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Identification of cytokine-induced nuclear factor-kappaB target genes in ovarian and breast cancer cells

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Abstract

NF-κB is a pleiotropic transcription factor controlling the expression of many genes and viruses. NF-κB plays a role in immune response, cellular adhesion or acute phase response. It also inhibits apoptosis and favors cancer cell survival. We studied the expression of genes controlled by NF-κB in ovarian and breast adenocarcinoma cancer cells. We stably transfected OVCAR-3 and MCF7 A/Z cells with an expression vector coding for the mutated inhibitor IκBα, which sequesters NF-κB in the cytoplasm. We stimulated control and IκBα expressing cells with IL-1β or TNF-α and extracted the RNA, which was reverse-transcribed and hybridized to DNA microarrays. Several of the genes identified were not known as NF-κB target genes. Among them, we confirmed the differential expression of *ephrin-A1* and *caveolin-1* by quantitative real-time polymerase chain reaction. Our results showed an NF-κB-dependent induction of *ephrin-A1* and *caveolin-1* mRNAs after stimulation with TNF-α and IL-1β, confirming that NF-κB controls target genes implied in tumor angiogenesis and cell transformation.

Keywords: NF-κB transcription; Differential expression; Cancer; Angiogenesis

1. Introduction

Nuclear factor kappaB is an ubiquitous transcription factor that regulates the expression of genes involved in cell proliferation, differentiation, immune response, inflammation and regulates apoptosis in normal and cancer cells [1]. The NF-κB proteins bind DNA as dimers and are sequestered in the cytoplasm of most cell types by inhibitory proteins [2,3]. These inhibitors, which belong to a family of proteins named IκB for inhibitory κB, mask the NF-κB nuclear localization signal and inhibit its DNA-binding activity. In response to a large variety of stimuli, including proinflammatory cytokines like IL-1β and tumor necrosis factor (TNF-α), the IκB inhibitor is rapidly phosphorylated by a kinase complex composed of three subunits (IKKα, β and IKKγ/NEMO) and targeted to rapid ubiquitin-dependent proteolysis [4], thus allowing NF-κB nuclear translocation, binding to specific sequence promoters and transcription of target genes.

Substantial evidence suggests that NF-κB may be involved in carcinogenesis [5]. Activation of NF-κB inhibits apoptosis induced by TNF-α, various anticancer drugs and radiation [6]. NF-κB activation may also promote cell proliferation [7–10]. The *c-rel* gene, encoding a component of NF-κB, is the human homologue of the *v-rel* oncogene that can directly transform cells *in vivo* and *in vitro* [6,11,12]. NF-κB constitutive activity has been observed in Hodgkin's disease cells and has been linked to a mutation in the gene encoding the IκBα inhibitor [7,13–16]. Chromosomal translocations or amplification of *Rel*/NF-κB genes leading to impaired control of NF-κB activity were linked to lymphomas or other human tumors [5]. Elevated NF-κB activities have been detected in a variety of tumors including hormone-dependent and -independent breast cancers as well as chemically induced breast cancers in rodent [5,17–20]. However, it is not clear whether the elevated

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Abbreviations: IKK, IκB kinase; IL-1β, interleukin-1β; TNF-α, tumor necrosis factor; NEMO, NF-κB essential modulator.

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level of NF-κB is just a marker of aberrant differentiation or plays a causative role in tumor development or progression by activating or repressing NF-κB target genes.

There is little constitutive detectable NF-κB activity in MCF7 A/Z breast and OVCAR-3 ovarian cancer cell lines, but exposure to IL-1β or TNF-α induced rapid and transient NF-κB nuclear DNA binding. In our experiment, complete inhibition of cytokine-induced NF-κB by stable transfection with a vector expressing a nondegradable mutant form of IκBα, where serine 32 and 36 are replaced by alanines, induces cell death in OVCAR-3 and MCF7 A/Z cells treated with TNF-α, but not in response to chemotherapeutic drugs. Thus, it seems that NF-κB activation has variable effect on cell mortality depending on the cell type, the stimulus, and the level of NF-κB inhibition [21,22]. Although several target genes are known, the identity of cytokine-induced and NF-κB-regulated genes in adenocarcinoma cells remains to be identified. To address this issue, we blocked NF-κB activation in IL-1β and TNF-α-exposed cells and analyzed gene expression by microarrays and subtractive hybridization. We identified several genes, which were not known as NF-κB target genes. We focused on two of them implied in oncogenesis: *ephrin-A1* and *caveolin-1* and analyzed their mRNA expression by quantitative real time RT–PCR.

2. Materials and methods

2.1. Reagents

The human recombinant IL-1β and TNF-α (specific activity>1.0×10^8 U/mg) were purchased from Roche. The protein synthesis inhibitor cycloheximide was purchased from Sigma. BAY11-7085 was purchased from Biomol.

2.2. Cell lines

HCT116 human colon carcinoma cells (ATCC CCL 247, ATCC) were grown in McCoy's 5A modified medium supplemented with 1% L-glutamine (200 mM), 10% (v/v) fetal bovine serum (Life Technologies), penicillin (100 IU/mL), and streptomycin (100 μg/mL). OVCAR-3 ovarian cancer cells (purchased from the ATCC) and MCF7 A/Z breast cancer cells (supplied by Prof. Mareel, University of Gent, Belgium) were maintained in RPMI 1640 medium supplemented with 10% fetal bovine serum, 1% L-glutamine (200 mM), penicillin (100 IU) and streptomycin (100 μg/mL). The OVCAR-3 cells were stably transfected either with an empty $pcDNA_3$ vector or with an expression vector coding for an IκBα protein mutated at serines 32 and 36 and linked to an HA epitope at its amino-terminal end. Other cell lines (HCT116 and MCF7 A/Z) expressing mutated IκBα protein were previously described [21,23]. All stably transfected cell lines were cultivated in the same medium as control cells supplemented with geneticin. The human dermal microvascular cell line HMEC-1 was cultivated in MDCB131 culture medium (Life Technologies) supplemented with 1 μg/mL hydrocortisone (Sigma), 10 ng/mL epidermal growth factor, 20% FCS, and ultra glutamine.

2.3. Protein extraction

Whole cell extracts were made by resuspending the cellular pellets in SDS 1%. The lysates were then boiled for 10 min. Nuclear extracts were prepared as previously described [24]. Cytoplasmic buffer contained 10 mM HEPES, pH 7.9, 0.1 mM EDTA, 2 mM $MgCl_2$, 10 mM KCl, 0.2% NP-40, and protease inhibitors (protease inhibitor kit, Roche). The pelleted nuclei were resuspended in nuclear buffer (20 mM HEPES, pH 7.9, 1.5 mM $MgCl_2$, 0.2 mM EDTA, 0.63 mM NaCl, 25% glycerol, protease inhibitors), incubated for 20 min at 4°, and centrifuged for 30 min at 13,200 *g*.

Protein amounts were quantified with the Micro BCA Protein Assay Reagent (Pierce), using a BSA standard solution as reference.

2.4. Electrophoretic mobility shift assays (EMSA)

Electrophoretic Mobility Shift Assays (EMSA) were performed as described [25]. The palindromic κB probe was 5′-TTGGCAACGGCAGGGGAATTCCCCTCTCCTTAGGTT-3′

2.5. Western blotting

Protein extracts (10 μg) were run on 12% SDS–PAGE gels. After transfer to an Immobilon™-P membrane (PVDF, Millipore), and blocking overnight with TBS–T buffer plus 5% nonfat dry milk, the membranes were incubated for 1 hr with the appropriate first antibody, washed and then incubated for 1 hr with the second horseradish peroxidase-conjugated antibody. The reaction was revealed with the enhanced chemoluminescence detection method (ECL kit, Amersham Pharmacia Biotech). Polyclonal anti-caveolin-1 antibodies were purchased from Santa Cruz Biotechnology.

2.6. Total RNA isolation

Total RNA was extracted using RNeasy columns from Quiagen, according to manufacturer's recommendations. After DNAse digestion, RNAs were eluted and quantified using a spectrophotometer (Gene Quant, Pharmacia).

2.7. Microarray analysis

For microarray analysis, cells were treated for 1 hr with IL-1β or TNF-α, 100 μg of total RNA were isolated, and

cDNAs were reverse-transcribed and cyanine-labeled. cDNAs from control OVCAR-3 and MCF7 A/Z cells were cyanine-5-labeled (Cy5), while cDNAs from IκB MT OVCAR-3 and IκB MT MCF7 A/Z were cyanine-3-labeled (Cy3). The cDNAs corresponding to each cell line were then mixed and hybridized to MICROMAX arrays (NEN), following manufacturer's recommendations. The fluorescence signal intensities and the Cy3/Cy5 ratios for each of the 2400 cDNAs were scanned (GSI Lumonics, Inc.) and analyzed by software (ImaGene; BioDiscovery, Inc.).

2.8. Quantitative real time RT–PCR

Each quantitative PCR reaction (20 μL) included 2 μL cDNA solution corresponding to the reverse transcription reaction of 2.5 ng of RNA, 2 μL 10X SYBR Green PCR mix buffer, 1.6 μL 25 mM $MgCl_2$ and 2 μL each primer (7 μM). The number of cycles was selected to allow linear amplification of the cDNAs under study. For quantitative PCR, the *GAPDH* housekeeping gene was used as a control. Quantification was performed with the Light Cycler™ PCR technology (Roche). The primer sequences were as follow : *caveolin-1*: 5′-AAGTAAAACTATATATCCATGCC-3′, and 5′CAGAAAGCTGCCTGGTATAT-3′; e*phrin-A1*: 5′-CCAGTCCAAGGACCAAGT-3′, and 5′-ATGTAGAACCGCACCT-3′: *GAPDH* 5′-ATGGGGAAGGTGAAGGTGGTC3′; and 5′-TGATGGCATGGACTGTGG-3′.

3. Results

3.1. Identification of NF-κB dependent genes by microarray analysis

Control ovarian OVCAR-3 and breast MCF7 A/Z cells, as well as cells transfected with the mutated form of IκBα were either exposed for 1 hr to TNF-α or IL-1β or left untreated. EMSA were performed with control and mutated IκBα-overexpressing cells, as shown in Fig. 1A. Cells were processed for total RNA extraction and resulting fluorescent labeled cDNA were hybridized to the array containing 2400 known human cDNAs.

Cy3 signal was derived from labeled cDNAs of IκB MT cells where NF-κB is inhibited and Cy5 signal was derived from labeled cDNAs of control cells. The criteria for inclusion of a cDNA in a group as either induced or repressed expression was whether the ratio Cy3/Cy5 was lower than 0.6 (induced) or greater than 2.0 (repressed). Microarray experiments were done in duplicate and we considered the genes which were found differentially expressed in both experiments. The NF-κB-dependent genes or their encoded protein are listed in Table 1. Treatment of OVCAR-3 cells with IL-1β during 1 hr resulted in gene expression changes of 21 genes from a total of 2400 genes. Eight of these genes were upregulated and 13 were downregulated by NF-κB.

In control cells, among the genes which were overexpressed as compared to the NF-κB-inhibited cells, we

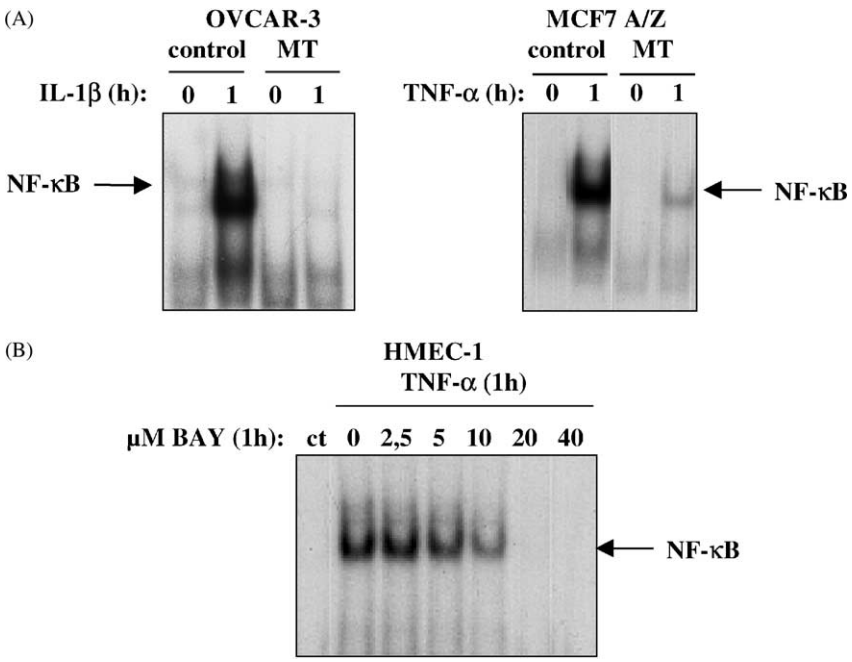

Fig. 1. IκBα expression and inhibition of NF-κB activation in mutated IκBα-overexpressing cells and BAY11-7085-treated cells. (A) NF-κB DNA binding was explored with nuclear extracts from unstimulated or respectively IL-1β- and TNF-α-treated control and IκBα-overexpressing (MT) OVCAR-3 and MCF7 A/Z epithelial cancer cells and a palindromic DNA-binding sequence of NF-κB. These experiments show that overexpression of a mutated nondegradable form of IκBα prevents NF-κB nuclear translocation. (B) HMEC-1 cells were treated with the IKK complex inhibitor, BAY-7085, before TNF-α stimulation to inhibit NF-κB activation. Different doses, ranging from 2.5 to 40 μM were used, and decrease of the NF-κB DNA-binding activity was monitored by electromobility shift assays. The quantitative RT–PCR experiments were performed with 20 μM inhibitor.

Table 1
NF-κB-dependent genes in IL-1β-treated OVCAR-3 cells

Gene Bank Accession number	Protein name	OVCAR-3 IL-1β	MCF7 A/Z TNF-α
Y00787	Monocyte-derived neutrophil chemotactic factor (MDNCF) (IL-8)	0.21	
M57730	B61(ephrin-A1)	0.26	0.29
X14454	Interferon regulatory factor 1(transcription factor)	0.26	0.39
X06990	Intercellular adhesion molecule-1 (ICAM-1).	0.30	0.47
U28838	Transcription factor TFIIIB 90 kDa subunit.	0.56	0.55
S76638	p50-NF-κB homolog	0.58	
M69043	MAD-3 encoding IkB-like activity	0.59	0.34
Z18951	Caveolin-1	0.62	
S62138	TLS/CHOP fusion protein	2.48	14.59
L23805	Alpha1E-catenin (cadherin-associated protein)	2.69	
M90657	Tumor antigen (L6)	2.71	
M17733	Thymosin-beta-4	2.74	
X68277	CL 100 protein tyrosine phosphatase	2.74	
X80909	Alpha-NAC (nascent polypeptide associated complex alpha subunit).	2.86	
L76191	Interleukin-1 receptor-associated kinase (IRAK)	2.88	
D23661	Ribosomal protein L37	3.03	
AB007893	KIAA0433	3.05	
D50683	TGF-βIIR alpha	3.40	
U66838	Cyclin A1	3.62	1.9
X16478	5′-Fragment for vimentin *N*-terminal fragment	3.64	
S78986	Id1 (Id1-a), transcription regulator helix-loop-helix protein	5.02	

Genes were considered as NF-κB induced or NF-κB repressed when the ratio IκB MT/CT was ≤0.6 or ≥2, respectively. The 0.6 ratio was chosen because this ratio is obtained in OVCAR-3 cells for *caveolin-1*, whose induction of mRNA transcription was checked in the control cells by quantitative RT–PCR. The ratio of 2 was chosen because it is the ratio obtained in MCF7 A/Z for *cyclin A1* whose expression of mRNA transcription was checked in the IκB MT cells by quantitative RT–PCR. The genes are ordered according to the increasing fold variation in gene expression observed in OVCAR-3 IκB MT cells. The number are representative of one out of two experiments. Ratios in the right column indicate that similar results were obtained in MCF7 A/Z cells after stimulation with TNF-α.

found these coding for *MDNCF* (*IL-8*) [26], *ICAM* [27], *IRF1* [28], *p50*/NF-κB1 [29], and *Mad-3*/IκBα [30], which are known NF-κB target genes [31–36]. NF-κB inhibition reduced the expression of *caveolin-1* [37] and *ephrin-A1* [38] mRNAs which were not known to be regulated by NF-κB. *Caveolin-1* gene was also found overexpressed in control cells by subtractive hybridization (Delhalle *et al.*, unpublished data). TFIIIB 90 kDa subunit [39] was also overexpressed in control cells. *Mad-3*/IκBα, *TFIIIB* 90 kDa subunit, *ICAM*, *IRF1* and *ephrin-A1* were also overexpressed in TNF-α-treated control MCF7 A/Z cells as compared to MT MCF7 A/Z cells.

When the ratio MT/CT (Cy3/Cy5) was greater than 2, we found 13 genes which were differentially expressed and downregulated by NF-κB. These genes code for proteins with distinct functions in cellular processes including cell adhesion (alpha1E-catenin [40]), cell cycle regulation (cyclin A1 [41]), signaling pathways (IRAK [42], TGF-βR [43], CL 100 tyrosine phosphatase [44]), cytoskeleton (thymosin-beta-4 [45], vimentin [46]), and transcription factors (alpha-NAC [47], Id1 [48], TLS/CHOP [49,50]). NF-κB inactivation also resulted in expression of ribosomal protein L37 [51] and tumor antigen L6 [52]. Two of these genes, *TLS*/CHOP and *cyclin A1*, were also found in TNF-α-stimulated MCF7 A/Z cells, with a respective MT/CT ratio of 14.59 and 1.90.

3.2. Quantitative RT–PCR analysis of ephrin-A1 and caveolin-1 mRNA expression

Among the genes overexpressed in control cell lines, we focused on two of them implied in oncogenesis, *ephrin-A1* and *caveolin-1,* and analyzed their mRNA expression by quantitative real time RT–PCR analysis using the *GAPDH* housekeeping gene as control. We compared control cells and NF-κB-inhibited cells. EMSA were performed with nuclear extracts from TNF-α-stimulated cells preincubated with NF-κB inhibitor (BAY11-7085) at increasing concentrations to verify that NF-κB activation is indeed blocked by this inhibitor (Fig 1B).

Ephrin-A1 is a membrane-bound ligand of the tyrosine kinase receptor EphA2. Eph receptors and their ephrin ligands are involved in the regulation of cell adhesion, cell proliferation, and tumor angiogenesis [53–55]. As shown in Fig. 2, TNF-α stimulation (1 and 2 hr) of the control cells induced a slight induction of the *ephrin-A1* mRNA in ovarian OVCAR-3 (2-fold) and colon HCT116 (2-fold) adenocarcinoma cells and a strong induction in breast MCF7 A/Z adenocarcinoma cells (10-fold). No mRNA induction was observed in cells expressing the mutated IκBα protein which inhibits NF-κB activation (Fig 2A, C, and E) or in cells pretreated with a specific NF-κB inhibitor (BAY11-7085) (data not shown). After IL-1β stimulation,

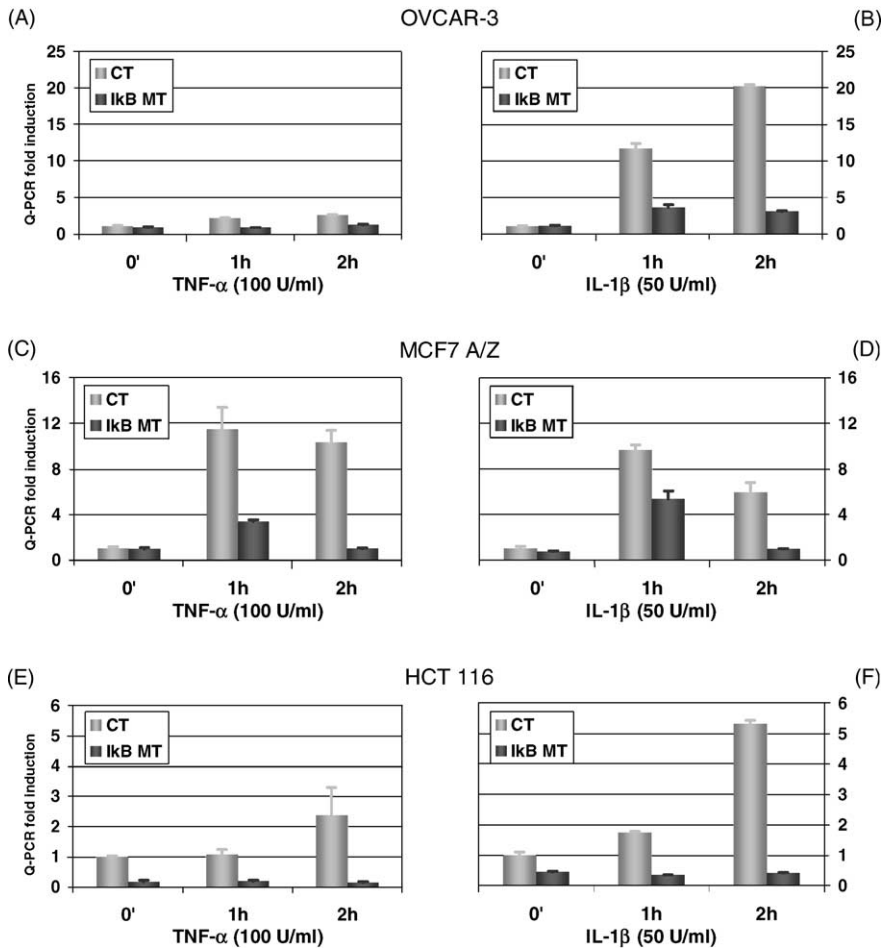

Fig. 2. Quantitative PCR analysis of *ephrin-A1* RNA transcripts in adenocarcinoma cells. The expression of *ephrin-A1* was analyzed by SYBR Green RT–PCR using the light cycler SYBR green master kit (Roche) as described in the experimental procedures. The histograms show the fold induction of *ephrin-A1* RNA in the control cell lines (CT) and in the cell lines overexpressing a mutated form of IκBα (IκB MT) in response to TNF-α or IL-1β stimulation (A–F). (A) and (B). OVCAR-3 cells treated with TNF-α (A) or IL-1β (B), (C) and (D). MCF7 A/Z cells treated with TNF-α (C) or IL-1β (D), and (E) and (F). HCT116 cells treated with TNF-α (E) or IL-1β (F). The level of *ephrin-A1* RNA transcripts was normalized by the level of *GAPDH* RNA transcripts. Experiments were performed in duplicate after cytokine treatment for the indicated times. The results are given as the mean ± SD.

quantitative RT–PCR analysis also showed a strong *ephrin-A1* mRNA induction in all the control cell lines and not in cells where NF-κB is inhibited by the surexpression of the mutated IκB (Fig 2B, D, and F) or by the treatment with BAY11-7085 (data not shown) confirming that *ephrin-A1* mRNA expression is under the control of NF-κB.

As ephrin-A1 seems to play a role in TNF-α-induced tumor neovascularization, we investigated whether *ephrin-A1* mRNA expression is regulated by NF-κB in HMEC-1 endothelial cells. As shown in Fig. 3, *ephrin-A1* mRNA is strongly induced by TNF-α or IL-1β treatment for 1 or 2 hr in HMEC-1 endothelial cells (16- and 10-fold induction, respectively). *Ephrin-A1* mRNA expression was blocked

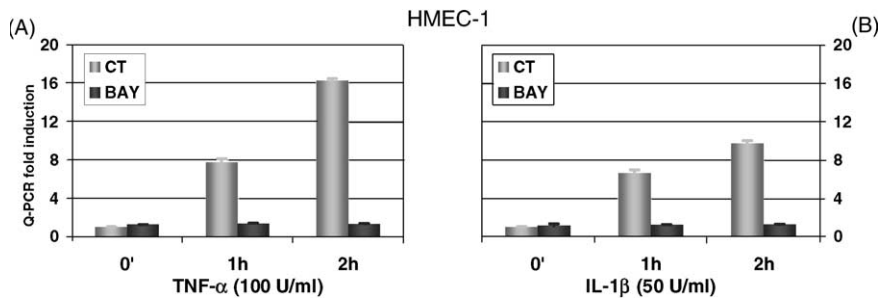

Fig. 3. Regulation of *ephrin-A1* mRNA expression in endothelial cells. The expression of *ephrin-A1* was analyzed by SYBR Green RT–PCR using the light cycler SYBR green master kit (Roche) as described in the experimental procedures. The histograms show the fold induction of *ephrin-A1* mRNA in the HMEC-1 cells either untreated (CT) or treated with BAY11-7085 at 20 μM before TNF-α (A) or IL-1β (B) stimulation.

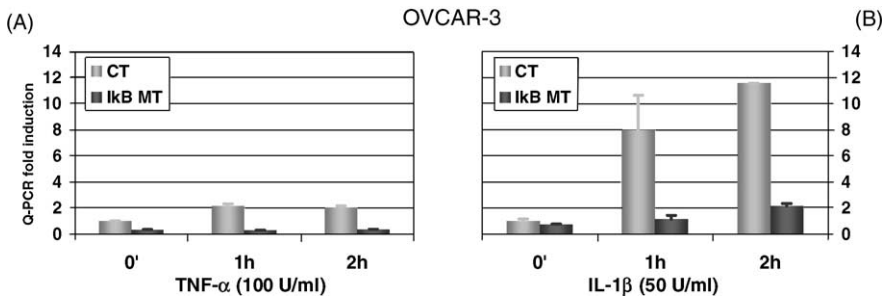

Fig. 4. Quantitative RT–PCR analysis of *caveolin-1* RNA transcripts. The histograms show the fold induction of *caveolin-1* RNA in the OVCAR-3 control cells (CT) and in the OVCAR-3 cells overexpressing a mutated form of IκBα (IκB MT) treated with TNF-α (A) or with IL-1β (B). The level of *caveolin-1* RNA transcripts was normalized to the level of *GAPDH* RNA transcripts.

by the NF-κB inhibitor BAY11-7085, indicating that NF-κB is a regulator of *ephrin-A1* mRNA expression in human endothelial HMEC-1 cells. These results are not in accordance with those reported in HUVEC cells (another endothelial cell line) where TNF-α-induced *ephrin-A1* mRNA expression was not regulated by NF-κB [56]. So far, the *ephrin-A1* promoter is not cloned, and putative binding sites for NF-κB or other transcriptional factors have not yet been identified.

Caveolin-1 is a member of the caveolin family of proteins which coat the cytoplasmic face of plasma membrane invaginations called caveolae. Caveolin-1 has been implicated as a regulator of various intracellular signaling pathways, and changes in *caveolin-1* expression or functions have been observed during cell differentiation, diseases such as diabetes, and oncogenesis. [57]. Caveolae play a role in transcytosis, virus and bacterial entry [58,59]. It has also been shown that *caveolin-1* expression may be upregulated in multidrug resistant cancer cells expressing the P-glycoprotein (reviewed in [60]).

As shown in Fig. 4, *caveolin-1* mRNA was weakly and strongly induced by TNF-α (Fig 4A) and IL-1β (Fig 4B), respectively, in control OVCAR-3 cells. NF-κB inhibition by surexpression of mutated IκBα resulted in a dramatic reduction of *caveolin-1* mRNA levels in response to TNF-α or IL-1β confirming that NF-κB is an important regulator

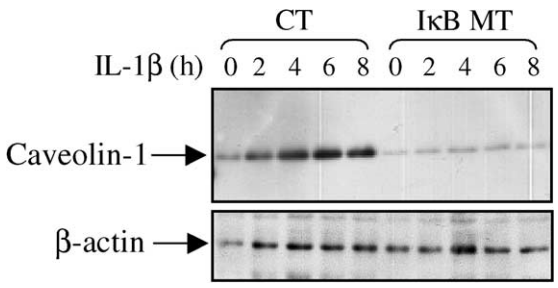

Fig. 5. Expression of caveolin-1 protein in OVCAR-3 cells. Equal amounts of total protein extracts (10 μg) from OVCAR-3 control cells (CT) and from OVCAR-3 cells overexpressing a mutated form of IκBα (IκB MT) were analyzed by immunoblotting for expression of *caveolin-1* after stimulation by IL-1β for the indicated times. Specific bands are indicated in the figure. β-Actin was used as loading control.

of cytokine-induced *caveolin-1* gene expression. The *caveolin-1* expression in response to IL-1β and TNF-α was analyzed in MCF7 A/Z and HCT116 cells, but no induction of mRNA transcription was observed. This reflects the results obtained with the microarrays, where induction of *caveolin-1* appeared only in OVCAR-3 cells.

Under the same conditions of treatment, protein cell extracts were used for Western blotting with anti-caveolin-1 antibody. We found that increased *caveolin* mRNA levels after IL-1β stimulation were correlated with increasing levels of caveolin-1 protein (Fig. 5). Caveolin-1 protein is not induced when NF-κB activation is blocked by the surexpression of the mutated IκBα.

4. Discussion

Using cDNA microarrays, we were able to identify genes that are differentially expressed in cancer cells under the control of NF-κB in response to proinflammatory cytokines. A total of 21 putative genes which are induced or repressed by NF-κB after cytokine treatment were identified. This method allowed to select genes which are up or downregulated by NF-κB and are implied in different cell functions like cell cycle regulation, signaling pathways, and angiogenesis.

We focused on two new NF-κB target genes (*ephrin-A1* and *caveolin-1*) and confirmed the NF-κB-induced mRNA expression by real time quantitative RT–PCR experiments. These results confirmed that NF-κB plays a role in cancer by the transcriptional activation of target genes implied in oncogenesis and cancer progression. Eph receptors and ephrin ligands can affect cell–matrix attachment by modulating integrin activity [61]. Ephrin-A1 promotes human umbilical vein endothelial cell assembly into a capillary-like structure in matrigel assays [62] and seems to be an important mediator of TNF-α-induced angiogenesis in rabbit cornea assays [63]. The ephrin-A1 ligand and its receptor, EphA2, play a role in human cancers by influencing tumor neovascularization, a process essential for tumor growth and malignant progression [55]. Expression of *ephrin-A1* is also upregulated by proinflammatory cyto-

kines during melanoma progression [64]. *Ephrin-A1* expression in tumor cells may influence the interactions of endothelial cells with the surrounding tumor cells. Thus *ephrin-A1* could be added to the list of NF-κB target genes involved in angiogenesis including *VEGF* and *IL-8* [65].

Caveolin-1 is a member of the caveolin family of proteins which coat the cytoplasmic face of plasma membrane invaginations called caveolae. *Caveolin-1* is located at the 7q31.1 subchromosomal locus in man, a known fragile site that is frequently deleted in a wide spectrum of human cancers [60]. *Caveolin-1* has been identified as one of over 20 candidate ''tumor suppressor'' genes that are downregulated in human breast cancer [66]. However, although most data were generated by *in vitro* overexpression studies for *caveolin-1*, an *in vivo* study using biopsy material actually linked the increased expression of *caveolin-1* with metastatic cancers, specifically the progression of prostatic cancer [67]. Caveolin-1 has even been identified as the only biomarker significantly upregulated in African-American prostate cancers [68]. It has been shown that upregulation of *caveolin-1* expression may occur in multidrug resistant cells [60], and *MDR1* (mdr1 mediator/P-glycoprotein) expression is regulated by NF-κB ([69] and Bentires-Alj *et al.*, submitted). Moreover, we have localized a consensual NF-κB DNA-binding site in the promoter sequence of the *caveolin-1* gene (data not shown). This transcriptional upregulation of *caveolin-1* in NF-κB-expressing cells might, thus, be part of a survival response of cancer cells.

Interestingly, our data also indicated that several genes are repressed by NF-κB in response to proinflammatory cytokines. Although NF-κB by itself positively regulates gene expression, it has been shown that it can interfere with other transcription factors activity [70,71].

For instance, NF-κB can inhibit *p53* activity by competing for co-activators [72,73]. We and others have reported that NF-κB downregulates *Bax* expression [72,74]. However, OVCAR-3 cells harbor a mutated *p53*, and this competition is unlikely to explain our data. NF-κB activity has also been recently reported to inhibit the JNK signal transduction pathway through the expression of *XIAP* or *GADD45* [75,76]. Although, only a few of the NF-κB repressed genes reported in the present paper are known to be regulated by *AP-1*, *SP1* [77], or *p53*, similar mechanisms could account for NF-κB-mediated inhibition of other transcription factors activity.

Our data thus demonstrated novel NF-κB target genes involved in cancer progression and indicate that NF-κB-dependent gene repression might be an important feature of NF-κB-expressing cancer cells.

Acknowledgments

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