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NF-κB and Epithelial to Mesenchymal Transition of Cancer

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Abstract During progression of an in situ to an invasive cancer, epithelial cells lose expression of proteins that promote cell–cell contact, and acquire mesenchymal markers, which promote cell migration and invasion. These events bear extensive similarities to the process of epithelial to mesenchymal transition (EMT), which has been recognized for several decades as critical feature of embryogenesis. The NF-κB family of transcription factors plays pivotal roles in both promoting and maintaining an invasive phenotype. After briefly describing the NF-κB family and its role in cancer, in this review we will first describe studies elucidating the functions of NF-κB in transcription of master regulator genes that repress an epithelial phenotype. In the second half, we discuss the roles of NF-κB in control of mesenchymal genes critical for promoting and maintaining an invasive phenotype. Overall, NF-κB is identified as a key target in prevention and in the treatment of invasive carcinomas. J. Cell. Biochem. 104: 733–744, 2008. © 2008 Wiley-Liss, Inc.

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Epithelial to mesenchymal transition (EMT), which has been recognized for several decades as critical for embryogenesis [Korsching et al., 2005], has recently been shown to also be relevant to cancer progression. During EMT of in situ cancer cells, expression of proteins that promote cell–cell contact such as E-cadherin and γ-catenin can be lost, and mesenchymal markers such as vimentin, fibronectin, N-cadherin and the metalloproteinases MMP-2 and MMP-9 can be acquired, resulting in enhanced ability for cell migration and invasion [Kang and Massague, 2004]. During embryonic development in *Xenopus*, *Drosophila* and *C. elegans*, EMT is regulated by a family of zinc finger master regulatory proteins, including Snail, Slug, Twist, ZEB1 and ZEB2/SIP1. In mammalian cells, this family has recently been implicated in repression of genes that promote an epithelial phenotype, thereby inducing transition to a mesenchymal phenotype.

Several studies have indicated that activity of the NF-κB transcription factor family is required for maintenance of an invasive phenotype in cancers induced either by Ras or carcinogen treatment or in sporadic breast cancer cells. Wirth and coworkers identified NF-κB as a central mediator of EMT in a mouse model of breast cancer progression [Huber et al., 2004]. Specifically, inhibition of NF-κB in Ras-transformed epithelial cells (EpRas cells) led to a 10-fold reduction in metastases to the lungs following tail vein injection into nude mice and to a 3-fold decrease in tumor weight in a mammary fat pad model. Our group demonstrated that inhibition of NF-κB activity reduced the invasive phenotype of 7,12-dimethylbenz(*a*)anthracene (DMBA) carcinogen-transformed mammary tumor cells driven by the NF-κB c-Rel subunit [Shin et al., 2006]. More recently, we have shown that the NF-κB

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subunit RelB promotes the more invasive phenotype of estrogen receptor (ER)α negative or low breast cancers [Wang et al., 2007].

In this review, we first briefly introduce the NF-κB family and then discuss the involvement of NF-κB in the control of master regulators of EMT. Then we describe studies elucidating the functions of NF-κB in transcription of mesenchymal genes encoding vimentin, MMP-2 and MMP-9, critical for promoting and maintaining a mesenchymal phenotype.

NF-κB FAMILY OF TRANSCRIPTION FACTORS AND CANCER

NF-κB is a structurally conserved family of dimeric transcription factors distinguished by the presence of an N-terminal 300 amino acid region, termed the Rel homology domain (RHD), which contains sequences mediating dimerization, DNA binding, nuclear localization, and interaction with inhibitory IκB proteins [Ghosh and Karin, 2002]. Mammals have five NF-κB members. The c-Rel, RelB, and p65 (also known as RelA) subunits are synthesized as mature products and contain a C-terminal transactivation domain. The p50 (NF-κB1) and p52 (NF-κB2) subunits are synthesized as longer precursors, p105 and p100, respectively, that require C-terminal proteolytic processing. Activation of the IKKα kinase has been implicated in processing of p100 and p105 to p52 and p50, respectively [Senftleben et al., 2001]. Although, p50 and p52 lack a C-terminal transactivation domain, they can transactivate when associated with Bcl-3 protein [Bours et al., 1993; Westerheide et al., 2001; Romieu-Mourez et al., 2003]. NF-κB factors bind DNA target sites as hetero- or homo-dimers and have different activities depending on subunit composition [Ballard et al., 1992; McDonnell et al., 1992; La Rosa et al., 1994; Lee et al., 1995; Baek et al., 2002; Jiang et al., 2002; Hoffmann et al., 2003; Bonizzi et al., 2004]. NF-κB controls genes encoding proteins involved in cell growth (e.g., Cyclin D1, c-Myc, Gro), adhesion (e.g., VCAM, ICAM), cell survival (e.g., Bcl-x_L, Bcl-2, and Bfl1/A1), and immune and inflammatory responses (e.g., IL-2, IL-6, IL-8).

While NF-κB factors are ubiquitously expressed, constitutive functional nuclear NF-κB activity occurs normally only in mature B-lymphocytes and a few other cell types. In most cells, inactive NF-κB proteins are sequestered in the cytoplasm in a complex with an inhibitor protein, termed IκB. NF-κB can be transiently activated to enter the nucleus by a variety of signals. In most cases, activation of NF-κB proceeds by activation of an IκB kinase (IKK) complex, which phosphorylates IκB and leads to its degradation. Two major pathways of post-translational activation of the NF-κB/IκB complexes have been established: the canonical and alternative pathways, leading to activation of p50/RelA or p50/c-Rel and p52/RelB or p50/RelB, respectively. More recently, we have described a transcriptional pathway mediated by p50/RelA or p50/c-Rel and AP-1 complexes, including c-Jun/Fra-2, which leads to de novo synthesis of the RelB protein [Mineva et al., 2007; Wang et al., 2007].

Almost a decade ago, high levels of nuclear NF-κB were unexpectedly found in human breast tumor cell lines, carcinogen-transformed mammary epithelial cells, and the majority of primary human and rodent breast tumor tissue samples [Nakshatri et al., 1997; Sovak et al., 1997]. It is now well established that primary human breast cancer tissues constitutively express functional c-Rel, RelA, RelB, p50, p52, or Bcl-3 [Sovak et al., 1997; Cogswell et al., 2000; Wang et al., 2007], as well as the IKKα or IKKβ kinases of the IKK NF-κB activating complex [Romieu-Mourez et al., 2001]. In addition, we have shown that the IKK-related kinase IKKε/i is active in breast cancer cells and regulates NF-κB activity and invasive phenotype [Eddy et al., 2005]. Amplification of the *IKBKE* gene has also been described in some breast cancers [Boehm et al., 2007]. Moreover, ectopic expression of c-Rel is sufficient to induce late onset mammary tumorigenesis in a transgenic mouse model, pointing to a potential causal role for aberrant NF-κB expression in human breast cancers [Romieu-Mourez et al., 2003]. ERα negative and low cancer cells in culture display high nuclear levels of NF-κB binding activity due, in part, to the lack of ERα-mediated inhibition of NF-κB [Nakshatri et al., 1997]. ERα has also been shown to inhibit de novo RelB synthesis and c-Rel binding and activity in breast [Belguise and Sonenshein, 2007; Wang et al., 2007] and other cancers [reviewed in Kalaitzidis and Gilmore, 2005]. Aberrant expression of one or more NF-κB subunit has now been reported in many solid cancers, including head and neck, prostate, lung and pancreatic adenocarcinomas, and hematopoietic malignancies

[reviewed in Rayet and Gelinas, 1999]. NF-κB has been shown to promote the transformed phenotype and survival of cancer cells, and these topics have been the subjects of several recent reviews [Gilmore et al., 2002; Gilmore, 2003; Basseres and Baldwin, 2006; Van Waes, 2007].

NF-κB IN REPRESSION OF AN EPITHELIAL PHENOTYPE

Several developmentally important transcription factors that induce EMT have been shown to repress epithelial gene expression. In the best-studied case, *E-cadherin* gene transcription is inhibited through specific E-boxes in the proximal promoter [Nieto, 2002; Peinado et al., 2004a]. Most prominent in this regulation are the Snail-related zinc-finger transcription factors Snail and Slug [Nieto, 2002]. Two members of the ZEB family, ZEB1 (or TCF8 and δEF1) and ZEB2 (also known as ZFXH1B and SMAD interacting protein 1 (SIP1)) have also emerged as important factors for the regulation of E-cadherin and EMT [Comijn et al., 2001; Eger et al., 2005]. The findings of Yang et al. [2004] added Twist, a basic helix–loop–helix (bHLH) transcription factor, to the growing list of developmental genes with a key role in repression of E-cadherin and induction of EMT [Kang and Massague, 2004; Yang et al., 2004]. Multiple lines of evidence indicate that these factors are regulated either directly or indirectly by NF-κB.

Snail

The Snail family of transcriptional repressors regulate various aspects of EMT during embryonic development as well as participate in tumor progression [Nieto, 2002]. During development in *Drosophila*, Snail proteins are involved in the ingression of the early mesodermal cells at gastrulation and in the delamination of the neural crest from the neural tube [Nieto, 2002]. Snail mutant mice die at gastrulation due to defective EMT, in part caused by persistent E-cadherin expression [Carver et al., 2001]. In mammalian cells, Snail has been shown to be a direct repressor of *E-cadherin* (*CDH1*) gene transcription and Snail expression induces a full EMT and increases migration/invasion in different physiological and pathological situations [Batlle et al., 2000; Cano et al., 2000; Peinado et al., 2004b]. Snail also downregulates the expression of additional epithelial genes, including *claudins*, *occludins*, and *muc1* [Guaita et al., 2002; Ikenouchi et al., 2003; Ohkubo and Ozawa, 2004; Martinez-Estrada et al., 2006], and induces the expression of genes that have been associated with a mesenchymal and invasive phenotype, including *fibronectin* and *metalloproteinase* (*MMP*)*-9* [Cano et al., 2000; Guaita et al., 2002; Jorda et al., 2005]. Snail expression has been detected in different invasive carcinoma and melanoma cell lines and, importantly, in invasive regions of squamous cell carcinomas and dedifferentiated ductal breast carcinomas and hepatocarcinomas [reviewed in Nieto, 2002; Peinado et al., 2004c], supporting a key role for Snail in induction of EMT and tumor invasion.

Snail activity is regulated at multiple levels. For example, selective phosphorylation by GSK3 leads to export of Snail from the nucleus and to its destruction via the ubiquitin-proteasome pathway [Zhou et al., 2004]. Mercurio and coworkers showed that GSK3 inhibition stimulates the transcription of the human gene encoding Snail (*SNAI1*) via NF-κB signaling [Bachelder et al., 2005]. In *Drosophila*, *Snai1* transcription is directly activated by the NF-κB homologue Dorsal [Ip et al., 1992]. Barbera et al. [2004] localized a region between −194 and −78 bp in the human *SNAI1* promoter, which is required for stimulation of *SNAI1* expression by ectopic co-expression of NF-κB p65 [Barbera et al., 2004]. More recently, NF-κB was identified as the upstream regulator of Snail expression during EMT of human mammary epithelial MCF10A cells overexpressing a constitutively active Type I insulin-like growth factor receptor (IGF-IR) [Kim et al., 2007]. Specifically, the induction of *SNAI1* mRNA levels during EMT can be reversed by inhibition of NF-κB signaling. Together, these observations support an important role of NF-κB in regulation of *Snai1* gene transcription.

Slug

Slug (Snail2) is a zinc-finger transcription factor in the Snail superfamily [Barrallo-Gimeno and Nieto, 2005]. Similar to *Snail*, *Slug* has been implicated in the control of gastrulation in invertebrates, and in emergence of the neural crest. Slug is also required for EMT of atrioventricular canal endothelial cells

[Romano and Runyan, 1999] and for mesoderm formation in the chick [Nieto et al., 1994]. Slug can also repress endogenous *E-cadherin* gene expression [Bolos et al., 2003], and cause desmosome dissociation [Savagner et al., 1997]. Slug also downregulates the expression of the epithelial *claudins* and *occludins* genes [Kajita et al., 2004]. In breast cancer cell lines, Slug levels were shown to correlate with loss of *E-cadherin* transcripts [Hajra et al., 2002], and with decreased expression of cytokeratins 8 and 19 [Tripathi et al., 2005]. Furthermore, elevated Slug expression is a marker of poor prognosis of breast [Martin et al., 2005], lung [Shih et al., 2005], colorectal [Shioiri et al., 2006] and pancreatic cancers [Hotz et al., 2007].

A recent article identified the *Slug* gene as a target of dioxin-activated aryl hydrocarbon receptor (AhR) [Ikuta and Kawajiri, 2006]. Historically, the AhR has been studied for its responsiveness to a variety of environmental chemicals (e.g., polycyclic aromatic hydrocarbons, PCBs, and dioxins) and its ability to transactivate genes encoding phase I cytochrome P-450 enzymes that metabolize endogenous substrates (e.g., 17β-estradiol) and some environmental chemicals into mutagenic intermediates [Safe and Krishnan, 1995; Spink et al., 2003]. However, more recent studies have indicated roles for the AhR, even in the absence of environmental ligands, in the transcriptional regulation of genes critical for growth [Ma and Whitlock, 1996; Elizondo et al., 2000; Tohkin et al., 2000; Abdelrahim et al., 2003; Patel et al., 2006], apoptosis [Elizondo et al., 2000; Patel et al., 2006; Wu et al., 2007], and in EMT and tumor progression [Mulero-Navarro et al., 2005]. For example, immortalized mouse mammary fibroblasts lacking AhR have impaired tumorigenicity in a subcutaneous mouse xenograph model [Mulero-Navarro et al., 2005], and DMBA treatment induced a more highly invasive phenotype in c-Rel-driven mammary tumor cell line [Shin et al., 2006]. Of note, we have recently shown that ectopic co-expression of c-Rel and the protein kinase CK2 in mammary epithelial cells induces AhR levels and Slug expression, which promote an invasive phenotype [Belguise et al., 2007]. Consistently, the mammary tumor cells from bitransgenic mice with enforced expression of c-Rel and CK2 displayed elevated levels of AhR and Slug and a highly invasive phenotype. Thus, NF-κB may indirectly regulate expression of Slug via the AhR, even in the absence of carcinogens.

ZEB1 and ZEB2

Members of the ZEB family, ZEB1 and ZEB2/SIP1, are important mediators of EMT [Comijn et al., 2001; Eger et al., 2005] that have been implicated in several different types of tumors [Rosivatz et al., 2002; Miyoshi et al., 2004; Elloul et al., 2005]. ZEB2/SIP1 knock-out mice display delamination arrest of cranial neural crest cells, resulting in the loss of migratory behavior of these cells [Van de Putte et al., 2003]. Furthermore, a role for upregulation of ZEB-family member during EMT was also demonstrated during tumor progression [Jechlinger et al., 2003]. Enhanced ZEB2 expression has to date been reported in a distinct set of cancers, including gastric, hepatocellular, ovarian and breast carcinomas [Rosivatz et al., 2002; Miyoshi et al., 2004; Elloul et al., 2005]. ZEB2 coordinately represses the transcription of epithelial cell junctional genes via direct interaction with ZEB2-binding sites within the promoter [Vandewalle et al., 2005]. ZEB1 has also been found to be upregulated during EMT, and its ectopic expression is sufficient to induce the downregulation of E-cadherin and ZO-1, disintegration of cell–cell junctions, and induction of mesenchymal marker proteins in epithelial cells [Eger et al., 2005].

NF-κB activation has been associated with the induction of ZEB1 and ZEB2 expression. MCF-10A cells stably expressing the NF-κB subunit p65 (MCF-10A/p65 cells) displayed elevated levels of expression of ZEB1 and ZEB2 compared to the parental MCF-10A line [Chua et al., 2007]. Moreover, in transient transfection assays, p65 increased *ZEB1* promoter activity [Chua et al., 2007]. Induction of ZEB1 and ZEB2 by NF-κB was also observed following treatment of MCF-10A cells with IL-1α or TNFα [Chua et al., 2007]. Thus, ZEB1 and ZEB2 may serve as key mediators of p65 NF-κB signaling during EMT.

Twist

The bHLH transcription factor Twist is essential for initiation of mesoderm development during gastrulation [Castanon and Baylies, 2002]. Increased Twist expression has been found in breast carcinomas [Watanabe et al.,

2004; Yang et al., 2004], as well as in several other cancer types, including melanomas [Hoek et al., 2004], gastric [Rosivatz et al., 2002], and prostate cancers [Kwok et al., 2005]. A role for Twist in cancer metastasis was reported in a breast cancer model, which suggested that Twist induces EMT and promotes tumor invasion [Yang et al., 2004]. Weinberg and coworkers found that inhibition of *Twist* expression diminished the metastatic potential of the 4T1 cell line [Yang et al., 2004]. Twist specifically mediated intravasation into the systemic circulation rather than survival of tumor cells. Ectopic Twist expression was sufficient to induce phenotypic and molecular hallmarks of EMT in MDCK cells and in immortalized human mammary epithelial cells. Twist repressed the *E-cadherin* promoter and gene transcription. Of note, an inverse correlation between high Twist expression and low E-cadherin levels was seen in human invasive lobular breast carcinomas [Yang et al., 2004]. Furthermore, elevated Twist expression has been correlated positively with an aggressive breast cancer phenotype and poor patient survival [Hoek et al., 2004; Yang et al., 2004; Kwok et al., 2005]. A recent study identified AKT2, a known metastasis gene, as a downstream target and functional mediator of Twist during cell migration and invasion [Cheng et al., 2007].

Twist is an evolutionary conserved NF-κB target gene [Wang et al., 1997; Kanegae et al., 1998; Takeda et al., 1999; Sosic and Olson, 2003; Sosic et al., 2003]. In *Drosophila*, *twist* is a direct transcriptional target of the NF-κB protein Dorsal [Jiang et al., 1991; Pan et al., 1991; Thisse et al., 1991]. Inhibition of NF-κB in mice by either expression of the super repressor form of IκBα or ablation of the IKKα kinase caused a dramatic impairment of Twist expression, and to morphogenetic defects in embryonal development [Kanegae et al., 1998; Takeda et al., 1999]. Moreover, *Twist* can be rapidly induced in mouse embryonic fibroblasts by TNF-α, and this induction is essentially absent in cells lacking the p65 NF-κB subunit [Sosic et al., 2003]. Interestingly, Pham and coworkers found that upregulation of Twist by chemotherapeutic agents in certain cancers depended on p65 NF-κB complexes, while in other systems, c-Rel-containing NF-κB complexes were sufficient to activate *Twist* gene transcription [Pham et al., 2007].

NF-κB IN THE INDUCTION OF MESENCHYMAL MARKERS

Vimentin

The *vimentin* (*VIM*) gene encodes a 56-kDa cytoskeletal protein that is part of the large intermediate filament (IF) gene family. Vimentin is the major IF protein found in mesenchymal cells. Because of the abundance of its expression, it had long been thought to be at least partially responsible for structural integrity of mesenchymal cells. Thus, it was surprising that knockout vimentin mice undergo normal embryonic development into adulthood and are able to produce offspring with no overt phenotype [Colucci-Guyon et al., 1994]. However, further analysis of vimentin −/− mice have shown significant impairment of wound healing in both embryonic and adult animals [Eckes et al., 2000], likely due to reduced migratory ability of vimentin −/− fibroblasts [Eckes et al., 1998]. These studies have solidified a functional role for vimentin in cellular migration in addition to its status as a mesenchymal marker.

Vimentin expression has often been described as the end stage progression in EMT, representing the completely dedifferentiated state in tumor cells that are highly proliferative and invasive. Consistent with this view, overexpression of vimentin in breast cancer cells in culture led to enhanced migration of breast cancer cells [Hendrix et al., 1996]. Conversely, knockdown of vimentin with antisense oligonucleotides reduced cell motility. In vivo expression of vimentin, however, is a rare occurrence even in invasive breast cancers. In a recent study using tissue microarray analysis, vimentin was found expressed in 21/272 breast cancer cases and correlated positively with tumor grade [Korsching et al., 2005]. Most were found to be Grade 3 invasive ductal carcinomas (19/21) but the majority (13/21) of these were associated with the ductal in situ component [Korsching et al., 2005], suggesting that the role of vimentin in EMT in vivo may be more complicated due to its possible involvement in progenitor cells.

The *VIM* promoter is comprised of multiple elements responsible for its transcriptional regulation. An NF-κB binding site has been implicated in growth factor responsiveness [Lilienbaum et al., 1990; Lilienbaum and Paulin, 1993], and in the induction of *VIM* mRNA in response to expression of the Human T-lympho-

trophic virus-1 (HTLV-1) Tax protein, a well-known activator of NF-κB [Lilienbaum et al., 1990]. Tax-induced activation of NF-κB leads to NF-κB binding to an element on the *VIM* promoter located between nucleotides −239 and −197 bp as judged by binding, competition EMSA, mutational analysis and reporter assays [Lilienbaum and Paulin, 1993]. Overexpression of the RelB in breast cancer cells induces vimentin expression, and a more mesenchymal phenotype [Wang et al., 2007]. Additionally, overexpression of a constitutively active form of p65 in MCF-10A breast cancer cells also results in increased expression of vimentin and to a more mesenchymal phenotype [Chua et al., 2007].

MMP-2 and MMP-9

Matrix metalloproteases (MMPs) are type IV collagenases, which increase cellular invasiveness and mobility. MMP-2 and MMP-9 are members of the family of 28 known Zn^{2+}- and Ca^{2+}-dependent extracellular matrix proteases. MMP-2 and MMP-9 degrade components of the extracellular matrix including denatured collagen, native type IV collagen, as well as collagen V/XI and elastin [Vu and Werb, 2000]. Of note, the rapid degradation of type IV collagen in the basement membrane allows for tumor invasion and metastasis. MMP-9, and to a lesser extent MMP-2, expression has been correlated with a poor prognoses in a number of cancers, including breast, bladder, and prostate [Davies et al., 1993b; Jones and Walker, 1997; Miyamoto et al., 2005]. MMP-9 expression in breast cancer also correlated with advanced tumor grade in that MMP-9 was found to be expressed in 11 of 11 grade III tumors [Davies et al., 1993a]. Of 43 total biopsies, a higher mean expression of MMP-9 was found in grade III breast tumors than in lower grade and benign tumors or normal mammary tissue [Davies et al., 1993a]. In a tissue microarray analysis of 131 patient cancer specimens, high expression of MMP-9 was associated with shortened relapse-free survival [Vizoso et al., 2007], consistent with its role as a type IV collagenase involved in tumor invasion and metastasis [Jones et al., 1999].

In early animal studies of metastasis, H-Ras-transformed rat embryo fibroblasts were found to express MMP-2 and MMP-9 and to display enhanced metastatic behavior in nude mouse models [Garbisa et al., 1987]. Addition of tissue inhibitor of MMP (TIMP) resulted in inhibition of the MMP-9 (92 kDa type IV collagenase). Furthermore, intravenously injected Ras-transformed rat embryo fibroblasts show a markedly reduced ability to metastasize to the lungs of nude mice after repeated recombinant intraperitoneal injection of TIMP [Alvarez et al., 1990]. Consistent with these observations, overexpression of MMP-9 was observed in the highly malignant H-Ras and v-*Myc* transformed rat embryo 2.10.10 cell line, and ribozyme-mediated degradation of MMP-9 resulted in reduced metastatic phenotype [Hua and Muschel, 1996]. Rat mammary adenocarcinoma cell clones, isolated for their ability to cause lung metastases after subcutaneous or intravenous injections, were also found to have high levels of type IV collagenase activity [Nakajima et al., 1987]. Kupferman and coworkers bred female homozygous mice transgenic for a fragment of the rabbit MMP-9 promoter (−522 to +12) linked to β-galactosidase, with males homozygous for MMTV polyoma middle T antigen, a transgene that leads to mammary tumorigenesis in female mice [Guy et al., 1992]. The resulting bitransgenic female progeny developed breast cancer displaying β-galactosidase expression, as a measure of MMP-9 reporter activity, only upon the formation of invasive carcinomas [Kupferman et al., 2000]. Thus, type IV collagenases are induced in metastatic cell types and are partly responsible for the metastatic phenotype.

NF-κB is responsible for activation of *MMP-9* promoter transcription [Himelstein et al., 1997]. Deletion or mutation of the NF-κB site at −599 bp resulted in a threefold reduction in reporter activity of a human *MMP-9*-CAT construct [Himelstein et al., 1997]. Consistent with these findings, the *MMP-9* promoter is activated in Bcl-2-overexpressing adriamycin-resistant cells; mutation of the −599 bp NF-κB site eliminates Bcl-2-mediated activation of the promoter. In addition, RelA was found to be the primary NF-κB factor that binds to this region [Ricca et al., 2000]. Similar findings were obtained in SK-N-SH cells, which undergo spontaneous phenotypic conversion from epithelial to neuroblastic phenotype, which in these cells represents a more migratory, invasive and tumorigenic phenotype [Farina et al., 1999]. In transient transfection assays, SK-N-SH cells displaying an epithelial phenotype failed to activate an *MMP-9*-CAT reporter

construct, while those displaying a neuroblast phenotype displayed an eightfold elevation in CAT activity [Farina et al., 1999]. In vitro footprint analysis revealed two regions on the *MMP-9* promoter relative to the transcriptional start site that were protected in neuroblastoma cells; −610 to −553 bp and −79 to −28 bp, which contained a putative NF-κB element and a putative SP-1 element, respectively. The functional role of the NF-κB site was confirmed when mutation of the element reduced activity of the −670-*MMP-9*-CAT promoter by 60% compared to the wild-type control [Farina et al., 1999]. Thus, NF-κB regulates *MMP-9* gene transcription through an element located approximately 600 bases upstream of the transcriptional start site.

NF-κB can indirectly regulate MMP-2 activity via control of an enzyme mediating post-translational processing. MMP-2 is synthesized in a precursor form, termed pro-MMP-2. To produce active MMP-2, the N-terminal pro-domain must be cleaved, which is done by a membrane type metalloprotease (MT-MMP) and by TIMP [Yoshizaki et al., 2002]. In dermal fibroblasts, NF-κB can increase MMP-2 activity by inducing the expression of MT-MMP rather than by acting directly on the *MMP-2* promoter [Han et al., 2001]. Blocking osteopontin-induced NF-κB activity with an IκB-α super-repressor also caused a reduction in MMP-2 activity in murine melanoma cells [Philip et al., 2001]. Thus, NF-κB indirectly regulates MMP-2 activity, which is distinct from the direct transcriptional regulation seen for MMP-9.

Consistent with the above data, transgenic mice overexpressing NF-κB p100/p52 under control of the β-lactoglobulin promoter display elevated MMP-9 and MMP-2 activity in the mammary gland [Connelly et al., 2007]. Although the mammary glands did not appear to undergo EMT, increased hyperplastic growth of mammary cells was found [Connelly et al., 2007]. Lastly, expression of a constitutively active IKKε/i in kidney epithelial cells caused a significant increase in MMP-9 expression [Boehm et al., 2007], consistent with its role in invasive phenotype [Eddy et al., 2005]. Conversely, osteoclasts deficient in IKKα displayed muted expression of MMP-9 in response to RANK activation also consistent with the requirement for NF-κB in regulation of MMP-9 [Chaisson et al., 2004].

CONCLUSIONS

NF-κB is at the center of multiple pathways that promote an invasive phenotype (see Fig. 1). Given that NF-κB also plays key roles in innate immunity, the challenge is to target these factors effectively in cancer. In a recent review [Gilmore and Herscovitch, 2006], 785 inhibitors of NF-κB were described. Introduction of some of these into the clinic will hopefully enable more effective treatment of patients with invasive cancers.

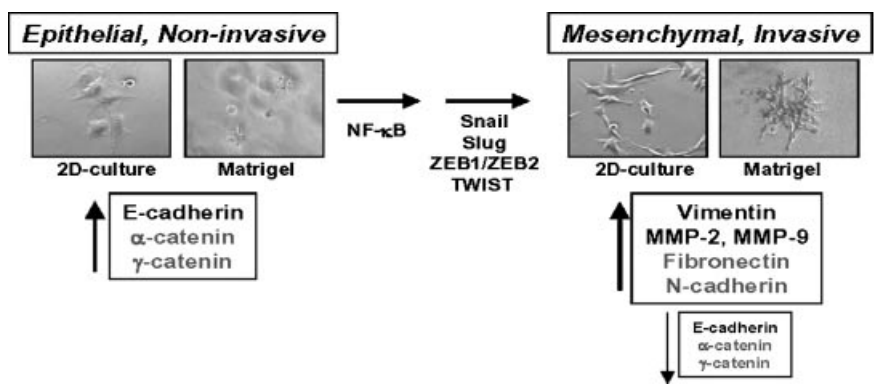

Fig. 1. The NF-κB family of transcription factors promotes a mesenchymal phenotype. Non-invasive epithelial cells typically express E-cadherin, α-catenin and γ-catenin. During transition to a more invasive mesenchymal phenotype, levels of these markers decrease while expression of vimentin, metalloproteinases MMP-2 and MMP-9, fibronectin and N-cadherin increases. NF-κB plays pivotal roles in both facets of this transition, either directly or indirectly. NF-κB induces expression of the master regulators Snail, Slug, ZIB1, ZIB2 and Twist, which repress expression of genes encoding epithelial markers such as *E-cadherin*. NF-κB also induces expression of mesenchymal markers Vimentin, MMP-2 and MMP-9, as explained in this review (known NF-κB regulated epithelial/mesenchymal markers are indicated in black font).

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