IKK-ε Coordinates Invasion and Metastasis of Ovarian Cancer

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Abstract
Inhibitor of IκB kinases (IKK) are key regulators of NF-κB signaling. Three IKK isoforms—α, β, and ε—have been linked to oncogenesis, yet the precise components of NF-κB signaling in ovarian cancer have not yet been dissected. We surveyed 120 ovarian cancer specimens for IKK-ε expression. Notably, cytoplasmic expression was elevated in metastatic lesions relative to primary tumors (P = 0.03). Therefore, we hypothesized that IKK-ε drives ovarian cancer metastasis. IKK-ε was identified previously as a breast cancer oncogene and was associated with poor clinical outcome in ovarian cancer. We now define an ovarian cancer–specific IKK-ε–regulated gene expression signature using stably expressed short hairpin RNA targeting IKK-ε. Pathway analysis of the signature indicated that IKK-ε regulates expression of genes involved in cell motility and inflammation. We further showed that IKK-ε depletion in metastatic ovarian cancer cell lines decreased growth, adhesion, and invasion. Consistently, human xenografts depleted of IKK-ε in mice showed decreased aggressiveness, whereas overexpression of IKK-ε in a less invasive ovarian cancer cell line increased metastasis in vivo. Taken together, these data provide evidence that IKK-ε is a key coordinator of invasion and metastasis programs in ovarian cancer. Inhibition of IKK-ε signaling thus emerges as a viable therapeutic strategy in women whose ovarian cancer shows aberrant activation of this pathway. Cancer Res; 72(21): 5494–504. ©2012 AACR.

Introduction
Ovarian cancer affects 20,000 women annually in the United States. The fractional death rate exceeds 50% due to late stage at diagnosis and eventual resistance to chemotherapy (1). Goals in the field include improved screening and diagnostics and improved therapy of advanced disease at the outset and for recurrence (2, 3). Recent molecular profiles of ovarian cancers reveal marked heterogeneity even within defined histologic subtypes. Gene expression defined subsets of patients presenting aggressive disease that respond differently to standard surgery and chemotherapy treatment (4). Attempts were made to correlate molecular signatures with better survival. The Cancer Genome Atlas (TCGA) 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 (5). These efforts have made clear that ovarian cancer is an extremely heterogeneous disease. A single approach to chemotherapy 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 treatments targeting such pathways to improve specific patient survival.

The involvement of NF-κB in cancer dissemination further makes it a logical target. Inhibitor of IκB kinase—α (IKK-α) promotes metastasis in prostate cancer, due to its ability to suppress Maspin expression (6). Activated IKK-α in prostate cancer cells' nuclei increased with advancing stage of prostate cancers, and inversely correlated with Maspin expression. We recently identified the NF-κB signaling pathway as a potential driver of ovarian cancer growth (7). We identified autonomous NF-κB signaling in a defined subset of ovarian cancer, driven by IKK-β and susceptible to therapeutic targeting of this kinase (8). However, the mechanism through which the NF-κB pathway affects the initiation, propagation, and dissemination of ovarian cancer is unknown. IKKs are key regulators of NF-κB signaling. Three IKK isoforms—α, β, and ε—have been linked to oncogenesis, yet the precise components of NF-κB signaling mechanisms in ovarian cancer have not yet been dissected.

A systems biology approach discovered that IKK-ε functioned as an oncogene in breast cancer (9). The IKK-ε locus (1q32) was found to be amplified in approximately 30% of human breast cancer specimens, and correlated with high expression of IKK-ε transcripts and protein. The oncogenic activity of IKK-ε was regulated through the transcription factor cRELI, indicating that the NF-κB pathway is a key downstream...
mediator of IKK-ε-induced transformation. Further evidence that IKK-ε acts through the NF-κB pathway in oncogenesis was derived from a positional scanning peptide library assay to identify substrates of IKK-ε (10). The deubiquitinating enzyme CYLD, a tumor suppressor and negative regulator of the NF-κB pathway, was of particular interest. IKK-ε exerted oncogenic pressure by inhibiting CYLD activity through phosphorylation, resulting in the blockage of the deubiquitination of TRAF2 and NEMO, positive regulators of the classical NF-κB pathway.

Recent work associated expression of IKK-ε with poor outcome in women with ovarian cancer (11). IKK-ε was found to be overexpressed and activated in primary ovarian cancer specimens and cell lines. Its overexpression in tumors was associated with high grade, advanced stage disease. In cell lines, IKK-ε promoted resistance to platinum-based chemotherapy, a class of agents included in the initial therapy of advanced ovarian cancer. A role for IKK-ε in ovarian cancer was implicated by these results, without definition of its mechanism and function. We therefore sought, in this study, to examine IKK-ε in ovarian cancer. Here, we have identified IKK-ε as a key coordinator of invasion and metastasis in ovarian cancer.

Materials and Methods

Patients and tissue microarray samples

Tissue microarrays (TMA) contained 2-mm cores (n = 270) from 120 ovarian carcinoma specimens (42 primary carcinomas, 78 solid metastases) operated at the Norwegian Radium Hospital (Oslo, Norway). Metastases were to the omentum (n = 46), peritoneum (n = 16), intestine (n = 12), lymph nodes (n = 4), or other sites (n = 2). Tumors were from 57 patients, of whom 38 had both primary carcinoma and 1 or more metastasis for evaluation, 4 had only primary carcinoma, and 14 had only 1 more metastasis. Tumors underwent microscopic confirmation of diagnosis, histologic type, and grade by a gynecopathologist (B. Davidson). Grading was according to the FIGO system. The Regional Committee for Medical Research Ethics in Norway approved the study. Antibodies and staining conditions were previously detailed (7).

Cell lines and culture conditions

Ovarian cancer cell lines CAOV3, A2780, MDA-Ovcar3, Skov3, A2780-1A9, and HeyA8 cells were a gift from Dr Elise Kohn (NCI/NIH, Bethesda, MD). Ovarian cancer cell lines IGROV1, Ovcar3, Ovcar4, Ovcar5, and Ovcar8 were obtained from the NCI-Frederick DCTD tumor/cell line repository. Low passage Caov3 cells were purchased from American Type Culture Collection (ATCC). All ovarian lines were cultured in RPMI plus 10% FBS (Hyclone) and standard antibiotics. Cultures were maintained at 37°C in a 5% CO2 atmosphere. The Kohn cell lines were authenticated in July 2009 at the Johns Hopkins University Fragment Analysis Facility (Baltimore, MD) using Promega PowerPlex 1.2 System to test for short tandem repeat markers (D16S539, D7S820, D13S317, D5S818, CSF1PO, TPOX, THO1, and vWA) and amelogenin for gender determination. Authenticity was confirmed against the ATCC database (12), CLIMA database (13), and NCI-60 database published data (14). ATCC lines and NCI-60 lines were authenticated at the source. Expression and short hairpin RNA (shRNA) constructs have been described previously and are detailed in the Supplementary Methods. Cell growth and invasion assays have been described previously and are detailed in the Supplementary Methods.

Western blot analysis

Protein was extracted from ovarian cancer cell lines using standard methods (8). Protein concentrations were estimated with BCA Protein Assay Kit (Thermo Scientific). SDS-PAGE and Western blot analysis were conducted using the NuPage system (Invitrogen) and the Supersignal Chemiluminescent Substrate system (Thermo Scientific), respectively. Antibodies are listed in the Supplementary Methods.

Mouse xenografts

Female Athymic Nu/Nu mice were obtained at 6 to 8 weeks of age from NCI-Frederick, and acclimated for 5 to 7 days. Care was provided in accordance to procedures in the Guide for the Care and Use of Laboratory Animals (NIH Publication No. 86-23, 1985). Experiments were carried out according to a protocol approved by the NCI Animal Care and Use Committee. Briefly, 1 million control shRNA or IKK-ε shRNA-expressing ovarian cells were injected intraperitoneally into groups of 10 mice; whole body weights were obtained 3 times per week for 5 weeks after injection. Ovcar5 and Ovcar8 cells were tested in 2 independent experiments. Caov3 (2 million cells) transduced with either empty vector, wild-type IKK-ε, or myristoylated IKK-ε were injected intraperitoneally into groups of 6 mice or PBS into 4 mice; whole body weights were obtained 3 times per week for 4 weeks after injection. Animals were sacrificed humanely and received complete necropsies, at which time wet organ weights, tissues, and samples were obtained. Immunohistochemistry for GFP expression (Abcam; Cat. No. ab290) and hematoxylin and eosin staining was conducted on paraffin-embedded, formalin-fixed sections, for evaluation of xenograft localization and morphology. Gross and microscopic pathology evaluation was conducted by M. Anver, and scored using the following definitions: Xenografts present in the peritoneal cavity from intraperitoneally injected cell lines involving omental fat are coded as "primary"; xenografts in the same mouse in other abdominal organs (liver, diaphragm, pancreas, other sites) are coded as "secondary." Xenografts were classified as "superficial" if they were implanted on the organ surface without disruption of the capsule, or "invasive" if they had disrupted the surface of the organ and were present within the organ parenchyma.

Gene expression analysis

Total RNA was isolated from 6 independent cultures of ovarian cells grown in 6-well plates using TRIzol (Invitrogen) according to manufacturer protocol. Details of analysis are provided in the Supplementary Methods. The following Gene Expression Omnibus datasets were analyzed: GSE9899, GSE18520 (15), and TCGA (16).

Quantitative PCR

Total RNA was isolated using RNeasy Mini Kit (Qiagen). One microgram of total RNA was converted to cDNA using iScript
cDNA Synthesis Kit (Bio-Rad). cDNA was diluted 1:5 in H2O for real-time PCR (QuantiTect SYBR Green PCR Kit; Qiagen), conducted in triplicates, by 7900HT Fast Real-Time PCR System (Applied Biosystems). Each mRNA expression level was normalized by that of GAPDH. Primers are listed in the Supplementary Methods.

Results

IKK-ε is more highly expressed in metastatic ovarian cancers compared with primary tumors

We surveyed 120 ovarian cancer specimens (42 primary carcinomas and 78 solid metastases) for expression of IKK proteins. Immunohistochemical analysis of 3 IKK proteins—IKK-α, IKK-β, and IKK-ε—showed that IKK-ε was the only isoform that was differentially expressed between primary tumors and metastatic lesions. Importantly, IKK-ε was significantly (P = 0.03) more frequently present in metastatic ovarian cancer cells relative to primary tumor sites (Fig. 1A and B and Supplementary Table S2), whereas IKK-α and IKK-β were evenly distributed between primary and metastatic tumors. TCGA measured gene expression and chromosomal amplifications or deletions in approximately 500 serous ovarian cancers that were stage IIIC at the time of initial diagnosis (5). These TCGA data show that at the time of initial diagnosis, IKK-ε is more frequently overexpressed than IKK-α or IKK-β in advanced ovarian cancer (Fig. 1C, left). Overexpression occurs at the transcriptional level, because there was no significant change in copy number in this dataset (Fig. 1C, right). These
findings prompted the hypothesis that IKK-ε promotes ovarian cancer metastasis.

**IKK-ε regulates expression of genes involved in cellular motility and inflammation**

Ovarian cancer cell lines, all derived from advanced cancers, consistently expressed IKK-ε (Fig. 2A). To dissect the functional role of IKK-ε in ovarian cancer, we screened 13 RNA interference constructs directed against IKK-ε, and selected the most effective shRNAs for further experiments (Supplementary Fig. S1A and S1B). Three shRNA constructs effectively depleted IKK-ε, but not IKK-β or IKK-α (Supplementary Fig. S2A and S2B). Knockdown was confirmed in 3 ovarian cancer cell lines after initial selection, and the stability of knockdown was reconfirmed after the selected cells were maintained in the absence of selection agent (Fig. 2B). Gene expression profiles of ovarian cancer cells depleted of IKK-ε identified molecules potentially causing the biologic phenotype observed in human tumors. We identified 278 genes regulated by selective depletion of IKK-ε with 2 individual shRNAs in 6 biologic replicates in Ovcar5 (Fig. 3A). The signature was further refined by examining the expression of these genes in primary ovarian cancer specimens (GSE9899). Seventeen genes were both downregulated after IKK-ε depletion and associated with elevated IKK-ε in primary ovarian cancers. These genes were associated with expression of IKK-ε itself, and were coregulated across 3 independent datasets (Fig. 3B and Supplementary Fig. S2C), suggesting a functional relationship in ovarian cancer. Strikingly, all 17 genes were overexpressed and/or amplified in the TCGA dataset (Supplementary Fig. S2D). Furthermore, the 17 IKK-ε signature genes were not equally represented among 6 previously defined molecular subgroups of ovarian cancer (17); the C1 and C2 subgroups had relatively high expression, whereas the C5 and C6 subgroups had low expression (Fig. 3C). This previously published classification identified C1 tumors as having a stromal signature, and the C2 type expressed an immune signature. Consistent with our finding, the previous observation also showed an enrichment of metastatic tumors within the C1 subgroup, with high desmoplasia. Importantly, Ingenuity pathway analysis identified inflammatory signaling as the most significantly regulated pathway (Fig. 3D). Accordingly, cellular movement and inflammation were the most significant networks affected by this set of genes (Fig. 3E). Seven of the 17 genes are known to function in metastasis and invasion. We chose these 7 genes to validate the microarray data. We verified their knockdown by quantitative PCR (qPCR) in 2 ovarian cancer cell lines (Supplementary Fig. S2E and S2F). Taken together, our analyses showed that IKK-ε modulates genes involved in cellular movement and inflammation, 2 functions critical for a metastatic phenotype.

**IKK-ε has a modest effect on ovarian cancer growth and invasion in vitro**

Depletion of IKK-ε by shRNA had a modest effect decreasing cell growth in vitro (Fig. 4A–C and Supplementary Fig. S3A). Interestingly, cell lines also showed a decrease in basement membrane invasion with IKK-ε depletion (Fig. 4D and Supplementary Fig. S3B). These results are consistent with the hypothesized role for IKK-ε–programmed events in regulating interactions of the tumor cell with its microenvironment. Therefore, we proceeded with in vivo xenograft experiments to assess the contribution of IKK-ε in a metastasis model.
IKK-ε promotes tumor growth, invasion, and metastasis in vivo

Ovarian cancer cell lines differed in their ability to form tumors in mice (Supplementary Fig. S4A). HeyA8, Ovcar8, and Ovcar5, expressing high levels of IKK-ε, were among the most aggressive tumors, whereas low passage Caov3 that expressed low IKK-ε was less malignant. Ovarian cancer cell lines Ovcar8 and Ovcar5 were stably transduced with shRNA constructs targeting IKK-ε or control and coexpressing GFP (Fig. 5A). Persistent knockdown of IKK-ε was measured at the midpoint of the xenograft time course (2.5 weeks), and maintenance of the shRNA construct in the ovarian cancer cells persisted until the end of the experiment (5 weeks) as evidenced by the coexpressed GFP protein. Cohorts of Ovcar5 or Ovcar 8 cells were tested in 2 independent experiments. Animal weight gain tended to be lower in mice inoculated with ovarian cancer cells.
IKK-ε in Ovarian Cancer Metastasis

depleted of IKK-ε, compared with controls, without reaching statistical significance (Supplementary Fig. S4B). Organ weights of liver, pancreas, and diaphragm were measured at the time of necropsy. Organ weights of animals that had been injected with IKK-ε-depleted Ovcar5 xenografts were lower than control xenografts, indicating that IKK-ε-deficient tumor cells had undergone less metastasis and invasion (Fig 5B). IKK-ε-depleted xenografts seemed markedly different from control cells on gross anatomic examination (Fig. 5C). Control cells were well attached and adhered to abdominal organs, whereas IKK-ε-depleted Ovcar8 cells floated in ascites fluid and cascaded out of the abdomen when the cavity was opened. Ovcar5 cells did not float but adhered less well to the organs (data not shown). In addition, more tumor nodules were visible in the secondary organ sites compared with the IKK-ε-depleted xenografts of both cell lines. Quantiﬁcation of metastatic xenograft invasion into solid organs showed decreased invasiveness of Ovcar5 cells that had been depleted of IKK-ε by either of the shRNA constructs (Fig. 5D). A similar pattern was observed in Ovcar8 cells (Supplementary Fig. S4C). Consistently, microscopy of tissue sections showed severe invasion into the organ parenchyma, whereas the GFP positive IKK-ε-depleted xenografts remained in the omental fat layer, outside of solid organs. Of note, expression of the shRNA construct was maintained over the 5-week interval in vivo, because GFP protein was present in the tumor cells. Xenografts expressing control shRNA showed higher capacity to invade solid organs, as compared with the IKK-ε-deficient cells, which generally remained adjacent to the solid organ.

**Figure 4.** IKK-ε modestly affects ovarian cancer growth and invasion *in vitro*. A–C, ovarian cancer cell lines Ovcar 5, Ovcar8, and HeyA8 were quantiﬁed by ﬂow cytometry analysis for GFP expression (coexpressed with shRNA construct) for 12 to 17 days after selection for IKK-ε depletion. Measurements are normalized to day 0 for each cell line. D, invasion through basement membrane was measured after depletion of IKK-ε and normalized to control shRNA for each cell line. Error bars represent SE.

**Overexpression of IKK-ε promotes metastasis to secondary organ sites in vivo**

As a complement to the loss-of-function shRNA model, we developed a gain-of-function system by overexpressing IKK-ε in the less invasive ovarian cancer cell line Caov3, which expresses endogenously low IKK-ε. We overexpressed the wild-type IKK-ε protein to mimic endogenous overexpression, and separately introduced the myristoylated version of IKK-ε, because this was previously determined to be oncogenic (9). Caov3 cells were stably transduced with either wild-type or myristoylated IKK-ε (Fig. 6A). Exogenous IKK-ε protein expression was conﬁrmed after selection and in cells cultured for 4 weeks without selection agent to verify maintenance of the transgene. Cells expressing wild-type IKK-ε showed a slight increase in invasion through basement membrane *in vitro* (Fig. 6B). Cells expressing myristoylated IKK-ε proliferated more;
however, fewer of these cells invaded, when compared with cells transfected with empty vector or wild-type IKK-ε, even without normalization to viability (Supplementary Fig. SHD). We queried by qPCR whether the 7 IKK-ε–related metastasis genes were upregulated upon introduction of IKK-ε (Fig. 6C). Five of the 7 genes were upregulated, supporting their functional relationship downstream of IKK-ε. IKK-ε–overexpressing Caov3 cells were injected into mice to determine their behavior in vivo. Mice inoculated with the Caov3 cells overexpressing wild-type IKK-ε gained weight at a significantly faster rate than the other groups (Fig. 6D). Xenograft present in the peritoneal cavity and omental fat from intraperitoneally injected cell lines was coded as primary. Xenografts in the same mouse in other abdominal organs and outside of the peritoneal cavity (liver, diaphragm, pancreas, other sites) were coded as secondary. Both wild-type and myristoylated IKK-ε resulted in more xenografts in secondary organ locations as compared with empty vector control (Fig. 6E). The IKK-ε–Myr acted differently in vivo, compared with in vitro invasion assays, suggesting that these assays are measuring different biologic processes and underscoring the importance of including both experiments. Tissue microscopy confirmed the increased ability of Caov3 cells containing the IKK-ε constructs to invade tissues (Fig. 6F).

Discussion

IKK-ε is an oncogene in breast cancer, and promotes chemoresistance in ovarian cancer (9, 11). Previous studies linked
IKK-ε to poor prognosis in patients with ovarian cancer (11). Its overexpression in ovarian cancer cell lines caused resistance to platinum chemotherapy, standard of care for initial treatment of advanced ovarian cancer. Our work presented herein suggests another mechanism for the poor prognosis conferred by high IKK-ε expression, namely an increased ability to disseminate. Our initial finding of IKK-ε overexpression in metastatic tumors, compared with primary tumors, was unexpected. Previous work in prostate cancer showed that IKK-ε was the differentially expressed isoform-promoting metastasis (6). Therefore, we expected a similar mechanism of IKK-related metastasis in ovarian cancer. Instead, IKK-α was equally expressed in most ovarian cancers examined. In contrast, IKK-ε was more highly expressed in metastatic ovarian
cancers, and showed uniformly low expression in primary sites of ovarian cancer. Most metastatic ovarian cancers, however, also showed low expression of IKK-ε, suggesting that this is not the sole mechanism promoting ovarian cancer metastasis. This is consistent with our previous findings of NF-κB activity in ovarian cancer, where IKK-β was active in a subset of tumors, but was not a uniform target for all ovarian cancers (7, 8). Interestingly, our results suggest that IKK-ε differentially influences functions of proliferation versus invasion in individual cell lines. We found a similar phenomenon with IKK-β in ovarian cancer (8) where the pattern of functions mediated by IKK-β was not uniform across the cell lines tested, indicating a differential predominance of each effect among distinct ovarian cancers. These findings underscore the heterogeneous nature of ovarian cancer, supporting the hypothesis that there are multiple molecularly defined subtypes of this disease. Future clinical studies with NF-κB–targeted agents should seek to identify biomarkers specific to IKK activity to appropriately allocate such therapies to patients whose tumors are more likely to respond.

We now show that IKK-ε coordinates genes promoting ovarian cancer metastasis. IKK-ε controlled genes involved in inflammation, cellular movement, and interaction with extracellular matrix, all functions linked to metastatic potential. IKK-ε had a moderate effect in maintaining cellular proliferation, but more prominently promoted invasive capacity of a subset of ovarian cancer cell lines.

The primary method of dissemination of epithelial ovarian cancer is through shedding from the primary tumor. Omental metastases occur in 70% to 80% of advanced ovarian cancers. In our mouse model, tumor was injected directly into the peritoneal cavity. Thus, the model mimics ovarian cancer that has intially shed. The omentum may play a role in trapping and destroying cancer cells (18). In a rat model, primary omentectomy followed by injection of tumor resulted in worse survival and more disseminated disease than in rats with an intact omentum (19). Because our xenografts are initially introduced into the peritoneal cavity of the mice, we coded our omental tumors the primary site and the extraomental tumors the secondary. Tumor that successfully moves past the omentum reflects its more aggressive biology. In mice, IKK-ε–deficient xenografts showed decreased tumor burden and invasion into secondary organs, supporting our hypothesis that IKK-ε promotes a program of metastasis in ovarian cancer. Conversely, exogenous overexpression of IKK-ε into a nonmetastatic cell line increased the cells’ ability to invade tissues and disseminate to secondary abdominal organs.

Several genes comprising the 17-gene ovarian cancer–specific IKK-ε signature are known to be involved in cellular invasion and metastasis. For example, overexpression of secretory leukocyte peptidase inhibitor (SLPI) increased tumor formation and dissemination in orthotopic mouse model of ovarian cancer, independent of its protease inhibition activity (20). Nicotinamide N-methyltransferase (NNMT) induced cellular migration and invasion in renal carcinoma cells and bladder cancer (21, 22). Fibronectin 1 (FN1) significantly increased migration of melanoma cells combined with peristin, osteoblast specific factor (POSTN) (23). Inhibin, beta A (INHBA) was a top-ranked gene in a metastasis-associated expression signature from multicancer computational analysis (24). Consistently, a previous analysis of ovarian carcinoma also identified NNMT, FN1, and INHBA as metastasis-associated genes (25). CYP1B1 was overexpressed in both primary and metastatic ovarian cancers (26). In addition, EPSTI1 was highly upregulated in invasive breast carcinoma compared with normal breast, suggesting its expression may be a crucial event in invasion and metastasis of cancer (27). The decreased metastatic potential in our IKK-ε–depleted xenografts are well explained by these genes’ function. The critical finding from our current analysis is that IKK-ε regulates these genes, as a metastasis-promoting cassette, in ovarian cancer. It is unlikely that any individual gene in the signature is responsible for the metastatic program induced by IKK-ε, but that coordinated expression of this gene cassette is required. However, the mechanism by which IKK-ε is activated in ovarian cancer is unknown, and is the subject of ongoing research.

Gene expression profiles previously defined 6 distinct subtypes of ovarian cancers (17). The ovarian cancer IKK-ε signature defined here was overrepresented in the C1 and C2 subtypes, suggesting that IKK-ε could drive the phenotype of these cancers. The C1 subtype included cancers with high stromal response and desmoplasia. Notably, the patients with the C1-type tumors had the shortest progression-free and overall survival. It is possible that IKK-ε contributes to the poor prognostic phenotype of the C1 subgroup by increasing the cancers’ metastatic potential, given our current findings in vitro and in vivo.

Conversely, 13 of the IKK-ε signature genes are underexpressed in the C5 subtype of ovarian cancers, which included mesenchymal-like tumors with a low immune signature. This inverse relationship with the C5 subgroup suggests that these genes may be required for an immune signature in ovarian cancer. Pathway analysis and networks corroborated this idea, because the genes are known to be instrumental in acute phase response, lymphotoxin β receptor, IL-8, and TNFR2 signaling pathways, all of which contribute to an inflammatory response network.

IKK-ε may therefore provide a therapeutic target for C1-type ovarian cancers. IKK-ε–specific inhibitors are not currently under clinical development, but it may be possible to therapeutically interfere with IKK-ε signaling upstream or downstream of IKK-ε target pathways. For example, TNFR can stimulate the growth and survival of some ovarian cancers, likely through activation of canonical NF-κB signaling. The same cytokine promotes apoptosis in other contexts. Cellular inhibitor of apoptosis protein-1 (cIAP1) acts as a critical switch to promote the prosurvival NF-κB pathway and prevent caspase activation (28). An early event in apoptosis is the release of second mitochondrial–derived activator of caspases (SMAC). In normal cells, SMAC promotes IAP degradation, thereby activating caspases and tipping the balance from survival to apoptosis. In tumor cells, however, apoptosis is deregulated because of insufficient amounts of SMAC (29). Thus, SMAC mimetics under clinical development could have selectively toxic activity against those molecularly defined IKKe-driven and/or C1-type ovarian cancers.
Understanding IKK-ε–regulated signaling in ovarian cancer will identify novel pathway interactions for context-specific therapeutics in the poor prognostic group of women whose ovarian cancers overexpress IKK-ε. Further clarification of the ovarian cancer–specific IKK-ε pathway is likely to provide several potential therapeutic avenues for individualized treatment of women with this molecularly defined variant of ovarian cancer.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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Grant Support
This work was supported by the Intramural Research Program, CCR, NCI (C. M. Annunziata), NIH Grant K99CA151746 (C. M. Annunziata), and the Norwegian Cancer Society and Health Region of South-Eastern Norway (to B. Davidson), and with Federal funds from the NCI, NIH, under contract number HHSN261200800001E.
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Received December 12, 2011; revised August 2, 2012; accepted August 20, 2012; published OnlineFirst August 31, 2012.

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