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► **To cite this version:**

Valentina Guarino, Maria Domenica Castellone, Elvira Avilla, Rosa Marina Melillo. Thyroid cancer and inflammation. *Molecular and Cellular Endocrinology*, 2010, 321 (1), pp.94. 10.1016/j.mce.2009.10.003 . hal-00582103

HAL Id: hal-00582103

<https://hal.science/hal-00582103v1>

Submitted on 1 Apr 2011

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Accepted Manuscript

Title: Thyroid cancer and inflammation

Authors: Valentina Guarino, Maria Domenica Castellone,
Elvira Avilla, Rosa Marina Melillo

PII: S0303-7207(09)00534-6
DOI: doi:10.1016/j.mce.2009.10.003
Reference: MCE 7349

To appear in: *Molecular and Cellular Endocrinology*

Received date: 7-7-2009
Revised date: 6-10-2009
Accepted date: 7-10-2009

Please cite this article as: Guarino, V., Castellone, M.D., Avilla, E., Melillo, R.M., Thyroid cancer and inflammation, *Molecular and Cellular Endocrinology* (2008), doi:10.1016/j.mce.2009.10.003

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Thyroid cancer and inflammation

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Key words: thyroid cancer, inflammatory cell, chemokine.

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Abstract

Some cancer types are strongly associated with chronic inflammatory or infectious diseases whereas others are not, but an inflammatory component is present in most human neoplastic lesions. This review focuses on various aspects of thyroid cancer and inflammation. The incidence of thyroid cancer, in particular of well-differentiated papillary thyroid carcinomas (PTCs), is increased in autoimmune thyroid diseases such as Hashimoto's thyroiditis. Thyroid cancer often has an inflammatory cell infiltrate, which includes lymphocytes, macrophages, dendritic cells and mast cells, whose role in thyroid cancer is still not completely understood. However, most experimental evidence suggests these cells exert a pro-tumorigenic function. Moreover, oncoproteins typically expressed in human PTCs, such as RET/PTC, RAS, and BRAF, trigger a pro-inflammatory programme in thyrocytes. These data suggest that inflammatory molecules are promising targets for thyroid cancer therapy.

Thyroid cancer

Thyroid cancer, although a rare disease, is the most frequent endocrine neoplasia, and its incidence is rapidly increasing. The papillary histotype is the most frequent. Thyroid cancer can derive from both the follicular and the parafollicular component of the thyroid gland. Three malignant lesions may derive from follicular cells: well-differentiated (WDTC), poorly differentiated (PDTC) and anaplastic (ATC) thyroid carcinomas. WDTCs are either papillary (PTC) or follicular (FTC). These two types are characterized by specific histological features, and by the maintenance of some differentiating features (**Kondo et al., 2006**). WDTCs have a favorable prognosis thanks to the efficacy of combined surgical and radioiodine-based therapy. Nevertheless, 5-10% of WDTCs progress to radioactive iodine refractory-disease. PDTCs and ATCs, which are thought to derive from pre-existing differentiated tumors, do not accumulate radioiodine and respond poorly to medical and surgical treatment (**Rosai et al., 1992**). Medullary thyroid carcinoma (MTC), which arises from the parafollicular C-cells of the thyroid, is generally treatable by surgery. However, novel chemotherapeutic agents like vandetanib, which target growth factor receptor, are showing promise in the treatment of MTC. This tumor can be inherited in the context of autosomal dominant MEN 2 (multiple endocrine neoplasia type 2) syndromes (MEN 2A, MEN 2B and familial medullary thyroid carcinoma [FMTC]) (**Roman et al., 2009**).

Non overlapping mutations of the RET, TRKA, RAS and BRAF genes are found in about 70% of PTCs. These genes encode activators of the mitogen-activated protein kinase (MAPK) cascade. RET encodes the tyrosine kinase receptor of growth factors belonging to the glial-derived neurotrophic factor (GDNF) family (**Manie et al., 2001**). RET/PTC rearrangements are thought to be early events in thyroid tumorigenesis since they are very frequent in clinically-silent small PTC (**Fusco et al., 2002**). Similar rearrangements of the high affinity receptor (TRKA) for nerve growth factor (NGF) are also found, albeit at a low prevalence, in human PTC (**Alberti et al., 2003**). Activating point-mutations in RAS small GTPases are found mainly in the follicular variant of PTC (PTC-FV) (**Zhu et al., 2003**). Point-mutations in BRAF are the most common genetic lesion

in these tumors (**Kimura et al., 2003; Xu et al., 2003; Soares et al., 2003; Cohen et al., 2003**).

BRAF is a member of the RAF family of serine/threonine kinases, which includes ARAF and RAF1, and it is a component of the RAF-MEK-ERK signaling module.

FTCs are characterized by RAS point-mutations (**Nikiforova et al., 2002**) or by the PAX8/PPAR rearrangement (**Kroll et al., 2000**). PDTC and ATC can derive from pre-existing WDTCs. RAS point-mutations and the V600E BRAF mutation are prevalent in PDTC and ATC (**Garcia-Rostan et al., 2003; Nikiforova et al., 2003; Soares et al., 2004; Begum et al., 2004**). Finally, p53 mutations are often found in ATC (**Donghi et al., 1993; Fagin et al., 1993**).

MTC can be sporadic or it could be one of the lesions that characterize the autosomal dominant MEN 2 syndromes (MEN 2A, MEN 2B and FMTC). MEN 2 syndromes are caused by germline point mutations that convert RET into a dominant oncogene (**Santoro et al., 1995**). In all MEN 2A cases, mutations target extracellular cysteine residues in RET. In more than 80% of cases, MEN 2B is caused by the Met918Thr substitution in the kinase domain of the receptor. Roughly 40% of sporadic MTC cases harbor point mutations in RET (**Leboulleux et al., 2004**).

Chronic inflammation and cancer

Inflammation is a physiological, protective process used by the organism in response to tissue damage. Several chemical signals initiate and sustain the inflammatory response whose aim is to fight pathogens and to repair tissue damage. Several cell types migrate to the sites of tissue damage. This migration is mediated by chemotactic and adhesion proteins, including members of the integrin and selectin family (**Coussens et al., 2002**). The first migrating cells are neutrophils, macrophages and mast cells, all of which secrete reactive oxygen species (ROS), vasoactive molecules, such as histamine and leukotrienes, and cytokines, chemokines and proteases that remodel the extracellular matrix (**de Visser et al., 2006**). Inflammation is an auto-limiting process; however, abnormal persistence of the stimuli that induced the inflammatory response or the failure

of the mechanisms that make it end, result in chronic inflammation (**Coussens et al., 2002**).

A functional relationship between chronic inflammation and cancer was first proposed by Virchow in 1863, and has been sustained by clinical and epidemiological evidence. The most compelling evidence is the association between: a) intestinal chronic inflammatory diseases (Crohn's disease and ulcerative rectocolitis) and adenocarcinoma of the colon; b) chronic HBV or HCV hepatitis and liver carcinoma; c) *Helicobacter pylori*-induced chronic gastritis and gastric carcinoma; d) asbestosis and mesothelioma; e) chronic obstructive pulmonary disease (COPD) and lung cancer; and f) chronic esophagitis and carcinoma of the esophagus (**Balkwill and Mantovani, 2001**). Moreover, a 40-50% reduction in the incidence of colorectal cancer is associated with the regular use of non-steroidal anti-inflammatory drugs, the COX enzymes that catalyze the synthesis of pro-inflammatory mediators, such as prostaglandins (**Baron and Sadler, 2000; Williams et al., 1999**).

Further support for the concept that inflammation can cause cancer comes from the observation that polymorphisms in genes encoding proinflammatory chemokines are associated with an increased risk of some forms of human cancer. For instance, IL1- β polymorphisms are associated with an increased incidence of gastric cancer (**Gianfagna et al., 2008**). Moreover, gastric-specific expression of this cytokine in transgenic mice can induce gastric inflammation and cancer (**Tu et al., 2008**).

Various infiltrating cells have been identified in tumors, namely, tumor-associated lymphocytes, tumor-associated macrophages (TAM), immature dendritic cells, mast cells and myeloid-derived suppressor cells. The presence of specific inflammatory-immune cells such as macrophages and mast cells in tumor site has been associated with a poor prognosis of the disease (**Fujimoto et al., 2009; Maltby et al., 2009**). Pollard and colleagues have shown that transgenic mice prone to breast cancer develop less proliferating and invasive tumors when crossed with mice deficient for CSF-1, a macrophage chemotactic factor (**Lin et al. 2001**). Other cell populations also contribute, via distinct mechanisms, to tumor growth. Indeed, mice deficient in mast cells or CD4+

T cells display a reduction in tumor incidence and progression in models of mice carcinogenesis (**Daniel et al., 2003; Soucek et al., 2007**). Among the cell populations recruited into tumor sites by cancer cells, a peculiar role is played by bone marrow-derived precursors. These include myeloid-derived suppressor cells that contribute to tumor-growth through tumor-induced immune tolerance (**Marigo et al., 2008**), and bone marrow-derived stem cells, which are thought to be recruited from the bloodstream and to repopulate areas of epithelial destruction. Indeed, these cells constitute all the metaplastic, dysplastic and neoplastic areas in the *Helicobacter*-induced gastric cancer model in mice. Again, IL1- β plays an important role in this process – indeed it seems to be required for both recruitment and activation of bone marrow-derived stem cells (**Houghton et al., 2004; Tu et al., 2008**).

Cancer cells secrete several cytokines and chemokines, thus sustaining cancer cell growth and recruiting leukocytes into tumor sites. Leukocytes physiologically secrete ROS and reactive nitrogen species to eliminate pathogens. However, these highly reactive metabolites induce the production of peroxynitrite and other mutagenic agents; therefore, they can induce “DNA damage”, i.e. mutations in proliferating cells (**Coussens et al., 2002**). Thus, in the case of persistent tissue damage, the O₂ and N highly reactive metabolites secreted by inflammatory cells induce point mutations, rearrangements and double-strand breaks in the DNA (**Colotta et al., 2009**). This results in a higher probability of oncogene activation or of tumor suppressor loss of function.

Proinflammatory cytokines produced in tumor sites are pivotal in cancer progression. The production of these cytokines by inflammatory and epithelial cancer cells depends mainly on the NF κ B transcription factor. Through conditional deletion of the NF κ B upstream activator IKK β , in myeloid and epithelial cells, it has been shown that NF κ B plays a major protumorigenic role in an experimental colitis-associated cancer (CAC) model (**Greten et al., 2004**). Among the cytokines induced by NF κ B, IL6 has been identified as one of the most relevant myeloid-derived factors that promote tumorigenesis. Moreover, activation of the IL6 pathway, in particular of the STAT3 transcription factor, which is a downstream mediator of IL6 activity, is necessary for CAC initiation

and progression (**Grivenikov et al., 2009. Bollrath et al., 2009**).

Thyroid cancer and autoimmune thyroid diseases

A link between thyroid cancer, in particular the PTC histotype, and autoimmune thyroid diseases (AITD) has long been recognized, although the precise relationship between the two diseases is still debated. This group of diseases includes Hashimoto's thyroiditis and Grave's disease. Hashimoto's thyroiditis (HT) is an autoimmune disorder in which the immune system reacts against a variety of thyroid antigens. The overriding feature of HT is the progressive depletion of thyroid epithelial cells, which are gradually replaced by mononuclear cell infiltration and fibrosis. Multiple immunologic mechanisms may contribute to the death of thyrocytes. Sensitization of autoreactive CD4+ T-helper cells to thyroid antigens appears to be the initiating event. Hashimoto's thyroiditis is characterized by proliferating nodules as well as by cytological alterations and nuclear modifications similar to those of papillary carcinomas (**Weetman, 2004**).

An epidemiological association has been identified between Hashimoto's thyroiditis and thyroid cancer (**Di Pasquale, 2001; Wirtschafter et al., 1997; Mechler et al., 2001; Segal et al., 1985; Eisemberg et al., 1989; Ott et al., 1987; Sclafani et al., 1993; Pisanu et al., 2003**). The increased incidence of carcinomas in patients with thyroiditis suggests thyroiditis might be a precancerous condition. Most thyroiditis-associated carcinomas are papillary; however, also follicular, anaplastic, medullary and squamous carcinomas have been reported in cases of thyroiditis. Thyroid follicular cells may have chromosomal defects, such as the RET/PTC rearrangement, which is the hallmark of many PTCs. Several authors found RET/PTC rearrangements in non neoplastic thyroid lesions, such as HT (**Wirtschafter et al., 1997; Sheils et al., 2000; Elisei et al., 2001**). Additional evidence implicating RET/PTC in the association between thyroiditis and cancer comes from the finding that patients exposed to radiation from the Chernobyl nuclear power plant disaster often develop not only RET/PTC-induced papillary tumors but also autoimmune thyroiditis (**Williams et al 2002**). Accordingly, transgenic mice engineered to express

RET/PTC develop papillary carcinomas and chronic thyroiditis (**Powell et al. 1998**). Finally, Wirtschafter and colleagues detected RET/PTC expression in about 90% of the cases of Hashimoto's thyroiditis they analyzed (**Wirtschafter et al., 1997**). However, these data partially contrast with other reports. Nikiforova and colleagues detected RET/PTC rearrangements only in PTCs not associated with Hashimoto's disease (**Nikiforova et al., 2002**). Rhoden et al. detected only a few follicular cells expressing very low levels of RET/PTC in Hashimoto's thyroiditis, which suggests that RET/PTC expression does not necessarily predict the development of PTC in patients with thyroiditis (**Rhoden et al., 2006**).

Two models can be hypothesized to explain the association between Hashimoto's thyroiditis and the RET/PTC rearrangement. The first suggests that inflammation might facilitate the rearrangement. According to this hypothesis, production of free radicals, cytokine secretion, cellular proliferation and other phenomena correlated with inflammation might predispose to the rearrangement of RET/PTC in follicular cells. In support of this hypothesis, it has been observed that the mutational rate in inflamed tissues is much higher than in normal tissues (**Colotta et al., 2009**). It is also possible that the inflammatory microenvironment supports thyrocyte survival in stress conditions. It is generally assumed that normal human epithelial cells do not tolerate oncogene expression because excessive growth signals induce DNA replication stress, which, in turn, induces oncogene-mediated senescence or apoptosis (**Lowe and Sherr, 2003; Halazonetis et al., 2008; Evan and D'adda di Fagagna, 2009**). Thus, evasion from apoptosis is required for neoplastic transformation and can occur through additional genetic lesions that lead to activation of anti-apoptotic pathways, such as those mediated by phosphatidylinositol 3-kinase. This concept is supported by the finding that the ectopic expression of the RET/PTC oncogene in a continuous rat thyroid cell line (PC Cl3) induces apoptosis (**Castellone et al., 2003; Wang et al., 2003**). This effect might be due to the strong RET-mediated mitogenic stimuli. It is possible that cytokines and chemokines released by the inflammatory tumoral stroma sustain the survival of those thyroid cells in which RET/PTC rearrangements randomly occur, thereby allowing the selection of clones that

acquire additional genetic lesions and thus become resistant to oncogene-induced apoptosis.

It has recently been suggested that tissue stem cells are the targets of malignant transformation. In the setting of chronic inflammation, the pool of resident or bone marrow-derived stem cells is thought to expand (Li et al., 2006). If this applies also to thyroid cancer, AITD-mediated inflammation could cause the expansion of stem cells in which a RET/PTC rearrangement has occurred.

An alternative hypothesis, which does not exclude the previous hypothesis, is that the RET/PTC rearrangement itself, and the consequent activation of the downstream signaling pathways can induce thyroid inflammation. Accordingly, RET/PTC induces the synthesis of many inflammatory proteins in epithelial thyroid cells (Table 1) and a severe inflammatory response is observed in TG-RET/PTC transgenic mice in which RET/PTC expression is confined to the thyroid gland (Powell et al., 2003; Melillo et al., 2005; Puxeddu et al., 2005; Pufnock and Rothstein, 2009). Indeed, RET/PTC transgenic mice develop PTCs and chronic thyroiditis. However, RET/PTC itself is not sufficient to induce complete Hashimoto's thyroiditis in these mice since this disease is characterized not only by lymphocytic infiltration, but also by a humoral autoimmune reaction that results in the production of autoantibodies against thyroid antigens, and by the formation of lymphoid follicles in the thyroid parenchyma. These features are not present in TG-RET/PTC transgenic animals. In conclusion, we favor the hypothesis that AITD creates a protumorigenic microenvironment in which the RET/PTC rearrangement is tolerated. The rearrangement itself then contributes to maintaining the inflammatory reaction.

Thyroid cancer and immune-inflammatory infiltrate

There are various reports of immune-inflammatory cell infiltrates in thyroid cancer. Not only are PTCs frequent in the context of Hashimoto's thyroiditis, but these carcinomas are often present with a remarkable lymphocytic infiltrate in the absence of the typical signs of autoimmune thyroiditis, such as autoantibody production and tertiary follicle formation. The prevalence of lymphocytic

infiltrate is generally significantly higher in patients with PTC than in patients with benign thyroid lesions (**Okayasu et al., 1997**), which indicates that these cells might favor cancer development. However, the presence of chronic lymphocytic thyroiditis in patients with PTC correlates with an improved prognosis (**Kebebew et al., 2001**). Moreover, thyroid carcinomas with a poor prognosis, such as PDTCs and ATCs, are characterized by a strongly reduced lymphocyte cell infiltrate compared with PTCs, thus suggesting that these cells may play a protective role in thyroid cancer (**Ugolini et al., 2007**). Macrophages and dendritic cells, mainly characterized by an immature phenotype, have been identified in human PTCs (**Scarpino et al., 2000**). Again, PDTCs and ATCs are characterized by a greatly reduced dendritic cell infiltrate with respect to PTCs, thus suggesting that these cell types also may play a protective role in thyroid cancer (**Ugolini et al., 2007**). Tumor-associated macrophages are generally considered protumorigenic (**Sica et al., 2008**). Ryder and colleagues found that the density of the TAM infiltrate was higher in PDTCs and ATCs than in PTCs and FTCs. Moreover, increased TAM infiltration in PDTCs was found to be positively correlated with capsule invasion, extrathyroidal extension and poor prognosis (**Ryder et al., 2008**). Taken together, these data support the concept that TAM may favor the malignant progression of thyroid cancer.

We compared the density of tryptase-positive mast cells in 96 PTCs versus normal thyroid tissue from 14 healthy individuals. Mast cell density was higher in PTCs than in control tissue. Mast cell infiltrate correlated with capsule invasion. We also observed that mast cells promoted proliferation, survival and the invasive ability of thyroid cancer cells, thereby contributing to thyroid carcinoma growth and invasiveness (**Melillo et al., unpublished observations**).

The presence of inflammatory cells has been evaluated in transgenic mice expressing RET/PTC3 in the thyroid gland by means of a tissue-specific promoter. In this system, mice develop PTC-like lesions, characterized by a leukocytic infiltrate, mainly constituted by macrophages (**Russell et al., 2004**). This effect seemed to be RET-specific because a similar oncogene, TRK-T1, which also derives from a rearrangement of a tyrosine kinase receptor with a

heterologous gene, did not have the same effect. Interestingly, the tumor incidence and burden in RET/PTC3 mice were influenced by the genetic background of the animals. In fact, tumors were significantly larger in C57BL/6 mice expressing RET/PTC3 than in C3H/HeJ animals. Cytokine expression was much higher in large tumors, suggesting that these molecules play a role in tumor growth. This effect could be due to the different polarization of TCD4⁺ cells and consequently to the distinct cytokine environment mounted by the C57BL/6 mice with respect to the C3H/HeJ animals. Taken together, these data demonstrate that the RET/PTC3 oncoprotein can induce recruitment of immune cells into tumor sites, and that cytokines produced in a tumor site play an important role in tumor progression.

In another set of experiments, Pufnock and Rothstein investigated whether RET/PTC3 is involved in the recruitment of other immune cell populations into tumor sites (**Pufnock and Rothstein, 2009**). In that study, RET/PTC3 and its mutant isoform (RET/PTC3 Y1062F), which is defective in the activation of the most relevant RET-mediated signaling pathways, were transduced into a mouse fibrosarcoma cell line. Tumors were induced by injecting RET/PTC3- and RET/PTC3 Y1062F-expressing cells in syngeneic mice. RET/PTC3 tumors were significantly larger than RET/PTC3 mutant tumors. CD4⁺ and CD8⁺ T cell infiltrate density was comparable in both tumor groups. However, RET/PTC3, but not RET/PTC3 Y1062F tumors, displayed a remarkable leukocytic infiltrate characterized by CD11b⁺, Gr1⁺ myeloid cells, previously described as innate suppressive inflammatory cells (**Marigo et al., 2008; Gabrilovich et al., 2009**). These data suggest that RET/PTC3-positive thyroid cancer, like other cancer types, can induce the recruitment of CD11b⁺, Gr1⁺ cells. These cells can mediate tumor escape from the immune response; this phenomenon is dependent on the integrity of the Y1062 residue of RET. Whether other mechanisms of immune evasion also operate in thyroid cancer is unknown. Interestingly, the BRAF-MAPK signaling pathway has been shown to induce the synthesis and secretion of immunosuppressive cytokines in melanomas (**Sumimoto et al., 2006**). These factors include IL-10, VEGF and IL6, which are cytokines that are produced also by thyroid cancer cells (**Stassi et al., 2003; Todaro et**

al., 2006; Russell et al., 2004; Shinohara et al., 2004; Belletti et al., 1999; Jo et al., 2006; Puxeddu et al., 2005).

Oncoprotein signaling in thyroid cancer and inflammation

The activation of RET in PTC derives from a genetic rearrangement between the RET tyrosine-kinase domain and heterologous genes. The consequence of these rearrangements is the constitutive activation of RET due to constitutive dimerization-oligomerization of the oncoprotein induced by the different RET-fused genes. In MEN2A and FMTC, RET activation is achieved by constitutive dimerization of RET through the replacement of an extracellular cysteine and the formation of disulfide bonds (Santoro et al., 2004). Differently, mutations that affect the intracellular domain induce kinase activation in the absence of dimerization, presumably through a modification of the kinase structure. For instance, the MEN2B M918T point mutation induces a change in substrate specificity with respect to MEN2A mutants, which is reflected in the different capability of MEN2B mutants to phosphorylate endogenous tyrosines and signaling adaptors. These differences can account, at least in part, for the phenotypic differences between the two syndromes. Indeed, although both MEN2A and MEN2B are characterized by the presence of pheochromocytomas and MTCs, MEN2B MTCs occur earlier and are more aggressive. Furthermore, MEN2B patients also present skeletal abnormalities and ganglioneuromas (Hansford et al., 2000).

Both point mutations and genetic rearrangements cause constitutive activation of the tyrosine kinase activity of RET in the absence of ligands. Several studies conducted in different laboratories have shown that activation of RET, either by physiological ligands or by oncogenic conversion, results in the phosphorylation of intracellular tyrosine residues that serve as docking sites for the recruitment of signaling adaptors. Among the tyrosines phosphorylated after RET activation, Y1062 has been shown to be important for RET-mediated biological activity in thyroid cells. Y1062 is a multi-docking site, being able to bind several PTB-containing adaptors, namely, ShcA, FRS2, IRS1,

ShcC and dok. Substitution of the Y1062 residue of Ret with a phenylalanine, both in the context of a wild-type and of an oncogenically activated receptor, abrogates Ret-mediated signal transduction and biological activities (**Takahashi, 2001**). We and others have shown that Ret can activate the MAPK pathway in a Y1062-RAS- and BRAF-dependent manner (**Kimura et al., 2003; Melillo et al., 2005**).

Several groups demonstrated that RET-induced transcriptional activity depends almost entirely on the integrity of the Y1062 residue and on activation of the RAS/BRAF/MAPK pathway. Russel and colleagues reported that the expression of the RET/PTC3 isoform in a rat thyroid cell line (PC Cl3) induced an increase in NFkB DNA-binding activity and a consequent increase in proinflammatory cytokine secretion (**Russell JP et al., 2003**). CXCL1/ Gro α , CCL2/mcp-1 and GM-CSF were up-regulated upon RET/PTC3 expression, and this increase depended on the integrity of residue 1062 of RET.

GDNF stimulation of the neuroectodermal tumor cell line SK-N-MC, ectopically expressing the human wild-type RET, induced the production of high levels of IL8. This cytokine has also been found in the TT human medullary thyroid carcinoma cell line carrying the RET/MEN2A oncogene and in the TPC1 human PTC cell line (**Iwahashi et al, 2002**). IL8 is a pro-inflammatory, mitogenic and proangiogenic chemokine that contributes to several human cancers (**Waug and Wilson, 2008**). Russel and colleagues found that RET/PTC3 caused the expression of the proinflammatory proteins IL-1 α , IL1 β , IL6 and IL24 in thyroid cancer cells (**Russell et al., 2004; Shinohara et al., 2004**). Similar results were obtained by Puxeddu and colleagues, who demonstrated that prostaglandin E2 (PGE2), microsomal prostaglandin E synthase1 (mPGES1), cyclooxygenase2 (COX2) and several other genes involved in immune response and inflammation were induced by RET/PTC3 in PC Cl3 cells (**Puxeddu et al., 2003; Puxeddu et al., 2005**).

To study the transcriptional changes associated with thyroid transformation, we used an oligonucleotide-based DNA microarray (Affymetrix) on PC Cl3 cells engineered to stably express wild-type and mutant isoforms of the oncogenic RET/PTC3 protein, or the HRAS (V12) or

BRAF(V600E) oncoproteins, respectively. When transfected cells were compared with the parental cells, it was evident that RET/PTC3 induced a complex pattern of gene expression that depended entirely on the integrity of the Y1062 residue. The HRAS (V12) and BRAF(V600E) oncoproteins each modified the expression of several genes, and 50% of these genes were common between the three oncoproteins. Furthermore, by using siRNA and pharmacological inhibitors, we showed that most of these gene changes depended on the ERK pathway (**Melillo et al., 2005**). Our gene expression profiling studies also revealed the induction of cytokine and chemokines and of their respective receptors in PC RET/PTC3 cells (**Fig. 1**). Indeed, we found that RET/PTC3 cells expressed high levels of osteopontin (OPN), VEGFA, CCL2, CXCL1 and CXCL10 (**Melillo et al., 2005**). Interestingly, CD44, CXCR2 and CXCR3, the receptors for OPN, CXCL1 and CXCL10, respectively, were also expressed by transformed cells. We also found the expression of CXCR4, the chemokine receptor for the chemokine CXCL12, in transformed cells with respect to the normal counterparts (**Castellone et al., 2004b**). These results were confirmed by Borrello and coworkers in human primary thyroid cells transduced with the RET/PTC oncogene (**Borrello et al., 2005**).

The proinflammatory properties of RET/PTC in the thyroid might have a dual effect: on one hand, molecules such as OPN, CXCL1, CXCL10, CCL2 and GM-CSF can influence the immune response to the tumor by recruiting and functionally regulating immune cells. For instance, transplantation of RET/PTC3-expressing thyrocytes into mice *in vivo* induced intense macrophage infiltrate and neovascularization followed by cell death (**Russell et al., 2003**). On the other hand, secreted cytokines and chemokines, such as OPN, CXCL1, CXCL10 and IL24, can act as autocrine growth and survival factors for thyroid tumor cells that express the cognate receptors on their plasma membrane (**Shinohara et al., 2004; Castellone et al., 2004a; Melillo et al., 2005**). These data are corroborated by studies conducted on human thyroid tumors. Indeed, it has been shown that human thyroid samples express most inflammatory genes (CXCR4, CD44, OPN, CXCL1, CXCL10 and SDF-1) (**Castellone et al., 2004; Guarino et al., 2005; De Falco et al., 2007; Melillo et al., 2005; Borrello et al., 2005**).

Other studies support the concept that thyroid cancer epithelial cells can produce inflammatory factors, and that these inflammatory factors can sustain the resistance of thyroid cancer cells to various apoptotic stimuli. Stassi and colleagues looked for cytokines involved in the resistance of thyroid cancer cells to chemotherapeutic agents and identified IL4 and IL10. These cytokines are typically secreted by T CD4⁺ cells polarized toward a T_H2 phenotype. T_H2 cytokines induce humoral immunity and promote thyrocyte survival in AITD by up-regulating levels of anti-apoptotic proteins such as Bcl2 and Bcl-XL (Stassi et al., 2003). Moreover, the authors demonstrated that IL4 and IL10 also induce resistance of thyroid cancer cells to FAS/FASL-mediated apoptosis. This effect is mediated by IL4 and IL10-induction of two anti-apoptotic proteins, namely cFLIP and PED/PEA15 (Todaro et al., 2006).

Conclusions

The relationship between inflammation and thyroid cancer is complex and still not completely understood. Like other cancer types, thyroid cancer is influenced by and modulates inflammation. Epidemiological and histological data indicate that thyroid cancer frequently occurs in the context of one of the most common AITDs, Hashimoto's thyroiditis, and that thyroid cancer is frequently infiltrated by inflammatory-immune cells.

The role of these cells is complex, and several studies indicate that, depending on the specific cell population, the effect can be either pro- or anti-tumorigenic. Specifically, the presence of lymphocytes, which belong to the adaptive branch of immunity, is significantly higher in neoplastic than in non neoplastic lesions. However, lymphocytic infiltration seems to confer protection against cancer progression. On the other hand, the presence of cells belonging to innate immunity, such as macrophages and mast cells, enhances tumor progression and is associated with an unfavorable prognosis. By activating the MAPK cascade, the oncogenes activated in thyroid carcinomas (i.e., RET/PTC, RAS and BRAF) can activate a cell-autonomous pro-inflammatory transcriptional

program that involves mainly cytokines, chemokines and their receptors (**Table 1**). These molecules exert two main effects in thyroid cancer. First, by acting in an autocrine fashion, they sustain most of the malignant phenotypic features of thyroid cancer cells namely, proliferation, survival and invasiveness. Second, by acting in a paracrine and, possibly, in an endocrine manner, they induce remodeling of the tumoral stroma by recruiting inflammatory, immune, endothelial and bone marrow-derived cells. In this way, cytokines can further enhance tumor progression through the release of mediators from infiltrating cells, by stimulating angiogenesis, and by inducing subversion of the anti-tumoral immune response. Based on these observations, we favor the concept that, at least in the full blown phase, thyroid cancer growth and progression are positively influenced by two major inflammatory components, one dependent on the cells that are present in the cancer stroma, the other dependent on activation, in epithelial cancer cells, of specific oncoprotein-mediated signaling. As a consequence, not only oncoproteins, but also inflammatory molecules are promising targets for novel thyroid cancer therapeutical strategies. In support of this, we have shown that the blockade of CXCR4 chemokine receptor inhibits the growth of ATC cell xenografts in immunodeficient mice (**De Falco et al., 2007**).

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Legends

Table 1. The most relevant inflammatory molecules in thyroid cancer cells

The official symbol and full name, Gene ID, and known function for each molecule are listed. References regarding their role in thyroid cancer are also reported.

Figure 1

Cytokines and chemokines secreted by RET/PTC-transformed PC CL3 rat thyroid cells: main biological effects

Transformation of PC CL3 rat thyroid cells through activation of the RET/PTC-RAS-BRAF-MAPK signaling pathway induces a proinflammatory program that includes cytokines, chemokines and their receptors. Selected mediators and their biological effects are indicated.

Table 1. Inflammatory molecules in thyroid cancer cells

Inflammatory molecules	Official symbol	Official full name	Gene ID	Function	References
M-CSF	CSF1	colony stimulating factor 1 (macrophage)	1435	Cytokine that controls the production, differentiation, and function of macrophages.	Borrello et al., 2005
G-CSF	CSF3	colony stimulating factor 3 (granulocyte)	1440	Cytokine that controls the production, differentiation, and function of granulocytes.	Borrello et al., 2005
GM-CSF	CSF2	colony stimulating factor 2 (granulocyte-macrophage)	1437	Cytokine that controls the production, differentiation, and function of granulocytes and macrophages.	Russell et al., 2003, 2004; Borrello et al., 2005
IL-1 α	IL1A	interleukin 1, alpha	3552	Member of the interleukin 1 cytokine family. Pleiotropic cytokine involved in various immune responses, inflammatory processes, and hematopoiesis. It is mainly produced by monocytes and macrophages.	Russell et al., 2003, 2004
IL-1 β	IL1B	interleukin 1, beta	3553	Member of the interleukin 1 cytokine family. It is produced by activated macrophages as a proprotein, which is proteolytically processed to its active form by caspase 1 (CASP1/ICE). This cytokine is an important mediator of the inflammatory response.	Russell et al., 2003, 2004; Borrello et al., 2005
IL-6	IL6	interleukin 6 (interferon, beta 2)	3569	Cytokine that functions in inflammation and the maturation of B cells. The protein is primarily produced at sites of acute and chronic inflammation, where it is	Russell et al., 2003, 2004; Puxeddu et al., 2005;

				secreted into the serum and induces a transcriptional inflammatory response.	
IL-8/CXCL8	IL8	interleukin 8	3576	Member of the CXC chemokine family. It is one of the major mediators of the inflammatory response. It is secreted by several cell types, functions as a chemoattractant, and is also a potent angiogenic factor.	Iwahashi et al., 2002; Borrello et al., 2005
IL-4	IL4	interleukin 4	3565	Pleiotropic cytokine produced by activated T cells. This cytokine is a ligand for interleukin 4 receptor.	Stassi et al., 2003; Todaro et al., 2006
IL-10	IL10	interleukin 10	3586	Cytokine produced primarily by monocytes and to a lesser extent by lymphocytes. It has pleiotropic effects in immunoregulation and inflammation. It down-regulates the expression of Th1 cytokines, and enhances B cell survival, proliferation, and antibody production.	Stassi et al., 2003; Todaro et al., 2006
IL-24	IL24	interleukin 24	11009	Member of the IL10 family of cytokines. It can induce apoptosis selectively in various cancer cells.	Shinohara et al., 2004
VEGFA	VEGFA	vascular endothelial growth factor A	7422	Member of the PDGF/VEGF growth factor family, acts on endothelial cells and mediates increased vascular permeability, induces angiogenesis, vasculogenesis and endothelial cell growth, promotes cell migration, and inhibits apoptosis.	Belletti et al., 1999
OPN/IL28/SPP1	SPP1	secreted phosphoprotein 1	6696	Cytokine secreted by immune and non immune cells. Involved in cell	Castellone et al., 2004a; Borrello et al., 2005

				migration and adhesion, bone metabolism and immune regulation of TH1 cells. Overexpressed in several cancer types.	
TNF α	TNF	tumor necrosis factor (TNF superfamily, member 2)	7124	Proinflammatory cytokine that belongs to the tumor necrosis factor (TNF) superfamily. It is mainly secreted by macrophages and is involved in the regulation of cell proliferation, differentiation, apoptosis, lipid metabolism, and coagulation.	Russell et al., 2003, 2004
CCL2/mcp1	CCL2	chemokine (C-C motif) ligand 2	6347	Small cytokine belonging to the CC chemokine family that displays chemotactic activity for monocytes and basophils but not for neutrophils or eosinophils. Ligand for the CCR2 receptor.	Russell et al., 2003, 2004; Puxeddu et al., 2005; Melillo et al., 2005
CCL20	CCL20	chemokine (C-C motif) ligand 20	6364	Small cytokine belonging to the CC chemokine family. It binds to the CCR6 receptor and is strongly chemotactic for lymphocytes and weakly attracts neutrophils. It is implicated in the formation and function of mucosal lymphoid tissues.	Borrello et al., 2005
CXCL1/GRO α	CXCL1	chemokine (C-X-C motif) ligand 1 (melanoma growth stimulating activity, alpha)	2919	Small cytokine belonging to the CXC chemokine family. It is strongly chemotactic for neutrophils and has been shown to be a major component required for serum-dependent melanoma cell growth. Ligand for the CXCR2 receptor.	Russell et al., 2003, 2004; Melillo et al., 2005
				Small cytokine belonging to the	

CXCL10/IP-10	CXCL10	chemokine (C-X-C motif) ligand 10	3627	CXC chemokine family, ligand for the receptor CXCR3. It has pleiotropic effects, including stimulation of monocytes, natural killer and T-cell migration, and modulation of adhesion molecule expression	Puxeddu et al., 2005; Melillo et al., 2005
CXCL12/SDF1	CXCL12	chemokine (C-X-C motif) ligand 12 (stromal cell-derived factor 1)	6387	Small cytokine belonging to the CXC chemokine which activate leukocytes and is often induced by proinflammatory stimuli. Ligand for the CXCR4 and the CXCR7 receptors.	Borrello et al., 2005
CXCR2	IL8RB	interleukin 8 receptor, beta	3579	Member of the G-protein-coupled receptor family. This protein is a receptor for interleukin 8 (IL8) and for other CXC-chemokines. It mediates neutrophil migration to sites of inflammation and has angiogenic effects.	Melillo et al., 2005
CXCR3	CXCR3	chemokine (C-X-C motif) receptor 3	2833	Member of the G-protein-coupled receptor family. It displays selectivity for three chemokines, CXCL10/IP10, Mig and I-TAC. It is prominently expressed in effector/memory T cells, and in T cells present in many types of inflamed tissues.	Melillo et al., 2005
CXCR4	CXCR4	chemokine (C-X-C motif) receptor 4	7852	Member of the G-protein-coupled receptor family. CXC chemokine receptor specific for stromal cell-derived factor-1. It acts with the CD4 protein to support HIV entry into cells and is also highly expressed in cancer cells.	Castellone et al., 2004b; Borrello et al., 2005

CD44	CD44	CD44 molecule (Indian blood group)	960	Cell-surface glycoprotein involved in cell-cell interactions, cell adhesion and migration. It is a receptor for hyaluronic acid (HA), osteopontin, collagens, and matrix metalloproteinases (MMPs). It participates in a wide variety of cellular functions including lymphocyte activation, recirculation and homing, hematopoiesis, and tumor metastasis.	Castellone et al., 2004a
mPGES-1	PTGES	prostaglandin E synthase	9536	This protein is a glutathione-dependent prostaglandin E synthase. The expression of this gene has been shown to be induced by the proinflammatory cytokine interleukin 1 beta (IL1B).	Puxeddu et al., 2003
COX2	PTGS2	prostaglandin- endoperoxide synthase 2 (prostaglandin G/H synthase and cyclooxygenase)	5743	It is the key enzyme in prostaglandin biosynthesis. This gene encodes the inducible isozyme. It is regulated by specific stimulatory events, suggesting that it is responsible for the prostanoid biosynthesis involved in inflammation and mitogenesis.	Puxeddu et al., 2003

RET/PTC-transformed thyroid cell

