 2 A schematic diagram to summarize the role of Nkx2-1 in the lung

Nkx2-1 activates the expression of selected genes in the thyroid, lung, and ventral forebrain, which have a close relationship with the development of the fetal and postnatal lung. *Nkx2-1* mutations can induce idiopathic pulmonary fibrosis (IPF), pulmonary infection, acute respiratory distress syndrome (ARDS), and diaphragmatic hernia, among other disease processes. *Nkx2-1* is highly expressed in lung cancer, especially in ADC and SCLC, while lower expression is found in SCC and LCLC. *Nkx2-1* is clinically applicable for lung cancer diagnosis, and as an indicator for therapy type and of prognosis. Understanding the role of *Nkx2-1* in lung cancer may open new avenues of research to improve the evaluation of the survival of the lung cancer patients and to develop novel effective therapeutic strategies

Acknowledgements

We thank Medjaden Bioscience Limited (Room 2001-4, China Insurance Group Building, 141 Des Voeux Road Central, Hong Kong, China) for assisting in the preparation of this manuscript.

References

Agoff, S.N., Lamps, L.W., Philip, A.T., Amin, M.B., Schmidt, R.A., True, L.D., Folpe, A.L., 2000. Thyroid transcription factor-1 is expressed in extrapulmonary small cell carcinomas but not in other extrapulmonarry neuroendocrine tumors. *Mod. Pathol.*, **13**(3):238-242. [doi:10.1038/modpathol.3880044]

Au, N.H., Cheang, M., Huntsman, D.G., Yorida, E., Coldman, A., Elliott, W.M., Bebb, G., Flint, J., English, J., Gilks, C.B., *et al*., 2004. Evaluation if immunohistochemical markers in non-small cell lung cancer by unsupervised hierarchical clustering analysis: a tissue microarray study of 284 cases and 18 markers. *J. Pathol.*, **204**(1):101-109. [doi:10.1002/path.1612]

Bachurski, C.J., Yang, G.H., Currier, T.A., Gronostajski, R.M., Hong, D., 2003. Nuclear factor I/thyroid transcription factor 1 interactions modulate surfactant protein C transcription. *Mol. Cell. Biol.*, **23**(24):9014-9024. [doi:10.1128/MCB.23.24.9014-9024.2003]

Bai, X.Y., Shen, H., 2008. Mutational analysis of thyroid transcription factor-1 gene (*TTF-1*) in lung carcinomas. *In. Vitro. Cell. Dev. Bio. Anim.*, **44**(1-2):17-25. [doi:10.1007/s11626-007-9062-0]

Barletta, J.A., Perner, S., Iafrate, A.J., Yeap, B.Y., Weir, B.A., Johnson, L.A., Johnson, B.E., Meyerson, M., Rubin, M.A., Travis, W.D., *et al*., 2009. Clinical significance of TTF-1 protein expression and *TTF-1* gene amplification in lung adenocarcinoma. *J. Cell. Mol. Med.*, **13**(8b):1977-1986. [doi:10.1111/j.1582-4934.2008.00594.x]

Barlési, F., Pinot, D., Legoffic, A., Doddoli, C., Chetaille, B., Torre, J.P., Astoul, P., 2005. Positive thyroid transcription factor 1 staining strongly correlates with survival of patients with adenocarcinoma of the lung. *Br. J. Cancer*, **93**(4):450-452. [doi:10.1038/sj.bjc.6602717]

Bejarano, P.A., Baughman, R.P., Biddinger, P.W., Miller, M.A., Fenoglio-Preiser, C., Al Kafaji, B., di Lauro, R., Whitsett, J.A., 1995. Surfactant proteins and thyroid transcription factor-1 in pulmonary and breast carcinomas. *Mod. Pathol.*, **9**(4):445-452.

Berghmans, T., Paesmans, M., Mascaux, C., Martin, B., Meert, A.P., Haller, A., Lafitte, J.J., Sculier, J.P., 2006. Thyroid transcription factor 1—a new prognostic factor in lung cancer: a meta-analysis. *Ann. Oncol.*, **17**(11):1673-1676. [doi:10.1093/annonc/mdl287]

Besnard, V., Xu, Y., Whitsett, J.A., 2007. Sterol response element binding protein and thyroid transcription factor-1 (Nkx2.1) regulate *Abca3* gene expression. *Am. J. Physiol. Lung Cell. Mol. Physiol.*, **293**(6):L1395-L1405.

[doi:10.1152/ajplung.00275.2007]

Bingle, C.D., 1997. Thyroid transcription factor-1. *Int. J. Biochem. Cell Biol.*, **29**(12):1471-1473. [doi:10.1016/S1357-2725(97)00007-1]

Bobos, M., Hytiroglou, P., Kostopoulos, I., Karkavelas, G., Papadimitriou, C.S., 2006. Immunohistochemical distinction between Merkel cell carcinoma and small cell carcinoma of the lung. *Am. J. Dermatopathol.*, **28**(2):99-104. [doi:10.1097/01.dad.0000183701.67366.c7]

Bohinski, R.J., di Lauro, R., Whitsett, J.A., 1994. The lung-specific surfactant protein B gene promoter is a target for thyroid transcription factor 1 and hepatocyte nuclear factor 3, indicating common factors for organ-specific gene expression along the foregut axis. *Mol. Cell. Biol.*, **14**(9):5671-5681. [doi:10.1128/MCB.14.9.5671]

Bohinski, R.J., Bejarano, P.A., Balko, G., Warnick, R.E., Whitset, J.A., 1998. Determination of lung as the primary site of cerebral metastatic adenocarcinomas using monoclonal antibody to thyroid transcription factor-1. *J. Neuro-oncol.*, **40**(3):227-231. [doi:10.1023/A:1006102607697]

Bruno, M.D., Bohinski, R.J., Huelsman, K.M., Whitsett, J.A., Korfhagen, T.R., 1995. Lung cell-specific expression of the murine surfactant protein A (*SP-A*) gene is mediated by interactions between the SP-A promoter and thyroid transcription factor-1. *J. Biol. Chem.*, **270**(12):6531-6536.

Cheuk, W., Kwan, M.Y., Suster, S., Chan, J.K., 2001. Immunostaining for thyroid transcription factor-1 and cytokeratin 20 aids the distinction of small cell carcinoma from Merkel cell carcinoma, but not pulmonary from extrapulmonary small cell carcinomas. *Arch. Pathol. Lab. Med.*, **125**(2):228-231.

Civitareale, D., Lonigro, R., Sinclair, A.J., di Lauro, R., 1989. A thyroid-specific nuclear protein essential for tissue-specific expression of the thyroglobulin promoter. *EMBO J.*, **8**(9):2537-2542.

Coleman, M.P., Forman, D., Bryant, H., Butler, J., Rachet, B., Maringe, C., Nur, U., Tracey, E., Coory, M., Hatcher, J., *et al*., 2011. Cancer survival in Australin, Canada, Denmark, Norway, Sweden, and the UK, 1995-2007 (the international Cancer Benchmarking Partnership): an analysis of population-based cancer registry date. *Lancet*, **377**(9760):127-138. [doi:10.1016/S0140-6736(10)62231-3]

Dave, V., Childs, T., Whitsett, J.A., 2004. Nuclear factor of activated T cells regulates transcription of the surfactant protein D gene (*Sftpd*) via direct interaction with thyroid transcription factor-1 in lung epithelial cells. *J. Biol. Chem.*, **279**(33):34578-34588. [doi:10.1074/jbc.M404296200]

Devriendt, K., Vanhole, C., Matthijs, G., de Zegher, F., 1998. Deletion of thyroid transcription factor-1 gene in an infant with neonatal thyroid dysfunction and respiratory failure. *N. Engl. J. Med.*, **338**(18):1317-1318. [doi:10.1056/NEJM199804303381817]

di Loreto, C., di Lauro, V., Puglisi, F., Damante, G., Fabbro, D., Betrami, C.A., 1997. Immunocytochemical expression of tissue-specific transcription factor-1 in lung carcinoma. *J. Clin. Pathol.*, **50**(1):30-32. [doi:10.1136/jcp.50.1.30]

di Loreto, C., Puglisi, F., di Lauro, V., Damante, G., Beltrami, C.A., 1998. TTF-1 protein expression in pleural malignant mesotheliomas and adenocarcinomas of the lung. *Cancer Lett.*, **124**(1):73-78. [doi:10.1016/S0304-3835(97)00466-7]

Doyle, D.A., Gonzalez, I., Thomas, B., Scavina, M., 2004. Autosomal dominant transmission of congenital hypothyroidism, neonatal respiratory distress, and ataxia caused by a mutation of *NKX2-1*. *J. Pediatr.*, **145**(2):190-193. [doi:10.1016/j.jpeds.2004.04.011]

Fabbro, D., di Loreto, C., Stamerra, O., Beltrami, C.A., Lonigro, R., Damante, G., 1996. *TTF-1* gene expression in human lung tumors. *Eur. J. Cancer*, **32**(3):512-517. [doi:10.1016/0959-8049(95)00560-9]

Fujita, J., Ohtsuki, Y., Bandoh, S., Ueda, Y., Kubo, A., Tojo, Y., Yamaji, Y., Ishida, T., 2003. Expression of thyroid transcription factor-1 in 16 human lung cancer cell lines. *Lung Cancer*, **39**(1):31-36. [doi:10.1016/S0169-5002(02)00390-2]

Gomez-Fernandez, C., Jorda, M., Delgado, P.I., Ganjei-Azar, P., 2002. Thyroid transcription factor 1: a marker for lung adenocarcinoma in body cavity fluids. *Cancer*, **96**(5):289-293. [doi:10.1002/cncr.10743]

Grasberger, H., Ringkananont, U., Lefrancois, P., Abramowicz, M., Vassart, G., Refetoff, S., 2005. Thyroid transcription factor 1 rescues PAX8/p300 synergism impaired by a natural PAX8 paired domain mutation with dominant negative activity. *Mol. Endocrinol.*, **19**(7):1779-1791. [doi:10.1210/me.2004-0426]

Hamdan, H., Liu, H., Li, C., Jones, C., Lee, M., de Lemos, R., Minoo, P., 1998. Structure of the human *Nkx2.1* gene. *Biochim. Biophy. Acta*, **1396**(3):336-348. [doi:10.1016/S0167-4781(97)00210-8]

Haque, A.K., Syed, S., Lele, S.M., Freeman, D.H., Adegboyega, P.A., 2002. Immunohistochemical study of thyroid transcription factor-1 and HER2/neu in non-small cell lung cancer: strong thyroid transcription factor-1 expression predicts better survival. *Appl. Immunohistochem. Mol. Morphol.*, **10**(2):103-109. [doi:10.1097/00022744-200206000-00002]

Harlamert, H.A., Mira, J., Bejarano, P.A., Baughman, R.P., Miller, M.A., Whitsett, J.A., Yassin, R., 1998. Thyroid transcription factor-1 cytokeratins 7 and 20 in pulmonary and breast carcinomas. *Acta Cytol.*, **42**(6):1382-1388. [doi:10.1159/000332172]

Harris, T., Pan, Q., Sironi, J., Lutz, D., Tian, J., Sapkar, J., Perez-Soler, R., Keller, S., Locker, J., 2011. Both gene amplification and allelic loss occur at 14q13.3 in lung cancer. *Clin. Cancer Res.*, **17**(4):690-699. [doi:10.1158/1078-0432.CCR-10-1892]

Hiramatsu, M., Ninomiya, H., Inamura, K., Nomura, K., Takeuchi, K., Satoh, Y., Okumura, S., Nakagawa, K., Yamori, T., Matsuura, M., *et al*., 2010. Activation status of receptors tyrosine kinase downstream pathways in primary lung adenocarcinoma with reference of *KRAS* and *EGFR* mutations. *Lung Cancer*, **70**(1):94-102.

[doi:10.1016/j.lungcan.2010.01.001]

Holzinger, A., Dingle, S., Bejarano, P.A., Miller, M.A., Weaver, T.E., di Lauro, R., Whitsett, J.A., 1996. Monoclonal antibody to thyroid transcription factor-1: production, characterization, and usefulness in tumor diagnosis. *Hybridoma*, **15**(1):49-53. [doi:10.1089/hyb.1996.15.49]

Hsu, D.S., Acharya, C.R., Balakumaran, B.S., Riedel, R.F., Kim, M.K., Stevenson, M., Tuchman, S., Mukherjee, S., Barry, W., Dressman, H.K., *et al*., 2009. Characterizing the developmental pathways TTF-1, NKX2-8, and PAX9 in lung cancer. *PNAS*, **106**(13):5312-5317. [doi:10.1073/pnas.0900827106]

Ikeda, K., Clark, J.C., Shaw-White, J.R., Stahlman, M.T., Boutell, C.J., Whitsett, J.A., 1995. Gene structure and expression of human thyroid transcription factor-1 in respiratory epithelial cells. *J. Biol. Chem*., **270**(14):8108-8114. [doi:10.1074/jbc.270.14.8108]

Islam, K.N., Mendelson, C.R., 2006. Permissive effects of oxygen on cyclic AMP and interleukin-1 stimulation of surfactant protein A gene expression are mediated by epigenetic mechanisms. *Mol. Cell. Biol*., **26**(8):2901-2912. [doi:10.1128/MCB.26.8.2901-2912.2006]

Iwatani, N., Mabe, H., Devriendt, K., Kodama, M., Miike, T., 2000. Deletion of *NKX2.1* gene encoding thyroid transcription factor-1 in two siblings with hypothyroidism and respiratory failure. *J. Pediatr*., **137**(2):272-276. [doi:10.1067/mpd.2000.107111]

Jiang, B., Wu, G.P., Zhao, Y.J., Wang, S.C., 2008. Transcription expression and clinical significance of *TTF-1* mRNA in pleural effusion of patients with lung cancer. *Diagn. Cytopathol*., **36**(12):849-854. [doi:10.1002/dc.20926]

Kaufmann, O., Dietel, M., 2000. Thyroid transcription factor-1 is the superior immunohistochemical marker for pulmonary adenocarcinoma and large cell carcinoma compared to surfactant proteins A and B. *Histopathplogy*, **36**(1):8-16. [doi:10.1046/j.1365-2559.2000.00801.x]

Kelly, S.E., Bachurski, C.J., Burhans, M.S., Glasser, S.W., 1996. Transcription of the lung-specific surfactant protein C gene is mediated by thyroid transcription factor 1. *J. Biol. Chem*., **271**(12):6881-6888. [doi:10.1074/jbc.271.12.6881]

Kendall, J, Liu, Q., Bakleh, A., Krasnitz, A., Nguyen, K.C., Lakshmi, B., Gerald, W.L., Powers, S., Mu, D., 2007. Oncogenic cooperation and coamplification of developmental transcription factor genes in lung cancer. *PNAS*, **104**(42):16663-16668. [doi:10.1073/pnas.0708286104]

Khoor, A., Byrd-Gloster, A.L., Nicosia, S.V., 2011. Expression of thyroid transcription factor-1 in malignant pleural effusions. *Pathol. Oncol. Res*., **17**(2):263-267. [doi:10.1007/s12253-010-9308-0]

Kimura, S., Hara, Y., Pineau, T., Fernandez-Salguero, P., Fox, C.H., Ward, J.M., Gonzalez, F.J., 1996. The T/ebp null mouse: thyroid-specific enhancer-binding protein is essential for the organogenesis of the thyroid, lung, ventral forebrain, pituitary. *Genes. Dev*., **10**(1):60-69. [doi:10.1101/gad.10.1.60]

Kolla, V., Gonzales, L.W., Gonzales, J., Wang, P., Angampalli, S., Feinstein, S.I., Ballard, P.L., 2006. Thyroid transcription factors in differentiating type II cells: regulation, isoforms, and target genes. *Am. J. Respir. Cell. Mol. Biol*., **36**(2):213-225. [doi:10.1165/rcmb.2006-0207OC]

Krude, H., Schutz, B., Biebermann, H., von Moers, A., Schnabel, D., Neitzel, H., Tonnies, H., Weise, D., Lafferty, A., Schwarz, S., *et al*., 2002. Choreoathetosis, hypothyroidism, and pulmonary alterations due to human NKX2-1 haploinsufficiency. *J. Clin. Invest*., **109**(4):475-480. [doi:10.1172/JCI200214341]

Kwei, K.A., Kim, Y.H., Girard, L., Kao, J., Pacyna-Gengelbach, M., Salari, K., Lee, J., Choi, Y.L., Sato, M., Wang, P., *et al*., 2008. Genomic profiling identifies *TITF1* as a lineage-specific oncogene amplified in lung cancer. *Oncogene*, **27**(25):3635-3640. [doi:10.1038/sj.onc.1211012]

Li, C., Cai, J., Pan, Q., Minoo, P., 2000. Two functionally distinct forms of Nkx2.1 protein are expressed in the pulmonary epithelium. *Biochem. Biophys. Res. Commun*., **270**(2):462-468. [doi:10.1006/bbrc.2000.2443]

Li, C., Zhu, N.L., Tan, R.C., Ballard, P.L., Derynck, R., Minoo, P., 2002. Transforming growth factor-β inhibits pulmonary surfactant protein B gene transcription through SMAD3 interactions with NKX2.1 and HNF-3 transcription factors. *J. Biol. Chem*., **277**(41):38399-38408. [doi:10.1074/jbc.M203188200]

Lin, S., Perl, A.K., Shannon, J.M., 2006. Erm/thyroid transcription factor 1 interactions modulate surfactant protein C transcription. *J. Biol. Chem*., **281**(24):16716-16726. [doi:10.1074/jbc.M602221200]

Lin, X., Saad, R.S., Luckasevic, T.M., Silverman, J.F., Liu, Y., 2007. Diagnostic value of CDX-2 and TTF-1 expression in separating metastatic neuroendocrine neoplasms of unknown origin. *Appl. Immunohistochem. Mol. Morphol*., **15**(4):407-414. [doi:10.1097/01.pai.0000210416.53493.0f]

Liu, C., Glasser, S.W., Wan, H., Whitsett, J.A., 2002. GATA-6 and thyroid transcription factor-1 directly interact and regulate surfactant protein-C gene expression. *J. Biol. Chem*., **277**(6):4519-4525. [doi:10.1074/jbc.M107585200]

Lotan, T.L., Ye, H., Melamed, J., Wu, X.R., Shih, IeM., Epstein, J.I., 2009. Immunohistochemical panel to identify the primary site of invasive micropapillary carcinoma. *Am. J. Surg. Pathol*., **33**(7):1037-1041. [doi:10.1097/PAS.0b013e3181962dcd]

Maeda, Y., Hunter, T.C., Loudy, D.E., Dave, V., Schreiber, V., Whitsett, J.A., 2006. PARP-2 interacts with TTF-1 and regulates expression of surfactant protein-B. *J. Biol. Chem*., **281**(14):9600-9606. [doi:10.1074/jbc.M510435200]

Maeda, Y., Chen, G., Xu, Y., Haitchi, H.M., Du, L., Keiser, A.R., Howarth, P.H., Davies, D.E., Holgate, S.T., Whitsett, J.A., 2011. Airway epithelial transcription factor NKX2-1 inhibits mucous cell metaplasia and Th2 inflammation. *Am. J. Respir. Crit. Care. Med*., **184**(4):421-429. [doi:10.1164/rccm.201101-0106OC]

Martins, S.J., Takagaki, T.Y., Silva, A.G., Gallo, C.P., Silva, F.B., Capelozzi, V.L., 2009. Prognostic relevance of

TTF-1 and MMP-9 expression in advanced lung adenocarcinoma. *Lung Cancer*, **64**(1):105-109. [doi:10.1016/j.lungcan.2008.07.017]

Missero, C., Pirro, M.T., Simeone, S., Pischetola, M., di Lauro, R., 2001. The DNA glycosylase T:G mismatch-specific thymine DNA glycosylase represses thyroid transcription factor-1-activated transcription. *J. Biol. Chem.*, **276**(36):33569-33575. [doi:10.1074/jbc.M104963200]

Myong, N.H., 2003. Thyroid transcription factor-1 (TTF-1) expression in human lung carcinomas: its prognostic implication and relationship with expressions of p53 and Ki-67 proteins. *J. Korea Med. Sci.*, **18**(4):494-500.

Nakamura, N., Miyagi, E., Murata, S., Kawaoi, A., Katoh, R., 2002. Expression of thyroid transcription factor-1 in normal and neoplastic lung tissues. *Mod. Pathol.*, **15**(10):1058-1067. [doi:10.1097/01.MP.0000028572.44247.CF]

Naltner, A., Ghaffari, M., Whitsett, J.A., Yan, C., 2000a. Retinoic acid stimulation of the human surfactant protein B promoter is thyroid transcription factor 1 site-dependent. *J. Biol. Chem.*, **275**(1):56-62. [doi:10.1074/jbc.275.1.56]

Naltner, A., Wert, S., Whitsett, J.A., Yan, C., 2000b. Temporal/spatial expression of nuclear receptor coactivators in the mouse lung. *Am. J. Physiol. Lung Cell. Mol. Physiol.*, **279**(6):L1066-L1074.

Oliveira, A.M., Tazelaar, H.D., Myers, J.L., Erickson, L.A., Lloyd, R.V., 2001. Thyroid transcription factor-1 distinguishes metastatic pulmonary from well-differentiated neuroendocrine tumors of other sites. *Am. J. Surg. Pathol.*, **25**(6):815-819. [doi:10.1097/00000478-200106000-00015]

Ordonez, N.G., 2000a. Thyroid transcription factor-1 is a marker of lung and thyroid carcinomas. *Adv. Anat. Pathol.*, **7**(2):123-127. [doi:10.1097/00125480-200007020-00007]

Ordonez, N.G., 2000b. Value of thyroid transcription factor-1, E-cadherin, BG8, WT1, and CD44S immunostaining in distinguishing epithelial pleural mesothelioma from pulmonary and nonpulmonary adenocarcinoma. *Am. J. Surg. Pathol.*, **24**(4):598-606. [doi:10.1097/00000478-200004000-00016]

Ordonez, N.G., 2000c. Value of thyroid transcription factor-1 immunostaining in distinguishing small cell lung carcinoma from other small cell carcinomas. *Am. J. Surg. Pathol.*, **24**(9):1217-1223. [doi:10.1097/00000478-200009000-00004]

Park, K.S., Whitsett, J.A., di Palma, T., Hong, J.H., Yaffe, M.B., Zannini, M., 2004. TAZ interacts with TTF-1 and regulates expression of surfactant protein-C. *J. Biol. Chem.*, **279**(17):17384-17390. [doi:10.1074/jbc.M312569200]

Pelosi, G., Fraggetta, F., Pasini, F., Maisonneuve, P., Sonzogni, A., Iannucci, A., Terzi, A., Bresaola, E., Valduga, F., Lupo, C., *et al*., 2001. Immunoreactivity for thyroid transcription facter-1 in stage I non-small cell carcinomas of the lung. *Am. J. Surg. Pathol.*, **25**(3):363-372. [doi:10.1097/00000478-200103000-00011]

Pohlenz, J., Dumitrescu, A., Zundel, D., Martine, U., Schonberger, W., Koo, E., Weiss, R.E., Cohen, R.N., Kimura, S., Refetoff, S., 2002. Partial deficiency of thyroid transcription factor 1 produces predominantly neurological defects in humans and mice. *J. Clin. Invest.*, **109**(4):469-473. [doi:10.1172/JCI200214192]

Puglisi, F., Barbone, F., Damante, G., Bruckbauer, M., di Lauro, V., Beltrami, C.A., di Loreto, C., 1999. Prognostic value of thyroid transcription factor-1 in primary, resected, non-small cell lung carcinoma. *Mod. Pathol.*, **12**(3):318-324.

Saad, R.S., Liu, Y.L., Han, H., Landreneau, R.J., Silverman, J.F., 2004. Prognostic significance of thyroid transcription factor-1 expression in both early-stage conventional adenocarcinoma and bronchioloalveolar carcinoma of the lung. *Hum. Pathol.*, **35**(1):3-7. [doi:10.1016/j.humpath.2003.08.011]

Saqi, A., Alexis, D., Remotti, F., Bhagat, G., 2005. Usefulness of CDX2 and TTF-1 in differentiating gastrointestinal from pulmonary carcinoids. *Am. J. Clin. Pathol.*, **123**(3):394-404. [doi:10.1309/UKN6PVRKXHG422DA]

Serls, A.E., Doherty, S., Parvatiyar, P., Wells, J.M., Deutsch, G.H., 2005. Different thresholds of fibroblast growth factors pattern the ventral foregut into liver and lung. *Development*, **132**(1):35-47. [doi:10.1242/dev.01570]

Shah, L., Walter, K.L., Borczuk, A.C., Kawut, S.M., Sonett, J.R., Gorenstein, L.A., Ginsburg, M.E., Steinglass, K.M., Powell, C.A., 2004. Expression of syndecan-1 and expression of epidermal growth factor receptor are associated with survival in patients with non small cell lung carcinoma. *Cancer*, **101**(7):1632-1638. [doi:10.1002/cncr.20542]

Srivastava, A., Hornick, J.L., 2009. Immunohistochemical staining for CDX-2, PDX-1, NESP-55 and TTF-1 can help distinguish gastrointestinal carcinoid tumors from pancreatic endocrine and pulmonary carcinoid tumors. *Am. J. Surg. Pathol.*, **33**(4):626-632. [doi:10.1097/PAS.0b013e31818d7d8b]

Stahlman, M.T., Gray, M.E., Whitsett, J.A., 1996. Expression of thyroid transcription factor-1 (TTF-1) in fetal and neonatal human lung. *J. Histochem. Cytochem.*, **44**(7):673-678. [doi:10.1177/44.7.8675988]

Stenhouse, G., Fyfe, N., King, G., Chapman, A., Kerr, K.M., 2004. Thyroid transcription factor 1 in pulmonary adenocarcinoma. *J. Clin. Pathol.*, **57**(4):383-387. [doi:10.1136/jcp.2003.007138]

Strickland-Marmol, L.B., Khoor, A., Livingston, S.K., Rojiani, A., 2007. Utility of tissue-specific transcription factors 1 and Cdx2 in determining the primary site of metastatic adenocarcinomas to the brain. *Arch. Pathol. Lab. Med.*, **131**(11):1686-1690.

Tan, D., Li, Q., Deeb, G., Ramnath, N., Slocum, H.K., Brooks, J., Cheney, R., Wiseman, S., Anderson, T., Loewen, G., 2003. Thyroid transcription factor-1 expression prevalence and its clinical implications in non-small cell lung cancer: a high-throughput tissue microarray and immunohistochemistry study. *Hum. Pathol.*, **34**(6):597-604. [doi:10.1016/S0046-8177(03)00180-1]

Tanaka, H., Yanagisawa, K., Shinjo, K., Taguchi, A., Maeno, K., Tomida, S., Shimada, Y., Osada, H., Kosaka, T.,

Matsubara, H., *et al*., 2007. Lineage-specific dependency of lung adenocarcinomas on the lung development regulator TTF-1. *Cancer Res*., **67**(13):6007-6011. [doi:10.1158/0008-5472.CAN-06-4774]

Toonen, R.F., Gowan, S., Bingle, C.D., 1996. The lung enriched transcription factor TTF-1 and the ubiquitously expressed proteins Sp1 and Sp3 interact with elements located in the minimal promoter of the rat Clara cell secretory protein gene. *Biochem*. *J*., **316**(Pt 2):467-473.

Wang, C.L., Yue, D.S., Zhang, Z.F., Zhan, Z.L., Sun, L.N., 2007. Value of thyroid transcription factor-1 in identification of the prognosis of bronchioloalveolar carcinoma. *Zhonghua Yi Xue Za Zhi*, **87**(33):2350-2354 (in Chinese).

Weidenfeld, J., Shu, W., Zhang, L., Millar, S.E., Morrisey, E.E., 2002. The WNT7b promoter is regulated by TTF-1, GATA6, and Foxa2 in lung epithelium. *J*. *Biol*. *Chem*., **277**(23):21061-21070. [doi:10.1074/jbc.M111702200]

Weir, B.A., Woo, M.S., Getz, G., Perner, S., Ding, L., Beroukhim, R., Lin, W.M., Province, M.A., Kraja, A., Johnson, L.A., *et al*., 2007. Characterizing the cancer genome in lung adenocarcinoma. *Nature*, **450**(7171):893-898. [doi:10.1038/nature06358]

West, H.L., Crowley, J.J., Vance, R.B., Franklin, W.A., Livingston, R.B., Dakhil, S.R., Giguere, J.K., Rivkin, S.E., Kraut, M., Chansky, K., *et al*., 2005. Advanced bronchioloalveolar carcinoma: a phase II trial of paclitaxel by 96-hour infusion (SWOG 9714): a Southwest Oncology Group study. *Ann*. *Oncol*., **16**(7):1076-1080. [doi:10.1093/annonc/mdi215]

Winslow, M.M., Dayton, T.L., Verhaak, R.G., Kim-Kiselak, C., Snyder, E.L., Feldser, D.M., Hubbard, D.D., DuPage, M.J., Whittaker, C.A., Hoersch, S., *et al*., 2011. Suppression of lung adenocarcinoma progression by Nkx2-1. *Nature*, **473**(7345):101-104. [doi:10.1038/nature09881]

Wislez, M., Antoine, M., Baudrin, L., Poulot, V., Neuville, A., Pradere, M., Longchampt, E., Isaac-Sibille, S., Lebitasy, M.P., Cadranel, J., 2010. Non-mucinomous and mucinous subtypes of adenocarcinoma with bronchioloalveolar carcinoma features differ by biomarker expression and in the response to gefitinib. *Lung Cancer*, **68**(2):185-191. [doi:10.1016/j.lungcan.2009.05.021]

Yan, C., Naltner, A., Conkright, J., Ghaffari, M., 2001. Protein-protein interaction of retinoic acid receptor α and thyroid transcription factor-1 in respiratory epithelial cells. *J*. *Biol*. *Chem*., **276**(24):21686-21691.

Yan, C., Naltner, A., Martin, M., Naltner, M., Fangman, J.M., Gurel, O., 2002. Transcriptional stimulation of the surfactant protein B gene by STAT3 in respiratory epithelial cells. *J*. *Biol*. *Chem*., **277**(13):10967-10972. [doi:10.1074/jbc.M109986200]

Yang, L., Yan, D., Bruggeman, M., Du, H., Yan, C., 2004. Mutation of a lysine residue in a homeodomain generatesdominant negative thyroid transcription factor 1. *Biochemistry*, **43**(39):12489-12497. [doi:10.1021/bi049283o]

Yang, M.C., Wang, B., Weissler, J.C., Margraf, L.R., Yang, Y.S., 2003. BR22, a 26 kDa thyroid transcription factor-1 associated protein (TAP26), is expressed in human lung cells. *Eur*. *Respir*. *J*., **22**(1):28-34. [doi:10.1183/09031936.03.00117702]

Yang, Y.S., Yang, M.C., Wang, B., Weissler, J.C., 2001. BR22, a novel protein, interacts with thyroid transcription factor-1 and activates the human surfactant protein B promoter. *Am*. *J*. *Respir*. *Cell*. *Mol*. *Biol*., **24**(1):30-37.

Yatabe, Y., Mitsudomi, T., Takahashi, T., 2002. TTF-1 expression in pulmonary adenocarcinomas. *Am*. *J*. *Surg*. *Pathol*., **26**(6):767-773. [doi:10.1097/00000478-200206000-00010]

Yi, M., Tong, G.X., Murry, B., Mendelson, C.R., 2002. Role of CBP/p300 and SRC-1 in transcriptional regulation of the pulmonary surfactant protein-A (SP-A) gene by thyroid transcription factor-1 (TTF-1). *J*. *Biol*. *Chem*., **277**(4):2997-3005. [doi:10.1074/jbc.M109793200]

Yoon, S.O., Kim, Y.T., Jung, K.C., Jeon, Y.K., Kim, B.H., Kim, C.W., 2011. TTF-1 mRNA-positive circulating tumors cells in the peripheral blood predict poor prognosis in surgically resected non-small cell lung cancer patients. *Lung Cancer*, **71**(2):209-216. [doi:10.1016/j.lungcan.2010.04.017]

Zhang, P., Han, Y., Huang, L., Li, Q., Ma, D., 2009. Expression and clinical significance of TTF-1 and P63 in NSCLC. *Chin*. *J*. *Lung Cancer*, **12**(9):995-999 (in Chinese).