Journal of Medical Sciences (2010); 3(1): 62-71

**Original Article**

Open Access

# The NF-κB Inhibitor IκBα Negates Colon Cancer Cell Migration, Invasion, Proliferation and Tumor Growth

**Samir Attoub[1,6], Rabah Iratni[2,5], Suhail Al-Salam[3], Khouloud Arafat[1], M.A.H Al Sultan[1], Nadia Al Marzouqi[1], Eric Bruyneel[4], Marc Bracke[4], Olivier De Wever[4] and Christian Gespach[6]**

[1]Department of Pharmacology & Therapeutics, Faculty of Medicine & Health Sciences, UAE University, P. O Box 17666, Al Ain, United Arab Emirates. [2]Department of Biology, UAE University, P. O. Box: 17551, Al Ain, United Arab Emirates, [3]Department of Pathology, Faculty of Medicine & Health Sciences, UAE University, P. O Box 17666, Al Ain, United Arab Emirates. [4]Laboratory of Experimental Cancer Research, University Hospital, De Pintelaan 185, B-9000 Gent, Belgium; [5]Institut Albert Bonniot, INSERM U823, University Joseph Fourier, Grenoble 1, Site Santé, 38042, Grenoble, Cedex 6, France and [6]INSERM U 673 and U938, Molecular and Clinical Oncology of Solid Tumors; University Pierre et Marie Curie Paris VI, Hôpital Saint-Antoine, 75571 Paris Cedex 12, France

**Abstract**

*It is now well accepted that the NF-κB pathways are involved in inflammatory diseases, cancer development and progression in human solid tumors. The NF-κB signaling element IκBα was shown to inactivates NF-κB activity through sequestration of this transcription factor in the cytoplasm. In the present study, we investigated the impact of the IκBα on the invasive growth of human colon cancer cells HCT8/S11 stably transfected by this endogenous NF-κB inhibitor. We report that IκBα ectopic expression inhibited NF-κB promoter activity induced by the Y527Fsrc oncogene, and reduced HCT8/S11 cell migration in wound healing assays. Our data show that IκBα abrogated collagen type I invasion induced by the trefoil factors TFF1 and TFF3, but was ineffective on the invasive phenotype determined by leptin. Moreover, IκBα reduced HCT8/S11 cell proliferation in vitro and the growth of their corresponding tumor xenografts established in the athymic mice. Taken together our data demonstrated that the intrinsic NF-κB inhibitor IκBα negates several transforming functions in human colon cancer cells. Our data provide the rationale for further preclinical and clinical studies based on therapeutic interventions targeting NF-κB pathway.*

**Keywords:** *NF-κB promoter, src, TFF, leptin, wound healing, collagen type I, Ki-67.*

**Correspondence**
SAMIR ATTOUB
Department of Pharmacology
and Therapeutics,
Faculty of Medicine & Health Sciences,
UAE University, P.O. Box 17666, Al Ain,
United Arab Emirates
Tel: +9713-7137-219
Fax: +9713-767-2033
E-mail: samir.attoub@uaeu.ac.ae

## Introduction

Cancer is the third leading cause of death in the United Arab Emirates (UAE) and other major World regions[1]. Despite recent progress in the treatment of human solid tumors and leukemia, new strategies leading to the design of targeted anticancer drugs are expected to improve cancer patient survival. Nuclear NF-κB is a key transcription factor involved in normal development, inflammatory diseases, and cancer progression[2]. In many types of cancers including breast and colon cancer, the transcription factor NF-κB is constitutively activated[3,4]. The activity of NF-κB is regulated by the IκB proteins, which bind NF-κB and retains it in the cytoplasm. Thus, IκBα-inhibitor of NF-κB, induces the formation of the inactive heterotrimeric complex comprising p50/p65

and IκBα. Upon cellular stimulation, IκB is phosphorylated by the IκB kinase (IKK), ubiquitinated and degraded by the proteasome, allowing the nuclear translocation of the p50/p65 NF-κB complex to regulate transcription of diverse genes encoding cytokines, growth factors, cell adhesion molecules, and regulators involved in survival and apoptosis[5,6]. Consistently, NF-κB activation is associated with transient and chronic inflammatory states linked to tumor promotion, progression and metastasis in several mouse models and clinical situations[7].

Loss-of-function mutations in IκB-α, resulting in high constitutive nuclear activity of NF-κB, are characteristic of Reed–Sternberg cells in Hodgkin lymphoma[8,9]. This implies a tumor suppression function for IκB. Moreover, defective IκBα were found in several solid tumors such as colon, breast, ovarian, pancreatic, bladder, prostate carcinomas and melanoma[10]. While ubiquitination-mediated proteolysis of IκBα by the 26S proteasome leads to NF-κB activation[11], proteolysis of IκBα by caspase 3 inhibited NF-κB activation instead. This proteolysis generated an N-terminal truncated protein that can still bind to NF-κB and is resistant to TNFα-induced phosphorylation and degradation[12]. Interestingly, phosphorylation of IκBα by IKK or replacement of Ser32 and Ser36 with glutamates to mimic their phosphorylation prevented caspase-mediated proteolysis[13]. It appears then that phosphorylation of IκBα by IKK serves for proteasome-dependent proteolysis of IκBα, and avoids generation of a repressor-like fragment of IκBα by caspases.

In the present study, we investigated the impact of IκBα ectopic expression on several transforming functions explored in the HCT8/S11 human colonic cancer cell line. We examined the behavior of IκBα -transfected HCT8/S11 cells on cell proliferation, migration and invasion assays monitored *in vitro* with the proinvasive agents trefoil factors TFF1, TFF3, and leptin[14,15]. In addition, we examined the influence of IκBα on the growth of HCT8/S11 tumor xenografts established in immunodeficient mice.

## Materials and Methods

### *Cell Culture, NF-κB Gene Reporter Assays, and Stable Transfections*

Human colorectal cancer cells HCT8/S11 were cultured in RPMI 1640 (Invitrogen, Cergy Pontoise, France) supplemented with 10% fetal bovine serum (Roche Molecular Biochemical's, Meylan, France). For transient transfections, HCT8/S11 colonic epithelial cells were seeded in six-well plates at a density of 75,000-200,000 cells/well. After overnight adhesion, cells were transfected with the Luciferase reporter constructs driven by NF-κB-dependent promoter, using the LipofectAMINE Reagent (Invitrogen). Where indicated, the transfection mixture was combined with plasmid vectors encoding src and IκBα. The src expression vector pSGT-srcY527F encoding the src oncogene and the corresponding control empty plasmid pSGT were provided by Dr. Serge Roche (CNRS UPR-1086 CRBM, Montpellier, France). Before assaying for luciferase activity, cells were washed with cold phosphate buffered saline (PBS) and scraped in the luciferase lysis buffer. Luciferase assays were performed using the Luciferase Assay System (Promega, Madison, USA). Values shown are mean ± SEM of at least three independent experiments, each performed in triplicate.

For stable transfections, HCT8/S11 cells were plated for 24h in 60 mm culture dishes and transfected by lipofection (LipofectAMINE Reagent Plus, Invitrogen), according to the manufacturer's instructions. Cells were transfected with 4μg of the expression vector pECFP-IκBα carrying the neomycin resistance gene. The expression vector pECFP-IκBα was a gift from Dr. Johannes A. Schmid (Department of Vascular Biology and Thrombosis Research, University Vienna, Austria). The next day, cultures were split into two 100-mm-diameter dishes and selected for ten days in the culture medium supplemented with 400 μg/ml of G418. Resistant colonies were ring-cloned as individual clones or pooled. Expression of the

transgene was determined by Western blotting using the IκBα mouse monoclonal antibody H4 raised against the GST-IκBα human fusion protein (Santa Cruz, CA, USA).

*Wound Healing and Invasion Assays*

Cells were grown in six-well culture dishes until confluence and then incubated for 10 min in Moscona buffer (137 mM NaCl, 2.68 mM KCl, 11.9 mM NaHCO3, 9.43 mM glucose, 0.176 mM NaH2PO4), pH7.4. The buffer was sterilized by filtration through a 0.22µm filters and stored at -20°C until use. A scrape was made through the confluent monolayer with a plastic pipette tip of 1mm diameter, the Moscona buffer was removed, and the dishes were washed twice and incubated at 37°C in fresh RPMI containing 10% fetal calf serum. At the underside of each dish, a mark was made at three arbitrary places where the width of the wound was measured with an inverted microscope (objective x 4). Migration was expressed as the average ± SEM of the difference between the measurements at time zero and the time points 4h, 6h, and 48h. The complete healing of the wound corresponds to 100%. The healing index corresponds to the % of the wound that is repaired per unit of time (h).

For invasion of collagen gels by HCT8/S11 and HCT8/S11- IκBα cells, six-well tissue culture dishes were filled with 1.35 ml of neutralized type I collagen (Upstate Biotechnology, Lake Placid, NY) and incubated overnight at 37°C to allow gelling. Parental and HCT8/S11- IκBα cells were harvested using Moscona buffer and trypsin/EDTA, and seeded on top of the collagen gels at the density of 300,000 cells per dish. Cultures were incubated for 24h at 37°C, in the presence or absence of the indicated agents. The number of invasive and superficial cells were counted in 12 fields of 0.157 $mm^2$ using an inverted microscope. The invasion index corresponds to the ratio of the number of cells invading the gel over the total number of cells counted in each field[16]. Recombinant human leptin was from R&D Systems Europe Ltd. (Oxon, UK). Purified recombinant human TFF1 and TFF3 were provided by Dr. Lars Thim and Pr. Bruce Westley.

*Cell Proliferation and Tumor Growth Assays*

Parental and stable HCT8/S11-IκBα cells were plated at the density of 25,000 cells into six-well tissue culture dishes supplemented with 10% FBS. At the time indicated, cancer cells were trypsinized, collected in 1 ml of medium, and counted by a Cell Coulter. Six-week-old athymic NMRI female nude mice (nu/nu, Elevage Janvier, France) were housed in filtered-air laminar flow cabinets and handled under aseptic conditions. Procedures involving animals and their care were conducted in conformity with Institutional guidelines that are in compliance with FMHS, national and international laws and policies. Parental and stable HCT8/S11-IκBα cells were injected s.c. into the lateral flank of athymic nu/nu mice (3 X $10^6$ cells in each xenograft, 7 to 8 animals in each group). Tumor volume (V) was calculated using the formula: $V = 0.4 \times a \times b^2$, with "a" being the length and "b" the width of the tumor. Tumor dimensions were measured with calipers every week for 9 weeks. Then, animals were sacrificed and the tumors excised, weighed and fixed for immuno-histochemical analysis. *For the immunohistochemical determination of the proliferating, five micrometer paraffin-embedded* tissue sections were deparaffinized, microwaved during 5 min for antigen retrieval, and then incubated with the mouse mAb against Ki-67 antigen (DAKO, Copenhagen, Denmark, clone MIB-1, dilution 1:50) for 1h at room temperature. The samples were then washed and incubated with secondary antibody for 1h at room temperature, followed by incubation with the streptavidin-peroxidase complex. Ten high-power fields (0.159 $mm^2$) per section from 4-5 tumor specimens of each group were examined microscopically. The average number of cells that stained positive for Ki-67 was evaluated in established control and IkBα -transfected HCT8/S11 xenografts.

*Statistical Analysis*

Data are means ± SEM for the number of experiments indicated. The statistical signifi-

cance between experimental values was assessed by the unpaired Student's t-test and P < 0.05 was considered to be statistically significant.

## RESULTS

### *Functional Expression of the IκB-α Transgene in HCT8/S11 Cells*

First, we examined the impact of the NF-κB inhibitor IκBα on the activity of the NF-κB reporter gene in HCT8/S11 human colon cancer cells. Transient transfections were performed using the pSGT-srcY572F vector encoding the oncogene src, together with the luciferase gene reporter construct driven by the NF-κB - dependent promoter. We have previously shown that this HCT8/S11 cell line exhibits a low level of src activity[17]. Consistently, introduction of the src oncogene induced a robust NF-κB activation (6.3 -fold) in HCT8/S11 cells**,** as compared with co-transfections performed with the control empty vector pSGT (*Fig. 1A*). As expected from previous studies showing the implication of src in NF-κB activation induced by the stress signals hypoxia and reactive oxy-

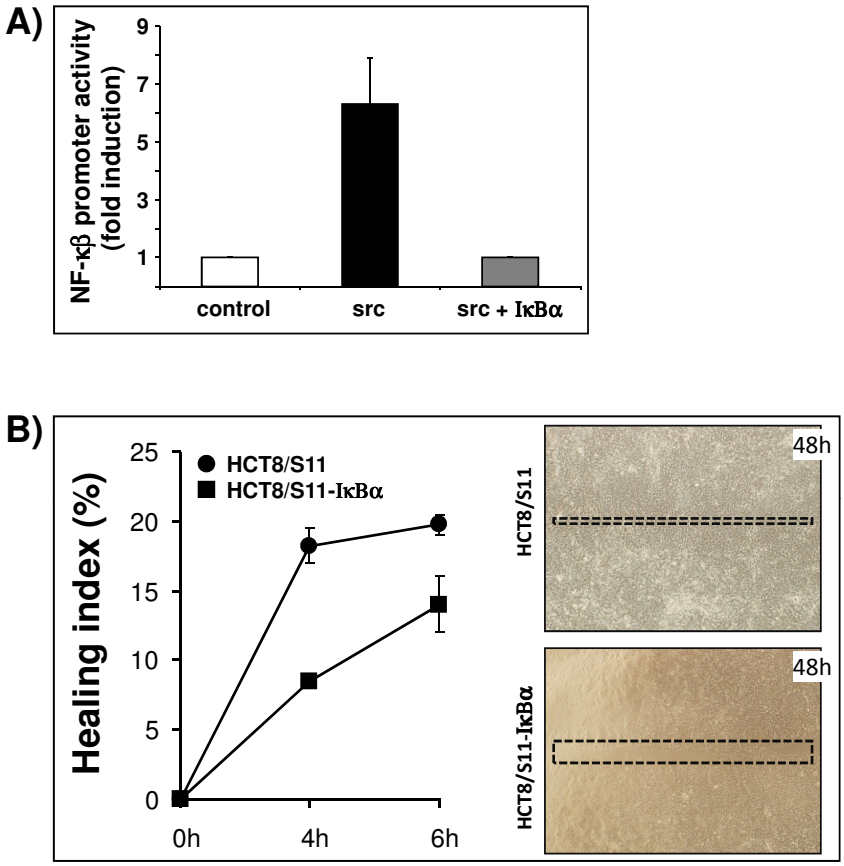


*Fig. (1).* ***IκBα down-regulates NF-κB promoter activity and wound healing in HCT8/S11 cells.***

***A.*** *Reversion by IκBα of src-induced NF-κB promoter transactivation. Human cancer cells HCT8/S11 were transiently transfected by luciferase reporter constructs driven by the NF-κB-dependent promoter, alone or combined with expression vectors encoding src alone or combined with the intrinsic NF-κB inhibitor IκBα. Data are means ± SEM of 3 independent experiments.* ***B****. Wounds were introduced in confluent monolayers of parental and stably transfected HCT8/S11- IκB αcells. Cells were then cultured at 37°C for 4, 6 and 48 h. The mean distance that cells traveled from the edge of the scraped area was measured in a blinded fashion, using an inverted microscope (4 x magnifications, insets). Data are means ± SEM of 3 independent experiments.*

gen species, as well as cellular adhesion to extracellular matrix components[18], over-expression of IκBα abrogated the direct activation of NF-κB induced by the src oncogene in HCT8/S11 cells.

Thus, our data validate the functional insertion of IκBα in our HCT8/S11 model and encouraged us to establish a stably transfected HCT8/S11 cell line expressing high levels of the endogenous NF-κB inhibitor IκBα.The resulting cell line was designated HCT8/S11- IκBα. Expression of the tagged IκBα transgene was confirmed by western blot (not shown).

*Impact of IκBα on Cancer Cell Migration, Invasion, Proliferation, and Tumor Xenograft Growth*

We next examined the effect of IκBα ectopic expression on cellular migration in HCT8/S11 cells. Using wound-healing experiments performed on sub-confluent cell cultures, we show that IκBα inhibited by 29-53% the migration of HCT8/S11- IκBα cells during the 4-6h short incubation time considered (*Fig. 1B, left*) suggesting that the inhibition of cellular migration is not due to inhibition of cell proliferation. Parental HCT8/S11 cells were able to achieve a complete wound healing within 48h, while IκBα cells were unable to fully colonize the wounds at this time period (*Fig. 1B, right*).

Although trefoil factors contribute to the immunity and mucosal protection in the normal gastrointestinal tract, it is now widely accepted that these secreted regulatory peptides are strongly induced during inflammatory diseases and cancer progression [19]. In agreement, TFFs play a pejorative role in several transforming functions associated with the neoplastic progression in cancer cells and tumors, including cellular scattering and invasion, survival and protection against anoïkis-apoptosis, adenoma-adenocarcinoma transition, and angiogenesis[20,21]. Indeed, we have previously shown that trefoil factors (TFF) and leptin induce the invasive phenotype in HCT8/S11 cells cultured on collagen type I gels[14,15]. As shown in *Fig. 2*, the leptin invasive phenotype was not affected by IκBα, while this NF-κB inhibitor selectively impaired the invasive responses elicited by the trefoil factors TFF1 and TFF3. To test the ability of IκBα to interfere with cancer cell proliferation, parental HCT8S/S11 cells and their IκBα transfected counterparts were compared for their growth rates. As shown in *Fig. 3A*, HCT8/S11- IκB cells

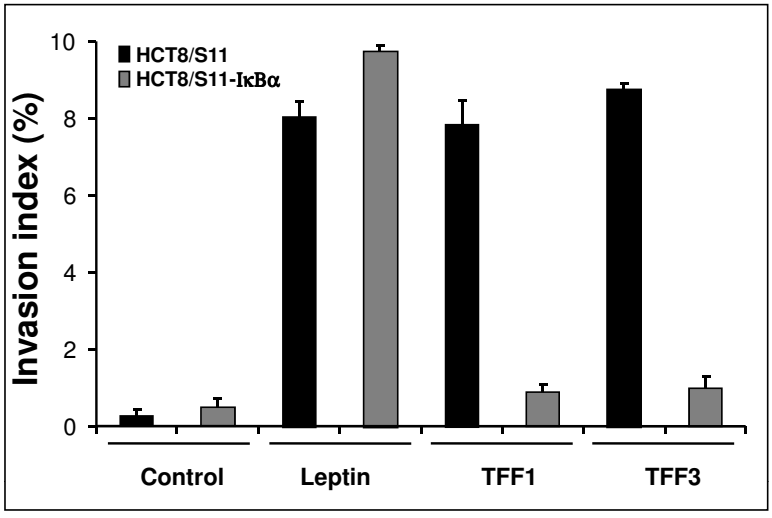


*Fig. (2).* ***IκBα ectopic expression selectively negates cellular invasion induced by pS2/TFF1 and intestinal trefoil factor /TFF3 in HCT8/S11 cells.***

*Cellular invasion induced by leptin (100 ng/ml), TFF1 (100nM) and TFF3 (100nM) was measured in parental HCT8/S11 cells (control) and stably transfected HCT8/S11- IκBα cells. Data are means ± SEM of 3 independent experiments.*

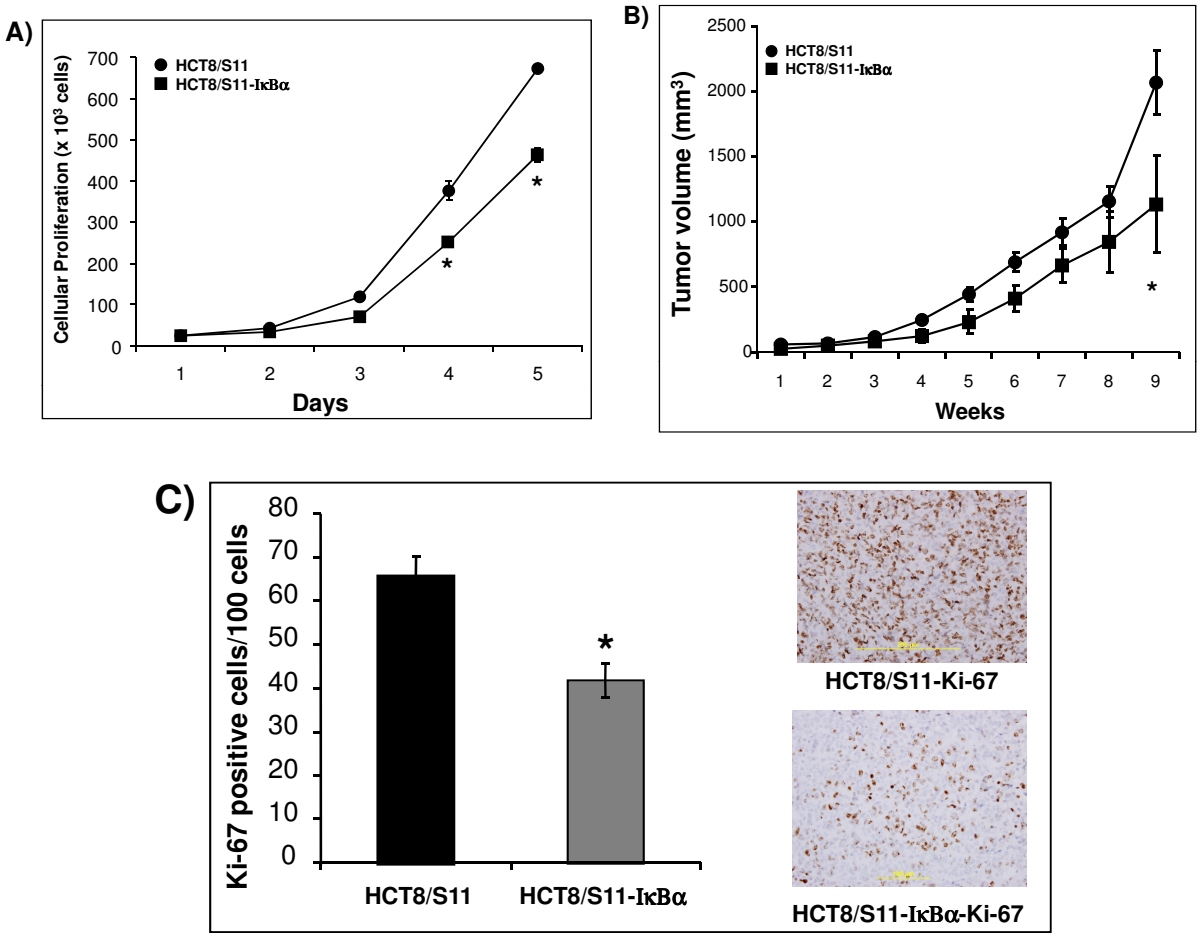


*Fig. (3).* ***Impact of IκBα ectopic expression on the proliferation of HCT8/S11 cancer cells in culture and on tumor xenografts in athymic mice.***

***A****. HCT8/S11 and HCT8/S11 IκBα stably transfected cells were seeded into six-well tissue culture dishes (25,000 cells /dish). Parental and IκBα -stably transfected cells were counted daily for 4 days. Data are means ± SEM of 3 independent experiments.* ***B****. Tumor growth assay in immunodeficient mice injected S.C. with HCT8/S11 or HCT8/S11-IκBα cells (*P < 0.05 versus HCT8/S11 xenografts).* ***C****. Immunohistochemical staining for Ki-67 expression in the corresponding HCT8/S11 and HCT8/S11-IκBα human colon cancer xenografts. Data are means ± SEM of 3 independent experiments. Note differences in Ki-67 proliferation signals between HCT8/S11 and HCT8/S11-IκBα human colon cancer xenografts (*P < 0.05 versus HCT8/S11 xenografts)*

exhibit slower proliferation rates, as shown at days 4 and 5 in culture (24 and 31 % inhibition, respectively). This interesting data prompted us to investigate the impact of IκBα ectopic expression on the growth of HCT8/S11 human colon tumor xenografts in immunodeficient mice. As shown in *Fig. (3B)*, IκBα ectopic expression led to significant inhibition (45%) of tumor growth at week 9 following heterotransplantation.

To test the role of IκBα on tumor cell proliferation in HCT8/S11 human colon xenografts, we performed a complementary study on the nuclear antigen Ki-67 by immunohistochemistry. This nuclear Ki-67 antigen, which is present in all phases of the cell cycle, except the Go phase, is considered as a classical marker in cells engaged in the cell proliferation cycle. There was a significant decrease in the mean number of Ki-67-positive cells in HCT8/S11- IκBα tumor xenografts (41.8 +/- 3.8 % *vs.* 65.8 +/- 4.6 %, P < 0.05), as compared to the controls at 9 weeks (*Fig. 3C*). Thus, the inhibition of tumor xenografts growth induced by IκBα appears to

be due, at least in part, to a direct effect on tumor cell proliferation.

## Discussion

Deregulated and constitutive activation of cell proliferation and survival signals constitute a critical mechanism underlying tumor development and cancer progression. Evidence suggests that aberrant activation of NF-κB and its downstream signaling pathways is responsible for the initiation of tumorigenesis including evasion from apoptosis, malignant transformation, sustained cell proliferation, metastasis, and angiogenesis[22]. A sustained activation of NF-κB contributes to the expression of proto-oncogenes c-myc and cyclin D1, which are responsible for both transformation initiation and tumorigenic proliferation[22].

In the present study, we have shown that ectopic expression of the intrinsic NF-κB inhibitor IκBα strongly inhibits NF-κB transactivation directly induced by src oncogene in HCT8/S11 human colon cancer cells. The src oncogene is known to be activated during the early and late stages of the neoplastic progression in human colon tumors[23]. Consistently, we demonstrated that blockade of the NF-κB pathways by IκBα led to the invalidation of several transforming functions in HCT8S/11 cells. We have shown that IκBα ectopic expression reduced the ability of HCT8/S11 cells to proliferate *in vitro* and *in vivo* as tumor xenografts in immunodeficient mice. Convergent data and reports also revealed that alterations in the adhesive properties of cancer cells are asso-

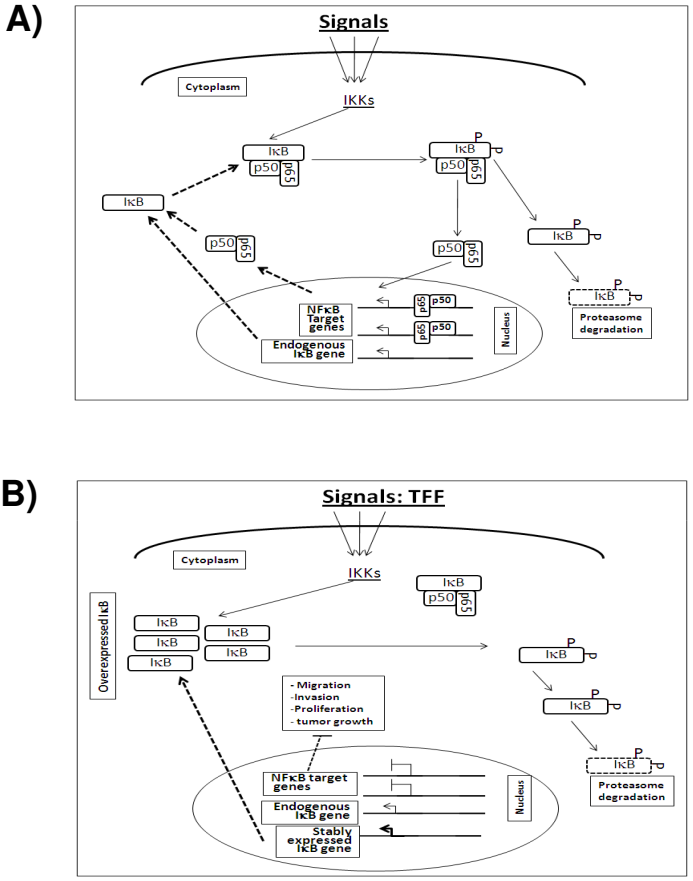


*Fig. (4).* ***Diagram summarizing the NF-κB pathway in a cancer cell before*** *(**A**) and following transfection with IκBα (**B**). Over-expression of IκB by stable transfection titrates IKKs out, leaving some IκB in the ternary complex unphosphorylated. This will prevent NF-κB nuclear translocation and thus maintaining a constitutive repression of the NF-κB responsive genes.*

ciated with invasive growth and tumor malignancy (data summarized in *Fig. 4*). Cellular adhesion to ECM components *via* their integrin receptors, intercellular adhesion, and activation of the actomyosin system in the cytoskeleton are known to play pivotal roles in the integration of the mechanisms leading to directed cellular movements during wound healing assays. Loss of E-cadherin and other intercellular adhesion molecules also correlate with cancer cell scattering, migration, and tumor cell invasion[23]. This loss of cell-cell adhesion appears to be a key event in acquisition of the invasive potential, because re-expression of E-cadherin suppresses the invasive growth of human tumors[24,25]. Consistently, it has been shown that NF-κB pathway can suppress E-cadherin expression *via* specific transcription repressors such as Snail, an NF-κB target gene [26,27]. Snail-induced E-cadherin loss and epithelial-mesenchymal transitions (EMT) are associated with the invalidation of the epithelial cell polarity, stimulation of cancer cell proliferation, and exacerbation of the invasive and metastatic potential in clinical human epithelial tumors[28,29]. Similarly, integrin ligation to ECM components was shown to activate the transcription factor NF-κB in multiple cell types[18]. Given the role of the NF-κB inhibitor IκBα in inhibiting HCT8/S11 cell migration in the present study, one can postulate that this pathway has potential roles in the regulation of intercellular and cell-matrix adhesion mechanisms, as well as activation of the cytoskeleton during cancer cell migration and invasion. In coherence with this prediction, we have shown here that IκBα ectopic expression selectively negated HCT8/S11 cancer cell invasion induced by TFF1 and TFF3, but not by leptin, in collagen type I gels. Consistently, several studies indicate that both TFF expression and biological activities are controlled by NF-κB since several NF-κB binding sites are localized in the promoter regions of the three TFF genes[19,29]. In contrast, blockade of the NF-κB pathways is not associated with the reversion of the leptin invasive potential in our study, suggesting that alternative proinvasive pathways induced by this cytokine are still operational under NF-κB blockade. Indeed, several studies have identified the multiplicity of the leptin receptor signaling networks connected with cancer cell invasion and tumor angiogenesis. These include the PI3-kinase /AKT axis, MAPK ERK1/2 cascade, the Rho-GTPases, the JAK2 /STAT3 transcription signals, as well as several crosstalks engaged with other transmembrane receptors[14]. These canonical and alternative leptin pathways are inherent to cancer cell proliferation, survival, invasion and metastasis, as well as tumor angiogenesis and metastasis.

We propose that the NF-κB oncogenic pathway can be targeted to halt tumor invasive growth and progression to the metastatic stages. Several studies have addressed the design of putative NF-κB inhibitors as pharmacologic therapeutic agents in cancer patients. Anti-inflammatory drugs and natural compounds, such as curcumin and transveratrol, inhibit NF-κB by interfering with IKK activity[30]. Finally, proteasome inhibitors were also shown to prevent NF-κB activation by blocking the degradation of IκB. In summary, the results of our study provide new insight into the development of therapeutic strategies against colon cancer promotion and progression, using NF-κB interfering drugs in combinations with other anticancer agents targeting selected oncogenic pathways or genotoxins that disrupt the functional integrity of the DNA.

**Acknowledgements**

This work was supported by Sheikh Hamdan Award to support medical research in UAE Grant No MRG-18/2005-2006, INSERM, the Belgische Federatie tegen Kanker, the FORTIS Verzekeringen and the Fund for Scientific Research Flanders (Brussels, Belgium). We thank Dr. Johannes Schmid for providing the IκBα expression vector.

**References**

1. Al-Shamsi SR, Bener A, Al-Sharhan M, Al-Mansoor TM, Azab IA, Rashed A, *et al*. Clinicopathological pattern of colorectal cancer in the United Arab Emirates. Saudi Med J 2003; 24: 518-22.

2. Karin M, Cao Y, Greten FR, Li ZW. NF-kappaB in cancer: from innocent bystander to major culprit. Nat Rev Cancer 2002; 2: 301-10.

3. Karin M. Nuclear factor-kappaB in cancer development and progression. Nature 2006; 441: 431-6.

4. Baud V, Karin M. Is NF-kappaB a good target for cancer therapy? Hopes and pitfalls. Nat Rev Drug Discov 2009; 8: 33-40.

5. Ghosh S, Karin M. Missing pieces in the NF-kappaB puzzle. Cell 2002; 109(Suppl): S81-96.

6. Karin M, Lin A. NF-kappaB at the crossroads of life and death. Nat Immunol 2002; 3: 221-7.

7. Luo JL, Kamata H, Karin M. IKK/NF-kappaB signaling: balancing life and death--a new approach to cancer therapy. J Clin Invest 2005; 115(10): 2625-32.

8. Cabannes E, Khan G, Aillet F, Jarrett RF, Hay RT. Mutations in the IkBa gene in Hodgkin's disease suggest a tumour suppressor role for IkappaBalpha. Oncogene 1999; 18(20): 3063-70.

9. Jungnickel B, Staratschek-Jox A, Brauninger A, Spieker T, Wolf J, Diehl V, *et al*. Clonal deleterious mutations in the IkappaBalpha gene in the malignant cells in Hodgkin's lymphoma. J Exp Med 2000; 191(2): 395-402.

10. Rayet B, Gelinas C. Aberrant rel/nfkb genes and activity in human cancer. Oncogene 1999; 18: 6938-47.

11. Karin M, Ben-Neriah Y. Phosphorylation meets ubiquitination: the control of NF- [kappa]B activity. Annu Rev Immunol 2000; 18: 621-63.

12. Reuther JY, Baldwin AS, Jr. Apoptosis promotes a caspase-induced aminoterminal truncation of IkappaBalpha that functions as a stable inhibitor of NF-kappaB. J Biol Chem 1999; 274: 20664-70.

13. Barkett M, Xue D, Horvitz HR, Gilmore TD. Phosphorylation of IkappaB-alpha inhibits its cleavage by caspase CPP32 in vitro. J Biol Chem 1997; 272(47): 29419-22.

14. Attoub S, Noe V, Pirola L, Bruyneel E, Chastre E, Mareel M, *et al*. Leptin promotes invasiveness of kidney and colonic epithelial cells via phosphoinositide 3- kinase-, rho-, and rac-dependent signaling pathways. FASEB J 2000; 14: 2329-38.

15. Emami S, Le Floch N, Bruyneel E, Thim L, May F, Westley B, *et al*. Induction of scattering and cellular invasion by trefoil peptides in src- and RhoA-transformed kidney and colonic epithelial cells. FASEB J 2001; 15: 351-61.

16. De Wever O, Hendrix A, De Boeck A, Westbroek W, Braems G, Emami S, *et al*. Modeling and quantification of cancer cell invasion through collagen type I matrices. Int J Dev Biol 2009 Epub ahead of print.

17. Rivat C, Le Floch N, Sabbah M, Teyrol I, Redeuilh G, Bruyneel E, *et al*. Synergistic cooperation between the AP-1 and LEF-1 transcription factors in activation of the matrilysin promoter by the src oncogene: implications in cellular invasion. FASEB J 2003; 17: 1721-23.

18. Courter DL, Lomas L, Scatena M, Giachelli CM. Src kinase activity is required for integrin alphaVbeta3-mediated activation of nuclear factor-kappaB. J Biol Chem 2005; 280: 12145-51.

19. Emami S, Rodrigues S, Rodrigue CM, Le Floch N, Rivat C, Attoub S, *et al*. Trefoil factor family (TFF) peptides and cancer progression. Peptides 2004; 25: 885-98.

20. Rodrigues S, Rodrigue CM, Attoub S, Flejou JF, Bruyneel E, Bracke M, *et al*. Induction of the adenoma-carcinoma progression and Cdc25A-B phosphatases by the trefoil factor TFF1 in human colon epithelial cells. Oncogene 2006; 25: 6628-36.

21. Perry JK, Kannan N, Grandison PM, Mitchell MD, Lobie PE. Are trefoil factors oncogenic? Trends Endocrinol Metab 2008; 19: 74-81.

22. Pikarsky E, Porat RM, Stein I, Abramovitch R, Amit S, Kasem S, *et al*. NF-kappaB functions as a tumour promoter in inflammation-associated cancer. Nature 2004; 431: 461-6.

23. Empereur S, Djelloul S, Di Gioia Y, Bruyneel E, Mareel M, Van Hengel J, *et al*. Progression of familial adenomatous polyposis (FAP) colonic cells after transfer of the src or polyoma middle T oncogenes: cooperation between src and HGF/Met in invasion. Br J Cancer 1997; 75: 241-50.

24. Rasbridge SA, Gillett CE, Sampson SA, Walsh FS, Millis RR. Epithelial (E-) and placental (P-) cadherin cell adhesion molecule expression in breast carcinoma. J Pathol 1993; 169: 245-50.

25. Frixen UH, Behrens J, Sachs M, Eberle G, Voss B, Warda A, *et al*. E-cadherinmediated cell-cell adhesion prevents invasiveness of human carcinoma cells. J Cell Biol 1991; 113: 173-85.

26. Chua HL, Bhat-Nakshatri P, Clare SE, Morimiya A, Badve S, Nakshatri H. NFkappaB represses E-cadherin expression and enhances epithelial to mesenchymal transition of mammary epithelial cells: potential involvement of ZEB-1 and ZEB-2. Oncogene 2007; 26: 711-24.

27. Sabbah M, Emami S, Redeuilh G, Julien S, Prevost G, Zimber A, *et al*. Molecular signature and therapeutic perspective of the epithelial-to-mesenchymal transitions in epithelial cancers. Drug Resist Updat 2008; 11: 123-51.

28. Batlle E, Sancho E, Franci C, Dominguez D, Monfar M, Baulida J, *et al*. The transcription factor snail is a repressor of E-cadherin gene expression in epithelial tumour cells. Nat Cell Biol 2000; 2: 84-9.

29. Chen YH, Lu Y, De Plaen IG, Wang LY, Tan XD. Transcription factor NF-kappaB signals antianoikic

function of trefoil factor 3 on intestinal epithelial cells. Biochem Biophys Res Commun 2000; 274: 576-82.

30. Holmes-McNary M, Baldwin AS Jr. Chemopreventive properties of transresveratrol are associated with inhibition of activation of the IkappaB kinase. Cancer Res 2000; 60:3477-83.