



**HAL**  
open science

## ROLE OF NF- $\kappa$ B IN THYROID CANCER

Francesco Pacifico, Antonio Leonardi

► **To cite this version:**

Francesco Pacifico, Antonio Leonardi. ROLE OF NF- $\kappa$ B IN THYROID CANCER. *Molecular and Cellular Endocrinology*, 2010, 321 (1), pp.29. 10.1016/j.mce.2009.10.010 . hal-00582105

**HAL Id: hal-00582105**

**<https://hal.science/hal-00582105>**

Submitted on 1 Apr 2011

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

## Accepted Manuscript

Title: ROLE OF NF- $\kappa$ B IN THYROID CANCER

Authors: Francesco Pacifico, Antonio Leonardi

PII: S0303-7207(09)00557-7  
DOI: doi:10.1016/j.mce.2009.10.010  
Reference: MCE 7356



To appear in: *Molecular and Cellular Endocrinology*

Received date: 29-6-2009  
Revised date: 21-9-2009  
Accepted date: 20-10-2009

Please cite this article as: Pacifico, F., Leonardi, A., ROLE OF NF- $\kappa$ B IN THYROID CANCER, *Molecular and Cellular Endocrinology* (2008), doi:10.1016/j.mce.2009.10.010

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

## ROLE OF NF- $\kappa$ B IN THYROID CANCER

Francesco Pacifico<sup>1</sup> & Antonio Leonardi<sup>2,3</sup>

<sup>1</sup>*Istituto di Endocrinologia e Oncologia Sperimentale, CNR, <sup>2</sup>Dipartimento di Biologia e Patologia Cellulare e Molecolare, “Federico II” University of Naples, Via Pansini, 5 - 80131 Naples, ITALY.*

<sup>3</sup>**Corresponding author.** Dipartimento di Biologia e Patologia Cellulare e Molecolare. “Federico II” University of Naples. Via Pansini 5, 80131 Naples, ITALY. Tel.:+39 081 7463048; Fax +39 081 7463308; e-mail: [leonardi@unina.it](mailto:leonardi@unina.it)

**Running title:** NF- $\kappa$ B and thyroid cancer.

**Keywords:** NF- $\kappa$ B; thyroid cancer; apoptosis; inflammation.

**Abbreviations:** NF- $\kappa$ B, Nuclear Factor-kappa B; I $\kappa$ B, Inhibitor of NF- $\kappa$ B; IKK, I $\kappa$ B kinase; TNF, Tumor necrosis factor; IAP, Inhibitor of apoptosis; c-FLIP, cellular FADD-like interleukin 1beta converting enzyme inhibitory protein; GADD, Growth arrest and DNA damage; NEMO, NF- $\kappa$ B essential modulator; TRAIL, TNF-related apoptosis-inducing ligand; PTEN, Phosphatase and tensin homologue; JNK, Jun N-terminal kinase; ROS, Reactive oxygen species; LT $\beta$ -R, Lymphotoxin beta-receptor; BAFF-R, B cell activation factor from the TNF family-receptor; NGAL, Neutrophil gelatinase-associated lipocalin; PPAR $\gamma$ , Peroxisome proliferator-activated receptor gamma; PTC papillary thyroid carcinoma; FTC follicular thyroid carcinoma; MTC medullary thyroid carcinoma; ATC anaplastic thyroid carcinoma.

**Abstract**

Thyroid cancer is the most common neoplasia of the endocrine system and accounts for approximately 1% of all newly diagnosed cancer cases. Its incidence has rapidly grown over the past few decades. Although most thyroid carcinomas are of the well-differentiated papillary histology, and respond well to treatment with surgical resection followed by radioactive iodine ablation, tumors with more aggressive phenotype, such as follicular, poorly differentiated, anaplastic, and medullary cancers, lead to almost 1,500 patient deaths annually. Therefore, understanding molecular mechanisms that regulate the biology of these carcinomas could be helpful to identify new molecules acting as novel targets for therapeutic intervention.

NF- $\kappa$ B has been recently shown to play an important role in thyroid cancer for its ability to control the proliferative and the anti-apoptotic signaling pathways of thyroid neoplastic cells. Oncogenic proteins RET/PTC, RAS and BRAF, that are involved in many aspects of thyroid carcinogenesis, can induce NF- $\kappa$ B activation in papillary, follicular, and medullary thyroid carcinomas, while constitutive de-regulated NF- $\kappa$ B activity has been found in anaplastic thyroid carcinomas. A number of NF- $\kappa$ B inhibitors have been demonstrated to induce anti-proliferative effects and/or massive apoptosis, especially in combination with radio- or chemo-therapy. The results obtained suggest that targeting NF- $\kappa$ B could be a promising strategy for advanced thyroid cancer treatment.

## Introduction

Thyroid carcinomas mainly comprise four types of tumors: papillary thyroid carcinoma (PTC), follicular thyroid carcinoma (FTC), both of which may be summarized as differentiated thyroid carcinoma, medullary thyroid carcinoma (MTC), and undifferentiated anaplastic thyroid carcinoma (ATC). PTC, FTC and ATC derive from the thyroid follicular epithelial cells, while MTC derives from the parafollicular C-cells (Sherman, 2003). PTC is the most common malignant thyroid neoplasm in countries with sufficient iodine diets, and comprises up to 80% of all thyroid malignancies. FTC is more common in regions with insufficient iodine diets and represents approximately 10-20% of all thyroid malignancies (Fagin and Mitsiades, 2008). The overall 5-10 year survival rate of patients with PTC is about 80-95%, while that of patients with FTC is about 70-95% (Gimm, 2001). The incidence of MTC is not well known because epidemiologic studies are rare. Generally, it is believed that MTC comprises about 5-10% of all thyroid malignancies (Gimm, 2001; Sherman, 2003). ATC is one of the most aggressive human malignancies, with a very poor prognosis. Although rare, accounting for up to 1-2% of clinically recognized thyroid cancers, the overall median survival is limited to months (Smallridge et al., 2009). Unfortunately, at the time of diagnosis most of ATC patients already show local and distant metastases, so that surgery, radiotherapy, and chemotherapy, based primarily on doxorubicin and cisplatin treatment, do not meaningfully improve survival of these patients. Consequently, there is a need for new diagnostic and therapeutic tools for the treatment of these tumors.

An emerging body of literature shows that NF- $\kappa$ B plays a role in thyroid cancer (Visconti et al., 1997; Ludwig et al., 2001; Russell et al., 2003; Vasudevan et al., 2004; Kato et al., 2006; Palona et al., 2006; Gombos et al., 2007; Gallel et al., 2008), especially of anaplastic type (Pacifico et al., 2004; Starenki et al., 2004a; Pacifico et al., 2007; Iannetti et al., 2008; Festa et al., 2009; Zhu et al., 2009). This is an important issue because NF- $\kappa$ B, and particularly the genes under its transcriptional regulation, could potentially become novel molecular targets in ATC therapy, given

the ability of NF- $\kappa$ B to control many aspects of thyroid cancer biology. This review will focus on these aspects, in particular on the molecular mechanisms by which NF- $\kappa$ B exerts its role in thyroid tumors.

## **NF- $\kappa$ B**

NF- $\kappa$ B is a family of transcription factors that plays a central role in the regulation of apoptosis, inflammation and immune response (Ghosh et al., 1998; Karin and Ben-Neriah, 2000; Tak et al., 2001). In mammals, the NF- $\kappa$ B family is composed of five members: RelA(p65), RelB, c-Rel, NF- $\kappa$ B1 (p50 and its precursor p100), NF- $\kappa$ B2 (p52 and its precursor p105) (Vallabhapurapu et al. 2009). These proteins form homodimers and heterodimers and their activity is regulated by two major pathways. The first pathway – known as the canonical pathway- regulates the activity of NF- $\kappa$ B dimers composed of RelA(p65), c-Rel and p50. These dimers are held in the cytoplasm in an inactive form bound to specific inhibitors known as inhibitor of  $\kappa$ B (I $\kappa$ B). Following different stimuli, such as cytokines, pathogens and pathogen-related factors, I $\kappa$ B is phosphorylated on specific serine residues, ubiquitinated and degraded through a proteasome dependent pathway. NF- $\kappa$ B dimers are then free to move to the nucleus and to transcribe target genes (Hayden et al. 2008). The second pathway affects NF- $\kappa$ B2, which preferentially dimerizes with RelB. Following triggering of some members of the TNF receptor superfamily, such as LT $\beta$ -R and BAFF-R, NF- $\kappa$ B2 is phosphorylated, ubiquitinated, and processed to form the mature form p52. RelB-p52 dimers then move to the nucleus, where they control transcription of genes involved in secondary lymphoid organ development and lymphocytes survival (Claudio et al., 2002; Kayagaki et al., 2002; Dejardin et al., 2002). The phosphorylation of the I $\kappa$ B and NF- $\kappa$ B2 is mediated by a high molecular weight complex, the I $\kappa$ B Kinase (IKK) complex. This complex is composed by two catalytic subunits, IKK $\alpha$  and IKK $\beta$ , and a regulatory subunit IKK $\gamma$ /NEMO (Ghosh et al. 2002). IKK $\alpha$  and IKK $\beta$  have distinct substrates. IKK $\beta$  directly phosphorylates I $\kappa$ B, then activating the canonical pathway, while

IKK $\alpha$  mediates the phosphorylation of NF- $\kappa$ B2 along with another protein kinase called NIK (Xiao et al., 2001; Senftleben et al., 2001). IKK $\gamma$ /NEMO does not have any enzymatic activity but its presence in the complex is absolutely necessary for a correct activation of the IKK complex, very likely because it serves to connect upstream signal mediators to the IKK (Yamaoka et al., 1998; Rothwarf et al., 1998). Once in the nucleus, NF- $\kappa$ B transcribes different genes controlling different aspects of cell physiology. Since many of these genes fall in the six categories characterizing a neoplastic cell (1. self-sufficiency in growth, 2. insensitivity to growth-inhibitory signals, 3. evasion of apoptosis, 4. limitless replicative potential, 5. sustained angiogenesis and 6. tissue invasion and metastasis) (Hanahan and Weinberg, 2000), it is not surprising that the NF- $\kappa$ B pathway is involved in cell transformation (Fig.1).

No information is available about the role played by NF- $\kappa$ B in thyroid gland development, and very little is known about the influence of NF- $\kappa$ B on the regulation of thyroid functions, as well as on the expression of thyroid specific genes, such as thyroglobulin, thyroid stimulating hormone-receptor, and thyroperoxidase. It has been reported that the murine Pax8 gene contains  $\kappa$ B sites, however no experimental evidence confirmed that these sites are functional (Okladova et al. 1997).

### **Role of NF- $\kappa$ B in thyroid cancer**

Several lines of evidence show that NF- $\kappa$ B plays a role in cancer. Its activity has been found constitutively elevated in many types of human tumors from either haematological or solid origin (Pacifico and Leonardi, 2006). The role of NF- $\kappa$ B in solid tumors has been well documented in several studies performed on primary tumors, and neoplastic cell lines derived from different human tissues. These studies show that the inhibition of constitutive NF- $\kappa$ B activity blocks the oncogenic potential of neoplastic cells by different ways: by sensitizing tumor cells to chemotherapeutic drug-induced apoptosis, by decreasing the highly proliferative rate which characterizes transformed cells, and by inhibiting tissue invasiveness and metastatic potential of

highly malignant cells (Karin et al., 2002). In addition, since it is now generally accepted that chronic inflammation contributes to the genesis of many solid tumors, such as gastric, colon and hepatic carcinomas, activation of NF- $\kappa$ B by the classical IKK $\beta$ -dependent pathway, is a crucial mediator of inflammation-induced tumor growth and progression in animal models of inflammation-associated cancer (Greten et al., 2004; Pikarsky et al., 2004).

The first evidence of NF- $\kappa$ B constitutive activation in thyroid carcinomas was reported by Visconti et al. in 1997, who described the presence of a strong de-regulated NF- $\kappa$ B activity in the nuclei of different thyroid cancer-derived cell lines. In this work the authors pointed out the requirement of NF- $\kappa$ B for the expression of neoplastic phenotype of thyroid carcinoma cell lines. Successively, Ludwig and co-workers demonstrated that NF- $\kappa$ B was strongly activated in tissue specimens from parafollicular C-cell carcinomas, and that RET oncogene needs NF- $\kappa$ B to exert its transforming activity in the MTC-derived TT cell line. Since then, a number of papers reported that different signaling pathways leading to the neoplastic transformation of thyroid cells, merge into NF- $\kappa$ B activation: the anti-apoptotic behaviour and invasiveness of thyroid cancer cells transformed by oncogenic BRAF is mediated by NF- $\kappa$ B activation (Palona et al., 2006); blocking PPAR $\gamma$  tumor suppressor promotes follicular thyroid carcinogenesis *via* NF- $\kappa$ B (Kato et al., 2006); constitutive activation of the PI3K/Akt pathway due to PTEN inactivation, accelerates thyroid cancer progression following increase of NF- $\kappa$ B activity (Guigon et al., 2009). It has also been reported that FOXO3 activity is negatively regulated by the PI3K/Akt pathway, suggesting that FOXO3 inactivation might cooperates with NF- $\kappa$ B in controlling survival of thyroid cancer cells (Guigon et al. 2009, Karger et al. 2009).

In addition to cell lines, additional studies have shown that primary human thyroid carcinomas exhibit high basal activity of NF- $\kappa$ B, especially in tissue specimens from ATC (Fig.2) (Pacifico et al., 2004; Mitsiades et al., 2006). The increased NF- $\kappa$ B activity detected in both cell lines and primary tumors, is not a simple epiphenomenon of the neoplastic transformation, but



rather one of the causes that contributes to the establishment of the neoplasia. In fact, inhibition of NF- $\kappa$ B by ectopic expression of a superrepressor form of I $\kappa$ B $\alpha$  in the ATC-derived cell line FRO, determines an increased susceptibility to chemotherapeutic drug-induced apoptosis, and the block of the oncogenic activity (Pacifico et al., 2004). The specific antisense oligonucleotides against RelA reduces the growth rate of different thyroid cell lines (Visconti et al., 1997 ). Several NF- $\kappa$ B inhibitory reagents have been demonstrated to promote thyroid cell death alone, or in combination with chemotherapy or radiation exposure (Starenky et al. 2004a; Starenky et al. 2004b; Mitsiades et al. 2006). These works indicate that NF- $\kappa$ B plays a central role in thyroid cancer being important to maintain the transformed phenotype, and to confer resistance to apoptosis. Much of the studies present in the literature, point to an exclusive role of the canonical pathway in thyroid tumorigenesis. However, constitutive processing of p100 is associated with the development of various lymphomas/leukemia, and with some solid cancer such as breast cancer and pancreatic cancer, then the possibility that the alternative pathway may also be involved in thyroid cancer needs to be explored (Cogswell et al. 2000, Chandler et al. 2004, Baud and Karin 2008).

### **NF- $\kappa$ B and apoptosis in thyroid cancer**

Cancer cells have the feature to circumvent apoptosis by up-regulating and/or down-regulating a number of genes involved in the control of programmed cell death. This property allows neoplastic cells to survive beyond their normal lifespan, providing protection against hypoxia as tumor mass expands, promoting angiogenesis and invasiveness during tumor progression, and allowing tumor cells to become resistant to radio- and chemo-therapy. Thus, deregulated apoptosis is a fundamental aspect of the biology of cancer.

The inhibition of NF- $\kappa$ B activity in mouse xenograft models of chemo-resistant tumors, provokes tumor regression by sensitizing them to chemo-therapeutic drug treatments (Greten et al., 2004). Consistent with this work, NF- $\kappa$ B inhibition in many human tumor-derived cell lines,

including malignant Reed-Sternberg (H-RS) cells of Hodgkin's disease, colon carcinoma cells, lung adenocarcinoma cells, Ewing sarcoma cells, prostate carcinoma cells, thyroid carcinoma cells, and many others, induces spontaneous apoptosis, or/and sensitizes them to killing by TNF $\alpha$ , TRAIL or anti-cancer drugs (Pacifico and Leonardi, 2006). Mechanisms for NF- $\kappa$ B-mediated protection from apoptosis are essentially based on its ability to activate the transcription of genes capable of suppressing cell death (Kucharczak et al., 2003). Genes controlled by NF- $\kappa$ B capable of controlling apoptosis may be classified in two major groups. 1) genes interfering with caspase activation and cytochrome c release, such as members of the mammalian Bcl-2 gene family, Bcl-xL and Bfl-1/A1, (Boise et al., 1993; Wang et al., 1999), and cellular inhibitors of apoptosis c-IAP1, c-IAP2, TRAF1 and TRAF2, the zinc-finger protein A20, c-FLIP, all of them able to suppress cell death induced by TNF $\alpha$ , death receptors or anti-cancer drugs (Deveraux et al., 1998; Yeh et al., 2000; Wang et al., 1998). 2) Genes controlling JNK activity. Belongs to this group the anti-apoptotic genes XIAP, and GADD45 $\beta$ , both of them implicated in the suppression of the prolonged, pro-apoptotic JNK activity (Tang et al., 2001; De Smaele et al., 2001). In addition to XIAP and GADD45 $\beta$  a similar mechanism of action is also displayed by Ferritin Heavy Chain (FHC), the principal site of storage of intracellular iron. FHC, by controlling free iron concentration inside the cell, also controls ROS production, responsible, in turn, to mediate sustained activation of the proapoptotic JNK pathway (Pham et al., 2004). All these anti-apoptotic proteins work in a coordinated manner to interfere with apoptosis at multiple steps along the apoptotic signalling cascade (Fig. 3).

The same antiapoptotic mechanisms also work in thyroid cancer cells, and contribute to the maintenance of the transformed phenotype. Numerous studies reported increased expression of the pro-survival members of the Bcl-2 gene family in both primary cancer and cell lines (Saltman et al., 2006; Smallridge et al., 2009). Indeed, Bcl-2 overexpression in thyroid carcinoma cell lines confers protection against several anticancer agents, and the use of small peptide that inhibits its antiapoptotic activity sensitizes thyroid cancer cell lines to sub-lethal concentration of

chemotherapeutic agents (Mitsiades et al. 2007). BRAF promotes its oncogenic activity in thyroid cancer cells through the NF- $\kappa$ B-mediated induction of c-IAP1, c-IAP2 and XIAP (Palona et al., 2006). These NF- $\kappa$ B regulated proteins are also highly expressed in ATC cell lines (Tirrò et al. 2006). Inactivation of PPAR $\gamma$  or PTEN proteins in FTC leads to the repression of critical genes involved in the control of apoptosis following NF- $\kappa$ B activation (Kato et al., 2006; Guigon et al., 2009).

Also in thyroid cells, the duration of JNK activity seems to be important to determine the susceptibility of thyroid cancer cells to apoptosis. Sustained JNK activation after exposure to UV increases apoptosis, while transient activation, for example the one induced by growth factors, does not (Shklyayev et al., 2001; Sautin et al., 2000). Inhibition of NF- $\kappa$ B, by stable transfection of a superrepressor form of I $\kappa$ B $\alpha$ , or by the use of the pharmacological inhibitor DHMED, increases JNK activity, rendering FRO cells susceptible to apoptosis. Administration of a specific JNK inhibitor blocks the apoptotic response (Pacifico et al., 2004; Starenki et al., 2004b). This effect seems to be mediated, at least partially, by the up-regulation of GADD45 $\beta$ , whose level paralleled the level of NF- $\kappa$ B. There is no evidence, so far, that also FHC is mediating the antiapoptotic activity of NF- $\kappa$ B in thyroid cancer. However, the recent finding that iron uptake mediated by NGAL, is important for survival of ATC cells, suggests that intracellular iron and ROS levels are important to control thyroid cancer cell apoptosis (Iannetti et al., 2008).

### **NF- $\kappa$ B links inflammation and cancer.**

Inflammation is central to control our fight against pathogens, and to regulate wound healing. These are very intricate biological responses that involve complex interactions among different cell types that regulate the expression of biological mediators promoting cell chemotaxis, cell migration, and cell proliferation. If inflammation is not ordered and timely, the resulting chronic inflammation can contribute to a variety of diseases including cancer. Several lines of

evidence, based on epidemiological and molecular studies, indicate that inflammation and cancer are closely linked: inflammatory diseases increase the risk of developing many types of cancer, including thyroid cancer; non-steroidal anti-inflammatory drugs reduce the risk of developing certain types of tumors, such as colon cancer; signaling pathways involved in inflammation act downstream to oncogenes (RAS, MYC, RET); a variety of inflammatory cells, chemokines, and cytokines are present in the microenvironment of tumors; targeting chemokines and cytokines, such as TNF $\alpha$  or IL-1 $\beta$ , as well as key transcription factors, such as NF- $\kappa$ B or STAT3, decreases the spread of cancer; transfer of inflammatory cells or over-expression of chemokines and cytokines promotes tumor development (Mantovani et al., 2008).

The interplay between epithelial and inflammatory cells is thought to be crucial for the genesis and the establishment of carcinomas. One of the main actors in the inflammatory process is NF- $\kappa$ B which, by regulating the expression and the function of different cytokines and chemokines in inflammatory cells, stimulates the growth and its own activity in epithelial cells. Thus, NF- $\kappa$ B establishes a network that, after prolonged time, can lead epithelial cells to undergo malignant transformation. Known examples are the *Helicobacter pylori*-induced gastric cancer (Keates et al., 1997), the colitis-associated cancer (Greten et al., 2004), and the cholangitis-derived hepatocarcinoma (Pikarsky et al., 2004). The data obtained suggest that the NF- $\kappa$ B pathway has multiple actions in tumor promotion, by preventing apoptosis of cells with malignant potential, and by stimulating the production of pro-inflammatory cytokines in the inflammatory cells infiltrating the tumor mass (Fig. 4). This model is consistent with the observed correlation between the number of inflammatory cells, level of cytokines, and tumor aggressiveness and prognosis in both human and mouse. However, the transformed cells themselves can contribute to the overall level of secreted pro-inflammatory cytokines, and to maintain the NF- $\kappa$ B pathway activated. In fact, it is possible to keep malignant cells in culture without the support of the innate immunity, and still detect constitutive activation of NF- $\kappa$ B.

A possible link between inflammation and cancer has been postulated in thyroid, too (Arif et al., 2002; Memon et al., 2004; Larson et al., 2007). In their work, Arif et al. showed an overlap in the morphological features, immunohistochemical staining pattern and, most importantly, molecular profile between papillary thyroid carcinoma and Hashimoto's thyroiditis (HT). Indeed, Memon and co-workers reported that women who had experienced episodes of postpartum thyroiditis, even with no previous thyroid disease, or X-ray exposure to the neck, were at a significantly increased risk of developing thyroid cancer. In a study conducted by Larson et al., patients with HT were three times more likely to have thyroid cancer, suggesting a strong link between chronic inflammation and cancer development. Interestingly, two studies have shown RET activation in a significant number of HT cases (Sheils et al., 2000; Sheils et al., 2002), whereas another study detected no RET rearrangements in HT (Nikiforova et al., 2002). Another group describes RET rearrangements in non-malignant thyroid tissue associated to lymphocytic thyroiditis (LT), whereas it was absent in the pure LT areas (Mechler et al., 2001). A very recent study shows that sixty-eight percent (15 of 22) of HT were positive by FISH analysis (Rhoden et al., 2006). The hypothetical association among RET, HT and thyroid cancer led to the suggestion of a possible pro-inflammatory role of RET in thyroid cells, that could predispose them to neoplastic transformation. This hypothesis is substantiated by the evidence that exogenous over-expression of oncogenic RET in primary human thyrocytes and rat thyroid cell lines, induces the expression of a distinct set of genes involved in inflammation and tumor invasion (Russell et al., 2003; Castellone et al. 2004; Borrello et al., 2005; Melillo et al. 2005). In addition, oncogenic RET enhances infiltration of lymphocytes and myeloid-derived cells in a transplantable tumor system and strongly stimulates tumor growth in immunocompetent mice (Pufnock and Rothstein, 2009).

During thyroiditis, in particular HT, thyroid gland parenchyma is progressively lost and replaced by cells of the inflammatory infiltrate that produce chemokines, cytokines, and growth factors, the majority of which are under NF- $\kappa$ B transcriptional control. The persistent stimulation of residual thyrocytes with such molecules could induce the activation of NF- $\kappa$ B in follicular cells,

thereby creating a functional network between epithelial thyroid cells and inflammatory cells. The microenvironment established by this interplay could strongly promote the onset of thyroid cancer, and could account for the progression of tumor growth. The microenvironment of primary tumor and lymph-node metastasis of patients diagnosed for PTC expresses the same inflammatory gene pattern produced by thyrocytes transfected with oncogenic RET (Castellone et al. 2004; Borrello et al., 2005; Melillo et al. 2005). Since oncogenic RET activates NF- $\kappa$ B, and the majority of pro-inflammatory molecules are under NF- $\kappa$ B transcriptional control, it is reasonable that NF- $\kappa$ B could be involved in the regulation of pro-inflammatory program of thyroid cells, and this event contributes to the onset of thyroid cancer. Interestingly, De Falco and co-workers found that ATC samples from human specimens, as well as ATC-derived cell lines, strongly express CXCR4, the chemokine receptor of stromal cell-derived factor (SDF)-1 (De Falco et al., 2007). Treatment of ATC cells with SDF-1 increases of the proliferative rate of cancer cells. This effect was blocked by a specific CXCR4 antagonist, and by CXCR4 RNA interference. Moreover, the CXCR4 antagonist effectively reduced tumor growth in nude mice inoculated with different ATC cells (De Falco et al., 2007). These results indicate that ATC cells promote their growth through the up-regulation of CXCR4/SDF-1 circuit in the tumor microenvironment. Also in this case, NF- $\kappa$ B could play a determinant role because it is able to regulate CXCR4 transcription (Helbig et al., 2003) and, therefore, it could drive this paracrine mechanism, determining the increase of cell proliferation and, as a consequence, of tumor growth. Accordingly to this paracrine model, we recently demonstrate that the strong constitutive NF- $\kappa$ B activity in ATC cells leads to the up-regulation of NGAL, a protein involved in inflammatory response, which is abundantly secreted in the extracellular milieu (Iannetti et al., 2008). The uptake of NGAL from culture medium allows the ATC cells to survive to serum withdrawal-induced apoptosis through an iron-dependent mechanism, thus enhancing tumor growth (Iannetti et al., 2008). In addition, these data also support the idea that the transformed cells themselves contribute to the overall level of secreted cytokines, chemokines and survival factors, and to maintain the NF- $\kappa$ B pathway constitutively activated.

### **NF- $\kappa$ B as therapeutic target in thyroid cancer.**

While differentiated PTC and FTC have generally a good prognosis after surgery and radio-iodine therapy, poorly differentiated MTC and undifferentiated ATC do not respond to neither radio-iodine treatment, nor conventional chemo-and radio-therapy. These tumors are obvious candidates for alternative therapeutic approaches, such as molecularly targeted therapy. The finding that NF- $\kappa$ B is one of the most important tumor-promoting machinery, prompted the search for drugs able to suppress NF- $\kappa$ B activity in order to improve chemo- and radio-therapy. It is possible to interfere with NF- $\kappa$ B activation at different levels, targeting components of its signalling cascade, such as the IKK complex, the I $\kappa$ B $\alpha$  inhibitory protein, the proteasome, the RelA subunit of the transcriptionally active heterodimers. An increasing number of compounds, able to block NF- $\kappa$ B have been tested, and have shown to suppress the growth of those cancer cells whose tumorigenicity depends on NF- $\kappa$ B activity (Karin et al 2002; Baud and Karin 2009). Some of these compounds have also been tested in ATC cells. The cell permeable peptide SN50, which blocks RelA nuclear translocation, has been found to promote cell death *in vitro* in combination with ionizing radiation (Starenki et al. 2004a). The dehydroxymethylepoxy quinomicin (DHMEQ) also blocks NF- $\kappa$ B nuclear translocation, and has been demonstrated to induce apoptosis *in vitro* and in nude mice xenografted with FRO cells (Starenki et al. 2004b). Interestingly, in addition to block NF- $\kappa$ B activation, DHMEQ treatment also induces sustained activation of the JNK pathway. This activation is, at least partially, responsible for the observed apoptosis, since administration of a specific JNK inhibitor suppressed the DHMEQ-induced apoptosis. These data further suggest that one of the ways used by NF- $\kappa$ B to protect cells from apoptosis is to terminate JNK activity. The proteasome inhibitor Bortezomib (Mitsiades et al. 2006), which is the only pharmacological inhibitor of NF- $\kappa$ B approved for treatment of some haematological malignancies, induces apoptosis in a panel of thyroid carcinoma cell lines originating from papillary, follicular, and anaplastic

carcinomas. Interestingly, Bortezomid induces apoptosis with an IC50 value within the range of clinically achievable concentration, suggesting the clinical use of Bortezomid alone, or in combination with conventional therapy (Mitsiades et al. 2006). However, one of the most problematic aspects of a cancer therapy based on inhibition of NF- $\kappa$ B activity is represented by the difficulty to find compounds which block the oncogenic activity of NF- $\kappa$ B, without interfering with its physiological roles in immunity, inflammation, and cellular homeostasis. Unfortunately, most of the drugs analyzed so far, also affect other cellular signaling pathways, thereby determining a number of highly toxic side effects. Much of the efforts are now concentrated on the identification of novel NF- $\kappa$ B targets specifically activated in tumors, but not in normal cells. Thus, the inhibition of these targets should block the oncogenic potential of NF- $\kappa$ B in cancer cells without affecting its role in normal tissues.

## Conclusions

In the last few years an enormous amount of basic and clinical observations strongly implicate NF- $\kappa$ B in a variety of human tumors, including thyroid cancer. The strongly deregulated NF- $\kappa$ B activity in human ATC represents a novel finding in the understanding of the molecular biology of thyroid cancer, that joins to those of RET, BRAF, PPAR $\gamma$ , PTEN, whose role has been well established in differentiated thyroid carcinomas. The future challenge is the comprehension of the molecular mechanisms mediating the oncogenic activity of NF- $\kappa$ B, in order to design compounds interfering with the anti-apoptotic, or the pro-survival signalling, without affecting its physiological role in immunity, inflammation and cellular homeostasis.



## References

- Arif, S., Blanes, A. and Diaz-Cano S. J. 2002. Hashimoto's thyroiditis shares features with early papillary thyroid carcinoma. *Histopathology* 41, 357–362.
- Baud V, Karin M.. 2009 Is NF-kappaB a good target for cancer therapy? Hopes and pitfalls. *Nat Rev Drug Discov.* 8(1):33-40.
- Boise, L. H., Gonzalez-Garcia, M., Postema, C. E., Ding, L., Lindsten, T., Turka, L. A., Mao, X., Nuñez, G. and Thompson, C. B.1993. Bcl-x, a bcl-2-related gene that functions as a dominant regulator of apoptotic cell death. *Cell* 74, 597-608.
- Borrello, M. G., Alberti, L., Fischer, A., Degl'innocenti, D., Ferrario, C., Gariboldi, M., Marchesi, F., Allavena, P., Greco, A., Collini, P., Pilotti, S., Cassinelli, G., Bressan, P., Fugazzola, L., Mantovani, A. and Pierotti, M. A. 2005. Induction of a proinflammatory program in normal human thyrocytes by the RET/PTC1 oncogene. *Proc. Natl. Acad. Sci. USA* 102, 14825-14830.
- Baud V, Karin M. 2009 Is NF-kappaB a good target for cancer therapy? Hopes and pitfalls  
*Nat Rev Drug Discov.* 8, 33-40
- Castellone, M.D., Guarino, V., De Falco, V., Carlomagno, F., Basolo, F., Faviana, P., Kruhoffer, M., Orntoft, T., Russell, J.P., Rothstein J.L., Fusco, A., Santoro, M., Melillo, R.M. 2004. Functional expression of the CXCR4 chemokine receptor is induced by RET/PTC oncogenes and is a common event in human papillary thyroid carcinomas. *Oncogene.* 23:5958-67.
- Chandler NM, Canete JJ, Callery MP. 2004 Increased expression of NF-kappa B subunits in human pancreatic cancer cells. *J Surg Res.* 118:9-14.
- Claudio, E., Brown, K., Park, S., Wang, H. and Siebenlist, U. 2002. BAFF-induced NEMO-independent processing of NF-kappa B2 in maturing B cells. *Nat. Immunol.* 3, 958-965.

- Cogswell, P.C., Guttridge, D.C., Funkhouser, W.K., Baldwin, A.S Jr. 2000 Selective activation of NF-kappa B subunits in human breast cancer: potential roles for NF-kappa B2/p52 and for Bcl-3. *Oncogene*. 19: 1123-1131.
- De Falco, V., Guarino, V., Avilla, E., Castellone, M. D., Salerno, P., Salvatore, G., Faviana, P., Basolo, F., Santoro, M. and Melillo, R. M. 2007. Biological role and potential therapeutic targeting of the chemokine receptor CXCR4 in undifferentiated thyroid cancer. *Cancer Res.* 67, 11821-11829.
- Dejardin, E., Droin, N. M., Delhase, M., Haas, E., Cao, Y., Makris, C., Li, Z. W., Karin, M., Ware, C. F. and Green, D. R. 2002. The lymphotoxin-beta receptor induces different patterns of gene expression via two NF-kappaB pathways. *Immunity* 17, 525-535.
- De Smaele, E., Zazzeroni, F., Papa, S., Nguyen, D. U., Jin, R., Jones, J., Cong, R. and Franzoso, G. 2001. Induction of gadd45beta by NF-kappaB downregulates pro-apoptotic JNK signalling. *Nature* 414, 308-313.
- Deveraux, Q. L., Roy, N., Stennicke, H. R., Van Arsdale, T., Zhou, Q., Srinivasula, S. M., Alnemri, E. S., Salvesen, G. S. and Reed, J. C. 1998. IAPs block apoptotic events induced by caspase 8 and cytochrome c by direct inhibition of distinct caspases. *EMBO J.* 17, 2215-2223.
- Fagin, J. A. and Mitsiades, N. 2008. Molecular pathology of thyroid cancer: diagnostic and clinical implications. *Best Pract. Res. Clin. Endocrinol. Metab.* 22, 955-969.
- Festa, M., Petrella, A., Alfano, S. and Parente, L. 2009. R-roscovitine sensitizes anaplastic thyroid carcinoma cells to TRAIL-induced apoptosis via regulation of IKK/NF-kappaB pathway. *Int. J. Cancer* 124, 2728-2736.
- Gallel, P., Pallares, J., Dolcet, X., Llobet, D., Eritja, N., Santacana, M., Yeramian, A., Palomar Asenjo, V., Lagarda, H., Mauricio, D., Encinas, M. and Matias-Guiu, X. 2008. Nuclear factor-kappaB activation is associated with somatic and germ line RET mutations in medullary thyroid carcinoma. *Hum. Pathol.* 39, 994-1001.
- Ghosh, S. and Karin, M. 2002. Missing pieces in the NF-kappaB puzzle. *Cell* 109, S81-S96.

- Ghosh, S., May, M. J. and Kopp, E. B. 1998. NF-kappa B and Rel proteins: evolutionarily conserved mediators of immune responses. *Annu. Rev. Immunol.* 16, 225-260.
- Gimm, O. 2001. Thyroid cancer. *Cancer Lett.* 163, 143-156.
- Gombos, K., Zele, E., Kiss, I., Varjas, T., Puskás, L., Kozma, L., Juhász, F., Kovács, E., Szanyi, I. and Ember, I. 2007. Characterization of microarray gene expression profiles of early stage thyroid tumours. *Cancer Genomics Proteomics* 4, 403-409.
- Greten, F. R. and Karin, M. 2004. The IKK/NF-kappaB activation pathway-a target for prevention and treatment of cancer. *Cancer Lett.* 206, 193-199.
- Guigon, C. J., Zhao, L., Willingham, M. C. and Cheng, S. Y. 2009. PTEN deficiency accelerates tumour progression in a mouse model of thyroid cancer. *Oncogene* 28, 509-517.
- Hanahan, D. and Weinberg, R. A. 2000. The hallmarks of cancer. *Cell* 100, 57-70.
- Hayden, M. S. and Ghosh, S. 2008. Shared principles in NF-kappaB signaling. *Cell* 132, 344-362.
- Helbig, G., Christopherson, K. W. II, Bhat-Nakshatri, P., Kumar, S., Kishimoto, H., Miller, K. D., Broxmeyer, H. E. and Nakshatri, H. 2003. NF- $\kappa$ B promotes breast cancer cell migration and metastasis by inducing the expression of the chemokine receptor CXCR4. *J. Biol. Chem.* 278, 21631-21638.
- Iannetti, A., Pacifico, F., Acquaviva, R., Lavorgna, A., Crescenzi, E., Vascotto, C., Tell, G., Salzano, A., Scaloni, A., Vuttariello, E., Chiappetta, G., Formisano, S. and Leonardi, A. 2008. The Neutrophil Gelatinase-Associated Lipocalin (NGAL), a NF- $\kappa$ B regulated gene, is a survival factor for thyroid neoplastic cells. *Proc. Natl. Acad. Sci. USA* 105, 14058-14063.
- Karger
- Karin, M. and Ben-Neriah, Y. 2000. Phosphorylation meets ubiquitination: the control of NF [kappa]B activity. *Annu. Rev. Immunol.* 18, 621-663.
- Karin, M., Cao, Y., Greten, F. R. and Li, Z. W. 2002. NF- $\kappa$ B in cancer : from innocent bystander to major culprit. *Nature Rev. Cancer* 2, 301-310.
- Kato, Y., Ying, H., Zhao, L., Furuya, F., Araki, O., Willingham, M. C. and Cheng, S. Y. 2006.

PPAR $\gamma$  insufficiency promotes follicular thyroid carcinogenesis via activation of the nuclear factor-kappaB signaling pathway. *Oncogene* 25, 2736-2747.

Kayagaki, N., Yan, M., Seshasayee, D., Wang, H., Lee, W., French, D. M., Grewal, I. S., Cochran, A. G., Gordon, N. C., Yin, J., Starovasnik, M. A. and Dixit, V. M. 2002. BAFF/BLyS receptor 3 binds the B cell survival factor BAFF ligand through a discrete surface loop and promotes processing of NF-kappaB2. *Immunity* 17, 515-524.

Keates, S., Hitti, Y. S., Upton, M. and Kelly, C. P. 1997. Helicobacter pylori infection activates NF-kappa B in gastric epithelial cells. *Gastroenterology* 113, 1099-1109.

Kondo, T., Ezzat, S. and Asa, S. L. 2006. Pathogenetic mechanisms in thyroid follicular cell neoplasia. *Nat. Rev.* 6, 292-306.

Kucharczak, J., Simmons, M. J., Fan, Y. and Gelinas, C. 2003. To be or not to be: NF- $\kappa$ B is the answer – role of Rel/NF- $\kappa$ B in the regulation of apoptosis. *Oncogene* 22, 8961-8982.

Larson, S. D., Jackson, L. N., Riall, T. S., Uchida, T., Thomas, R. P., Qiu, S. and Evers, B. M. 2007. Increased incidence of well-differentiated thyroid cancer associated with Hashimoto thyroiditis and the role of the PI3k/Akt pathway. *J. Am. Coll. Surg.* 204, 764-773.

Ludwig, L., Kessler, H., Wagner, M., Hoang-Vu, C., Dralle, H., Adler, G., Böhm, B. O. and Schmid, R. M. 2001. Nuclear factor-kappaB is constitutively active in C-cell carcinoma and required for RET-induced transformation. *Cancer Res.* 61, 4526-4535.

Mantovani, A., Allavena, P., Sica, A. and Balkwill, F. 2008. Cancer-related inflammation. *Nature* 454, 436-444.

Mechler, C., Bounacer, A., Suarez, H., Saint Frison, M., Magois, C., Aillet, G. and Gaulier, A. 2001. Papillary thyroid carcinoma: 6 cases from 2 families with associated lymphocytic thyroiditis harbouring RET/PTC rearrangements. *Br J Cancer.* 85, 1831-1877.

Melillo, R. M., Castellone, M. D., Guarino, V., De Falco, V., Cirafici, A. M., Salvatore, G., Caiazzo, F., Basolo, F., Giannini, R., Kruhoffer, M., Orntoft, T., Fusco, A. and Santoro, M. 2005. The RET/PTC-RAS-BRAF linear signaling cascade mediates the motile and

- mitogenic phenotype of thyroid cancer cells. *J. Clin. Invest.* 115, 1068-1081.
- Memon, A., Radovanovic, Z. and Suresh, A. 2004. Epidemiological evidence for a link between postpartum thyroiditis and thyroid cancer. *Eur. J. Epidemiol.* 19, 607-609.
- Mitsiades, C. S., McMillin, D., Kotoula, V., Poulaki, V., McMullan, C., Negri, J., Fanourakis, G., Tseleni-Balafouta, S., Ain, K. B. and Mitsiades N. 2006. Antitumor effects of the proteasome inhibitor bortezomib in medullary and anaplastic thyroid carcinoma cells in vitro. *J. Clin. Endocrinol. Metab.* 91, 4013-4021.
- Mitsiades CS, Hayden P, Kotoula V, McMillin DW, McMullan C, Negri J, Delmore JE, Poulaki V, Mitsiades N. 2007. Bcl-2 overexpression in thyroid carcinoma cells increases sensitivity to Bcl-2 homology 3 domain inhibition. *J Clin Endocrinol Metab.*92:4845-52.
- Nikiforova, M. N., Caudill, C. M., Biddinger, P. and Nikiforov, Y. E. 2002. Prevalence of RET/PTC rearrangements in Hashimoto's thyroiditis and papillary thyroid carcinomas. *Int. J. Surg. Pathol.* 10, 15-22.
- Pacifico, F., Mauro, C., Barone, C., Crescenzi, E., Mellone, S., Monaco, M., Chiappetta, G., Terrazzano, G., Liguoro, D., Vito, P., Consiglio, E., Formisano, S. and Leonardi, A. 2004. Oncogenic and anti-apoptotic activity of NF-kappa B in human thyroid carcinomas. *J. Biol. Chem.* 279, 54610-54619.
- Pacifico, F. and Leonardi, A. 2006. NF-kappaB in solid tumors. *Biochem. Pharmacol.* 72, 1142-1152.
- Pacifico, F., Paolillo, M., Chiappetta, G., Crescenzi, E., Arena, S., Scaloni, A., Monaco, M., Vascotto, C., Tell, G., Formisano, S. and Leonardi, A. 2007. RbAp48 is a target of nuclear factor-kappaB activity in thyroid cancer. *J. Clin. Endocrinol. Metab.* 92, 1458-1466.
- Palona, I., Namba, H., Mitsutake, N., Starenki, D., Podtcheko, A., Sedliarou, I., Ohtsuru, A., Saenko, V., Nagayama, Y., Umezawa, K. and Yamashita, S. 2006. BRAFV600E promotes invasiveness of thyroid cancer cells through nuclear factor kappaB activation. *Endocrinology* 147, 5699-5707.

- Pham, C. G., Bubici, C., Zazzeroni, F., Papa, S., Jones, J., Alvarez, K., Jayawardena, S., De Smaele, E., Cong, R., Beaumont, C., Torti, F. M., Torti, S. V. and Franzoso, G. 2004. Ferritin heavy chain upregulation by NF-kappaB inhibits TNFalpha-induced apoptosis by suppressing reactive oxygen species. *Cell* 119, 529-542.
- Pikarsky, E., Porat, R. M., Stein, I., Abramovitch, R., Amit, S., Kasem, S., Gutkovich-Pyest, E., Urieli-Shoval, S., Galun, E. and Ben-Neriah, Y. 2004. NF- $\kappa$ B functions as a tumour promoter in inflammation-associated cancer. *Nature* 431, 461-466.
- Pufnock, J. S. and Rothstein, J. L. 2009. Oncoprotein signaling mediates tumor-specific inflammation and enhances tumor progression. *J. Immunol.* 182, 5498-5506.
- Rhoden, K. J., Unger, K., Salvatore, G., Yilmaz, Y., Vovk, V., Chiappetta, G., Qumsiyeh, M. B., Rothstein, J. L., Fusco, A., Santoro, M., Zitzelsberger, H. and Tallini, G. 2006. RET/papillary thyroid cancer rearrangement in nonneoplastic thyrocytes: follicular cells of Hashimoto's thyroiditis share low-level recombination events with a subset of papillary carcinoma. *J. Clin. Endocrinol. Metab.* 91, 2414-2423.
- Rothwarf, D. M., Zandi, E., Natoli, G. and Karin, M. 1998. IKK-gamma is an essential regulatory subunit of the IkappaB kinase complex. *Nature* 395, 297-300.
- Russell, J. P., Shinohara, S., Melillo, R. M., Castellone, M. D., Santoro, M. and Rothstein, J. L. 2003. Tyrosine kinase oncoprotein, RET/PTC3, induces the secretion of myeloid growth and chemotactic factors. *Oncogene* 22, 4569-4577.
- Saltman, B., Singh, B., Hedvat, C. V., Wreesmann, V. B. and Ghossein, R. 2006. Patterns of expression of cell cycle/apoptosis genes along the spectrum of thyroid carcinoma progression. *Surgery* 140, 899-905.
- Sautin, Y., Takamura, N., Shklyayev, S., Nagayama, Y., Ohtsuru, A., Namba, H. and Yamashita, S. 2000. Ceramide-induced apoptosis of human thyroid cancer cells resistant to apoptosis by irradiation. *Thyroid* 10, 733-740.
- Senftleben, U., Cao, Y., Xiao, G., Greten, F. R., Krähn, G., Bonizzi, G., Chen, Y., Hu, Y., Fong, A.,

- Sun, S. C. and Karin, M. 2001. Activation by IKK $\alpha$  of a second, evolutionary conserved, NF-kappa B signaling pathway. *Science* 293, 1495-1499.
- Sheils, O. M., O'Leary, J. J., Uhlmann, V., Lättich, K. and Sweeney, E. C. 2000. ret/PTC-1 Activation in Hashimoto Thyroiditis. *Int. J. Surg. Pathol.* 8, 185-189.
- Sheils, O., Smyth, P., Finn, S., Sweeney, E. C. and O'Leary, J. J. 2002. RET/PTC rearrangements in Hashimoto's thyroiditis. *Int. J. Surg. Pathol.* 10, 167-168.
- Sherman, S. I. 2003. Thyroid carcinoma. *The Lancet* 361, 501-511.
- ShklyaeV, S. S., Namba, H., Mitsutake, N., Alipov, G., Nagayama, Y., Maeda, S., Ohtsuru, A., Tsubouchi, H. and Yamashita, S. 2001. Transient activation of c-Jun NH<sub>2</sub>-terminal kinase by growth factors influences survival but not apoptosis of human thyrocytes. *Thyroid* 11, 629-636.
- Smallridge, R. C., Marlow, L. A. and Copland, J. A. 2009. Anaplastic thyroid cancer: molecular pathogenesis and emerging therapies. *Endocr. Relat. Cancer* 16, 17-44.
- Starenki, D., Namba, H., Saenko, V., Ohtsuru, A. and Yamashita, S. 2004. Inhibition of nuclear factor-kappaB cascade potentiates the effect of a combination treatment of anaplastic thyroid cancer cells. *J. Clin. Endocrinol. Metab.* 89, 410-418.
- Starenki, D. V., Namba, H., Saenko, V. A., Ohtsuru, A., Maeda, S., Umezawa, K. and Yamashita, S. 2004. Induction of thyroid cancer cell apoptosis by a novel nuclear factor kappaB inhibitor, dehydroxymethylepoxyquinomicin. *Clin. Cancer Res.* 10, 6821-6829.
- Tak, P. P. and Firestein, G. S. 2001. NF-kappaB: a key role in inflammatory diseases. *J. Clin. Invest.*, 107, 7-11.
- Tang, G., Minemoto, Y., Dibling, B., Purcell, N. H., Li, Z., Karin, M. and Lin, A. 2001. Inhibition of JNK activation through NF-kappaB target genes. *Nature* 414, 313-317.
- Tirrò, E., Consoli, M. L., Massimino, M., Manzella, L., Frasca, F., Sciacca, L., Vicari, L., Stassi, G., Messina, L., Messina, A. and Vigneri, P. 2006. Altered expression of c-IAP1, survivin, and Smac contributes to chemotherapy resistance in thyroid cancer cells. *Cancer Res.* 66,

4263-4272.

- Vallabhapurapu, S. and Karin, M. 2009. Regulation and function of NF-kappaB transcription factors in the immune system. *Annu. Rev. Immunol.* 27, 693-733.
- Vasudevan, K. M., Gurumurthy, S. and Rangnekar, V. M. 2004. Suppression of PTEN expression by NF-kappa B prevents apoptosis. *Mol. Cell. Biol.* 24, 1007-1021.
- Visconti, R., Cerutti, J., Battista, S., Fedele, M., Trapasso, F., Zeki, K., Miano, M. P., de Nigris, F., Casalino, L., Curcio, F., Santoro, M. and Fusco, A. 1997. Expression of the neoplastic phenotype by human thyroid carcinoma cell lines requires NFkappaB p65 protein expression. *Oncogene* 15, 1987-1994.
- Yamaoka, S., Courtois, G., Bessia, C., Whiteside, S. T., Weil, R., Agou, F., Kirk, H. E., Kay, R. J. and Israël, A. 1998. Complementation cloning of NEMO, a component of the IkappaB kinase complex essential for NF-kappaB activation. *Cell* 93,1231-1240.
- Yeh, W. C., Itie, A., Elia, A. J., Ng, M., Shu, H. B., Wakeham, A., Mirtsos, C., Suzuki, N., Bonnard, M., Goeddel, D. V., and Mak, T. W. 2000. Requirement for Casper (c-FLIP) in regulation of death receptor-induced apoptosis and embryonic development. *Immunity* 12, 633-642.
- Wang, C. Y., Mayo, M. W., Korneluk, R. G., Goeddel, D. V. and Baldwin, A. S. Jr. 1998. NF-kappaB antiapoptosis: induction of TRAF1 and TRAF2 and c-IAP1 and c-IAP2 to suppress caspase-8 activation. *Science* 281, 1680-1683.
- Wang, C. Y., Guttridge, D. C., Mayo, M. W. and Baldwin, A. S. Jr. 1999. NF-kappaB induces expression of the Bcl-2 homologue A1/Bfl-1 to preferentially suppress chemotherapy-induced apoptosis. *Mol. Cell. Biol.* 19, 5923-5929.
- Xiao, G., Cvijic, M. E., Fong, A., Harhaj, E. W., Uhlik, M. T., Waterfield, M. and Sun, S. C. 2001. Retroviral oncoprotein Tax induces processing of NF-kappaB2/p100 in T cells: evidence for the involvement of IKKalpha. *EMBO J.* 20, 6805-6815.
- Zhu, W., Ou, Y., Li, Y., Xiao, R., Shu., Zhou, Y., Xie, J., He, S., Qiu, P. and Yan, G. 2009. A small



molecule triptolide suppresses angiogenesis and invasion of human anaplastic thyroid carcinoma cells via down-regulation of the nuclear factor-kappa B pathway. *Mol. Pharmacol.* 75, 812-819.

Accepted Manuscript

## FIGURE LEGENDS

**Figure 1.** *NF- $\kappa$ B contributes to cancer development through regulation of different genes involved in oncogenesis*

According to Hanahan and Weinberg, six essential alterations in cell physiology characterize a tumor cell: self-sufficiency in growth, insensitivity to growth-inhibitory signals, evasion of apoptosis, limitless replicative potential, sustained angiogenesis and tissue invasion and metastasis. Many of the genes able to mediate such effects are under transcriptional control of NF- $\kappa$ B.

**Figure 2.** *NF- $\kappa$ B constitutive activity in primary human anaplastic thyroid carcinomas*

NF- $\kappa$ B staining of primary human anaplastic thyroid carcinomas (B-D) and normal thyroid tissue (A). Note the strong nuclear staining in transformed cells compared to the normal counterpart.

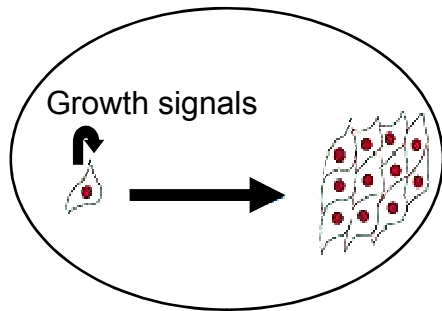
**Figure 3.** *NF- $\kappa$ B controls transcription of different genes interfering with different steps in the apoptotic process.*

NF- $\kappa$ B drives transcription of genes controlling Caspase activation (c-IAP, c-FLIP), release of cytochrome C from the mitochondria (A1, Bcl-xL), NF- $\kappa$ B activation (TRAFs, A20), JNK activation (XIAP, GADD45 $\beta$  and Ferritin Heavy chain).

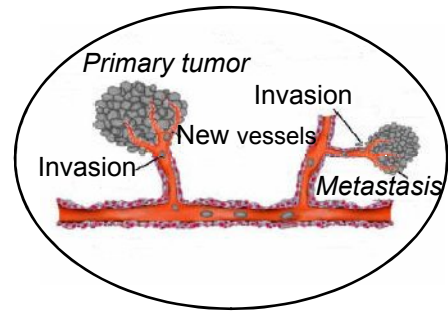
**Figure 4.** *Mechanisms by which NF- $\kappa$ B contributes to cancer development during inflammation.*

The interplay between epithelial and inflammatory cells is thought to be crucial for the genesis and the establishment of carcinomas. One of the main actors in inflammatory process is NF- $\kappa$ B which, by regulating the expression and the function of different cytokines and chemokines in inflammatory cells, stimulates the growth and its own activity in epithelial cells. Thus, NF- $\kappa$ B establishes a network that can sustain cell proliferation and resistance to apoptosis.

**Self-sufficiency in growth signals**  
**Insensitivity to anti-growth signals**  
**Limitless replicative potential**



**Sustained angiogenesis**  
**Tissue invasion and metastasis**



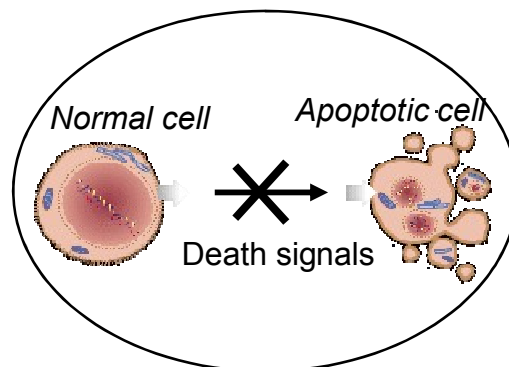
*Cyclin D1, CDK2, c-Myc,  
 TNF, IL-1, IL-6, RbAp48*

*VEGF, MMPs, uPA, VCAM-1,  
 ICAM-1, ELAM-1, CXCR4*

**NF- $\kappa$ B**

*Bcl-2 family, GADD45 $\beta$ ,  
 FHC, NGAL*

*TRAF-1, TRAF-2, XIAP,  
 cIAP-1, cIAP-2, c-FLIP*



**Evading apoptosis**

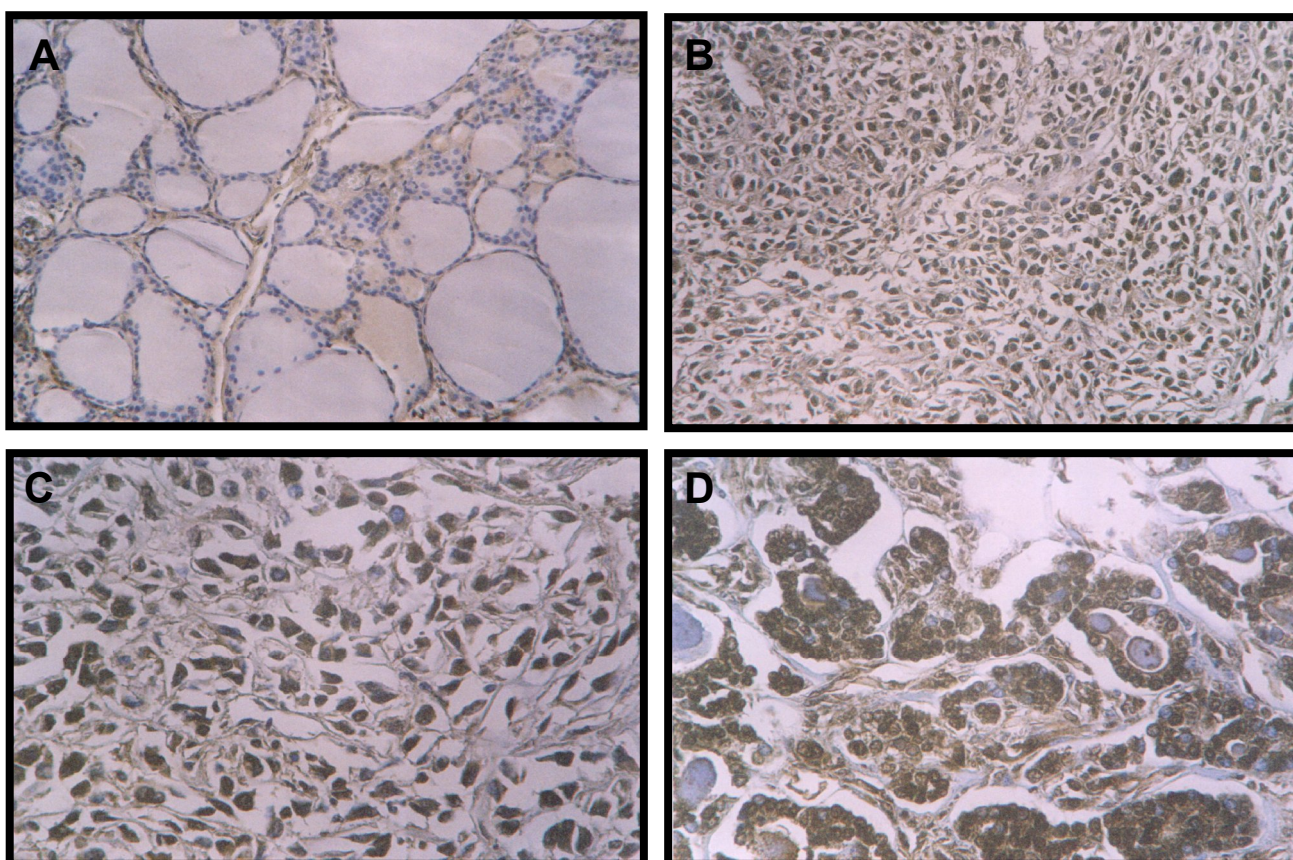


Figure 3

