Review Article

Thyroid-Specific Transcription Factors and Their Roles in Thyroid Cancer

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Homeodomain, forkhead domain, and paired domain-containing transcription factors play a major role in development, tissue-specific gene expression, and tissue homeostasis in organs where they are expressed. Recently, their roles in stem cell and cancer biology are emerging. In the thyroid, NKX2-1, FOXE1, and PAX8 transcription factors are responsible for thyroid organogenesis and expression of thyroid-specific genes critical for thyroid hormone synthesis. In contrast to their known roles in gene regulation, thyroid development and homeostasis, their involvement in stem cell, and/or cancer biology are still elusive. In order to further understand the nature of thyroid cancer, it is critical to determine their roles in thyroid cancer.

1. Introduction

Tissue-specific transcription factors play a pivotal role in regulating expression of tissue-specific genes, thereby controlling the function, homeostasis, and differentiation of tissue where they are expressed. Their altered expression due to gene mutation, deletion, amplification, and/or epigenetic modification, and/or posttranslational modification can change the cell fate and perturb metabolism and differentiation status, leading to various clinical conditions. Since both cell proliferation and differentiation are involved in the process of normal and cancer development, it is not surprising that genes critical for development play an important role in oncogenesis. Transcription factors, containing the homeobox [1, 2], forkhead domain (FOX) [3], and paired domain (PAX) [4], that are among those expressed tissue-specifically that play a critical role in tissue homeostasis and development, can also have roles in carcinogenesis. Thyroid is an organ in which the homeodomain, forkhead domain, and paired domain-containing transcription factors all play major roles in tissue-specific gene expression and thyroid development. The current view on the roles of thyroid-specific transcription factors in thyroid cancer will be summarized below.

2. Thyroid-Specific Transcription Factors

The three distinct thyroid-specific transcription factors are critical for the function of thyroid: NKX2-1 (also called TTF1, TITF1, T/EBP, or NKX2.1) [5, 6], FOXE1 (also called TTF2 or TITF2) [7], and PAX8 [8] (Table 1). They are members of the homeodomain, forkhead box, and paired box family of transcription factors, respectively, and regulate genes encoding thyroglobulin, thyroid peroxidase, thyrotropin receptor, and sodium/iodide symporter, proteins critical for thyroid hormone synthesis [5–7, 9–14]. They are also essential for thyroid development [15–17]; *Nkx2-1*-null mice are born without the thyroid (agenesis) [16], while *Pax8*-null mice are severely hypothyroidism with rudimental thyroid remnant [17]. *Foxe1*-null mice have either agenesis or thyroid ectopy [15]. These transcription factors are responsible for the athyreosis, hypothyroidism, and/or ectopic thyroid, which provide crucial clues to their roles in thyroid dysgenesis in humans [18]. In addition to thyroid, NKX2-1 is expressed in lung primordium and ventral forebrain [16], PAX8 in developing kidney [8], and FOXE1 in the floor of the foregut and the craniopharyngeal ectoderm including Rathke's pouch during development [15]. At later stages, FOXE1 is expressed in the secondary palate, definitive

TABLE 1: Thyroid-specific transcription factors and thyroid cancers.

	Thyroid phenotype in null mice	Gene requirement	Expression in thyroid cancer	Common variants/Gene mutations for thyroid cancer predisposition	Other specifics
NKX2-1	Athyreosis	Proliferation and survival of thyroid follicular cells and C cells	Level correlates with the degree of differentiation	14q13.3: PTC, FTC A339V: PTC	Gene mutation increases thyroid cell proliferation Lineage-specific oncogene amplified in lung cancer
PAX8	Athyreosis, rudimental thyroid remains	Proliferation and survival of thyroid follicular cells	Correlation between expression level and differentiation not clear	PAX8/PPARγ fusion protein: FTC	Expression found in cancers of other tissues such as kidney, Müllerian system, and ovary
FOXE1	Athyreosis or ectopia	Migration of thyroid primordium	Level correlates with the degree of differentiation	rs965513 on 9q22.33: PTC, FTC, radiation-induced PTC rs1867277 ($-283G > A$): PTC	LOH at D9S180 on 9q22.3: frequently found in skin SCC 16 Ala variant: associated with SCC

PTC: papillary thyroid carcinoma, FTC: follicular thyroid carcinoma, SCC: squamous cell carcinoma, and LOH: loss of heterozygosity.

choanae, whiskers, and hair follicles [19]. Some structures derived from these areas are also defective in respective null mice. Thus, *Nkx2-1*-null mice also have severely hypoplastic lung, defective hypothalamus, and pituitary agenesis [16]. *Foxe1*-null mice have cleft palate [15]. Similar to the defects found in the *Nkx2-1*-null mice and sometimes more manifested in humans, various mutations in the *NKX2-1* gene result in the Brain-Thyroid-Lung syndrome, which is characterized by benign hereditary chorea, congenital hypothyroidism, and respiratory diseases [20–22]. Mutations in the *FOXE1* gene are responsible for syndromic congenital hypothyroidism dysgenesis, cleft plate, and spiky hair [22–24].

2.1. NKX2-1

2.1.1. NKX2-1 and Cancer. Due to the nature of tissue-specific expression, NKX2-1 is expressed in human thyroid and lung cancers [25–28]. In particular, NKX2-1 is highly expressed in human lung adenocarcinomas and small cell carcinomas (~60–90%) [25, 26, 29]. NKX2-1 has been widely used as a marker for the diagnosis of primary and metastatic lung cancer [30] and as a prognostic indicator for survival [26, 31, 32]. In fact, *NKX2-1* is a lineage-specific oncogene amplified in lung cancers and the survival of a subset of adenocarcinoma cells depends on the sustained expression of NKX2-1 [33–35]. However, no mutations in the *NKX2-1* gene are described in any adenocarcinomas examined in these studies. Patients with adenocarcinomas that lack NKX2-1 expression or have NKX2-1 expression accompanied by *NKX2-1* gene amplification tend to have

a significantly worse prognosis than patients with NKX2-1 expression and no *NKX2-1* gene amplification [32].

In contrast to the expression in lung, NKX2-1 is expressed at lower levels in malignant thyroid as compared to normal thyroid [36]. The level of expression is significantly correlated with the progressive dedifferentiation and increase of malignancy of thyroid tumors [27]. Thus, the expression is generally found in the order of follicular thyroid adenoma > follicular thyroid carcinoma > papillary thyroid carcinoma > medullary thyroid carcinoma > anaplastic thyroid carcinoma [27, 37–39]. These studies use immunohistochemical analysis of primary thyroid tissues, and low or no expression of NKX2-1 is found in anaplastic thyroid carcinomas. Using RT-PCR, NKX2-1 expression is reported in some anaplastic thyroid carcinoma-derived cell lines [40, 41]. The latter studies present different results for the expression of NKX2-1 within the same cell line, suggesting the controversial nature of NKX2-1 expression. In order to explain the loss of NKX2-1 expression in most of undifferentiated thyroid carcinomas and cell lines, epigenetic silencing of the *NKX2-1* gene through DNA hypermethylation and histone H3 modification has been suggested [40]. Further studies are required to obtain clear understanding of the relationships in between expression of NKX2-1, differentiation status of tissues and primary carcinomas versus cell lines, and the mechanisms underlying the loss of NKX2-1 expression in malignancy.

A genome-wide association study (GWAS) revealed the predisposition of common variants on 9q22.33 and 14q13.3 to both papillary and follicular thyroid cancers. The gene nearest to the 9q22.33 is *FOXE1,* and among the genes located at the 14q13.3 locus is *NKX2-1* [42], suggesting

potential roles for these two thyroid-specific transcription factors in thyroid cancers. A germline mutation of *NKX2-1* gene leads to a mutant NKX2-1 protein (A339V) that has impaired transactivation of thyroid-specific genes such as thyroglobulin, thyrotropin receptor, and PAX8, while the expression is associated with the increased cell proliferation, thyrotropin-independent growth, and enhanced activation of survival signaling molecules such as Stat3 and Akt as compared to wild-type protein [43]. A population study demonstrated that the *NKX2-1* A339V mutant contributes to predisposition of maltinodular goiter and/or papillary thyroid carcinomas and to the pathogenesis of papillary thyroid carcinomas [43].

2.1.2. Nkx2-1 Thyroid-Specific Conditional Knockout Mouse as a Model to Study Thyroid Carcinogenesis. *Nkx2-1(fl/fl);TPO-Cre* thyroid-specific conditional knockout mouse provides an animal model to study the role of NKX2-1 in adult thyroid, which circumvents the problem of immediate neonatal lethality of *Nkx2-1*-null mouse [44]. In the *Nkx2-1(fl/fl);TPO-Cre* mouse, the recombination of *Nkx2-1* floxed gene occurs at the rate of ~50%, resulting in *Nkx2-1* thyroid-specific conditional hypomorphic mouse [45]. These mice exhibit either atrophic/degenerative thyroids with frequent presence of adenomas and extremely high TSH levels, or thyroids with reduced numbers of extremely dilated follicles having more number of follicular cells than usual within a follicle. The atrophic/degenerative thyroid mostly consists of atrophic/degenerative follicles, in which many follicular cells frequently have lost NKX2-1 expression, suggesting that the loss of NKX2-1 may be the cause of atrophic/degenerative follicular cells [45]. These findings further suggest that NKX2-1 is required for the maintenance of ordered architecture and function of the differentiated thyroid [45].

In chemical carcinogenesis bioassays using the genotoxic mutagen N-bis(2-hydroxypropyl)-nitrosamine (DHPN) followed by sulfadimethoxine (SDM) as a promoter, the *Nkx2-1(fl/fl);TPO-Cre* mice developed significantly higher incidence of adenomas as compared with wild-type or *Nkx2-1*-heterozygous mice [46]. In contrast, with the non-genotoxic carcinogen amitrole (3-amino-1,2,4-triazole), all three genotype groups of mice developed adenomas at similar incidence. Surprisingly, no gene mutation was identified in any adenoma-developed thyroids. The increased incidence of adenomas in the *Nkx2-1(fl/fl);TPO-Cre* mice after genotoxic carcinogen exposure may be partially explained by more than a twofold higher cell proliferation rate found in these mouse thyroids as compared to those of wild-type or *Nkx2-1*-heterozygous mice. These results may be analogous to human exposure to genotoxic mutagens or radiation, which could cause somatic mutation of *NKX2-1* gene → inactivation of *NKX2-1* gene → degeneration of thyroid follicular cells → increased cell proliferation → augmentation of the damage occurred in DNA, and/or chromosomes by genotoxic mutagens or radiation exposure, ultimately leading to cancer [46].

2.2. FOXE1 and Cancer. The human *FOEX1* gene is located on chromosome 9q22.3 [47]. The loss of heterozygosity of marker D9S180 from this chromosomal area is frequently observed in squamous cell carcinomas of skin, suggesting the presence of tumor suppressor gene in this genomic region [48]. The common variant rs965513 on 9q22.33 contributes to an increased risk of papillary and follicular thyroid cancer [42]. Further, a high incidence of *FOXE1* gene promoter methylation is found in cutaneous squamous cell carinomas (SCC) [49], pancreatic cancers [50], and breast cancers [51]. FOXE1 protein has a polyalanine tract starting at the 13th amino acid residue from the end of the forkhead domain, which stretches from 12 to 17 residues with the 14 alanine stretch at the highest frequency [47]. The less common variant (allele 16) is associated with SCC, suggesting that the more common variant (allele 14) may be protective against developing SCC [52].

Similar to NKX2-1, FOXE1 expression is found in various thyroid cancers [38, 53]. The level of expression correlates with their differentiation status as seen with NKX2-1, and anaplastic thyroid carcinoma has very little expression of FOXE1 [38, 53]. The candidate gene association study revealed that the variant rs1867277 (−283G > A) located in the *FOXE1* 5′ UTR is associated with papillary thyroid cancer susceptibility through recruitment of USF1/USF2 transcription factors to the −283A allele, which affects gene expression [54]. FOXE1 is required for thyroid cell precursors to migrate into the underlying mesenchyme from the thyroid bud [15, 55]. Although the exact mechanism for the enhanced transcription of *FOXE1* gene leading to increased susceptibility to papillary thyroid cancer remains unknown, the enhanced expression of FOXE1 in thyroid carcinomas could be related to a motile advantage of malignant thyroid cells [54].

Radiation exposure causes papillary thyroid cancer as revealed by various studies after the Chernobyl accident [56]. Genome-wide association studies (GWAS) employing Belarusian patients and control subjects demonstrated that the variant rs965513 on 9q22.33 is significantly associated with the radiation-induced papillary thyroid cancer [57]. This variant was identified together with NKX2-1, as those having the strongest rink to papillary and follicular thyroid cancers [42]. Although *Foxe1* thyroid conditional null mice are currently not available, they would be a useful model to understand the role of FOXE1 in the pathogenesis of thyroid cancer.

2.3. PAX8

2.3.1. PAX8 and Cancer. PAX8 is a crucial transcription factor for organogenesis of the thyroid, kidney, and Müllerian system [8, 58]. PAX8 is expressed in normal as well as neoplastic renal tissues, and in Wilms' tumors [58, 59]. PAX8 is a useful marker for Müllerian carcinomas [60] and ovarian cancer [61, 62] and can be used to distinguish ovarian serous tumors from malignant mesothelioma [61–64] or from other metastatic tumors such as breast and colon [63, 64].

PAX8 is expressed in various thyroid cancers; however, the pattern of expression is somewhat controversial;
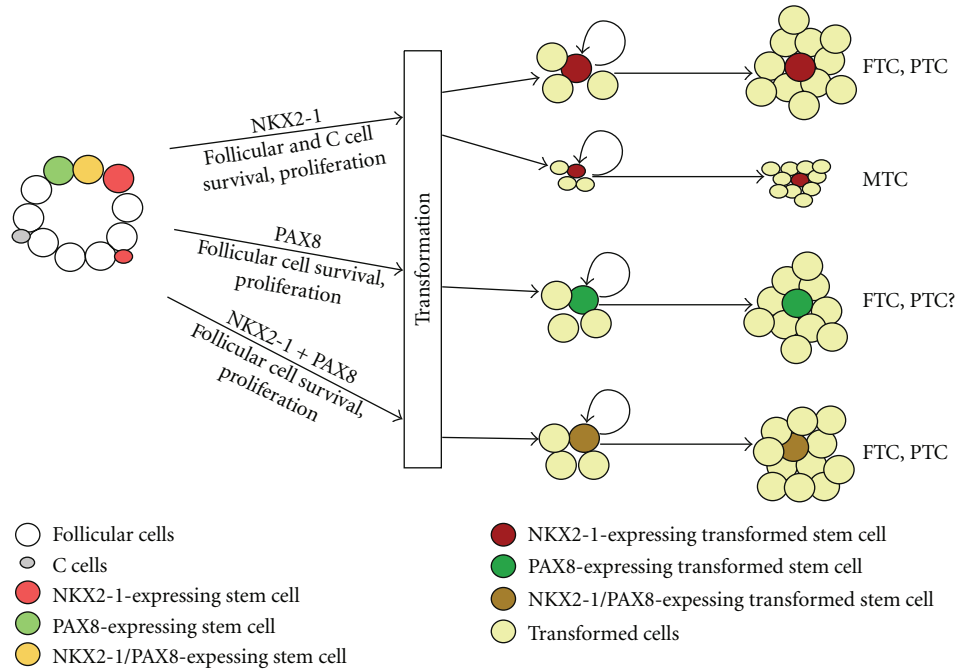
Figure 1: Possible involvement of NKX2-1 and/or PAX8 in the maintenance and/or activity of stem cells of the thyroid. Transformation of NKX2-1 and/or PAX8-expressing stem cells leads to thyroid cancer. Transformed NKX2-1 and/or PAX8-expressing stem cells self-renew and proliferate to produce cancer. Upon becoming tumorigenic, most of transformed cells may lose NKX2-1 and/or PAX8 expression. FTC: follicular thyroid carcinoma, PTC: papillary thyroid carcinoma, and MTC: medullary thyroid carcinoma.

one study showed that the nuclear PAX8 staining is correlated with the thyroid differentiation phenotype as seen with NKX2-1 and FOXE1 [27], while others demonstrated that PAX8 is a useful marker for the diagnosis of anaplastic carcinomas [38]. More studies are required to determine the expression pattern and the role of PAX8 in thyroid cancers, including the use of *Pax8* thyroid-conditional null mice.

2.3.2. PAX8/PPARγ Fusion Oncogene in Thyroid Cancer. The chromosomal translocation of the 2q13-qter region to 3p25 results in an in-frame fusion protein (PPFP) between most of the coding sequence of PAX8 and the entire translated reading-frame of the nuclear receptor-family member peroxisome proliferator-activated receptor gamma (PPARγ) ([65] reviewed in [66, 67]). The PPFP has several different PAX8 breakpoints while the PPARγ breakpoint seems to be constant [65, 67, 68]. This fusion protein is more prevalently expressed in follicular thyroid carcinomas (36%, reviewed in [69]); however, follicular adenomas (11%), follicular variant of papillary carcinoma (16%), and Hürthle cell carcinoma (2%) also express PPFP [68–70]. PPFP has been proposed to be an early follicular thyroid carcinoma-specific oncogene [65, 71]. Several *in vitro* studies demonstrated that PPFP has oncogenic activity such as increased cell cycle transition, reduced apoptosis, and enhanced growth [71], which is partly due to PPFP's dominant negative activity to suppress wild-type transcriptional activities of PPARγ [65, 71, 72], the suggested tumor suppressor [73, 74]. PPFP can also work as a dominant negative inhibitor of wild-type

PPARγ *in vivo* [75]. Further studies are required to establish the mechanisms for the PPFP-mediated tumorigenesis.

3. Thyroid-Specific Transcription Factors, Cancer, and Stem Cells

Normal embryogenesis is believed to share many of the same pathways as neoplasia, such as Wnt/β-catenin, Hedgehog, and Notch pathways. These signaling pathways are also involved in the maintenance and/or activity of stem cells, while their dysregulation plays a role in tumorigenesis (reviewed in [76–80]). It is increasingly recognized that homeobox proteins including PAX proteins play a critical role in stem cell maintenance [4, 81]. PAX3 or PAX7 is essential for generating the cell pool of muscle progenitors from which satellite cells derive [82]. Overexpression of PAX3 and 7 is frequently found in pediatric soft-tissue malignant tumor rhabdomyosarcomas [83, 84]. PAX6 is essential for maintenance of the multipotency of retinal progenitor cells [85]. On the other hand, *HOX* genes are expressed in hematopoietic cells in a stage- and lineage-specific manner, and are implicated in leukemogenesis [81]; for instance, HOXA10 is a critical regulator for haematopoietic stem cells, and erythroid and megakaryocyte development [86], while HOXA9 is required for normal hematopoietic stem cell function [87]. The involvements of other homeobox genes in the maintenance of stem cells are described in various tissues including brain [88] and kidney [89]. In the prostate, NKX3-1, another member of the *NKX* gene family, is required for stem cell maintenance [90]. The targeted deletion of *Pten*,

a tumor suppressor gene in castration-resistant NKX3-1-expressing cells, results in rapid carcinoma formation after androgen-mediated regeneration [90].

The three transcription factors, NKX2-1, FOXE1, and PAX8, are critical for normal embryogenesis and appear to play a role in tumorigenesis in various tissues where they are expressed, including the thyroid. By analogy to other homeodomain/PAX proteins, it is likely that NKX2-1 and PAX8 may be involved in the maintenance and/or activity of stem cells in the thyroid, dysregulation of which may lead to thyroid cancer (Figure 1). Currently, it is not clear whether FOX transcription factors are involved in stem cell maintenance/activity [3]. Knockout mouse studies demonstrated that in the absence of NKX2-1, primordium cells to both thyroid follicular and C cells disintegrate during thyroid organogenesis [16, 91], while PAX8 is required for the survival of follicular cells [17]. It would be interesting to determine whether NKX2-1 and/or PAX8-expressing stem/progenitor cells exist that can rapidly form cancers upon targeted disruption of a tumor suppressor gene in cell pools, similar to that seen with NKX3-1. In this regard, NKX2-1 in lung cancers may be more analogous to this scenario since NKX2-1 is a lineage-specific oncogene and is required for survival of a subset of adenocarcinoma cells [33–35].

4. Conclusions

It appears that most transcription factors, if not all, that are critical for developmental process are involved in the maintenance and/or activity of stem cells, whose dysregulation results in cancers. Currently, it is entirely unknown whether and/or how the thyroid-specific transcription factors NKX2-1, FOXE1, and PAX8 are related to stem/progenitor cells of the thyroid that may lead to cancer when dysregulated. Identification/characterization of thyroid stem/progenitor cells, their relation to the expression of NKX2-1, FOXE1, and/or PAX8, and more detailed characterization of various thyroid cancers and/or cancer cells, particularly in relation to the expression of these transcription factors, are urgently required in order to better understand the roles of NKX2-1, FOXE1, and PAX8 in thyroid cancer.

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