The Thyroid Gland: A Crossroad in Inflammation-Induced Carcinoma? An Ongoing Debate with New Therapeutic Potential

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Abstract: Chronic infection and inflammation contribute to around 25% of cancer cases worldwide. While a direct link between several types of human malignancies and inflammation has now been established, in particular at the gastrointestinal level, the relationship between inflammation and thyroid cancer and the pathophysiology of chronic inflammation that induces papillary thyroid carcinoma (PTC) are still subjects of debate. However, several epidemiological and morphological studies have strongly suggested an increased risk of PTC in patients with Hashimoto’s thyroiditis (HT). As in HT, an intense immune infiltrate is associated with certain PTC and might play a critical role in the regulation of carcinogenesis and in carcinoma progression. Proinflammatory molecules, such as cytokines and chemokines, which are produced by immune infiltrate in the tumor microenvironment, contribute to the regulation of key cellular processes for cancer onset and progression, in particular for tumor cell proliferation, apoptosis, autophagy, angiogenesis and metastasis. Molecular studies have identified activation of the RET/PTC rearrangement-induced MAPK signaling pathway as the driving force in the development of PTC in the context of HT. These genetic alterations may be favored by chronic inflammation. In this regard, the RET oncoprotein and its downstream effectors, such as those implicated in the activation of the MAPK pathway, as well as inflammatory molecules of the tumor microenvironment could be promising molecular targets for new therapeutic strategies for thyroid cancer. This review focuses on the complex link between thyroid cancer and chronic inflammation and highlights the different current hypotheses regarding the role of the immune cell microenvironment in the initiation and progression of PTC.

Keywords: Inflammation, thyroid, cancer, carcinogenesis, Hashimoto’s thyroiditis.

INTRODUCTION

In the second half of the 19th century, Virchow suggested that cancers tended to arise at sites of chronic inflammation [1]. Since then, epidemiological studies have confirmed the correlation between cancer and chronic inflammation, in particular for the gastrointestinal tract, where the link between chronic active inflammation and the risk of digestive carcinoma onset is now well-established [1-3]. Inflammation involves a well-coordinated response of different types of immune cells belonging to the innate or adaptive immune system. It is noteworthy that these cells are implicated in all stages of cancer development, from initiation of carcinogenesis to promotion of neoplastic, tumor progression and metastasis [2-4].

Thyroid carcinoma is the most common endocrine malignancy. Its incidence is increasing, particularly in Western Europe and North America [5, 6]. Some concordant epidemiological data suggest a strong correlation between thyroid carcinoma and chronic inflammatory diseases of the thyroid gland, particularly between papillary thyroid carcinoma (PTC) and Hashimoto’s thyroiditis (HT) [7-11]. Similarly, a link between HT and thyroid lymphoma has been established [12, 13]. At the molecular level, activation of the RET/PTC/RAS/BRAF/MAPK pathway, which induces both a proinflammatory and a protumorigenic thyroid program, is a possible mechanism linking chronic inflammation with carcinogenesis [14-17].

In this review, we first present some general considerations regarding the different molecular pathways linking possibly inflammation and oncogenesis. We then focus on the connection between PTC and chronic inflammatory processes, in particular those observed in carcinomas occurring in HT. We go on to describe the cellular and molecular mechanisms which are currently suspected to be involved in this phenomenon. Finally, we discuss the new therapeutic strategies used to target both thyroid tumor cells and the associated inflammatory tumor microenvironment.

CHRONIC INFLAMMATION AND CANCER: GENERAL CONSIDERATIONS

The connection between inflammatory immune responses and tumorigenesis has been extensively investigated during the past decade and some of the underlying mechanisms have been elucidated. In this regard, chronic inflammatory states are thought to contribute to approximately 20 – 25% of all human malignancies [18]. Liver and gastrointestinal cancers are among the best examples of this connection where there is an association between hemochromatosis, viral hepatitis B or C and liver cancer, chronic gastric infection with Helicobacter pylori and gastric cancer, and Crohn’s disease or ulcerative colitis and colon cancer [18].

Inflammation is a complex and auto-regulated phenomenon that involves different cells types, cytokines and chemokines following infection or injury by exogenous or endogenous means. Macrophages, neutrophils, mast cells, dendritic cells and natural killer cells belonging to the innate immune system initiate the inflammatory process by secreting cytokines and chemokines and releasing matrix-remodeling proteases and reactive oxygen and nitrogen species [18-20]. Furthermore, these cells are able to recruit and...
activate cells of the adaptive immune system like T-CD4+ or CD8+ and B-lymphocytes, which require antigen specificity. Control of the inflammatory process involves different molecules such as prostaglandin E2, transforming growth factor-β (TGF-β), reactive oxygen and nitrogen species which play a dual role in promoting and suppressing inflammation. Apoptosis of inflammatory cells, followed by their phagocytosis and the production of anti-inflammatory mediators such as TGF-β by apoptotic cells contribute to the resolution of inflammation [18, 19]. The deregulation of this complex mechanism or the persistence of the factors responsible for tissue damage can lead to chronic inflammation.

Sites of chronic inflammation are characterized by a rich lymphocyte, plasma cell, neutrophil and macrophage infiltrate and by the production of a large amount of various growth factors, cytokines, chemokines and oxygen and nitrogen reactive species [18, 19]. This microenvironment may contribute to the persistence and aggravation of tissue damage. Indeed, reactive oxygen and nitrogen species produced by macrophages and neutrophils to fight infection may become deleterious in the context of an ongoing inflammatory process by generating highly mutagenic agents such as peroxynitrite, which cause point-mutations, rearrangements and double-strand breaks in the DNA of proliferating epithelial and stroma cells [19, 21]. This latter phenomenon is associated with an increased risk of oncogene activation and tumor suppressor gene loss of function which may lead to neoplastic transformation [19].

Sustained tissue damage induces proliferation of epithelial and stromal cells, which is generally associated with metaplasia, a reversible change in cell type. In this context of intensive cell proliferation, dysplasia, a precancerous state characterized by an accumulation of atypical cells, may occur [19]. Houghton et al. have demonstrated, in a mouse model of Helicobacter pylori-induced gastric cancer, that bone marrow-derived cells were recruited at the site of chronic inflammation to repopulate the stomach and subsequently progress through metaplasia and dysplasia to intraepithelial cancer [22]. These immature bone marrow-derived cells are considered to be more pliable and sensitive to mutagenic agents than epithelial cells originating from the site of chronic inflammation. Consequently, they are more susceptible to neoplastic transformation [19].

Dysregulation of cell proliferation and secretion of chemokines and cytokines by tumor cells recruit a large variety of leukocytes into the tumor microenvironment. This immune cell infiltrate may act to suppress tumor growth. However, there are also several lines of evidence indicating that it may contribute to tumor progression, particularly in inflammation-induced cancers [19]. Tumor-associated macrophages (TAM) play a key role in this phenomenon as a high density infiltrate of TAM has been correlated to poor prognosis in clinical studies [19, 23, 24]. TAM can exhibit paradoxical roles in cancer because of their functional plasticity, resulting in the polarized expression of either pro- or anti-tumor functions [25]. Classical TAM activation (TAM1) induces a polarized type 1 immune response. TAM1 are generally considered to be potentiating effector cells which kill tumor cells. In contrast, several molecular signals present in the tumor microenvironment can induce distinct TAM functions (TAM2) characterized by a polarized type 2 immune response and pro-tumoral actions [25]. TAM2 may promote tumor progression by different mechanisms. They release matrix metalloproteinases (MMP-2 and MMP-9) that facilitate the breakdown of the basement membrane, a process required for invasion and migration of tumor cells [26]. They induce angiogenesis, which is necessary for tumor growth and metastasis, by secreting proangiogenic factors such as vascular endothelial growth factor (VEGF) and endothelin-2 [26, 27]. They also release a large number of growth factors such as epidermal growth factor receptor (EGFR) family ligands that enhance cell proliferation and migration [28, 29]. Furthermore, by secreting interleukin (IL)-10 and prostaglandin E2, they help to induce immune tolerance to tumor development [30]. Similarly, a recent study demonstrated that a subpopulation of tumor-associated neutrophils (TAN) can promote tumor progression (TAN2), whereas another population of neutrophils had an anti-tumor action (TAN1) [20].

The role of other immune cells in tumor development, and particularly that of T-lymphocytes, is debatable. Indeed, several studies have shown a protective role for a lymphocytic infiltrate against tumor progression, particularly in thyroid cancer. In contrast, other studies have demonstrated a correlation between a T-lymphocyte infiltrate and poor prognosis, for example, in renal cancers [31, 32]. In patients with colorectal cancer, there is still some debate about the role of a T-lymphocyte infiltrate [33-35]. Moreover, Galon et al. demonstrated that immunological data (the type, density, and location of immune cells within the tumor samples) are a better predictor of survival than histopathological data obtained with currently used methods to stage colorectal cancer [36].

In the tumor microenvironment, a wide variety of cytokines, chemokines and growth factors are secreted either by tumor or stroma cells, or by leukocytes recruited at the site of tumor development [19]. These molecules play a critical role in the regulation of tumor growth, invasion and metastasis. Tumor necrosis factor (TNF)-α is a cytokine secreted mainly by activated-macrophages or neutrophils and by tumor cells and has both anticancer and procarcinogenic actions [36]. On the one hand, it has been shown that a high dose of TNF-α induced the destruction of the tumor vasculature and then resulted in tumor necrosis. On the other hand, this cytokine exhibits antiapoptotic and proinflammatory activities through the induction of nuclear factor-κB (NF-κB) [36, 37]. Indeed, NF-κB, one of the main TNF-α downstream effectors is a transcription factor targeting inflammatory, antiapoptotic and cell proliferation regulatory genes. Hence, TNF-α is recognized as one of the key cytokines linking inflammation to cancer [19].

Among the other molecules of interest in chronic inflammation and cancer development, inducible nitric oxide synthase (iNOS), cyclooxygenase-2 (COX-2), hypoxia-inducible factor-1α (HIF-1α) and their related signaling pathways have been extensively studied [19]. iNOS is a downstream effector of various proinflammatory cytokines such as TNF-α and IL-1β that catalyze NO production. NO accumulation may lead to DNA damage and p53 mutations [38, 39]. Furthermore, NO has been shown to regulate angi-
ogogenesis, leukocyte adhesion and metastasis [19, 40]. The iNOS/NO signaling pathway is also able to induce COX-2 expression, which exhibits proinflammatory effects through the production of prostaglandins [40]. COX-2 is overexpressed in a wide variety of human malignancies and is implicated in cellular proliferation, anti-apoptotic activity, angiogenesis and metastasis [41-43]. Moreover, several population-based studies have shown that nonsteroidal anti-inflammatory drugs, which inhibit COX-2 activity, might reduce the risk of various cancers [44, 45]. HIF-1α is activated by hypoxia, which is a common phenomenon both in chronic inflammation and in the tumor microenvironment [19]. HIF-1α facilitates cancer development by inducing a metabolic shift in tumor cells, resulting in an enhancement of the glycolytic activity, and by promoting angiogenesis through the secretion of VEGF [46].

**THE ASSOCIATION BETWEEN THYROID INFLAMMATION AND THYROID CANCER: AN ONGOING DEBATE**

**Thyroid Cancer and Molecular Pathways**

Well-differentiated thyroid carcinomas which derive from follicular cells, including papillary and follicular carcinomas represent 90 to 95% of thyroid cancers. Their incidence is increasing, particularly in the United States and in Western Europe [6]. They usually have a good prognosis with only 5% of the patients dying from progression of their disease. This is mainly due to effective surgery combined with radioiodine-based therapy. Nevertheless, 10 to 20% of patients develop recurrence, which often correlates to the presence of radioiodine-refractory tumor cells [47]. Poorly-differentiated and anaplastic thyroid carcinomas are uncommon thyroid tumors which may arise from pre-existing differentiated tumors and present very aggressive behavior. Unlike well-differentiated carcinomas, they do not respond to radioiodine treatment because of a loss in the avidity of the tumor cells for radioactive iodine [48]. Medullary thyroid carcinomas arise from parafollicular C-cells in the thyroid. They secrete calcitonine which is a useful biomarker for both diagnosis and post-therapeutic follow-up [49]. This tumor appears most often as a sporadic event. Nevertheless, it may also be inherited in the context of familial medullary thyroid carcinomas or autosomal dominant multiple endocrine neoplasia type 2 syndromes [50, 51]. Surgery is the cornerstone of curative treatment of such tumors. Some molecular targeted therapies, and particularly vandetanib, which targets the epidermal growth factor receptor pathway and/or angiogenesis, are currently being developed for metastatic patients [50, 51].

Evaluation of the prognosis for patients with thyroid cancer is mainly based upon classical clinicopathological factors. Indeed, advanced patient age, male gender, large tumor size, extrathyroidal extension, lymph node metastasis and distant metastasis are associated with worse prognosis [52, 53]. Furthermore, for PTC, the histological subtype also has an important impact on tumor prognosis. Tall cell PTC is generally considered to be the most aggressive subtype followed by conventional PTC, and then by follicular variant PTC [54, 55]. At a molecular level, the RET/PTC/RAS/BRAF/MAPK pathway plays a critical role in thyroid cancer development and progression [55]. The MAPK cascade is a classical conserved intracellular signaling pathway involved in the regulation of cellular proliferation, differentiation, apoptosis and survival. Constitutive activation and deregulation of this pathway has been found in numerous human malignancies [56]. Physiologically, the tyrosine kinase membrane receptor RET can be stimulated by various growth factors and consecutively activates the membrane-bound small G-protein RAS. RAS then interacts with and activates RAF protein kinase. In mammals, RAF has three isoforms: A-RAF, B-RAF (BRAF) and C-RAF. In many cells, and particularly in follicular cells, BRAF is the most abundant and potent activator of the MAPK cascade [55]. Activated BRAF phosphorylates and activates MEK1 and MEK2, which in turn activates ERK 1 and ERK2. Ultimately, this process alters the expression of various genes regulating key cellular functions such as proliferation, differentiation, and apoptosis [55].

Aberrant activation of the MAPK pathway, through activation of genetic alterations, is commonly found in thyroid cancers. The different types of RET/PTC chromosomal rearrangements, between the 3’ portion of the RET gene and the 5’ portion of an unrelated gene, produce an aberrant RET/PTC protein with ligand-independent activation of its intracellular tyrosine kinase domain. This phenomenon, resulting in constitutive activation of the MAPK pathway, has been frequently described in occult small PTC and would seem, therefore, to be an early event in thyroid tumorigenesis [55, 57-59].

Activating point-mutations in RAS small GTPases are also a common cause of aberrant activation of the MAPK pathway and have been described in numerous human malignancies [60]. In thyroid cancers, they mainly occur in the follicular variant of PTC, in follicular carcinomas, and in poorly-differentiated or anaplastic carcinomas [57].

Somatic mutations in the BRAF gene have been described in several types of human cancer [61]. The mutant BRAF protein is characterized by elevated kinase activity and activates the MAPK pathway independently of RAS. The most common BRAF mutation is a point mutation at residue 600 of the BRAF protein called the BRAFV600E mutation, and is highly prevalent in PTC, principally with a papillary architecture [55, 61, 62]. This mutation has also been found in poorly-differentiated and anaplastic thyroid carcinomas [63]. Furthermore, several authors have shown that the discovery of the BRAFV600E mutation in tissue or fine-needle aspirations of patients with PTC was associated with a worse prognosis [55, 64]. The requirement for BRAF in RET/PTC-mediated MAPK activation in thyroid cells has been tested functionally by Mitsutake et al. in the RET/PTC3-expressing thyroid PCCL3 cell line [65]. The authors demonstrated that the selective knockdown of BRAF prevented ERK phosphorylation and the subsequent downregulation of the sodium iodide symporter, a gene that confers key biological effects of RET/PTC in PTCs [65]. In addition, rearrangements of the NTRK1 gene on chromosome 1 can be detected in a fraction of PTCs [66, 67]. Although occurring less frequently than RET rearrangements and the BRAFV600E mutation, rearrangements in NTKRI provide a
very interesting model for studying the molecular basis of thyroid carcinogenesis. In this regard, experimental evidence strongly suggests that TRK oncogenes exert a direct role and represent an early event in the process of thyroid carcinogenesis [66, 67]. However, the number of PTC carrying TRK oncogenes so far identified is limited, certainly because in some studies the genotyping of PTCs is restricted to RET rearrangement and BRAF mutation analysis.

Other genetic alterations that do not directly affect the activity of the MAPK pathway, such as the PAX8/PPARγ rearrangement and mutations of the tumor suppressor gene p53, have been found, respectively, in follicular and anaplastic thyroid carcinomas, but are not commonly encountered in PTC [57, 68-70].

Autoimmune Thyroid Diseases

A link between autoimmunity and cancer has long been recognized and several studies have shown that a history of autoimmune disease increases the risk of cancer [71]. Indeed, lupus patients exhibit a 2-fold increased risk of cancer and patients with a history of autoimmune disease have a 2.4-fold excess risk of esophageal cancer and a 2-fold increased risk of pancreatic cancer [72]. These data must be interpreted with caution as some treatments for autoimmune diseases may themselves increase the risk of cancer [72]. While autoimmunity has always been linked to immune dysregulation, cancer development is viewed as progressive accumulation of genetic abnormalities in malignant cells, with the immune system allowing or even promoting its progression [73, 74].

HT is an autoimmune inflammatory disease characterized by an immune system reaction against various thyroid antigens. Histologically, there is widespread lymphocyte infiltration and a progressive depletion in thyroid epithelial cells, which are replaced by fibrosis. This thyroiditis affects approximately 5% of the population and usually occurs between the 4th and the 6th decade, more frequently in women [75]. Patients with HT are usually asymptomatic, and some patients develop goiter with or without hypothyroidism [76].

Table 1. Different Studies Involving a Link And/Or an Absence of Link Between Hashimoto’s Thyroiditis (HT) and Papillary Thyroid Carcinoma (PTC)

<table>
<thead>
<tr>
<th>Author, year, [Ref]</th>
<th>Comments</th>
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<tbody>
<tr>
<td>Ott, 1985, [83]</td>
<td>Increased prevalence of HT in thyroid cancer patients</td>
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<tr>
<td>Singh, 1999, [80]</td>
<td>Increased prevalence of HT in patients with PTC</td>
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<tr>
<td>Kebebew, 2001, [85]</td>
<td>HT-associated PTC correlated with younger age, female gender and multicentric tumors</td>
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<tr>
<td>Pisanu, 2003, [10]</td>
<td>Increased prevalence of HT in WDTC patients</td>
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<tr>
<td>Cipolla, 2005, [79]</td>
<td>Increased prevalence of HT in patients with PTC and increased prevalence of PTC in patients with HT</td>
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<tr>
<td>Larson, 2007, [75]</td>
<td>Increased prevalence of WDTC in patients with HT</td>
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<tr>
<td>Repplinger, 2008, [84]</td>
<td>Increased prevalence of PTC in female patients with HT</td>
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<tr>
<td>Prasad, 2004, [90]</td>
<td>Similar immunophenotypic changes in HT and PTC</td>
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<tr>
<td>Matsumoto, 2006, [91]</td>
<td>Similar immunophenotypic changes in HT and PTC</td>
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<tr>
<td>Anderson, 2009, [87]</td>
<td>Similar immunophenotypic changes in HT and PTC</td>
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<tr>
<td>Wirtschafter, 1997, [7]</td>
<td>RET/PTC rearrangement found in most patients with HT with or without PTC</td>
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<tr>
<td>Mechler, 2001, [8]</td>
<td>RET/PTC (1 or 3) rearrangement found in familial PTCs associated with HT</td>
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<td>Kang, 2007, [98]</td>
<td>RET/PTC-dependent activation of the MAPK pathway in HT-associated PTC and in HT</td>
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<tr>
<td>Muzza, 2009, [97]</td>
<td>RET/PTC rearrangement without a BRAF mutation in HT-associated PTC and in HT</td>
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<tr>
<td>Burstein, 2004, [100]</td>
<td>HT and PTC initiated by the same population of pluripotent p63-positive embryonal stem cell remnants</td>
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<tr>
<td>Crile, 1978, [82]*</td>
<td>Incidence of PTC not increased in the context of HT</td>
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<tr>
<td>Holm, 1985, [81]*</td>
<td>Incidence of PTC not increased in the context of HT</td>
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<tr>
<td>Intidhar Labidi, 2006, [87]*</td>
<td>Incidence of PTC not increased in the context of HT</td>
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<tr>
<td>Del Rio, 2008, [88]*</td>
<td>No significant difference in clinical and histological characteristics between patients with HT-associated PTC and PTC without HT</td>
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<tr>
<td>Matesa-Anic, 2009, [86]*</td>
<td>No correlation between HT and PTC on cytological material</td>
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<tr>
<td>Nikiforova, 2002, [102]*</td>
<td>Absence of RET/PTC rearrangement in HT-associated PTC and in HT</td>
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<tr>
<td>Rhoden, 2006, [103]*</td>
<td>Low level of RET/PTC rearrangement in HT and PTC</td>
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<tr>
<td>Nakazawa, 2009, [104]*</td>
<td>RET/PTC rearrangement identified only in a small proportion of tumor cells in RET/PTC-positive PTC</td>
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<tr>
<td>Nasr, 2009, [93]*</td>
<td>Absence of a BRAF mutation in HBME1+ and CK19+ atypical cell clusters in HT</td>
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WDTC: Well-differentiated thyroid carcinoma.
*Studies that did not support an association between HT and PTC.
The majority of thyroid lymphomas, which generally belong to two histological subtypes: extranodal marginal zone B-cell lymphoma of mucosa-associated lymphoid tissue (MALT) and diffuse large B-cell lymphoma, arise against a background of HT [12, 13]. Nevertheless, as some studies failed to find a significant relationship between HT and thyroid lymphoma, the correlation between these two diseases is still subject to debate [77, 78]. Finally, another controversial association is the correlation between HT and PTC. There are epidemiological, histological and molecular data that suggest a significant association between these two diseases [75, 79, 80] (Table 1). Nevertheless, there are also several studies that do not support this association [81, 82] (Table 1). Although an increased dietary iodine intake is one of the potential explanatory factors for the distinctly increased incidence of HT and PTC, a different etiologic mechanism should probably be considered, however, to account for the complex relationship between these two diseases [11].

The Link Between Hashimoto’s Thyroiditis (HT) and Papillary Thyroid Carcinoma (PTC) Onset: The Pros and Cons

Several epidemiological studies have established an increased risk of PTC in patients with HT [8, 10, 75, 80, 83]. Ott et al. reported a 38% incidence of HT in 161 patients treated for thyroid cancer [83]. Similarly, Singh et al. found that the prevalence of HT is significantly higher in patients with PTC with an overall odds ratio of 1.89 [80]. In a more recent study on 812 patients who underwent thyroid resection, Larson et al. reported that patients with HT were approximately 3 times more likely to have well-differentiated thyroid cancer as compared to patients with no HT [75]. Repplinger et al. also demonstrated that HT was associated with an increased risk of developing PTC and that female patients with HT undergoing thyroidectomy were 30% more likely to have PTC [84]. In a series of 136 patients with PTC, Kebebew et al. showed that patients with coexisting PTC and HT were younger, more likely to be female and more likely to have multicentric tumors compared to patients without HT [85]. This increased incidence of PTC in patients with HT suggests that HT might be a precancerous condition promoting PTC development. Therefore, more aggressive surveillance for PTC may be indicated in patients with thyroid nodules arising in a context of HT [84]. Nevertheless, some studies failed to demonstrate any correlation between HT and PTC [81, 82, 86-88]. Matesa-Anic et al. did not find a significant relationship between these two diseases on examination of cytologic material from more than 10 000 patients with ultrasound-guided fine needle aspiration cytology of the thyroid [86]. Intidhar-Labidi et al. did not report an increased incidence of thyroid cancer among 78 patients with pathological diagnosis of HT [87]. In the same way, a study comparing 2 groups of patients with PTC with or without associated-HT, Del Rio et al. did not find any significant difference between these 2 groups of patients in their clinical characteristics and in the histological parameters of tumor aggressiveness [88]. These authors concluded that HT may have a minimum or an absence of impact in the development of PTC.

At a histological level, there are several similarities between HT and PTC. Proliferating nodules, cytological and nuclear alterations in association with strong lymphoid infiltrates composed of B and T lymphocytes, similar to those of PTC, are commonly encountered in HT [89] (Fig. 1). Furthermore, focal PTC-like immunophenotypic changes such as galectin 3, CITED1, cytokeratin 19 (CK19), HBME1 and fibronectin 1 expression may be found in HT, suggesting the possibility of early, focal premalignant transformation in some cases of HT [90]. Interestingly, Matsumoto et al. have shown that Niban, a recently identified molecular marker of renal carcinogenesis, was overexpressed in thyroid tumors, particularly in those with an oxyphilic cytoplasm, and in HT, mainly in scattered cells with oxyphilic cell metaplasia [91]. Similarly, Anderson et al. demonstrated that the expression of CD98, a component of a cell surface amino acid transporter, was decreased in inflamed areas of both HT and PTC compared to normal follicular cells, follicular adenoma or carcinoma, multinodular goiter and Grave’s disease [92]. Since CD98 binds and activates β1-integrin, thus promoting anchorage-independent growth, they conclude that this down-regulation of CD98 could explain, in part, the good prognosis of PTC, particularly in the context of HT. In contrast, a recent study by Nast et al. suggested that HBME1+ and CK19+ atypical cell clusters, found in HT, could not be considered to be preneoplastic as they did not harbor the BRAF mutation [93]. Nevertheless, this conclusion should be interpreted with caution since, as mentioned later, the BRAF mutation is not commonly encountered in HT-associated PTCs.

HT is characterized by a lymphocytic infiltrate which is also frequently found in PTC even in the absence of associated HT [57]. This lymphocytic infiltrate is more frequent and more intense in PTCs than in benign thyroid tumors [94]. Indeed, a peritumoral lymphocytic infiltrate or focal lymphocytic thyroiditis, without the hallmark of true HT, is commonly encountered in PTCs [11] (Fig. 1). Interestingly, Reines et al. suggested that autoimmune diseases, such as HT, could be seen as a sustained systemic response to threatened neoplastic transformation [95]. However, there is still no clear evidence to show whether this PTC-induced immune reaction leading to lymphocytic infiltration of the thyroid gland can promote the development of HT in its proper clinical and pathologic expression [11]. As a lymphocytic infiltrate is generally found in the peritumoral environment of PTC, it has been suggested that thyroid lymphocytic infiltration favors the development of PTC [11]. On the other hand, there are also several lines of evidence that this lymphocytic infiltrate plays a protective role against thyroid cancer. Firstly, PTCs arising in the context of HT exhibit a better prognosis than PTCs without chronic lymphocytic thyroiditis [80, 85]. Indeed, Singh et al. reported in a meta-analysis on HT and PTC, that the presence of HT positively correlated to disease-free and overall survival [80]. Secondly, in the absence of typical signs of HT, poorly-differentiated and anaplastic thyroid carcinomas, which are associated with a worse prognosis, generally exhibit a reduced lymphocytic infiltrate compared to PTCs [31]. These aggressive histological subtypes of thyroid cancers are also associated with a reduced dendritic cell infiltrate and an increased TAM2 infiltrate compared to PTCs [19, 23, 24, 31, 96].
The different studies exploring the relationship between chronic lymphocytic thyroiditis and thyroid cancer are listed in Table 1.

CORRELATION BETWEEN THYROID CARCINOMA DEVELOPMENT AND HASHIMOTO’S THYROIDITIS: POTENTIAL MOLECULAR MECHANISMS INVOLVED IN INITIATION OF CARCINOGENESIS

Activation of the MAPK signaling pathway through RET/PTC rearrangements seems to be the key molecular event linking PTC and HT. A recent study, conducted by Muzza et al. on a large series of PTCs with or without thyroiditis, showed that the RET/PTC rearrangement was more often present in PTCs associated with autoimmunity, whereas the BRAFV600E mutation was encountered more frequently in PTCs alone. The RET/PTC rearrangement was also found in 41% of non-neoplastic thyroiditis tissues [97]. Similarly, Kang et al. reported that the expression of the RET, nuclear-localized RAS and ERK proteins was greatly enhanced in PTC and HT oxyphil cells, but that no BRAF exon 15 or N-RAS exon 2 mutations were found in HT oxyphil cells [98]. Sargent et al. also demonstrated that BRAF...
mutations were uncommon in HT-associated PTC and absent in non-neoplastic nuclear atypia of thyroiditis [99]. Interestingly, p63 proteins, which are p53 homologs regulating squamous stem cell commitment, have been detected in PTC, HT and in embryonic remnants found sporadically in the fully-developed thyroid. Burstein et al. have suggested therefore that HT and PTC might be linked etiologically because both disorders might be initiated by the same population of pluripotent p63-positive embryonal stem cell remnants [100, 101]. Wirtschafter et al. detected the expression of the RET/PTC1 and RET/PTC3 oncogenes in 95% of patients with HT even in the absence of histological evidence of PTC, suggesting that multiple independent occult tumors might exist in this type of patient [7]. In contrast, Nikiforova et al. found no RET/PTC rearrangement in patients with HT or with PTC arising in the context of HT [102]. These authors concluded that if the association between HT and thyroid cancer existed, its molecular basis was different from the RET/PTC rearrangement. As suggested by Rhoden et al., these contrasting results might be explained by the variability in RET/PTC expression and, more probably, in the sensitivity of the techniques used to identify the RET/PTC rearrangement [103]. Indeed, in a study investigating the frequency of the RET/PTC rearrangement in patients with HT or thyroid carcinoma using different techniques of identification (interphase fluorescence in situ hybridization, real-time RT-PCR, RT-PCR after laser capture microdissection), Rhoden et al. found a low-level of RET/PTC recombination in non-neoplastic follicular cells in HT and in a subset of PTC. Furthermore, they demonstrated that the identification rate of the RET/PTC rearrangement was strongly technique dependent [103]. Similar results were reported in a study by Nakazawa et al. for patients with PTC [104]. These authors demonstrated that the RET/PTC rearrangement rate was dependent on the technique used for identification (FISH or RT-PCR) and occurred only in a small percentage of tumor cells. Therefore, they suggested the possibility of a RET/PTC rearrangement as a “passenger” abnormality rather than a “driver” of oncogenic mutation in thyroid carcinogenesis [104].

Among the different types of RET/PTC rearrangements, RET/PTC3 has been implicated in the development of an unusual solid subtype of PTC which prevails in children exposed to radiation from the Chernobyl nuclear power plant disaster. Interestingly, Powell et al., who generated transgenic mice expressing human RET/PTC3 exclusively in the thyroid, have shown that these mice also develop solid tumor variants of PTC [105]. Similarly, Santoro et al. examined 106 cases of PTC in children and demonstrated that two rearrangements resulting from inversion of part of chromosome 10 (PTC1 and PTC3) accounted for the majority of the RET rearrangements identified, with PTC1 being associated with PTC of the classic and diffuse sclerosing variants and PTC3 with the solid/follicular variant [106]. In an interesting study on 71 cases of PTC in survivors of the atomic bomb, Hama-tani et al. reported that, contrary to the BRAF (V600E) mutation, RET/PTC rearrangements showed an increased frequency with increased radiation dose, and that PTC patients harboring RET/PTC rearrangements developed this cancer earlier than did patients with the BRAF(V600E) mutation [107].

Oncogene expression normally induces apoptosis since excessive growth and proliferation signals, such as those induced by the RET/PTC rearrangement, are not tolerated. Castellone et al. demonstrated that the expression of RET/PTC oncogenes induces apoptosis of rat thyroid PC CL 3 cells [108]. This phenomenon was explained by the chronic activation of the RET kinase, constitutively phosphorylated on tyrosine 1062, which transmitted both mitogenic and proapoptotic signals to thyroid cells. Therefore, additional signals promoting survival of thyroid cells are also necessary to induce tolerance to RET/PTC oncogene activity. Cytokines and chemokines released into the inflammatory tumor microenvironment play a crucial role in delivering anti-apoptotic signals and in inducing survival of thyroid cells expressing RET/PTC [57]. For example, IL-6 is a cytokine produced at the site of chronic inflammation and is known to induce anti-apoptotic effects in tumor cells through overexpression of anti-apoptotic genes Bcl-2 and Bcl-XL [18, 109]. Furthermore, Stassi et al. reported that autocrine production of IL-4 and IL-10 by thyroid tumor cells induced resistance to chemotherapeutic drug cytotoxicity and to FAS/FAS-ligand-mediated apoptosis [110]. This effect was linked to the induction by IL-4 and IL-10 of two anti-apoptotic proteins, cFLIP and PED/PEA15, in thyroid tumor cells [111]. The important role of survival and anti-apoptotic signaling pathways in linking inflammation and thyroid cancer has also been suggested in a study from Larson et al. [75]. In this study, the authors demonstrated overexpression of p-Akt, Akt1 and Akt2 in regions of HT and thyroid cancer compared to regions of normal surrounding tissue [75]. They concluded that the phosphatidylinositol 3-kinase (PI3K)/Akt signaling pathway, which is known to promote survival in tumor cells, was a possible molecular mechanism accounting for the link between HT and thyroid cancer [75]. Interestingly, Kim et al. showed that activation of the signal transducer and activator of transcription 3 (STAT3) in the RET/PTC-expressing PTC cell line TPC-1 was dependent on the interaction between the RET/PTC oncoprotein and phospholipase D, which is known to regulate cell survival through activation of the PI3K/Akt signaling pathway [112].

While chronic inflammation can promote thyroid cancer development, thyroid cancer cells can, in turn, contribute to maintain an inflammatory microenvironment through the secretion of proinflammatory factors. Several studies have demonstrated that the RET/PTC rearrangement induced secretion of various inflammatory proteins by thyroid epithelial cells [14, 15, 17, 113]. In a recent study, Muzza et al. showed increased expression of inflammatory molecules such as CCL20, CXCL8 and L-selectin, in PTC [97]. Increased NFκB DNA-binding activity and subsequent overexpression of several proinflammatory proteins such as CXCL1/Groα, CCL2/mcp-1 and GM-CSF has been reported by Russel et al., in the rat thyroid cell line PC C13 expressing RET/PTC3 [14]. Accordingly, they also demonstrated, in vivo, that RET/PTC3-transgenic thyroid tissue produced inflammatory molecules such as IL-1α, IL-1β, IL-6, TNF-α and COX2 [15]. Similar results have also been reported by Puxeddu et al. who demonstrated that RET/PTC3 induced expression of a panel of inflammatory mediators such as CXCL10, IL-6, prostaglandin E2, microsomal prostaglandin E synthase 1 and COX2 [17, 113]. The RET/PTC-induced secretion of inflammatory factors by thyroid tumor cells is
mediated by the activation of the RET/PTC/RAS/RAF/MAPK signaling pathway. Indeed, like the RET/PTC rearrangement, the activating mutations in the BRAF or RAS oncogenes are also able to activate a common transcriptional program in thyroid cells that includes upregulation of the CXCL1 and CXCL10 chemokines [114]. Several authors have shown that these inflammatory molecules not only sustain the inflammatory reaction but were also implicated in an autocrine loop acting on thyroid tumor cells that express their specific receptors [114-116]. Castellone et al. found that RET/PTC-positive human thyroid cancer cell lines and human thyroid carcinomas express CXCR4, the receptor for the chemokine CXCL12/SDF-1α/β. They also found that the CXCR4/SDF-1α receptor-ligand pathway was used by thyroid tumor cells to proliferate, survive and migrate [116]. Similarly, Guarino et al. showed that osteopontin (OPN), a secreted glycoprotein, was overexpressed in thyrocytes transformed by the RET/PTC oncogene and that the prevalence and intensity of OPN staining significantly correlated with the presence of lymph node metastases and tumor size [117]. They also demonstrated that OPN stimulated the invasiveness of thyroid cancer cells through stimulation of its receptor (CD44) which was also expressed by thyroid tumor cells.

The different cytokines and chemokines secreted in the tumor microenvironment by either tumor cells or infiltrating immune cells may contribute to thyroid cancer progression via different mechanisms. They promote tumor cell proliferation and migration, angiogenesis and metastasis [57]. IL-8 is a chemokine known to contribute to human cancer progression through its potential function as a mitogenic, angiogenic or motogenic factor [18]. Iwahashi et al. reported that the secretion of IL-8 was under the control of the RET/PTC/RAS/RAF/MAPK pathway and that this cytokine was overexpressed in human PTC [118]. Similarly, COX2, which is known to promote cellular proliferation, survival, angiogenesis and metastasis in several human malignancies is overexpressed both in thyroid cancers and in HT [41-43, 119].

Another important role of several cytokines in thyroid cancer progression is the induction of immune tolerance to the tumor. Sumimoto et al. demonstrated that the MAPK pathway mediates melanoma cell evasion of the immune system through the secretion of the immunosuppressive soluble factors interleukin IL-10, VEGF or IL-6 [120]. These factors are also secreted by thyroid tumor cells and may act in the induction of immune evasion of RET/PTC-positive thyroid cancers [15, 110, 111]. Pfannkuck et al. reported that tumors expressing the functional, but not mutant, form of RET/PTC3 showed enhanced infiltration of CD8(+) lymphocytes, myeloid-derived CD11b(+)Gr1(+) cells and enhanced growth in immunocompetent mice [121]. In contrast, they found that RET/PTC3 signaling mutant-expressing tumors maintained enhanced infiltration of CD8(+) lymphocytes but did not enhance recruitment of CD11b(+)Gr1(+) cells and showed decreased tumor incidence. They concluded that the RET/PTC3 oncprotein was able to recruit and activate innate and adaptive immune cells, resulting in enhanced tumor progression [121].

The activation of the RET/PTC/RAS/BRAF/MAPK signaling pathway through RET/PTC rearrangement is therefore the main molecular link between HT and PTC. Several authors have established that this activation was dependent on the integrity of the Y1062 residue of RET [122, 123]. Indeed, Melillo et al. demonstrated that the docking protein FRS2 is tyrosine-phosphorylated by ligand-stimulated and constitutively activated oncogenic forms of RET through the binding of the phosphotyrosine-binding domain of FRS2 to pY1062 [123]. They also established that oncogenic RET/PTC proteins were associated with FRS2 constitutively, leading to tyrosine phosphorylation of FRS2, MAPK stimulation and cell proliferation. Similarly, Pfannkuck et al. reported that tumor cells expressing the mutant isoform Y1062F of RET/PTC3 were unable to recruit innate suppressive inflammatory cells, and, therefore, to induce tumor escape from the immune response [121].

In summary, a RET/PTC rearrangement-dependent activation of the MAPK pathway is generally found both in HT and PTC (Fig. 2). Chronic inflammation may favor this molecular event which, in turn, induces a pro-inflammatory program in thyroid cancer cells (Fig. 2). The chemokines and cytokines, released in the tumor microenvironment by thyroid cancer cells and leukocytes, deliver proliferation, survival and anti-apoptotic signals to tumor cells and promote angiogenesis and metastasis, finally leading to tumor progression.

**CORRELATION BETWEEN THYROID CANCER AND CHRONIC INFLAMMATORY PROCESSES: IMPLICATIONS FOR NEW THERAPEUTIC STRATEGIES?**

As the constitutive activation of the RET/PTC/RAS/BRAF/MAPK pathway is considered to be the driving force in the development of PTC in patients with HT, the selective targeting of one of the components of this critical signaling pathway should offer a promising therapeutic approach. Carmignago et al. have shown that RET kinase inhibitors such as pyrazolopirimidine derivatives are able to prevent the growth of two human PTC cell lines harboring the RET/PTC rearrangement [124]. In another study, they demonstrated that sorafenib (BAY 43-9006), a multikinase inhibitor, inhibited the growth of RET/PTC-positive thyroid cancer cell lines both in vitro and in vivo [125]. They also reported that sorafenib was active on cells harboring the mutations RET(V804L) and RET(V804M) of the RET kinase that are known to confer resistance to anilinoquinazolines and pyrazolopyrimidines. In two recent phase II clinical trials on patients with metastatic and iodine-refractory thyroid carcinoma, sorafenib has shown promising clinical activity with an acceptable safety profile [126, 127]. Several phase III clinical trials are currently ongoing to determine the benefit of sorafenib in this type of patient.

Some specific BRAF kinase inhibitors have been recently developed and seem more effective than sorafenib on thyroid cell lines harboring the BRAF(V600E) mutation [55, 128]. Nevertheless, as mentioned above, BRAF activating mutations are rarely encountered in HT-associated PTCs, where the RET/PTC rearrangement is the most frequent genetic alteration [97].

As MEK inhibitors are able to suppress MAPK pathway signaling, they are considered to be very promising therapeutic
agents in several human malignancies, including thyroid cancers. Numerous preclinical studies have demonstrated that MEK inhibitors preferentially inhibited BRAF or Ras mutation-harboring cancer cells [55, 129-131]. Although MEK inhibitors are able to inhibit ERK1/2 phosphorylation in both RET/PTC rearrangements and BRAF mutation-positive thyroid cancer cells, the effects of MEK inhibitors on cell proliferation and apoptosis are encountered essentially in PTC harboring BRAF mutations [131]. Indeed, Liu et al. showed that cell proliferation was potently inhibited by the MEK inhibitor CI-1040 in thyroid cancer cells harboring BRAF or RAS mutations but not in cells harboring a RET/PTC rearrangement or wild-type alleles [130]. More recently, the same authors have reported comparable results with the new-generation MEK inhibitor PD0325901 [132]. Interestingly, although there was no inhibition of the proliferation of RET/PTC1-harboring cells by PD0325901 itself, this inhibition could be induced or significantly potentiated by concurrent inhibition of the PI3K or NF-kB pathway. This may be explained by the fact that RET/PTC rearrangements are coupled to these multiple signaling pathways, which are important for proliferation of cancer cells. Consequently, it is not surprising perhaps that the MEK inhibitor alone did not show significant inhibition on proliferation and some other cellular events of RET/PTC1-harboring thyroid cancer cells [132]. Nevertheless, in a phase II clinical trial on patients with iodine-131 refractory progressive PTC treated with AZD6244, a MEK inhibitor, Lucas et al. reported promising clinical results with a mean progression free survival greater than one year [133]. Thus, inhibition of the different components of the MAPK signaling pathway in combination with the blockage of other critical signaling pathways for thyroid cancer development, survival regulation, apoptosis or inflammation appears to offer a promising therapeutic approach. In this regard, Cabanillas et al. evaluated the effect of sorafenib (a BRAF, RET and VEGFR inhibitor) in association with tipifarnib, a farnesyl transferase inhibitor of activation of Ras oncoproteins, in patients with metastatic thyroid cancer. In this phase I study, these authors reported promising clinical activity (progression free survival of 20 months), along with a good safety profile [134]. Moreover, several authors have demonstrated that overexpression of the chemokine receptors CCR3, CCR7 and CXCR4 was a potential biological marker of tumor aggressiveness in patients with PTC [135, 136]. Interestingly, De Falco et al. reported that AMD3100, a specific antagonist of CXCR4, inhibited the growth of undifferentiated thyroid cancer cells, both \textit{in vitro} and \textit{in vivo}, suggesting a potential therapeutic benefit of CXCR4 targeting in patients with thyroid cancer [137].

**CONCLUSION**

A close relationship exists between inflammation and thyroid cancer. Most epidemiological and histological studies have demonstrated an increased risk of PTC, in patients with HT, one of the most common autoimmune thyroid diseases. At the molecular level, activation of the MAPK signaling pathway through RET/PTC rearrangement is considered to be the driving force in the development of HT-associated PTC. Chronic inflammation in the thyroid gland can probably favor the appearance of this genetic alteration through the secretion of a high level of mutagenic agents, such as reactive oxygen and nitrogen species. Furthermore, inflammation creates a specific microenvironment in which the expression of oncoproteins such as RET/PTC by tumor
cells is tolerated. In turn, activation of the RET/PTC/RAS/ BRAF/MAPK signaling pathway elicits a proinflammatory program inducing secretion of various cytokines and chemokines by thyroid cancer cells. These molecules can act on tumor cells themselves, contributing to a malignant phenotype by activation of key signaling pathways regulating cell proliferation, survival, migration and invasion. They can also act on the tumor microenvironment, recruiting immune cells, inducing angiogenesis and metastasis. Thus, chronic lymphocytic thyroiditis might favor PTC onset which, in turn, sustains the inflammatory reaction and increases lymphocytic infiltration in the tumor microenvironment. Chemokines released by both thyroid cancer epithelial cells and lymphocytes can act through an autocrine or paracrine loop on thyroid cancer epithelial cells, promoting proliferation, survival and metastasis (Fig. 2). Nevertheless, this inflammation-associated carcinogenesis, which is mainly driven by RET/PTC rearrangement-activation of the MAPK pathway, leads to tumors harboring a less aggressive phenotype than those arising in the absence of a context of inflammation and characterized essentially by BRAF mutation-dependent activation of the MAPK pathway. Finally, in the development of future therapeutic agents for patients with thyroid cancer, the inflammatory tumor microenvironment, like the different components of the RET/PTC/RAS/BRAF/MAPK signaling pathway, should also be taken into consideration.

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