CHAPTER 1

General introduction
THE THYROID

The thyroid is a butterfly-shaped endocrine gland that consists of two lobes that are connected by the isthmus. The thyroid gland is located in the anterior neck, just below the larynx, and around the trachea (Figure 1). The gland produces several hormones that are released into the circulation, and are vital for metabolism throughout the body. The thyroid consists of large colloid-filled follicles that are surrounded by follicular cells (Figure 1), and a relatively small number of parafollicular C-cells that lie in between them.

The thyroid hormones triiodothyronine (T3) and thyroxine (T4) are synthesized within the follicles and follicular cells. Synthesis and secretion of T3 and T4 are regulated by thyroid stimulating hormone (TSH), which is under control of the hypothalamic-pituitary axis. Via the sodium-iodide symporter (NIS), iodide (I⁻) is trapped in the follicular cell. The iodide is subsequently oxidized and coupled to tyrosine residues of thyroglobulin (Tg, a large thyroid-specific protein), to produce monoiodotyrosines and diiodotyrosines. Coupling of the tyrosine residues results in formation of the thyroid hormones T3 and T4, which are stored in the follicles. These processes are catalyzed by thyroid peroxidase. As a result of TSH stimulation, colloid droplets containing Tg-bound T3 and T4 are endocytosed. In the follicular cells, these colloid drops are hydrolyzed, and T3 and T4 can subsequently be released into the circulation. More than 99% of circulating thyroid hormones are bound to proteins, whereas the unbound (free) fraction is biologically active. Although T3 is the major biologically active hormone, the thyroid secretes primarily T4, which is in part converted to T3 in peripheral tissues by deiodinases. The parafollicular C-cells produce calcitonin, a hormone involved in calcium homeostasis.
THYROID CANCER

Thyroid carcinoma is the most common endocrine malignancy, with approximately 700 new cases in The Netherlands, and an estimated 62,450 cases in the United States for 2015. Around 70% of patients is female. The incidence of thyroid carcinoma increased over the past decades, and is now estimated to be the fifth most common cancer for women. Cancer develops as a consequence of genetic and epigenetic alterations. In the past years much research has been performed on mutation analyses in thyroid carcinoma, that mostly occur in the RAS/RAF/MEK/ERK (MAPK pathway) and PI3K/AKT pathways and result in enhanced cell proliferation, survival, and migration (Figure 2). Furthermore, epigenetic alterations that affect gene expression but not the DNA sequence itself, have been found to be involved in thyroid carcinoma. DNA methylation, i.e. the addition of a methyl group to a cytosine, is one of the best characterized epigenetic modifications. Two main alterations in DNA methylation have been found in cancer: 1) hypermethylation of regulatory elements such as gene promoters, and 2) global DNA hypomethylation of mainly repetitive DNA elements. Hypermethylation of a gene promoter leads to silencing of gene expression, which has been found to affect the tumor suppressor gene PTEN in thyroid tumors, as well as genes involved in thyroid differentiation function. Global DNA hypomethylation leads to genomic instability and is commonly involved in carcinogenesis, although the role in thyroid cancer remains unclear.

Differentiated thyroid carcinoma (DTC) develops from the follicular cells and consists of the papillary (PTC) and follicular (FTC) subtypes, and is the most common thyroid cancer type as it accounts for approximately 90% of cases. For PTC, the V600E mutation in the BRAF gene is most common; it occurs in 45 - 60% of tumors. Furthermore, RET fusions (7-15%), and RAS mutations (~13%) are regularly found in PTC. For FTC, mutations in RAS and PAX8-PPARG are most common (~40% and ~35%, respectively). Furthermore, activating mutations in PI3K, leading to AKT activation, or inactivating PTEN mutations

![Signal transduction pathways involved in thyroid cancer. Abbreviations: RTKs = receptor tyrosine kinases.](image-url)
occur in approximately 8% of FTCs. Survival is favorable for most patients with DTC with a 10-year relative survival (i.e., the survival adjusted for normal life-expectancy) of 95%.Most DTCs retain characteristics of normal thyrocytes: they have the ability to produce thyroglobulin, take up iodine via the NIS, and are under influence of TSH, as TSH receptors are widely expressed. When tumors dedifferentiate, the functionality of normal thyrocytes, including the ability to take up iodine, is lost.

The remaining 10% of thyroid cancers consists of medullary (MTC), poorly differentiated (PDTC), and anaplastic thyroid carcinomas (ATC). MTC originates from the parafollicular C-cells, which do not have the ability to take up iodine, and are unresponsive to TSH. The 5-year relative survival is approximately 85%. Like DTC, PDTC and the undifferentiated ATC arise from follicular cells. These carcinomas, however, are far more aggressive, and arise after an accumulation of several mutations. Especially the anaplastic variant is extremely aggressive as patients have a median survival of 5 to 6 months.

**CLINICAL PRESENTATION AND DIAGNOSIS**

Patients with thyroid cancer are usually asymptomatic, despite that they present with a palpable thyroid nodule. Although unusual, patients with FTC can present with complaints from distant metastases, such as a pathological fracture from bone metastases, whereas PTC metastasizes mainly to the neck lymph nodes. Some patients with MTC suffer from diarrhea and flushes as a result of calcitonin overproduction, and patients with ATC can present with complaints related to local invasive growth of the tumor in the trachea, esophagus or recurrent laryngeal nerve. After history taking and physical examination, a non-functioning palpable thyroid nodule of 1 cm or larger (or a suspicious lesion < 1 cm) is evaluated using thyroid ultrasonography and fine needle aspiration cytology (FNAC). PTC, MTC and ATC can be diagnosed with FNAC, whereas no distinction can be made between a benign or malignant follicular lesion since capsular or vascular invasion of the tumor cannot be determined using cytology. In case of a positive or suspicious FNAC, or when a follicular neoplasm is found, surgery is performed to obtain histology for final diagnosis.

**TREATMENT**

**Initial treatment**

Treatment decisions for thyroid cancer patients should always be taken during a multidisciplinary consultation. For patients with DTC, initial treatment generally consists of a total thyroidectomy, accompanied by a central and/or lateral neck lymph node dissection in case of suspected or proven lymph node metastases. Postoperatively, patients are staged according to the TNM classification. Patients receive radioiodine ablation therapy four to six weeks after surgery, to destroy remnant benign or malignant thyroid tissue. To provide adequate uptake of radioiodine in thyroid remnants, therapy takes place under endogenous TSH stimulation and an iodine-deficient diet (initiated one week prior to ablation therapy). Alternatively, recombinant human TSH (rhTSH) can be used for low-risk DTC patients and selected patients who do not tolerate hypothyroidism induced by endogenous TSH stimulation.
Using post-radioiodine therapy whole body scintigraphy, and a single-photon emission computed tomography (SPECT)/CT scan, thyroid remnants and neck lymph node or distant metastases can be visualized. After radioiodine ablation, patients start with thyroid hormone replacement or suppression therapy. Substitution with thyroid hormone is vital, as the entire thyroid is removed by surgery and radioiodine treatment. With thyroid hormone suppression therapy (THST), supraphysiological doses of thyroid hormone are administered to suppress TSH. Rationale of this is that TSH can stimulate growth of (metastatic) tumor cells.\(^7\) In the UMCG, patients use triiodothyronine (T3) during initial treatment, and switch to levothyroxine (T4) during follow-up.

For patients with MTC, initial treatment consists of a total thyroidectomy and a central, and if indicated, a lateral neck dissection.\(^9\) Postoperatively patients are staged according to the TNM classification,\(^25\) and start thyroid hormone substitution therapy. There is no role for radioiodine ablation, or THST. Also for ATC, surgery is the primary treatment modality.\(^27\) Due to typically extensive local disease, pre-operative radiological assessment is necessary to evaluate the extent of invasion in surrounding tissues. If possible, invaded structures are resected along with the thyroid. When the tumor is unresectable, external beam radiotherapy can be used as initial therapy, which can be succeeded by surgery. By definition, ATCs are classified as T4 tumors.\(^25\)

**Treatment for persistent, recurrent or metastatic disease**

In case of persistent, recurrent, or metastatic disease, additional surgery, and for patients with DTC repeated radioiodine therapies, are considered. If patients are not eligible for this, other (combinations of) treatments can induce local control and palliation, but not cure. Available options include external radiotherapy on the neck or distant metastases, radiofrequency ablation for hepatic metastases, and embolization for bone metastases.\(^26-30\) A new development is the use of tyrosine kinase inhibitors (TKIs).\(^31\) Most TKIs target multiple cell signaling transduction pathways at multiple sites. Sorafenib for example inhibits BRAF, RET and the vascular endothelial growth factor (VEGF) receptor, that promote tumorigenesis by inducing cell growth, proliferation, and angiogenesis. For patients with advanced DTC, the TKIs sorafenib and lenvatinib are registered,\(^32\) whereas for advanced MTC patients, registered TKIs are cabozantinib and vandetanib.\(^33\)

**STAGING AND FOLLOW-UP**

For patients with DTC, follow-up is initiated when remission is reached. Patients are considered to have a low risk of recurrence in case of a minimally invasive FTC, or a T1 or T2 follicular or classical PTC in the absence of -or with limited- neck lymph node metastases (located in level VI, without extranodal growth).\(^26\) Furthermore, low-risk patients are defined to have a detectable thyroglobulin (but no thyroglobulin antibodies) before thyroid surgery, no extrathyroidal iodine uptake after radioiodine treatment, and are finally designated to have a low recurrence risk in case of a TSH stimulated thyroglobulin below 1 ng/ml 6 - 12 months after initial treatment. All other patients are assigned to have a high risk according to the Dutch guidelines,\(^26\) whereas the American guidelines also distinguish an intermediate risk group that includes patients with a microscopic invasive tumor (T3), and more extensive neck metastases (that
are under 3 cm). Although most recurrences occur within several years after diagnosis, some appear decades later. Long-term follow-up is therefore indicated for high-risk patients, whereas a follow-up of 5 years seems sufficient for DTC patients with a low risk according to the most recent Dutch guidelines. Thyroglobulin measurements are the cornerstone of follow-up. Because of the thyroid-specific origin, thyroglobulin can act as a sensitive tumor marker in the absence of thyroglobulin-antibodies. Sensitivity is further increased under endogenous TSH stimulation after thyroid hormone withdrawal (THW), or use of rhTSH, although the added value of stimulation decreased since the introduction of highly sensitive thyroglobulin assays. Furthermore, neck palpation, ultrasonography (with FNAC if indicated), and diagnostic radioiodine-131 scintigraphy are essential tools during follow-up. In case recurrent or metastatic disease is suspected, further imaging using chest X-ray, computed tomography (CT), magnetic resonance imaging (MRI), or nuclear imaging such as 18F-fluorodeoxyglucose positron emission tomography (FDG-PET) is indicated.

Until 2007, lifelong THST was applied to all patients with DTC. Because of the a growing awareness of adverse effects of THST on the cardiovascular system and bone marrow density, and moreover, the emergence of studies that indicated that THST does not improve outcome in low-risk patients, guidelines were adjusted. According to the latest Dutch guidelines, THST with a TSH target < 0.1 mU/L remains indicated for high-risk patients with persistent or metastatic disease, whereas for low-risk patients, a TSH in the low-normal range (0.5 - 2.0 mU/L) is advocated after initial treatment. For patients with an initial high risk who are cured after therapy, THST with a target < 0.5 mU/L can be applied in the first two years, and tapered thereafter. Intermediate risk patients are no longer separately mentioned in this guideline, and are included in the high-risk category.

Follow-up for patients with MTC includes serum calcitonin and carcinoembryonic antigen (CEA) measurements. These tumor markers can early indicate recurrent disease, and are reason for a diagnostic workup when elevated or increasing. In the unusual case that ATC patients get disease-free, follow-up using total body CT/MRI and FDG-PET is advised as long as the patient desires ongoing aggressive treatment.

LONG-TERM EFFECTS OF TREATMENT

The large majority of DTC patients is successfully treated and remains in long-term remission. For these patients, it is therefore very important to preserve quality of life, and prevent late adverse effects of treatment. THST can adversely affect the cardiovascular system, as diastolic and systolic cardiac dysfunction, decreased arterial elasticity, and a prothrombotic profile have been reported in association with THST in DTC patients. However, data on clinical overt adverse cardiovascular effects remain scarce. Furthermore, many patients experience oral complaints such as pain and swelling of salivary glands, or complaints of a dry mouth (xerostomia), as a result of a radioiodine-induced inflammatory reaction of the salivary glands (sialoadenitis). Although this can result in significant morbidity, and may lead to chronic salivary gland dysfunction, the precise effect of radioiodine on the salivary glands remains poorly understood. Similarly, the long-term effects of high-dose radioiodine treatment on bone marrow function remain unclear. In Chapter 2 a more extensive overview of (long-term) effects of treatment for...
DTC patients is provided. In the past years this topic received more attention, but ongoing evaluation is needed to address the specific long-term treatment effects and its outcome and causes.

AIMS AND OUTLINE OF THE THESIS

In this thesis we aimed to study the long-term adverse effects of conventional treatment modalities for patients with DTC, and to assess new developments in thyroid cancer treatment and the understanding of molecular pathways.

In Chapter 2 an overview of the most clinically relevant adverse effects of radioiodine treatment and THST is presented, and the trend towards less aggressive treatment for low-risk DTC patients is outlined.

The next section consists of Chapters 3, 4 and 5, which comprise several cohort studies on adverse cardiovascular effects of DTC treatment. Especially THST, which gives rise to a (subclinical) hyperthyroid state, has been related to adverse cardiovascular effects. Studies on hard endpoints such as (cardiovascular) mortality and events were, however, lacking. In Chapter 3, we aimed to evaluate whether there is an increased risk of cardiovascular and all-cause mortality in DTC patients, and to explore the association between TSH level and outcome parameters. A retrospective cohort study was performed, in which the risk of cardiovascular and all-cause mortality was compared between 524 DTC patients treated in the UMCG between 1980 and 2010, and 1572 age- and sex matched controls. Furthermore, the association between outcome and TSH level during follow-up was assessed within the DTC cohort. In Chapter 4, we aimed to study whether DTC patients have an increased risk of atrial fibrillation, and whether incident atrial fibrillation is associated with TSH level, radioiodine treatment or neck radiotherapy. For this study the cohort of DTC patients and controls from Chapter 3 was used, except for study subjects that had AF at baseline. The incidence of AF was compared between patients with DTC and controls, and within the DTC cohort the associations between DTC treatments and AF were studied. In Chapter 5, we aimed to compare the cardiac damage marker N-terminal pro brain natriuretic peptide (NT-proBNP) between DTC patients and controls. Furthermore, we aimed to assess whether NT-proBNP could act as a prognostic marker for cardiovascular events and all-cause mortality during follow-up for patients with DTC. NT-proBNP measurements were performed in frozen serum samples dating from 2004-2008 from DTC patients in follow-up at the UMCG. Subsequently, these NT-proBNP measures were compared to those of controls, and related to cardiovascular events and all-cause mortality occurring during follow-up.

The subsequent section consists of Chapters 6 and 7, which includes the effects of radioiodine therapy on oral health and bone marrow function. Salivary glands contain the sodium-iodide symporter (NIS) and therefore have the ability to take up radioiodine, which can lead to an inflammatory reaction in the salivary glands. This can give rise to painful, swollen glands, and eventually complaints of a dry mouth in case of chronic salivary gland dysfunction. However, the precise effects of radioiodine on saliva secretion, composition and oral complaints are incompletely understood. Moreover, as yet we are unable to early identify patients at risk for salivary gland damage. In Chapter 6 we aimed to evaluate whether radioiodine treatment affects whole and glandular saliva flow rates and composition, and whether it induces xerostomia. Furthermore, we aimed to study whether radioiodine uptake on diagnostic scans is associated with saliva flow rate alterations after radioiodine treatment. Therefore, a
multicenter prospective study was performed in which a total of 75 patients were included. A total of 67 patients completed both study visits (before and 5 months after radioiodine treatment). In addition to salivary glands, the bone marrow can be affected by radioiodine. Data on this issue on both the short- and long-term in a large, general DTC population are, however, lacking. In Chapter 7 we aimed to evaluate the short- and long-term effects of radioiodine treatment on bone marrow function in DTC patients, and define characteristics of patients at risk for impaired bone marrow function post-radioiodine treatment. We retrospectively studied bone marrow function as reflected by peripheral blood counts in 331 DTC patients who were treated with radioiodine between 1989 and 2013. Additionally, we identified characteristics of DTC patients at risk for post-radioiodine therapy thrombocytopenia.

The last section consists of Chapters 8 and 9, in which we focus on new developments in thyroid cancer treatment, and the understanding of molecular thyroid cancer pathways. TKIs comprise a promising new class of systemic therapy for patients with advanced thyroid cancer. Many papers on different TKIs appeared over the past years, but a comprehensive overview of (adverse) treatment effects that can be expected upon treatment initiated was lacking. In Chapter 8 we therefore aimed to systematically summarize response and toxicity induced by tyrosine kinase inhibitors in patients with thyroid cancer. We performed a systematic review and meta-analysis on responses and toxicities of small-molecule TKIs in patients with thyroid carcinoma. We eventually included 22 studies, reporting on 1435 thyroid cancer patients. Summary estimates for the different response and toxicity endpoints were reported. Another promising development is the rapidly evolving field of thyroid cancer (epi)genetics, which contributes to an improved understanding of the molecular pathways involved in thyroid cancer. Local gene promoter hypermethylation (which leads to gene silencing) and global DNA hypomethylation of repetitive DNA elements (which leads to genome instability) are two epigenetic hallmarks of cancer that have not been well characterized in thyroid cancer. In Chapter 9, we studied whether global Alu hypomethylation (a surrogate marker for global DNA hypomethylation) is increased in primary tumors of patients with low-risk and distant metastastic DTC, pediatric PTC, and PDTC/ATC. Furthermore, we assessed whether global Alu hypomethylation alters in distant metastatic tissues as compared to the primary tumor, and whether global DNA hypomethylation can act as a marker for thyroid cancer-related and all-cause mortality. Therefore, we included 90 tumors of patients with several thyroid cancer risk categories, together with 20 matched distant metastases, and 20 normal thyroid tissues. Extracted DNA was analyzed using the Quantification of Unmethylated Alu (QUALu) technique.

In Chapter 10 a summary, and general discussion of the thesis are provided, and future perspectives are discussed.
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