Thyroid Cancer—Risks and Causes

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Abstract

The incidence of thyroid cancer has almost doubled in recent years and over 60,000 people will be diagnosed in the US in 2015. While the prognosis for most such patients is excellent, a significant proportion die of thyroid cancer from local tumor progression and above all from metastases. Here we review the different types of thyroid cancers and their molecular changes with a special emphasis on the currently known susceptibility and precipitating factors. With the recent clinical introduction of tyrosine kinase inhibitors for the treatment of metastatic thyroid cancer it is clear that a simple cure is not at hand and further understanding of the molecular mechanisms of these tumors is urgently needed.

Thyroid cancer is the most common endocrine cancer, representing about 1 % of all malignancies diagnosed worldwide, with approximately 600,000 men and women alive in the US who have a history of cancer of the thyroid (http://seer.cancer.gov /statfacts/html/thyro.html). Despite its prevalence, the mortality from thyroid cancer remains relatively low at ~0.5 per 100,000 population per year as a result of the availability of effective therapies for most types of the disease. The exception to this is the anaplastic subtype, which is ranked among the most deadly of all human cancers.

While the incidence of many cancers has remained stable or even decreased in recent years, thyroid cancer incidence has significantly increased with an annual percentage change of 6.4 % for males and females in the US over the period 1997–2010 (http://seer.cancer.gov/statfacts/html/thyro.html) (*see Figure 1A*). There is variation between countries that could be explained by differences in environmental exposure, genetic factors, or access to healthcare (*see Figure 1B*). It is estimated that 60,000 men and women (15,000 men and 45,000 women) will be diagnosed with the disease in 2014 and almost 2,000 will die. The reasons for this increased incidence are controversial and most likely multifactorial. Increased detection of preclinical stage tumors of small size would appear to be an obvious cause with the introduction and widespread use of thyroid sonography and increased use of aspiration biopsies of small tumor as suggested by Davies and Welch.[1,2] This explanation for earlier diagnosis and identification of small tumors is supported by the lack of increase in thyroid cancer mortality. But many experts claim that the rising incidence of thyroid cancer cannot be explained by improvements in screening and detection,[3–5] as an increase has occurred in large tumors especially papillary thyroid cancer but no significant change for the follicular,

medullary, or anaplastic histotypes.[1,5] A true increase could be the result of a change in exposure to an unidentified risk factor in the environment or in our lifestyle. To date, ionizing radiation is the best-established risk factor for thyroid cancer, as a result of data obtained from nuclear incidents such as the Chernobyl radioiodine releases and from Hiroshima and Nagasaki. Many studies have focused on other possible risk factors, which we will review.

Patients die of thyroid cancer from local tumor progression and above all from metastases. Epithelial to mesenchymal transition (EMT) is thought to play a major role in tumor genesis/invasion and spread of metastases in many cancers including thyroid cancer. The relationship between cancer cells, EMT, and cancer stem cells (CSCs) is poorly understood although it has been shown that certain epithelial cancer cells that pass through EMT acquire CSC properties including thyroid cancer cells.[6,7] Determining the mechanisms by which known risk factors such as EMT initiation could potentially lead to the development of new specific targets for thyroid cancer.

The goal of this review, therefore, is to summarize the risk factors and susceptibilities for thyroid cancer in order to lead to the development of new and more-effective therapeutic targets. Moreover, detailed examination of population-level risk factors can help identify and support prevention efforts to reduce the disease burden.

The Different Types of Thyroid Cancer
Overview

Thyroid cancers are still categorized by their histologic appearance and their natural history (*see Table 1*). The two most common forms of

Table 1: The Different Subtypes of Thyroid Cancer

Subtype	Percentage of Cases	Prognosis	Differentiation	Characteristics
Papillary	80–85	Good: up to stage II 5-year survival is 100 %	Well differentiated	Tumor cell form finger-like or papillary structures
Follicular	10–15	Good: up to stage II 5-year survival is 100 %	Well differentiated	Tumor cells have follicles that are similar to normal thyroid follicles
Medullary	3–4	Good: up to stage II 5-year survival is 98 %	Well differentiated	Tumors arise from the parafollicular C cells of the thyroid gland that produce calcitonin and secrete this peptide into the bloodstream. Tumor cannot concentrate radioiodine, do not secrete thyroglobulin and are unresponsive to serum thyroid-stimulating hormone level
Anaplastic	1–2	Very poor: 5-year survival is only 9 %	Undifferentiated, with marked epithelial to mesenchymal transition	Tumor cells do not resemble normal thyroid cells or form follicles. Unresponsive to radioactive iodine, serum thyroid-stimulating hormone level and all currently available treatment modalities

TSH = thyroid-stimulating hormone

differentiated thyroid cancer are the papillary and follicular types. The papillary form tends not to metastasize outside the neck compared with follicular thyroid cancers, which have a propensity to spread further. This histologic differentiation is now supported by molecular studies (*see below*). The vast majority of thyroid tumors arise from thyroid follicular epithelial cells but the 3–5 % of medullary cancers originate from the C cells, which secrete calcitonin and are outside the purview of this review.

The well-differentiated thyroid cancers often have an indolent clinical course with low morbidity and mortality. With some exceptions, these are among the most curable of cancers and, as a consequence, patients are sometimes ill advised that thyroid cancer is not a serious problem. Although anaplastic thyroid cancer (ATC), which is highly aggressive and accounts for ~50 % of all thyroid cancer-related deaths, remains uncommon, more malignant forms of differentiated thyroid cancer exist, such as the tall cell variant of papillary thyroid cancer[8] and the Hurtle cell follicular cancers,[9,10] which can be difficult to treat.

Histologic Characteristics of Thyroid Cancer

Papillary carcinomas have characteristic nuclear features,[11] with large, ovoid, crowded, ground-glass 'Orphan Annie eyed' nuclei and nuclear grooves with pseudo inclusions. The demonstration of invasion is not required for diagnosis; therefore, this tumor is often diagnosable by fine-needle aspiration (FNA) and cytologic examination. Mitoses are usually sparse. The papillae are usually arborizing, with a delicate fibro vascular core.

Follicular carcinomas are malignant epithelial tumors showing follicular cell differentiation and often lacking the diagnostic nuclear features of papillary thyroid carcinoma.[11] Follicular neoplasms demonstrate signs of vascular or capsular invasion, but neither architectural nor cytologic atypia are reliable criteria of malignancy. Therefore, the distinction from follicular adenoma cannot be easily based on FNA cytology resulting in the appellation 'follicular neoplasm.'

ATCs exhibit wide variations in appearance with several morphologic patterns recognized and many tumors manifesting a mixed morphology.[12] A common morphologic presentation, and one that is most easily recognized as an anaplastic carcinoma of thyroid, is that of the biphasic spindle and giant cell tumor.[13] All variations of anaplastic carcinoma of

Figure 1: A) The Thyroid Cancer Epidemic
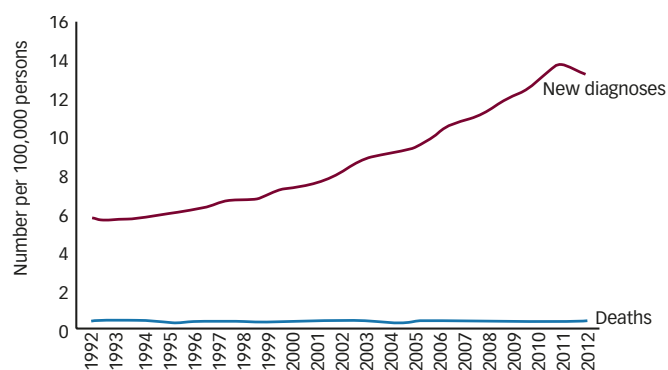
Figure 1: B) Geographic Variation of Thyroid Cancer

Source: http://seer.cancer.gov/statfacts/html/thyro.html
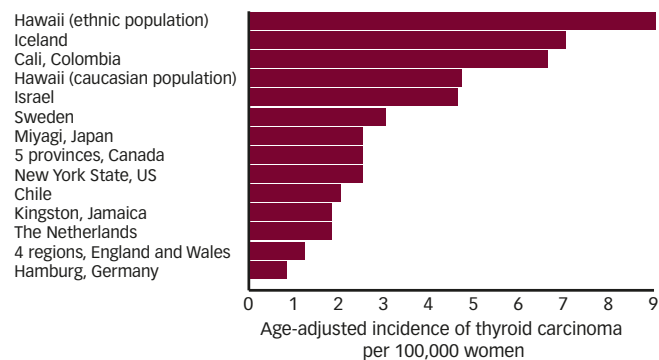
the thyroid are highly proliferative with numerous mitotic figures and atypical mitoses.[14] There is usually extensive necrosis with inflammatory infiltrate, and, in some cases, the necrosis may be so widespread that the only viable tumor is preserved around blood vessels. Macrophages form a major component of the inflammatory infiltrate.[15] In approximately 50 % of cases, histologic examination of ATC identifies a component of papillary, follicular, or poorly differentiated carcinoma, or patients have a history of previously resected well-differentiated or poorly differentiated thyroid carcinoma.[12,16,17] This supports the notion that ATC can arise by dedifferentiation of a preexisting well-differentiated thyroid carcinoma.

Thyroid Cancer

Figure 2: The Major Signaling Pathways in Thyroid Cancer[88]
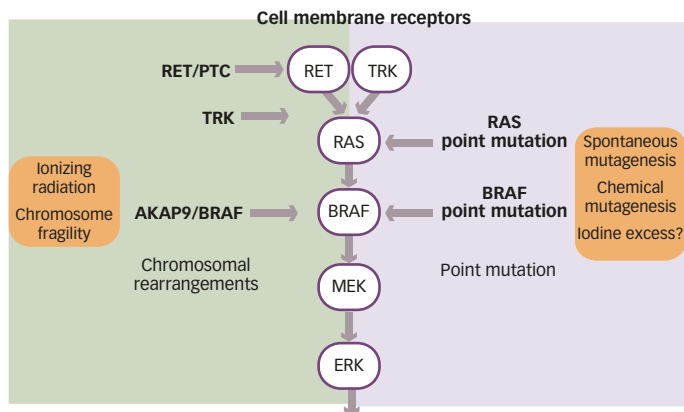
Adapted from Nikiforov et al.[88] AKAP9 = A-kinase anchor protein 9; RET = rearranged during transfection proto-oncogene; PTC = papillary thyroid cancer; TRK = tropomyosin-receptor kinase; RAS = RAS protein; BRAF = B-type Raf kinase; MEK = MEK protein; ERK = ERK protein.

Table 2: Prevalence of Chromosomal Rearrangements and Point Mutations in Sporadic and Radiation-induced Papillary Thyroid Carcinomas[87]

Genetic Change	Sporadic (%)	Radiation-induced (%)
RET/PTC rearranged	10–40	50–85
TRK rearranged	<5	6
BRAF rearranged	1	1
BRAF mutation	40–45	0-4
RAS mutation	10–15	0

Adapted from Gandhi et al.[87] BRAF = B-type Raf kinase; PTC = papillary thyroid cancer; RAS = RAS protein; RET = rearranged during transfection proto-oncogene; TRK = tropomyosin-receptor kinase.

Table 3: Risk Factors for Thyroid Cancer

Female gender

Familial or genetic factors

Radiation

An increased thyroid-stimulating hormone level

Iodine deficiency

Autoimmune thyroid disease

Toxic chemical exposures

Chromosomal and Genetic Alterations
Mutations and Gene Fusions

While the final population of cancer cells may be composed of a dominant clone accompanied by many other clones formed during the development of the neoplasm,[18] it appears to be the activation of the mitogen-activated protein kinases (MAPK) and phosphinoside 3 kinase–AKT kinases (PI3K–AKT) signaling pathways, which are important for thyroid cancer initiation and progression (*see Figure 2*).

In papillary thyroid cancer, activation of the MAPK signaling pathway occurs via two main mechanisms: recombination events (often called chromosomal rearrangements) and point mutations (*see Table 2*) and these, often mutually exclusive, mutations are found in almost 70 % of papillary cancers. Chromosomal rearrangements such as rearranged during transfection proto-oncogene/papillary thyroid cancer (RET/PTC), PAX8/

PPARγ, and B-type Raf kinase/A-kinase anchor protein 9 (BRAF/AKAP₉) have been associated with exposure to ionizing radiation and likely represent fragile sites on the chromosome. Point mutations, such as those in the *RAS* and *BRAF* genes, lack this association with radiation exposure and probably develop as a result of environmentally induced or stochastic mutagenesis. As discussed below, dietary iodine excess may be one important factor, by inducing prolonged thyroid-stimulating hormone (TSH) stimulation, as well as exposure to certain chemical elements, such as those present in higher concentrations in drinking water from volcanic areas.

In contrast to the papillary form of cancer, follicular thyroid cancers are more associated with expression of a mutated RAS oncogene (in 40 %) and/or a translocation causing a PAX8/PPARγ fusion protein to be expressed (in 30 %) both of which enhance unregulated cell growth.[19]

Using Molecular Markers of Thyroid Neoplasia

With the advances in our understanding of the genetic contribution to thyroid cancer, molecular markers have been used to predict the malignancy of thyroid tumors. Both BRAF and TERT promoter mutations have been associated with a worse prognosis.[20] A variety of markers can now be used to improve cancer diagnosis in FNA samples from thyroid nodules and to aid tumor prognostication. Different approaches have been used—micro RNA assessments, gene profiling, and point mutation detection and all have advantages and disadvantages. Molecular testing is most beneficial for thyroid FNA samples with indeterminate cytology, where it may resolve the diagnosis in a significant number of cases.[21] Nevertheless, molecular analysis, like cytology, has limitations and error rates of at least 5 % remain using each of the available techniques leaving clinical acumen of great importance.

The Risk Factors for Thyroid Cancer

The risk factors for thyroid cancer are noted in *Table 3*.

Susceptibility Genes

Thyroid cancer is believed to have one of the highest inherited risks of any cancer indicating the presence of an important heritable predisposition in many patients. However, only about 5–10 % of thyroid cancers are considered truly familial in nature with an afflicted first degree relative so the vast majority of tumors are still considered sporadic. Multiple potential regions for harboring responsible genes have been reported, suggesting that multiple susceptibilities are associated with thyroid cancer development.[22] From genome-wide association (GWAS) studies and direct association studies at least four single-nucleotide polymorphisms (SNPs) in four distinct genes have been reproducibly associated with thyroid cancers: Papillary Thyroid Carcinoma Susceptibility Candidate 3 (PCTSC3) (on 14q13-3), which is a long noncoding RNA that acts as a tumor suppressor; the fork head box E1 gene (FoxE1, formerly TTF2) (on 9q22.33), which plays a crucial role in thyroid morphogenesis; the disrupted in renal carcinoma 3 gene (DIRC3) (on 2q35), which is thought to be associated with chromatin remodeling and stress response signals; and neurogulin 1 (NRG1) (on 8p12), which mediates cell–cell interactions and induces the growth and differentiation of epithelial cells.[23–25] These associations provide potential mechanisms leading to thyroid cell malignancy.

Familial nonmedullary thyroid cancer is mostly of the papillary histotype and a recent study showed that the 0–84-year lifetime cumulative risk for

thyroid cancer (CRTC) in female relatives of a patient with papillary cancer was 2 %, representing a threefold increase over the general population risk (women—sliced inverse regression [SIR]=2.9, 95 % confidence interval [CI] 2.4 to 3.4; men—SIR=2.5, 95 % CI 1.9 to 3.3). When there were ≥2 papillary cancer patients diagnosed at age <60 years in a family, CRTC for female relatives was 10 % (male 24 %).[26] Hence, our current understanding of the transmission of these tumors appears to be autosomal, polygenic, and dominant but with incomplete penetrance.

The familial form of papillary thyroid cancer possesses more aggressive features than the sporadic disease, as indicated by increased metastases and higher rates of reoperation and/or requiring additional radioactive iodine therapy.[27] Therefore, a prompt recognition of the familial nature of the disease may improve the mortality by providing earlier diagnosis and treatment in similarly affected family members. Several rare familial syndromes, including Gardner's syndrome and Cowden disease, have also been associated with an increased prevalence of thyroid cancer.[22–26]

Radiation

Radiation is a well-known carcinogen, interacting with DNA to produce a range of mutations at sensitive sites. Irradiated cells also show genomic instability, as do adjacent nonirradiated cells (the bystander effect). Ionizing radiation is the best-established risk factor for thyroid cancer in the human population when exposure occurs during childhood, a time when the thyroid appears to be very radiosensitive. Most of the reported exposure has been in the form of radioiodine from the environment or from medical procedures such as computed tomography (CT) scanning.

Radioiodine Exposure

We have known since the 1940s that large doses of radioiodine kill the thyroid gland and cause thyroid failure. This is the rationale behind such a treatment for patients with overactive thyroid glands and for those with residual thyroid cancer. However, thyroid carcinoma was demonstrated to be significantly more prevalent among patients who were heavily exposed to ionizing radiation at the time of the atomic bombings in 1945.[28] Moreover, it is exposure to low-dose radiation that is associated with malignant changes and this became most obvious after the Chernobyl disaster in 1986 (*see Figure 3*).[29] During such radiation exposure the increase in thyroid carcinoma, attributable to the very large amounts of iodine 131 released, was first noticed in children with a strong relationship between young age at exposure and risk for developing papillary thyroid carcinoma.[30] A pooled analysis based on five cohorts and two case-control studies and involving 58,000 exposed individuals, 61,000 unexposed, 700 thyroid cancers, and three million person-years, found a significant dose-related risk for exposure during childhood[31] and demonstrated that susceptibility to radiation-induced thyroid cancer is inversely related to age at exposure.

Direct Thyroid Exposure

The use of CT scans in the US has increased more than threefold since 1993 to approximately 70 million scans annually.[32] In level I healthcare countries, CT scans have been estimated to account for 7.9 % of diagnostic radiology examinations, but 47 % of the cumulative effective dose from diagnostic radiology procedures.[33] Medical and dental X-Rays have also specifically increased thyroid exposure, but represent only 11 % of the cumulative effective dose. However, to date, there is no direct evidence of CT scan radiation affecting the incidence of thyroid cancer in children but

Figure 3: Thyroid Cancer Incidence Rate Among those Exposed as Children and Adolescents (Age Under 18 Years in 1986) in Belarus
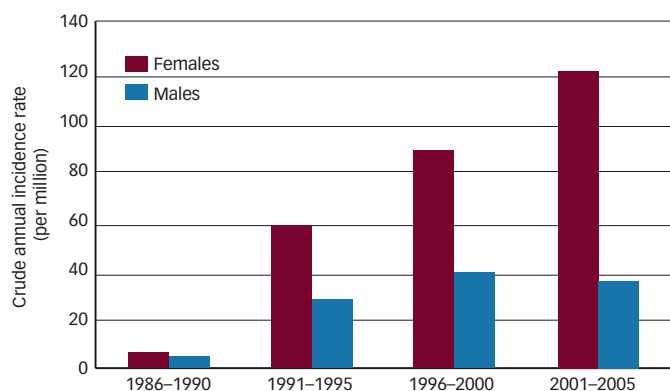
Reproduced with permission from Fushiki.[29]

projected cancer risks have estimated that about 1,000 thyroid cancers a year could be caused by such CT scans during childhood in the US.[33] However, the observation that the prevalence of RET/PTC rearrangements, associated with thyroid cancer after radiation exposure (*see Table 2*), has been decreasing in recent decades does not support this view.

Mechanisms of Radiation Damage

Direct radiation-damage at the cellular level is now better understood in terms of gene responses. The response to radiation may involve stress and inflammatory response genes, damage recognition and signaling genes, signal transfer and cell cycle control genes in addition to programmed cell death genes, and genes responsible for DNA damage and repair (http://www.ncbi.nlm.nih.gov/books/NBK13393/).

As discussed earlier, we also know that several types of chromosomal rearrangements that occur in thyroid cancer are more common in radiation-associated tumors and that the RET/PTC rearrangement can be induced experimentally by exposing human thyroid cells to ionizing radiation.[34] The opposite is true for point mutations involving the BRAF and RAS genes, which are rare in radiation-related tumors but common in the general population. Among papillary carcinomas found in atomic bomb survivors in Japan (where doses received by the thyroid gland were calculated with high precision), a strong positive correlation was found between increased frequency of RET/PTC rearrangement and increased radiation dose, whereas the frequency of BRAF point mutations showed an inverse correlation with the radiation dose.[35] After the Chernobyl accident, most early cases were solid papillary thyroid cancers with RET-PTC-3 rearrangements while later cases were classic papillary thyroid cancer with RET-PTC-1 rearrangements. Small numbers of many other RET rearrangements have occurred in 'Chernobyl' papillary thyroid cancers and also some rearrangements of BRAF (*see Table 2*).

Why Children?

The high susceptibility of young children to radiation can be explained by a higher proliferative activity of thyroid cells during intrauterine development and childhood. However, information on thyroid cancer risks associated with *in utero* exposure is insufficient to draw conclusions. Data on adult exposure are also limited and not entirely consistent. Exposure

to ionizing radiation in adulthood was positively associated with thyroid cancer among women A-bomb survivors, although the risk was lower than for those exposed to radiation in childhood.[36]

Thyroid Imaging with Radioiodine 131

The annual number of thyroid examinations using radioiodine is currently five per 1,000 individuals worldwide, so this matter is of public health importance in view of the international trend of increasing population exposure to medical diagnostic sources of radiation. However, there are no data suggesting that diagnostic imaging can be correlated with thyroid cancer. There are data from patients receiving larger doses for treatment of thyroid cancer and Graves' disease, which may correlate with long-term cancer development in a variety of organs,[37,38] but the increased risk appears to be quite small.[39]

Dietary Iodine Intake

Iodine deficiency influences thyroid function directly, as well as indirectly, through a reduction in the level of thyroid hormones and a consequent rise in TSH secretion, which is a major growth factor for thyroid follicular cells. While severe iodine deficiency remains the most common cause of thyroid failure in the world, chronic milder iodine deficiency may also be an important risk factor for follicular thyroid cancer.

Animal experiments have demonstrated a clear increase in thyroid cancer after prolonged iodine deficiency and increased TSH levels[40] although it has not been demonstrated in humans. In fact, some studies have suggested that iodine supplementation programs may increase the incidence of papillary thyroid cancer.[41,42] Increased surveillance and improvement in the quality of diagnostic tools are the most logical explanation for such data. Iodine intake has been shown to influence the thyroid cancer histotype distribution, rather than the overall incidence, with more follicular and fewer papillary carcinomas in iodine-deficient areas.

When iodine prophylaxis is introduced, average serum TSH decreases and the papillary:follicular ratio increases. The iodine-associated shift from a follicular to a papillary histotype may be due to the frequency of the BRAF (V600E) mutation, a typical molecular alteration in papillary thyroid cancer whose prevalence has increased.[43] BRAF-positive papillary thyroid cancers were significantly more frequent in Chinese regions with a high iodine intake than in control areas.[42] Although a causal relationship between iodine intake and BRAF mutation has not been proved, the worldwide increase in iodine intake and the parallel increase in the prevalence of BRAF-positive papillary thyroid cancers[43] might reflect the role of iodine excess in the generation of such BRAF point mutations although a biologic explanation remains to be determined.

Combined Iodine Deficiency and Radioiodine Exposure

Data from radioiodine exposure downwind of Chernobyl has suggested that those children from iodine-deficient areas were more likely to develop thyroid cancer than those from iodine replete areas.[44] This may have resulted secondary to the increased uptake of iodine 131 in the presence of iodine deficiency. Although the prevalence of iodine deficiency has dramatically decreased in most developed countries it remains rampant in many parts of the world. These data, therefore, have public health implications regarding stable iodine supplementation in iodine-deficient populations. Such

supplementation may substantially reduce the risk for thyroid cancer related to radioactive iodine exposure in childhood, which may occur after radiation accidents or during medical diagnostic and therapeutic procedures.

Other Environmental Exposures

There are no widely recognized and confirmed environmental causes of thyroid cancer other than ionizing radiation and dietary iodine intake. Solvent exposure, especially occupational exposure and particularly benzene and formaldehyde used in the shoemaking and rubber industries, fungicides such as dioxin, polychlorinated biphenyls (PCB), hexachlorobenzene (HCB), polybrominated diphenyl ether (PBDE), and high consumption of green tea have all been associated with thyroid cancer but the evidence remains contradictory.[45,46]

Some epidemiologic studies have shown an increased incidence of thyroid cancer in volcanic areas, but the mechanism by which a volcanic environment increases the incidence of thyroid cancer is not known. For example, the incidence of papillary, but not follicular or medullary, thyroid cancers was significantly increased in the Catania province of Italy, where levels of many elements (including boron, iron, manganese, and vanadium) in the drinking water exceeded maximum admissible levels and was in sharp contrast with Sicily. Papillary tumors from patients in Catania were found to more frequently carry the BRAF V600E gene mutation (52 % of 106 tumors) than tumors from patients in Sicily (33 % of 205 tumors),[47] reflecting the worldwide pattern of thyroid cancer increase.

Female Gender

We do not know for sure why the risk for thyroid cancer is two to four times more frequent in females than in males. In fact, all thyroid diseases are more common in women. This susceptibility of women to thyroid disease in general is presumed to have a genetic causation but does not predispose to any particular phenotype. The relationship between menstrual and reproductive factors with thyroid cancer has been studied extensively, but studies have shown mixed results.[48] It has been proposed that estrogen may contribute to the difference in incidence although there remains no strong evidence for this except the presence of estrogen receptors in thyroid cells.[49,50] Estrogen has the capacity to initiate cell proliferation and this has been shown even in stem cells. However, there has been no direct association with menarche, pregnancy, and oral contraceptive use. Thyroid cancer in pregnancy has been reported to have both a worse prognosis than in nonpregnant women and the same prognosis.[51,52]

Body Weight

Worldwide, the obesity rate has been dramatically increasing over the past 30 years along with the incidence of thyroid cancer—and of course many other factors. Several studies, including meta analyses,[53] have suggested an association between an elevated body mass (BMI) and cancer. This relationship may prove to be related to the increased prevalence of diabetes in the obese population since there is now abundant evidence relating diabetes to increased cancer rates.[54] The role of insulin-like growth factor I (IGF1), an insulin agonist, has been invoked as having a major role in stimulating thyrocyte proliferation and differentiation, as well as regulating thyroid gene expression.[55,56] Hence, it has been suggested that insulin resistance and hyperinsulinemia, rather than metabolic derangement, may be the risk factor for thyroid cancer. Indeed, insulin resistance was shown in one study to be present in 50 % of papillary thyroid cancer patients

versus 10% of euthyroid, control, patients.[57] Whether physical activity has an effect on thyroid cancer is unknown: some studies showed a positive association;[58] others no association.[59] Other potential mechanisms for this relationship include changes in sex steroids and adipokines that may stimulate thyroid cell proliferation and suppress apoptosis.

Thyroid-stimulating Hormone and Thyroid Cancer

TSH, acting through the TSH receptor, is the major stimulator of postdifferentiation thyroid cell growth and function. There is good evidence that TSH primes thyroid cells to undergo cell cycle progression in response to growth factors, primarily IGF-I or insulin. It has been demonstrated that well-differentiated thyroid cancers retain the expression of many of their TSH receptors, thus providing the rationale for TSH suppression as a treatment for patients with differentiated thyroid cancer. Many reports have shown an improved survival in differentiated thyroid cancer patients treated with suppressive doses of levothyroxine. Furthermore, several recent large epidemiologic studies have shown a strong association between serum TSH levels and risk for malignancy in thyroid nodules.[60–63] A fourfold increase in thyroid cancer was found in patients whose TSH levels were in the upper quartile of the normal range compared with those in the lower quartile of the normal range using both univariate and multivariate analyses. The risk for advanced stage thyroid cancer is also increased in patients with higher TSH levels.[62] A large series of more than 10,000 patients with thyroid nodules who underwent FNA supports this correlation, finding that patients with T3–T4 stage disease showed significantly higher TSH levels in comparison with patients who had T1-T2 stages.[61]

There is also robust experimental evidence that TSH is important in the initiation and progression of thyroid cancer.[64] It has been well demonstrated that the TSH signaling pathway predisposes thyroid cells to BRAF-induced transformation in mice, presumably via the cyclic adenosine monophosphate (cAMP) signaling pathway (*see Figure 4*). These findings provide impetus for further delineation of the signaling effectors that mediate this interaction and may provide future strategies for cancer prevention and therapy distinct from the MAPK pathways.

## Autoimmunity and Thyroid Cancer

Graves' Disease

There is a paucity of information on the relationship between thyroid cancer and Graves' disease. While the influence of TSH receptor antibodies on stimulating cancer metastases is well recognized there are few studies of thyroid cancer prevalence in the Graves' disease population. Those available suggest an increased risk for ~1.3.[65,66]

Hashimoto's Thyroiditis

By contrast, there have been many studies of patients with Hashimoto's disease (autoimmune thyroiditis), which have also shown an increased risk. The frequency of Hashimoto's thyroiditis has increased in the last 2 decades, paralleling the increased incidence of thyroid cancer and female patients with Hashimoto's thyroiditis undergoing thyroidectomy were 30 % more likely to have thyroid cancer than expected.[62]

There is evidence that the frequency of papillary thyroid cancer in patients with Hashimoto's autoimmune thyroiditis may be partly related to increased serum TSH,[67] but there is increasing evidence of an association between thyroid lymphocytic and macrophage infiltrations and thyroid cancer,

Figure 4: Thyroid-stimulating Hormone Receptor Dependence of Murine Papillary Thyroid Cancer in a Mutate Braf Model
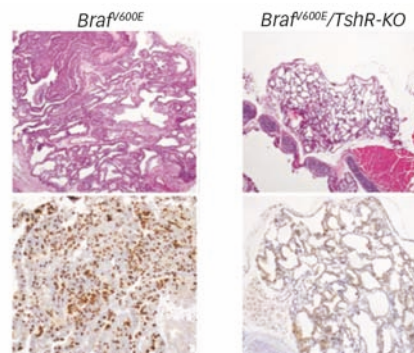
Braf^V600E thyroids are larger/disrupted compared with the Braf^V600E/TSHR-KO that lack the characteristics of papillary cancer. Ki67 staining is only intense in the presence of the TSHR. Reproduced from Franco et al.[64]

Figure 5: A Thyroid Follicle Differentiated from Embryonic Stem Cells[89]
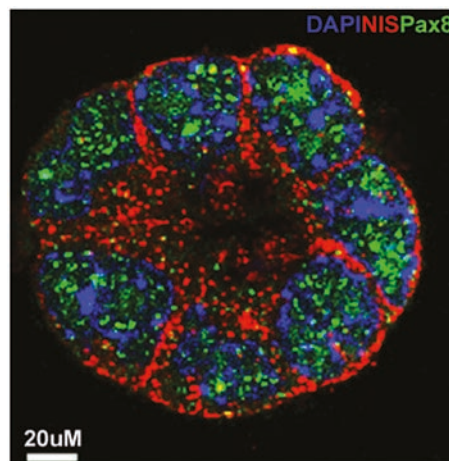
Immunodetection of paired box gene 8 (Pax8) (green) and sodium/iodide symporter (NIS) (red) expression in a thyroid neofollicle derived from differentiated Pax8+/Nkx2-1+ EB cells at day 21. Reproduced from Ma et al.[89]

which may apply to both Hashimoto's and Graves' diseases. While many studies based on FNA biopsy data have shown no association,[68–71] studies based on thyroidectomy specimens, including a retrospective cohort analysis of 2,500 patients, have shown that elevated levels of thyroglobulin antibodies were associated with an increased risk for thyroid cancer (odds ratio [OR]=1.57; CI=1.11–2.23)[62,72,73] and that any nodules present are more likely to be malignant.[74] By contrast, a case-control study comparing patients with benign thyroid nodular disease and patients with papillary thyroid cancer suggested that cancer patients with Hashimoto's thyroiditis had a better prognosis.[75] Nevertheless, the literature mostly supports a real association that is likely related to chronic thyroid inflammation since cellular stress responses are easily detectable in thyroid tissues from patient with autoimmune thyroid disease. Such stress responses confer an increased risk for tissue specific malignant transformation.[76]

The characteristics of the immune response may play a major role in this unclear relationship since there is also evidence of increased tolerance

and active avoidance of tumor immunity when certain T cell subsets are present[77] while the presence of macrophages is associated with decreased patient survival from thyroid cancer.[15] Hence, although there may be more thyroid cancers in this population it may have a better prognosis.[78] Much remains to be learned about this relationship including the alternative possibility that cancer may trigger autoimmunity.[79]

Thyroid Nodules

The presence of intrathyroidal multinodularity is often said not to increase the risk for thyroid cancer (see Guidelines for the management of thyroid nodules[80]) compared with solitary thyroid nodules and the data indicate a similar prevalence in both situations. Theoretically, each nodule has its own risk and, therefore, the more nodules the more risk. While there are no data to suggest that previously benign lesions progress directly to thyroid carcinoma, the exception to this is the development of the rare ATC which often is found within a multinodular gland and can be seen developing within a nodule usually in an elderly patient. There are also a number of well-researched clinical risk factors for the presence of a malignant rather than a benign thyroid nodule that will not be discussed in depth here. These include size, firmness, and age of the patient and ultrasound characteristics.

Cellular Origin of Thyroid Cancer and the Role of Stem Cells

Thyroid cancer must be derived from a normal cell, either a differentiated cell, or a thyroid stem or progenitor cell, subjected to external influences or stochastic mutations. Well-differentiated thyroid cancer cells may transform into more undifferentiated thyroid cancer cells through the sequential accumulation of additional genetic mutations. Although CSCs are now recognized as important components in carcinogenesis, and may form the basis of many tumor types,[72] including thyroid cancer, their origin has been in some dispute. Recent data in the mouse is highly suggestive that such CSCs are indeed derived from normal thyroid cells.[7] However, it is also important to note that stem-like cells may be induced by the phenomenon of EMT and such cells can be seen in thyroid cancers.[81] Cells undergoing EMT show loss of the morphologic features of epithelium, loss of intracellular adhesion molecules such as E-cadherin, loss of apico-basal polarity, and gain of mesenchymal markers, such as vimentin and gain of high motility and invasive capacities,[82,83] and show additional characteristics of stemness. In order to develop metastases, cancer cells must first undergo EMT and acquire these characteristics. EMT-inducers include inflammatory and immune cells and their secretions, hypoxia from local necrosis, and even the local stroma and extracellular matrix, which may contain a multitude of growth factors, all of which contribute to initiation of EMT allowing cells to become pro-metastatic. We know that transforming growth factor beta (TGFβ) is a potent inducer of the Snail transcription factor, which is intimately involved in EMT induction, and that Snail factors repress E-cadherin transcription directly,[84] activate the transcription of vimentin, and has been shown to accelerate thyroid papillary carcinoma development in mice.[85] Since EMT appears to be a major player in the development of local and distant tumor aggression, the development of small molecule intervention could become a treatment for the extreme forms of thyroid cancer based on the thyroid-specific inhibition of EMT.

The identification of stem-like cells within differentiated thyroid tissues has been definitive,[86] but their isolation and characterization from adult tissues has been more difficult. Therefore, much recent work has been based on embryonic stem (ES) cells or on determining whether CSCs vary among the different type of thyroid cancer. Markers for their identification include CD133, high intracellular aldehyde dehydrogenase (ALDH) activity, and stage-specific embryonic antigen 1 (SSEA-1).[86,87] Cells isolated from thyroid cancers expressing high ALDH activity were able to be expanded indefinitely *in vitro* as spheroids that retained tumorigenic potential when transplanted into immune-compromised mice.[88] Of importance has been the observation that the cells with a stem-like phenotype are capable of resisting conventional chemotherapy, thus leading to disease relapse even when the primary lesion has been eradicated.[84,85] Hence, the existence of CSCs might help explain recurrences after successful radiotherapy or chemotherapy since many current cancer therapeutics have been developed based on killing differentiated cancer cells. This logic suggests that the characterization of thyroid CSCs may provide not only insight into thyroid oncogenesis but also new specific targets for chemotherapy. Furthermore, advances in the differentiation of embryonic stem cells into functional thyroid follicles offers additional opportunities for exploring cancer neogenesis (*see Figure 5*).[89] ■

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