Activation of NF-κB Signaling by Inhibitor of NF-κB Kinase β Increases Aggressiveness of Ovarian Cancer

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Abstract
The NF-κB family of transcription factors has been implicated in the propagation of ovarian cancer, but the significance of constitutive NF-κB signaling in ovarian cancer is unknown. We hypothesized that constitutive NF-κB signaling defines a subset of ovarian cancer susceptible to therapeutic targeting of this pathway. We investigated the biological relevance of NF-κB in ovarian cancer using a small-molecule inhibitor of inhibitor of NF-κB kinase β (IKKβ) and confirmed with RNA interference toward IKKβ. We developed a gene expression signature of IKKβ signaling in ovarian cancer using both pharmacologic and genetic manipulation of IKKβ. The expression of IKKβ protein itself and the nine-gene ovarian cancer–specific IKKβ signature were related to poor outcome in independently collected sets of primary ovarian cancers (P = 0.02). IKKβ signaling in ovarian cancer regulated the transcription of genes involved in a wide range of cellular effects known to increase the aggressive nature of the cells. We functionally validated the effect of IKKβ signaling on proliferation, invasion, and adhesion. Downregulating IKKβ activity, either by a small-molecule kinase inhibitor or by short hairpin RNA depletion of IKKβ, blocked all of these cellular functions, reflecting the negative regulation of the target genes identified. The diversity of functions controlled by IKKβ in ovarian cancer suggests that therapeutic blockade of this pathway could be efficacious if specific IKKβ inhibitor therapy is focused to patients whose tumors express a molecular profile suggestive of dependence on IKKβ activity.

Introduction
Ovarian cancer is the fifth most common form of cancer in women in the United States, resulting in more than 15,000 deaths per year, making it the most lethal gynecologic cancer in this country (1). The majority of deaths are attributed to serous carcinoma, most commonly identified at an advanced stage with metastases present beyond the ovaries, which precludes curative treatment. Goals in the field include improved screening and diagnostics and, until they result in early detection, improved therapy of advanced disease at the outset and for recurrence (2, 3). Gene expression profiling studies have identified subsets of patients presenting aggressive disease who respond differently to standard surgery and chemotherapy treatment (4). Attempts were made to identify molecular signatures of patients that correlate with better survival. The Cancer Genome Atlas project has rapidly and comprehensively advanced the molecular profiling of ovarian cancer through large-scale gene expression profiling, comparative genomic hybridization, single-nucleotide polymorphism analysis, and gene exon sequencing (http://cancergenome.nih.gov). Analysis of these efforts has made clear that ovarian cancer is an extremely heterogeneous disease. A single approach to chemotherapy for all ovarian cancer patients is unlikely to achieve similar success across all patients. Therefore, there is pressing need to identify the molecular etiology driving defined subgroups of ovarian cancers and to develop alternate treatments targeting such pathways to improve specific patient survival.

The involvement of NF-κB in cancer dissemination makes it a logical target (5). The NF-κB family of transcription factors is ubiquitously expressed and has been studied extensively in lymphoid development and lymphoid malignancies. These transcription factors have been implicated in the propagation of solid tumors (6). Although found in ovarian cancer, the significance and the mechanism of constitutive NF-κB signaling in ovarian cancer are unexplored. We hypothesized that constitutive NF-κB signaling defines a subset of ovarian cancer susceptible to therapeutic targeting of this pathway.

Constitutive NF-κB signaling has been identified in tumors of epithelial origin, including breast, colon, lung, and ovarian carcinomas (6). A systems biology approach integrating three global screening techniques discovered inhibitor of NF-κB (IκB) kinase ε (IKKe), a cytosolic kinase involved in triggering
NF-κB signaling, as an oncogene in breast cancer (7). Recent work suggested the importance of these transcription factors in the propagation of ovarian cancer cell lines (8). We recently showed the NF-κB family proteins are expressed and coregulated in primary ovarian cancer tumors, and overexpression of the NF-κB subunit p50 at diagnosis conveyed poor outcome.1

Having established the coordinate presence of the NF-κB machinery in ovarian cancers, we sought to modulate its activity. The NF-κB family consists of five subunits that join into active dimers. Homodimers or heterodimers form the active transcription factor complex. IκBs are tagged for degradation through the proteasome on specific inducible phosphorylation by IKKs (9). Therefore, targeted inhibition of IKKs could isolate NF-κB as a mechanism for the growth and survival of ovarian cancers. Iκκβ was the focus of the current investigation in ovarian cancer. Here, we examined the biological relevance of NF-κB in ovarian cancer using a small-molecule inhibitor of IKKβ, ML120b (Millennium Pharmaceuticals), and confirmed with RNA interference using stably expressed short hairpin RNA (shRNA) molecules toward IKKβ.

Materials and Methods

Reagents. Lysophosphatidic acid (LPA) was purchased from Avanti Polar Lipids. Before use, LPA was dissolved in PBS containing 1% fatty acid–free bovine serum albumin. ML120b [N-(6-chloro-7-methoxy-9H-β-carbolin-8-yl)-2-methylnicotinamide], a β-carboline compound, was obtained from Millennium Pharmaceuticals (10). IKK-2 inhibitor IV [5-(p-fluorophenyl)-2-ureido[thiophene-3-carboxamide], a ureidothiophene carboxamide compound, and IKK-2 inhibitor VI [5-phenyl-2-ureido]thiophene-3-carboxamide], a ureido-thiophencarboxamide compound, were purchased from EMD Biosciences. Each compound is reportedly highly specific to IKKβ kinase inhibition (10, 11). Puromycin, DMSO, MTT, 2,3-bis[2-methoxy-4-nitro-5-sulfophenyl]-2H-tetrazolium-5-carboxanilide inner salt (XTT), phenazine methosulfate, noble agar, and mouse monoclonal antibody to β-αβ-2 – tubulin were purchased from Sigma-Aldrich. Antibodies to IKKα were from Cell Signaling Technology, Inc.; IKKβ from Abcam; and IKKε from Millipore.

Patients and samples. The Regional Committee for Medical Research Ethics in Norway approved this study. Tissue microarrays (TMA) containing 2-mm cores (n = 270) from 119 ovarian carcinoma specimens (42 primary carcinomas, 77 solid metastases) operated on at the Norwegian Radium Hospital were additionally studied. Tumors were predominantly (>80%) of the serous type. Metastases were to the omentum (n = 46), peritoneum (n = 15), intestine (n = 12), lymph nodes (n = 2), or various other sites (n = 2). Tumors were from 56 patients, of whom 38 had both primary carcinoma and one or more metastasis for evaluation, 4 had only primary carcinoma, and 14 had only one or more metastasis. Tumors underwent microscopic confirmation of diagnosis, histologic type, and grade by a gynecopathologist (B.D.). Grading was according to the International Federation of Gynecologists and Obstetricians system.

Gene expression profiles were obtained from http://www.ncbi.nlm.nih.gov/geo/ (4, 12). The probe sets in the IKKβ signature are CLDN1, 218182_s_at; CXCL1, 204470_at; CXCL2, 209774_s_at; interleukin-8 (IL-8), 211506_s_at; INSIG1, 201627_s_at; ITGB6, 208083_s_at; PTGER2, 206631_at; PTGS1, 215813_s_at; and SOD2, 215078_at.

Immunohistochemistry. Expression was scored based on the number of positive cells in a sample rather than the intensity of staining to minimize bias due to technical variation across samples. Staining extent was scored on a scale of 0 to 4, as follows: 0 = no staining, 1 = 1% to 5%, 2 = 6% to 25%, 3 = 26% to 75%, 4 = 76% to 100% stained tumor cells.

Statistical analysis. Statistical performance was applied using the SPSS-PC package (version 15.0). Probability of <0.05 was considered statistically significant. Analysis of the association between IKKβ expression by immunohistochemistry and clinicopathologic parameters was undertaken using the two-sided χ2 test. Progression-free survival (PFS) and overall survival (OS) were calculated from the date of diagnosis to the date of recurrence/death or last follow-up. Univariate survival analyses of PFS and OS were executed using the Kaplan-Meier method and log-rank test. Expression categories in the latter test were grouped based on staining extent above or below 25% so as to allow for a sufficient number of cases to be included in each category.

Cell lines and culture. Ovarian cancer cell lines CAOV3 and SKOV3 were obtained from the American Type Culture Collection (ATCC), A2780 cell line was a gift from Tito Fojo [National Cancer Institute (NCI), Bethesda MD], HeyA8 was a gift from Gordon Mills (M.D. Anderson Cancer Center, Houston, TX), and human vascular endothelial cells (HMVEC) were obtained from Cascade Biologics (Invitrogen). OVCAR5, OVCAR8, and IGROV1 cells were from the NCI-Frederick Developmental Therapeutics Program tumor/cell line repository (Frederick, MD). All lines were cultured in RPMI 1640 plus 10% fetal bovine serum (HyClone) and standard antibiotics. Cell lines were authenticated in July 2009 at the Johns Hopkins University Fragment Analysis Facility (Baltimore, MD) using Promega PowerPlex I.2 System to test for eight STR markers (D16S539, D7S820, D13S317, D5S818, CSF1PO, TPOX, THO1, and vWA) and amelogenin for gender determination. Authenticity was confirmed against the ATCC database (http://www.atcc.org/CulturesandProducts/CellBiology/STRProfileDatabase/tabid/174/Default.aspx), CLIMA database (http://bioinformatics.istge.it/clima/), and NCI-60 database published data (13).

Cell growth assays. Attached ovarian cancer cell growth was assessed using MTT and XTT as described (14). Plates were incubated for up to 10 days, and inhibitors or vehicle were replenished every 3 to 4 days. Anchorage-independent cell growth was assessed using XTT as described (15). Briefly,
cells were seeded in 96-well plates precoated with 0.5% soft agar in 0.3% soft agar/10% RPMI 1640 at a density of 1 to 2,000 cells/50 µL/well and incubated overnight. Cell density in treated wells was expressed as a percent of vehicle-treated control wells. Experiments included duplicate samples and were repeated at least three times.

**Cell invasion assays.** The ability of ovarian cancer cells to migrate and invade through basement membrane extract was measured using Cultrex 96-well BME Cell Invasion Assays (Trevigen) according to the manufacturer’s specifications. Briefly, cells suspended in serum-free RPMI 1640 were plated in coated chambers in the presence or absence of 25 µmol/L ML120b and allowed to migrate toward RPMI 1640 containing 10% FCS as a chemoattractant for 24 hours. Cells that had migrated through BME chambers in IKKβ shRNA-inhibited/control shRNA samples, ML120b-treated, or vehicle-treated samples were stained with calcein and solubilized; numbers were assessed by measuring fluorescence. Migrated cell numbers in triplicate samples were reported as percent control.

**Heterotypic cell adhesion assays.** The attachment of ovarian cancer cells to HMVEC cells was assessed using the CytoSelect Tumor-Endothelium Adhesion Assay (Cell Biolabs, Inc.) according to the manufacturer’s specifications. Briefly, ovarian cells preexposed to ML120b or vehicle control for 48 to 72 hours were labeled with a fluorescent cytotracker dye in serum-free RPMI 1640 and allowed to adhere to a monolayer of HMVEC cells in a 96-well plate. Plates were incubated for 90 minutes, followed by gentle serial washing to dislodge unattached cells. Cells were solubilized and supernatant fluorescence was measured in a fluorometer (Tecan). Cell number from triplicate samples was reported as percent control.

**IKKβ shRNA depletion.** IKKβ shRNA retroviral constructs have been previously described (16). Two rounds of viral supernatants were applied to ovarian cancer cell lines over the course of 48 hours, followed by incubation with growth medium for 24 hours and selection with 2 µg/mL puromycin for 4 days. Selected cells were used as RNA sources for microarray analysis and all other assays.

**Western analysis.** Protein from nuclei and cytoplasm was extracted from ovarian cells using the Nuclear/Cytosol Fractionation kit according to the manufacturer’s protocol (Biovision), and concentrations were estimated with BCA Protein Assay kit (Thermo Scientific). SDS-PAGE and Western analysis were performed using, respectively, the NuPage system (Invitrogen) and the SuperSignal Chemiluminescent Substrate system (Thermo Scientific).

**RNA isolation and microarray analysis.** Total RNA was isolated from six independent cultures of ovarian cells using Trizol (Invitrogen) according to the manufacturer’s protocol. Samples were submitted to the Laboratory of Molecular Technology Microarray Core, Science Applications International Corporation/NCI-Frederick, and RNA quality was checked on an Agilent Bioanalyzer; only samples having a high quality score (RNA integrity number > 9) were used for microarray analysis. All replicates of experimental samples were prepared, labeled, and hybridized to Affymetrix H133 Plus 2.0 gene chips and scanned on a GeneChip scanner 3000 (Affymetrix). Data analysis was performed using the RNA normalization algorithm of BRB-Array Tools version 3.7 (Biometrics Research Branch, NCI, publicly available at http://linus.nci.nih.gov/BRB-ArrayTools.html). Genes showing minimal variation across the set of arrays were excluded from the analysis. Genes whose expression differed by at least 1.5-fold from the median in at least 10% of the arrays or within the 25th percentile of the log-ratio variation were retained. Class comparison was used to identify genes that were differentially expressed among control and test groups using a random variance t test. Gene expression differences were considered statistically significant if their P value was < 0.001. Data sets containing significant genes deregulated in ML120b-treated samples and in shRNA-induced samples were compared using the Compare function of Ingenuity Pathways Analysis (Ingenuity Systems, http://www.ingenuity.com).

**Quantitative real-time PCR.** cDNA was synthesized from total RNA using SuperScript II RT (Invitrogen). PCR was performed using SYBR PCR Master Mix (Applied Biosystems) on an ABI 7500 thermal cycler. B2M expression was used as an

![Figure 1. IKKβ protein expression is associated with poor OS in ovarian cancer.](http://www.ingenuity.com)
internal control to normalize between samples. Primer sequences are as follows: IKKB5, 5′-TCCGATGGCACAATCAGGAAC-3′ (forward) and 5′-TCCAGGCACCACCGCTCTC-3′ (reverse); ITGB6, 5′-GAAGACTGCCTGCTTATTGGACCTC-3′ (forward) and 5′-TGCTGGGGTATCACACCTTTCG-3′ (reverse); CLDN1, 5′-CCATCGTCAGCACTGCCCTG-3′ (forward) and 5′-AGGACATCCACAGCCCCTCG-3′ (reverse); IL-8, 5′-TCAGAGACAGCAGAGCACACAAGC-3′ (forward) and 5′-CACACATGGGTATCGCTCTCG-3′ (reverse); CXCL2, 5′-TGCTCAACCCCGCATCGC-3′ (forward) and 5′-CTTCAGGAACAGCCACAAATGG-3′ (reverse).

Primers for B2M and PTGS1 were purchased from http://RealTimePrimers.com.

Cytokine analysis. Cells stably expressing IKKβ shRNA or a control shRNA were seeded in a 96-well format at a density of 1,000 per well. After 72 hours, cells were serum starved for 24 hours in RPMI 1640–0.5% FCS and then stimulated with 10 μmol/L. LPA in medium containing 0.5% FCS. Supernatants were harvested 18 hours later. Cells treated with IKK-2 inhibitor IV (2 μmol/L) were handled similarly, except treatment was begun 48 hours before supernatant collection. IL-8 concentration was measured using the Quantikine Human CXCL8/IL-8 ELISA (R&D Systems) as per the manufacturer’s instructions. Values were normalized to cell density as determined by XTT.

Results

IKKβ protein expression is associated with poor OS in ovarian cancer. IKKβ expression was explored by immunohistochemistry in 119 tumors composed of 42 primary carcinomas and 77 solid metastases on TMA. Of the 270 tissue cores, 261 were informative. IKKβ was expressed in the cytoplasmic location in 89 of 119 tumors (Fig. 1A), with the following staining extent: score = 1: 27 tumors; score = 2: 14 tumors; score = 3: 23 tumors; score = 4: 25 tumors. Expression in primary carcinomas and metastases was comparable (data not shown). High expression of IKKβ, as indicated by >25% of cells expressing IKKβ, was associated with poor OS in primary carcinomas (median survival, 20 mo versus 31 mo; P = 0.023; Fig. 1B). IKKβ was not differentially expressed in
tumors based on stage, grade, or level of residual disease at the time of initial surgery (Supplementary Table).

**IKKβ regulates critical target genes in ovarian cancer.** Ovarian cancer cell line CAOV3 expresses a moderate level of IKKβ protein compared with most ovarian cancer cell lines tested (Supplementary Fig. S2A). CAOV3 was treated with a highly specific IKKβ small-molecule inhibitor ML120b, and gene expression was profiled to measure changes in target genes specifically regulated by IKKβ. Differentially expressed genes were selected by class comparison analysis using BRB-Array Tools (Fig. 2A). The same cells were subjected to RNA interference with two individual shRNAs directed against IKKβ to confirm the target gene list (Fig. 2B; Supplementary Fig. S1). The overlapping set of nine genes, eight downregulated and one upregulated, create a novel ovarian cancer–specific signature of IKKβ-regulated genes. The individual genes were validated by quantitative PCR after either small-molecule IKKβ inhibition or shRNA-mediated IKKβ depletion (Fig. 2C; Supplementary Fig. S1).

The gene set was then evaluated in two independently collected cohorts of ovarian tumor specimens profiled at the time of initial surgical resection (4, 12). Both of these data sets are shown in Figure 3.

**Figure 3.** IKKβ target genes are coregulated in primary ovarian cancers. A, Affymetrix U133plus2.0 gene expression profiling data from 185 purified cancer cell populations harvested from untreated patients with ovarian cancer (4). Samples are ranked according to the average expression of the nine IKKβ target genes. Expression was centered based on the median value. Correlation of each gene with the signature average is noted. B, expression of the nine-gene IKKβ signature was evaluated in an independently collected data set of ovarian cancers profiled on Affymetrix U133A gene expression arrays (12). Correlation was strongest with seven of the nine genes. C, patients from data set 1 were separated into two groups based on the median expression of the nine-gene IKKβ signature. OS is plotted. D, patients from data set 2 were separated into two groups based on the median expression of the genes in the IKKβ signature, and OS is again plotted.
sets are publicly available at http://www.ncbi.nlm.nih.gov/geo/. The nine genes in the ovarian cancer–specific IKKβ signature were highly correlated with each other, suggesting that they are coregulated in ovarian cancer (Fig. 3A and B). The expression of IKKβ gene itself was highly correlated with the group of target genes ($R = 0.20$, $P = 0.006$; data not shown), suggesting a positive relationship between expression of IKKβ and its activity as represented by downstream target gene regulation. Elevated IKKβ target gene expression was statistically related to worse outcome in the data sets of women with high-grade, stage III or IV ovarian cancer at diagnosis ($P = 0.02$; Fig. 3C). Patient samples were separated into the high or low cohort based on the median expression of the ovarian cancer IKKβ signature within the group. Patients whose tumors showed activation of IKKβ, based on expression of the signature above the median, had a lower median survival than those who showed signature expression below the median. A second previously published data set, independently collected and composed of tumor samples from women with ovarian cancer of any stage, was used to validate these findings. A similar trend was observed in the second data set, without reaching statistical significance ($P = 0.09$; Fig. 3D). The more modest effect in the independent analysis of the second data set may be due in part to the different characteristics of the patients. The first data set includes a more homogeneous set of only high-grade (3), late-stage (IIIC or IV) cancers, whereas the second set includes all stages and all grades.

**IKKβ mediates properties of aggressiveness in ovarian cancer.** Inhibition of IKKβ differentially suppressed growth of ovarian cancer cell lines. A panel of six different ovarian cancer cell lines was treated with three different small-molecule inhibitors of IKKβ (Fig. 4). Each compound is reportedly highly specific to IKKβ kinase inhibition (10, 11). All three inhibitors resulted in a similar pattern of anchorage-independent growth inhibition. The cell lines CAOV3, IGROV1, and A2780 showed at least a 40% to 60% decrease in viability with IKKβ inhibition, whereas the cell lines OVCAR5, OVCAR8, and HeyA8 were less susceptible.

Blockade of IKKβ by either small-molecule inhibition (Fig. 5A, left) or targeted RNA interference (Fig. 5A, right) decreased invasiveness of most ovarian cancer cell lines. Baseline invasion varied modestly between the cell lines and generally correlated with IKKβ protein expression level (Supplementary Fig. S2). A decrease in invasiveness was also observed in OVCAR8 and OVCAR5, cell lines that were insensitive to the growth-inhibitory effects of the pathway inhibition. This is consistent with the expression patterns shown in the primary tumors (Fig. 3A), where the genes were highly correlated across the entire data set, but individual patients did not have exactly the same pattern in every case.

The attachment of ovarian cancer cells to HMVECs represents an early function required for initiation of tumor dissemination. Such heterotypic cell adhesion was assessed in the presence of small-molecule IKKβ inhibition or with IKKβ knockdown by shRNA. Heterotypic adhesion of tumor cells to endothelial cells was decreased in the absence of IKKβ signaling by either small-molecule inhibition (Fig. 5B, left) or shRNA depletion of IKKβ (Fig. 5B, right).

Similarly, secretion of IL-8 was decreased after IKKβ inhibition by either method (Fig. 5C). IL-8 gene expression was regulated by IKKβ (Fig. 2) and contributed to the ovarian cancer IKKβ signature (Fig. 3). Two of the cell lines (A2780 and IGROV1) did not secrete IL-8 at baseline, consistent with prior studies, and therefore, this property was not
affected by IKKβ blockade (data not shown). LPA is a serum cytokine that is upregulated in ovarian cancer. LPA is known to stimulate IL-8 secretion (17), and the LPA-induced rise in IL-8 secretion was attenuated by IKKβ inhibition by either small molecule or shRNA (Fig. 5C). Again, the range of biological effect downstream of IKKβ signaling in the cell lines mirrors the individual patient samples, which showed varying levels of the nine IKKβ target genes.

Figure 5. IKKβ mediates properties of aggressiveness in ovarian cancer. A, invasion through basement membrane was measured after interruption of IKKβ signaling with the small-molecule inhibitor (left) or the shRNA directed to IKKβ (right). B, adhesion of ovarian cancer cells to endothelial cells was measured after interruption of IKKβ signaling with the small-molecule inhibitor (left) or the shRNA directed to IKKβ (right). C, IL-8 secretion was measured after interruption of IKKβ signaling with the small-molecule inhibitor (left) or the shRNA directed to IKKβ (right).
Discussion

We have shown here that the expression of IKKβ protein itself and the ovarian cancer–specific signature of IKKβ-regulated genes defined a subset of ovarian cancer susceptible to therapeutic targeting of this pathway. The gene signature regulated by IKKβ in cell lines was related to poor outcome in independently collected sets of primary ovarian cancers, suggesting that IKKβ activity contributes to an aggressive phenotype of this disease. The genes regulated by IKKβ signaling in ovarian cancer are involved in a wide range of cellular effects that increase the aggressive nature of the cells, including proliferation, invasion, adhesion, and IL-8 secretion (Fig. 6). Overactivation of these cellular activities is consistent with the poor prognostic association in patients. We functionally validated the effect of IKKβ signaling on stimulating proliferation, invasion, and adhesion in ovarian cancer cell lines. IKKβ activity promoted all of these cellular functions in ovarian cancer cell lines, reflecting its modulation of the target genes identified.

This ovarian cancer–specific IKKβ gene signature was developed using an unbiased approach of global gene expression profiling after pharmacologic or genetic manipulation of IKKβ in ovarian cancer cell lines. Therefore, it reflects downstream effects of IKKβ activity and represents the functions that are regulated by IKKβ in these ovarian cancer cells. An overlapping but not identical gene list was recently reported to be IKKβ dependent in breast cancer cell lines when the E3 ligase KEAP1 was silenced, allowing IKKβ activity to ensue (18). The breast cancer KEAP1-IKKβ gene list was selected based on genes previously known to be NF-κB targets and lends further confidence to the independently generated signature that we have discovered in ovarian cancer.

The significance of IKKβ activity in ovarian cancer has been characterized by demonstration of its role in multiple biological events necessary for the composite activity of ovarian cancer proliferation and dissemination. Although each cellular process interrogated was blocked by 50% or less in most of the cell lines, such modest effects on individual cellular properties, when taken as a whole, have the potential to result in even more profound suppression of tumor growth in vivo. Interestingly, the pattern of functions mediated by IKKβ was not uniform across the cell lines tested, indicating a differential predominance of each effect among distinct ovarian cancers. For example, the IGROV1 and A2780 cell lines express very little of the IKKβ target gene IL-8, yet they showed sensitivity to IKKβ inhibition in the phenotypes of proliferation and invasion, likely affected by changes in the IKKβ targets CLDN1 and CXCL1 (also known as growth-related oncogene; refs. 19, 20). In other cell lines such as OVCAR5 and OVCAR8, IL-8 was expressed downstream of IKKβ, yet the proliferative rate was less influenced by IKKβ. These findings underscore the heterogeneous nature of ovarian cancer, supporting the hypothesis that there are multiple molecularly defined subtypes of this disease.

It is also interesting to note that the small-molecule inhibitor of IKKβ kinase activity showed a slightly different pattern of effect on the panel of ovarian cancer cell lines compared with the shRNA depleting IKKβ protein. One potential reason for this difference could lie in a differential effect caused by inhibiting the kinase activity of the IKKβ protein versus taking away the entire protein from the cell.
This might suggest that the protein can have some function independent of its kinase activity, perhaps as a scaffold for other signaling cascades in the cell.

Diverse mechanisms can trigger NF-κB signaling, and extensive cross talk exists between major signaling pathways within a cell that can augment the NF-κB signaling cascade (21). Activation of NF-κB in ovarian cancer could be provided by signals in the tumor microenvironment. Inflammatory cytokines and growth factors have been associated with NF-κB activation. One potential cause of IKKβ activation may derive from microenvironmental or paracrine influences such as LPA (22). LPA is found at high levels in the serum of ovarian cancer patients and is known to activate several cell signaling cascades promoting cancer cell survival and proliferation (23). Our results confirmed the role of IKKβ in relaying LPA-induced signaling in ovarian cancer.

Downstream of cell surface receptors and immediately upstream of IKKβ, many different signaling nodes influence the kinase activity of the IKK complex. Interaction with the stroma, via intercellular adhesion molecules or integrins, may be amplified or perpetuated by mutations in NF-κB activators such as IKKα or by loss of NF-κB attenuators such as CYLD (24). We previously identified diverse genetic abnormalities that activated IKKβ in multiple myeloma (9). These mechanisms included inactivation of negative regulators CYLD, BIRC2/3, or TRAF3 or overactivation of positive regulators NIK or CD40. Additionally, RAS signaling may activate IKKβ and NF-κB through TBK1 (25, 26). Potential activators of IKKβ do not appear in the signature that we present in the current work due to the manner in which the signature was generated. Activators of IKKβ are of great interest in the context of ovarian cancer, and disease-specific dysregulation of such pathways is the subject of ongoing investigations.

The diversity of functions controlled by IKKβ in ovarian cancer has implications for treatment of this malignancy. Small-molecule inhibitors of IKKβ are under preclinical development, yet, like many kinase inhibitors tried thus far in ovarian cancer, they may not exhibit a strong anticancer effect as a single agent in most forms of ovarian cancer. Therefore, IKKβ inhibitor therapy, alone or in combination with chemotherapy, should be focused to patients whose tumors express a molecular profile suggestive of dependence on IKKβ activity. This profile could then be monitored in the tumor during therapy for validation of drug effect or mechanisms of resistance.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Grant Support

Intramural Program of the Center for Cancer Research, NCI (C.M. Annunziata and E.C. Kohn); Marshar Rivkin Foundation for Ovarian Cancer Research (C.M. Annunziata); and Norwegian Cancer Society and Health Region of South-Eastern Norway (B. Davidson).

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Received 10/22/2009; revised 02/24/2010; accepted 03/17/2010; published OnlineFirst 04/27/2010.

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