Pathogenesis of cancers derived from thyroid follicular cells

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Abstract

The genomic simplicity of differentiated cancers derived from thyroid follicular cells offers unique insights into how oncogenic drivers impact tumour phenotype. Essentially, the main oncoproteins in thyroid cancer activate nodes in the receptor tyrosine kinase–RAS–BRAF pathway, which constitutively induces MAPK signalling to varying degrees consistent with their specific biochemical mechanisms of action. The magnitude of the flux through the MAPK signalling pathway determines key elements of thyroid cancer biology, including differentiation state, invasive properties and the cellular composition of the tumour microenvironment. Progression of disease results from genomic lesions that drive immortalization, disrupt chromatin accessibility and cause cell cycle checkpoint dysfunction, in conjunction with a tumour microenvironment characterized by progressive immunosuppression. This Review charts the genomic trajectories of these common endocrine tumours, while connecting them to the biological states that they confer.

Introduction

Cancers derived from thyroid follicular cells are classified into five main types: papillary thyroid carcinoma (PTC) (representing 65–93% of all thyroid cancers worldwide)[1], follicular thyroid carcinoma (FTC) (6–10%)[2–4], oncocytic thyroid carcinoma (OC) (3–7%)[5], poorly differentiated thyroid carcinoma (PDTC) (0.5–2%)[6] and anaplastic thyroid carcinoma (ATC) (1%)[7]. PTC, FTC and OC are generically termed differentiated thyroid carcinomas (DTCs), most patients with which present with localized disease and have a 5-year survival rate of >98% (ref. 8), by contrast to PDTC, which has a 5-year survival rate of 76% (ref. 9). The 2022 WHO classification of thyroid tumours introduced a new intermediate clinical entity — differentiated high-grade thyroid carcinoma — to define DTCs with a high mitotic rate and/or tumour necrosis, as these have a 5-year survival rate comparable with that of PDTC[9,10]. ATC is an extremely aggressive form of the disease. Until recently, patients with ATC had a dismal median overall survival of 4 months, although this has improved markedly since 2018 with the FDA approval of new oncoprotein-targeted treatments coupled

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to evidence that immunotherapies may confer additional benefit[11–13]. Thyroid parafollicular or C cells are a neuroendocrine lineage that gives rise to medullary thyroid cancers, which account for <5% of all thyroid cancers. This is a distinct entity that has been recently reviewed elsewhere[14,15] and will not be discussed further in this Review.

The incidence of thyroid cancer in the USA was approximately 14.6 per 100,000 in 2022, with an estimated total of 43,800 cases[8]. Furthermore, there had been a steep rise in thyroid cancer incidence in the past three decades mostly owing to overdiagnosis of small PTCs, part of a large subclinical reservoir of tumours that would have otherwise mostly not caused disease or mortality[16]. However, incidence rates since 2013 have stabilized and may have even begun to fall[8], probably owing to greater awareness of the dangers of overdiagnosis, as well as a recent reassessment by pathologists of the histological features that merit a diagnosis of thyroid cancer[17–19]. Active surveillance of patients with PTCs measuring <1 cm, termed papillary microcarcinomas, shows that most do not grow after prolonged monitoring, particularly in older subjects[20,21]. The genomic and transcriptomic characterization of papillary microcarcinomas has so far not identified distinct differences with larger tumours that progress or metastasize to regional lymph nodes[22]. Whether the trend towards quiescence of many PTCs is due to cell autonomous events, such as oncogene-induced senescence[23,24], or to host effects, is unknown.

Constitutively active mutant effectors in the MAPK pathway are the major drivers of thyroid cancers across the entire spectrum of the disease with the notable exception of OCs (Box 1). Since the topic of thyroid cancer pathogenesis was last reviewed in depth[7,25,26], there has been an explosion of new information on the genomics of all forms of sporadic[2] as well as radiation-induced thyroid cancer. Comprehensive next-generation sequencing studies of PDTC and ATC performed in the past decade show that these aggressive tumours arise from DTCs through the acquisition of mutations that drive tumour immortalization, activate the PI3K pathway, disable key cell cycle checkpoints and/or disrupt chromatin[27–30]. This is supported by evidence that genomic lesions that are clonal in advanced disease can be found in rare subsets of DTCs as subclonal events[28,31]. How each of these 'disease progression' pathways alter tumour biology and, in some cases, therapeutic dependencies are a major focus of this Review.

Thyroid cells are highly specialized, with their primary function being the biosynthesis and secretion of the thyroid hormones. This exquisitely regulated process is crucial for organismal homeostasis and, in essence, endows thyroid cells with the capacity to actively transport and incorporate iodide into thyroid hormones[32]. As a consequence, radioactive iodine (RAI) therapy is the most prescribed treatment for thyroid cancer, and its efficacy is contingent on the cancer cells retaining some ability to metabolize iodine. The genetic drivers of thyroid cancer impinge on this process in distinct ways, which is elaborated on in this Review. Although we do not address the recent therapeutic advances in detail, we discuss key therapeutic vulnerabilities and how thyroid cancer cells adapt to the selective inhibition of the common truncal drivers of the disease, as well as present new information on the transcriptional and compositional heterogeneity of these tumours, which is central to understand the complex interactions that drive thyroid cancer pathogenesis.

Genetic predisposition to thyroid cancer

Although more than 90% of thyroid cancers are sporadic[33], genome-wide association studies of large cohorts identified low penetrance genetic variants associated with non-medullary thyroid cancers, most with moderate effect sizes[34,35]. Among all cancer types, those of the thyroid have among the highest genetic risk, which in Icelandic and European populations extends beyond the nuclear family[36]. Genetic variants on chromosomes 9q22.33 and 14q13.3 show the highest association in Europeans[34,37] and were also linked with thyroid cancer in Korean populations[38]. The gene encoding the thyroid-specific transcription factor forkhead box E1 (*FOXE1*) is positioned close to the variants on 9q22.33. Single nucleotide polymorphisms (SNPs) in and around the *FOXE1* gene were also strongly associated with thyroid cancer in Spanish and Italian populations[37], as well as in patients with radiation-induced thyroid cancer[39]. Notably, a risk variant within the *FOXE1* 5′-untranslated region impacts *FOXE1* transcription by recruiting a transcriptional complex that is distinct from that which binds to the wild-type allele[37]. Moreover, NK2 homeobox 1 (*NKX2-1*), which also encodes a thyroid lineage transcription factor, is within a gene block lying in proximity to the other main susceptibility locus on 14q13.3. The 9q22.33 and 14q13.3 SNPs are also associated with low serum levels of thyroid-stimulating hormone (TSH; also known as thyrotropin)[34]. Hence, these thyroid cancer susceptibility alleles may impact the TSH signalling axis in thyroid cells through perturbations in the regulatory control of key lineage transcription factors.

The strongest association with thyroid cancer in Korean populations has been identified with variants of the neuregulin-1 (*NRG1*) gene on 8p12 (ref. 38), also identified in Europeans[35]. Consistent with this observation, NRG1 expression is increased in thyroid cancer tissues harbouring the risk variant alleles[38]. NRG1 is the ligand for human epidermal growth factor receptor 3 (HER3; also known as ERBB3), a member of the epidermal growth factor family of receptor tyrosine kinases (RTKs), which, as discussed later in this Review, has a central role in adaptive resistance of thyroid cancers to MAPK pathway inhibitors[40,41].

Predisposition to familial non-medullary thyroid carcinoma is common, with up to 9% of patients having a first-degree relative with the disease[33]. Infrequently, they may arise as components of recognized cancer predisposition syndromes conferred by pathogenic germline mutations of *PTEN*, adenomatous polyposis coli (*APC*), *DICER1*, which encodes an endoribonuclease, *PRKAR1A*, which encodes the regulatory R1α subunit of the cAMP-dependent protein kinase A (PKA) or *WRN*, which encodes the Werner syndrome helicase (Table 1) (reviewed elsewhere[42,43]). More commonly, the familial susceptibility occurs as an isolated event only impacting risk of thyroid cancer. However, owing to the high frequency of microcarcinomas in the general population, co-occurrence within families may be a fortuitous event rather than one determined by genetic predisposition. Numerous candidate low penetrance susceptibility variants have been identified in families with familial non-medullary thyroid carcinoma[33,42,44]. This information is not yet clinically applicable for screening purposes because most variants have not been fully validated and the segregation of variant alleles within families is confounded by the low penetrance of the disease[42].

Radiation-induced thyroid cancer

Exposure to ionizing radiation during childhood increases the risk of developing PTC later in life, as demonstrated by the strong dose–response relationship between radiation dose to the thyroid and thyroid cancer incidence in individuals exposed to radioiodine isotopes during childhood following the Chernobyl nuclear reactor accident[45]. Whole-genome sequencing of a large series of post-Chernobyl thyroid cancers showed strong radiation dose-dependent increases in clonal small deletions and simple and balanced structural variants bearing genomic hallmarks of DNA repair by non-homologous end joining. By contrast, there was no impact of radiation on the frequency of single nucleotide substitutions[46]. Consistent with this, there was a radiation dose-dependent increase in the frequency of fusions versus driver mutations[46], primarily of *RET*, neurotrophic tyrosine kinase receptor 1 (*NTRK1*), *NTRK3*, *BRAF* and anaplastic lymphoma kinase (*ALK*), as previously reported in smaller cohorts of radiation-exposed patients[47–50]. Indeed, *RET* fusions can be induced by exposure of human thyroid cells to ionizing radiation in vitro[51]. The propensity for radiation to induce *RET* rearrangements may be facilitated by the close spatial proximity of the chromosomal regions involved in the recombination events during interphase in normal human thyroid cells[52]. Contrary to previous reports[53,54], there is no evidence for a transcriptomic signature of radiation-induced thyroid cancer. Gene expression and epigenomic changes show strong associations with the tumour driver but not with radiation-dose exposure[46].

Cell of origin of thyroid cancers

Two major competing hypotheses have been advanced to define the subpopulation of cells that can give rise to the different histological types of thyroid cancer. A multistep carcinogenesis model suggests that undifferentiated thyroid carcinomas derive from well-differentiated tumours via the sequential accumulation of genetic mutations[55]. Alternatively, thyroid cancer cells are proposed to originate from transformation of fetal thyroid cells at various stages of differentiation, with ATC, PTC and FTC arising from thyroid stem cells, thyroblasts and pro-thyrocytes, respectively[56]. A recent study investigated the transformation efficiency of thyroid cancer oncogenes introduced by CRISPR–Cas9 editing into either thyroid progenitor cells (TPCs) or mature thyrocytes derived from human embryonic stem cells and found that oncoproteins transformed TPCs with much greater efficiency than mature thyrocytes[57]. Although these data suggest that thyroid progenitors may be more susceptible to transformation, they do not negate the need for sequential genetic events for disease progression, as ATCs developed only in the TPCs when *TP53* was co-mutated with either *BRAF* or RAS[57].

Drivers of differentiated thyroid cancers

PTC and FTC are prototypical cancers driven by oncoproteins that signal in part through the canonical MAPK pathway. Mutations of genes encoding proteins in this pathway are almost invariably clonal and drive the majority of DTCs, including 89% of PTCs[58,59]. However, a subset of tumours harbour oncoproteins that induce tumorigenesis through

distinct mechanisms. Although they are comparatively infrequent, they provide unique insights into the biology of thyroid cancer and are also discussed.

Thyroid tumorigenesis by mutant effectors of the MAPK pathway

The Cancer Genome Atlas (TCGA) study of PTC showed that 59% of the 496 samples harboured activating *BRAF* mutations, almost all resulting in the V600E substitution[58]. Consistent with previous reports[60,61], these were mutually exclusive with RAS mutations, which were present in 13% of tumours (*NRAS* ≫ *HRAS* > *KRAS*) and with the 16% of cases harbouring fusion oncogenes, primarily of the RTKs *RET*, *NTRK3*, *NTRK1* and *ALK*[58]. Generically, these recombinant fusion oncoproteins consist of N-terminal domains contributed by the upstream gene partner, coupled to the kinase domain of the respective RTK[62]. The N-terminal fragments usually contain coiled-coil motifs that drive dimerization and autophosphorylation of tyrosine residues in the kinase domain, leading to constitutive downstream signalling. *BRAF* was also activated through gene fusions in 2.3% of tumours, some of which were confirmed to selectively overexpress the BRAF kinase domain[58]. RTK fusions are particularly prevalent in sporadic paediatric PTCs[63].

PTCs have a low overall frequency of non-synonymous somatic mutations (0.41 per Mb) when compared with other cancer types[64,65]. Moreover, >70% of PTCs lack copy number gains or losses[58,66]. The overall genomic simplicity of PTCs allows for remarkably clear associations between driver mutations and gene expression profiles, signalling pathway transcriptional outputs, proteomic changes, differentiation states and histological characteristics. Although both RAS mutants and BRAF-V600E activate the MAPK pathway, they do so to different degrees. Mutant RAS constitutively signals through RAF dimers, which are subject to negative feedback by ERK, resulting in an attenuation of the flux through the pathway. By contrast, BRAF-V600E signals as a monomer and is partially unresponsive to negative feedback by ERK, resulting in a higher MAPK signalling pathway output[67]. Consistent with this, *BRAF*-mutant PTCs are transcriptionally distinct from their RAS-mutant counterparts[58]. This prompted the creation of a transcriptional score to distinguish them from each other – the BRAF–RAS score (BRS), which is a 71-gene signature that quantifies the extent to which a particular tumour transcriptionally resembles either a $BRAF^{V600E}$-mutant or a *RAS*-mutant tumour[58]. There is a highly significant correlation between the BRS and a thyroid differentiation score (TDS), which is a set of 16 genes that encode proteins that regulate iodine metabolism: that is, RAS-like tumours have a higher TDS than $BRAF^{V600E}$-like tumours[58] (Fig. 1). Furthermore, the application of the BRS and the TDS to thyroid cancers driven by other oncoproteins can be instructive, as tumours with a high TDS tend to respond to RAI therapy[68]. Genetically engineered mouse models of $Braf^{V600E}$-induced thyroid cancer closely phenocopy the histological characteristics of human PTC, have a high MAPK pathway transcriptional output and are associated with a marked inhibition of differentiated thyroid function, manifesting as a decrease in iodine uptake and thyroid hormone biosynthesis[69–71]. RAS-like PTCs mostly retain the structure of the thyroid follicle, a characteristic feature of the non-infiltrative follicular variant of PTC (FV-PTC), which tend to be encapsulated and rarely develop nodal metastases[72]. By contrast, $BRAF^{V600E}$-like tumours have the classical or tall cell variant (TCV) histology, are frequently invasive and have a tropism to regional lymph nodes[58].

The association of BRAF-V600E with tumour invasiveness may also be a consequence of its higher MAPK signalling flux, which leads to transcriptional activation of genes encoding proteins involved in extracellular matrix (ECM) remodelling, including matrix metalloproteinase 3 (MMP3), MMP9 and MMP13 (ref. 73). Similar relationships among BRAF-V600E, ERK signalling, invasiveness and expression of MMP1 have been shown in human melanomas[74,75]. It is also plausible that the degree of pathway flux may be a determining factor in cell transformation. Activation of high output MAPK signalling through endogenous expression of $Braf^{V600E}$ or overexpression of RET and $NTRK1$ fusions is sufficient to drive thyroid tumorigenesis in mice[69,70,76–78] and zebrafish[79]. By contrast, thyrocyte-specific endogenous expression of $Hras^{G12V}$ (ref. 80) or $Kras^{G12D}$ (ref. 81) in mice induces cell hyperplasia and requires cooperative events to induce transformation[80–84].

TCV-PTC is a distinct subtype of DTC that has a disease-specific survival rate of 82%, when compared with a population of stage-matched and treatment-matched classical PTC, which has a disease-specific survival rate of 98% (ref. 85). This subtype almost universally harbours the BRAF-V600E mutant, but by contrast to classical PTC, they have abundant mitochondria with a high frequency of homoplasmic or heteroplasmic mutations in mitochondrial DNA-encoded genes for oxidative phosphorylation (OXPHOS) complex I subunits, analogous to those present in OC[86] (Box 1). How putative OXPHOS dysfunction may modify BRAF-V600E-induced PTC tumorigenesis is unknown.

Drivers in the TSH receptor–cAMP signalling pathway

Thyroid cells are under the regulatory control of TSH for growth and expression of genes required for thyroid hormone biosynthesis[87]. The TSH receptor (TSHR) is a G protein-coupled seven-transmembrane receptor, which upon binding to its ligand engages with multiple G protein subtypes[88]. Signalling through $G_s\alpha$ has a dominant role in mediating TSH action on cell growth and gene expression by activating adenylyl cyclase and the cAMP signalling pathway[89]. This is best exemplified by activating mutations of $TSHR$[90,91] or $GNAS$[92], the gene encoding $G_s\alpha$, which gives rise to autonomously functioning thyroid nodules (AFTNs) that, when they reach a critical size, cause hyperthyroidism. Furthermore, these gain-of-function mutations in $TSHR$ and $GNAS$, which activate adenylyl cyclase, are genetic hallmarks of AFTN, consistent with the stimulatory effects of cAMP on cell growth and thyroid hormone biosynthesis. AFTNs are usually benign and rarely progress to thyroid cancer[93]. However, activating $TSHR$ mutations are present in 0.5% of PTCs[58] and 10% of FTCs[94]. TSHR-mutant FTCs may cause hyperthyroidism, particularly those with a high metastatic burden[95,96]. Preliminary evidence from a small series of patients suggests that a high mutant $TSHR$ allelic ratio may favour malignant transformation[95]. Interestingly, a recurrent activating mutation of the enhancer of zeste homologue 1 ($EZH1$) gene, which encodes a catalytic subunit of the chromatin remodelling polycomb repressor complex 2 (PRC2), is co-mutated with $TSHR$ or $GNAS$ in a subset of AFTNs[97]. Expression of the EZH1-G571R mutant promotes histone H3 lysine 27 (H3K27) trimethylation and induces cyclin D1 expression and ultimately thyroid cell growth. It is unclear, however, whether the co-mutation pattern is enriched in FTCs[97].

Germline loss-of-function mutations of *PRKAR1A* confer predisposition to benign and malignant thyroid tumours, as well as tumours of other endocrine cell types as part of the Carney complex[98] (Table 1). Loss of function of this regulatory protein results in decreased basal activity of PKA, but markedly increases cAMP-stimulated PKA levels[98]. Thyroid-specific homozygous deletion of *Prkar1a* in mice results in a high penetrance of FTC and hyperthyroidism[99]. Despite this, to our knowledge, no somatic mutations of *PRKAR1A* have been reported in PTC[58] or FTC[94] to date. Isolated cases of PDTC and ATC with somatic truncation mutations or deep deletions of *PRKAR1A* have been reported, but their role in transformation has not been firmly established[100,101].

Interactions between the MAPK and cAMP signalling pathways in thyroid cancer

Constitutive MAPK pathway activation through *RET* fusions or BRAF-V600E disrupts the TSHR–adenylyl cyclase–PKA signalling axis at multiple nodes, primarily by decreasing the expression of TSHR and inhibiting the catalytic activity of adenylyl cyclase[24,102] (Fig. 2). This is the simplest explanation for the significant inverse correlation between TDS and BRS in PTCs from the TCGA study[58]. The functional corollary of this reciprocal relationship is that it can be reversed by RAF and/or MEK inhibitors in cell lines[103,104], *Braf* V600E-induced PTCs in mice[31,71,105] and in subsets of patients with *BRAF*[V600E] or RAS-mutant thyroid cancers[106–108], leading to a rescue of expression of differentiated thyroid genes. This includes genes encoding the thyroid lineage transcription factors paired box 8 (PAX8) and forkhead box E1 (FOXE1) and the key genes they regulate, that is, sodium iodide symporter (*NIS*), dual oxidase 1 (*DUOX1*) and *DUOX2* and thyroid peroxidase (*TPO*), the protein product of which is required for iodine oxidation and incorporation into tyrosine residues of thyroglobulin (*TG*). As this gene expression programme governs the ability of thyroid cancer cells to incorporate radioactive iodine, treatment of *RET*-mutant, *NTRK*-mutant, *RAF*-mutant and RAS-mutant thyroid cancers with selective MAPK pathway inhibitors has been shown to enhance clinical responses to iodine-131 in pilot phase II clinical trials and in index cases[106–112].

Other inputs may have important roles in mediating the effects of MAPK pathway activation on thyroid-specific functional properties. For instance, transforming growth factor β (TGFβ) also impairs TSH-induced expression of the thyroid differentiation genes *Tg* and *Nis* in rat thyroid cell lines[113,114]. Oncogenic BRAF indirectly leads to the engagement of TGFβ or activin signalling[115], driven in part through activation of a TGFβ autocrine loop[114]. This results in phosphorylation of SMAD, which binds to PAX8 and impairs its transactivation of *Nis*, and presumably of other PAX8-regulated genes required for thyroid hormone biosynthesis[116]. This circuit is reversed by treatment with potent MEK inhibitors, whereas TGFβ and/or activin receptor antagonists are insufficient to rescue thyroid differentiation in *Braf*[V600E]-driven PTCs in mice[115], indicating that the impairment of differentiated thyroid function by constitutive MAPK signalling is in part independent of SMAD activation.

Genomic fusions of nuclear receptors and lineage transcription factors

Approximately 1% of PTCs and 8–30% of FTCs are driven by *PAX8*–peroxisome proliferator-activated receptor –(*PPARG*) fusions[94,117], which consist of the coding region of all but the extreme C terminus of the thyroid lineage transcription factor PAX8, fused

to the entire coding sequence of PPARG, a nuclear receptor that among other functions governs the gene expression programme of adipogenesis[118]. Targeted expression of the *PAX8–PPARG* fusion to thyroid cells of transgenic mice induces thyroid cell hyperplasia, whereas in the context of homozygous *Pten* loss, the PAX8–PPARG fusion drives locally invasive thyroid cancers with lung metastases[119].

The PAX8 and PPARG components of the fusion retain their respective DNA-binding domains and appear to activate distinct transcriptional programmes. PAX8–PPARG chromatin immunoprecipitation-sequencing of mouse $PAX8–PPARG^{Thy}$;$Pten^{Thy-/-}$ cancers showed that binding sites were enriched for motifs of both PAX8 and PPARG, commonly in close vicinity to transcription start sites[120]. The PPARG component of PAX8–PPARG likely accounts for the regulation of genes involved in lipid metabolism, cell cycle and motility. Interestingly, treatment of tumour-bearing $PAX8–PPARG^{Thy}$;$Pten^{Thy-/-}$ mice with the PPARG agonist pioglitazone induced transdifferentiation of cancer cells into adipocytes and a profound reduction in tumour size[119]. Accordingly, a patient with PAX8–PPARG-driven metastatic thyroid cancer had an impressive response to this PPARG agonist[121]. Dysregulated expression of PPARG has also been described in a radiation-induced PTC and a sporadic FTC harbouring cAMP-responsive element-binding protein 3-like protein 2 (*CREB3L2*)–*PPARG* fusions[49,122], suggesting that overexpression of this nuclear receptor may have a key role in driving tumorigenesis in this subset of tumours.

The PAX8 component of PAX8–PPARG likely accounts for its binding to motifs adjacent to genes regulating the cellular response to hypoxia and cytoskeletal structure[120]. In this regard, PAX8 has a critical role in the determination of thyroid cell polarity and assembly of the thyroid follicle, acting primarily through regulation of cadherin-16 (*CDH16*)[123], which encodes an adhesion protein that is uniquely expressed in thyroid and kidney cells – both lineages in which PAX8 has an essential role in development[124]. Consistent with this, PAX8–PPARG-driven PTCs retain the follicular architecture of the normal thyroid gland[125]. Furthermore, the expression of PAX8 and CDH16 is higher in FV-PTCs, which retain follicular structures, than in classical and TCV-PTCs, which do not[58], suggesting that this pathway may be an important determinant of the distinctive histological architecture of the major variants of DTCs (Fig. 1).

Genomic dysregulation of mRNA translation

A subset of PTCs from the TCGA study were found to have recurrent mutations of the X chromosome copy of the gene eukaryotic translation initiation factor 1A (*EIF1AX*), a component of the 43S translation preinitiation complex[58]. They have also been described in benign thyroid adenomas and FTC[126,127]. In PTC, they are frequently the sole tumour driver, whereas in PDTC and ATC, *EIF1AX* mutations are almost invariably associated with RAS mutations[28,84,127]. *EIF1AX* mutations in thyroid cancer primarily affect the N-terminal domain of the encoded protein, as well as a splice site within the C terminus[28]. The *EIF1AX*-A113 splice mutation is found almost exclusively in thyroid cancer and is enriched in more advanced disease[28]. EIF1A mutants promote preferential translation of activating transcription factor 4 (ATF4), which orchestrates adaptive responses to cellular stress. ATF4 in turn induces the expression of the GADD34 phosphatase (also known as

protein phosphatase 1 regulatory subunit 15A), which dephosphorylates eIF2A and leads to a global increase in protein synthesis (Fig. 3). ATF4 also induces MYC, which together promotes the expression of amino acid transporters, thus increasing amino acid supply and driving activation of mTOR[84]. Mutant RAS, which as mentioned is co-mutated with *EIF1AX* in advanced thyroid cancers, stabilizes the MYC protein, likely explaining the vulnerability of RAS and *EIF1AX* co-mutated cancers to bromodomain-containing protein 4 (BRD4) inhibitors, which target *MYC* transcription[84]. Interestingly, *EIF1AX* mutations in uveal melanomas, in which they were originally described, show mutual exclusivity with *MYC* amplifications, consistent with a common requirement for transformation[128].

Thyroid cancer progression

The role of immortalization in thyroid cancer progression

The genetic drivers of DTCs described earlier likely have a role in tumour initiation, as they are clonal in early-stage DTCs and remain truncal during the evolution of the disease[58,59,129]. An important transitional step in disease progression includes genomic events that promote tumour cell immortalization, in particular the highly prevalent genetic alterations of telomerase reverse transcriptase (*TERT*)[130]. The telomerase complex is active during embryogenesis, but is silenced postnatally in somatic cells[131]. Continued cell replication requires a mechanism to repair critically shortened telomeres to protect chromosomes from undergoing end-to-end fusions, which in turn would cause telomere crisis and apoptosis[132]. Reactivation of telomerase is the most common strategy preventing this from happening in cancer cells[133]. Recurrent hotspot mutations of the *TERT* promoter were first described in patients with melanoma[134,135], and later in numerous other cancer types, including those of the thyroid[136–139]. The base substitutions in the *TERT* proximal gene promoter generate de novo consensus binding sites for the E-twenty-six (ETS) family of transcription factors, which have a substantial role in mediating the transcriptional output of the MAPK signalling pathway[135]. The ETS family members, GA-binding protein α (GABPA) and GABPB, transactivate the mutant *TERT* promoter and have been proposed to have a selective role in inducing *TERT* mRNA in thyroid cancers with coexisting *BRAF* and *TERT* promoter mutations[140]. However, GABP mRNA levels in PTC are low compared with other members of this family, and it may be that multiple ETS family members, including GABP, contribute to the transcriptional regulation of TERT[141–143]. Hypermethylation of a CpG island in the distal *TERT* gene promoter region has been implicated as a mechanism of increased TERT expression in cancer, which is in contrast to the canonical role of CpG island hypermethylation as a repressor of gene transcription[144]. A recent analysis of 23 cancer lineages, including thyroid, found that hypomethylation at a CpG region flanking the *TERT* transcription start site is also strongly associated with active transcription and bound by the histone activation marks acetylated H3K9 (H3K19ac) and H3K14ac[145,146].

Recurrent *TERT* promoter mutations, aberrant methylation and possible copy number gains are associated with worse clinical outcomes in patients with DTC[130,147]. *TERT* promoter mutations are subclonal in DTC with a prevalence of 9% and become clonal in PDTC and ATC, where they are found in 40% and 73% of cases, respectively, consistent with a key role in tumour microevolution[28]. The combination of *BRAF*[V600E] and *TERT* has been

highlighted as being particularly deleterious, although *TERT* defects are also enriched in PDTCs and ATCs harbouring RAS or RTK fusion drivers[28,29,148]. Other components of the telomerase complex, such as the telomerase RNA component, the non-coding RNA template for telomere replication, are also expressed at higher levels in advanced disease[130], consistent with the assumption that the catalytic activity of TERT is primarily involved in telomere elongation. Whether one of the proposed non-canonical effects of TERT[149] is also involved in disease progression remains to be demonstrated. There is no evidence that alternate mechanisms of telomere elongation have a major role in thyroid cancer, or that mutations that can favour this process, such as those of the α-thalassemia/mental retardation syndrome X-linked (ATRX)–death domain-associated protein 6 complex (DAXX) complex and the histone variant H3.3, are prevalent in this disease.

Genetic events associated with progression to PDTCs and ATCs

There have, to date, been no large-scale, TCGA-like efforts to investigate the integrated genomic and transcriptomic landscapes of human PDTC and ATC. Despite this, a picture of the major categories of genetic lesions associated with progression has emerged on the basis of smaller whole-genome, whole-exome or cancer-exome series[27–30,150–152].

PDTCs are characterized by a solid (sheets of neoplastic epithelial cells), trabecular ('beams') of neoplastic epithelium separated by connective tissue) or insular (tightly packed islands or nests of neoplastic epithelial cells) histological growth pattern, a high mitotic rate and areas of necrosis and vascular invasion. They metastasize at a distance in 40–70% of the cases, and by contrast they infrequently invade regional lymph nodes when compared with classical or TCV-PTCs[153]. RAS mutations are the primary oncogenic driver, present in 30–45% of the cases, with *RET* and *ALK* fusions accounting for an additional 15% (refs. 28,154). Although the precise sequence of genetic events is unclear, *TERT* promoter mutations likely enable their more aggressive phenotype, presumably by allowing the accumulation of additional genetic lesions. High-grade *BRAF*-mutant PTC with a high mitotic rate and areas of necrosis have a comparable disease-specific mortality to RAS-mutant PDTC[9]. They also have a high frequency of *TERT* genetic defects and acquire mutations in the same category of genes found in RAS-mutant PDTC, but retain histological features of PTC, tend to be locally invasive and have a tropism for regional lymph nodes.

ATCs are the most aggressive forms of thyroid cancer. Although there has been meaningful progress in treatments for patients with ATC, the 5-year survival of patients with ATC presenting with metastatic disease is less than 10% (ref. 8). This subtype are rapidly growing, locally invasive tumours that frequently have lymph node and distant metastases at presentation or that develop during the course of the disease. The histology is pleomorphic (mixtures of neoplastic epithelial and myoepithelial/stromal components in various patterns) with cells of sarcomatoid, giant cell and epithelial appearance present at varying degrees. ATCs also have a high mitotic rate and areas of necrosis[10]. Similar to DTCs, they have a high frequency of MAPK pathway driver alterations[28]. Loss-of-function mutations of *TP53* are a hallmark of the disease, such that ATC can be recapitulated with high penetrance in genetically engineered mouse models with thyroid targeted deletions of *Trp53* combined

with either activating mutations of *Braf* and *Hras* or expression of *RET* fusions[83,155–157]. The major pathways involved in disease progression are discussed subsequently.

The PI3K pathway in advanced thyroid cancer

Germline mutations of *PTEN*, which encodes a phosphatase for phosphatidylinositol 3,4,5-trisphosphate (PtdIns(3,4,5)P$_3$), and less frequently of *PIK3CA* (which encodes the PI3K catalytic subunit, p110α) and *AKT1*, can predispose affected individuals to develop thyroid neoplasms[158,159]. However, somatic mutations of these genes are not commonly involved in sporadic thyroid cancer initiation[58,94]. Thyrocyte-specific expression of *PIK3CA*[H1074R] is insufficient to induce thyroid cell transformation in mice[160]. Furthermore, homozygous loss of *Pten* increases cell proliferation and leads to goitre and development of benign thyroid adenomas in mice[161]. Interestingly, induction of TSH by treatment with methimazole and sodium perchlorate (which increase TSH secretion by decreasing thyroid hormone synthesis) failed to stimulate further growth of *Pten* null thyrocytes in vivo[161], suggesting that the proliferative effects of TSH may be mediated in part through activation of the PI3K–AKT pathway, possibly through autocrine stimulation by insulin-like growth factor 1 (IGF1)[162,163].

Nevertheless, mutations of effectors in the PI3K–AKT–mTOR pathway are more prevalent in advanced thyroid cancers (ATC > PDTC > PTC)[28,164,165]. Loss-of-function *PTEN* mutations tend to co-occur with RAS or *NF1* mutations in PDTC and ATC, whereas *PIK3CA* mutations are more commonly seen in concert with *BRAF*[V600E]. Although these combinatorial relationships are not absolute, their role in thyroid cell transformation is supported by experiments in genetically engineered mouse models. Thyroid-specific expressions of *Kras*[G12D] or *Pten* loss alone are insufficient to induce thyroid cancer, but their combined effects lead to metastatic FTCs and decreased survival[166]. By contrast, the combination of *Braf*[V600E] with either *Pten* loss or expression of *PIK3CA*[H1074R] induces a more severe phenotype, including early transition from PTC to ATC[160]. *BRAF*-mutant and *PIK3CA*-mutant human ATC cell lines show enhanced sensitivity to the combination of MEK and pan-class 1 PI3K inhibitors compared with the respective mono-therapies, indicative of novel dependencies developing during tumour progression[160]. However, combined blockade of the MAPK and PI3K pathways with selective inhibitors in patients with cancer causes substantial toxicities[167,168]. The constitutive activation of MAPK and PI3K pathways converges to inhibit the heterotrimeric tuberous sclerosis complex (TSC), the GTPase activating protein that lies upstream of the small GTPase RHEB, leading to mTOR complex 1 (mTORC1) activation, a key node controlling cell growth through its effects on protein, lipid and nucleotide biosynthesis[169]. A small subset of ATCs harbour inactivating mutations of *STK11* (also known as *LKB1*)[28], a serine/threonine kinase. When STK11 is inactivated, it fails to phosphorylate AMP-activated protein kinase α, leading to inactivation of TSC2 and de-repression of mTORC1 (ref. 170). Therefore, mTORC1 inhibition with rapamycin analogues may be particularly beneficial for some patients with tumours harbouring mutant *MTOR*, *RHEB*, *TSC1* or *TSC2* (refs. 171,172), but this may not be the case for those that harbour oncoproteins that lie upstream of TSC[173].

Mice harbouring a germline inactivating mutation of the ubiquitously expressed nuclear receptor thyroid hormone receptor β (TRβ) (known as the $TR\beta^{PV/PV}$ mouse) develop metastatic FTC[174]. Loss-of-function mutations of TRβ result in a state of thyroid hormone resistance in humans[175] and in mice. Although mutations of nuclear thyroid hormone receptors do not have a role in human thyroid cancer pathogenesis, this is one of the only mouse models in which a single engineered mutant gene causes metastatic thyroid cancer, and as such it has attracted considerable interest, particularly as a vehicle to investigate the role of the PI3K pathway in promoting tumour metastases. $TR\beta^{PV/PV}$ mice develop unrestrained elevated levels of TSH because of resistance to the negative feedback of thyroid hormone on pituitary thyrotrophs, which is required but not sufficient for thyroid cell transformation[176]. The unbound wild-type TRβ was previously shown to stimulate AKT–mTOR signalling by binding to the p85 regulatory subunit of PI3K in fibroblasts[177]. This non-canonical effect may account in part for the transforming effects of mutant TRβ in thyroid cells, as it was found to have higher binding affinity to p85 than wild-type TRβ, leading to constitutive phosphorylation of AKT and a therapeutic vulnerability to pan-PI3K inhibitors[178,179]. Germline deletion of *Akt1* attenuates both tumour growth and metastatic spread in $TR\beta^{PV/PV}$ mice, whereas *Akt3* and to a lesser extent *Akt2* knockouts have a more selective inhibitory effect on the development of lung metastases[180,181]. A caveat is that the biological consequences of expression of mutant TRβ and deletion of *Akt* isoforms cannot be attributed with certainty to cell autonomous effects in thyroid cells as they are mutated in the germline in these aforementioned genetically engineered mouse models and therefore impact numerous cell lineages.

Loss of cell cycle and DNA damage response checkpoints

Mutations of *TP53* are the genetic hallmark of ATC. They are extraordinarily rare in PTC (0.7%)[58], where they tend to be subclonal[28], uncommon in PDTC (9%) but present in 63–73% of ATCs[28,29,182]. *TP53* mutant ATC foci have been seen to arise adjacent to PTCs that are wild-type for this gene, further documenting its importance in tumour microevolution[129]. A pan-cancer study identified *TP53* mutations as the genomic event that is most strongly associated with copy number changes[183]. Potential explanations for this include loss of the regulatory role of p53 in maintaining the integrity of the spindle assembly checkpoint, which when disrupted leads to an increased rate of tetraploidy and chromosome mis-segregation[184]. Thus, *TP53* loss promotes aneuploidy because it facilitates the generation of chromosome abnormalities and prevents the culling of cells, which have undergone abnormal mitoses[185].

Genomic instability caused by *Trp53* loss in mice does not follow a random, chaotic process. Instead, it causes a series of evolutionary changes that begin with deletion events, which are later followed by copy number gains and amplifications[186]. Although the sequence of events after *TP53* loss in ATC is not known, these tumours are commonly aneuploid and harbour recurrent specific copy number losses and gains, some of which predict for worse disease outcomes, such as gain of chromosome 1q and 20q, or loss of 13q[28,187]. In addition, through the loss of the critical role of p53 in the response to DNA damage, where it helps mediate DNA repair or removal of damaged cells by apoptosis, *TP53* mutations also likely favour the accumulation of oncogenic mutations. p53 is also critical in constraining the activation

of the MAPK pathway by BRAF or RAS oncoproteins, by triggering cell cycle arrest and senescence if the pathway output exceeds a critical threshold[188–190]. Removal of this constraint by *TP53* loss in ATCs is associated with a greater MAPK pathway transcriptional output[155,156]. In addition, lack of p53 has been shown to activate the MAPK pathway in 'RAS-less' mouse embryonic fibroblasts, presumably through induction of RAF kinase activity[191]. Regardless of the underlying mechanism, potent activation of MAPK signalling by enforced overexpression of *BRAF*[V600E] in thyroid cells in vivo has important phenotypic consequences, such as driving the production of inflammatory cytokines by tumour cells, which in turn promotes the recruitment of macrophages to the tumour microenvironment (TME)[192]. Accordingly, ATCs are characterized by their heavy immune infiltrate, primarily consisting of tumour-associated macrophages that form a dense interconnected network that is in close contact with the cancer cells, and likely has a critical role in their biology[193,194].

In an analysis of a cancer-exome sequencing panel of 196 ATCs, Pozdeyev et al.[29] categorized this tumour subtype into four main mutational clusters. Cluster 2 was characterized by loss-of-function alterations of cyclin-dependent kinase inhibitor 2A (*CDKN2A*) and *CDKN2B*, which were primarily deep deletions, as well as driver mutations associated with either PTC or FTC development, and cluster 4 by mutations of *RB1*. Hence, besides the defects of *TP53*, which impact the integrity of the DNA damage checkpoint, loss of function of genes that lead to impairment of the G1-to-S phase transition is a common feature of ATC[182,195].

Chromatin remodelling gene defects in advanced thyroid cancers

A forward genetic screen using Sleeping Beauty (SB) transposon mutagenesis to introduce insertional disruptions randomly into the genome of *Hras*[G12V] mutant mouse thyroid follicular cells identified genes that cooperate with RAS to induce PDTC, which primarily clustered in genes associated with chromatin remodelling functional nodes and the PI3K pathway[80]. Consistent with this, mutations of genes encoding subunits of the SWI/SNF chromatin remodelling complexes, the histone lysine methyltransferases KMT2A, KMT2C and KMT2D and the histone acetyltransferases CREB-binding protein (CBP) and P300 are enriched in PDTCs and ATCs compared with PTCs[28,29,196]. SWI/SNF complexes are preferentially targeted to enhancers distal to the transcription start sites of genes, many of which are linked to developmental processes and lineage specification[197]. Recurrent mutations in many of the subunits of the SWI/SNF complexes occur in ~20% of all human cancers across multiple tumour lineages[198,199]. SWI/SNF complexes are antagonistic with the activity of PRC2. Therefore, loss of SWI/SNF complex function can promote oncogenesis via derepression of PRC2 and upregulation of a stem cell-associated programme[200]. Thyroid-specific homozygous loss of the SWI/SNF complex subunit genes AT-rich interactive domain-containing 1A (*Arid1a*), *Arid2* or SWI/SNF-related matrix-associated actin-dependent regulator of chromatin subfamily B member 1 (*Smarcb1*) in mouse *Braf*[V600E]-driven tumours promotes disease progression to PDTC and ATC. Loss of function of each of these SWI/SNF complex subunit genes leads to distinct and more compact chromatin states and loss of thyroid differentiation. Although treatment of mouse *Braf*[V600E]-mutant PTC with RAF or MEK inhibitors can partially restore differentiation, this is prevented by coexisting SWI/SNF complex subunit mutations, rendering them

insensitive to redifferentiation therapies aimed to restore radioiodine responsiveness. This was also noted in a small series of patients in clinical trials of redifferentiation therapy, in which metastatic DTC or PDTC tumours with *SMARCB1*, *ARID1A* or *ARID2* mutations failed to regain RAI avidity in response to RAF or MEK inhibitors[31] (Fig. 4). However, these patient studies were based on a small number of cases and hence the findings will need to be confirmed in larger trials. The functional consequences of other epigenetic lesions, including those of the KMT2 gene family, have not been investigated so far in thyroid cancer.

Heterogeneity of thyroid cancers

DTCs can be clonally heterogeneous, whereby mutations co-occurring with a primary driver can lead to the emergence of a dominant subclone that outcompetes other cells[201]. High depth genomic sequencing studies show that many of the mutations leading to disease progression are subclonal in PTCs, including mutations in *TERT*, *TP53* and those of genes encoding subunits of SWI/SNF complexes, and become clonal in PDTCs or ATCs[28,31,196]. Clonal diversity predicts an increased risk of subsequent disease progression[201]. Cancer cells can also diverge from each other through development of distinct transcriptional states, which are not necessarily governed by genomic events. A single-cell RNA-sequencing (scRNA-seq) study of 11 tissue samples from patients with PTC at different stages of disease progression defined three transcriptional states of malignant thyrocytes along the course of disease progression[202]: follicular-like, partial epithelial-to-mesenchymal (EMT)-like and dedifferentiation-like. The follicular-like state represents normal and premalignant thyrocytes that have retained a high degree of thyroid differentiation (high TDS) and are RAS-like. Partial EMT-like cells, which derived from primary tumours and lymph node metastases, exhibited partial upregulation of transcriptional drivers of EMT, induction of genes encoding proteins related to the ECM, downregulation of thyroid-specific epithelial genes and preserved expression of other epithelial markers. Finally, cells in a dedifferentiated-like state derived from samples with progressive disease. They expressed very low levels of thyroid-specific epithelial markers, had high levels of *SOX4*, *GATA2* and *MYC* mRNAs and were enriched for oncogenic and immune-related signalling pathways. A separate scRNA-seq study explored the evolutionary trajectory during progression from PTC to ATC and in the process identified a subset of PTC cells with gain of transcriptional signatures of EMT, mTORC1 activation and negative regulation of p53 that represented an intermediate state between PTC and ATC[203]. The drivers of this transcriptional microevolution in thyroid cancer are unknown. Although key genetic subclones could contribute to transcriptional heterogeneity, it can also be driven by alternate mechanisms, including distinct metabolic and epigenetic states, as well as interactions with other cell types in the TME[204].

The immune tumour microenvironment

The TME is a complex ecosystem composed of tumour cells, immune cells, fibroblasts, endothelial cells and non-cellular components, primarily consisting of ECM and soluble mediators (cytokines, chemokines and growth factors)[205]. A detailed review of the impact of the immune TME on thyroid cancer biology is beyond the scope of this Review.

However, it should be noted that the dynamic interplay between tumour and immune cells has a crucial role in tumour initiation and progression in several cancer types[205,206], including those of the thyroid[207], in which cancer cells generate an immunosuppressive and tolerogenic microenvironment. The immune landscapes of DTCs, PDTCs and ATCs are very distinct, although there is limited information on their functional implications[208]. CD4+ T helper cells are enriched in low-grade DTC[208], whereas an abundance of regulatory T (T_{reg}) cells correlates with recurrent disease and the presence of nodal metastases[209,210]. Consistent with this, CD8+ cytotoxic T lymphocytes (CTLs) are enriched in those DTCs with favourable prognostic features, whereas CD8+, granzyme B− T cells, a marker of T cell exhaustion, are associated with tumour recurrence[209,211]. T cells in advanced DTCs also show activation of immune checkpoints, including the PD1–PDL1 axis, cytotoxic T lymphocyte-associated antigen 4 (CTLA4), T cell immunoreceptor with immunoglobulin and ITIM domains (TIGIT) and T cell immunoglobulin mucin receptor 3 (TIM3)[212–214]. Moreover, ATCs are heavily infiltrated with immune cells, including higher levels of T_{reg} cells, CD4+ T cells, CD8+ T cells and natural killer (NK) cells compared with DTCs, with many of these cells expressing markers of dysfunction[203]. Overall, the evolution of the immune landscape from PTC to ATC is marked by progressive immunosuppression[203]. However, caution is needed in the interpretation of the studies reporting these findings, as most were based on limited numbers of tissue samples or on RNA-based analyses.

Myeloid cell infiltration likely has an important role in driving the immunosuppressive TME. Following conditional activation of $BRAF^{V600E}$ in mouse thyroid glands, there is an increased expression of the myeloid cell chemoattractants macrophage colony-stimulating factor 1 (CSF1) and CC-chemokine ligand 2 (CCL2). This is followed by the development of PTCs that are densely infiltrated with tumour-associated macrophages expressing CSF1 receptor (CSF1R) and CC-chemokine receptor 2 (CCR2), the respective receptors for these cytokines. Targeting CCR2-expressing cells during mutant BRAF induction reduced macrophage density and impaired PTC development[192]. A switch from pro-inflammatory M1-like macrophages in PTCs to pro-tumorigenic M2-like macrophages in ATCs suggests that the latter may have a particularly prominent role in thyroid cancer progression[193,203,215]. BRAF-V600E-expressing PTCs may also drive recruitment of myeloid-derived suppressor cells (MDSCs) through activation of the developmental transcription factor TBX3 in the cancer cells and induction of CXC-chemokine receptor 2 (CXCR2) ligands[216,217]. MDSCs are also enriched in ATCs[218] and can dampen antitumour immunity by expressing immune checkpoint molecules, depleting amino acids required for T cell responses, and by producing deleterious nitric oxide and reactive oxygen species[219]. MDSCs can also favour tumour progression via non-immunological mechanisms, such as supporting angiogenesis, promoting tumour stemness and aiding the formation of the pre-metastatic niche[220]. Consistent with the latter, circulating levels of MDSCs are increased in patients with ATC[221].

The production of interferon γ is largely restricted to T lymphocytes and NK cells, and its downstream signalling induces expression of major histocompatibility complex I (MHCI) and MHCII in immune and non-immune cells[222], which is required to initiate adaptive immune responses[223]. Tumour antigens are taken up by dendritic cells and cross-presented to prime CD8+ T cells. In a second step, antigens are presented by tumour

cells to the primed CD8^{+} T cells, leading to tumour cell killing[224]. Tumours invoke multiple mechanisms to evade immune recognition via both these steps, for instance, by modulating antigen expression or disrupting the antigen processing and presentation pathway. In this regard, MHCI expression is decreased by MAPK activation in cancer cells[225], thus repressing a central component in the activation of adaptive immune responses to tumour cells. This is also true for MHCII expression in human BRAF-mutant PTC cells[226]. Moreover, RAF kinase inhibitors restored MHCII expression and CD4^{+} T cell infiltration in mouse BRAF-mutant PTCs and sensitized tumours to treatment with an anti-PD1 antibody[226].

Genomic events associated with metastatic thyroid cancer

As is the case for most cancer types, mortality in thyroid cancer is primarily due to metastatic disease. The metastatic process arises from subsets of cancer cells endowed with features that allow them to undergo intravasation, survive in the circulation, extravasate and successfully home at a distant site, where they may remain dormant for extended periods[227,228]. Although many of the biological requirements for cancer cells to successfully undergo this process have been identified[227,228], how these apply to different lineages and how oncogenic drivers affect them is poorly understood. The properties that confer thyroid cancer cells with an ability to metastasize are mostly unknown, as are the signals that allow them to home at different sites, exit dormancy and develop clinical metastatic disease. The MSK-MET pan-cancer genomic profiling of 25,000 patients, which included a representative number of patients with PTC and PDTC, identified tumour cell lineage-specific genetic mutations associated with metastatic disease[229]. PTC metastases were more chromosomally unstable than primary tumours, as determined by the fraction of the genome altered, had a higher tumour mutation burden and more frequent *TERT* promoter mutations. In addition to these features, *CDKN2A* deletions and mutations of the RNA splicing factor RNA-binding 10 (*RBM10*) were more common in PTC with a high metastatic burden. Consistent with this, RBM10 loss in thyroid cancer has been shown to alter the ratio of cassette exon inclusion events in a subset of transcripts that encode proteins that regulate interactions between the ECM and the cytoskeleton, leading to RAC activation, which in turn favours increased cell movement and metastatic competence[230]. In terms of metastatic tropism, *BRAF*[V600E]-mutant tumours are less likely to metastasize to bone, whereas those with chromosome 22q loss, which is commonly associated with RAS mutations, more frequently metastasize to this site[229]. Chromosome 22q loss of heterozygosity (LOH) is present in 14% of PTCs, mostly in FV-PTC, consistent with the association with RAS mutations[58]. Candidate cancer genes on 22q include checkpoint kinase 2 (*CHEK2*), *NF2* and *SMARCB1*. In the TCGA study of PTCs, *CHEK2* mutations were significantly associated with chromosome 22q LOH[58]. Arm-level chromosome 22q LOH is enriched in PDTC and ATC, in which it is found in 35% and 25% of tumours, respectively[28].

Therapeutic strategies for thyroid cancer

Radioiodine therapy

RAI is the most commonly prescribed treatment for thyroid cancer. The rationale for this approach is predicated on its relative selectivity for thyroid cells, as incorporation of iodide into cells is mediated through the sodium iodide symporter, the expression of which is restricted to very few sites other than the thyroid[32]. It is used in the adjuvant setting and to treat metastatic disease[231]. However, there are currently no data from prospective randomized clinical trials supporting the efficacy of RAI therapy. Despite this, it has been used empirically for several decades, particularly following surgery for thyroid cancers presenting with extensive lymph node metastases, on the basis of data from prospective observational trials and retrospective registry studies[232,233]. However, this approach needs to be interpreted in the context of the genetics of DTC, most of which are driven by $BRAF^{V600E}$, which have a high MAPK pathway transcriptional output and a low TDS, which in most cases predicts for refractoriness to RAI. This has been demonstrated in mouse models[71,105] and in patient series[234]. Consistent with this, a recent study of patients with metastatic thyroid cancer who had major responses to RAI showed that these responses were universally associated with RAS-like tumours, whose MAPK pathway output is attenuated by negative feedback and which have a high TDS[68]. By contrast, RAI-refractory cancers in this study were enriched for $BRAF^{V600E}$, chromosome 1q amplification and concomitant mutations of genes encoding PI3K pathway effectors and RNA splicing factors[68]. Several phase II, open-label clinical trials have demonstrated that a short course of RAF and/or MEK inhibitors can restore RAI avidity and response to RAI therapy in approximately 30% of patients with RAI-refractory metastatic thyroid cancer driven by mutant $BRAF$ or RAS or RTK fusions[106–112]. The long-term impact of these redifferentiation therapies on progression-free survival and overall survival has not been established.

Angiogenesis inhibitors

First-line systemic therapies for metastatic DTC, PDTC and OC are the multikinase inhibitors lenvatinib and sorafenib[235,236]. The efficacy of these drugs is mostly due to their antiangiogenic activity through inhibition of the kinase activity of vascular endothelial growth factor receptors (VEGFRs) and other endothelial cell receptors[237,238], rather than through inhibitory effects on mutant oncoproteins[235,239,240]. Angiogenesis has an important role in tumour initiation, progression and metastasis[241], and yet inhibitors of this process are only effective in a limited number of tumour types[242]. Their efficacy is believed to be due in part to restricting the supply of oxygen and nutrients to the tumour. The neovasculature can also secrete angiocrine factors that regulate tumour growth[243]. Which of these mechanisms drive the remarkable responses of DTC to lenvatinib, in particular, is unknown[235]. Notably, normal thyroid follicular cells are closely juxtaposed to an endothelial capillary network, which is required for thyroid follicle formation through production of exosomes[244,245], conceivably generating a dependency that is maintained during tumorigenesis.

Therapeutic targeting of tumour drivers

As discussed throughout this Review, the drivers of recurrent and metastatic RAI-refractory thyroid cancer are primarily oncogenic kinases and small GTPases. Tumours are dependent

on these truncal driver mutations for viability as genetic or pharmacological inhibition of their activity has been shown to suppress cancer cell growth in preclinical models. Furthermore, many of them can be targeted effectively with selective small molecule inhibitors[71,83,155,246]. Highly selective RET and NTRK inhibitors induce durable responses in patients with metastatic thyroid cancers harbouring the respective fusions and are FDA-approved for this indication[247,248]. On the basis of studies in RET and NTRK fusion-driven non-small-cell lung cancers, the primary mechanisms of acquired resistance are due to on-target solvent front mutations in the fusion kinase domains[249,250]. Furthermore, adaptive resistance to RET kinase inhibitors through off-target fibroblast growth factor receptor activation has been shown in a zebrafish model of RET fusion-driven thyroid cancer[79], and it is possible that analogous mechanisms may also operate in human tumours. Although considerable progress has been made with small molecule targeting of specific KRAS mutants[251] and of HRAS[252], this is of marginal relevance to RAI-refractory thyroid cancers as there are as yet no drugs specifically targeting $NRAS^{Q61R}$, the most prevalent small GTPase mutation in this disease.

Lineage-specific adaptation of BRAF-mutant cancers to MAPK pathway inhibition

BRAF-V600E mutations are highly prevalent in melanomas and thyroid cancers and are also present in a subset of colorectal cancers[60,253]. On the basis of the premise that the viability of all three lineages would be dependent on this tumour driver, there was an initial expectation that all of these tumour types might respond to an equivalent degree to RAF and/or MEK kinase inhibitors. Much to the contrary, responses differ markedly between these tumour types, with thyroid and in particular colorectal cancers showing lower overall response rates compared with melanomas[254–256]. This is likely because RAF and MEK inhibitors relieve distinct negative feedback inputs upstream of BRAF-V600E in these cancer cell types, leading to reactivation of MAPK signalling. In colorectal cancer cells, activation of the epidermal growth factor receptor (EGFR) ensues after MAPK pathway inhibitor treatment, whereas in thyroid cancer cells, the resistance to the RAF inhibitor vemurafenib is primarily due to activation of NRG1–HER3 or HER2 (also known as ERBB2) signalling through relief of negative feedback[40]. The clinical relevance of this adaptive resistance mechanism in thyroid cancer is supported by data from a phase I trial showing a 69% response rate to the combination of the RAF kinase inhibitor dabrafenib and the HER kinase inhibitor lapatinib in patients with BRAF-mutant metastatic DTC[257]. It is intriguing to consider whether germline thyroid cancer predisposition alleles mapping close to the *NRG1* gene locus and associated with higher NRG1 expression may underpin this lineage-specific response[38]. A recent study showed that dysregulated activation of Yes-associated protein (YAP), a transcriptional co-activator that executes the growth-promoting effects of HIPPO pathway inhibition, governs the expression of genes in the NRG1–HER3 or HER2 pathway and amplifies resistance to MAPK pathway blockade in BRAF-mutant thyroid cancers and melanomas[41] (Fig. 5).

Therapy for ATC

Surgery, when feasible, followed by chemoradiotherapy is the current standard of care for these rapidly growing inflammatory cancers[258]. The prognosis of patients with ATC with distant metastases (stage IVc) is poor. Approximately 45% of ATCs have $BRAF^{V600E}$

mutations[28,29], and fortunately, *BRAF*-mutant ATCs show significant responses to the RAF kinase inhibitor vemurafenib[259]. As thyroid cancers develop strong adaptive resistance to RAF kinase inhibition through relief of negative feedback[40], a combination of RAF and MEK kinase inhibitors is a logical choice to potently inhibit the MAPK pathway. An open-label phase II study of patients with $BRAF^{V600E}$-mutant ATC treated with a combination of dabrafenib and the MEK inhibitor trametinib showed a remarkable overall response rate of 69% (refs. 12,260). By contrast, the overall response rate of $BRAF^{V600E}$-driven DTC to the same combination was only 30% (ref. 261). This was unexpected as ATCs have a more aggressive biological behaviour. In addition, compared with DTCs, ATCs are heavily infiltrated with macrophages and MDSCs[193,194] and enriched for NK cells and CD4^{+} and CD8^{+} T cells, although these latter cell types express markers of exhaustion[203]. How MAPK pathway inhibitors modulate this complex cellular network to drive robust antitumour responses is a subject of active investigation. However, there is encouraging evidence that relief of T cell suppression with the PD1 inhibitor spartalizumab induces clinical responses in patients with ATC expressing high levels of PDL1 (ref. 262).

Conclusions

As outlined in this Review, the main genomic defects involved in the evolution of thyroid cancers have now been largely identified. The oncoproteins that drive tumour initiation are mostly either oncogenic kinases or small GTPases, each of which is associated with distinct phenotypic features that are recapitulated quite faithfully in genetically engineered mouse models. Despite their apparent genomic simplicity, DTC cells within a tumour exhibit distinct transcriptional states. Whether these are stable or transitional and whether they result from cell autonomous events or arise in response to cues from the TME is unknown. Comprehensive integrated genomic, epigenomic and transcriptomic analyses of PDTC and ATC are still lacking, as most information has been derived from relatively small series studies.

Current understanding of the genomic characteristics of the different types of thyroid cancer has yielded major improvements in our ability to diagnose the disease with a high degree of accuracy in fine needle biopsies of thyroid nodules, resulting in a meaningful decrease in unnecessary surgeries[263,264]. The evidence that constitutive activation of MAPK signalling suppresses differentiated thyroid function has clinical implications, as small molecule MAPK pathway inhibitors sensitize a subset of patients with metastatic thyroid cancer to RAI therapy[106–112]. Although therapeutic targeting of BRAF or RTK-mutant ATCs is effective in treating the disease[110,260], patients ultimately develop resistance[265,266]. Advanced thyroid cancers are complex and heterogeneous. Further progress will require a deeper understanding of their biology. Single-cell and spatial integrated 'omic' approaches in patient samples and preclinical models may help devise new mechanism-based approaches to activate the antitumour immune response and target specific cancer cell states or other elements of the TME.

Acknowledgements

Glossary

Adaptive resistance

MAPK pathway oncoproteins activate downstream signalling networks, which in turn elicit negative regulatory events designed to dampen pathway output. Upon targeted inhibition of the oncoprotein with small molecules, the network adapts by relieving this negative feedback, causing intrinsic resistance to their action

Adipogenesis

Differentiation process through which mesenchymal cells commit to preadipocytes, and these in turn differentiate into adipocytes

Angiocrine factors

Growth factors, trophogens or membrane-bound paracrine factors supplied by endothelial cells to regulate neighbouring cell growth and homeostasis

Capsular invasion

Invasion of tumour through the entire capsule distinguishes malignant from benign follicular-patterned thyroid neoplasms

Cassette exon

Intervening exon between two other exons from the mature mRNA sequence that can be either included or skipped to generate two distinct protein isoforms

Goitre

Abnormally enlarged thyroid gland

Hyperthyroidism

Syndrome associated with excessive production of thyroid hormones by thyroid cells

Non-homologous end joining

Pathway that repairs DNA double-stranded breaks without the need for a homologous template to ligate the break ends

Non-synonymous somatic mutations

Mutations acquired postnatally that change the amino acid sequence of a protein

Oncogene-induced senescence

Antiproliferative effects of oncoproteins mediated by a DNA damage response to DNA hyper-replication

Open-label clinical trials

A type of study in which health providers and study subjects are aware of the treatment or drug being given

Oxyphil cells
Cells with an eosinophilic cytoplasm containing abundant, abnormally large, mitochondria and large centrally located nuclei

Radioactive iodine (RAI) therapy
Administration of iodine-131 to ablate thyroid tissue. In patients with thyroid cancer, it is used to destroy the remnants of thyroid after thyroidectomy as adjuvant therapy or to treat RAI-avid metastases

RAI avidity
Refers to the cellular property of incorporating and retaining radioactive iodine isotopes

Sleeping beauty (SB) transposon
Composed of a Sleeping Beauty transposase and a synthetic DNA transposon designed to integrate into the genome of vertebrates to introduce new phenotypes and identify the genes responsible for them

SWI/SNF chromatin remodelling complexes
Multisubunit protein complexes that elicit a DNA-stimulated ATPase activity that destabilizes histone–DNA interactions to mobilize nucleosomes, which increases accessibility of transcription factors to chromatin to activate or repress gene expression

Telomere crisis
When telomeres become critically short, they are unable to protect chromosome ends from the DNA damage response and repair pathways. Telomere shortening can enable cancer growth through telomere crisis, a state of extensive genomic instability causing translocations, amplifications and deletions

Tetraploidy
A cell containing four homologous copies of all chromosomes

Thyroglobulin
A glycoprotein produced by thyroid cells that is secreted into the lumen of the follicle. Tyrosine residues in thyroglobulin incorporate iodine through the action of thyroid peroxidase, which upon cleavage and coupling in lysosomes gives rise to thyroid hormones

Thyroid follicular cells
The major cell type of the thyroid gland, derived from the endoderm, and responsible for the production and secretion of the thyroid hormones thyroxine and triiodothyronine

Thyroid parafollicular or C cells
Neuroendocrine cells of the thyroid, which secrete calcitonin, a hormone that helps control the level of calcium in blood

Thyroid-stimulating hormone

(TSH). Glycoprotein hormone secreted by the pituitary gland that binds to the TSH receptor on thyroid cells to stimulate cell growth and expression of iodine metabolism genes

Thyrotrophs

Pituitary cells that secrete TSH

Tumour microevolution

Cancer is commonly understood to develop as a microevolutionary process, whereby a mutation initially confers a cell with a growth advantage allowing it to clonally expand. Sequential acquisition of new mutations in turn provides further stepwise fitness to the emerging clones

Uniparental disomy

(UPD). Refers to the presence of two copies of a chromosome (chromosomes) derived from a single parent. In cancer, it manifests as large blocks of homozygosity with normal copy number

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Box 1

Mitochondrial defects and near haploidization of the nuclear genome in oncocytic carcinomas

Until 2018, oncocytic carcinomas (OCs) of the thyroid, formerly known as Hürthle cell neoplasms, were considered to be variants of follicular adenoma or carcinoma. However, they are now recognized as a distinct entity that accounts for about 3–7% of all malignancies arising from thyroid follicular cells[5]. They are characterized histologically by a follicular-patterned architecture of oxyphil cells containing abundant mitochondria, a large nucleus and a prominent nucleolus[271], whereas the presence of capsular invasion or vascular invasion distinguishes advanced (widely invasive OC) from indolent (minimally invasive OC) tumours[272]. Through genomic analysis, it was revealed that OCs are a distinct entity, characterized by mitochondrial DNA mutations that disrupt the electron transport chain, particularly in genes encoding complex I subunits[273,274]. By contrast to the mitochondrial DNA mutations observed in other cancer types, which are usually present in only a subset of the mitochondria, and are thus termed heteroplasmic mutations[275], those in OC tend to be present in virtually all mitochondria — that is, they are homoplasmic — indicating that they must confer OC cells with a fitness advantage[276,277]. These mutations render cells defective in mitochondrial respiration and reliant on aerobic glycolysis, a vulnerability that could be exploited therapeutically in the future[278]. Aside from the mitochondrial defects, comprehensive whole-exome studies of OCs identified global loss of heterozygosity (LOH), either through near-haploid genomes (depicted as chromosomes with blue shading in the figure) or by genome duplication with uniparental disomy (UPD), which spares only chromosomes 5, 7 and 12 (refs. 276,277) (see the figure). The functional implications of the global LOH are not fully understood. However, a recent integrative analysis of genomic, transcriptomic and metabolomic data on OCs showed that global LOH or UPD may account for the presence of an immunosuppressive tumour microenvironment, primarily via depletion of T helper cells[279]. Recurrent mutations in genes such as *NRAS*, death domain-associated protein 6 (*DAXX*), cyclin-dependent kinase inhibitor 1A (*CDKN1A*), *NF1*, *TP53* and eukaryotic translation initiation factor 1A X isoform (*EIF1AX*) and the telomerase reverse transcriptase (*TERT*) promoter are also relatively frequent events in OCs[276,277]. ATP, ATP synthase protein; CO, cytochrome *c* oxidase subunit; *CYB*, cytochrome B; *MT-TL1*, mitochondrially encoded tRNA leucine 1; ND, NADH dehydrogenase subunit.
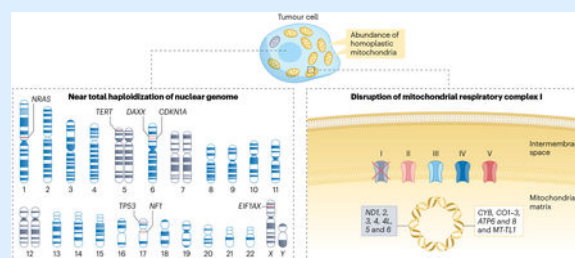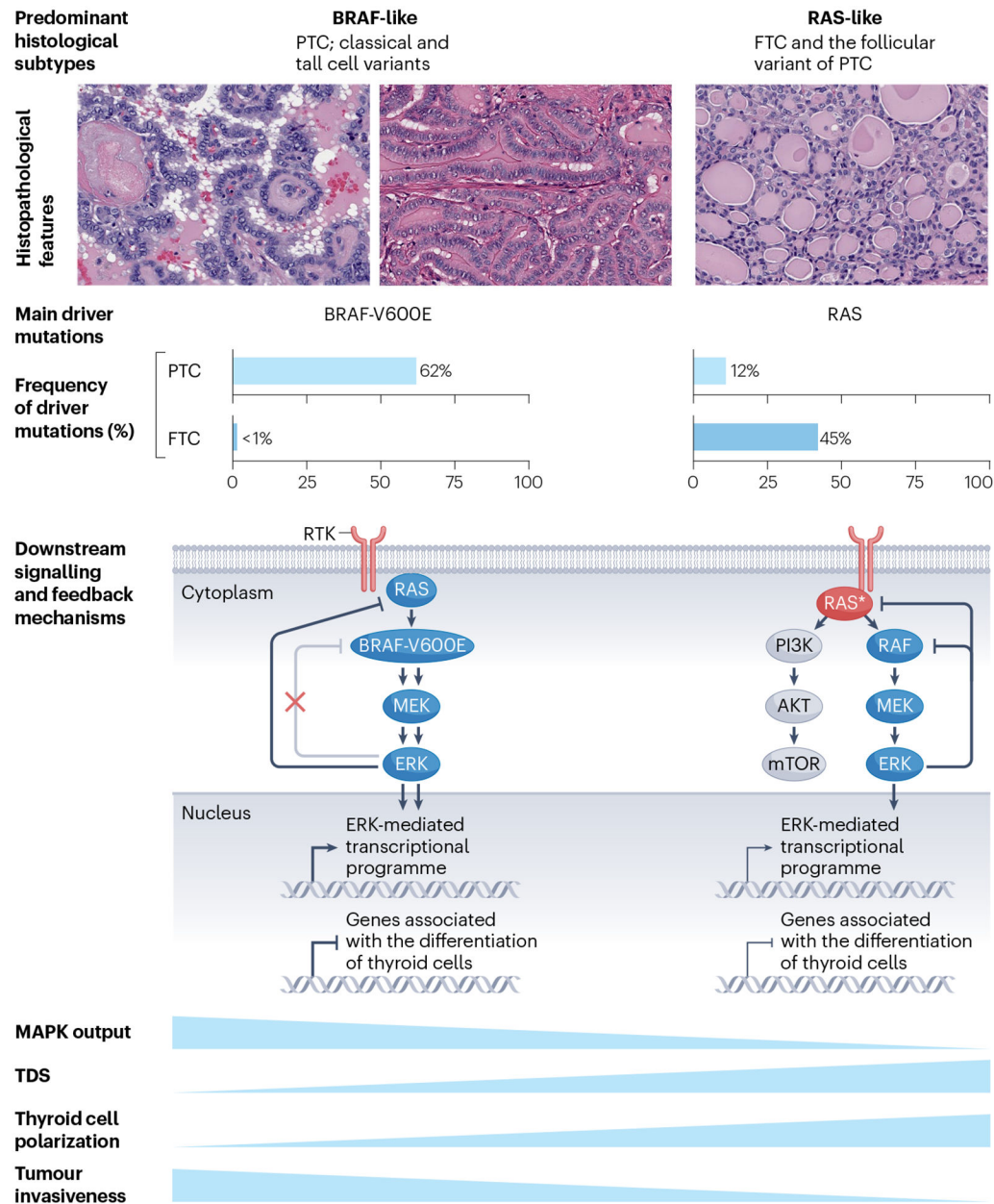
Fig. 1 |. Characteristics of BRAF-like and RAS-like differentiated thyroid cancers.
Differentiated thyroid carcinomas can be categorized transcriptomically as either BRAF-like or RAS-like on the basis of their similarity to papillary thyroid carcinomas (PTCs) harbouring either $BRAF^{V600E}$ or RAS mutations, respectively. The $BRAF^{V600E}$ mutation occurs in 62% of PTCs, particularly in the classical and tall cell variants of the disease. By contrast $NRAS$, $HRAS$ and $KRAS$ mutations are frequent events in follicular thyroid carcinoma (FTC) (45%) and in the follicular variant of PTC[29,58,94,152,267–269]. The characteristic histological feature of BRAF-like tumours is the presence of papillae, consisting of epithelial cells arranged around a fibrovascular core, whereas RAS-like tumours tend to retain the structure of the thyroid follicle. Mutant $BRAF$ and RAS activate MAPK signalling but do so to different degrees (the asterisk in the figure represents the

mutational activation of RAS). The MAPK signalling flux induced by mutant RAS is dampened by negative feedback of activated ERK on CRAF, which disrupts RAF dimer signalling. BRAF-V600E signals as a monomer and is therefore unresponsive to the negative feedback by activated ERK on CRAF and hence has a higher MAPK pathway output. The expression of thyroid lineage transcription factors and of genes required for iodide uptake and metabolism is inhibited by MAPK signalling. The former is quantified by the thyroid differentiation score (TDS), which is lower in BRAF-like than in RAS-like tumours. Expression of the thyroid transcription factor paired box 8 (PAX8) and cadherin 16 (CDH16), which control correct apical–basal polarization of thyroid cells, is lower in BRAF-like than in RAS-like differentiated thyroid carcinomas. The BRAF-V600E mutation also induces expression of matrix metalloproteinases, rendering *BRAF*-mutant tumours more invasive than their RAS-mutant counterparts. RTK, receptor tyrosine kinase. Histological images courtesy of Bin Xu and Ronald Ghossein.
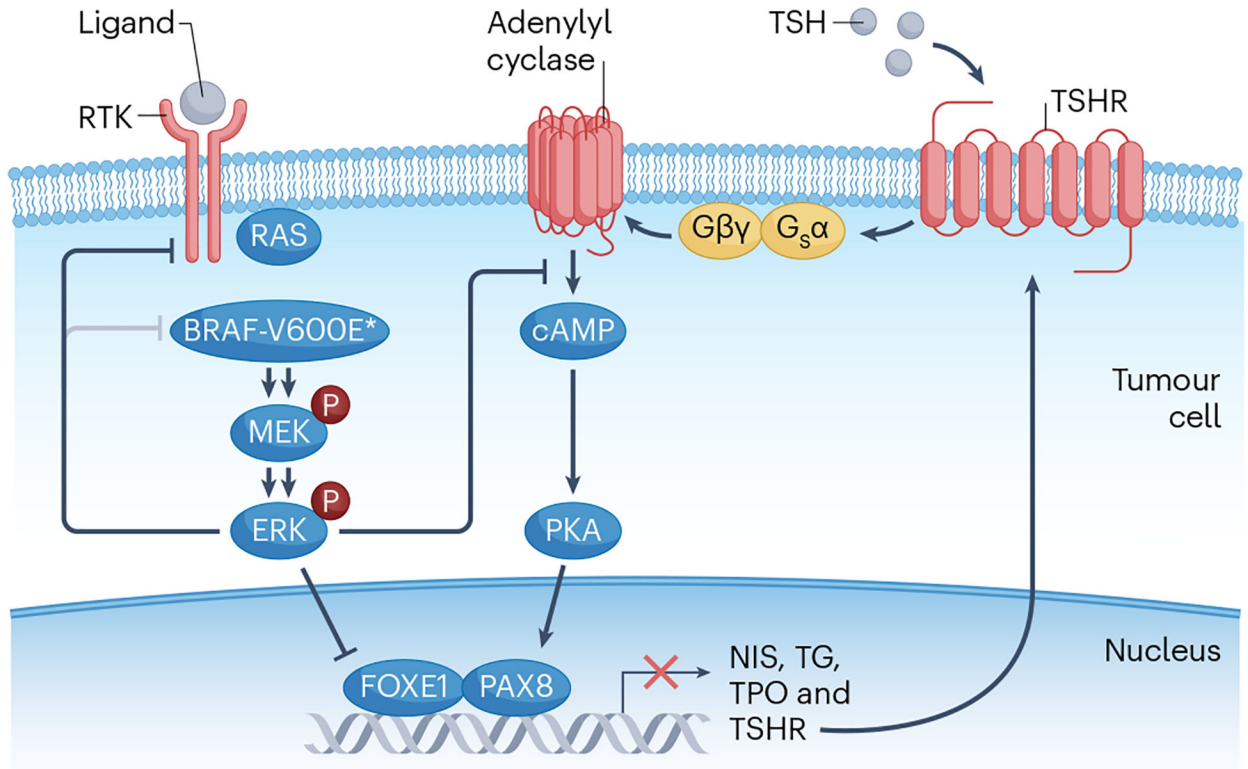
Expression of genes associated with the differentiation of thyroid cells

Fig. 2 |. Interactions between MAPK and cAMP signalling in thyroid cancer cells.
Thyroid cells are under the regulatory control of thyroid-stimulating hormone (TSH), acting through the TSH receptor (TSHR) to signal primarily through $G_s\alpha$ and adenylyl cyclase to increase intracellular cAMP levels. cAMP in turn activates protein kinase A (PKA), which phosphorylates substrates to ultimately regulate the growth and differentiated function of thyroid cells in part through inducing expression of the lineage transcription factors paired box 8 (PAX8) and forkhead box E1 (FOXE1), which regulate the expression of genes required for thyroid hormone biosynthesis (for example, sodium iodide symporter (*NIS*), thyroglobulin (*TG*), thyroid peroxidase (*TPO*) and *TSHR*). Activation of the MAPK pathway, through receptor tyrosine kinase (RTK) fusions, or RAS or BRAF mutations, impairs TSH–cAMP signalling at various nodes in the pathway[24,102,105,270]. This takes place by repressing expression of TSHR and inhibiting the catalytic activity of adenylyl cyclase, thus impairing PAX8-mediated and FOXE1-mediated transcription and inhibiting differentiated function in thyroid cancer cells. The figure illustrates the functional consequences of the BRAF-V600E mutation, highlighted with an asterisk.
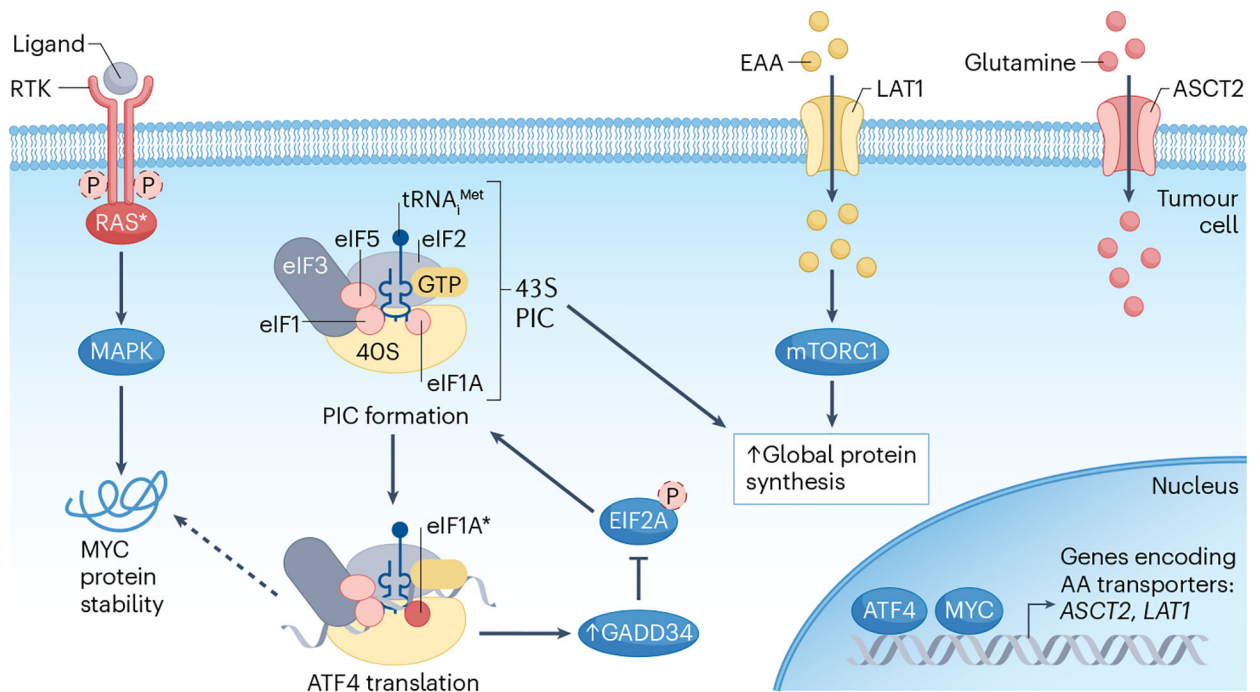
Fig. 3 |. Mechanisms of mutant eIF1A and RAS cooperation in thyroid tumorigenesis.
Regulation of translation determines the levels of proteins synthesized by their
corresponding mRNAs. Once the small 40S ribosomal subunit binds to the 5′-untranslated
region of mRNAs, the preinitiation complex (PIC) containing the initiator methionyl tRNA$_i$
(tRNA$_1$^{Met}) initiates the scanning process for the AUG initiation codon. The eukaryotic
translation initiation factor 1A (eIF1A) is a component of the PIC that is mutated in thyroid
cancer (indicated by an asterisk). eIF1A mutants have a higher affinity for other subunits
of the PIC, leading to its stabilization and to the illegitimate translation of activating
transcription factor 4 (ATF4). ATF4 then induces dephosphorylation of the translation
initiation factor eIF2A (which loads the tRNA$_1$^{Met} onto the 40S small ribosomal subunit).
This takes place via a GADD34-mediated negative feedback pathway, leading to enhanced
ternary complex loading to form the PIC and to an increase in protein synthesis. RAS
mutants (indicated by an asterisk) in turn stabilize MYC, an effect augmented by the
EIF1AX-A113 splice mutant, the most common somatic variant in thyroid cancer. ATF4
and MYC induce expression of amino acid (AA) transporters and cooperate to activate
mTOR complex 1 (mTORC1), further augmenting global protein synthesis[84]. ASCT2,
alanine/serine/cysteine/threonine transporter 2; EAA, essential amino acid; LAT1, L-type
amino acid transporter 1; RTK, receptor tyrosine kinase; ternary complex: a complex of
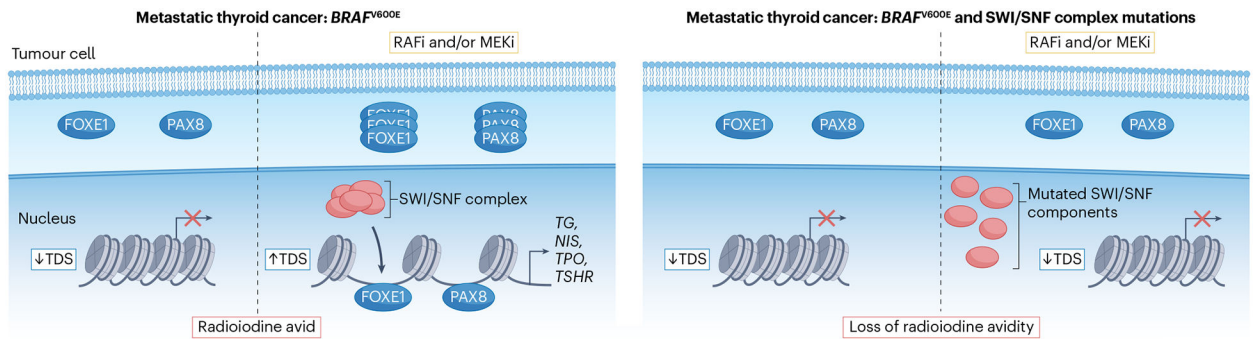GTP-bound eIF2A and the tRNA$_1$^{Met}.

Fig. 4 |. Epigenetic reprogramming in thyroid cancer progression and differentiation.
Loss-of-function recurrent mutations of chromatin-modifying genes, including those encoding subunits of the SWI/SNF complexes, are enriched in advanced thyroid cancers[28]. Left: *BRAF*^V600E-mutant metastatic thyroid cancer cells have decreased expression of the lineage transcription factors forkhead box E1 (FOXE1) and paired box 8 (PAX8), and impaired accessibility to their respective DNA-binding sites required to induce expression of thyroid differentiation genes. RAF and/or MEK inhibitors (RAFi and MEKi, respectively) restore expression of PAX8 and FOXE1, which in concert with SWI/SNF complexes promote an open chromatin state enabling expression of iodine metabolism genes and restoration of response to radioactive iodine (RAI) therapy. Right: BRAF-mutant tumours with coexisting SWI/SNF complex subunit mutations fail to remodel chromatin and enable thyroid transcription factor accessibility to DNA to regulate expression of thyroid differentiation genes following treatment with MAPK pathway inhibitors, rendering metastases refractory to RAI. NIS, sodium iodide symporter; TDS, thyroid differentiation score; TG, thyroglobulin; TPO, thyroid peroxidase; TSHR, thyroid-stimulating hormone receptor.
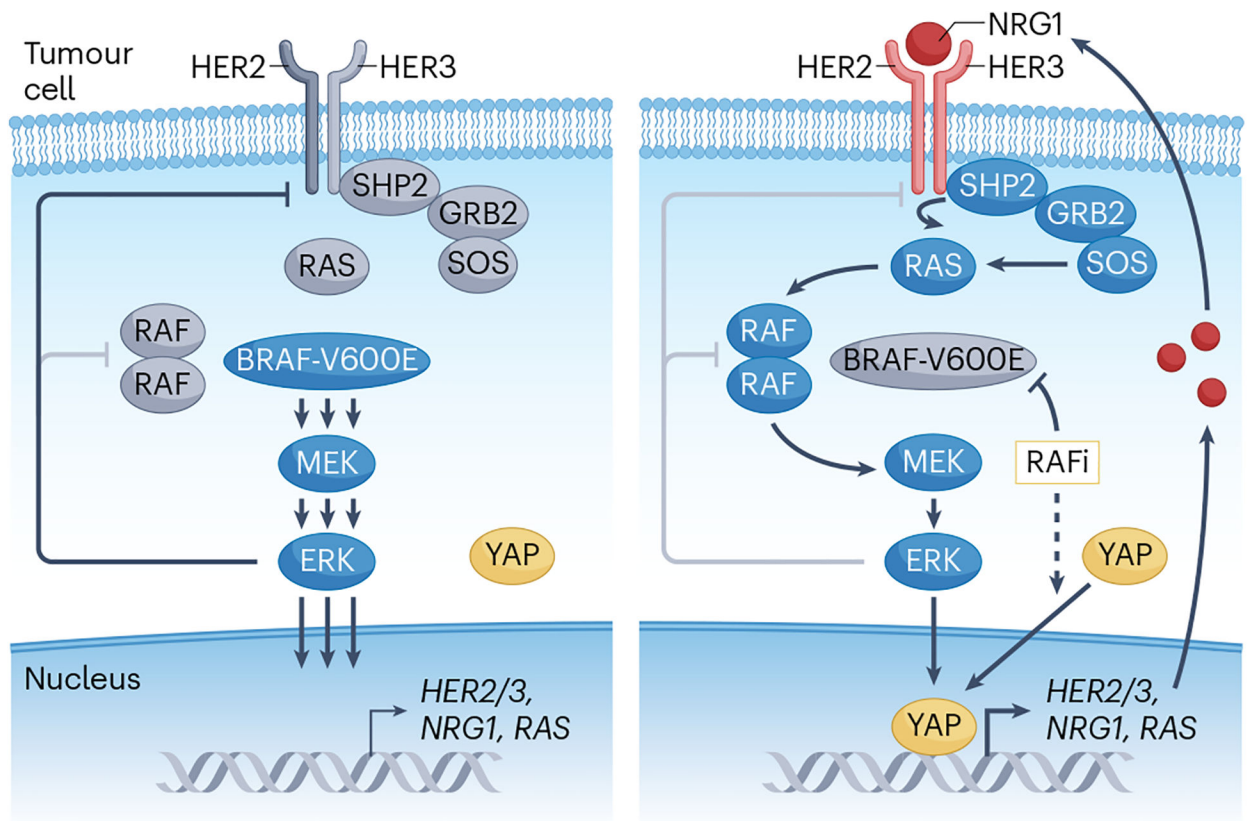
Fig. 5 |. Adaptive responses of BRAF-mutant thyroid cancers to RAF kinase inhibitors.
Left: BRAF-V600E hyperactivates the MAPK pathway because it constitutively signals
as a monomer and is unresponsive to negative feedback by ERK on RAF dimers. In
this context, expression of the receptor tyrosine kinases (RTKs) human epidermal growth
factor receptor 2 (HER2) and HER3 is attenuated and RAS-GTP levels are low. Right:
RAF kinase inhibitors suppress MEK and ERK phosphorylation and induce Yes-associated
protein (YAP) nuclear translocation and activation. The mechanism by which RAF kinase
inhibitors promote YAP nuclear translocation is unknown. Under this therapeutic pressure,
YAP activates the transcription of genes encoding neuregulin-1 (NRG1), HER2, HER3
and RAS. NRG1 is the ligand for HER3, and upon its activation of the HER3/HER2
heterodimer, downstream signalling via Src homology region 2 (SH2)-containing protein
tyrosine phosphatase 2 (SHP2), growth factor receptor-bound protein 2 (GRB2) and SOS
induces RAS activation, resulting in adaptive resistance to RAF kinase inhibition.

Table 1 |

Molecular and clinical features of familial syndromes causing follicular cell–derived thyroid neoplasms

Familial syndrome	Genes	Inheritance	Thyroid phenotype penetrance	Thyroid phenotype distribution	Specific PTC subtype	Other phenotypes
PTEN hamartoma tumour syndrome	*PTEN*	Autosomal dominant	~80%	MNG/FA: ~50% PTC: ~30% FTC: ~20% ATC: ~1%	Both classic and FV-PTC	Mucocutaneous lesions, genital pigmentation, breast cancer, endometrial carcinoma, macrocephaly, autism spectrum disorder
Familial adenomatous polyposis	*APC*	Autosomal dominant	~16%	PTC: 100%	Exclusively cribriform	Colorectal adenomatous polyps, colorectal cancer, desmoid tumours, hepatoblastoma, medulloblastoma
Carney complex	*PRKAR1A*	Autosomal dominant	~60%	MNG/FA: ~60% PTC: ~15% FTC: ~25%	Predominantly FV-PTC	Pigmentation in skin and mucosa, multiple myxomas, primary pigmented nodular adrenocortical disease, Sertoli cell tumours, growth hormone-secreting pituitary adenoma, breast ductal adenoma, osteochondromyxoma
Werner's syndrome	*WRN*	Autosomal recessive	~18%	PTC: ~40% FTC: ~50% ATC: ~10%	Predominantly FV-PTC	Premature greying and/or thinning of scalp hair, atherosclerosis, type 2 diabetes, cataracts, short stature, melanoma, meningioma, soft-tissue sarcomas, leukaemia, osteosarcomas
DICER1 syndrome	*DICER1*	Autosomal dominant	~45%	MNG/FA: ~90% PTC: ~5% FTC: ~5%	Both classic and FV-PTC	Pleuropulmonary blastoma, pulmonary cysts, cystic nephroma, ovarian tumours, ciliary body medullo-epithelioma, nasal chondro-mesenchymal hamartoma, embryonal rhabdo-myosarcoma, pituitary blastoma, pineoblastoma, central nervous system sarcoma, presacral malignant teratoid tumour[42,43]

Distributions of pathogenic mutations in all syndromes are typically loss-of-function events consistent with the tumour suppressor roles of the affected gene products. Thyroid lesions in patients with DICER1 syndrome typically display a germline loss of function in the *DICER1* gene and a somatic second-hit missense mutation affecting the RNase IIIb domain of the protein. ATC, anaplastic thyroid carcinoma; FA, follicular adenoma; FTC, follicular thyroid carcinoma; FV-PTC, follicular variant of papillary thyroid carcinoma; MNG, multinodular goitre; PTC, papillary thyroid carcinoma.