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NKX2-1/TTF-1: An Enigmatic Oncogene that Functions as a Double-Edged Sword for Cancer Cell Survival and Progression

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Emerging evidence indicates that NKX2-1, a homeobox-containing transcription factor also known as TTF-1, plays a role as a "lineage-survival" oncogene in lung adenocarcinomas. In T cell acute lymphoblastic leukemia, gene rearrangements lead to aberrant expression of NKX2-1/TTF-1. Despite accumulating evidence supporting its oncogenic role, it has become apparent that NKX2-1/TTF-1 expression also has biological and clinical functions in the opposite direction that act against tumor progression. Herein, we review recent findings showing these enigmatic double-edged characteristics, with special attention given to the roles of NKX2-1/TTF-1 in lung development and carcinogenesis.

Oncogenic Involvement of NKX2-1/TTF-1

Emerging evidence suggests that "lineage-specific addiction" to survival mechanisms that are programmed for developmental roles in normal progenitor cells of particular lineages may exist in cancer cells. The transcription factor MITF in melanoma is considered to be an archetypal prototype (Garraway and Sellers, 2006), whereas survival of lung cancers with neuroendocrine (NE) features such as small-cell lung cancer (SCLC) is dependent on continued expression of ASH1, a transcription factor indispensable for pulmonary NE cell development (Nishikawa et al., 2011; Osada et al., 2005, 2008). Thyroid transcription factor 1 (TTF-1), also known as NKX2-1, is a homeobox-containing transcription factor essential for the development of the lung and thyroid as well as a restricted part of the brain (Stanfel et al., 2005), and a series of peripheral lung cells defined as the terminal respiratory unit (TRU) is under the control of this master regulator. About 70% of adenocarcinomas express NKX2-1/TTF-1 independent of disease stage and retain features of the TRU to a certain extent (Yatabe et al., 2002). These TRU-type adenocarcinomas exhibit a distinctively higher prevalence of *EGFR* mutations, disproportionately high occurrence in females and nonsmokers, and characteristic expression profiles; in fact, *p53* and *KRAS* mutations are inversely associated with NKX2-1/TTF-1 expression (Takeuchi et al., 2006; Yatabe et al., 2005). We and others have previously found that NKX2-1/TTF-1-positive lung adenocarcinomas are dependent on sustained expression of NKX2-1/TTF-1 and sometimes even exhibit focal copy-number increases (Figure 1; Table 1) (Kendall et al., 2007; Kwei et al., 2008; Tanaka et al., 2007; Weir et al., 2007). Intriguingly, *Nkx2-1/Ttf-1* transgenic mice exhibit hyperplasia of type II alveolar cells (Wert et al., 2002). In addition, NKX2-1/TTF-1 is prominently expressed in lung epithelial cells undergoing regeneration (Stahlman et al., 1996). Furthermore, haploinsufficiency of *Nkx2-1/Ttf-1* was recently reported to reduce tumor formation in transgenic mice expressing mutant EGFR (Maeda et al., 2012).

Several lines of evidence suggest possible oncogenic involvement of *NKX2-1/TTF-1* in other types of cancers. In addition to the lung, the thyroid is another organ that expresses NKX2-1/TTF-1. A germline missense mutation of *NKX2-1/TTF-1* that results in a valine substitution for alanine at codon 339 has been identified in families affected by multinodular goiter and papillary thyroid carcinoma (Ngan et al., 2009). It is of note that the SNP rs944289, which maps close to *NKX2-1/TTF-1*, was shown to be significantly associated with increased risk of thyroid cancer (Gudmundsson et al., 2009), although the mechanistic link remains to be elucidated. Rearrangements of *NKX2-1/TTF-1* with T cell receptor or immunoglobulin heavy-chain loci were recently identified in T cell acute lymphoblastic leukemia (T-ALL), suggesting a role in the pathogenesis of hematopoietic malignancies (Homminga et al., 2011). Rearrangements and ectopic expression of *NKX2-2* and *NKX2-5*, both homeobox-containing transcription factors closely related to *NKX2-1/TTF-1*, have also been reported in a subset of T-ALL (Homminga et al., 2011; Nagel et al., 2003). These data strongly suggest an oncogenic role for NKX2-1/TTF-1 as well as other members of the NK2 family, not only in lung and thyroid cancers but also in hematopoietic malignancies. On the other hand, *NKX2-8*, residing in close proximity to *NKX2-1*, exhibits loss of heterozygosity and reduced expression in lung squamous cell carcinomas (Harris et al., 2011), suggesting distinct modes of involvement.

Enigma Surrounding NKX2-1/TTF-1 in Tumor Biology

Despite its role as a lineage-survival oncogene in lung adenocarcinomas, NKX2-1/TTF-1 expression is also known to be associated with favorable prognosis in affected patients (Anagnostou et al., 2009). Evidence to explain this paradox has recently emerged (Figure 2). For example, we found that *MYBPH* is directly transactivated by NKX2-1/TTF-1 and inhibits phosphorylation of the myosin regulatory light chain via direct interaction with ROCK1, which is a prerequisite process for acquisition of assembly competence (Hosono et al., 2012b). In addition, MYBPH directly binds to and inhibits assembly of nonmuscle myosin heavy chain IIA (Hosono et al., 2012a), thereby conferring

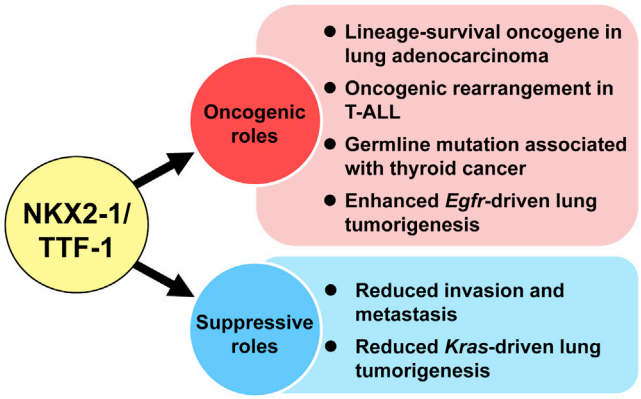

Figure 1. Double-Edged Characteristic of NKX2-1/TTF-1
NKX2-1/TTF-1 has shown both oncogenic and inhibitory activities in cancer development and progression.

firm inhibition of actomyosin assembly by two distinct mechanisms and consequently reducing cell motility, invasion, and metastasis. These apparently deleterious effects in lung adenocarcinoma progression appear to be negated by frequent promoter DNA methylation of *MYBPH*. The epithelial tight-junction protein OCLN as well as two other epithelial tight-junction proteins, CLDN1 and CLDN18, were also shown to be transcriptionally activated by NKX2-1/TTF-1 (Niimi et al., 2001; Runkle et al., 2012). These findings indicate that genes implicated in regulation of cytoskeletal and cell-cell organization are prime transcriptional targets of TTF-1, which negatively affects cell motility, invasion, and metastasis and is also conceivably involved in lung morphogenesis and regeneration after lung injury. In addition, downregulation of *Nkx2-1/Ttf-1* has been shown to lead to eventual derepression of *Hmga2* and acquisition of metastatic ability in a mouse model of lung adenocarcinoma with conditionally activated *Kras* and loss-of-function *p53* mutant alleles (Snyder et al., 2013; Winslow et al., 2011). Interestingly, haploinsufficiency or conditional knockout of *Nkx2-1/Ttf-1* was recently reported to enhance development of invasive *Kras*-driven mucinous lung adenocarcinoma (Maeda et al., 2012; Snyder et al., 2013), in contrast to suppressing *Egfr*-driven lung tumorigenesis (Maeda et al., 2012). Loss of Nkx2-1/Ttf-1 appears to induce the mucin-producing phenotype through consequential release of Foxa1/Foxa2, transcription factors known to physically interact and cooperate with Nkx2-1/Ttf-1, onto de novo binding sites near gastrointestinal differentiation-related genes including Hnf4α, which critically regulates the differentiation program (Snyder et al., 2013). Along this line, it is notable that human invasive mucinous adenocarcinomas of the lung almost invariably express HNF4α, and have exhibited a significant association with negative TTF-1 expression and positive KRAS mutation status (Kunii et al., 2011). Although epithelial-to-mesenchymal transition (EMT) is linked with cancer progression, NKX2-1/TTF-1 represses TGF-β-induced EMT by alleviating TGF-β-mediated induction of Snail and Slug, as well as by reducing TGF-β production (Saito et al., 2009). Conversely, TGF-β represses NKX2-1/TTF-1 by induction of miR-365 (Qi et al., 2012). Thus, accumulated evidence points to the notion that NKX2-1/TTF-1 plays a double-edged role in cancer.

NKX2-1/TTF-1-Mediated Lineage-Survival Signaling

Despite the requirement for sustained NKX2-1/TTF-1 expression in the survival of lung adenocarcinoma cells, NKX2-1/TTF-1 itself cannot be considered as a molecular target for treating this devastating cancer because of its indispensable roles in normal lung physiology, such as the production and secretion of surfactant proteins. Thus, elucidation of how NKX2-1/TTF-1 mediates survival signals has long been anticipated. In this regard, we recently found that NKX2-1/TTF-1 directly transactivates the receptor tyrosine kinase *ROR1*, which in turn sustains a favorable balance between prosurvival PI3K-AKT and proapoptotic p38 signaling, in part through ROR1 kinase-dependent c-Src activation as well as kinase activity-independent sustainment of EGFR-ERBB3 association, ERBB3 phosphorylation, and consequential PI3K activation (Yamaguchi et al., 2012). These findings may underlie the molecular basis for the functional interrelationship between NKX2-1/TTF-1 and EGFR. Consistently, NKX2-1/TTF-1 expression is significantly associated with *EGFR* mutations in lung cancer tissues (Takeuchi et al., 2006; Yatabe et al., 2005), and *Nkx2-1/Ttf-1* haploinsufficiency reduces mutant *Egfr*-driven lung tumorigenesis (Maeda et al., 2012). It is also of particular interest from a clinical point of view that ROR1 inhibition appears to be effective for treatment of lung adenocarcinomas carrying various gefitinib-resistance mechanisms, such as secondary EGFR mutations and HGF overexpression, because the existence of such diverse mechanisms makes it difficult to predict which should be targeted to prevent expansion of resistant clones. This molecule with possible druggability, namely a cell-surface receptor with a

Table 1. Alterations of the NK2 Family in Human Cancers

NK2 Family	Aberrations	Organ Sites	Cancer Types	References
NKX2-1	amplification	lung	adenocarcinoma	Tanaka et al., 2007
	amplification	lung	adenocarcinoma	Kendall et al., 2007
	amplification	lung	adenocarcinoma	Weir et al., 2007
	amplification	lung	adenocarcinoma	Kwei et al., 2008
	germline mutation	thyroid	multinodular goiter, papillary adenocarcinoma	Ngan et al., 2009
	rearrangement	hematopoietic	T cell acute lymphoblastic leukemia	Homminga et al., 2011
NKX2-2	rearrangement	hematopoietic	T cell acute lymphoblastic leukemia	Homminga et al., 2011
NKX2-5	rearrangement	hematopoietic	T cell acute lymphoblastic leukemia	Nagel et al., 2003
NKX2-8	loss of heterozygosity	lung	squamous cell carcinoma	Harris et al., 2011

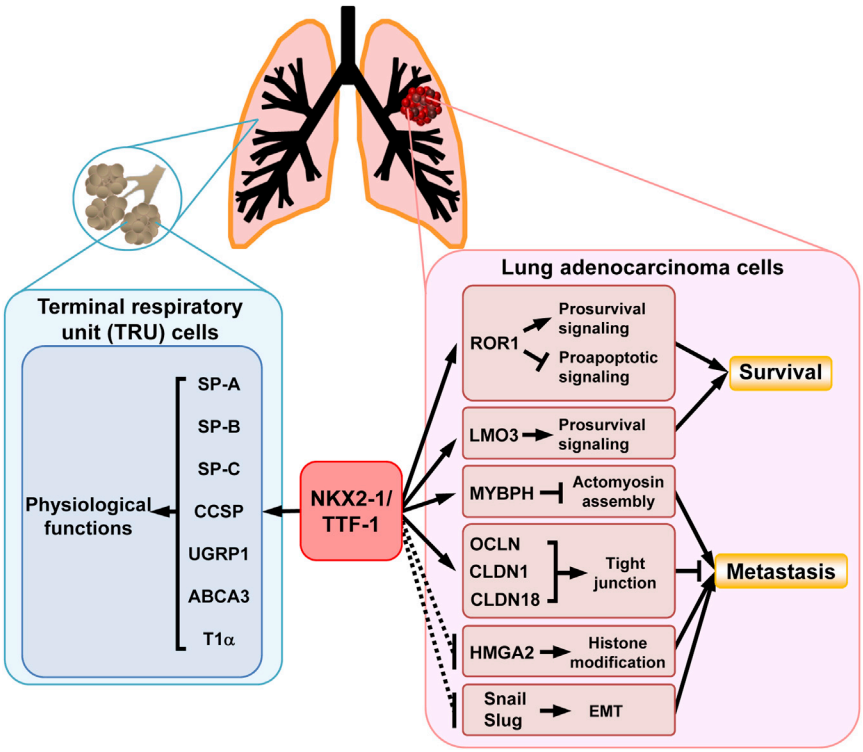

Figure 2. NKX2-1/TTF-1-Mediated Transcriptional Regulation and Consequences in Normal and Cancer Cells of the Lung

NKX2-1/TTF-1 is required for maintenance of physiological lung functions in addition to its developmental roles. The oncogene plays a role as a lineage-survival oncogene in lung adenocarcinomas, whereas it also inhibits invasion, metastasis, and progression, paradoxically conferring better prognosis. Solid and dashed lines represent direct and indirect regulation, respectively.

tyrosine kinase domain, may thus be considered to be an "Achilles' heel" in lung adenocarcinomas, and future development of therapeutic means is greatly anticipated to reduce the intolerable death toll from currently "hard-to-cure" lung adenocarcinomas. In addition to *ROR1*, *LMO3*, a paralog of the *LMO1* and *LMO2* oncogenes in T-ALL, was recently identified as an additional direct transcriptional target for mediating survival signals (Watanabe et al., 2013). NKX2-1/TTF-1 appears to cooperatively transactivate *LMO3* together with FOXA1, whereas *LMO3* knockdown induced apoptosis in a lung adenocarcinoma cell line. However, ectopic overexpression of *LMO3* failed to overcome *NKX2-1/TTF-1* knockdown-induced apoptosis, suggesting the existence of additional crucial targets for lineage-survival signaling in lung adenocarcinoma cells.

Developmental Roles of NKX2-1/TTF-1 in Relation to Cancer Biology

During embryonic lung development, temporal-spatial expression of NKX2-1/TTF-1 is tightly regulated. NKX2-1/TTF-1 expression is first detected in the ventral foregut endoderm during a very early stage and then becomes abundantly expressed in virtually all cells in the progenitor of the trachea arising from the lung primordium. As subsequent branching morphogenesis proceeds, NKX2-1/TTF-1 expression is progressively restricted to distal airway cells and finally confined to epithelial cells in the TRU (Stahlman et al., 1996; Yatabe et al., 2002). A lung rudiment in *Nkx2-1/Ttf-1* knockout mice exhibited proximal, albeit abnormal, airway characteristics, suggesting its dispensable nature in specification of the lung primordium and proximal lung morphogenesis (Minoo et al., 1999). In contrast, this oncogene was shown to be strictly required for distal lung morphogenesis (Yuan et al., 2000). *NKX2-1/TTF-1* critically translates instructive morphogenic signals from the surrounding mesenchyme into transcriptional regulation of its targets, which are mediated by factors including fibroblast growth factors, Sonic hedgehog, and bone morphogenetic proteins. In humans, *NKX2-1/TTF-1* haploinsufficiency confers the rare autosomal-dominant disorder benign hereditary chorea as well as brain-lung-thyroid syndrome, which is manifested by chorea, hypothyroidism, and infantile respiratory distress (Inzelberg et al., 2011). Human *NKX2-1/TTF-1* haploinsufficiency might be associated with lung tumorigenesis in context-dependent and subtype-specific manners, as reported in mice (Maeda et al., 2012; Snyder et al., 2013). Unfortunately, no comprehensive epidemiologic data on the predisposition to lung cancers in affected individuals have been presented.

In addition to lung adenocarcinoma, it is interesting to note that NKX2-1/TTF-1 is frequently detected in SCLCs, which usually arise in the proximal airway, a region that normally lacks NKX2-1/TTF-1 expression. Because NKX2-1/TTF-1 expression is seen in the lung primordium, this phenomenon may reflect an atavistic, yet committed, state of SCLCs, which is consistent with its lack of expression in small-cell carcinomas arising from other organs, despite the similar characteristics of small and round morphology and NE properties. Future study comparing NKX2-1/TTF-1 target gene regulation between adenocarcinomas and those in small-cell carcinomas of various organs, including the adult and developing fetal lungs, would likely shed light on both similarities and distinctions with regard to its functional roles.

Regulation of NKX2-1/TTF-1 and Context Dependence

The 42 kD major isoform is encoded by mRNAs harboring exons 2 and 3, whereas the 44 kD minor isoform is encoded by all three exons (Figure 3). The proximal major promoter contains a TATA-like element and binding sites for FOXA1 (also known as HNF-3α), FOXA2 (HNF-3β), and GATA6, all of which are known to be crucially involved in lung development (Costa et al., 2001). The minor distal promoter is regulated by SP1 and SP3. NKX2-1/TTF-1 directly transactivates multiple genes implicated to have physiological lung functions, including SP-A, SP-B, SP-C, CCSP (also known as CC10, uteroglobin, or secretoglobin),

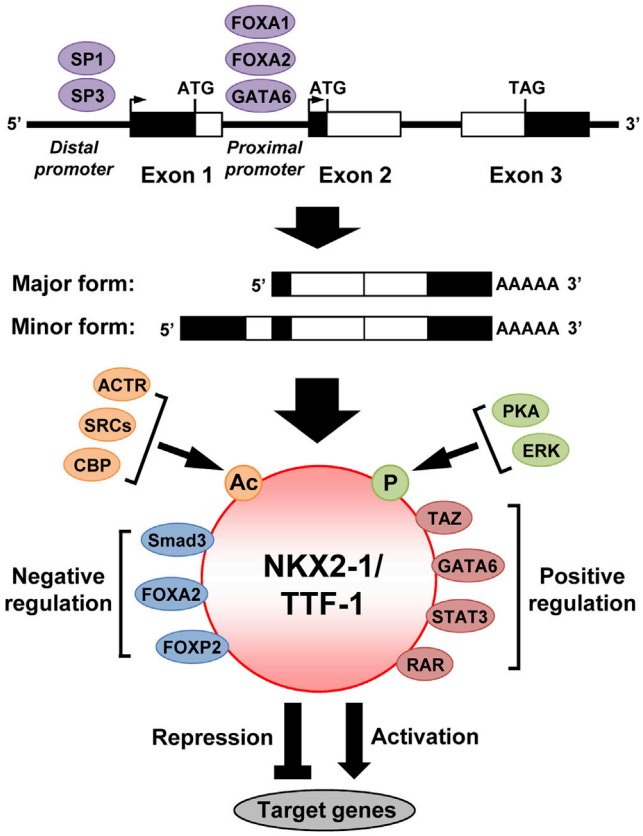

Figure 3. Regulatory Mechanisms of NKX2-1/TTF-1
NKX2-1/TTF-1 is transcribed from two distinct promoters under the influence of various transcription factors. Its transcriptional regulatory activities are modulated in a context-dependent manner, possibly by cooperating transcription factors as well as protein modifications. Ac, acetylation; P, phosphorylation.

UGRP1, and ABCA3. NKX2-1/TTF-1 also transactivates the functions of HOP, an HDAC-dependent negative regulator of NKX2-1/TTF-1 (Yin et al., 2006), as well as T1α, a type I pneumocyte-specific marker (Ramirez et al., 1997). CLDN18 and OCLN tight-junction proteins MYBPH, LMO3, and ROR1 have recently been identified as targets for their roles in cancer, as discussed above. Transcription factors that interact and cooperate with NKX2-1/TTF-1 include FOXA2 (Minoo et al., 2007), FOXP2 (Zhou et al., 2008), GATA6 (Liu et al., 2002), STAT3 (Yan et al., 2002), and RAR (Yan et al., 2001). Furthermore, Smad3 and TAZ modulate the transcriptional activity of NKX2-1/TTF-1 via their binding in a negative and positive manner, respectively (Li et al., 2002; Park et al., 2004). Posttranslational modifications are also important as a regulatory mechanism of NKX2-1/TTF-1 functions. Multiple nuclear coactivators, including ACTR, p160 steroid receptor coactivators, and p300/CBP, acetylate NKX2-1/TTF-1 (Yang et al., 2004), whereas NKX2-1/TTF-1 is also regulated through its phosphorylation, positively by PKA (Yan and Whitsett, 1997) and negatively by ERK (Missero et al., 2000). In addition, Smad3 physically interacts with NKX2-1/TTF-1 and inhibits NKX2-1/TTF-1-mediated transcription from the *SP-B* promoter lacking a Smad binding site (Li et al., 2002).

Recent ChIP-seq and ChIP-chip analyses have revealed a large number of additional potential transcriptional targets of NKX2-1/TTF-1 (Maeda et al., 2012; Tagne et al., 2012; Watanabe et al., 2013), with experimental validation of the induction of LMO3, E2F3, and cyclins B1 and B2, as well as repression of MUC5A, FGFR1, and MET. It is notable that NKX2-1/TTF-1 appears to be associated with and affect promoters via not only its canonical binding sites but also by the AP-1, forkhead, and nuclear hormone receptor-binding motifs. Therefore, downstream targets appear to be regulated by NKX2-1/TTF-1 in a context-dependent manner, possibly reflecting the expression of its cofactors.

Accumulating evidence, as noted above, implicates opposing roles of NKX2-1/TTF-1 in lung cancer development, which may also be the case in thyroid tumors and hematopoietic malignancies. NKX2-1/TTF-1 expression is absolutely required for peripheral lung development and differentiation, whereas its level in lung adenocarcinoma is associated with but not deterministic of differentiated morphologies (Takeuchi et al., 2006; Yatabe et al., 2002). It would be interesting to investigate whether any similarities and/or distinctions exist in the regulation of downstream targets by this enigmatic oncogene in cancer cells as well as in normal development, with special attention given to context dependence.

Conclusions and Future Perspectives

NKX2-1/TTF-1 has long been a focus of research in the field of lung and thyroid physiology, whereas emerging evidence has called attention to its roles in cancer. This oncogene appears to function as a double-edged sword in the pathogenesis of lung adenocarcinoma and possibly in other tumors as well. A future rigorous search for additional downstream molecules is warranted to gain a more complete picture of NKX2-1/TTF-1-centered regulatory networks in order to take advantage of its Jekyll-and-Hyde characteristics. It should also be kept in mind that current understanding of the regulatory web surrounding this enigmatic transcription factor may be oversimplified, as the same architecture may not exist in normal and cancerous states, or even among individual tumors. For example, a sizable fraction of NKX2-1/TTF-1-positive human lung adenocarcinomas are negative for surfactant proteins, which are authentic targets for transcriptional activation in normal lungs, and/or positive for HMGA2, a target for transcriptional repression. Opposing effects of *Nkx2-1/Ttf-1* haploinsufficiency in transgenic mice carrying mutant *Kras* and *Egfr* also suggest its multifaceted nature. Thus, the NKX2-1/TTF-1 regulatory networks present in cells in both normal and cancerous conditions may well be quite complex and context dependent, and likely require a radically different approach to elucidate. Along this line, a cancer systems biology approach with the aid of ever-increasing computing power may help to reveal a path to resolve this challenge, ultimately allowing an opportunity to take advantage of the double-edged characteristics of NKX2-1/TTF-1 in patients affected by cancer.

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