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Current Management of Medullary Thyroid Cancer

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Key Words. Thyroid neoplasm • Medullary thyroid cancer • Multiple endocrine neoplasia • RET protein

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LEARNING OBJECTIVES

After completing this course, the reader will be able to:

1. Evaluate a patient with a new diagnosis of medullary thyroid cancer.
2. Use genetic testing for the ret proto-oncogene and assess how the location of the mutation affects the risks for the patient.
3. Select among the surgical treatment options for patients with medullary thyroid cancer, including the optimal surgical treatment for patients with palpable disease as well as those patients who are found to be genetic carriers.

ABSTRACT

Medullary thyroid cancer accounts for 5%–10% of all thyroid cancers. The majority of medullary thyroid cancers are sporadic, but 20% of cases are a result of a germline mutation in the ret proto-oncogene. Hereditary medullary thyroid cancer can be seen as part of the multiple endocrine neoplasia syndrome type 2A or 2B or as part of familial medullary thyroid cancer. This article discusses the current methods available for the diagnosis and evaluation of a patient with suspected medullary thyroid cancer. The management of medullary thyroid cancer is predominantly surgical excision, consisting of a total thyroidectomy and lymph node dissection. The extent and timing of surgical excision are discussed. Systemic therapeutic options are limited for medullary thyroid cancer, but several therapeutic targets show promise for the development of new therapies in the future. The Oncologist 2008;13:539–547

INTRODUCTION

Medullary thyroid cancer (MTC) currently accounts for 5%–10% of all thyroid cancers. The clinical course of MTC varies from an extremely indolent tumor that can go unchanged for years to an aggressive variant that is associated with a high mortality rate. The majority of MTCs are sporadic, but approximately 20% of MTCs are a result of a germline genetic mutation in the rearranged during transfection (ret) proto-oncogene. Hereditary MTC can be seen in isolation (familial medullary thyroid cancer [FMTC]) or as part of the multiple endocrine neoplasia (MEN) syndrome type 2 (2A or 2B).

MTC is a malignancy of the parafollicular C cells of the thyroid. The C cells are of neural crest origin and migrate to the
ultimobranchial body and are incorporated into the thyroid when the ultimobranchial body fuses with the median thyroid anlage. C cells are located throughout the thyroid gland, but the majority of C cells are located at the junction of the upper third and lower two thirds of the thyroid gland; hence, this is the most common location of MTC. C cells secrete a variety of peptides and hormones, with calcitonin being the most common. Serum calcitonin levels are elevated in patients with MTC and can be used to confirm the diagnosis as well as to follow patients longitudinally for recurrence.

**DIAGNOSIS**

Most patients diagnosed with sporadic MTC present with a palpable neck mass. MTC tends to present in the fifth or sixth decade of life and there is a slight female preponderance. Sporadic MTC is usually unifocal. These tumors tend to be located in the posterior thyroid, and therefore can compress or invade local structures causing hoarseness, dysphagia, or respiratory difficulty. High levels of circulating calcitonin levels can also cause symptoms including flushing, diarrhea, and weight loss. Lymph node involvement is seen in 35%–50% of patients at initial diagnosis. Distant metastases are present in 10%–15% of patients at the time of diagnosis. The most common locations for metastatic disease include the mediastinum, liver, lungs, and bone.

Most patients with hereditary disease are now identified through genetic testing of at-risk family members. Family members of patients with a germline mutation of the ret gene have a 50% chance of inheriting the mutation. If patients are identified to be genetic carriers, their lifetime risk for malignancy approaches 100%. Hereditary disease tends to present at an earlier age than sporadic disease and commonly presents with multifocal and bilateral involvement.

The diagnosis of MTC is most frequently obtained from a fine-needle aspiration (FNA) of a new thyroid nodule. On FNA, MTC is characterized by the presence of stromal amyloid and the absence of thyroid follicles. FNA cannot always distinguish MTC based on the appearance of the cells alone, so the diagnosis is typically confirmed through the use of immunohistochemistry. Another useful technique that has recently been described is to measure the calcitonin level of the washout fluid from an FNA. This technique appears to be even more sensitive than cytology with immunohistochemistry [1, 2]. Grossly, MTCs are white or gray in color and are very firm to palpation (Fig. 1). Histologically, MTCs form nests of uniform cells that are characterized by the deposition of stromal amyloid (Fig. 2 and Fig. 3). C-cell hyperplasia, defined as more than six C cells per follicle or >50 C cells per low power field, is seen...
in many patients with hereditary disease and is felt to be a precursor of malignant transformation. While C-cell hyperplasia is associated with malignancy in hereditary disease, its significance in nonhereditary disease is uncertain.

If a patient has a clinical history or FNA that is suspicious for MTC, the serum calcitonin level can be useful to confirm the diagnosis. Calcitonin levels may be slightly elevated in a small percentage of normal patients, but most patients with an elevation >100 pg/ml have a diagnosis of MTC. The degree of calcitonin elevation correlates well with tumor volume. Nodal metastases start emerging at basal calcitonin levels of 10–40 pg/ml (normal range, <10 pg/ml). Distant metastases are typically associated with a calcitonin level >150 pg/ml and frequently >1,000 pg/ml.

Carcinoembryonic antigen (CEA) has also been proven to be a useful tumor marker in patients with MTC. CEA levels are elevated in >50% of patients with MTC. A preoperative serum CEA level >30 ng/ml is highly predictive of the inability to cure a patient with operative intervention [3]. CEA levels >100 ng/ml are highly associated with extensive lymph node involvement and distant metastasis. An increasing CEA level in the presence of a stable calcitonin level can be a sign of dedifferentiation of the tumor and is associated with a worse prognosis.

A neck ultrasound should be performed as part of the initial evaluation of any patient with a new diagnosis of MTC. The ultrasound can be used to look for additional thyroid tumors as well as the presence of suspicious neck lymphadenopathy. A contrast-enhanced computed tomography (CT) of the chest, mediastinum, and abdomen is also recommended as part of the metastatic evaluation of a patient with an initial diagnosis of MTC.

FAMILIAL DISEASE

While the majority of MTC is sporadic, approximately 20% of cases are a result of a hereditary form of the disease. The hereditary forms of MTC are MEN-2A, MEN-2B, and FMTC. All these disorders are inherited in an autosomal dominant pattern, and have variable penetrance. MEN-2A is the most common disorder and accounts for 75% of hereditary MTCs.

MTC is present in >95% of cases of MEN-2A and is typically multifocal and bilateral. The age at onset varies with the specific genetic mutation, but it usually presents in early adulthood. Pheochromocytomas can be seen in up to 50% of cases and they are frequently multifocal and associated with adrenal medullary hyperplasia. Pheochromocytomas can be screened for using either plasma metanephrines or 24-hour urine collections for catecholamines and metanephrines. If identified, pheochromocytomas should be treated and resected prior to proceeding with a neck operation. Preoperative alpha blockade should be initiated and blood pressure normalized prior to proceeding with a laparoscopic adrenalectomy. Hyperparathyroidism occurs in 20%–35% of patients with MEN-2A. Screening for hyperparathyroidism can be performed with serum calcium and parathyroid hormone levels. While historically felt to be a result of hyperplasia, parathyroid disease is frequently very asymmetric and may be a result of a single enlarged gland. Patients should be treated as any other patient with primary hyperparathyroidism, with removal of only the grossly abnormal parathyroid glands. Some variants of MEN-2A are also associated with either cutaneous lichen amyloidosis or Hirschsprung’s disease. Most of the mortality associated with MEN-2A is from MTC; therefore, early recognition and treatment are essential.

In MEN-2B, nearly 100% of patients develop MTC. MTC develops at a very young age (infancy) and has a very aggressive course. Because of the early age at onset and the frequent delay in diagnosis, patients with MEN-2B are rarely cured of their disease. Pheochromocytomas are again seen in 50% of patients, but no patients develop hyperparathyroidism. A distinguishing feature of MEN-2B is the development of diffuse ganglioneuromas of the lips, tongues, eyelids, and gastrointestinal tract. These patients have a characteristic appearance, including a marfanoid habitus, everted eyelids, and thick lips. These patients also have problems with megacolon, skeletal abnormalities, and markedly enlarged peripheral nerves. Because of the aggressive nature of MTC in these patients, many die at a young age. Therefore, most of the MEN-2B diagnoses seen today are de novo germline mutations.

FMTC occurs when families develop only MTC. Because there is significant overlap in the genetic mutations that lead to either FMTC or MEN-2A, the definition of FMTC is strict. In order to consider a family to have FMTC, and not MEN-2A, there must be no evidence of either pheochromocytoma or hyperparathyroidism in >10 carriers and multiple members need to be affected after the age of 50. Since MTC is often the first manifestation of MEN-2A, with pheochromocytomas lagging significantly behind, distinguishing between MEN-2A and FMTC can be difficult.

GENETIC TESTING

The genetic mutation in familial MTC is in the ret proto-oncogene, which is mapped to chromosome 10q11.2. The ret gene encodes a transmembrane tyrosine kinase receptor. Because ret is a proto-oncogene, only a single point mutation is required for malignant transformation. The first germline mutation of the ret gene was identified in patients in 1993. The currently known mutations encode >95% of
cases of hereditary MTC. The most common mutation in MEN-2A is in codon 634, occurring in 80% of patients. The codon most frequently associated with MEN-2B is a codon 918 mutation.

Approximately 20% of patients with MTC have a germline mutation in the ret proto-oncogene. Even patients with apparently sporadic disease have a 6%–10% chance of having a germline ret mutation. Therefore, all patients with a diagnosis of MTC should undergo genetic testing.

The significance of a genetic mutation for a patient and their family cannot be underestimated. It is important that, prior to screening for genetic mutations, patients receive appropriate genetic counseling. The risks and benefits of genetic testing should be carefully discussed with the patient and their family. Once a patient is found to be positive for a ret mutation, they must be carefully counseled regarding the risks to additional family members. At-risk family members need to be identified and should undergo genetic testing, because patients that are identified as ret mutation carriers can be offered a prophylactic thyroidectomy.

Among ret mutations, there is significant variation in the aggressiveness of the MTC that develops. Currently, ret mutations are classified into three groups based on the level of risk (or aggressiveness) for MTC (Table 1) [4–7]. Level 3 mutations (codon 883, 918, and 922) have the most aggressive course, with metastatic disease presenting in the first years of life. Because of the high risk for malignancy at an early age, thyroidectomy is recommended within the first 6 months of life and preferably within the first month of life [4]. Level 2 ret mutations (codon 611, 618, 620, and 634 mutations) are considered high risk for MTC and the current recommendation is that these patients undergo thyroidectomy before the age of 5 years [4]. Level 1 ret mutations (codon 609, 768, 790, 791, 804, and 891) are still considered high risk for MTC, but are the lowest risk of the ret mutations. MTC in these patients tends to develop later in life and takes on a more indolent course. Because clinically apparent disease is rarely reported prior to 10 years of age, many recommend waiting until then to perform a thyroidectomy. However, there remains variability and unpredictability in some families; hence, many surgeons recommend treating all patients with MEN-2A the same and performing their prophylactic operation by the age of 5 years whenever possible.

**Surgical Treatment**

**Treatment of Clinically Evident Disease**

Patients who have clinically evident disease are best treated with a minimum of a total thyroidectomy and bilateral central neck dissection. Ipsilateral lateral neck dissection should be added if the primary tumor is >1 cm in size or there is evidence of positive nodes in the central neck. A contralateral lateral neck dissection should be considered in patients with bilateral tumors or extensive lateral adenopathy on the side of the primary tumor (Fig. 4). Unfortunately, despite aggressive surgical resection of all neck lymph nodes, only 32% of patients with nodal disease at the time of their operation have undetectable calcitonin levels postoperatively [8].

Central neck nodal disease is present in up to 81% of patients with palpable tumors [9]. The addition of a central neck dissection results in a cure rate that is higher than that seen with a thyroidectomy alone in patients with clinically evident MTC [10]. A central neck dissection consists of a complete clearing of all lymph nodes and fibrofatty tissue from the level VI compartment. Level VI extends from the hyoid bone superiorly to the sternal notch inferiorly; laterally it is bound by the carotids. A level VII dissection, which includes removing the lymph nodes between the sternal notch and the innominate vessels, may be required in patients with significant level VI disease or locally ad-

| Table 1. ret mutations associated with hereditary MTC |  |
|----------------------|------------------|------------------|
| Risk level for MTC   | Codon mutation   | Age of prophylactic surgery |
| Level 3 (highest)    | 883              | Within the first 6 months |
|                      | 918              | of life (preferably in the first month) |
|                      | 922              |                                |
| Level 2 (higher)     | 611              | By age 5                |
|                      | 618              |                                |
|                      | 620              |                                |
|                      | 634              |                                |
| Level 1 (high)       | 609              | By age 5–10             |
|                      | 630              |                                |
|                      | 768              |                                |
|                      | 790              |                                |
|                      | 791              |                                |
|                      | 804              |                                |
|                      | 891              |                                |


Abbreviations: MTC, medullary thyroid cancer; ret, rearranged during transfection proto-oncogene.
vanced tumors. A level VI lymphadenectomy requires careful dissection of the recurrent laryngeal nerve along its entire length; it also requires meticulous dissection of the parathyroid glands. Many surgeons argue that it is impossible to do a complete central neck dissection without removing the parathyroids and/or their blood supply. Some surgeons routinely remove the parathyroid glands with the specimen and then carefully dissect them free from the nodal tissue and autotransplant them. If patients have sporadic MTC, FMTC, or MEN-2B then the autotransplant can be performed in the sternocleidomastoid. In patients with MEN-2A, because of the risk for hyperparathyroidism in the remnant, the parathyroid tissue should be autotransplanted to the nondominant forearm. Placement of the autograft in the forearm facilitates the workup and management of any hyperparathyroidism that may develop. Autotransplanted parathyroid glands usually do not function for 4–8 weeks, so calcium and vitamin D replacement is required during this time.

The role of routine lateral dissection in MTC is less clear. Ipsilateral lateral nodal metastases are present in 14%–80% of patients [9, 11] and contralateral lateral nodal metastases have been reported in 19%–49% of patients [8, 9]. Because there is a high incidence of lymph node disease, even in tumors <1 cm, some surgeons advocate a bilateral lateral neck dissection for all patients with MTC [8, 9]. Because there are risks associated with a bilateral neck dissection, many surgeons advocate a more selective approach to the lateral neck, especially when the primary tumor is <1 cm. Because preoperative neck ultrasound is highly sensitive for detecting lateral lymphadenopathy, an ipsilateral lateral lymphadenectomy can be added when the ultrasound or physical exam suggests the presence of lateral lymphadenopathy.

**Prophylactic Surgery**

Prophylactic surgery removes the at-risk organ prior to its developing clinically significant disease. When determining the timing of prophylactic surgery, it is important to balance the risk of clinically significant disease with the risks of operative intervention. In hereditary MTC, there is a clear age-related progression from C-cell hyperplasia to MTC and ultimately to nodal spread. The *ret* mutations associated with hereditary MTC are listed in Table 1 with guidelines as to when to perform a prophylactic thyroidectomy for each mutation.

The extent of surgery that is necessary in the prophylactic setting has been debated. Everyone agrees that, at a minimum, all patients should undergo a total thyroidectomy. The debate involves whether or not a central neck lymphadenectomy should be performed. Advocates of routine central neck dissection argue that, even in screened patients, clinically occult disease with nodal metastasis can be present in 6% of patients [5]. They argue that the best opportunity to cure a patient is at their initial operation. With the use of routine autotransplantation of the parathyroid glands, the long-term complications of a central neck dissection can be minimized. Opponents of routine central

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**Figure 4.** Treatment algorithm for clinically apparent medullary thyroid cancer.

Abbreviations: CEA, carcinoembryonic antigen; CT, computed tomography; PTH, parathyroid hormone; US, ultrasound.
neck dissection argue that, while nodal disease has been seen in the occult setting, it is very rare in children <10 years of age [5]. They suggest that a more selective approach can be performed, using preoperative ultrasound and tumor markers to further risk stratify patients. With a normal preoperative ultrasound and serum calcitonin (basal and/or stimulated) and CEA levels, the risk for occult nodal disease is very low and the potential benefits of a prophylactic neck dissection are outweighed by the risks for permanent hypoparathyroidism. In order to minimize the risks of this prophylactic operation, it is essential that these procedures be performed only by experienced surgeons.

**Postoperative Surveillance**

Patients with disease confined to the thyroid gland, without nodal disease, have a very low risk for recurrence and rarely die from their disease [12]. However, many patients with MTC have nodal disease at presentation, and these patients have a very high risk for developing recurrent or persistent disease. Therefore, they must be followed closely postoperatively.

Follow-up should start 2–3 months postoperatively by obtaining new baseline calcitonin and CEA levels. Patients who have undetectable calcitonin levels postoperatively can be followed with annual measurements of serum calcitonin and CEA. Routine cervical ultrasound can be added, but is of no proven benefit. If there is a rise in serum markers, then additional imaging can be pursued as indicated. Thyroid hormone replacement is required after a total thyroidectomy; however, thyroid-stimulating hormone suppression is not indicated in patients with MTC. Patients with hereditary disease need to be screened annually for the development of pheochromocytoma and hyperparathyroidism.

**Prognosis**

Overall, the prognosis for patients with MTC is good. The 10-year survival rate for patients with MTC is 75%–85% [13–15]. Approximately half of the patients with MTC present with disease localized to the thyroid gland, and these patients have a 10-year survival rate of 95.6% [14]. One third of patients present with locally invasive tumors or clinically apparent spread to the regional lymph nodes. Patients with regional disease have a 5-year overall survival rate of 75.5%. Distant metastases are present in 13% of patients at initial diagnosis and portend a poor prognosis, with a 10-year survival rate of only 40%.

**Persistent/Recurrent Disease**

Recurrent disease develops in approximately 50% of patients with MTC. Calcitonin and stimulated calcitonin levels are very sensitive ways for detecting either residual or recurrent disease. When the postoperative calcitonin level is elevated, a careful metastatic evaluation must be performed prior to proceeding with operative exploration. Neck reoperations are associated with significant risks. Therefore, reoperation should be pursued only if there is a significant likelihood of benefiting the patient. If a patient has had an inadequate initial operation or is found to have only locoregional disease, then surgical resection should be pursued. Patients with tracheal or mediastinal invasion can die from local compression/invasion if the disease is not resected. Therefore, if patients develop symptomatic locoregional recurrence, even in the setting of metastatic disease, they should be offered surgical resection when feasible and external beam radiation therapy when surgery is not possible [16].

Several studies have confirmed that, with good patient selection, neck reoperation can normalize calcitonin levels in about one third of patients [17, 18]. The keys to successful neck reoperation are careful patient selection and recognition of metastatic disease. Evaluation of metastatic disease can include anatomic imaging with neck ultrasound, CT scan of the chest and abdomen, and magnetic resonance imaging of the neck and mediastinum. Functional imaging, such as positron emission tomography and metaiodobenzylguanidine (MIBG) scans, have also been used to localize metastatic disease in patients with MTC. Often, metastatic disease to the liver takes on a miliary pattern and is not detectable by conventional imaging. Therefore, some have advocated the use of more invasive studies, such as selective venous sampling or diagnostic laparoscopy, as part of the metastatic workup [19, 20].

**Radiation Therapy**

External beam radiation therapy does not currently play a significant role in the treatment of patients with MTC. However, radiation therapy has been applied to help palliate local disease when surgery is not a feasible option. Radiation therapy has also been used to palliate bony metastases. Given the high risk for cervical recurrence in these patients, especially those with microscopic residual disease, nodal involvement, or extraglandular spread, some have advocated for postoperative treatment with external beam radiation therapy. There have been several studies examining the role of adjuvant external beam radiation therapy in MTC. One study found that high-risk patients treated with surgery plus external beam radiation therapy had a recurrence rate of 14%, compared with 48% in the surgery-alone group [21]. While that study suggests that a subset of patients may benefit from adjuvant external beam radiation therapy, this question needs to be further evaluated.
External beam radiation therapy causes extensive scarring and fibrosis within the neck, making future surgical interventions both difficult and potentially dangerous. Because the benefits of radiation therapy are not clear, and its use limits future surgical intervention, it should be reserved for cases of known residual disease in which complete surgical resection is not possible.

Radioactive iodine is part of the standard treatment for papillary thyroid cancer, but since C cells are not of thyroid follicular origin, radioactive iodine is not taken up in the C cells and radioactive iodine treatment plays no role in the management of MTC.

**SYSTEMIC THERAPY**

Patients with metastatic disease can have significant symptoms from calcitonin excess and may benefit from medical treatment with somatostatin analogues. These patients may also benefit from cytoreductive surgery of unresectable disease. Procedures to decrease the tumor burden, including resection and ablation, may provide patients with significant symptomatic relief [16].

Conventional chemotherapy has shown limited efficacy in patients with MTC. Complete responses are very rare and partial responses have been seen in less than one third of patients. The side-effect profile of chemotherapy is often substantial, making this an unappealing option for many patients. Single-agent regimens using doxorubicin, dacarbazine, capecitabine, and 5-fluorouracil have been reported, with partial response rates up to 24%–29% [22]. Newer chemotherapeutic agents, such as irinotecan (a topoisomerase I inhibitor) and 17-AAG (a heat shock protein 90 inhibitor), are currently being evaluated in phase II clinical trials.

With the discovery of the *ret* proto-oncogene and its integral role in the pathogenesis of MTC, a new class of therapies has been developed, aimed at the molecular pathways central to the development and progression of MTC. *RET* is part of the receptor tyrosine kinase family and has been shown to signal through multiple downstream pathways, including the extracellular signal–related kinase (ERK), phosphatidyl-inositol 3-kinase (PI3K)/Akt, p38 mitogen-activated protein kinase (MAPK), and c-Jun N-terminal kinase pathways [23]. While present investigations and therapies aim to block the tyrosine kinase at the receptor level, there is significant potential for developing more focused therapies as we gain a better understanding of the critical downstream targets of these receptors (see future therapies).

Recently, a new class of drugs that act as tyrosine kinase inhibitors was discovered. The first commercially available receptor tyrosine kinase inhibitor was imatinib mesylate (Gleevec®; Novartis Pharmaceuticals Corporation, East Hanover, NJ), which has been used successfully in the treatment of chronic myelogenous leukemia and gastrointestinal stromal tumors. An initial phase II study with imatinib in MTC showed limited efficacy in patients with MTC [24].

Many of the tyrosine kinase inhibitors that are now being investigated inhibit multiple receptors, including RET, the epidermal growth factor receptor, and vascular endothelial growth factor (VEGF). Vandetanib (Zactima®; AstraZeneca Pharmaceuticals, Wilmington, DE), a RET inhibitor, is currently being evaluated in a multicenter phase II clinical trial for patients with hereditary MTC. Preliminary results have been presented in abstract form and reveal a 20% partial response rate and a 30% stable response rate by CT imaging, with a dramatic decrease in tumor markers [25]. Motesanib diphosphate (AMG 706; Amgen, Thousand Oaks, CA) is a multikinase inhibitor that targets the VEGF, platelet-derived growth factor, RET, and Kit receptors. It is currently being evaluated in both advanced differentiated thyroid cancer and advanced MTC. Interestingly, many of these new therapies lead to dramatic reductions in calcitonin levels almost immediately, suggesting that tumor markers may not be a reliable way to monitor tumor response to therapy.

**FUTURE THERAPIES**

Several signal transduction pathways have been implicated as contributing to the growth and hormone production of MTC tumors. These include the PI3K–Akt, MAPK, and Notch-1–hairy enhancer of split (HES)-1–achaeate-scute complex like (ASCL)-1 signaling pathway, and the glycogen synthase kinase-3 (GSK-3) pathway [26–33]. Thus, a potential therapeutic target could be manipulation of these various cellular signaling pathways.

**The PI3K–Akt Pathway**

Activation of the PI3K–Akt pathway appears to play an important role in the development and progression of thyroid tumors, and this activation might be a result of loss of expression of the phosphatase and tensin homologue (*PTEN*) gene, a tumor-suppressor gene in various cancers [34]. Despite the central role for PI3K–Akt pathway activation in thyroid tumorigenesis, little is known about the molecules that mediate this pathway in regulating the growth of MTC tumors. Recently, it was shown that inhibition of the PI3K–Akt pathway by a well-known inhibitor, LY294002, resulted in a reduction in MTC cellular growth and neuroendocrine tumor markers. The reduction in growth is mediated by apoptosis [26]. In addition, an Akt inhibitor, KP372–1, has been shown to inhibit cell proliferation and induce apoptosis in thyroid cancer cells [34].
The Notch-1–HES-1–ASCL-1 Signaling Pathway

The Notch-1 pathway is a highly conserved pathway throughout the animal kingdom that regulates cellular differentiation, development, proliferation, and survival in a variety of contexts. Activated Notch-1 binds with the DNA-binding protein complex CSL (core binding factor 1, Su (H), and Lag-1), resulting in activation of various target genes such as that encoding HES-1 [35]. Notch-1 signaling is very minimal or absent in neuroendocrine tumors such as small cell lung cancer, carcinoids, and human MTC tissues and MTC cells, and activation of Notch-1 signaling results in a reduction in tumor cell growth [29]. Activation of doxycycline-inducible Notch-1 in MTC cells significantly reduced the growth of MTC cells, and the growth reduction was dependent on the level of Notch-1 protein [27]. Notch-1 also regulates the calcitonin level in a dose-dependent manner. Furthermore, the levels of reduction in growth and hormone production depend on the amount of Notch-1 protein present in the cell [27]. These observations clearly demonstrate that Notch-1 signaling pathway proteins are conserved in MTC cells and support the idea that activation of Notch-1 signaling may be a potential target to treat patients with MTC tumors.

The Raf-1–Mitogen-Activated Extracellular Protein Kinase–ERK Pathway

Despite several findings and new insights into this signaling pathway, the role of the Raf-1–mitogen-activated extracellular protein kinase (MEK)–ERK pathway in cancer cells remains controversial yet interesting. Activation of the Raf-1 pathway in MTC cells by expression of estradiol-inducible estrogen receptor fused with the catalytic domain of the Raf-1 fusion protein led to a reduction in calcitonin and chromogranin A [33], and most importantly, led to a significant growth suppression [36]. Further, it has been shown that growth inhibition by Raf-1 activation in the MTC-TT cell line induces an autocrine–paracrine protein, leukemia inhibitory factor, and this alone could mediate differentiation and cell growth inhibition [36]. Activation of the Raf-1 pathway in these cells also led to inactivation of GSK-3β by phosphorylation at Ser-9 [30]. These findings indicate that Raf-1 activation not only activates its own Raf-1–MEK–ERK pathway but also crosstalks with other pathways, which in turn could possibly regulate growth.

GSK-3 as a Potential Target for MTC Growth Regulation

GSK-3 is a serine/threonine protein kinase that was first described as playing a role in the regulation of glycogen synthesis [37]. GSK-3 β, an isoform of GSK-3, is involved in many cellular processes, including metabolism, embryonic development, and cell differentiation, proliferation, and survival [30, 38–43]. GSK-3α has been shown to be involved in the regulation of cellular proliferation [44, 45]. In contrast to other kinases, GSK-3β is highly active and non-phosphorylated in unstimulated cells, and becomes inactivated by phosphorylation in response to signaling cascades [37]. GSK-3β regulates other molecules such as β-catenin, MAPK kinase 1, ERK-1/2, c-Myc, c-Jun, murine double minute 2, Mcl-1, and heat shock factor by phosphorylation, and therefore modulates diverse intracellular signaling pathways that are known to play key roles in cancer biology. Recently, we have shown that inactivation of GSK-3β with lithium chloride resulted in MTC differentiation and cell growth inhibition [30]. Based on these studies, a clinical trial using lithium, a noncompetitive inhibitor of GSK-3 with a well-established safety profile, was initiated for patients with metastatic MTC.

AUTHOR CONTRIBUTIONS

Conception/design: Rebecca S. Sippel, Herbert Chen
Collection/assembly of data: Rebecca S. Sippel
Data analysis and interpretation: Rebecca S. Sippel, Muthusamy Kunimalaiyaan, Herbert Chen
Manuscript writing: Rebecca S. Sippel, Muthusamy Kunimalaiyaan
Final approval of manuscript: Rebecca S. Sippel, Muthusamy Kunimalaiyaan, Herbert Chen

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