**Molecular Pathways**

**Targeting Vascular Endothelial Growth Factor Receptor in Thyroid Cancer: The Intracellular and Extracellular Implications**

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**Abstract**

Our understanding of the molecular pathophysiology of differentiated thyroid cancer (DTC) has developed considerably over the last 10 years. Aberrant signaling through B-Raf and Akt has been implicated in the tumorigenesis of DTC. Moreover, these highly vascular tumors have proven to be sensitive to the inhibition of vascular endothelial growth factor receptor (VEGFR-2). It is likely that the multikinase inhibitors, sorafenib, sunitinib, axitinib, and motesanib, whose targets include VEGFR-2, exert their effects primarily through inhibition of endothelial cells. However, as VEGFR-2 is expressed on DTC cells, these compounds may have direct antitumor action. This review will discuss the key signaling pathways involved in thyroid cancer and their implications for targeted therapy.

**Background**

Thyroid cancer accounts for up to 95% of primary cancers involving the endocrine system. It has been estimated that 37,340 new cases of thyroid cancer were diagnosed in 2008 (1, 2). Papillary (PTC) and follicular (FTC) carcinomas, including Hürthle cell carcinoma (HCC), arise from thyroid epithelial cells and together make up differentiated thyroid cancer (DTC). However, epithelial cells also give rise to anaplastic thyroid cancer (ATC), a rare and extremely aggressive type of thyroid cancer that is lethal within a year in the majority of cases. Medullary thyroid carcinoma (MTC) arises from the parafollicular cells that produce calcitonin. Immune and stromal cells give rise to lymphomas and sarcomas and are treated accordingly.

DTCs, which account for approximately 90% of thyroid cancers, are generally indolent malignancies (3, 4), in which 80 to 90% of patients survive to 10 years or more (5). For most DTCs, definitive surgical resection with total thyroidectomy is indicated followed by postoperative radioiodine ablation with 131I (RAI) to destroy residual thyroid tissue (6). Treatment strategies for recurrent disease depend on the site of recurrence. Local recurrences are treated with re-excision, subsequent RAI, or both. Pulmonary metastases, the most common site of distant recurrence, are often treated with RAI, whereas bone metastases are often treated with surgery or external beam radiation. Unfortunately, a significant number of these will become RAI unresponsive, either because there is lack of uptake (iodine nonavid disease) or because they have progressed in spite of adequate RAI doses (RAI refractory). Until recently, results using systemic therapy with cytotoxic drugs for progressive, metastatic disease not controlled by surgery or RAI have been disappointing, and so patients have been managed symptomatically. Numerous studies in the 1980s examined the treatment of metastatic thyroid cancer with doxorubicin as both a single agent and combination therapy (7, 8), whereas a more recent trial examined a potential role for taxanes (9). The only FDA-approved, systemic treatment for advanced, refractory DTC is doxorubicin (10). However, using conventional dosing and imaging, response rates are disappointing (~5%; ref. 11). Due to the low response rate and significant toxicity, doxorubicin is rarely used, and the majority of advanced DTC patients are managed symptomatically. Over the last 3 years, new targeted agents have been shown to have significant clinical activity and, as a result, are creating a paradigm shift for the management of these patients (12).

**Role of Intracellular Signaling in Thyroid Cancer and Associated Endothelial Cells**

Intracellular signaling pathways involving Ras, B-Raf, and Akt are critical to the molecular pathophysiology of DTC (Fig. 1). Ras and Raf are central mediators of the MAP-kinase signaling pathway (13), and, as such, mediate cell survival and proliferation. Of note, Ras activates both Raf and PI3K as well as many other downstream effector molecules (13). Though several Raf mutations have been reported in DTC [e.g., *BRAF* T599I-VKSR (del 600-603); ref. 14], the most common (approximately 80-90%) is...
the BRAF T17699A (V600E) mutation that results in constitutive activation of the kinase, present in 30 to 70% of PTC (15–18). Additionally, the multiple isoforms of RAS (H-, K-, and N-RAS) may harbor mutations in DTC (19). Estimates of the prevalence of RAS mutations in thyroid tumors vary considerably but are generally reported to be in the range of 25% to 30% (15, 20). Further studies show that genetic alteration in RAS and RAF in DTC is associated with higher risk phenotypes, more aggressive disease, and poorer overall survival (21, 22). Mutations that alter intracellular signaling, such as RAS and RAF, generally occur in a mutually exclusive fashion.

More recently, increased activity of the PI3 kinase (PI3K)/Akt pathway has been shown to play a role in the tumorigenesis and progression of DTC. PI3K/Akt signaling promotes tumor cell survival and proliferation (23–25). PI3K is activated by various membrane growth factor receptors such as epidermal growth factor receptor (EGFR), vascular endothelial growth factor receptor (VEGFR), and platelet-derived growth factor receptor (PDGFR). Activating mutations in PI3K have been identified in ATC cell lines (26), and increased expression has been seen in PTC tissue (23). In thyroid cancer cells, PI3K, through its p110 catalytic subunit, activates phosphatidylinositol-4,5-bisphosphate (PIP2) to phosphatidylinositol-3,4,5-trisphosphate (PIP3). PIP3 in turn participates in the phosphorylation of Akt to its active state with a phosphoinositide-dependent kinase, PDK-1 (23, 27). Akt activates the mammalian target of rapamycin (mTOR). The PI3K/Akt pathway is antagonized by the tumor suppressor gene PTEN, whose gene product dephosphorylates PIP3 thus interrupting PI3K signaling. Akt expression also has been shown to lead to the expression of HIF1α, raising the possibility that Akt might play a role in tumor angiogenesis (28, 29). Akt is overexpressed in sporadic thyroid cancers (30) and induces apoptosis in thyroid cancer cell lines (31). Point mutations in PIP3 are found in ATC cell lines, and PIP3 has been found to be overexpressed in PTC tumor specimens (26).

Genetic alterations involving RET have long been implicated in the molecular pathophysiology of sporadic PTC. The proto-oncogene RET encodes a receptor tyrosine kinase involved in cell survival (32). More than 15 translocations of the C terminus of RET with various N-terminus genes and their promoters have been found to form chimeric oncogenes in PTC (32). The most common of these are RET/PTC1 and RET/PTC3. The prevalence of these events has been estimated to be as high as 43% in PTC (15). Interestingly, RET/PTC rearrangements have been shown to signal through Ras, B-Raf, and Akt (33–36), once again highlighting the importance of these two signaling pathways in thyroid cancer.

Considerable evidence now points to the effectiveness of targeting angiogenesis to arrest tumor progression and control disease. VEGFR signals via both Akt and Raf in endothelial cells (Fig. 1). VEGF receptors are members of a
family of transmembrane tyrosine kinase receptors that mediate signal transduction from extracellular signaling ligands, like vascular endothelial growth factor (VEGF), to intracellular signaling cascades. VEGFR-2, which is expressed on endothelial cells (37) is thought to be among the most important mediators of angiogenesis and is activated primarily by the ligand VEGF-A165 (37, 38). When activated, VEGFR-2 activates Raf and the mitogen-activated protein kinase (MAPK) pathway via phospholipase C-γ (PLC-γ) in the endothelial cell (39). PI3K, also activated by VEGFR-2, leads to the activation of Akt in endothelial cells (38). The result of activation of these intracellular signaling cascades is the promotion of endothelial cell survival, proliferation, and migration—key steps in tumor angiogenesis (40). The importance of VEGFR-2 and angiogenesis in thyroid cancer tumor progression is supported by a number of observations the first of which is that thyroid cancers are highly vascular. Second, studies have shown that VEGF expression correlates with risk for the development of metastatic disease in PTC (41). Last, as will be described below, VEGFR-2 inhibition is the most effective new therapeutic strategy developed to date in the treatment of these tumors (22, 42, 43).

Clinical-Translational Advances

The lack of effective systemic treatments for advanced DTC combined with the knowledge that aberrant intracellular signaling plays a central role in thyroid cancer tumorigenesis present a unique opportunity for new targeted agents, many of which are multikinase inhibitors (MKI; ref. see Table 1). Only gefitinib, of EGFR, has been found to have relatively little effect on DTC (44). Common to all MKIs that have shown activity in DTC is VEGFR-2 inhibition (38). Anti-angiogenic agents that predate the kinase inhibitors also have shown activity in thyroid cancer as was the case with combretastatin A4 (45). However, the ease of administration, MKIs are oral drugs, combined with their relatively tolerable toxicity profiles make MKIs the preferred strategy for first-line systemic therapy. Although most MKIs target VEGFR on endothelial cells, differential therapeutic efficacy in DTC may relate to the unique molecular inhibition profile of each drug. Furthermore, VEGFR-1 and VEGFR-2 are expressed on tumor cells in thyroid cancer and other solid tumor malignancies (46–48). Although downstream effectors of VEGFR-2 signaling in thyroid cancer are not known, BRAF and Akt are likely to be involved (Fig. 1).

The MKIs exert their effects through competitive allosteric inhibition of the intracellular ATP-binding site of VEGFR-2 or other target kinases (49). VEGFR-2 is taken to be the key MKI therapeutic target on the basis of a number of factors. First, it is central to the process of angiogenesis (50). Additionally, preclinical research has shown a relationship between alteration of circulating VEGF and soluble VEGFR-2 levels and antitumor activity (51, 52). Finally, it is a target shared by all active drugs in this class. Sorafenib was first examined and is now U.S. Food and Drug Administration (FDA)-approved in the treatment of metastatic renal cell carcinoma (49, 53, 54). Sorafenib is an oral inhibitor of several tyrosine kinases including VEGFR-2, -3, PDGFR, RET, and B-Raf (55). Sorafenib first showed clinical activity in the treatment of TC in an open-label, single-arm phase II clinical trial (22). Sixty percent of patients had PTC, and at the time of the interim analysis the median progression-free survival (PFS) was 79 weeks overall and 84 weeks for patients with DTC. Seven patients (23%) had a partial response, and 16 (53%) had stable disease for an overall clinical benefit rate of 77%. A second phase II clinical trial confirmed the activity of sorafenib in patients with DTC (56). Sunitinib, an oral MKI with selectivity for all isoforms of VEGFR, PDGFR, c-KIT, and RET also has been FDA-approved for the treatment of metastatic renal cell carcinoma (57) and has shown activity in DTC (58). Additional clinical trials are underway examining the activity of sunitinib in DTC (58). The National Comprehensive Cancer Network (NCCN) has recommended that patients with metastatic DTC in need of systemic treatment be enrolled on clinical trials examining small molecule compounds. In the event that such trials are not available, the NCCN recommends that patients be treated with FDA-approved and available agents such as sorafenib or sunitinib (59).

Two additional MKIs in development, but not currently available, have shown promise for patients with DTC. Axitinib (AG-013736) is an oral multikinase inhibitor that selectively inhibits all isoforms of VEGFR potently but is less selective for PDGFR and c-KIT. In a phase II clinical trial, 18 patients (30%) experienced a partial response, and another 23 patients (38%) experienced stable disease (42). The authors estimated the median PFS to be approximately 79 weeks, and they found axitinib to be

| Table 1. Targets of kinase inhibitors for metastatic thyroid cancer |
|-----------------|--------|--------|--------|--------|--------|--------|--------------------|
| **Compound**    | VEGFR  | BRAF   | PDGFR  | KIT    | RET    | EGFR   | **Other**          |
| Sorafenib (Nexavar) | +     | +     | +     | +     | +     |        | FLT-3             |
| Sunitinib (Sutent)      | +     | +     | +     | +     | +     |        |                   |
| Vandetinib (Zactima)    | +     | +     | +     |        |        | +     |                   |
| Axitinib (AG-013736)    | +     | +     | +     | +     | +     |        |                   |
| Motesanib (AMG-706)     | +     | +     |        | +     |        | +     |                   |
well-tolerated with hypertension as the most common National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE) grade 3 toxicity (12%). Fifty percent of patients had PTC, but all subtypes of thyroid cancer enjoyed a clinical benefit with treatment. Motesanib diphosphate (AMG 706) is an oral inhibitor of all isoforms of VEGFR, PDGFR, and c-KIT that has a lower overall response rate than the other MKIs (14%), but a higher stable disease rate (67%). Median PFS for AMG 706 was 40 weeks (95% confidence interval, 32-50 weeks).

Several additional compounds are in development and have the potential for therapeutic efficacy in thyroid cancer. Vandetanib, a small molecule inhibitor of EGFR, VEGFR-2, -3, and RET, is currently in development in the phase II setting in medullary thyroid cancer (60, 61). Pazopanib (VEGFR1-3, c-kit, and PDGFR), AZD6244 (MEK inhibitor), XL281 (BRAF, CRAF inhibitor), PLX4032 (highly selective BRAF inhibitor), and others are under investigation (62–67). The potential for improved responses with the selective inhibition of BRAF remains to be determined.

It is worth noting that the clinical benefits associated with VEGFR-2 inhibition seem to be greater than those observed in the setting of other metastatic solid tumor malignancies (e.g., colorectal, breast, non-small cell lung cancer). When added to chemotherapy, bevacizumab extended overall survival in general by about 25%, in some cases more, in some cases less (68–72). Similarly, sunitinib extended overall survival by approximately 25% as compared with interferon alpha in the treatment of metastatic renal cell carcinoma (57). It is difficult to compare the impact of MKIs in the treatment of DTC to these other tumor types. In the case of the former group, there are large, randomized phase III clinical trials measuring survival, whereas only phase II clinical trials have been completed in DTC. Nevertheless, the percentage of patients with DTC experiencing a clinical benefit on MKIs is on the order of 80%, and median PFS, as described above, has been quite long, 79 to 84 weeks. This is considerably longer than that observed for other solid tumor malignancies, and it is longer than the median survival of 15 to 20 months reported from small clinical trials of single-agent doxorubicin in the treatment of DTC (73, 74).

Conclusions

Increased intracellular signaling through VEGFR-2, MAP-kinase, and Akt is a prominent feature of thyroid cancer tumor and endothelial cells. This attribute has allowed significant inroads into the treatment of patients with advanced disease. Targeted inhibition of VEGFR-2 in endothelial cells, and, potentially thyroid cancer cells, has lead to considerable advances in therapeutic efficacy in DTC over the last 3 years. How the presence of genetic alterations in individual tumors affects the therapeutic index of these tumors is a subject for further studies including future large scale phase III trials of these agents. Sequential and combination therapy is the likely next step in improving outcome for patients that do not respond and for the development of second-line therapy for patients that develop resistance. Given that there is no standard of care for patients with advanced DTC and that these agents are becoming increasingly available off label, a randomized, placebo-controlled trial with a cross-over design will likely be the best approach to enable the recruitment and retention of patients on study, but it will make overall survival endpoints elusive if not impossible to obtain. In spite of these difficulties, these agents provide advanced thyroid cancer patients with well-tolerated therapies that offer prolonged progression-free survival.

Disclosure of Potential Conflicts of Interest

M. Brose, consultant, Onyx Pharmaceuticals, Bayer Healthcare. The other authors disclosed no potential conflicts of interest.

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