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Thyroid Follicular Cell Neoplasms in Multistep Carcinogenesis

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Abstract

The present review summarizes current knowledge on the alterations of molecular genetics and epigenetics of sporadic thyroid follicular cell neoplasms and the relationship of the alterations with multistep carcinogenesis theory. Approximately 50% of follicular thyroid carcinomas have mutations in *RAS* family genes or *PAX8/PPARγ* gene rearrangements. These mutations are found in a mutually exclusive manner, which suggests that follicular thyroid carcinomas develop *via* two different initiating mechanisms. *BRAF* gene mutations and *RET/PTC* and *NTRK1* rearrangements that involve the mitogen-activated protein kinase pathway are found in the majority of papillary thyroid carcinoma. These mutations are also found in a mutually exclusive manner and contribute to the initiation of the transformation from normal follicular cells to papillary thyroid carcinoma. *TP53* mutations are almost exclusively found in poorly differentiated carcinoma and undifferentiated carcinoma and play an essential role in tumor progression. Aberrant methylation of oncogenes and suppressor oncogenes such as the *PTEN* gene also causes tumor progression. Growth factors (FGF, FGFR, MET, EGFR and VGEF), cell cycle regulators (cyclin D1, RB, p16, p21 and p27) and adhesion molecules (E-cadherin and fibronectin) activate the mitogenic signaling pathways and have impacts on the initiation, promotion and progression of thyroid carcinomas.

Keywords: Thyroid, follicular cells, carcinoma, gene, multistep carcinogenesis

Introduction

Two theories have been proposed regarding the thyroid follicular cell carcinogenesis: the fetal cell carcinogenesis theory proposed by Takano T and the more common multistep carcinogenesis theory by Vogelstein B et al. (1-6). As predisposing conditions for thyroid follicular cell carcinomas, various risk factors including nutritional (iodine intake), environmental (radiation exposure) and genetic (inherited tumor syndromes and familial non-medullary thyroid carcinoma) backgrounds have been reported. According to the multistep carcinogenesis theory, carcinogenesis is a sequential process involving alterations of multiple genes and epigenetics (4-6). The number of genes and the number of corresponding lesions have not been fully elucidated in thyroid follicular cell carcinogenesis, while as many as 10 or more sequential alterations have been shown to occur in colorectal carcinoma, in accordance with the multistep carcinogenesis theory (7-16). Analysis of thyroid carcinomas has disclosed accumulation of the changes of growth factors, cell cycle regulators and adhesion molecules, which activate mitogenic signaling pathways, in addition to genetic and epigenetic alterations of multiple genes. The present review summarizes current knowledge on the alterations of molecular genetics and epigenetics of sporadic (non-hereditary) thyroid follicular cell neoplasms.

Table 1: Hereditary (familial) thyroid carcinomas

C Cell Origin

A) Syndromic types
   Multiple endocrine neoplasia type 2 (MEN 2a/2b) (*RET* gene)

B) Non-syndromic types
   Familial Medullary Thyroid Carcinoma (*RET* gene)

Follicular Cell Origin

A) Syndromic types
   1. Familial adenomatous polyposis (FAP) (*APC* gene)
   2. Cowden syndrome (PTEN hamartoma tumor syndrome) (*PTEN* gene)
   3. Multiple endocrine neoplasia type 1 (MEN 1) (*MEN1* gene)
   4. Carney complex (*PRKAR1A* gene)
   5. Werner syndrome (*WRN* gene)
   6. Li-Fraumeni syndrome (*TP53* gene)

B) Non-syndromic types
   Familial non-medullary thyroid carcinoma
   1. Familial papillary thyroid carcinoma (PTC) with papillary renal cell neoplasia (1q21)
   2. Familial PTC with oxyphilia (19p13.2)
   3. Familial PTC without oxyphilia (19p13.2)
   4. Familial multinodular goiter with PTC (14q)
   5. Familial PTC (2q21)

1. Hereditary Thyroid Carcinomas

Hereditary (familial) thyroid carcinoma can arise from both follicular cells and C cells of thyroid (Table 1). The majority of

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Table 2: Genetic alterations in thyroid neoplasms and Ki-67 proliferation index*

Genetic changes/ Histo-types	*RET/PTC* rearrangements	*BRAF* (V600E) mutations	*RAS* mutations	*PAX8/PPARγ* rearrangements	*CTNNB1* (β catenin) mutation	*TP53* mutations	*Ki-67 Labeling Index*
FA	0%	0%	17-43%	8%	0%	0%	<3%
PTC	13-43%	30-69%	0-21%	0.3%	0%	0-5%	<10%, usually <5%
FTC	0%	0%	40-53%	39%	0%	0-9%	<10%, usually <5%
PDC	0-13%	0-13%	18-55%	0%	25%	17-38%	10-30%
UC	0%	10-35%	4-60%	0%	66%	60-88%	>30%, usually >50%

FA: Follicular adenoma; PTC: Papillary thyroid carcinoma; FTC: Follicular thyroid carcinoma; PDC: Poorly differentiated carcinoma; UC: Undifferentiated carcinoma; *modified from Kondo T et al., Nikiforov Y et al., Soares P et al., Omur O et al., Freitas BC et al. and Kakudo K et al.

thyroid carcinomas of follicular cell origin are non-hereditary (sporadic), however a 3% to 10% risk of well-differentiated thyroid carcinoma (WDC) has been documented in first-degree relatives of patients with WDC (17-22). WDC can be a minor component of several known hereditary tumor syndromes, such as Cowden syndrome (*PTEN* gene), familial adenomatous polyposis (*APC* gene), Carney complex (*PRKAR1α* gene) and Werner syndrome (*WRN* gene) (7, 13, 19, 21, 22). Inactivation of the *PTEN* gene through methylation has recently been found to be a common molecular event in follicular neoplasms (23-27). However, these responsible gene mutations are rare in sporadic PTC and follicular thyroid carcinoma (FTC) (28). In addition to those hereditary cancer syndromes, several susceptible gene loci have been identified in familial non-medullary thyroid carcinomas of non-syndromic type. Chromosomal locations identified in patients with familial thyroid carcinomas are 1q21, 2q21, 14q, and 19p13.2 (Table 1) (19, 21). However, majority of the sporadic thyroid carcinomas do not harbor allelic losses and DNA copy number changes at these loci (29-35).

2. Genetic Alterations in Sporadic Thyroid Neoplasms

PTC and FTC are WDC of follicular cell origin and constitute more than 90% of all types of thyroid malignancy. Although they are derived from the same follicular cells, they have different morphological features, biological behaviors and genetic alterations (7-14, 21, 33, 36). Four different gene mutations have been identified in PTC and FTC, including *BRAF* and *RAS* point mutations, and *RET/PTC* and *PAX8/PPARγ* rearrangements. Poorly differentiated carcinoma (PDC) and undifferentiated carcinoma (UC) may occur either *de novo* or as a progression from WDC, and the minor components of PTC or FTC in PDC and UC are accepted as evidence of progression from WDC to PDC or UC (7, 11, 12, 16, 21, 37). Overexpression of cyclin D1, decreased expression of p27, and inactivation of *PTEN*, *CTNNB1* (β-catenin) and *TP53* genes have been reported to be responsible for the progression from WDC to PDC or UC (8, 11, 16, 24, 37-45) (Fig. 1).

a. Molecular Genetics of Follicular Neoplasms (Follicular Tumors)

Follicular neoplasms are a group of thyroid tumors that display a follicular growth pattern, and include follicular adenoma (FA), FTC and follicular variant of PTC. Gain-of-function mutations of *RAS* genes (*NRAS*, *KRAS* and *HRAS*) have been reported in all types of follicular neoplasm at various frequencies (46-55). Their prevalence is higher in FTC than in PTC, and in FTC than in FA (Table 2) (46-55). Some investigators concluded that *RAS* mutations may be important genetic alterations in the initiation of thyroid follicular cell carcinogenesis because these mutations are present in all types of follicular neoplasm including FA, FTC, PDC and UC (47), while others concluded that *N-RAS* codon 61 mutation in FTC causes tumor progression and could be an important predictive factor for distant metastasis and a higher risk of anaplastic transformation (49, 50, 52, 54, 55). The peroxisome proliferator-activated receptor-gamma (*PPARγ*) is a transcription factor essential for thyroid gland development, and the paired-box gene 8 (*PAX8*) is a member of the steroid/thyroid nuclear receptor family, and their fusion gene (*PAX8/PPARγ* rearrangement) has been found in FA and FTC, but not in PTC, PDC and UC (9, 56-58) (Table 2). The two most common mutations in FTCs, *PAX8/PPARγ* rearrangements and *RAS* point mutations, are seen in a mutually exclusive manner, which indicates that FTC could be initiated by two different mechanisms: either *RAS* point mutations or *PAX8/PPARγ* rearrangements (9, 51).

b. Molecular Genetics of Papillary Thyroid Carcinoma

PTC is the most common histological type of thyroid malignancy globally and accounts for approximately 88% of all thyroid carcinomas in Japan (21, 36, 59). The most common genetic alterations such as in *BRAF* and *RAS* genes found in PTC are gain-of-function mutations that activate the mitogen-activated protein kinase (MAPK) pathway, which is responsible for thyroid tumorigenesis. The hotspot mutation, *BRAF(V600E)*, is frequently detected in PTC (36-69%), in contrast to its absence in follicular neoplasms (benign FAs and malignant FTCs) (60-66) (Table 2). *BRAF(V600E)* mutation has been reported to be highly prevalent in PTC with a papillary and mixed papillary and follicular growth pattern, and *BRAF(K601E)* mutation is restricted to the follicular variant of PTC, suggesting possible genotype-phenotype correlations (62, 67). High frequency of *BRAF(V600E)* mutation has been reported in PTCs from early to late advanced stages and Ugolini et al. reported that *BRAF(V600E)* mutation was found in 17.6% of incidentally identified micro-PTCs and 38.3% of clinically identified micro-PTCs, indicating that this mutation is an early event in PTC tumorigenesis (14, 68-71). While *BRAF* mutations are found in up to 13% of PDC and 35% of UC, it is concluded that *BRAF* mutation-positive PTC may be more prone to PDC or UC transformation than PTC without *BRAF* mutation (66, 67-74). As *BRAF* mutation is highly prevalent (95%) in metastatic tumors from radioactive iodine-refractory PTC, these studies concluded that this mutation has prognostic implications (69, 71-75). However, conflicting data have been reported by some other investigators and *BRAF(V600E)* mutation has been suggested as a secondary subclonal change rather than a primary event in thyroid carcinogenesis (76-78).

The *RET* proto-oncogene is located on chromosome 10q11.2 and encodes a cell membrane receptor tyrosine kinase. *RET/PTC* rearrangements are reported in sporadic PTC and postulated to be important genetic alterations in thyroid tumorigenesis (79-84). More than 10 *RET/PTC* chimeric rearrangements have been described in sporadic PTC (7, 10, 13, 16, 84). The reported frequency in PTC is widely distributed from 10% to 40% in adult patients (7-16, 84). A higher frequency (60%-70%) of *RET* rearrangements, particularly *RET/PTC3*, has been reported in pediatric patients with radiation history (84). A relatively high incidence of *RET/PTC* rearrangements has also been reported in early-stage small PTCs. These findings suggest that this genetic alteration occurs as an early event in PTC tumorigenesis (82, 83). The absence of *RET/PTC* rearrangements in most PDC and UC also suggests that *RET/PTC* rearrangements have a minor role in tumor progression, which means a lower risk of anaplastic transformation in PTC with *RET/PTC* rearrangements (7, 17, 65, 67, 68).

RET/PTC rearrangement, and *RAS* and *BRAF* mutations collectively account for 60%-70% of PTC and appear to be mutually exclusive. Kimura et al. emphasized the pivotal role of this kinase cascade in PTC development (65). However, Nakazawa et al. suggested that the *RET/PTC* rearrangement in PTC is not a driver mutation because the PET/PTC signal was found in only a minor population of tumor cells using *in situ* hybridization on cytological smear samples (86). Furthermore, *RET/PTC* rearrangement was found in benign thyroid nodule and their exact roles in thyroid carcinogenesis become unclear (87-89).

TRK rearrangements involve another receptor tyrosine kinase gene, *NTRK1* (1q22), and these rearrangements have been reported in up to 10%-15% of PTCs (90-93). Musholt et al. reported 12.6% of *TRK* rearrangements in 199 PTCs and the PTC with *TRK* rearrangement is associated with a higher (60%) local recurrence and a higher (27%) tumor-related mortality rate than in the PTC without *TRK* rearrangements (93).

c. Molecular Genetics of Poorly Differentiated Carcinoma and Undifferentiated Carcinoma

UC is a rare and highly aggressive thyroid carcinoma, with a mean survival of less than 6 months after diagnosis and PDC is an aggressive thyroid carcinoma with its morphological and behavioral features between WDC and UC, and often shows anaplastic transformation (7, 8, 11, 12, 16, 21, 36, 44, 94, 95). In the multistep carcinogenesis theory, UC is postulated to be derived from preexisting low-grade WDCs through the gain of additional mutations, such as loss of *TP*53 and further loss of tumor suppressors (7, 8, 11, 12, 16, 21, 36, 44, 68, 94-97). Soares et al. screened for and found *BRAF* mutations in 6/17 (35%) cases of UC and 1/3 UC-derived cell line, but none in insular-type PDC (68). They concluded that UC may progress from *BRAF(V600E)* mutated PTC and insular-type PDCs are more closely related to FTC than PTC (11, 68). There are many studies analyzing the genetic alterations and molecular profiles of UC, the end step in the thyroid multistep carcinogenesis; many of them conclude that *BRAF*-mutated UCs are often associated with *BRAF*-mutated PTC as a precursor lesion and progression from PTC to UC could be favored by further *TP53* mutation and SOX2 expression, entering the final stage of progression of UC (7, 11-16, 41, 66, 69, 96). The *TP53* tumor suppressor gene is located on chromosome 17p13 and encodes a nuclear transcription factor related to the cell cycle, DNA repair and apoptosis. *TP53* point mutations are almost exclusively found in PDC and UC (17%-38% of PDC and 60%-88% of UC) and are regarded as a late event in the multistep carcinogenesis theory in contrast to that they are observed in the early phase of colorectal carcinogenesis (Table 2) (4-6, 11-13, 16, 37-45). Its deadly biological behavior and high proliferation rate (Ki-67 labeling index more than 30%), as well as genetic instability and marked aneuploidy, are attributed to accumulation of these genetic and epigenetic alterations (7, 98-100).

β-catenin is a cytoplasmic protein encoded by the *CTNNB1* gene located on chromosome 3p22-3p21.3. Point mutations in the *CTNNB1* gene have been reported in 25% of PDC and 66% of UC (Table 2), but none in WDCs (PTC and FTC) (103). Constitutive activation of the Wnt-signaling pathway by mutated *β-catenin* may play an important role in the progression from WDCs to PDC and UC (11, 103-106).

Murugan AK and Xing M reported two novel gain-of-function mutations of the *ALK* gene in 11% of UCs but no mutations in WDCs (107). Combination of *PI3K/Akt* genetic alterations (*PTEN, RAS, ALK*) with a *BRAF* mutation has been shown to be present in more aggressive thyroid tumors such as UC, and genetic or epigenetic alterations that activate both MAPK and PI3K/Akt pathways have been shown to be present in most cases of UC (42, 75, 108, 109). Other genes and pathways important for the development of PDC and UC may include the *LRP1B* gene located at 2q21, a susceptibility locus for familial non-medullary thyroid carcinomas (110). Hypermethylation of many tumor suppressor genes and multiple allelic losses, which may be linked to the inactivation of tumor suppressor genes, have been reported in thyroid carcinomas with poor outcome (30-32, 108, 110-121).

3. Molecular Changes in the Progression of Thyroid Carcinomas

The progression of thyroid carcinomas after initiation and promotion involves complex genetic and epigenetic alterations and mutations, and the epigenetic alterations in PDC and UC are far from being completely clarified (110-119). Growth factors (FGF, FGFR, MET, EGFR and VGEF) involved in the PI3K-Akt-mTOR pathway have been reported to have an important role in tumor development and progression in thyroid follicular cell carcinogenesis (15, 27, 33, 42, 109, 119-127). Liu et al. found copy number gains in *EGFR, PDGFR* (*α* and β), *VEGFR1, VGEFR2, KIT, MET, PIK3* (*a* and *b*) and *PDK1* genes, which may play important roles in the tumor progression to UC (42). Cell cycle regulators such as retinoblastoma (Rb), p21, p27 and TP53 also play important roles in the progression of thyroid carcinogenesis (49, 100, 126, 127). Adhesion molecules (β-catenin and E-cadherin) induce signal transduction through the Wnt signal pathway. Decreased E-cadherin expression is often found in UC, which activates β-catenin transcription activity by increasing the pool of β-catenin to migrate to the nucleus. Fibronectin is another adhesion molecule involved in the progression of thyroid carcinoma and the up-regulation of fibronectin has been demonstrated in thyroid carcinomas (33, 128).

4. Thyroid Carcinomas with Metastasis

Metastatic disease is the most common and important immediate cause of death in patients with thyroid carcinomas, either WDC or UC (21, 36, 44, 94, 126, 127). From the multistep carcinogenesis theory, FTC with metastasis should have some additional genetic alterations compared with those without metastasis, and this should also be the case for PTC. These genetic events in either PTC or FTC are far from being completely clarified. *N-RAS* codon 61 mutation has been reported to be an

important predictive genetic alteration for distant metastasis in PTC (128) and FTC (49, 50, 54, 55). However, these genetic changes are observed in cases without invasion or metastasis, and the genetic alterations that specifically linked to invasion and metastasis have not been fully elucidated in thyroid carcinomas. Many studies have postulated that accumulation of the genetic and epigenetic alterations rather than a single genetic event are responsible for tumor progression from non-invasive to invasive or from non-metastasizing to metastasizing (15). A common hypermethylation of *hMLH1* (DNA repair gene) has been reported to be associated with lymph node metastasis in PTC with *BRAF* mutation (113). *BRAF(V600E)* mutation has been reported to promote the progression and aggressiveness of PTC by down-regulation of tumor suppressor genes and up-regulation of cancer-promoting molecules (VEGF, matrix metalloproteinases, nuclear transcription factor kappa B and c-met) (112). VEGF-D has been reported to play an important role in lymph node metastasis *via* lymphangiogenesis in PTC, and Nakamura Y et al. showed that up-regulation of VEGF-D expression by nitric oxide in a cultured PTC cell line and the high level of nitrotyrosine (a biomarker for peroxynitrate formation from nitric oxide) immune expression was significantly correlated with lymph node metastasis (121, 129). Zuo M et al. showed that *S100A4* was one of the overexpressed genes in a human thyroid carcinoma clone with a high incidence of lung metastasis using microarray analysis of gene expression profiling in their animal transplantation model (130). Zuo M et al. further reported a significantly higher level of S100A4 transcript in metastatic tumors than in the primary tumors (131). There are several genes (*Nm23-H1*, *KiSS-1*, *RCAN1* and *KAI1*) that act as suppressors in tumor metastasis, and Phay JE et al. concluded that the loss of expression or function of such metastasis suppressor genes may play an important role in the development of metastasis (15). Since distant metastasis is one of the most important prognostic factors in thyroid carcinomas, identification of those genetic and epigenetic events could reveal new molecular targets for treating advanced thyroid carcinomas (126, 127).

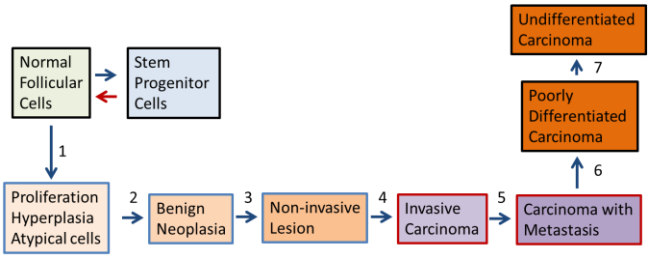

Figure 1: Thyroid neoplasia and multistep carcinogenesis theory.
PTC lineage: 1: unknown, 2: unknown, 3: *RET* rearrangement or *BRAF* mutation, 4: unknown, 5: cyclin D1 overexpression and p27 down regulation, 6: *TP53*, *PTEN* and *CTNNB1* mutation, 7: *TP53*, *PTEN*, *ALK*, *PIK3CA*, *LRP1B* and *CTNNB1* mutation.
FTC lineage: 1: unknown, 2: *RAS* mutation or *PPARG* rearrangement, 3: RAS mutation or PPARG rearrangement, 4: RAS mutation or *PPARG* rearrangement, 5: *N-RAS* codon 61 mutation, 6: *TP53*, *PTEN* and *CTNNB1* mutation, 7: *TP53*, *PTEN*, *ALK*, *PIK3CA*, *LRP1B* and *CTNNB1* mutation.

5. Multistep Carcinogenesis Theory

A simplified model of the multistep carcinogenesis theory is shown in Figure 1 and the known genetic changes of thyroid carcinomas discussed in the present review are incorporated into the figure legends (Fig. 1). Thyroid tumors develop as a result of the sequential accumulation of genetic and epigenetic alterations involved in the control of cell proliferation, cell differentiation or cell death, according to the multistep carcinogenesis theory. Additional epigenetic alterations also superimpose on those genetic changes in this model (15, 16, 109-117). One of the difficulties in this model is the lack of a clear cut separation of all histological types of thyroid carcinomas into corresponding tumor stages (stages 0, I, II, III and IV, or early non-invasive neoplasm, invasive carcinoma confined to thyroid gland, invasive carcinoma with local metastasis and invasive carcinoma with distant metastasis). This is because the age of patients is an important factor in the staging of thyroid carcinomas in most clinical guidelines and all patients younger than 45 years old are classified into stage I regardless of invasion and metastasis, and all UC patients are classified into stage IV even without invasion and metastasis (21, 36, 132). As a result, most of the literature on thyroid carcinogenesis handled thyroid carcinomas in histologic groups, such as PTC group, FTC group, PDC group and UC group, rather than the stage of tumors. Therefore, in this review, multistep theory and related genetic alterations are explained separately in the PTC lineage and the FTC lineage in the figure legends (Fig. 1). Other problems for understanding thyroid carcinogenesis are 1) so-called mutation-negative thyroid carcinomas (about one-third of PTC and FTC could not be explained by known genetic mutations) and 2) *TP53* mutation is restricted in PDC and UC as a late genetic event in thyroid carcinogenesis, in contrast to the *TP53* mutation in colorectal cancer (it occurs at an early phase of progression from adenoma to carcinoma in colorectal carcinoma) (4-6, 11). There are still many unanswered questions in this multistep theory when it is applied to thyroid follicular cell carcinogenesis.

6. A Missing Link in Multistep Carcinogenesis

There are several points that do not fit the multistep carcinogenesis theory in thyroid follicular cell neoplasms, and they are discussed in detail by Takano et al. elsewhere in this special issue of the Journal of Basic & Clinical Medicine (3). Another problem in explaining thyroid neoplasms using the multistep carcinogenesis model is a lack of known precursor lesions of thyroid carcinomas between normal follicular cells and early WDCs, a missing link in the multistep carcinogenesis of thyroid follicular cell carcinogenesis. Although FA is described as a candidate precursor lesion for thyroid carcinomas in many reviews and textbooks, prophylactic surgery is not recommended for patients with FA owing to infrequent progression from benign FA to malignant WDC (7, 8, 10, 13-16, 21, 51, 132-137). Furthermore, from many studies on how to separate malignant lesions (FTC and PTC) from FA using genetic markers, they act as if they are distinct lesions rather than a lesion with genetic continuity (134-143). In the PTC chapter of WHO blue book, it is explained that there is no known precursor lesion for PTC, and in the FTC chapter, it is stated that no definite precursor lesions of FTC are known (36). The possible precursor lesions of thyroid carcinomas, such as small papillary carcinoma and borderline lesions between normal follicular cells and WDCs including FA have been reviewed elsewhere (144-146).

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