

# Nuclear Factor- $\kappa$ B in Thyroid Carcinogenesis and Progression: a Novel Therapeutic Target for Advanced Thyroid Cancer

## ABSTRACT

Apoptosis is an essential physiological process of elimination of destined cells during the development and differentiation or after damage from external stresses such as ionizing radiation or chemotherapeutic agents. Disruption of apoptosis is proved to cause various diseases including cancer. Among numerous molecules involved in diverse anti- or pro-apoptotic signaling pathways, NF- $\kappa$ B is one of the key factors controlling anti-apoptotic responses. Its anti-apoptotic effect is thought to be mediated through not only transcriptional activation of dependent genes but also by crosstalking with the JNK pathway. Oncogenic proteins such as Ret/PTC, Ras and BRAF can induce NF- $\kappa$ B activation making it an important change in thyroid cancer. A number of specific or non-specific NF- $\kappa$ B inhibitors have been tried to take over the cascade in *in vitro* and *in vivo* experiments. These agents can induce massive apoptosis especially in combination with radio- or chemotherapy. Current results suggest that the inhibition of the NF- $\kappa$ B may be a promising strategy for advanced thyroid cancer treatment but further investigations are warranted to develop specific and clinically effective NF- $\kappa$ B inhibitors in future. (**Arq Bras Endocrinol Metab 2007;51/5:843-851**)

**Keywords:** Thyroid cancer; Apoptosis; NF- $\kappa$ B; Molecular target therapy

## RESUMO

### Fator Nuclear- $\kappa$ B na Carcinogênese de Tireóide e sua Progressão: um Novo Alvo Terapêutico para Câncer da Tireóide Avançado.

A apoptose é um processo fisiológico essencial destinado a eliminar células durante o desenvolvimento e diferenciação ou após danos decorrentes de estresses externos com a radiação ionizante ou agentes quimioterápicos. Distúrbios na apoptose têm sido demonstrados como causadores de várias doenças, incluindo câncer. Entre as inúmeras moléculas envolvidas nas várias vias de sinalização anti- ou pró-apoptóticas, NF- $\kappa$ B é um dos fatores-chave que controlam as respostas anti-apoptóticas. Acredita-se que seu efeito anti-apoptótico seja mediado não apenas pela ativação transcricional de genes dependentes mas também por *crosstalking* com a via JNK. Proteínas oncogênicas como Ret/PTC, Ras e BRAF podem induzir ativação de NF- $\kappa$ B promovendo importante transformação no câncer da tireóide. Uma série de inibidores específicos e não-específicos do NF- $\kappa$ B tem sido usada em experimentos *in vitro* e *in vivo* procurando inibir a cascata. Esses agentes podem induzir apoptose maciça especialmente em combinação com radio ou quimioterapia. Resultados atuais sugerem que a inibição de NF- $\kappa$ B pode ser uma estratégia promissora no tratamento do câncer da tireóide avançado, mas novas investigações são necessárias para desenvolver inibidores específicos e clinicamente efetivos do NF- $\kappa$ B. (**Arq Bras Endocrinol Metab 2007; 51/5843-851**)

**Descritores:** Câncer da tireóide; Apoptose; NF- $\kappa$ B; Terapia de alvo molecular

## perspectiva

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**A**POPTOSIS, OR PROGRAMMED CELL DEATH, is an active process of cell destruction which requires the activation of genetic and signaling suicidal programs manifesting in membrane blebbing, DNA fragmentation, shrinking and condensation of the cell and its organelles (1,2). This process is obligatory in various physiological and pathological conditions in multicellular organisms. An appropriate balance between apoptosis and cell proliferation is essential in the development and differentiation in the body. It also contributes to normal tissue functioning by the elimination of “worn” cells or those damaged by various external stresses such as ionizing radiation, UV, chemical substances and some cytokines. Considering an indispensable importance of the process in the body, one can easily imagine that the dysfunction or defect of the apoptotic machinery may result in disease.

This review describes how deregulation of apoptosis is involved in thyroid carcinogenesis. Particular attention is given to the nuclear factor kappa B (NF- $\kappa$ B), as a key regulatory molecule in anti-apoptotic pathways and a new possible therapeutic molecular target for advanced thyroid cancer.

**APOPTOSIS IN THYROID CANCER CELLS**

Proteins encoded by oncogenes or tumor suppressor genes are an integral part of carcinogenesis and tumor progression. Among them, many molecules, e.g. Bcl-2 and p53, play an important role in the regulation of apoptosis. It has long been speculated that carcinogenesis starts when inadequate apoptosis occurs due to dysregulation of pro-apoptotic or anti-apoptotic intracellular signaling (3). The acquired resistance to an insult in cancer cells would contribute to escaping from apoptotic process, which could be switched on by cytokines released by immunocompetent cells and malnutrient or anoxic condition arising during the tumor growth. Furthermore, in the cancer treatment setting, the aberrant apoptotic signaling might result in the resistance to chemo- or radiation therapy. This knowledge naturally brought up the idea that the restoration of functional apoptosis in cancer cells could be a useful therapeutic means, which can enhance the efficacy of treatment.

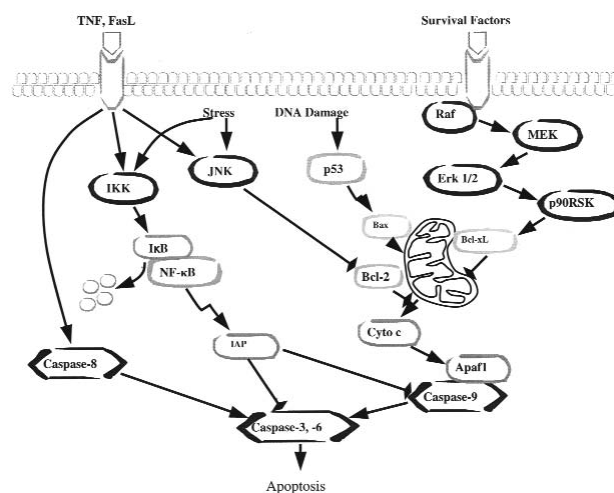
Advanced thyroid cancers, such as undifferentiated and anaplastic thyroid carcinomas, are clinically characterized by the rapid growth, concomitant inflammation and resistance to conventional therapy. Consistent with these clinical observations, anaplastic thyroid cancer cells have been found to be extremely

resistant to apoptosis after exposure to ionizing radiation *in vitro* compared with normal thyroid cells (4). Furthermore, thyroid cancer cells also show unusual responses to TNF family proteins TNF- $\alpha$ , FasL and TRAIL, which were originally identified as proteins that kill tumor cells. Of them, only TRAIL can induce cell death whereas TNF- $\alpha$  and FasL fail to do so. Since these cytokines are usually produced by macrophages or lymphocytes, the phenomenon suggested that thyroid cancer cells might not be efficiently eliminated through anti-tumor immune response (5-8).

**NF- $\kappa$ B AS A KEY-REGULATORY MOLECULE OF ANTI-APOPTOTIC SIGNALING**

Cell fate is determined by numerous molecules in diverse intracellular pro-apoptotic and anti-apoptotic pathways (figure 1). Among those molecules, the transcription factor NF- $\kappa$ B is well recognized as a central activator of the anti-apoptotic cascades in response to external stimuli or intrinsic immune reactions (9).

There are five known members of the NF- $\kappa$ B family: NF- $\kappa$ B1 (p50/p105), NF- $\kappa$ B2 (p52/p100), c-Rel, RelB, and RelA (p65), each distinguished by its Rel homology domain, which mediates DNA binding and dimerization. Usually ubiquitous NF- $\kappa$ B dimers interact with inhibitory factors known as the I $\kappa$ B proteins (I $\kappa$ B $\alpha$ , I $\kappa$ B $\beta$  and I $\kappa$ B $\epsilon$ ), binding to which retains the complex in the cytoplasm. Phosphorylation of I $\kappa$ B proteins by I $\kappa$ B kinases (IKK $\alpha$  and IKK $\beta$  and the associated modulatory protein IKK $\gamma$  or NEMO) results in



**Figure 1.** The NF- $\kappa$ B cascade and its interaction with pro- and anti-apoptotic pathways.

their rapid ubiquitination and proteolysis by the 26S proteasome (10-12). The degradation of I $\kappa$ B proteins liberates the NF- $\kappa$ B complex, which is subsequently translocated from the cytoplasm to the nucleus. Then, NF- $\kappa$ B activates the expression of a wide spectrum of genes involved in inflammation, immune response, cell proliferation and resistance to apoptosis (13). The anti-apoptotic genes transcriptionally activated by NF- $\kappa$ B include the cellular inhibitors of apoptosis (*c-IAP1*, *c-IAP2* and *XIAP*), TNF receptor-associated factors (*TRAF1* and *TRAF2*), *Gadd45 $\beta$* , the *Bcl-2* homologue *A1/B $\beta$ -1*, *IEX-IL* and *Bcl-xL* (14,15). There are two types of NF- $\kappa$ B activation pathways. The classical pathway, which is mainly mediated by the p50/p65 and p52/c-Rel dimers, is responsible for inhibition of apoptosis under most conditions including ionizing radiation, UV, cytotoxic agents and cytokines (16). Another is the alternative pathway, which depends on the selective activation of the p52/RelB dimer after processing of the NF- $\kappa$ B2/p100 precursor protein triggered by certain members of the TNF cytokine family (17).

### **CROSTALK BETWEEN THE NF- $\kappa$ B AND JNK PATHWAYS**

JNK, a member of the MAP kinase superfamily (18), is involved in stress-induced apoptosis via the mitochondrial pathway (19). In contrast, some studies have suggested that activation of JNK contributes to cell survival (18). A number of studies helped to dissect this problem by showing that the duration of JNK activation affects its role in apoptosis. It was found that prolonged, but not transient, JNK activation promotes cell death induced by external stimuli such as TNF- $\alpha$  (20,21), although sustained JNK activation alone is insufficient to induce cell killing (21). These data suggested that JNK may cooperate with other pro- or anti-apoptotic signals to evoke apoptosis. Several groups have demonstrated a crosstalk between the NF- $\kappa$ B and JNK pathways seen as an inhibitory effect of NF- $\kappa$ B on TNF- $\alpha$ -induced apoptosis due to the suppression of JNK activity (20-22). The findings implied that the balance between JNK and NF- $\kappa$ B activities is crucial to determine the cell fate, i.e. survival or death, in response to external stimuli (23,24). Indeed, the prolonged JNK activation occurs in the cells that cannot activate NF- $\kappa$ B by external stimuli. Suppression of NF- $\kappa$ B by p65 ablation, forced expression of IKK $\beta$  or super-repressor mutant form of I $\kappa$ B $\alpha$ , mut-I $\kappa$ B $\alpha$ , leads to persistent JNK signaling following TNF- $\alpha$  treatment.

Two NF- $\kappa$ B-dependent factors that mediate JNK activity have been identified so far (20,21). One is GADD45 $\beta$ , which activates a selective positive JNK regulator MKK7/JNKK2. Suppression of the latter in fibroblasts abolishes JNK induction by TNF- $\alpha$  (18,19, 24) and blocking of MKK7 alone seems to be sufficient to account for the specific and near-complete inhibition of the JNK cascade. Another molecule, XIAP, which participates in the negative modulation of JNK activity, has been identified in the study of murine embryonic fibroblasts deficient in either IKK $\beta$  or p65 (25).

Overall, data indicate that the mechanism by which NF- $\kappa$ B affects cell survival and death is influenced by the NF- $\kappa$ B-dependent factors' ability to control JNK activation. Particularly, the pro-survival effect of NF- $\kappa$ B depends on the prevention of prolonged JNK activation (26,27).

### **JNK ACTIVITY IN THYROID CELLS**

JNK activity was examined in thyroid cells after various external stimuli including ionizing radiation, UV, ceramide, and growth factors (28-30). The result showed that each of the stimuli led to different duration of JNK activation. We have demonstrated that sustained JNK activation after exposure to UV or ceramide correlated with cell death, whereas its transient activation after either X-ray irradiation or treatment by growth factors did not (29,30). Moreover, transient activation of JNK in thyroid cells may be mediated by the activation of protein kinase C (PKC)-MKK7-JNK cascade after radiation exposure (31). Despite a high basal level of *GADD45* mRNA in thyroid cancer cells harboring *TP53* gene mutation, its level is not augmented following irradiation (4). Thus, it is possible that the aberrant GADD45 response might result only in the transient JNK activation contributing to cell survival. Since ionizing radiation is the only known etiological factor of papillary thyroid cancer, it could be speculated that the resultant transient JNK activation after radiation exposure results in an incomplete elimination of damaged cells that might eventually manifest in thyroid carcinogenesis.

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

The viral oncoprotein v-Rel had been identified as the causative agent of acute avian leukemia before discovery of NF- $\kappa$ B molecules (32). After NF- $\kappa$ B family proteins have been identified, many studies demonstrated the

aberrant regulation of NF- $\kappa$ B signaling in a variety of human cancers including leukemia, lymphoma, head and neck squamous carcinoma, and ovarian, prostate, colon and breast cancers (33-35). The genes encoding NF- $\kappa$ B family members p52/p100, c-Rel, p65 and I $\kappa$ B-like protein Bcl-3 are frequently rearranged or amplified in human lymphoma and leukemia, and inactivating mutations of I $\kappa$ B $\alpha$  gene can cause Hodgkin's lymphoma (16,36,37). Moreover, most oncogene products, including the Tax protein of human T-cell leukemia virus type 1 (HTLV-1), Bcr-Abl, Her-2/Neu and oncogenic variants of Ras, can induce overexpression of p65 in cancer cells (38) and NF- $\kappa$ B activation (36,37,39,40). The activation of NF- $\kappa$ B can contribute to the oncogenesis in several ways: by driving cell proliferation (41) perhaps through increased transcription of cyclin D1, which mediates G1/S progression of the cell cycle (13), by enhancing cell survival (42), and by promoting angiogenesis and metastasis (43). The *in vivo* studies discovered a link of NF- $\kappa$ B activation with inflammation-associated tumor promotion, progression and metastasis in mouse models (44,45). For example, the role of the IKK $\beta$ -dependent NF- $\kappa$ B activation in cancer initiation was demonstrated in an experiment in which inactivation of IKK $\beta$  in enterocytes resulted in a dramatic decrease in the number of tumor foci due to increased apoptosis (45). In another study, the inflammatory process triggered chronic activation of NF- $\kappa$ B in hepatocytes and resulted in cholestatic hepatitis followed by the development of hepatocellular carcinoma in the Mdr2-deficient mice. Suppression of the chronic NF- $\kappa$ B activation at later stage of carcinogenesis resulted in the apoptosis of inflamed hepatocytes and a failure to progress to malignancy (46). The critical role of NF- $\kappa$ B activation in the inflammation-driven tumor progression was also shown in a colon and mammary cancer xenograft model (44). Cancer cells were inoculated into syngeneic immunocompetent mice to form metastases in the lungs. Once metastases were established, mice were given a sublethal dose of LPS to elicit systemic inflammation that promoted tumor growth. Inhibition of NF- $\kappa$ B in cancer cells converted LPS-induced tumor growth to tumor regression without any effect on the metastatic ability to the lung.

### NF- $\kappa$ B AND MAPK PATHWAY IN PAPILLARY THYROID CANCER

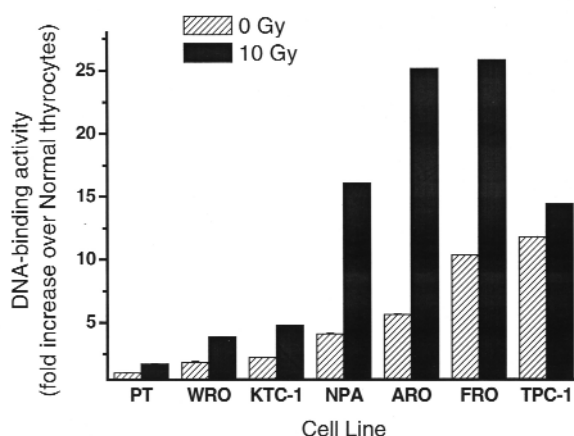
Several studies have suggested that the increased NF- $\kappa$ B activity is associated with thyroid carcinogenesis and tumor progression. A significant increase of p65 mRNA

and protein expression, compared to normal thyroid cells, was found in thyroid cancer cell lines (47). Consistent with the elevated expression level, NF- $\kappa$ B DNA binding and reporter assays showed the increased transcriptional activities in the cultures of thyroid cancer cells (48,49) (figure 2). The activation of NF- $\kappa$ B was also observed in the papillary, follicular, and anaplastic cancer tissue specimens by immunohistochemical staining using an anti-p65 antibody (49,50).

The products of *RET/PTC*s, activated mutant *RAS* and *BRAF<sup>V600E</sup>* genes involved in pathogenesis of papillary thyroid carcinoma can potentially activate the MAPK pathway (51-53). In its turn, activated MAPK has been shown to induce activation of NF- $\kappa$ B signaling and associated NF- $\kappa$ B-mediated transcriptional activity (54,55). In addition, we have observed that degradation of I $\kappa$ B $\alpha$  takes place shortly after ectopic accumulation of the BRAF<sup>V600E</sup> protein, resulting in the activation of NF- $\kappa$ B signaling via MEK-ERK independent pathway (56). In line with these findings, previous study demonstrated that oncogenic Ret-induced NF- $\kappa$ B activity depends on IKK-mediated I $\kappa$ B $\alpha$  degradation and requires functional Ras, Raf and MEKK1 in a medullary thyroid cancer cell line TT, and that NF- $\kappa$ B activation is not accomplished by MEK/ERK activation (57).

### NF- $\kappa$ B AND PI3K PATHWAYS IN THYROID CANCER

PI3-kinase pathway is an important signaling cascade that regulates cell survival and apoptosis. Cell stimulation with various growth factors or cytokines recruits a



**Figure 2.** Thyroid cancer cell lines display the higher basal level and more potent induction of NF- $\kappa$ B DNA-binding activity compared with cultured primary thyrocytes (PT) after exposure to 10 Gy of ionizing radiation.



lipid second messenger phosphatidylinositol 3,4,5-triphosphate [PI(3,4,5)P<sub>3</sub>] from membrane by the action of PI3-kinase, which then activates a serine/threonine kinase Akt/PBK. Akt/PBK relays signal to a number of molecules involved in cell proliferation, resistance to apoptosis and angiogenesis. The sequential activation of PI3K-Akt/PBK is negatively regulated by the PTEN phosphatase.

Akt/PBK and PTEN have been implicated in follicular thyroid cell carcinogenesis. Germline mutations in the *PTEN* gene have been associated with Cowden's syndrome, an autosomal dominant multiple hamartoma syndrome in which more than 50% of patients develop follicular thyroid cancer (58). The abnormal activation of Akt/PBK is often found in thyroid cancer harboring *RAS* mutation or *RET/PTC* rearrangements (59-61). The pro-survival ability of Akt/PBK is likely to be mediated through increased NF- $\kappa$ B activity (62). It has been shown that the inhibition of endogenous Akt by overexpression of PTEN resulted in the decrease of NF- $\kappa$ B transcriptional activity and sensitization of cells to TNF-induced apoptosis (63). Interestingly, PTEN itself is downregulated by p65 but not p50 subunit, forming a negative regulatory loop between PTEN and NF- $\kappa$ B. Therefore, the findings suggest that depressed PTEN or activated Akt/PBK in cancer cells might contribute to carcinogenesis by activation of the NF- $\kappa$ B pathway.

### **NF- $\kappa$ B ACTIVATION BY PAX8-PPAR $\gamma$ AND CYTOKINES IN THYROID CANCER**

*PAX8-PPAR $\gamma$*  fusion gene was initially identified in follicular thyroid tumors (64). When fused to PAX8, PPAR $\gamma$  not only loses the ability to stimulate thiazolidinedione-induced transcription, but also inhibits intrinsic PPAR $\gamma$  transcriptional activity. In an *in vivo* model study, in the progeny from the cross of PPAR $\gamma^{+/-}$  and TR $\beta^{PV/+}$  mice, the reduced PPAR $\gamma$  protein led to the activation of NF- $\kappa$ B, resulting in the elevation of cyclin D1 level and decreased apoptosis (65).

Similar to oncogenes, some cytokines have been associated with thyroid carcinogenesis through NF- $\kappa$ B activation. For example, overexpression of TGF- $\beta$  is often observed in thyroid cancer cells. The study suggested that TGF- $\beta$  induces apoptosis in normal thyroid cells via p27 reduction, but NF- $\kappa$ B activation in neoplastic thyroid cells blocks the apoptotic effect (66).

In case of TNF- $\alpha$ , which cannot induce cell death in thyroid cancer cells as mentioned above, resistance to apoptosis is thought to be due in part to NF- $\kappa$ B activation (67).

### **NF- $\kappa$ B AND THYROID CANCER PROGRESSION**

The close interrelation between oncogenes and NF- $\kappa$ B activity raises the possibility that NF- $\kappa$ B activity not only promotes cell survival in early stage of thyroid carcinogenesis, but also may facilitate tumor progression and metastasis. Supportive to the idea, NF- $\kappa$ B inhibition by overexpression of I $\kappa$ B $\alpha$  in a lung cancer cell line was found to decrease the frequency of metastases in model experiments (68).

The activated NF- $\kappa$ B transcriptionally controls *Cox-2*, *ICAM-1* and *matrix metalloproteinase 9* (*MMP-9*) genes, whose products are known to correlate with metastasis, poor prognosis and reduced disease-free interval (69). Accordingly, we recently found that forced expression of *BRAF<sup>V600E</sup>* by an adenovirus vector in wild-type *BRAF* thyroid cancer cells could elevate MMP-1 and MMP-9 levels in parallel with NF- $\kappa$ B activation, resulting in the increased cancer cells migration through extracellular matrix that could be reverted by NF- $\kappa$ B inhibition (56).

### **NF- $\kappa$ B AS A THERAPEUTIC TARGET IN CANCER**

A number of studies have been conducted to examine whether suppression of NF- $\kappa$ B activity promotes apoptosis in cancer cells. A super-suppressor mutant form of I $\kappa$ B $\alpha$  or a dominant-negative form of IKK can block the NF- $\kappa$ B pathway and enhance cell killing (55,70). The specific antisense oligonucleotides against NF- $\kappa$ B p65 gene greatly reduced the ability of two undifferentiated thyroid cancer cell lines to form colonies in soft agar and reduced their growth rate (47). RNA interference (siRNA) specific to p65 with or without concomitant administration of a chemotherapeutic agent, irinotecan, significantly impaired drug-induced NF- $\kappa$ B activation, enhanced apoptosis, and reduced colony formation in soft agar in HCT116 colon cancer cell line (71).

Among the drugs known to be beneficial in cancer treatment, some can exert a nonspecific inhibitory effect on NF- $\kappa$ B. Nonsteroidal anti-inflammatory drugs (NSAID) such as aspirin and sulindac sulfide have been shown to inhibit initiation and/or progression of certain malignancies including colorectal cancer (72). At least to some extent, the anti-tumor effects of NSAIDs could be attributed to the suppression of NF- $\kappa$ B activity due to the inhibition of IKK $\beta$ -dependent phosphorylation of I $\kappa$ B $\alpha$  (73). Arsenic trioxide (ATO), proteasome inhibitors, flavonoids, cyclopentenone prostaglandins, and glucocorticoids also render the inhibitory effect on NF- $\kappa$ B (74-76). Interestingly, with

regard to ATO, which is clinically applied for treatment of promyelocytic leukemia and multiple myeloma (77-79), it has been shown that the anticancer effect is achieved not only because of NF- $\kappa$ B suppression but also through upregulation of JNK activity.

Further studies, however, have found that clinical use of NF- $\kappa$ B inhibitors in cancer therapy may not be as effective as expected. For example, it was shown that the inhibition of NF- $\kappa$ B alone can retard cancer cell growth, but not necessarily leads to massive cell death in most solid cancers because of the coexistence of other, sometimes aberrant, NF- $\kappa$ B-independent anti- or pro-apoptotic pathways (40,80).

Yet, even though an NF- $\kappa$ B inhibitor *per se* may have a moderate potential to induce apoptosis, it might play an apoptosis-permissive role in the cells treated with cytokines, chemotherapeutic drugs or by ionizing radiation, which induce NF- $\kappa$ B activation increasing the resistance to an insult (40). Indeed, inhibition of NF- $\kappa$ B by expression of the mutant I $\kappa$ B $\alpha$  or other NF- $\kappa$ B inhibitors has resulted in model experiments in a dramatic improvement of the apoptotic response to ionizing radiation or to drugs as compared with the control cells (14).

### NF- $\kappa$ B CASCADE INHIBITION AS A NEW THERAPEUTIC APPROACH TO ADVANCED THYROID CANCER

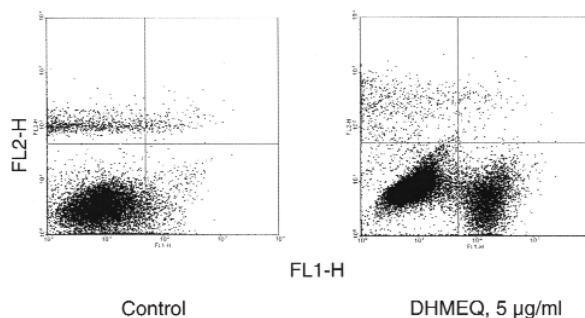
Advanced thyroid cancers are well known to be refractory to conventional chemo- or radiation therapy and usually result in a fulminant fatal outcome. Therefore, effective therapeutic modalities are being continuously sought for to achieve better treatment results. Since the increased NF- $\kappa$ B activity at least partly reinforces the intrinsic radio- or chemo-resistance of thyroid cancer cells, its inhibition could be a potential novel therapeutic approach. Effects of inhibition of the NF- $\kappa$ B pathway were experimentally examined in one study in an anaplastic thyroid cancer cell line stably expressing mutant I $\kappa$ B $\alpha$ . The mutant I $\kappa$ B $\alpha$  could enhance sensitivity to drug-induced apoptosis, led to the loss of cells' ability to form colonies in soft agar and to give rise tumors in nude mice (49). Of note, whereas parental anaplastic thyroid cancer cell line displayed a very low JNK expression, inhibition of NF- $\kappa$ B by the mutant I $\kappa$ B $\alpha$  restored its levels. In line with these results, the beneficial effects of NF- $\kappa$ B inhibition by mutant I $\kappa$ B $\alpha$  were found in the *in vivo* models of anaplastic thyroid carcinoma. The most pronounced inhibitory effect on the tumor growth after radiation therapy was observed in the animals with mutant I $\kappa$ B $\alpha$ -harboring tumors (figure 3).

Several NF- $\kappa$ B inhibitory reagents have been tested for the effectiveness in anaplastic thyroid cancer cells. The cell-permeable peptide SN50, which acts as an inhibitor of NF- $\kappa$ B nuclear translocation, markedly promoted cell death *in vitro* in combination with radiation exposure (48). Bortezomib, a proteasome inhibitor, also induced apoptosis in anaplastic thyroid cancer cell lines accompanied by the decrease in I $\kappa$ B degradation and increase of p53 expression and JNK activity (50).

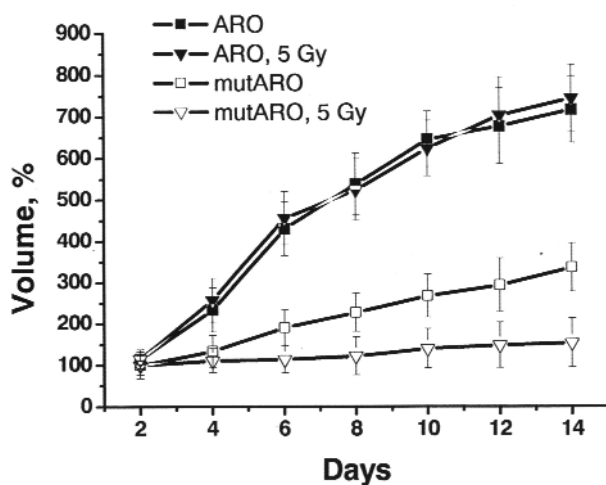
Recently, a number of low molecular weight NF- $\kappa$ B inhibitors have been designed as anticancer, immunosuppressant and anti-inflammatory agents. Most of them target factors upstream I $\kappa$ B (81). One such compound, dehydroxymethylepoxy quinomicin (DHMEQ), a derivative of the antibiotic epoxyquinomicin C (82), has been tested in anaplastic thyroid cancer cells (83). After DHMEQ treatment, we observed the activation of caspases followed by massive apoptosis of thyroid cancer cells both *in vitro* and *in vivo* (figure 4). The mechanism of induction of apoptosis by DHMEQ was proved to be the inhibition of NF- $\kappa$ B translocation to the nucleus, the decrease of expression level of several downstream anti-apoptotic proteins and the prolonged JNK activation.

### CONCLUSION

Accumulated somatic mutations in oncogenes or tumor-suppressor genes in thyroid cancer cells cause resistance to apoptosis by unbalancing anti- and pro-apoptotic pathways. NF- $\kappa$ B is a key molecule to induce the dominance of the former thus contributing to tumor growth and progression. The sustained elevation of NF- $\kappa$ B activity is often found in thyroid can-



**Figure 3.** Effect of NF- $\kappa$ B inhibition on an *in vivo* model tumor growth with or without radiation therapy. In this experiment, an anaplastic thyroid cancer cell line ARO was stably transfected with a super-repressor mutant I $\kappa$ B $\alpha$ -expressing plasmid (mutARO). Animals from each group were exposed to 5Gy of X-Ray on day 14 after inoculation of cell suspensions. The graph shows the dynamics of tumor growth; the error bars represent SEs.



**Figure 4.** Apoptotic cell death in thyroid cancer cells treated with an NF- $\kappa$ B inhibitor DHMEQ. In the flow cytometry analysis, the Annexin V/propidium iodide staining reveals massive apoptosis in anaplastic thyroid cancer cell line FRO after treatment with 5 $\mu$ g/ml of DHMEQ for 24 hours.

cer and it can be further augmented by chemo- or radiation-therapy, perhaps accruing the therapy-resistant phenotype of advanced cancers. Preliminary experimental studies show that the inhibition of NF- $\kappa$ B or interference with the pathway might be a promising treatment strategy for thyroid cancer, especially when combined with conventional chemo- and radiation therapy. Further extended large-scale experiments to discover specific NF- $\kappa$ B inhibitors and clinical studies to confirm their safety and effectiveness in the patients with advanced thyroid cancer are urgently needed.

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