REVIEW

Molecular mechanisms of resistance to kinase inhibitors and redifferentiation in thyroid cancers

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Abstract

Protein kinases play critical roles in cell survival, proliferation, and motility. Their dysregulation is therefore a common feature in the pathogenesis of a number of solid tumors, including thyroid cancers. Inhibiting activated protein kinases has revolutionized thyroid cancer therapy, offering a promising strategy in treating tumors refractory to radioactive iodine treatment or cytotoxic chemotherapies. However, despite satisfactory early responses, these drugs are not curative and most patients inevitably progress due to drug resistance. This review summarizes up-to-date knowledge on various mechanisms that thyroid cancer cells develop to bypass protein kinase inhibition and outlines strategies that are being explored to overcome drug resistance. Understanding how cancer cells respond to drugs and identifying novel molecular targets for therapy still represents a major challenge for the treatment of these patients.

Key Words
- differentiated thyroid cancers
- anaplastic thyroid carcinoma
- BRAF
- RAS
- kinase inhibitors
- drug resistance
- NIS

Introduction

Thyroid cancer is the most common endocrine malignancy, with an estimated 44,280 cases in the United States in 2021 (Siegel et al. 2021). They originate from two types of endocrine cells: the thyrocytes, or follicular cells, or the parafollicular C cells. Our understanding of the molecular pathways involved in thyroid tumorigenesis has greatly improved due to the identification of several oncogenic alterations and the availability of agents to target them (Nikiforov & Nikiforova 2011). These alterations include point mutations as well as gene translocations and their chimeric fusion products, gene amplifications, and deletions. Many of these alterations result in oncogenic drivers that promote cell proliferation, motility, and metastasis (Fagin & Wells 2016). They often directly or indirectly increase the activity of protein kinases, which are critical for many cellular functions, including regulation of the cell cycle. The identification of these mutations has led to the development of new targeted therapies, mostly protein kinase inhibitors (PKIs), for advanced thyroid cancers (Sipos & Ringel 2022). So far, four of the FDA-approved PKIs for thyroid cancers are antiangiogenic multi-kinase inhibitors and five are mutation-selective. These novel drugs have revolutionized the treatment of patients with advanced thyroid cancers (Cabanillas et al. 2019). Kinase inhibitors have shown good efficacy in controlling the disease and are better tolerated than cytotoxic therapies. However, despite an early response, many patients will show transient benefit and
progress under treatment, and tumor-specific mortality is nearly universal. The mechanisms leading to acquired drug resistance are far from being completely understood. In this review, we summarize the properties and function of protein kinases and kinase inhibitors, and how these drugs are used to treat thyroid cancers. We then describe some mechanisms of resistance recently identified and which strategies were used to overcome the challenges.

**Protein kinases**

**Protein kinases and their dysregulation**

Protein kinases are enzymes that covalently add phosphate groups to selected proteins. They catalyze the transfer of phosphate from ATP to specific amino acid residues of target proteins in a process called phosphorylation. Phosphorylation is considered one of the most abundant post-translational modifications of proteins and usually modulates their function by adjusting their activity, modifying their cellular location, or altering their association with other proteins (Seek 2021). The majority of protein kinases are serine/threonine kinases, which phosphorylate the hydroxyl groups of serines and threonines in their target proteins. Most other kinases are tyrosine kinases, which catalyze the addition of a phosphate group to tyrosine residues. Up to 30% of all human proteins may be modified by kinase activity, and kinases are known to regulate many cellular processes and pathways, especially those involved in signal transduction. Protein kinases may be located at the cell membrane and function as receptors, such as growth factor receptors (EGFR, MET, KIT). They may also reside in the cytoplasm, where their activation is dependent on upstream signaling molecules such as RAS and protein kinases functioning in signaling cascades (Fig. 1). Phosphorylation through protein kinases regulates key cellular processes that include gene expression, proliferation, differentiation, motility, membrane transport, metabolism, and apoptosis. Therefore, the predominance of dysregulation of protein kinases activity in many cancers is not surprising (Brognard & Hunter 2011).

Alterations of protein kinase activities are indeed a frequent component of the pathogenesis of solid and hematologic malignancies. These alterations are caused by a variety of mechanisms, such as genomic rearrangements (chromosomal translocations and fusions) (Nikiforov 2002, Hallberg & Palmer 2013), gene amplification or deletions (Lopez-Gines et al. 2010, Rodriguez-Antona et al. 2010, Cicenas et al. 2018), and missense point mutations (Lahiry et al. 2010). Missense point mutations often arise in the kinase domain of receptor tyrosine kinases or in cytoplasmic serine/threonine protein kinases. For example, a sporadic point mutation in the BRAF gene, which belongs to the MAPK pathway (Fig. 1), can constitutively activate the kinase to drive cell proliferation continually, without the presence of growth factors (Wan et al. 2004, Xing 2005). Most BRAF-activating missense point mutations are found in exons 11 and 15 of the gene, within the kinase domain of the protein. In particular, the c.1799A mutation is present in over 80% of all BRAF-mutated cancers (Davies et al. 2002). The protein resulting from this mutation is a substitution of valine for glutamic acid at codon 600 (V600E), which leads to constitutive activation of BRAF and its downstream targets MEK and ERK. Activating BRAF mutations are found in 40–60% of melanoma and 50–60% of papillary thyroid carcinoma (PTC) (Fig. 2B) (Xing et al. 2005, Long et al. 2011, Cancer Genome Atlas Research Network 2014). BRAF mutations have also been frequently reported in colorectal cancers (Barras 2015). An example of gene rearrangement/fusion in thyroid cancers is illustrated by somatic juxtapositions of 5′ activating sequences from other genes with 3′ sequences of the RET or NTRK genes encoding the tyrosine kinase domain (Fig. 1). Sporadic RET and NTRK rearrangements occur in approximately 16% of thyroid cancers (Nikiforov 2002, Park et al. 2022, Ullmann et al. 2022) (Fig. 2B). In addition to mutations affecting DNA sequences, epigenetic events can also increase or modify the expression of many receptor tyrosine kinases such as EGFR, ERBB2, and MET in many solid tumors (Hoque et al. 2010, Ogunwobi et al. 2013) and untimely activation of the kinases p38, MEK, ERK, AURKA, and AKT due to phosphorylation is well known (Cicenas et al. 2018).

**Targeting protein kinases**

Small molecule kinase inhibitors have been very efficient in targeting specific mutations driving tumorigenesis. These inhibitors are generally categorized according to their mechanism of action. They are classified as type I, II, III (allostERIC), IV (substrate directed), V (bivalent), and VI (covalent) inhibitors (Bhullar et al. 2018, Cicenas et al. 2018, Martinez et al. 2020) (Table 1). Type I inhibitors compete for the substrate and bind the DFG (Asp-Phe-Gly) motif in the ATP-binding pocket of the active kinase when the Asp residue is oriented toward bound ATP (‘in’ conformation) (Peng et al. 2013). Such is the case for crizotinib, dasatinib, erlotinib, lapatinib, pazopanib, sunitinib, vemurafenib, and dabrafenib. Type II inhibitors
Figure 1
Overview of the MAPK and AKT signaling pathways. The main protein kinases driving these pathways are depicted. Kinase inhibitors that are presently FDA-approved for thyroid cancers are highlighted.

A
Hürthle (~3%)

B
Estimated Mutation Frequency in Thyroid Carcinoma (COSMIC)

Figure 2
Clinical and molecular spectrum of thyroid cancers. (A) Incidence of the main types of thyroid cancers (data from Fagin & Wells 2016). (B) Distribution of the top eight mutated genes in thyroid cancers (from COSMIC 2022).
<table>
<thead>
<tr>
<th>Class</th>
<th>Mechanism of action</th>
<th>Names</th>
<th>Target protein kinases</th>
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<tbody>
<tr>
<td><strong>Type I inhibitors</strong></td>
<td>Compete for the substrate and binds in the ATP-binding pocket of the active kinase conformation (DFG-Asp ‘in’)</td>
<td>Bosutinib</td>
<td>BCR-ABL, Src</td>
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<td></td>
<td>Cabozantinib</td>
<td>VEGFR, PDGFR, KIT, MET, FLT3, RET</td>
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<td>Ceritinib</td>
<td>ALK-EML4</td>
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<td>Crizotinib</td>
<td>ALK-EML4, MET, ROS-1</td>
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<td>Dabrafenib</td>
<td>BRAF&lt;sup&gt;V600E&lt;/sup&gt; mutant kinase</td>
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<td>Gefitinib</td>
<td>EGFR</td>
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<td></td>
<td>Papozanib</td>
<td>VEGFR, PDGFR, KIT, FGFR3</td>
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<td>Vandetanib</td>
<td>VEGFR, RET, MET</td>
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<td>Vemurafenib</td>
<td>BRAF&lt;sup&gt;V600E&lt;/sup&gt; mutant kinase</td>
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<td>Encorafenib</td>
<td>BRAF&lt;sup&gt;V600E&lt;/sup&gt; mutant kinase</td>
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<td>Larotrectinib</td>
<td>NTRK fusions</td>
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<td></td>
<td>Entrectinib</td>
<td>NTRK fusions</td>
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<td><strong>Type II inhibitors</strong></td>
<td>Compete for the substrate and binds in the ATP-binding pocket of the inactive kinase conformation (DFG-Asp ‘out’)</td>
<td>Imatinib</td>
<td>BCR-ABL, KIT, PDGFR</td>
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<td></td>
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<td>Sorafenib</td>
<td>VEGFR, PDGFR, BRAF, FLT3, RET, KIT</td>
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<td></td>
<td>Axitinib</td>
<td>VEGFR</td>
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<td><strong>Type III (allosteric inhibitors)</strong></td>
<td>Occupy a site next to the ATP-binding pocket so that both ATP and the allosteric inhibitor simultaneously bind to the protein kinase. Offer more selectivity against the targeted kinases and avoid the development of ATP-binding site gatekeeper mutations</td>
<td>Trametinib</td>
<td>MEK1/2</td>
</tr>
<tr>
<td><strong>Type IV (substrate-directed inhibitors)</strong></td>
<td>Bind to a site distal from the ATP pocket and induce a conformational change. Offer more selectivity against the targeted kinases</td>
<td>Cobimetinib</td>
<td>MEK1/2</td>
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<td></td>
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<td>Selumetinib</td>
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<td>Binimetinib</td>
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<td>GnF2</td>
<td>BCR-ABL</td>
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<td>ONO12380</td>
<td>BCR-ABL</td>
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<td><strong>Type V (bivalent inhibitors)</strong></td>
<td>Consist of a small molecule that targets the ATP-binding site coupled to a peptide representing the substrate targeted by the specific kinase</td>
<td>Lenvatinib</td>
<td>VEGFR&lt;sup&gt;1–3&lt;/sup&gt;, FGFR&lt;sup&gt;1–4&lt;/sup&gt;, PDGFR&lt;sub&gt;α&lt;/sub&gt;, KIT, RET</td>
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<td>ARC-1411</td>
<td>PKA</td>
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<td>ARC-3140</td>
<td>CK2</td>
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<td>ERK5.1</td>
<td>ERK5</td>
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<td></td>
<td></td>
<td>Rapalink-1</td>
<td>mTORC1</td>
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<td></td>
<td>BI 2536</td>
<td>PLK1</td>
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<td></td>
<td></td>
<td>BI 53</td>
<td>c-SRC</td>
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<td></td>
<td></td>
<td>Afatinib</td>
<td>EGFR</td>
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<td></td>
<td></td>
<td>Ibrutinib</td>
<td>BTK</td>
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<tr>
<td><strong>Type VI (covalent inhibitors)</strong></td>
<td>Irreversibly bind the targeted kinase</td>
<td>Neratinib</td>
<td>HER1, HER2, HER3, HER4</td>
</tr>
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</table>
bind to the ATP pocket of the inactive kinase (DFG-Asp ‘out’ conformation) (imatinib, sorafenib, axitinib, nilotinib). The disadvantages of type I and II inhibitors reside in their binding to the ATP-binding site, which is conserved in protein kinases. Therefore, developing inhibitors that are specific is particularly challenging (Table 1). This lack of specificity increases the potential for off-target side effects, in particular cardiotoxicity. In contrast, type III inhibitors such as the MEK1/2 inhibitors trametinib and cobimetinib occupy highly specific allosteric sites close to the ATP-binding pocket and therefore are thought to be more selective than type I and II inhibitors (Zhao et al. 2017). This design might also prevent the occurrence of ‘gate-keeper’ mutations, which can obstruct drug binding by modulating access to the kinase ATP-binding site. More recently, efforts have been made to develop inhibitors that bind to unique structural domains outside the ATP-binding pocket. This led to the synthesis of type IV inhibitors, which usually induce a conformational change disrupting interactions of the kinases with their downstream targets (JNK, ERK1/2 inhibitors) (Martinez et al. 2020). This approach again offers more selectivity by blocking only the kinase function associated with a particular disease, while preserving other kinase functions that have potential benefits. To further increase selectivity, type V inhibitors, or bivalent inhibitors, were developed that can target both the ATP-binding site and a unique structural feature found on the kinase (lenvatinib and specific c-SRC, ERK5 and PLK1 inhibitors) (Okamoto et al. 2015, Lee et al. 2021) (Table 1). Finally, type VI inhibitors bind covalently and irreversibly to their target for a longer duration of the effect (Weisner et al. 2019, Martinez et al. 2020). This is the case for afatinib (an EGFR inhibitor), ibrutinib (a BTK inhibitor), and neratinib (a HER1-HER4 inhibitor).

### Thyroid cancers

The thyroid lies within the neck and consists of two connected lobes. The functional unit of the gland is the spherical thyroid follicle lined with thyrocytes, or follicular cells, that secrete triiodothyronine (T3) and thyroxine (T4). T3 and T4 have a wide range of effects including control of metabolism, regulation of the cardiac rate, and control of growth during development. Parafollicular cells, or C cells, are located adjacent to the thyroid follicles and secrete calcitonin, which contributes to the control of blood calcium levels. The most common primary thyroid cancers originate from thyrocytes and are classified as differentiated thyroid cancers (DTCs).

DTCs include PTC, follicular thyroid carcinoma (FTC), and Hürthle cell thyroid carcinoma (HTC). DTCs usually harbor single driver mutations in the RAS or BRAF gene, which are mutually exclusive. Poorly differentiated thyroid carcinoma (PDTC) and anaplastic thyroid carcinoma (ATC) are rare and aggressive tumors that also originate from thyrocytes (Fig. 2A). They both show a de-differentiated phenotype; however, ATCs harbor a higher number of mutations than PDTCs and PTCs (Landa et al. 2016). Medullary thyroid carcinomas (MTCs) are tumors derived from neuroendocrine parafollicular C-cells and are often driven by a RET receptor mutation. In thyroid cancers, most somatic driver mutations are found in receptor tyrosine kinases or in serine/threonine kinases belonging to the MAPK signaling pathway, regardless of the histologic subtype (Fig. 1).

### Molecular pathology of thyroid cancers

PTCs represent about 80% of thyroid cancers and often harbor somatic mutations in the MAPK pathway (Fig. 2A and B). Activation of the pathway is mainly due to BRAF or RAS mutations, or re-arrangements of the RET or NTRK genes. Primary PTCs often display single mutations, such as the BRAFV600E mutation mentioned earlier (60% of the cases), and these tumors harbor one of the lowest mutation densities of cancers that have been studied according to whole-exome sequencing (WES) data (Fagin & Wells 2016). About 10–20% of thyroid cancers are due to RET mutations or rearrangements, and another 10–20% are due to RAS point mutations (Fig. 2B). In classical PTCs, RET fusions account for 6–8% of the cases (RET/PTC rearrangements) and the presence of RAS mutations is low (1–6%) (Cancer Genome Atlas Research Network 2014, Song & Park 2019). If the PTC tumor is small and localized within the thyroid, the prognosis is excellent, especially in younger patients. However, larger tumors that may extend outside the thyroid and have invaded lymph nodes and/or distant organs carry a poorer prognosis.

FTCs account for ~12% of thyroid cancers (Fig. 2A and B). FTCs usually harbor NRAS, HRAS, or KRAS gene mutations (40–50% of the cases). Another common mutation is the PAX8/PPARG rearrangement (30–35% of the cases), while point mutations in the PTEN and PIK3CA genes are observed in about 5–10% of the cases. The prognosis of FTC depends on the age of the patients, the size and stage of the tumors, the completeness of surgery, the presence of distance metastases, and the responsiveness to radioactive iodine therapy (Sobrinho-Simoes et al. 2011).
HTCs amount to 2–3% of thyroid cancers (Fig. 2A and B). HTCs exhibit a more aggressive behavior compared with PTC and FTC and the frequency of distant metastases is high (McFadden & Sadow 2021). A fraction of HTCs harbor mutations in the RAS, TP53, PTEN, E1F1AX, and PAX8-PPARG genes, which are often found in other thyroid cancers. Mutations in the TERT promoter, as well as the DAXX and ATRX genes that cause alternate telomere elongation, are found in more aggressive forms of HTCs (Ganly et al. 2018). In addition, an interesting feature of the HTCs is their accumulation of abnormally shaped mitochondria, probably due to mutations in the mitochondrial DNA leading to impairments in proteins of the electron transport chain, in particular subunits of the complex I and III (Bonora et al. 2006). Further, chromosome gains and losses are often observed, with near haploidization in certain cases, or selective gains of chromosomes 7, 12, and 17 (Ganly et al. 2018).

PDTCs are more aggressive than PTC and FTC tumors and represent approximately 2–3% of thyroid cancers. Radioiodine therapy is of limited benefit and these tumors can readily metastasize to the neck lymph nodes, lungs, and bones. They are associated with a mean survival of 3.2 years (Landa et al. 2016). Molecular pathology data indicate that these tumors retain the driver mutations seen in differentiated PTCs or FTCs (BRAFV600E or RAS) and that they have acquired additional alterations such as PTEN loss of function (Volante et al. 2021). In addition, while TERT promoter mutations are found in about 5–15% of PTCs and FTCs, this mutation is significantly more frequent in PDTCs (20–50%) (Volante et al. 2021). Thus, the acquisition of a TERT promoter mutation appears to be a key transitional step in the microevolution of these tumors (Landa et al. 2016).

ATCs are rare but are among the most aggressive tumors known, with historic median survival of 3–5 months, although more recent studies have demonstrated improved survival (Maniakas et al. 2020). The current accepted model considers that ATCs derive from PTCs, FTCs, or HTCs through the acquisition of additional genomic alterations (Fig. 3). BRAF, RAS, and RET mutations are prevalent; however, de novo acquisition of TERT promoter, PIK3CA,
and \(TP53\) mutations (70% of the cases) are a hallmark of these malignancies (Landa et al. 2016, Pozdyev et al. 2018, Qin et al. 2021). Mutations in the \(PTEN\) and \(NFI\) genes are also occasionally seen. In general, ATC tumors harbor a higher mutation burden than PTC. ATC tumors easily invade neck structures, metastasize to distant organs, and are resistant to conventional chemotherapy and radiation therapy. Capdevilla and colleagues sought to understand more in detail how, and if, ATCs truly derive from PTCs by performing WES of 14 ATC samples, including from 3 patients with concomitant ATC and PTC (Capdevila et al. 2019). Interestingly, in the cases of concomitant ATC and PTC, most mutations identified in the ATC component differed from the ones in PTC. Phylogenetic tree analyses indicated that ATC and PTC might diverge early in tumor development and evolve independently. Mutational analysis of 44 ATCs and 401 PTCs/FTCs from public databases confirmed that most of the high incidence lesions were not the same between ATCs and PTCs or FTCs. For example, despite the fact that \(BRAF\) and \(NRAS\) are frequently mutated in these tumor types, \(BRAF\) mutations have a higher incidence in PTC (60%) than ATC (37%), and \(NRAS\) mutations are more frequent in FTC (30–45%) than ATC (21%), supporting early divergence.

MTCs originate from the interstitial C cells of the thyroid and account for 2–3% of thyroid cancers. Most cases are sporadic but familial cases exist that are inherited in an autosomal dominant manner and account for approximately 20% of the cases. Familial MTCs are most of the time associated with gain of function germline \(RET\) receptor mutations. Somatic \(RET\) mutations occur often in sporadic MTCs (30–60%), but \(RAS\) mutations can also be found. Evidence suggests that \(RET\) and \(RAS\) mutations very rarely overlap (Ciampi et al. 2019). Further, \(RET\) mutations are generally associated with an elevated risk for metastasis, tumor recurrence, and patient mortality while the association of \(RAS\) mutations with tumor aggressiveness in sporadic MTC is unclear (Vuong et al. 2018).

Molecular targeted therapies in thyroid cancers

While more than 90% of localized, well-differentiated carcinomas of the thyroid such as PTC and FTC can be cured by surgery combined with thyroid-stimulating hormone (TSH)-suppressive thyroid hormone therapy and occasional use of radioactive iodine, cancers that persist or recur following these regimens have a poorer prognosis. Cytotoxic chemotherapy or external beam radiotherapy shows poor efficacy in these patients, since MTC tumor cells have a neuroendocrine origin, they do not respond to radioiodine or TSH suppression. Therefore, the advent of therapies targeting growth factor receptors, or constitutively activated protein kinases, represented a welcomed additional option. This led to significant improvements in the treatment of advanced cases of radioiodine refractory PTC and FTC, as well as PDTC and ATC. In the United States, there are currently eight drugs and one drug combination that are FDA-approved for advanced thyroid cancers. Three antiangiogenic drugs, sorafenib, lenvatinib, and cabozantinib, are FDA-approved for DTCs (Brose et al. 2014, 2021, Schlumberger et al. 2015). The combination of dabrafenib and trametinib is FDA-approved for \(BRAF^{V600E}\)-mutated ATC (Subbiah et al. 2018b, 2022, Wang et al. 2019a). The NTRK inhibitors larotrectinib and entrectinib are approved for NTRK fusion solid tumors, regardless of tumor histology. In MTCs, two antiangiogenic drugs, vandetanib and cabozantinib, are FDA-approved. Finally, the selective RET inhibitors pralsetinib and selpercatinib are approved for \(RET\)-altered thyroid cancers (Wells et al. 2012, Elisei et al. 2013, Subbiah et al. 2018a,c) (Fig. 1). All these drugs have resulted in remarkable improvement in progression-free survival, in particular in ATC. The anti-angiogenic drugs inhibit VEGFR activity, but they are not specific; whereas some secondary targets may also be anti-angiogenic, such as FGFR, others can lead to ‘off-target’ toxicities (Table 1). This lack of target specificity might cause side effects, such as fatigue, diarrhea, and weight loss, as well as poor wound healing, hypertension, and congestive heart failure. However, the selective RET inhibitors pralsetinib and selpercatinib are new-generation drugs that show a safer toxicity profile probably due to lower inhibition of other tyrosine kinases, including VEGFR (Subbiah et al. 2020, Wirth et al. 2020).

The majority of mutations found in thyroid cancers affect components of the MAPK pathway, with most alterations found in the \(RAS\) and \(BRAF\) genes (Fig. 2B). Targeting \(RAS\) mutations in cancers has proven challenging, but recent advances in targeting \(KRAS^{G12C}\) with inhibitors such as sotorasib and adagrasib have been promising (Huang et al. 2021). These drugs form an irreversible, covalent bond with the cysteine residue of \(KRAS^{G12C}\), holding the protein in its inactive form and blocking downstream signaling. Unfortunately, \(KRAS^{G12C}\) mutations are very rare in thyroid cancers. As mentioned earlier, 60% of PTCs and 37% of ATCs harbor a \(BRAF\) mutation, most often the \(BRAF^{V600E}\) mutation. Dabrafenib is a \(BRAF^{V600E}\)-specific drug, which is FDA-approved alone or in combination with an MEK inhibitor such as trametinib for melanoma (Long et al. 2014). This combination was recently approved for most solid tumors.
in patients who have progressed following prior treatment and have no satisfactory alternative treatment options. In thyroid cancers, dabrafenib is only approved for BRAFV600E-mutated ATCs in combination with trametinib (Subbiah et al. 2018b). The combination dabrafenib–trametinib is also used as neoadjuvant therapy followed by surgery in cases of advanced unresectable disease (Wang et al. 2019a). This regimen represents significant progress in the treatment of ATC as long-term survival of these patients is now significantly increased (Maniakas et al. 2020, Subbiah et al. 2022).

Resistance to kinase inhibitors

Despite a large amount of promising data and early therapeutic successes, blocking signaling pathways with kinase inhibitors appears to be ultimately ineffective and most patients progress. To overcome this issue, second-generation BRAF and MEK inhibitors are presently studied in thyroid cancers, such as encorafenib (type I BRAF inhibitor) and binimetinib (type III MEK inhibitor), which showed significant benefit in metastatic colorectal cancer and advanced melanoma (Table 1) (Dummer et al. 2018, Tabernero et al. 2021). Many tumors display multiple coexisting cellular subclones that confer a high degree of heterogeneity; therefore, single-targeting approaches are limited because new clones may be selected that become dominant. Inherent genomic instability, such as found in ATC, predisposes the tumor cells to rapidly acquire additional alterations that increase their fitness in a changing microenvironment. Efforts have been made to combine targeted therapies with immune checkpoint inhibitors. In DTCs, treatments with immunotherapy are associated with low response rates (Mehnert et al. 2019). Side effects of immunotherapy have led to treatment discontinuations in RET-mutated tumors (Hegde et al. 2020). In ATC, where both PD-L1 positive cells and tumor-infiltrating lymphocytes are present in high frequency, kinase inhibitors combined with immunotherapy are useful only in a subset of patients, but the reasons are unclear (Iyer et al. 2018, Wang et al. 2019a, Capdevila et al. 2020).

Resistance to VEGFR inhibitors

Molecular-targeted therapies inhibiting the VEGFR tyrosine kinase have dominated the field of DTC therapeutics. Because this approach also targets other receptor tyrosine kinases such as RET, FGFR, PDGFR, KIT, and MET, it is likely to remain important in the treatment of this disease. Although some patient subsets show prolonged overall survival (Elisei et al. 2013, Brose et al. 2017), rates of cure are low, responses are only partial, and patients remain on drug for prolonged periods of time causing long-term toxicity. Acquired resistance to anti-VEGF therapy often involves escape mechanisms that activate parallel signaling pathways. For example, upregulation of fibroblast growth factor (FGF2) signaling is often observed in anti-VEGF-resistant tumors, especially in tumors that are exposed to a hypoxic environment (Casanovas et al. 2005). While preclinical studies showed that dual blockade of VEGF and FGF signaling pathways was beneficial, this did not translate in clinical studies (Semrad et al. 2017). Acquired resistance to sorafenib can also be due to activation of the PI3K/AKT pathway or reactivation of the JAK-STAT pathway and STAT3 phosphorylation (Zhu et al. 2017). Further, epigenetic mechanisms might also explain sorafenib resistance. For example, the group of Wang and colleagues demonstrated that miR-124 and miR-506 were significantly reduced in the serum of sorafenib-resistant thyroid cancer patients and that their common target, EZH2, was upregulated in these tumors and thyroid cancer cell lines in comparison with controls (Wang et al. 2019b). EZH2 is a well-known histone modification factor that represses the transcription of specific genes. Its upregulation is associated with poor clinical outcomes in many cancer types and EZH2 inhibitors are presently tested in clinical trials (Duan et al. 2020, Li et al. 2021a). Acquired resistance to lenvatinib in thyroid cancers has been also studied, mainly in preclinical models (Khan et al. 2019, Bertol et al. 2022). Khan and colleagues performed long-term cultures of ATC cells exposed to lenvatinib for 72 days. They demonstrated significant changes in lenvatinib-resistant cells toward a mesenchymal morphology. Transcriptomic and proteomic analyses uncovered upregulation of SNAIL and ZEB1 genes/proteins, activation of pro-survival signaling pathways, activation of the nuclear exporter protein exportin 1 (XPO1), and activation of the RAC/RHO GTPase effector p21 activated kinases (PAK). When combined with lenvatinib, XPO1 and PAK4 inhibitors showed antitumor activity that was superior to lenvatinib alone, both in vitro and in subcutaneous xenografts (Khan et al. 2019). This and similar studies may pave the way for future novel therapies.

Resistance to BRAFV600E inhibitors

Acquired (or secondary) resistance to BRAFV600E inhibitors presents a significant therapeutic challenge
in thyroid cancer patients. Vemurafenib and dabrafenib monotherapies increase progression-free survival, but responses are incomplete (Kim et al. 2013, Dadu et al. 2015, Falchook et al. 2015, Brose et al. 2016). Resistance mechanisms have been extensively studied in melanoma and colon cancers. Sequence analysis of the mutated \textit{BRAF} gene in vemurafenib-resistant melanoma samples demonstrated that secondary mutations within this gene are rare (Nazarian et al. 2010). Only one case of an additional mutation in the kinase domain was found (Wagenaat et al. 2014). Therefore, other mechanisms, including the activation of parallel signaling pathways, may be responsible for acquired resistance.

**Overexpression of membrane receptors**

Several studies have now demonstrated that cancer cells adapt to \textit{BRAF} inhibitor therapies by overexpressing growth factor receptors at their surface. This is often the case for KIT, MET, EGFR, and PDGFRβ. This increase in expression may be epigenetically directed since no secondary activating mutation nor DNA amplification of these genes were detected (Nazarian et al. 2010, Corcoran et al. 2012, Prahallad et al. 2012). Corcoran and colleagues showed that in colorectal cancers, resistance to vemurafenib was mediated by the epigenetic upregulation of EGFR, which reactivated RAS and CRAF, and the downstream phosphorylation of ERK1/2 (Corcoran et al. 2012). Similarly, Montero-Conde and colleagues demonstrated that \textit{BRAF}-mutated PTC cell lines acquire resistance to vemurafenib by overexpressing the ERBB or FGF receptor families, therefore reactivating the MAPK pathway downstream of activated RAS (Montero-Conde et al. 2013). Resistance to vemurafenib in melanoma can also be caused by overexpression of EPHA2, a member of the Ephrin receptor family of tyrosine kinases (Miao et al. 2015). These mechanisms of resistance driven by membrane receptor overexpression imply that the corresponding ligands must be present in sufficient quantity in the cellular microenvironment for their activation. Often, ligand production is indeed upregulated in an autocrine manner in cancer cells, and experimental data have shown that most cells can bypass receptor tyrosine kinase inhibitors by simply exposing them to one or more receptor tyrosine kinase ligands (Wilson et al. 2012). For example, exposure to hepatocyte growth factor from the microenvironment will promote intrinsic resistance to \textit{BRAF} inhibitors in melanoma, colorectal cancer, and glioblastoma cells overexpressing the MET receptor (Straussman et al. 2012). These results underscore the importance of the cellular microenvironment for drug resistance.

**Gene amplification**

Gain of WT gene copy numbers has been associated with resistance to \textit{BRAF} inhibitors. For example, several copies of the gene \textit{MCL1}, a BCL2 family member and apoptosis modulator, have been associated with resistance to vemurafenib treatment in PTC (Duquette et al. 2015). Our group recently demonstrated that resistance to \textit{BRAF} inhibitors can also be caused by acquired chromosomal polyploidy. In one case of PTC that dedifferentiated into ATC during dabrafenib treatment, we observed triploidy of chromosome 7, which harbors the \textit{EGFR}, \textit{RAC1}, \textit{MET}, and \textit{BRAF} genes. Copy numbers and expression of these protooncogenes were consequently increased in the dedifferentiated sample, probably causing tumor progression (Bagheri-Yarmand et al. 2021). It is now evident that overexpression of any non-mutated receptor tyrosine kinase has the potential to reactivate the MAPK pathway (Yadav et al. 2012) or the PI3K pathway (Atefi et al. 2011, Chapman et al. 2011, Greger et al. 2012, Delmas et al. 2015, Byeon et al. 2017) provided that the ligand is present in sufficient quantity.

**Point mutations**

Acquired resistance to \textit{BRAF} inhibitors in melanoma and colorectal cancers has often been attributed to secondary mutations in the \textit{NRAS} or \textit{KRAS} genes (Nazarian et al. 2010, Kopetz et al. 2015, Monsma et al. 2015). Our group sought to identify the acquisition of secondary resistance mechanisms in thyroid cancers by exposing \textit{BRAF}\textsubscript{V600E}-mutated PTC cells to long-term treatment with vemurafenib for a period of 5 months (Danysz et al. 2016). This treatment induced upregulation of the MET, EGFR, and ERBB receptors and activation of the PI3K/AKT and MAPK pathways, as previously described by others (Montero-Conde et al. 2013, Byeon et al. 2017). Using WES, we also identified a subpopulation of cells harboring a secondary \textit{KRAS} mutation (\textit{KRAS}\textsubscript{G12D}), conferring them significant proliferative and invasive advantages. These data, obtained with a cell line, were later validated by the observation that about 50% of our \textit{BRAF}\textsubscript{V600E}-mutated patients progressing under vemurafenib or dabrafenib treatment (with/without MEK inhibitors) harbored a secondary \textit{RAS} mutation (\textit{NRAS} or \textit{KRAS}) after WES analysis (Table 2) (Owen et al. 2019, Cabanillas et al. 2020). These studies were the first to establish that acquired resistance to kinase inhibitors in thyroid cancers can be \textit{RAS}-driven. In addition to \textit{RAS} point mutations, secondary mutations that possibly conferred drug resistance were often found in the \textit{PTEN}, \textit{NF1}, \textit{NF2}, \textit{TP53}, and \textit{CDKN2A} genes (Table 2). We recently analyzed a
**Table 2**  Mutations in PTC, PDTC, and ATC patients at baseline and at progression.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Histology</th>
<th>Mutation(s) at baseline</th>
<th>Kinase inhibitor(s)</th>
<th>Immune checkpoint inhibitor</th>
<th>Additional mutations at progression</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>PTC</td>
<td>BRAF^V600E</td>
<td>D+T</td>
<td>ND</td>
<td>BRCA^D237A, PTEN^S60*</td>
</tr>
<tr>
<td>2</td>
<td>ATC</td>
<td>BRAF^V600E, PIK3CA^E525K, TP53^G331*</td>
<td>D+T</td>
<td>ND</td>
<td>CDKN2A^D84N, TP53^R273H</td>
</tr>
<tr>
<td>3</td>
<td>PDTC</td>
<td>BRAF^V600E</td>
<td>D</td>
<td>ND</td>
<td>KIT^M541L, NF1^R1276Q</td>
</tr>
<tr>
<td>4</td>
<td>ATC</td>
<td>BRAF^V600E, PIK3CA^E525K</td>
<td>V+T</td>
<td>ND</td>
<td>PIK3CA^E545K, TP53^R175H, EGFR^G325S</td>
</tr>
<tr>
<td>5</td>
<td>ATC</td>
<td>BRAF^V600E, PIK3CA^E525K</td>
<td>D+T</td>
<td>ND</td>
<td>MET^R1007K, NOTCH1^P213R, NOTCH1^E360K</td>
</tr>
<tr>
<td>6</td>
<td>ATC</td>
<td>BRAF^V600E, TP53^R175H</td>
<td>D</td>
<td>ND</td>
<td>KIT^M541L, NF1^R1276Q</td>
</tr>
<tr>
<td>7</td>
<td>ATC</td>
<td>BRAF^V600E, TP53^R175H</td>
<td>D+T</td>
<td>ND</td>
<td>PIK3CA^E545K, TP53^R175H, EGFR^G325S</td>
</tr>
<tr>
<td>8</td>
<td>ATC</td>
<td>BRAF^V600E, ATM^Q516V</td>
<td>D+T</td>
<td>ND</td>
<td>KIT^M541L, NF1^R1276Q</td>
</tr>
<tr>
<td>9</td>
<td>PDTC</td>
<td>BRAF^V600E, PIK3CA^E545K</td>
<td>V+C</td>
<td>A</td>
<td>NRAS^G12V</td>
</tr>
<tr>
<td>10</td>
<td>PDTC</td>
<td>BRAF^V600E, PIK3CA^E545K</td>
<td>V+C</td>
<td>A</td>
<td>NRAS^Q61K</td>
</tr>
<tr>
<td>11</td>
<td>ATC</td>
<td>BRAF^V600E, PIK3CA^E545K</td>
<td>V+C</td>
<td>A</td>
<td>NRAS^Q61K</td>
</tr>
</tbody>
</table>

A, atezolizumab; C, cobimetinib; D, dabrafenib; ND, no immunotherapy; P, pembrolizumab; T, trametinib; V, vemurafenib.
secondary mutation in the RAC1 gene, RAC1P34R, found in a lymph node metastasis of a PTC patient who progressed under dabrafenib treatment. A cell line was established that showed dabrafenib resistance in vitro, and sensitivity to the drug was restored with a RAC1 activity inhibitor (Bagheri-Yarmand et al. 2021). RAC1 is a small GTPase involved in cell motility and cytoskeletal reorganization, as well as cell proliferation through its canonical targets, PAK1-3 (Mack et al. 2011). The RAC1P29S mutation, which affects the same protein domain as RAC1P34R, is a known driver mutation in primary and drug-resistant melanoma (Watson et al. 2014, Cannon et al. 2020). Similar to RAS, efforts to target RAC1 itself have been challenging; therefore, approaches to target downstream effectors such as PAK-3 are underway. Understanding tumor heterogeneity and mutational patterns emerging under drug pressure is therefore fundamental to improving therapies.

Super-enhancers

Enhancers are short sequences of DNA that are located near or far from promoter regions and can be bound by specific transcription factors to increase transcription. In a broad range of human cell types, super-enhancers (SEs) are large clusters of enhancers with high levels of transcription factor binding (Tang et al. 2020). SEs are critical for driving the expression of genes that control cell identity. They also regulate and increase the expression of key oncogenes such as c-MYC in many tumor cells, and their development drives drug resistance (Li et al. 2021b). Therefore, disrupting SE structure or inhibiting SE protein co-factors can be utilized as therapeutic strategies. For example, BET bromodomain inhibitors can repress c-MYC expression by decreasing the binding of bromodomain-containing-protein 4 to c-MYC SE regions (Delmore et al. 2011). SE-dependent transcription also utilizes cyclin-dependent kinase 7 (CDK7) at the initiation complex (TFIIH), which can be inhibited by the covalent inhibitor THZ1 (Chipumuro et al. 2014). Inhibitors of BET and CDK7 were recently tested in preclinical studies and significantly reduced tumor growth in cancer cell lines and tumor-bearing mice (Mertz et al. 2011, Nakamura et al. 2017). The effects of selective CDK7 and BET inhibitors in advanced solid tumors are now studied in clinical trials. In thyroid cancer cells, BET inhibitors repressed MYC expression in ATC, and the CDK7 inhibitor THZ1 repressed RET expression in MTC cells (Valentiaga et al. 2018, Cao et al. 2019, Zhu et al. 2019).

Overcoming resistance

Additional kinase inhibitors

Overcoming acquired resistance in BRAF-mutated PTC and ATC has been difficult because targeting MEK downstream of BRAFV600E with MEK inhibitors such as selumetinib, cobimetinib, and trametinib, alone or in combination with BRAF inhibitors, rarely prevents progression (Table 2) (Wagle et al. 2014, Fofaria et al. 2015, Cabanillas et al. 2020). In addition, BRAF and/or MEK inhibitors might reactivate ERK1/2 activation/phosphorylation through parallel signaling pathways (Samatar & Poulikakos 2014). Therefore, recent efforts have focused on targeting ERK1/2 itself (Germann et al. 2017, Roskoski 2019). Drugs showing promising preclinical activities such as BVD-523 (ulixertinib) are still in clinical trials, but the FDA recently granted expanded access to ulixertinib for treatment of stage IIb through Stage IV BRAF-mutant melanoma. Unfortunately, resistance to ERK1/2 inhibitors has already been identified, with activation of MEK5-ERKS as a compensatory mechanism (Tubita et al. 2021).

In a recent study, Iyer and colleagues showed that ATC patients treated with lenvatinib, or dabrafenib combined with trametinib, showed additional response when given the PD-L1 inhibitor pembrolizumab after initial progression. Therefore, in certain cases, immunotherapy can be used as an effective therapy to improve the benefits of kinase inhibitors (Iyer et al. 2018).

Redifferentiation therapies

The uptake of iodine by thyroid follicular cells is mediated by a sodium iodide symporter (NIS) located in the basolateral membrane of follicular cells. NIS spatiotemporal expression and function depends on a number of modulators. TSH (thyrotropin) upregulates NIS expression and stimulates its transport to the plasma membrane (Kogai et al. 1997, Riedel et al. 2001). PIGU, a subunit of GPI transamidase that attaches GPI-anchors to proteins, ensures proper anchoring of NIS at the endoplasmic reticulum, and therefore at the plasma membrane after its vesicular transport, itself stimulated by ARF4 (Eisenhaber et al. 1998, Amit et al. 2017, Fletcher et al. 2020). These NIS effectors are counterbalanced by inhibitors of NIS function, expression, and localization. For example, at the plasma membrane, the NIS inhibitor PTG-binding factor (PBF) inhibits the uptake of iodide by binding NIS (Smith et al. 2009). Further, β-catenin seems to control the availability of NIS since its overexpression leads to the decrease of NIS expression and...
its transfer from the plasma membrane to an intracellular location close to the nucleus \cite{Lan2017a,Lan2017b}. In thyroid cancer cells, the balance between positive and negative NIS modulating factors is dysregulated. Cellular dedifferentiation, which increases with the accumulation of \textit{BRAF}, \textit{RAS}, \textit{TEKT}, or other gene mutations, correlates with the downregulation of NIS expression at the plasma membrane and its relocation in the cytoplasm, which is not its proper functional location \cite{Tavares2018,Martin2019}. Thus, redifferentiation therapies often aim at redistributing NIS properly at the plasma membrane to improve NIS-mediated RAI treatment.

Increasing the level of serum thyrotropin (TSH) before RAI therapy, either by inducing hypothyroidism or by administering recombinant human TSH, will significantly increase the uptake of RAI in differentiated tumors with intact iodine uptake mechanisms \cite{Klubo-Gwiezdzinska2012,Haugen2016}. However, additional modalities are required to re-differentiate tumors that have lost the capability to uptake iodine. Approaches have been attempted to increase NIS expression at the plasma membrane. For example, retinoic acid (RA) has been used to redifferentiate cancer cells for decades. RA is the bioactive metabolite of vitamin A and plays a critical role in cellular differentiation during mammalian development. RA indeed may increase the expression of NIS mRNA in human FTC cells \cite{Schmutzler2002,Lan2017a}; however a recent meta-analysis reported that only a minority of patients with RAI-refractory DTC responded to RA treatment \cite{Pak2018}. This poor response might be explained by the fact that RA treatment induces growth inhibition through retinoic acid receptor beta in cells where it is expressed, rather than through an increase of NIS at the plasma membrane \cite{Elisei2005}. Similarly, PPAR\textgamma agonists and histone deacetylase inhibitors, which showed good effects in preclinical studies, offered disappointing results in clinical trials \cite{Park2005,Woyach2009,Rosenbaum-Krumme2012,Sherman2013,Cheng2016,Nilubol2017}. Further, PBF phosphorylation levels could be reduced using a c-SRC kinase inhibitor, at least \textit{in vitro} \cite{Smith2013}. Other preclinical studies indicated that treatment with MEK inhibitors rescued PIGU expression in PTC cells \cite{Amit2017}. Recently, Fletcher and coworkers demonstrated that clotrimazole, an antifungal medication, and ebastine, an antihistaminic, also enhanced RAI uptake in normal mouse thyrocytes and human thyroid cancer cells due to their allosteric inhibition of valosin-containing protein (VCP/p97). They also demonstrated the importance of ARF4 in the trafficking of NIS to the plasma membrane \cite{Fletcher2020}. Further, clinically relevant redifferentiation was previously obtained in small cohorts of patients treated with MAPK pathway/\textit{BRAF} inhibitors. In particular, dabrafenib, trametinib, and selumetinib seemed to improve the iodine uptake rate of thyroid cancer cells by improving NIS expression and function \cite{Ho2013,Rothenberg2015,Jaber2018,Dunn2019,Lebouleux2021}. However, a recently completed phase III clinical trial showed that the addition of selumetinib to adjuvant RAI did not significantly improve the complete remission rate in DTC patients with metastatic disease \cite{Ho2022}. Finally, redifferentiation of NTRK-rearranged, RAI-resistant thyroid tumors was possible with larotrectinib \cite{Groussin2022}, and selpercatinib restored iodine uptake in almost all metastases in a patient with a \textit{RET} rearrangement/fusion as a sole mutation \cite{Groussin2021}. While these results are encouraging, they were obtained from case studies. Therefore, these drugs should not yet be used as routine short-term pretreatments before RAI in DTCs.

**Conclusions**

Protein kinases play critical roles in signaling pathways that transfer information from receptors located at the plasma membrane down to the nucleus, eventually modifying and adapting cell behavior to the environment. Mutational changes can dysregulate the function of many of these kinases, which become constitutively active and lead to cancer cell proliferation and metastases. Targeted therapies use kinase inhibitors that often target the ATP-binding pocket. These novel drugs are better tolerated than chemotherapies. However, resistance to these inhibitors is a challenge that will need to be overcome. Multiple molecular strategies will be required to thoroughly understand mechanisms of resistance and devise effective targeted treatments, including disruption of the transcriptional machinery of specific genes and cellular redifferentiation. Finally, there is also a need to identify biomarkers that will help predict response and resistance to improve the selection of an optimal targeted therapy for each patient.

**Declaration of interest**

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