



Donna Morrison. *Great Blues*. Pastel on clayboard, 24" × 28".

Ongoing research to increase our understanding of the mechanisms involved in thyroid tumor progression will lead to improved treatment of patients with this disease.

Genetic Considerations in Thyroid Cancer

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Background: Recent molecular studies have described a number of abnormalities associated with the progression and dedifferentiation of thyroid carcinoma. These distinct molecular events are often associated with specific stages of tumor development. A better understanding of the mechanisms involved in thyroid cancer pathogenesis may help to translate these discoveries toward improvements in patient care.

Methods: We reviewed the literature on the molecular pathogenesis of thyroid cancer and compared clinical, histopathologic, and genetic features important in defining the disease process.

Results: The progression of thyroid cancer from well-differentiated to poorly differentiated and undifferentiated carcinoma represents a biological continuum. Specific genetic events serve as early initiating and late triggering events. Poorly differentiated thyroid carcinomas occupy an intermediate position in this progression model.

Conclusions: With sophisticated genetic tools generating a wealth of information, we have gained better insight into the mechanisms driving thyroid tumor progression. Recognition of these features is crucial to the management of patients with thyroid cancer. Novel treatments are being designed based on our enhanced understanding of this disease process.

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Abbreviations used in this paper: WDTC = well-differentiated thyroid carcinoma, ATC = anaplastic thyroid carcinoma, PDTC = poorly differentiated thyroid carcinoma, PTC = papillary thyroid carcinoma.

Introduction

Thyroid cancer is the most common endocrine malignancy and accounts for the majority of endocrine cancer-related deaths each year.^{1,2} Accumulating evidence indicates that follicular cell-derived thyroid carcinomas constitute a biological continuum progressing from the highly curable well-differentiated thyroid carcinoma (WDTC) to the often fatal undifferentiated or anaplastic thyroid carcinoma (ATC).^{3,4} Poorly differentiated thyroid carcinoma (PDTC) and aggressive variants of WDTC, such as tall cell and columnar cell, frequently serve as intermediates in this progression model.^{5,6}

Medullary thyroid cancer arises from the parafollicular or C cells and is not of follicular cell origin. This review focuses on thyroid carcinomas of follicular cell origin.

Clinical, epidemiologic, and pathologic evidence supports the concept of stepwise progression and dedifferentiation.⁷ For example, the gradual loss of papillary and follicular growth patterns and the simultaneous increase in a solid growth pattern, with increased mitoses, necrosis, and nuclear pleomorphism, are often observed in aggressive thyroid carcinomas.⁸ A majority of these tumors exhibit residual foci of differentiated thyroid carcinoma.

The true incidence of histologic dedifferentiation is difficult to assess. Metastatic or recurrent thyroid cancer is often not surgically managed; therefore, the functional and histologic dedifferentiation of a well-differentiated, "low risk" neoplasm evolving into a "high-risk" and potentially lethal malignancy is probably not reported accurately. Furthermore, this transformation can evolve over years or decades, making patient follow-up difficult.

Although observations strongly support a progression concept, little is known regarding the possible mechanisms underlying this process. Since aggressive carcinomas, such as PDTC and ATC, result in significant thyroid cancer-related morbidity and mortality, it is important to identify and appreciate the molecular factors that may play a role in driving the dedifferentiation of WDTC. These factors affecting tumor proliferation, cellular immortalization, and death may serve as potential therapeutic targets. During the past decade we have witnessed an explosion of genetic information, and a large body of information has been generated on the molecular alterations involved in thyroid carcinomas of follicular origin. This review introduces some of the genetic abnormalities that have been identified and also attempts to develop a framework for understanding how they contribute to the transformed phenotype.

RET/PTC Rearrangements

The *RET* (rearranged during transfection) protooncogene is a 21-exon gene located on the proximal long arm of chromosome 10 that encodes a tyrosine kinase receptor. It is involved in the regulation of growth, survival, differentiation, and migration of cells of neural crest origin. It is not normally expressed in the follicular cell.⁹ Rearrangements of the *RET* gene, known as *RET/PTC* rearrangements, occur in papillary thyroid carcinoma (PTC). The unique spatial proximity of translocation-prone gene loci, which may be preferentially occurring in thyrocytes in their mitotic interphase, favors *RET* gene rearrangements.^{10,11} This may help explain why *RET* rearrangements are specific for thyroid tumors.^{12,13} Although more than 10 rearrangements have been

Table 1. — Summary of the Common *RET/PTC* Rearrangements and Their Resulting Chimeric Products

<i>RET/PTC</i> Rearrangements	Chimeric Product
<i>RET/PTC1</i>	10q paracentric inversion; H4 (ubiquitously expressed gene of unknown origin)
<i>RET/PTC2</i>	t(10;17)(q11.2;q23); regulatory subunit of cAMP-dependent protein kinase A
<i>RET/PTC3</i>	intrachromosomal rearrangements; ELET, a gene of unknown function

described, *RET/PTC1*, *RET/PTC2*, and *RET/PTC3* account for most of the rearrangements found in PTC.^{14,15} In each of these rearrangements, the upstream (5') component of a "housekeeping" (or ubiquitously expressed) gene drives the expression of the tyrosine kinase domain of *RET* (Table 1). Expression of the *RET/PTC* chimeric proteins is facilitated by the heterologous promoters provided by the fused genes and results in constitutive, ligand-independent activation of *RET* receptor tyrosine kinase in papillary cancer cells.¹⁶⁻¹⁸

In the adult population, the *RET* rearrangements have been found in 2.6% to 34% of PTC.¹⁹⁻²⁸ However, in the pediatric population, *RET* rearrangements — specifically, *RET/PTC1* and *RET/PTC3* — have been found in up to 80% of the cases.^{29,30} Initial studies showed that this was especially true in cancers from children exposed to radiation after the Chernobyl nuclear accident or to external irradiation for treatment of benign diseases of the head and neck.^{23,31-33} Recent reviews show that the *RET/PTC* rearrangements occur commonly in pediatric PTC regardless of radiation history. It is therefore probably an event associated with young age, although young age is particularly susceptible to development of PTC after radiation exposure.³⁴

There is evidence to support the belief that *RET/PTC* rearrangements represent early genetic changes leading to the development of PTC.^{27,35} Several studies have shown that *RET/PTC* rearrangements are associated with PTC that lacks evidence of progression to PDTC or ATC.^{12,36} A recent study from Santoro et al³⁷ showed that less than 10% of PDTCs were positive for *RET/PTC* rearrangements. They concluded that PTCs with *RET/PTC* rearrangements have a relatively low potential for progression to PDTC or ATC.

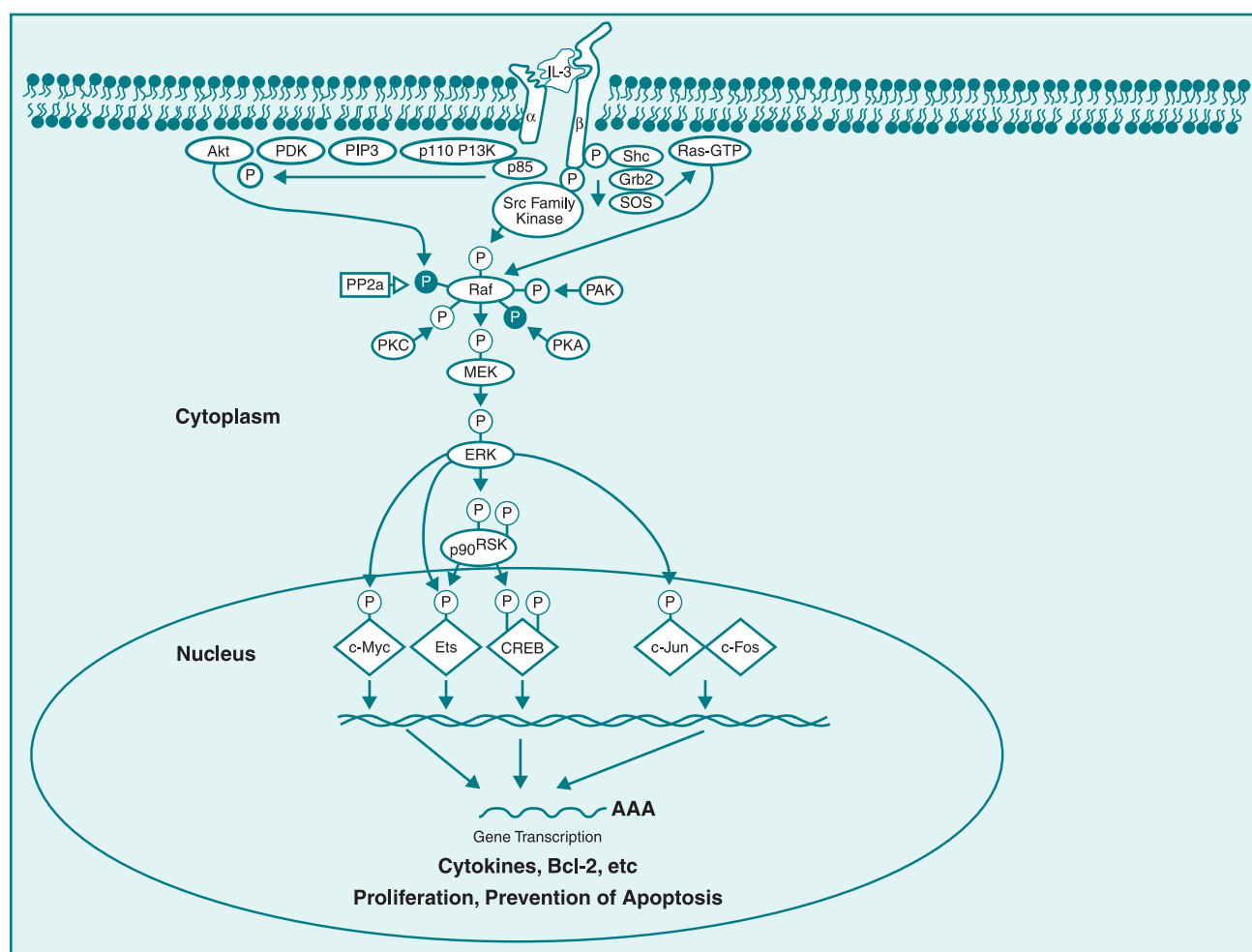
The recent success in the treatment of chronic myelogenous leukemia with imatinib mesylate, an inhibitor of constitutively activated ABL kinase, has generated considerable interest in developing therapeutic protein kinase inhibitors. Recently, compounds have been identified that exhibit significant inhibitory activity on *RET* kinase.³⁸ These new class of drugs may prove to be clinically beneficial for patients with *RET*-induced thyroid carcinomas.

RAS Mutations

Three *RAS* genes, *H-RAS*, *K-RAS*, and *N-RAS*, synthesize a family of 21-kDa proteins that play an important role in tumorigenesis.³⁹ The *RAS* proteins exist in two different forms: an inactive form that is bound to guanine diphosphate (GDP) and an active form that exhibits guanosine triphosphatase (GTPase) activity. Their function is to convey signals originating from tyrosine kinase membrane receptors to a cascade of mitogen-activated protein kinases (MAPK). This activates the transcription of target genes involved in cell proliferation, survival, and apoptosis.³⁹ Oncogenic *RAS* activation results from point mutations, affecting the GTP-binding domain (codons 12 or 13) in exon 1 or the GTPase domain (codon 61) in exon 2, which fix the protein in the activated state and thus resulting in chronic stimulation of downstream targets, genomic instability, additional mutations, and malignant transformation.⁴⁰

The *RAS* mutations are among the most common mutations found in transformed cells. Mutations in all

three cellular *RAS* genes have been identified in benign and malignant thyroid tumors. They seem to be common in follicular carcinoma, PDTC, and ATC and occur less frequently in PTC.⁴¹⁻⁴⁷ The role of oncogenic *RAS* in thyroid tumor progression is unclear. Some studies have shown a similar prevalence of *RAS* mutations in benign and malignant thyroid neoplasms, suggesting that *RAS* activation may represent an early event.^{41,48} Other studies have shown that *RAS* mutations, specifically mutations at codon 61 of *N-RAS*, are involved with tumor progression and aggressive clinical behavior.^{47,49-54} Transgenic mice with thyroid-specific mutant *RAS* expression develop thyroid hyperplasia and carcinoma.⁵⁵ A recent study by Garcia-Rostan et al⁵⁴ demonstrated that the presence of *RAS* mutations predicted a poor outcome for WDTC independent of tumor stage. Furthermore, they found that PDTC and ATC often harbor multiple *RAS* mutations. These mutations probably represent an intermediate event in the progression of thyroid carcinoma.



Overview of the Ras/Raf/MEK/ERK pathway. This illustrates how the Raf/MEK/ERK pathway is regulated by Ras as well as other kinases. From Chang F, Steelman LS, Lee JT, Shelton JG, Navolanic PM, Blalock WL, Franklin RA, McCubrey JA. Signal transduction mediated by the Ras/Raf/MEK/ERK pathway from cytokine receptors to transcription factors: potential targeting for therapeutic intervention. *Leukemia*. 2003;17:1263-1293. Reprinted with permission of Nature Publishing Group. www.nature.com/

BRAF Mutations

The most recent and major development in the field of thyroid cancer genetics has been the identification of the BRAF-activating point mutation as the most common molecular defect in PTC.³⁴ There are three isoforms of the serine-threonine kinase RAF in mammalian cells: ARAF, BRAF, and CRAF or RAF-1. CRAF is expressed ubiquitously, whereas BRAF is expressed predominantly in hematopoietic cells, neurons, and testes.⁵⁶ BRAF is also the predominant isoform in thyroid follicular cells.³⁴

As with *RET/PTC* rearrangements and *RAS* mutations, most of the genetic alterations in thyroid cancer exert their oncogenic effect at least partially through the activation of the MAPK pathway. The RAF isoforms activate the mitogen-activated protein (MAP) kinase/extracellular signal-regulated kinase (ERK) kinase (MEK) cascade (Figure).⁵⁷ This is a key component in the MAPK pathway, which activates the transcription of target genes involved in cell proliferation, survival, and apoptosis (Table 2).⁵⁷ When constitutively activated, the MAPK pathway leads to tumorigenesis.⁵⁸ Among the three forms of RAF kinases, BRAF, with its gene located on chromosome 7, is the most potent activator of the MAPK pathway.^{59,60}

The BRAF-activating point mutation in thyroid cancer is almost exclusively a thymine-to-adenine transversion at position 1799 (T1799A) in exon 15. This leads to a valine-to-glutamate substitution at residue 600 (V600E) and subsequent constitutive activation of the BRAF kinase.^{61,62} The initial discovery of *BRAF* mutations indicated a high prevalence of this event in malignant melanoma, colorectal carcinoma, and ovarian carcinoma.⁶² Recent studies have reported a prevalence of *BRAF* mutation in 29% to 83% of PTC, making it the most common oncogene identified in sporadic forms of PTC.^{34,63-71}

In all the studies published to date, *BRAF* mutation (V600E) has been found only in PTC, PDTC and ATC.³⁴ It is not seen in follicular carcinoma or benign thyroid neoplasms. Some studies have shown *BRAF* mutation in follicular adenomas and follicular variant of PTC; however, the mutation is not V600E.^{64,72,73} The high frequency and specificity of *BRAF* mutation suggest that this mutation may play a fundamental role in the initiation of PTC tumorigenesis. PTCs with *BRAF* mutation have distinct phenotypic and biologic properties. They seem to behave more aggressively and carry a poorer prognosis.⁷⁰ The tall cell variant, an aggressive variant of PTC, usually harbors the *BRAF* mutation.^{34,66,74} PTCs with *BRAF* mutation present more commonly at an advanced stage, usually with extrathyroidal extension. Some authors suggest that this may reflect the age of the patient and not the presence of a *BRAF* mutation.⁷³ However, others have shown that PTC with *BRAF* mutation displays an increased inci-

Table 2. — Growth-Related Target Genes of *Raf*-Induced Transcription Factors

Transcription Factor	Target Genes
NF-κB	IL-2 IL-6 TNFα TNFβ GM-CSF PDGF VEGF <i>c-myc</i> IκBα JunB Cyclin D ₁ p53 Bcl-X _L FasL
CREB	Cyclin A Cyclin D ₁ c-Fos Bcl-2
AP-1	GM-CSF IL-2 IL-3 IL-4 IL-5 TNFα p16 ^{Ink4a} p19 ^{ARF} p53 Cyclin D ₁ Cyclin A FasL
Ets	p53 c-Fos NF-κB Cyclin D ₁ Rb p21 ^{Cip1} Bcl-2 Bcl-X _L Fas GM-CSF IL-3 PDGF HB-EGF
c-Myc	p19 ^{ARF} Cdk1 Cdk4 Cyclin A Cyclin D ₁ Cyclin D ₂ Cyclin E p53 TRAP1 p27 ^{Kip1} p21 ^{Cip1} p15 ^{Ink4b} cdc25 PDGF-βR

From Chang F, Steelman LS, Lee JT, Shelton JG, Navolanic PM, Blalock WL, Franklin RA, McCubrey JA. Signal transduction mediated by the Ras/Raf/MEK/ERK pathway from cytokine receptors to transcription factors: potential targeting for therapeutic intervention. *Leukemia*. 2003;17:1263-1293. Reprinted with permission of Nature Publishing Group. www.nature.com/

dence of locoregional recurrence and a decreased response of the recurrent tumor to radioactive iodine.^{66,68,75,76} The *BRAF* mutation has also been found in approximately 15% of PDTC and ATC.^{66,68} The *BRAF* mutation-positive ATC is likely derived from *BRAF* mutation-positive PTC, as suggested by the coexistence of PTC and ATC in the same tumor, both of which harbored the *BRAF* mutation.^{34,66,70,77,78} Further studies have shown that a subset of papillary microcarcinomas harbor the *BRAF* mutation, indicating that this oncogene may be activated during tumor initiation.⁶⁶ These data suggest that *BRAF* mutation may be a tumor-initiating early event in PTC and thus is associated with tumor dedifferentiation.

An elegant study by Knauf et al⁷⁹ provides the most convincing evidence to support a role of *BRAF* mutation in the initiation and progression of PTC. They showed that transgenic mice with thyroid specific expression of mutated *BRAF* developed PTC that transitioned to PDTC. These findings provide molecular evidence for the stepwise progression of PTC to PDTC and ATC.

PAX8-PPAR γ Rearrangement

The *PAX8* gene encodes a transcription factor essential for the genesis of thyroid follicular cell lineages and regulation of thyroid specific gene expression. The peroxisome proliferator-activated receptor γ (PPAR γ) is a member of the nuclear hormone receptor superfamily that includes thyroid hormone, retinoic acid, and androgen and estrogen receptors.⁸⁰ The *PAX8-PPAR γ* rearrangement leads to in-frame fusion of exon 7, 8, or 9 of *PAX8* on 2q13 with exon 1 of *PPAR γ* on 3p25.⁸¹ The exact mechanism by which this rearrangement imparts a carcinogenic phenotype is not fully understood. It appears as though the *PAX8-PPAR γ* chimeric protein inactivates the wild-type PPAR γ , which is a putative tumor suppressor.^{81,82}

As with *RAS* mutations, *PAX8-PPAR γ* rearrangement has also been shown to be involved in the development of thyroid follicular carcinoma. The *PAX8-PPAR γ* rearrangement is found in follicular thyroid carcinoma and in the follicular variant of PTC, where it occurs in approximately 33% of all tumors.^{7,83-87} The rearrangement has also been shown to occur in follicular adenomas and is not specific for carcinoma.⁸³ The role of this rearrangement in the progression and dedifferentiation of follicular thyroid cancer to PDTC and ATC has not been well defined.

p53 Inactivation

The *p53* gene encodes a nuclear transcription factor that plays a central role in the regulation of cell cycle,

DNA repair, and apoptosis.⁸⁸ As the policeman of the genome, *p53* is overexpressed after cellular exposure to DNA-damaging agents and causes transient cell cycle arrest, presumably to allow for DNA repair.⁸⁹ However, if the damage is severe, it initiates apoptosis to prevent replication of the flawed cell.⁷ Cells with impaired *p53* function are likely to accumulate genetic damage and are at a selective advantage for clonal expansion. Alterations in the *p53* tumor suppressor gene by inactivating point mutations, usually involving exons 5–8, or by deletion result in progressive genome destabilization, additional mutations, and propagation of malignant clones. This represents the most frequent genetic damage in human cancer, usually occurring as a late tumorigenic event.⁸⁰

Among thyroid tumors, *p53* mutations are generally restricted to PDTC and ATC, unlike the mutations discussed above.^{90,91} Point mutations of *p53* occur in approximately 60% of ATC and in 25% of PDTC.⁹⁰⁻⁹⁶ Moreover, in tumors with both well-differentiated and anaplastic components, *p53* mutations were present only in the anaplastic component.^{90,94,97} These findings are consistent with the hypothesis that *p53* inactivation likely serves as a second hit, triggering tumor dedifferentiation and progression to PDTC and ATC.

Experimental studies have shown that loss of *p53* results in progressive dedifferentiation of thyroid tumors. Transgenic mice with thyroid-specific *RET/PTC* rearrangements developed PTC, but when crossed with *p53* $-/-$ mice, the progeny succumbed to rapidly growing PDTC and ATC.^{98,99} Conversely, the recovery of wild-type *p53* in cultured ATC cells resulted in the re-expression of thyroid-specific genes and the re-ability to respond to thyroid-stimulating hormone.^{100,101} It is unlikely that *p53* mutation is an initiating event in PDTC or ATC; it is likely a late event that contributes to the evolution of the transformed phenotype.

Other Molecular Factors

Beta-catenin, a cytoplasmic protein encoded by the *CTNNB1* gene, plays an important role in E-cadherin-mediated cell-cell adhesion. It is also an integral intermediate in the wingless (Wnt) signaling pathway.¹⁰² Point mutations in exon 3 of the gene stabilize the protein and make it insensitive to its degradation by the adenomatous polyposis coli (APC) multiprotein complex. This results in the accumulation of beta-catenin and the constitutive activation of target gene expression. Beta-catenin upregulates the transcriptional activity of Cyclin D1, *C-MYC*, *C-JUN*, and other genes.⁷

Point mutations in exon 3 have been reported in up to 25% of PDTC and 66% of ATC but not in WDTC.^{103,104} The data suggest that the role of beta-catenin mutations in thyroid carcinoma probably represents a late event in

the tumor progression model, likely to trigger directly the process of dedifferentiation.

Mutations of the tumor suppressor gene *PTEN* have been identified in up to 25% of sporadic follicular adenomas and carcinomas but rarely in PTC.¹⁰⁵⁻¹⁰⁷ *PTEN* has been shown to negatively regulate the PI3-kinase/AKT pathway. Germline *PTEN* gene mutations have been identified in patients with Cowden syndrome, which is an autosomal dominant condition characterized by multiple hamartomas of skin, intestines, breast, and thyroid. *PTEN* +/- transgenic mice have been shown to develop thyroid tumors.¹⁰⁸

Gene expression profiling is offering additional evidence for support of the thyroid carcinoma progression model. If WDTC, PDTC, and ATC are linked in a progressive relationship, then a sequential increase in genomic complexity is anticipated from WDTC to ATC. Direct genetic comparison of WDTC, PDTC, and ATC may help decipher molecular factors associated with thyroid tumor progression. Using comparative genomic hybridization (CGH), our laboratory has previously shown that the chromosomal complexity sequentially increases and that the chromosomal abnormalities can be grouped into three categories — early, intermediate, and late — as tumors progress from WDTC to PDTC to ATC.¹⁰⁹ More recently, using cDNA microarray and CGH technology, we identified *MUC1*, a cell surface glycoprotein, as an independent prognostic marker for PTC.¹¹⁰ Experimental studies have validated the role of *MUC1* in promoting an aggressive phenotype in PTC.¹¹¹ Current investigations include defining the role of *MUC1* as a therapeutic target for aggressive PTC.

Conclusions

No absolute proof exists to show that the transition from benign to premalignant, malignant, and finally invasive and metastatic thyroid cancer occurs in a predictable fashion. The presence of intermediate phenotypes is consistent with this concept. Multiple molecular abnormalities have been described in association with the progression of normal follicular thyroid cells to benign adenomas, well-differentiated thyroid tumors, poorly differentiated thyroid tumors, and ultimately ATC. However, the progression is not necessarily linear. This is exactly where genomic and proteomic studies can help identify those WDTCs that may have already acquired the necessary genetic changes to express a highly malignant phenotype. Some genetic changes, such as *BRAF* mutation, may represent early or initiating events, whereas others, such as *p53* mutation or loss, probably represent late events and serve to dedifferentiate tumors to PDTC and ATC. Furthermore, molecular factors can be specific to a certain tumor subtype. For example, *RAS* mutations are more common in follicular carcinomas.

Identification of new molecular factors involved in the progression of thyroid cancer may potentially serve as prognostic markers or therapeutic targets. With the advent of large-scale, high throughput genomic screening, an abundance of data will be collected that may provide a better understanding of the pathogenesis of thyroid carcinoma.

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