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Molecular pathogenesis and mechanisms of thyroid cancer

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Abstract

Thyroid cancer is a common endocrine malignancy. There has been exciting progress in understanding its molecular pathogenesis in recent years, as best exemplified by the elucidation of the fundamental role of several major signalling pathways and related molecular derangements. Central to these mechanisms are the genetic and epigenetic alterations in these pathways, such as mutation, gene copy-number gain and aberrant gene methylation. Many of these molecular alterations represent novel diagnostic and prognostic molecular markers and therapeutic targets for thyroid cancer, which provide unprecedented opportunities for further research and clinical development of novel treatment strategies for this cancer.

Thyroid cancer is a common endocrine malignancy that has rapidly increased in global incidence in recent decades[1,2]. In the United States, the average annual increase in thyroid cancer incidence of 6.6% between 2000 and 2009 is the highest among all cancers[2]. Although the death rate of thyroid cancer is relatively low, the rate of disease recurrence or persistence is high, which is associated with increased incurability and patient morbidity and mortality[3].

There are several histological types and subtypes of thyroid cancer with different cellular origins, characteristics and prognoses[4] (TABLE 1). There are two types of endocrine thyroid cells — follicular thyroid cells and parafollicular C cells — from which thyroid cancers are derived. Follicular thyroid cell-derived tumours, including papillary thyroid cancer (PTC), follicular thyroid cancer (FTC), poorly differentiated thyroid cancer (PDTC) and anaplastic thyroid cancer (ATC), account for the majority of thyroid malignancies. PTC and FTC are collectively classified as differentiated thyroid cancer (DTC). Parafollicular C cell-derived medullary thyroid cancer (MTC) accounts for a small proportion of thyroid malignancies[2]. The primary molecular mechanism underlying MTC tumorigenesis is the aberrant activation of RET signalling (which is caused by *RET* mutations[5]), which are not present in follicular thyroid cell-derived tumours. The molecular pathogenesis of follicular thyroid cell-derived tumours is the focus of this Review.

Conventional surgical thyroidectomy with adjuvant ablation by radioiodine treatment has been the mainstay of treatment for follicular thyroid cell-derived cancer, but it is often not curative. The recent progress in understanding the molecular pathogenesis of thyroid cancer has shown great promise for the development of more-effective treatment strategies for thyroid cancer. This has mainly resulted from the identification of molecular alterations,

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including the genetic and epigenetic alterations of signalling pathways — such as the RAS–RAF–MEK–MAPK–ERK pathway (MAPK pathway) and the PI3K–AKT pathway — which is reshaping thyroid cancer medicine. This Review discusses the recent remarkable progress in understanding the molecular pathogenesis and mechanisms of thyroid cancer.

Common genetic alterations in thyroid cancer

Gene mutations

Numerous genetic alterations that have a fundamental role in the tumorigenesis of various thyroid tumours have been identified (TABLE 2). A prominent example is the T1799A transverse point mutation of *BRAF*, which results in the expression of BRAF-V600E mutant protein and causes constitutive activation of this serine/threonine kinase[6–11]. *BRAF*^{V600E} mutation occurs in approximately 45% of PTCs[12]. There are also a few rare types of *BRAF* mutations identified in PTC, which mostly affect nucleotides around codon 600 and constitutively activate the BRAF kinase[13,14]. The requirement for BRAF-V600E to maintain tumour growth was initially demonstrated in a xenograft tumour model[15]. A previous comprehensive multicentre study demonstrated a strong association of *BRAF*^{V600E} with poor clinicopathological outcomes of PTC, including aggressive pathological features, increased recurrence, loss of radioiodine avidity and treatment failures[16]. This was subsequently confirmed in numerous other studies, although, perhaps owing to variations in study design, some studies showed inconsistent results, as summarized and discussed in large meta-analyses that uniformly support the aggressive role of *BRAF*^{V600E} mutation in PTC[17,18]. *BRAF*^{V600E}-transgenic mice also developed aggressive PTC[19–21]. Interestingly, some human PTC tumours have recently been found to exhibit intra-tumour heterogeneity in the *BRAF* genotype — with a minority of cells harbouring *BRAF*^{V600E} and the majority harbouring wild-type *BRAF*[22]. This raises an interesting ‘chicken and egg’ puzzle of whether BRAF-V600E initiates PTC tumorigenesis or instead *BRAF*^{V600E} occurs following the development of a thyroid tumour[23]. Although it is possible that *BRAF*^{V600E} could be a secondary genetic event in PTC tumorigenesis as previously proposed[24], an alternative possibility is that once PTC is initiated by *BRAF*^{V600E}, secondary oncogenic alterations could take over to drive the tumorigenesis of PTC such that *BRAF* mutation is no longer selected for[23].

Second in prevalence to *BRAF* mutations in thyroid cancer are RAS mutations. RAS is in its active state when bound with GTP. The intrinsic GTPase of RAS hydrolyses GTP and converts RAS into an inactive GDP-bound state, thus terminating RAS signalling. RAS mutations cause the loss of its GTPase activity, thus locking RAS in a constitutively active GTP-bound state. There are three isoforms of RAS: HRAS, KRAS and NRAS, and *NRAS* is predominantly mutated in thyroid tumours, mostly involving codons 12 and 61. Although RAS is a classical dual activator of the MAPK and PI3K–AKT pathways, RAS mutations seem to preferentially activate the PI3K–AKT pathway in thyroid tumorigenesis, as suggested by the preferential association of RAS mutations with AKT phosphorylation in thyroid cancers[25,26]. The common occurrence of RAS mutations in follicular thyroid adenoma (FTA), a presumed premalignant lesion, suggests that activated RAS may have a role in early follicular thyroid cell tumorigenesis. However, additional genetic alterations other than RAS mutation are apparently required to transform FTA into thyroid cancer. This is consistent with studies in which the expression of mutant HRAS was induced in normal thyroid cells *in vitro*, which resulted in only well-demarcated and differentiated colonies with phenotypes consistent with FTA[27] and cell proliferation with normal differentiation[28]. More direct evidence has come from transgenic mouse studies in which conditional physiological expression of a KRAS mutant in the thyroid gland could not transform thyroid cells, but concurrent KRAS mutant expression and *Pten* deletion induced a rapid occurrence of aggressive FTC[29].

Mutations or deletions of the tumour suppressor gene *PTEN* are the classical genetic alterations that activate the PI3K–AKT pathway and are the genetic basis for follicular thyroid cell tumorigenesis in Cowden's syndrome[30]. Mutations of *PIK3CA*, which encodes the p110 catalytic subunit of PI3K, are also common in thyroid cancer, particularly FTC, PDTC and ATC[25,26,31–35]. As in other human cancers[36], activating *PIK3CA* mutations in thyroid cancer occur in exon 9 and exon 20. *AKT1* mutations were found in metastatic thyroid cancers in one study, and their functional relevance remains to be established[35].

Other important genes that are mutated in thyroid tumorigenesis include catenin (*CTNNB1*)[37,38], *TP53*[39,40], isocitrate dehydrogenase 1 (*IDH1*)[41,42], anaplastic lymphoma kinase (*ALK*)[43] and epidermal growth factor receptor (*EGFR*)[44]. The preferential occurrence of these mutations in PDTC and ATC, which are the most aggressive thyroid cancers, suggests that they may have a role in the progression and aggressiveness of thyroid cancer. In Hürthle-cell thyroid carcinoma (HCTC), mutations of NADH dehydrogenase (ubiquinone) 1 subcomplex 13 (*NDUFA13*; also known as *GRIM19*) are fairly common[45]. Unlike other types of thyroid cancers, HCTC does not harbour classical genetic alterations, such as *BRAF* and RAS mutations or *RET*–PTC [46,47], but commonly harbours DNA haploidization in recurrent disease[47].

Gene amplifications and copy-number gains

Oncogenic gene amplification or copy-number gain are additional prominent genetic mechanisms in thyroid tumori-genesis (TABLE 3). This is particularly the case for genes encoding receptor tyrosine kinases (RTKs)[26]. Copy-number gains are also common for the genes encoding PI3K–AKT pathway members, including *PIK3CA*, *PIK3CB*, 3-phosphoinositide-dependent protein kinase 1 (*PDPK1;* also known as *PDK1)*, *AKT1* and *AKT2* (REFS 25,26,32). Overall, genetic copy-number gains of these genes are more prevalent in ATC than in DTC, suggesting that these genetic alterations are important for the progression and aggressiveness of thyroid cancer. Copy-number gain of these genes in ATC could occur either through genetic amplification or chromosomal instability and aneuploidy. An important and expected consequence of these copy-number gains is the activation of downstream signalling pathways, as is suggested by the association of copy-number gains of genes encoding RTKs with increased phosphorylation of AKT and ERK in thyroid cancer[26].

Many of the genes with copy-number gains are proto-oncogenes. A mechanism for them to contribute to thyroid tumorigenesis is through increased protein expression and consequent aberrant activation of the signalling pathways in which they are involved[25,26,33]. It is interesting to note that in DTCs, *PIK3CA* mutation is mutually exclusive with *PIK3CA* copy-number gain[31,33], suggesting that either of these genetic alterations is sufficient to promote thyroid tumorigenesis through the PI3K–AKT pathway. However in aggressive undifferentiated ATC, *PIK3CA* mutations and amplifications often occur simultaneously in the same tumour[26,33], which is a mechanism through which mutant *PIK3CA* can be amplified to drive the progression and aggressiveness of thyroid cancer.

Gene amplification or copy-number gain of the IQ-motif-containing GTPase-activating protein 1 (*IQGAP1*) is another important genetic event that has been recently discovered in thyroid cancer[48]. IQGAP1 is a multifunctional scaffold protein that has an important role in human tumorigenesis[49]. *IQGAP1* amplification was found to increase protein expression and to be associated with invasiveness of thyroid cancer cells. Interestingly, coexistence of *IQGAP1* copy-number gain and $BRAF^{V600E}$ mutation was particularly associated with a high incidence of recurrent PTC[48].

Gene translocations

Gene translocation resulting in oncogenic rearrangements in thyroid cancer is best exemplified by *RET*–PTC. There are more than 10 types of *RET*–PTC translocation, as determined by the types of partner genes, and the most common types are *RET*–PTC1 and *RET*–PTC3[50–52]. *RET* is a proto-oncogene encoding an RTK. *RET*–PTC occurs as a consequence of genetic recombination between the 3 tyrosine kinase portion of *RET* and the 5 portion of a partner gene, such as the coiled-coil domain-containing gene 6 (*CCDC6*; also known as *H4)* in *RET*–PTC1 and nuclear receptor co-activator 4 (*NCOA4*; also known as *ELE1*) in *RET*–PTC3 (REFS 53,54). Spatial contiguity of *RET* and the partner gene in the nucleus is a structural basis for the formation of *RET*–PTC rearrangements[55]. The rearrangements result in ligand-independent dimerization and constitutive tyrosine kinase activity of RET. *RET*–PTC can occur in benign FTA and follicular variant PTC (FVPTC), but more commonly in classical PTC[56]. A recent study found a correlation between the presence of *RET*–PTC and a high growth rate of benign thyroid tumours[57]. However, the role of RET–PTC in early thyroid tumorigenesis is unclear. RET–PTC is a classical oncoprotein that activates the MAPK and PI3K–AKT pathways[58,59]. RET–PTC activates both pathways by recruiting signalling adaptors to phosphorylated Tyr1062 on the intracellular domain of the RET fusion protein[60]. It is thus not surprising that *RET*–PTC occurs in FTA and FVPTC; these are follicular thyroid tumours in which the PI3K–AKT pathway has a primary role in tumorigenesis[61].

The paired box 8 (*PAX8*)–peroxisome proliferator-activated receptor- (*PPARG*) fusion gene (*PAX8–PPARG*) is another prominent recombinant oncogene in thyroid cancer, occurring in up to 60% of FTC and FVPTC[62–64]. *PAX8–PPARG* also occurs in FTA[64], although, like *RET*–PTC, its prevalence is low in benign thyroid tumours and its oncogenic role is unclear. PAX8–PPAR exerts a dominant-negative effect on the wild-type tumour suppressor PPAR and also transactivates certain PAX8-responsive genes[65]. Interestingly, the expression of PPAR was dramatically reduced in FTC that developed in the TR PV mouse model — which expresses a knock-in dominant-negative mutant of human thyroid hormone receptor- (TR) — resulting in follicular thyroid tumorigenesis[66]. An *AKAP9–BRAF* fusion gene resulting in activation of the BRAF kinase also occurs in ionizing-radiation-induced PTC[67], but not in sporadic PTC[68]. Unlike *RET*–PTC, the importance of *AKAP9–BRAF* in thyroid tumorigenesis is probably limited owing to its rarity.

Aberrant gene methylation

Aberrant gene methylation is an epigenetic hallmark of human cancer — which is also common in thyroid cancer[69] — that usually silences a gene when occurring in the promoter regions. $BRAF^{V600E}$ mutation was found to be associated with hypermethylation of several tumour suppressor genes, including tissue inhibitor of metalloproteinases 3 (*TIMP3*), *SLC5A8*, death-associated protein kinase 1 (*DAPK1*) and retinoic acid receptor- (*RARB*)[70]. A recent DNA methylation microarray study revealed broad hypermethylation of genes throughout the genome driven by BRAF-V600E signalling in PTC cells[71]. Interestingly, this study also revealed a large range of genes throughout the genome that, driven by BRAF-V600E, became hypomethylated and hence overexpressed. These hypermethylated or hypomethylated genes have important established metabolic and cellular functions. Thus, alterations in gene methylation coupled to BRAF-V600E and probably to other oncoproteins represent a prominent epigenetic mechanism in thyroid tumorigenesis.

Promoter methylation of *PTEN* is also common in FTC and ATC[72–74], which is consistent with the loss of expression of *PTEN* that is found in a subset of these thyroid cancers[73,75,76]. *PTEN* methylation is associated with genetic alterations of the PI3K–AKT pathway in thyroid tumours, including mutations of various isoforms of RAS, *PIK3CA* mutation and

amplification, and *PTEN* mutations[72]. This is consistent with a model in which aberrant activation of the PI3K–AKT pathway, driven by activating genetic alterations, causes aberrant methylation and hence silencing of *PTEN*, which in turn leads to failure to terminate the PI3K–AKT signalling and creates a self-amplifying loop[72].

Despite the identification of these common genetic alterations in thyroid cancers, it is important to note that approximately 30–35% of DTCs do not harbour known genetic alterations, and so further investigation is required to identify the underlying genetic backgrounds.

Altered signalling pathways in thyroid cancer

The MAPK signalling pathway

The MAPK pathway has a fundamental role in the regulation of cell proliferation and survival and in human tumorigenesis (FIG. 1). The importance of this pathway has been well established in thyroid tumorigenesis, particularly for PTC[77,78]. In thyroid cancer, the MAPK pathway is driven by activating mutations, including *BRAF* and RAS mutations, by *RET*–PTC and, in some cases, by the recently discovered *ALK* mutations[43].

MAPK-mediated thyroid tumorigenesis involves a wide range of secondary molecular alterations that synergize and amplify the oncogenic activity of this pathway, such as genome-wide hypermethylation and hypomethylation[71]. Additionally, upregulation of various well-established oncogenic proteins can occur. These include chemokines[79,80], vascular endothelial growth factor A (VEGFA)[81], MET[82,83], nuclear factor- B (NF- B)[84], matrix metalloproteinases (MMPs)[79,84,85], prohibitin[86], vimentin[87], hypoxia-inducible factor 1 (HIF1)[88], prokineticin 1 (PROK1; also known as EG-VEGF)[89], urokinase plasminogen activator (uPA) and its receptor (uPAR)[90,91], transforming growth factor- (TGF)[92–94] and thrombospondin 1 (TSP1)[93]. These proteins are all established players in human tumorigenesis through a variety of mechanisms that drive cancer cell proliferation, growth, migration and survival, as well as tumour angiogenesis, invasion and metastasis.

Many of these proteins are key constituents of the unique extracellular matrix (ECM) microenvironments that have been proposed to be important in the pathogenesis of thyroid cancer driven by oncoproteins such as BRAF-V600E[95]. It is now clear that the ECM micro-environment does not only function as a structural support for the cellular elements of cancer, but it also has a profound impact on the behaviours of cancer cells, including their viability, proliferation, adhesion and motility. Thyroid cancer cells and stromal cells, such as fibroblasts and macrophages, produce proteins that form paracrine and autocrine signalling loops. For example, BRAF-V600E-mediated MAPK pathway activation promotes the release of TSP1 into the ECM where it interacts with and modulates other proteins. These include integrins and non-integrin cell-membrane receptors, matrix proteins, cytokines, VEGFA and MMPs, which in turn activate signalling in thyroid cancer cells and promote tumour progression and metastasis[93,95]. Another example is the tumour-promoting cytokine TGF , which is released into the ECM owing to the activation of the BRAF-V600E–MAPK pathway[92,94]. Cytokines can create an inflammatory microenvironment that may produce reactive oxygen species and oxidative stress, which may in turn stimulate the MAPK pathway and augment thyroid tumorigenesis[96]. Thus, secondary molecular alterations in the tumour ECM microenvironment, triggered by the aberrant signalling of the MAPK pathway, have an important role in the pathogenesis of thyroid cancer.

In terms of stromal cells in the tumour micro-environment, an important role of tumour-associated macrophages is that they produce cytokines such as TGF . The role of stromal fibroblasts in thyroid tumori-genesis seems to be affected by the expression patterns of the

two fibroblast growth factor receptor 2 (FGFR2) isoforms, FGFR2-IIIb and FGFR2-IIIc, in cancer cells and fibroblasts: co-implantation of thyroid cancer cells and fibroblasts expressing the same type of FGFR2 isoform did not affect xenograft tumour progression, whereas co-implantation of thyroid cancer cells and fibroblasts that respectively expressed FGFR2-IIIb and FGFR2-IIIc resulted in increased tumour progression[97]. These results highlight the importance and complexity of cellular and molecular constituents in determining the impact of the microenvironment on thyroid cancer cell behaviour.

The PI3K–AKT signalling pathway

The PI3K–AKT pathway also has a fundamental role in thyroid tumori-genesis[61,98] (FIG. 2). Early evidence to support the role of this pathway in thyroid tumorigenesis came from the finding that Cowden's syndrome (which is caused by germline mutations of *PTEN*) was associated with FTA and FTC[99]. A functional role for the PI3K–AKT pathway in sporadic thyroid tumorigenesis was initially revealed by the finding of increased expression and activation of AKT in thyroid cancers, particularly FTC[100–102]. Among the three AKT isoforms, AKT1 and AKT2, but not AKT3, were found to be robustly expressed and activated in thyroid cancer, suggesting a particularly important role for these two isoforms in thyroid tumorigenesis[101].

In the TR PV transgenic mouse model — in which the PI3K–AKT pathway is activated, which involves the interaction of TR PV with the p85 regulatory subunit of PI3K — FTC spontaneously developed[103,104], and the activation and nuclear localization of AKT1 was observed[105]. Human tumour studies suggested that the invasiveness and metastasis of FTC promoted by the PI3K–AKT pathway particularly involved the activation and nuclear localization of AKT1 (REF. 102). This is consistent with the occurrence of *AKT1* mutations in metastatic thyroid cancers[35]. Furthermore, *Akt1* ablation delayed or prevented tumour progression, vascular intravasation and distant metastasis of FTC in TR PV mice[106]. The role of AKT2 in thyroid tumorigenesis has also been demonstrated *in vivo*, as the occurrence of thyroid tumours was reduced in *Pten*$^{+/-}$*Akt2*$^{-/-}$ mice[107]. Thus, whereas the MAPK pathway has a central role primarily in PTC, the PI3K–AKT pathway has such a role primarily in FTC and its invasion and metastasis. This is consistent with genetic alterations in the PI3K–AKT pathway occurring most frequently in FTC, whereas those in the MAPK pathway occur most frequently in PTC[17,18,61,78,98]. The inhibition of thyroid cancer cell proliferation by PI3K–AKT pathway inhibitors was dependent on the presence of genetic alterations in the PI3K–AKT pathway[108,109], suggesting that thyroid cancer cells harbouring such genetic alterations have become dependent on the over-activation of this pathway.

Vascular invasiveness and capsular invasiveness are defining histopathological features of FTC[110]. Genetic alterations in the PI3K–AKT pathway are far more common in FTC than in its precursor, FTA[31,33]. It is therefore conceivable that overactivated PI3K–AKT signalling promotes the conversion of FTA to FTC by conferring tumour cell invasiveness. There are also secondary molecular alterations, albeit limited in number so far, that are robustly induced by PI3K–AKT signalling and that have an important role in the pathogenesis of FTC; these include alterations of the WNT– catenin[111], HIF1 [112,113], FOXO3 (REF. 114) and NF- B[114] pathways.

The NF-κB signalling pathway

The NF- B pathway has an important role in the regulation of inflammatory responses that are linked to tumorigenesis[115] (FIG. 1). Increased NF- B activation in thyroid cancer cell lines and tissues has long been documented[116–118]. Recent studies demonstrate that the NF- B pathway controls proliferative and anti-apoptotic signalling pathways in thyroid cancer cells[119,120]. The NF- B pathway is involved in upregulating the expression of several

oncogenic proteins that are also upregulated by the MAPK pathway. Furthermore, RET–PTC, RAS and BRAF-V600E — members of the MAPK pathway — can cause activation of the NF- B pathway in thyroid cancers[119]. Interestingly, NF- B signalling was upregulated by expression of BRAF-V600E[121,122], but not by expression of a constitutively active mutant of MEK in NIH3T3 cells[123], suggesting a direct coupling of BRAF-V600E to the NF- B pathway. Through a mechanism that is not yet specifically defined, BRAF-V600E was shown to cause I B degradation (and consequent activation of NF- B) independently of MEK–ERK signalling in thyroid cancer cells[84]. This dual coupling of BRAF-V600E to the NF- B pathway and to the MEK–ERK pathway is consistent with the finding that simultaneously suppressing the two pathways using NF- B and MEK inhibitors synergistically inhibited the proliferation of thyroid cancer cells harbouring a BRAF-V600E mutation[124].

The RASSF1–MST1–FOXO3 signalling pathway

RASSF1 is a member of the RAS association domain family, which, in response to apoptotic signalling, associates with and activates mammalian STE20-like protein kinase 1 (MST1; also known as STK4)[125]. Activated MST1 phosphorylates Ser207 in the forkhead domain of the forkhead transcription factor FOXO3. This phosphorylation disrupts the interaction of FOXO3 with 14-3-3 proteins in the cytoplasm and promotes FOXO3 translocation to the nucleus, where it promotes the transcription of pro-apoptotic genes[126] (FIG. 1). Thus, this RASSF1–MST1–FOXO3 pathway has an important tumour-suppressive role by promoting apoptosis.

Hypermethylation of the promoter of *RASSF1A* is common and is associated with its silencing in thyroid cancers[127,128]. This occurs even in benign FTA, albeit to a lesser extent, suggesting that impairment of the RASSF1–MST1–FOXO3 pathway is involved in early thyroid tumorigenesis[128].

Activation of the RASSF1–MST1–FOXO3 signalling pathway promoted thyroid cancer cell apoptosis[21]. Interestingly, this study demonstrated that BRAF-V600E, but not wild-type BRAF, directly interacted with the carboxyl terminus of MST1 and inhibited its kinase activities, resulting in decreased FOXO3 transactivation. This was independent of MEK–MAPK signalling. This suggests that, in addition to the classical coupling to MEK–MAPK, negative regulation of RASSF1–MST1–FOXO3 is a mechanism that is involved in BRAF-V600E-driven thyroid tumorigenesis. This unique mechanistic model explains the finding that thyroid cancers that developed in $BRAF^{V600E}Mst1^{-/-}$ transgenic mice were more aggressive than those that developed in $BRAF^{V600E}$ mice[21]. The direct interaction of BRAF-V600E with MST1 to prevent RASSF1-mediated MST1 activation also provides an explanation for the mutually exclusive occurrence of $BRAF^{V600E}$ mutation and *RASSF1A* hypermethylation in thyroid cancer[128], as either event may be sufficient to inactivate RASSF1–MST1–FOXO3 signalling. Therefore, BRAF-V600E is coupled independently to three major pathways: MEK–MAPK, RASSF1–MST1–FOXO3 and NF- B, and thus represents a unique and powerful oncogenic mechanism in thyroid tumorigenesis (FIG.1). The activation of the PI3K–AKT pathway, such as that induced by *PTEN* inactivation, can also downregulate the activities of the RASSF1–MST1–FOXO3 pathway in FTC[114]. This involves AKT-mediated phosphorylation of FOXO family members, which causes their translocation from the nucleus to the cytoplasm, where they are sequestered by 14-3-3 proteins, thus reducing the transcription of pro-apoptotic genes[129] (FIG. 2).

The WNT–β-catenin signalling pathway

The WNT– catenin pathway has a well established role in the regulation of cell growth and proliferation, as well as in stem cell differentiation, and its constitutive activation is

commonly found in human cancers[130]. -catenin, when upregulated by upstream WNT signalling, is translocated into the nucleus and transcribes various tumour-promoting genes. In thyroid cancer, activation of WNT– -catenin signalling is often caused by activating mutations of *CTNNB1* (which encodes -catenin), particularly in PDTC and ATC[37,38]. Furthermore, the expression of -catenin was higher in ATC than in DTC[131]. Thus, the WNT– -catenin pathway seems to have a particularly important role in thyroid tumour aggressiveness.

Aberrant activation of WNT– -catenin signalling often occurs as a consequence of the activation of the PI3K–AKT pathway in thyroid cancers[132,133]. This occurs through glycogen synthase kinase 3 (GSK3), which is directly phosphorylated — and hence inactivated — by AKT[134]. As GSK3 promotes the degradation of -catenin, its inactivation results in the upregulation of WNT– -catenin signalling (FIG. 2). Interestingly, RET–PTC can activate the WNT– -catenin pathway by activating the PI3K–AKT pathway and also by directly phosphorylating -catenin in thyroid cancer cells[58].

The HIF1α pathway

It has long been known that hypoxia is a strong stimulus of cancer metabolism, growth and progression. HIF1 is a key mediator of the response to hypoxia, in which it binds to HIF1 (also known as ARNT) to form the HIF1 transcription factor that induces the expression of various genes associated with cell metabolism and tumour angiogenesis[135]. Angiogenesis, which is a key step in the progression of solid tumours, is a common response to intratumoural hypoxia. This process is promoted by VEGFA, the expression of which is strongly upregulated by HIF1. HIF1 is not expressed in normal thyroid tissues but is expressed in thyroid cancers, particularly in aggressive types, such as ATC, and this is consistent with a role in thyroid cancer progression[112,136]. The oncogene *MET*, which is another target of HIF1, is also abundantly expressed in association with upregulated HIF1 in thyroid cancer[136]. Interestingly, HIF1 can be upregulated in thyroid cancer by both the PI3K–AKT[112,113] and the MAPK pathways[88], thus contributing to the effects of these two pathways on thyroid tumour progression.

The thyroid-stimulating hormone receptor signalling pathway

Through activation by thyroid-stimulating hormone (TSH), the TSH receptor (TSHR) has a fundamental role in the regulation of thyroid cell proliferation, differentiation and function, as well as in the development of the thyroid gland[137]. TSHR is a guanine-nucleotide-binding G-protein-coupled receptor that triggers two intracellular signalling pathways: G_s -mediated adenylyl cyclase–cyclic AMP (cAMP) signalling and the G_q- or G_{11}-mediated phospholipase C -inositol 1,4,5-trisphosphate–intracellular Ca^{2+} signalling. FTC spontaneously developed in TR PV mice, but when these mice were crossed with *Tshr*$^{-/-}$ mice, no thyroid cancer developed[138]. This study thus elegantly demonstrates the requirement of TSHR signalling in thyroid carcinogenesis in this mouse model. Similar findings were reported in another mouse model in which thyroid-specific knock-in of *Braf*^{V600E} (LSL-*Braf*^{V600E}thyroid peroxidase (*Tpo*)-Cre) caused the development of aggressive PTC, and crossing these mice with *Tshr*$^{-/-}$ mice resulted in a failure to develop thyroid cancer[139]. In this study, thyroid-specific deletion of *Gnas* (which encodes G_s) in LSL-*Braf*^{V600E} *Tpo*-Cre mice attenuated thyroid tumour formation. Interestingly, there is a clinical association of higher serum levels of TSH with a higher risk for malignancy of thyroid nodules in humans[140–142].

However, it is not clear whether TSHR signalling is required directly for the initiation of thyroid cancer or whether it is simply required, as would be expected physiologically, for the TSHR-dependent generation of thyroid cells, from which an oncogene-driven thyroid

cancer would originate. Overactivation of TSHR signalling, such as that achieved through activating mutations in TSHR or $G_{s\alpha}$, is well known to cause benign hyperfunctional FTA[143,144]. These tumours are almost never malignant, which suggests that TSHR signalling may be protective against malignant transformation of thyroid cells. Consistently, low serum levels of TSH are associated with common genetic variants that predispose to an increased risk of thyroid cancer[145,146]. It therefore seems that the TSH–TSHR system has a dichotomous role in the development of thyroid cancer: it may suppress malignant transformation of thyroid cells and hence suppress the occurrence of thyroid cancer, but it may promote the growth and progression of thyroid cancer once it has been initiated by oncogenic alterations.

Progressive molecular alterations

Progressive accumulation of genetic alterations

The accumulation of genetic alterations during thyroid tumour progression is probably best exemplified by genetic alterations to members of the PI3K–AKT pathway[61]. Mutations in members of this pathway, including those in RAS (particularly *NRAS*), *PIK3CA* and *PTEN*, as well as *PIK3CA* amplifications, increasingly occur from FTA to FTC and to ATC[26,31–33,147]. Hypermethylation of the *PTEN* promoter also progressively increases in frequency from FTA to FTC and to ATC[72]. The coexistence of these genetic and epigenetic alterations also increasingly occurs from low-grade to high-grade thyroid tumours[31,33]. Genetic or epigenetic defects of *PTEN* can occur simultaneously with activating mutations of other genes in the PI3K–AKT pathway; in such cases, the PI3K–AKT pathway can presumably remain maximally activated[26,33,72]. Indeed, deletion of *Pten* accelerated the progression of FTC in TR PV mice[114].

The coexistence of multiple genetic alterations to members of the MAPK pathway also occurs, as exemplified by the simultaneous presence of $BRAF^{V600E}$ mutation, RAS mutations and *RET*–PTC in aggressive recurrent PTC and ATC[26,148,149]; these alterations are otherwise mutually exclusive in well-differentiated thyroid cancers[7,9]. It is thus compelling to propose that the process of thyroid cancer progression is one of progressive accumulation of multiple genetic alterations, which synergistically cooperate to amplify their oncogenicity.

Cooperation of the MAPK and PI3K–AKT pathways

The MAPK and PI3K–AKT pathways are primarily involved in differentiated PTC and FTC, respectively, and the simultaneous activation of both pathways becomes more frequent as the grade of thyroid tumours increases[26,31–33,35]. One study analysed 24 genetic alterations in the major genes of the MAPK and PI3K–AKT pathways in 48 ATC samples and found that the majority (81%) of the samples harboured genetic alterations that could potentially activate both pathways[26]. The coexistence of phosphorylated ERK and AKT was also common in ATC but not in DTC[26]. Thus, genetic alterations that activate the MAPK and PI3K–AKT pathways are an important mechanism that drives the progression of thyroid cancer. In this model, as illustrated in FIG. 3, when a genetic alteration occurs in the MAPK pathway it drives tumorigenesis of the thyroid cell towards PTC; when a genetic alteration occurs in the PI3K–AKT pathway it drives tumorigenesis towards FTA and FTC. As genetic alterations accumulate and both pathways become activated, the tumour progresses into PDTC and ATC, which is a process that may be further accelerated by additional or secondary genetic alterations, including mutations in *TP53*, *CTNNB1* and *ALK*.

This model is supported by studies of transgenic mice in which the deletion of *Pten* and knock-in of $Kras^{G12D}$ (to activate both MAPK and PI3K–AKT pathways), but not either genetic manipulation alone, caused the development of aggressive thyroid cancer[29].

Similarly, this phenomenon is observed in melanoma. Transgenic mice with induced expression of $BRAF^{V600E}$ in melanocytes to activate the MAPK pathway alone developed only melanocytic hyperplasias, but the expression of $BRAF^{V600E}$ with *Pten* deletion (to also activate the PI3K–AKT pathway) caused melanoma with a rapid onset of metastasis[150]. Thus, dual activation of the MAPK and PI3K–AKT pathways may be a common mechanism for promoting tumour progression.

Impairment of the iodide-handling machinery

The main and unique function of follicular thyroid cells is to use iodide to synthesize thyroid hormone[151,152] (FIG. 4). In this process, iodide is transported into the cell through the sodium–iodide symporter (NIS) that is located in the basal membrane. At the apical membrane, pendrin transports iodide out of the cell into the lumen of the thyroid follicle. In the lumen of the thyroid follicle, iodide undergoes oxidation by TPO and is incorporated into tyrosine residues of thyroglobulin (TG), which is later cleaved through proteolysis to produce thyroid hormones. This process is upregulated by TSH-mediated activation of TSHR. This is the biological basis for the conventional radioiodine treatment of thyroid cancer, but the iodide-handling machinery is often impaired, particularly in advanced thyroid cancers, thus making radioiodine treatment ineffective.

Aberrant activation of the MAPK pathway has a crucial role in the impairment of the iodide-handling machinery[17]. $BRAF^{V600E}$ mutation is associated with the loss of radioiodine avidity and with radioiodine treatment failure in PTC[16,35,153–155]. Consistently, $BRAF^{V600E}$ mutation is highly prevalent (78–95% of cases) in recurrent radioiodine-refractory PTC[16,35,149,155], in contrast with the lower prevalence of $BRAF^{V600E}$ mutation (45% of cases) in primary PTC[12]. Numerous studies have reported an association of $BRAF^{V600E}$ mutation with decreased or absent expression of thyroid iodide-handling genes: *NIS*, *TSHR*, *TPO*, *TG* and *SLC26A4* (which encodes pendrin) in thyroid cancer[82,153,154,156–160]. A direct role of $BRAF^{V600E}$ in the downregulation of thyroid hormone synthesis pathway genes was demonstrated by showing that induced expression of BRAF-V600E in thyroid cells impaired the expression of almost all of these genes, which was restored by ceasing the expression of BRAF-V600E or by suppressing the MAPK pathway with a MEK inhibitor[161]. Similarly, in thyroid cells expressing *RET*–PTC1, *NIS* expression was increased following treatment with a MEK inhibitor[162]. Suppression of BRAF-V600E in mouse models of thyroid cancer also restored the expression of thyroid iodide-handling genes and radioiodine uptake[163]. This aberrant gene silencing by BRAF-V600E may involve alterations to histone acetylation at gene promoters[164,165]. An autocrine loop involving TGF is another mechanism[92]: BRAF-V600E promotes the secretion of TGF which, through the activation of SMADs and consequent impairment of the thyroid-gene transcription factor PAX8, is a potent repressor of NIS in thyroid cells[166].

Activation of the PI3K–AKT pathway was also shown to downregulate the iodide-handling machinery in thyroid cells both *in vitro* and *in vivo*[167,168]. Inhibition of the PI3K–AKT pathway induced NIS expression and iodide uptake in human thyroid cancer cells[164,169]. In addition to NIS, suppression of the PI3K–AKT pathway also induced the expression of *TSHR*, *TPO* and *TG* in human thyroid cancer cells, which was enhanced by treatment with histone deacetylase inhibitors, and radioiodine uptake was correspondingly robustly induced[164]. This could be augmented by co-treatment with TSH[164]. The involvement of both MAPK and PI3K–AKT pathways in the silencing of thyroid iodide-handling genes is consistent with the accumulation of genetic alterations in both pathways as thyroid tumours progress, during which there is an increasing loss of radioiodine avidity.

Translational promises in thyroid cancer

Remarkable advances in the translation of molecular findings in thyroid cancer to the clinic have occurred recently[170]. For example, because of its cancer specificity, the detection of *BRAF*^{V600E} mutation in fine-needle aspiration biopsy (FNAB) samples was initially tested for the evaluation of thyroid nodules[171,172], which has now found increasing clinical use[170,173]. The diagnostic utility of detecting *RET*–PTC using FNAB has also been intensively investigated[170]. The combinatorial use of genetic markers identified in FNAB samples, including *BRAF* mutation, RAS mutations, *RET*–PTC and *PAX8–PPARG*, has been shown to improve the diagnostic accuracy for thyroid nodules that are otherwise diagnostically indeterminate by conventional cytology assessment, although this diagnostic approach may need further improvement[174,175]. Applying genomic information to address clinical issues of thyroid neoplasia has also shown promise. For example, a gene-expression classifier (with high sensitivity and negative predictive power) is being used in the clinic to assist the diagnostic evaluation of indeterminate thyroid nodules[176].

The prognostic application of *BRAF*^{V600E} mutation has also become part of the clinical management of PTC[170,173]. A large, multicentre study demonstrated a strong association of *BRAF*^{V600E} mutation with patient mortality from PTC[177]. Large studies have demonstrated that *BRAF*^{V600E} is strongly associated with poor clinico-pathological outcomes even in conventionally low-risk patients[16,178–181]. *BRAF*^{V600E} detected in FNAB samples preoperatively predicted poor clinicopathological outcomes of PTC[180,182,183] and even predicted lymph node metastasis (as determined by prophylactic central neck dissection in patients without preoperative evidence of lymph node metastasis)[183]. It is thus recommended that preoperative testing of *BRAF*^{V600E} in FNAB samples be used for assisting risk stratification and defining surgical and medical treatments for patients with PTC[170]. Owing to their association with a worse prognosis of PDTC, RAS mutations are also promising prognostic markers for this type of thyroid cancer[184–186]. It has been recently shown that RAS mutations are also associated with poor clinicopathological outcomes of FTC[187] but this needs to be evaluated further.

Many components of the MAPK and PI3K–AKT pathways, from RTKs in the cell membrane to the various downstream signalling relay molecules, such as BRAF, MEK, AKT and mTOR, are therapeutic targets that are being actively tested for the treatment of thyroid cancer[170]. Novel small-molecule protein-kinase inhibitors have shown promise in clinical trials on thyroid cancer, including axitinib[188], sorafenib[189–191], motesanib[192] and pazopanib[193]. A promising therapeutic strategy is genetic-based targeting of thyroid cancer[194], as supported by many preclinical studies demonstrating the selective inhibition of *BRAF*^{V600E}-mutant thyroid cancer cells by various MEK inhibitors[124,195–199] and BRAF-V600E-specific inhibitors[200,201]. The latter include PLX4032 (also known as vemurafenib), which has been recently approved by the US Food and Drug Administration (FDA) for the treatment of *BRAF*^{V600E}-positive melanoma[202]. Some of these MEK and BRAF-V600E inhibitors are currently being clinically tested for thyroid cancer. Therapeutic targeting of the PI3K–AKT pathway may also be genetically guided, as genetic alterations that activate this pathway confer thyroid cancer cells with remarkably increased sensitivities to AKT and mTOR inhibitors[108,109]. This therapeutic strategy for thyroid cancer may prove to be useful and should be tested clinically, as encouraged by a recent clinical trial showing that mutations in the PI3K–AKT pathway significantly increased the response rate of breast and gynaecological cancers to inhibitors of the PI3K–AKT pathway[203].

The involvement of multiple signalling pathways in aggressive thyroid cancer suggests that it may be necessary to target them simultaneously for effective treatment. Indeed, single agent clinical trials of various kinase inhibitors in thyroid cancer have generally shown only

partial responses. Several recent preclinical studies testing the combination of MEK or BRAF-V600E inhibitors with PI3K, AKT or mTOR inhibitors showed synergistic effects in inhibiting the proliferation and promoting apoptosis of thyroid cancer cells[124,199,204,205]. Synergistic effects of simultaneously targeting the MAPK and PI3K–AKT pathways were even more pronounced in cells that harboured genetic alterations in both pathways[199]. It has been recently demonstrated that simultaneously inhibiting the MAPK, PI3K–AKT and histone deacetylase pathways could more robustly induce the expression of thyroid iodide-handling genes (and thus increase radioiodine uptake) in thyroid cancer cells compared with inhibiting each pathway alone, and this could be enhanced by co-treatment with TSH[164]. Therefore, to restore radioiodine avidity by simultaneously targeting multiple signalling pathways is another promising area of translational research on thyroid cancer.

Concluding remarks

Remarkable progress in understanding the molecular pathogenesis of thyroid cancer has been made in recent years, as exemplified by the elucidation of the fundamental role of signalling pathways, such as the MAPK and PI3K–AKT pathways. Activation of these pathways, often in close connection and cooperation, constitute the primary oncogenic mechanism that promotes the development and progression of thyroid cancer. Central to this mechanism are the many powerful oncogenic alterations that drive these pathways. Important secondary molecular derangements driven by the overactivation of these pathways have also been increasingly uncovered, which synergize and amplify oncogenic signalling in thyroid tumorigenesis. This understanding of the molecular pathogenesis of thyroid cancer has now opened unprecedented opportunities for the development of novel clinical strategies for the management of thyroid cancer.

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Glossary

Differentiated thyroid cancer	(DTC). Collectively includes both papillary thyroid cancer (PTC) and follicular thyroid cancer (FTC), which are histologically differentiated, by comparison with poorly differentiated thyroid cancer (PDTC) and undifferentiated anaplastic thyroid cancer (ATC)
Radioiodine treatment	A classical treatment for patients with thyroid cancer. It is used after total thyroidectomy and consists of treating patients with the radioiodine isotope ^{131}I, which emits high-energy □particles and takes advantage of the unique function of thyroid follicular cells to accumulate iodide as a substrate for the synthesis of thyroid hormone
MAPK pathway	This pathway has a fundamental role in the regulation of cell growth, proliferation, apoptosis and metabolic activities, through regulating the expression of various genes
PI3K–AKT pathway	This pathway also has a fundamental role in the regulation of cell growth, proliferation, apoptosis and metabolic activities, through regulating the expression of various genes

Pten	A tumour suppressor gene, the protein product of which converts phosphatidylinositol (3,4,5)-trisphosphate (PIP3) to phosphatidylinositol (4,5)-trisphosphate (PIP2), counteracting the conversion of PIP2 to PIP3 by PI3K and thus terminating PI3K–AKT signalling
Cowden's syndrome	Also known as Cowden's disease or multiple hamartoma syndrome. A rare, autosomally inherited disorder that is caused by mutations or defects in the tumour suppressor gene *PTEN* and is characterized by multiple tumour-like growths called hamartomas and an increased risk of certain cancers, such as breast cancer and follicular thyroid cancer
Copy-number gain	A genetic abnormality in which the copy number of a chromosomal region or gene is more than the normal two copies (one paternal allele and one maternal allele), which occurs through the amplification of a local region of DNA within a chromosome, or through aneuploidy, in which multiple copies or fragments of identical chromosomes are present
Receptor tyrosine kinases	(RTKs). These are typically growth-promoting transmembrane receptors that transduce extracellular signalling into intracellular signalling through the activation of their cytoplasmic kinase domain in response to extracellular signals such as ligand-binding
Gene translocation	A genetic rearrangement in which a chromosomal fragment is translocated to another chromosome where it is not normally located, which may create a recombinant gene product with new function or uncontrolled function (compared with the original gene product)
Follicular thyroid tumours	Collectively includes follicular thyroid adenoma (FTA), follicular thyroid cancer (FTC) and follicular variant papillary thyroid cancer (FVPTC); they share common dense follicular cell architectures
Gene methylation	An epigenetic covalent addition of a methyl group to the fifth carbon of the cytosine residue in a CpG dinucleotide, typically in CpG islands (that is, CpG-rich regions) in the 5 □flanking promoter regions of genes. Such methylation is usually closely associated with chromatin remodelling and silencing of the corresponding genes. Capsular invasiveness, A phenomenon in which thyroid cancer invades the connective tissue capsule that surrounds the tumour, which is a defining feature of progression to malignancy from a benign thyroid tumour

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At a glance

- Thyroid cancer is a common endocrine malignancy, and exciting progress has occurred in recent years in understanding its molecular pathogenesis.
- Genetic and epigenetic alterations are the driving forces of thyroid cancer. Examples of these alterations include mutations in *BRAF* (*BRAF*^{V600E}), RAS, *PIK3CA*, *PTEN*, *TP53*, β-catenin (*CTNNB1*), anaplastic lymphoma kinase (*ALK*) and isocitrate dehydrogenase 1 (*IDH1*), translocations (*RET*–PTC and paired box 8 (*PAX8*)–peroxisome proliferator-activated receptor-γ (*PPARG*)) and aberrant gene methylation.
- At the core of the molecular pathogenesis of thyroid cancer is the uncontrolled activity of various signalling pathways, including the MAPK, PI3K–AKT, nuclear factor-κB (NF-κB), RASSF1–mammalian STE20-like protein kinase 1 (MST1)–forkhead box O3 (FOXO3), WNT–β-catenin, hypoxia-inducible factor 1α (HIF1α) and thyroid-stimulating hormone (TSH)–TSH receptor (TSHR) pathways.
- The progression of thyroid cancer is a process of accumulation of genetic and epigenetic alterations with corresponding progressive derangements of signalling pathways. These are accompanied by numerous secondary molecular alterations, both in the cell and in the tumour microenvironment, which, acting in cooperation, amplify and synergize their impacts on thyroid tumorigenesis.
- Aberrant silencing of thyroid iodide-handling genes and consequent loss of radioiodine avidity of thyroid cancer promoted by BRAF-V600E is a unique molecular pathological process in thyroid cancer, which causes the failure of radioiodine treatment.
- The recent molecular findings provide unprecedented opportunities for further research and clinical development of novel molecular-based treatment strategies for thyroid cancer.

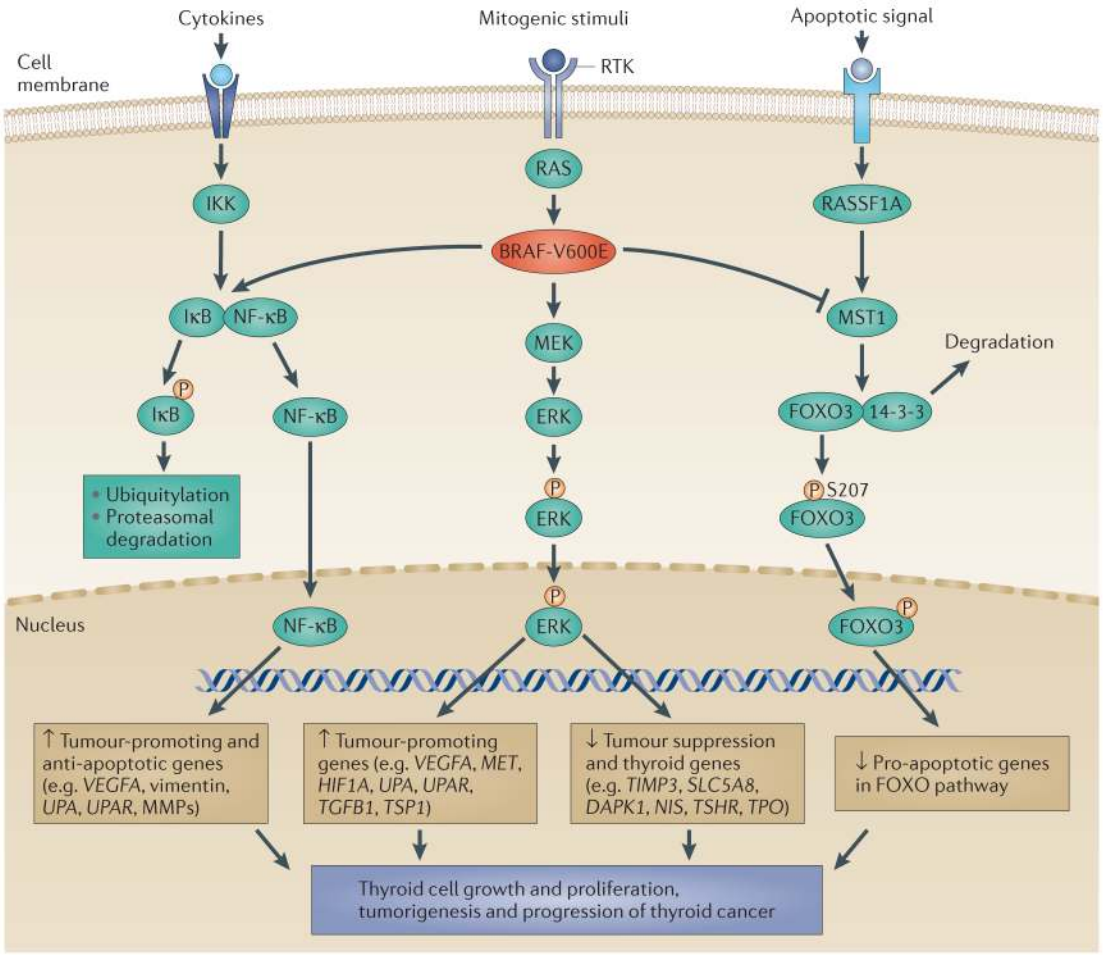

Figure 1. The MAPK and related pathways in thyroid cancer

Shown in the middle of the figure is the classical MAPK pathway leading from an extracellular mitogenic stimulus that activates a receptor tyrosine kinase (RTK) in the cell membrane, to RAS, RAF (shown as BRAF-V600E), MEK and ERK. ERK is activated by phosphorylation (P) and enters the nucleus where it upregulates tumour-promoting genes and downregulates tumour suppressor genes and thyroid iodide-handling genes. On the left side of the figure is the nuclear factor- B (NF- B) pathway, in which extracellular stimuli activate the pathway by acting on receptors in the cell membrane, leading to activation of the inhibitor of B (I B) kinase (IKK), resulting in the phosphorylation of I B. Phosphorylated I B becomes dissociated from NF- B, which is normally bound with I B in a complex and sequestered in the cytoplasm. Phosphorylated I B undergoes ubiquitylation and proteasomal degradation. Free NF- B then enters the nucleus to promote the expression of tumour-promoting genes. Through an undefined mechanism that is independent of MEK signalling, BRAF-V600E promotes the phosphorylation of I B and the release of NF- B, thus activating the NF- B pathway. Shown on the right side of the figure is the RASSF1–mammalian STE20-like protein kinase 1 (MST1)–forkhead box O3 (FOXO3) pathway. Activated by extracellular pro-apoptotic stimuli through membrane receptors, RASSF1A activates MST1. Activated MST1 then phosphorylates FOXO3 on Ser207. The resulting phosphorylated FOXO3 becomes dissociated from 14-3-3 proteins in the cytoplasm. 14-3-3 proteins undergo proteasomal degradation, and phosphorylated FOXO3 enters the nucleus to promote the expression of pro-apoptotic genes in the FOXO pathway. BRAF-V600E directly interacts with and inhibits MST1 and prevents its activation by RASSF1A, thereby

suppressing the pro-apoptotic signalling of the FOXO3 pathway. The downward arrow for the FOXO activities shown in the nucleus indicates this negative effect of BRAF-V600E on pro-apoptotic genes, which are normally upregulated by the RASSF1A–MST1–FOXO3 pathway. The triple independent coupling of BRAF-V600E to the pathways shown here represents a unique and powerful mechanism of thyroid tumorigenesis driven by BRAF-V600E. *DAPK1*, death-associated protein kinase 1; *HIF1A*, hypoxia-inducible factor 1 □; MMP, matrix metalloproteinase; *NIS*, sodium–iodide symporter; *TGFB1*, transforming growth factor □; *TIMP3*, tissue inhibitor of metalloproteinases 3; *TPO*, thyroid peroxidase; *TSHR*, thyroid-stimulating hormone receptor; *TSP1*, thrombospondin 1; *UPA*, urokinase plasminogen activator; *UPAR*, urokinase plasminogen activator receptor; *VEGFA*, vascular endothelial growth factor A.

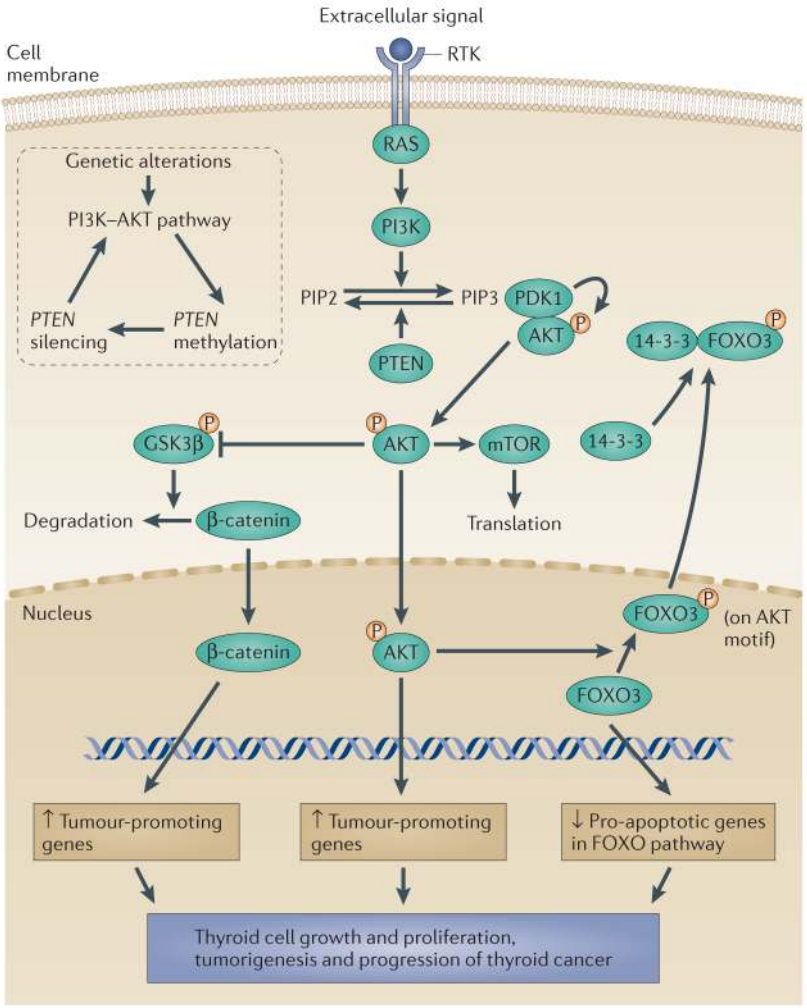

Figure 2. The PI3K–AKT and related pathways in thyroid cancer

Extracellular signals activate receptor tyrosine kinases (RTKs) in the cell membrane, leading to the activation of RAS and subsequent activation of PI3K. Activated PI3K catalyses the conversion of phosphatidylinositol (4,5)-bisphosphate (PIP2) to phosphatidylinositol (3,4,5)-trisphosphate (PIP3). PIP3 activates 3-phosphoinositide-dependent protein kinase 1 (PDK1; also known as PDPK1), which consequently associates with AKT and leads to phosphorylation (P) and activation of AKT by PDK1. Phosphorylated AKT, which is an activated form of AKT, enters the nucleus where it induces tumour-promoting genes. In the cytoplasm, phospho-AKT also activates other signalling molecules or pathways, a prominent one being the mTOR pathway, which has an important role in tumorigenesis by promoting translation. Phospho-AKT can also directly phosphorylate glycogen synthase kinase 3 (GSK3) and consequently inactivates it. GSK3 normally inhibits catenin, thus the effect of phospho-AKT is to relieve GSK3 mediated suppression of catenin. Consequently, catenin can enter the nucleus where it promotes the expression of tumour-promoting genes. In the nucleus, phospho-AKT can phosphorylate forkhead box O3 (FOXO3) on its AKT-specific motif. Such phosphorylated FOXO3 is translocated out of the nucleus to the cytoplasm where it binds 14-3-3 proteins to be sequestered in the cytoplasm, thus terminating the pro-apoptotic activities of the FOXO3 pathway. The downward arrow for the FOXO activities in the nucleus in the figure indicates this negative effect of AKT on pro-apoptotic genes in the FOXO pathway, which would otherwise be upregulated by the

FOXO3 pathway. This unique coupling of phospho-AKT to the three pathways provides a powerful driving force for thyroid tumorigenesis. The major negative regulatory mechanism of the PI3K–AKT pathway is PTEN, which is a phosphatase that converts PIP3 to PIP2, thus terminating the activation of the pathway. The inset shows the self enhancement mechanism of PI3K–AKT signalling in which genetic-alteration-driven activation of the pathway causes *PTEN* methylation and silencing with consequent loss of termination of the signalling, thus maintaining the pathway in full and constitutive activation.

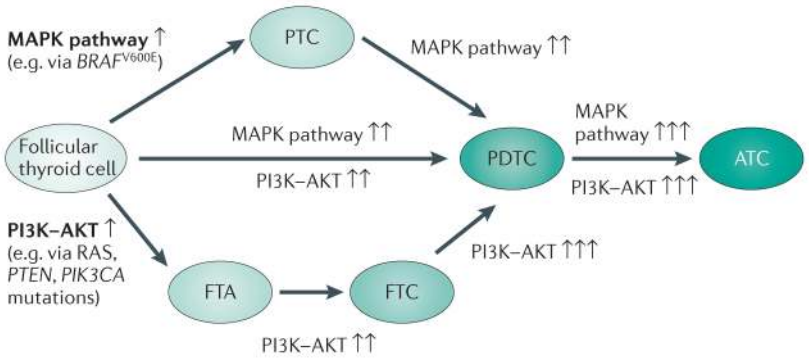

Figure 3. Model of the progression of thyroid tumorigenesis driven by the MAPK and PI3K–AKT pathways

Activation of the MAPK pathway by genetic alterations, such as the *BRAF*^{V600E} mutation, primarily drives the development of papillary thyroid cancer (PTC) from follicular thyroid cells. By contrast, activation of the PI3K–AKT pathway by genetic alterations, such as mutations in RAS, *PTEN* and *PIK3CA*, primarily drives the development of follicular thyroid adenoma (FTA) and follicular thyroid cancer (FTC) from follicular thyroid cells. Conversion from FTA to FTC is largely due to increasing activation of the PI3K–AKT pathway. As genetic alterations accumulate and intensify the signalling of each of the two pathways, PTC and FTC can progress to poorly differentiated thyroid cancer (PDTC). When both pathways are fully activated through accumulated genetic alterations, conversion from PDTC to anaplastic thyroid cancer (ATC) is strongly facilitated. It is also possible that PDTC and ATC can both develop *de novo* directly from follicular thyroid cells, and that ATC can develop from PTC or FTC if appropriate genetic alterations occur. Many secondary molecular alterations also progressively accumulate and synergize with the two pathways in driving the progression of thyroid tumorigenesis, as discussed in the text (not shown in the figure). The increasing number of vertical arrows and colour intensity of the ovals symbolize the increasing genetic alterations and signalling of the two pathways as thyroid tumorigenesis progresses. The figure is modified from REF.33 © (2007) American Association for Cancer Research.

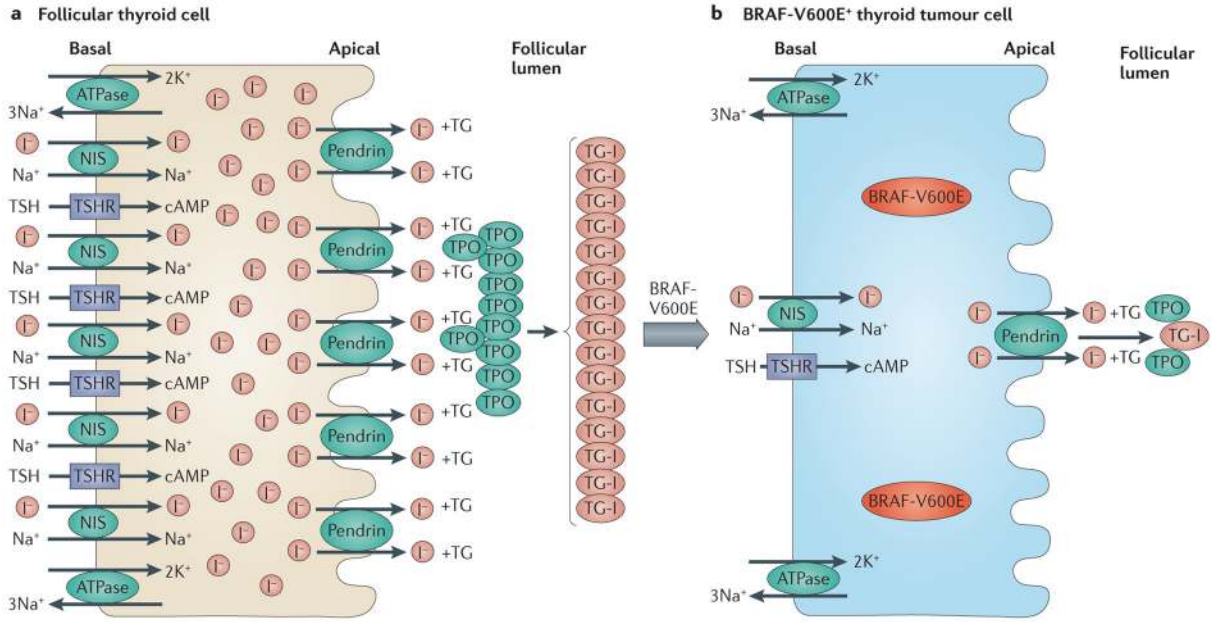

Figure 4. Iodide-handling machinery in the thyroid cell and its silencing by BRAF-V600E

a | A follicular thyroid cell is shown, which abundantly expresses the molecules involved in the uptake and metabolism of iodide (I^-), including the sodium–iodide symporter (NIS) in the basal membrane, which transports I^- coupled with Na^+ into the cell from the extracellular compartment. I^- is then transported into the follicular lumen via pendrin in the apical membrane where it is oxidized by thyroid peroxidase (TPO) and incorporated into tyrosine amino-acid residues in thyroglobulin (TG) to form iodinated TG (TG-I) for the synthesis of thyroid hormone. The whole process is upregulated by cyclic AMP (cAMP) signalling that is triggered by the binding of thyroid-stimulating hormone (TSH) to its receptor (TSHR) in the membrane. With normal expression and function of this system, I^- is abundantly taken up and accumulated in the follicular thyroid cell and in the follicular lumen. **b** | BRAF-V600E, through activating the MAPK pathway in thyroid cancer, causes the silencing of thyroid-specific genes and shuts off the iodide-handling machinery. Consequently, I^- uptake is reduced in the thyroid cell and is sparsely accumulated in the follicular lumen. For simplicity, much molecular detail is omitted.

Table 1

Thyroid tumours and their characteristics

Tumour type	Cell of origin	Prevalence (% of thyroid cancers)	Standard care and prognosis	Characteristics
FTA	Follicular thyroid cells (which produce thyroid hormone and thyroglobulin)	This is a benign lesion	Conservative monitoring; thyroidectomy if symptomatic	Common benign thyroid tumour; similar architecture to FTC, but typically encapsulated; lacking capsular or vascular invasion; lacking metastasis; lacking nuclear features of PTC
PTC *	Follicular thyroid cells	80–85	Thyroidectomy and, in selected cases, radioiodine ablation (novel drugs for resistant disease); good overall prognosis	Well differentiated, with papillary architecture and characteristic nuclear features that include enlargement, oval shape, elongation, overlapping and clearing, inclusions and grooves; propensity for lymphatic metastasis; PTC subtypes include conventional PTC (CPTC), follicular-variant PTC (FVPTC), tall-cell PTC (TCPTC) and a few rare variants
FTC *	Follicular thyroid cells	10–15	Thyroidectomy and radioiodine ablation (novel drugs for resistant cases); good overall prognosis	Well differentiated, hypercellular, microfollicular patterns, lacking nuclear features of PTC; vascular or capsular invasion; propensity for metastasis via the blood stream; Hürthle cell thyroid cancer is a unique subtype of FTC that accounts for 2–3% of thyroid cancers and is characterized by large, mitochondria-rich oncocytic cells and dense nuclei and nucleoli, as well as a high propensity for metastasis and a poor prognosis
PDTC	Follicular thyroid cells	5–10	Surgery, radioiodine (in selected cases), chemotherapy, radiotherapy, novel drugs; poor prognosis	Poorly differentiated, often overlapping with PTC and FTC; intermediate aggressiveness between differentiated and undifferentiated thyroid cancers
ATC	Follicular thyroid cells	2–3	Surgery, chemotherapy, radiotherapy, novel drugs, palliative care; highly and rapidly lethal	Undifferentiated; admixture of spindle, pleomorphic giant and epithelioid cells; extremely invasive and metastatic; highly lethal; may occur *de novo* or derive from PTC, FTC or PDTC
Medullary thyroid cancer	Parafollicular C cells (which produce calcitonin)	2–3	Surgery, chemotherapy, radiotherapy, novel drugs (for example, vandetanib)	Moderate aggressiveness, high propensity for lymphatic metastasis; *RET* mutation; occurring in familial, MEN2 or sporadic forms
Primary lymphoma of the thyroid gland	Lymphocytes	<1	Chemotherapy	Unusual and uncommon type of lymphoma
Metastatic cancer from other organs	Non-thyroid origin	<1	Thyroidectomy in selected cases; treatment of original cancer	Most commonly metastasized from renal and breast cancers; characteristics of original cancer

ATC, anaplastic thyroid cancer; FTA, follicular thyroid adenoma; FTC, follicular thyroid cancer; MEN2, multiple endocrine neoplasia type 2; PDTC, poorly differentiated thyroid cancer; PTC, papillary thyroid cancer.

* PTC and FTC are collectively classified as differentiated thyroid cancer (DTC).

Table 2

Gene mutations in thyroid tumours

Mutations	Types of thyroid tumours	Approximate prevalence (%)*	Primary signalling pathways affected	Functional impact on the protein and tumour	Refs
BRAF^{V600E}	CPTC	45	MAPK	Activating; promoting tumorigenesis, invasion, metastasis, recurrence and mortality	6–12, 16–18
	FVPTC	15			
	TCPTC	80–100			
	ATC	25			
BRAF^{K601E}	FVPTC	5	MAPK	Activating; probably similar to *BRAF*^{V600E}	14
HRAS, *KRAS*, *NRAS*	FTA	20–25	MAPK and PI3K–AKT	Activating; promoting tumorigenesis, invasion and metastasis of PDTC and FTC	26,31,33, 35,148, 184–187, 206–211
	FTC	30–45			
	FVPTC	30–45			
	PDTC	20–40			
	ATC	20–30			
PTEN (mutation)	FTA	0	PI3K–AKT	Inactivating the gene but activating the PI3K pathway; promoting tumorigenesis and invasiveness	26,31–33, 212
	FTC	10–15			
	ATC	10–20			
	PTC	1–2			
PTEN (deletion)	FTC	30	PI3K–AKT	Inactivating the gene but activating the PI3K pathway; promoting tumorigenesis and invasiveness	212
PIK3CA	FTA	0–5	PI3K–AKT	Activating; promoting tumorigenesis and invasiveness	25,26, 31–35
	FTC	5–15			
	ATC	15–25			
	PTC	1–2			
AKT1	Metastatic cancer	15	PI3K–AKT	Unclear; seems to favour metastasis	35
CTNNB1	PDTC	25	WNT– catenin	Activating; promoting tumour progression	37,38
	ATC	60–65			
TP53	PDTC	25	p53-coupled pathways	Inactivating; promoting tumour progression	39,40
	ATC	70–80			

Mutations	Types of thyroid tumours	Approximate prevalence (%)*	Primary signalling pathways affected	Functional impact on the protein and tumour	Refs
IDH1	FTC	5–25	IDH1-associated metabolic pathways	Inactivating; impact on tumours is unclear	41,42
	FVPTC	20			
	CPTC	10			
	ATC	10–30			
ALK	ATC	10	MAPK and PI3K–AKT	Activating; probably promoting tumour progression	43
EGFR	CPTC	5	MAPK and PI3K–AKT	Activating; impact on tumours is unclear	44
NDUFA13 (also known as *GRIM19*)	HCTC	15	Component of complex I of the mitochondrial respiratory chain	Presumably inactivating; affecting mitochondrial metabolism and cell death	45

ALK, anaplastic lymphoma kinase; ATC, anaplastic thyroid cancer; CPTC, conventional PTC; *CTNNB1*, catenin; *EGFR*, epidermal growth factor receptor; FTA, follicular thyroid adenoma; FTC, follicular thyroid cancer; FVPTC, follicular-variant PTC; HCTC, Hürthle cell thyroid cancer; *IDH1*, isocitrate dehydrogenase 1; *NDUFA13*, NADH dehydrogenase (ubiquinone) 1 subcomplex 13; PDTC, poorly differentiated thyroid cancer; PTC, papillary thyroid cancer; TCPTC, tall-cell PTC.

* The values represent estimated overall prevalence of the indicated mutations.

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Table 3

Copy-number gains in thyroid cancer*

Gene	Prevalence in ATC cases (%)	Prevalence in FTC cases (%)
EGFR	19/41 (46.3)	19/59 (32.2)
PDGFRA	11/46 (23.9)	4/52 (7.7)
PDGFRB	14/37 (37.8)	8/59 (13.6)
VEGFR1	20/44 (45.5)	26/59 (44.1)
VEGFR2	8/46 (17.4)	2/52 (3.8)
KIT	10/46 (21.7)	6/61 (9.8)
MET	5/42 (11.9)	5/58 (8.6)
PIK3CA	18/47 (38.3)	15/63 (23.8)
PIK3CB	16/42 (38.1)	25/55 (45.5)
PDPK1 (also known as *PDK1*)	8/40 (20)	14/58 (24.1)
AKT1	9/48 (18.8)	5/61 (8.2)
AKT2	0/44 (0)	13/58 (22.4)

ATC, anaplastic thyroid cancer; *EGFR*, epidermal growth factor receptor; FTC, follicular thyroid cancer; *PDGFR*, platelet-derived growth factor receptor; *PDPK1*, 3-phosphoinositide dependent protein kinase 1; *VEGFR*, vascular endothelial growth factor receptor.

*From REF. 26. Copy-number gains of some of these genes in thyroid cancers were also investigated in the studies reported in REFS 25,31–33, which showed comparable prevalences.