

Review

## Transcriptional Regulation of Chemokine Expression in Ovarian Cancer

Bipradeb Singha, Himavanth R. Gatla and Ivana Vancurova \*

Department of Biological Sciences, St. John's University, 8000 Utopia Parkway, New York, NY 11439, USA; E-Mails: bipradeb.singha10@my.stjohns.edu (B.S.); himavanthreddy.gatla12@my.stjohns.edu (H.R.G.)

\* Author to whom correspondence should be addressed; E-Mail: vancuroi@stjohns.edu; Tel.: +1-718-990-6409.

Academic Editor: Jürg Bähler

Received: 8 December 2014 / Accepted: 9 March 2015 / Published: 17 March 2015

---

**Abstract:** The increased expression of pro-inflammatory and pro-angiogenic chemokines contributes to ovarian cancer progression through the induction of tumor cell proliferation, survival, angiogenesis, and metastasis. The substantial potential of these chemokines to facilitate the progression and metastasis of ovarian cancer underscores the need for their stringent transcriptional regulation. In this Review, we highlight the key mechanisms that regulate the transcription of pro-inflammatory chemokines in ovarian cancer cells, and that have important roles in controlling ovarian cancer progression. We further discuss the potential mechanisms underlying the increased chemokine expression in drug resistance, along with our perspective for future studies.

**Keywords:** chemokines; interleukin-8; NFκB; ovarian cancer; transcriptional regulation

---

### 1. Introduction

Chemokines are a family of cytokines that induce chemotaxis of target cells. Though they were originally discovered for their ability to induce leukocyte migration into the infected or injured sites, more recently, it became clear that they could also promote cancer progression [1–9]. In addition to inducing tumor cell proliferation, angiogenesis and metastasis, chemokines and their receptors regulate tumor cell differentiation and survival. Currently, the human chemokine network includes more than

45 known chemokines and 20 chemokine receptors. Based on the number and spacing of conserved N-terminal cysteine residues that form disulfide bonds, chemokines are divided into four groups: (X)C, CC, CXC, and CX3C [10–12].

Epithelial ovarian cancer (EOC) is among the leading causes of cancer death in women. Since most ovarian cancers relapse and become drug-resistant, the survival rates remain low. Progression of ovarian cancer (OC) has been associated with the increased expression and release of pro-inflammatory chemokines, which contribute to ovarian cancer development through their induction of tumor cell proliferation, survival, migration, and angiogenesis [13–15]. The chemokine expression by ovarian cancer cells is controlled at several levels that include transcriptional regulation, post-transcriptional regulation and regulation of mRNA stability, translation, and mechanisms regulating the cytokine intracellular storage, transport, and release. Table 1 summarizes chemokines produced by ovarian cancer cells. Several excellent reviews have addressed the physiological and cellular functions of these chemokines in ovarian cancer [9,16,17]. Thus, in this review, we focus instead on the main mechanisms that regulate transcription of these chemokines in ovarian cancer cells.

**Table 1.** Chemokines released by ovarian cancer cells.

Systematic Name	Alternate Human Names	Tissue/Cells	Reference
<b>CCL2</b>	Monocyte chemoattractant protein 1 (MCP-1)	Tumor biopsies, serum and ascites	Negus <i>et al.</i> , 1995 [18] Milliken <i>et al.</i> , 2002 [19]
<b>CCL5</b>	RANTES	Tumor ascites, plasma and peritoneal fluid	Milliken <i>et al.</i> , 2002 [19] Negus <i>et al.</i> , 1997 [20]
<b>CCL11</b>	Eotaxin	Primary ovarian cancer cells obtained from ascites	Levina <i>et al.</i> , 2009 [21] Nolen <i>et al.</i> , 2010 [22]
<b>CCL25</b>	Thymus expressed chemokine (TECK)	Tumor tissue	Singh <i>et al.</i> , 2011 [23]
<b>CCL28</b>	Mucosae-associated epithelial chemokine (MEC)	Tumor tissue	Facciabene <i>et al.</i> , 2011 [24]
<b>CXCL1</b>	Growth-regulated protein $\alpha$ (GRO- $\alpha$ )	Plasma and tumor ascites	Lee <i>et al.</i> , 2006 [25] Yang <i>et al.</i> , 2006 [26]
<b>CXCL2</b>	Growth-regulated protein $\beta$ (GRO- $\beta$ )	Ovarian cancer cell lines	Son <i>et al.</i> , 2007 [27] Kavandi <i>et al.</i> , 2012 [28]
<b>CXCL8</b>	Interleukin 8 (IL-8)	Tumor tissue, ascites, serum and cyst fluid	Lee <i>et al.</i> , 1996 [29] Xu <i>et al.</i> , 1999 [30]
<b>CXCL12</b>	Stromal cell-derived factor (SDF-1)	Tumor biopsies, tissues and ascites	Zou <i>et al.</i> , 2001 [31] Scotton <i>et al.</i> , 2002 [32]
<b>CXCL16</b>	Transmembrane chemokine CXCL16	Epithelial ovarian carcinoma tissue	Guo <i>et al.</i> , 2011 [33] Gooden <i>et al.</i> , 2014 [34]
<b>CX3CL1</b>	Fractalkine	Epithelial ovarian carcinoma tissue	Gaudin <i>et al.</i> , 2011 [35]
<b>XCL1/2</b>	Lymphotactin	Tumor ascites and ovarian cancer cell lines	Kim <i>et al.</i> , 2012 [36]

## 2. Mechanisms Regulating Chemokine Transcription in Ovarian Cancer Cells

### 2.1. Chemokine Regulation by NFκB and Epigenetic Acetylation

Chemokines are regulated at the transcriptional level by binding of transcription factors and repressors to gene promoter and enhancer regions. The transcription factors that control the expression of most inflammatory chemokines include the nuclear factor-κB (NFκB), activator protein-1 (AP-1) and the signal transducers and activators of transcription (STAT) family. The NFκB activity is constitutively increased in aggressive ovarian cancers, and inhibition of NFκB signaling suppresses angiogenesis and tumorigenicity of ovarian cancer cells and increases their sensitivity to chemotherapy and apoptosis [37–40]. The underlying mechanisms likely involve the NFκB-regulated chemokine expression, since several studies have demonstrated that the expression of CCL2, CXCL1, CXCL2, and IL-8/CXCL8 is mediated by NFκB in ovarian cancer cells [28–30,41].

The increased activity of NFκB in ovarian cancer cells is mediated by enzymes of the IκB kinase (IKK) complex, which phosphorylate the NFκB inhibitory protein, IκBα, resulting in IκBα proteasomal degradation and nuclear translocation of NFκB subunits [42–45]. In addition to phosphorylating IκBα, IKKs can also phosphorylate the NFκB subunits, particularly p65 [46]. While the cytoplasmic degradation of IκBα, resulting in the nuclear translocation of NFκB subunits, represents a general step in NFκB activation, the specificity of NFκB-regulated responses is mediated by the subunit composition of NFκB complexes and their post-translational modifications [47,48].

In addition to transcription factor binding to promoter sequences, chemokine expression is regulated by epigenetic modifications that include histone modifications as well as post-translational modifications of transcription factors, particularly the p65 subunit of NFκB. It is believed that while histone acetylation and acetylation of transcription factors induced by histone acetyl transferases (HATs) generally promotes transcriptional activation, hypoacetylation induced by histone deacetylase (HDAC) activity is associated with transcriptional repression. Since hypoacetylation of tumor suppressor genes by HDACs has been linked to tumor development, HDACs inhibitors are now being evaluated for their therapeutic effects in cancer, including ovarian cancer [49–51]. Clinical studies using HDAC inhibitors in the treatment of ovarian cancer are summarized in the recent elegant review by Khabele [52]. Numerous studies have shown that HDACs regulate chemokine expression in different cell types [53–58]; however, their role in the regulation of chemokine expression in ovarian cancer has yet to be documented.

### 2.2. Chemokine Modulation by Hypoxia and Metabolism

Ovarian cancer tissues and ascites are characterized by decreased oxygen content, which stabilizes the α-subunit of the transcription factor hypoxia-inducible factor-1 (Hif-1) [59]. Hif-1 responds to hypoxia by increasing the transcription of genes that promote survival in low-oxygen conditions, thus promoting angiogenesis and oncogenesis. Indeed, the increased expression of Hif-1 has been detected in epithelial ovarian cancer, and correlates with poor prognosis [60–62]. Hypoxia induces IL-8 [30], CXCL12 [63], and CCL28 [24] expression in ovarian cancer cells. The seminal study by Xu *et al.* [30] demonstrated that hypoxic conditions increase the IL-8 expression in ovarian cancer cells by increasing NFκB and AP-1 binding to IL-8 promoter. The mechanisms of how hypoxia increases the NFκB-dependent IL-8 transcription involve activation of the transforming growth factor beta-activated

kinase 1 (TAK1), resulting in increased IKK activation, and p65 NF $\kappa$ B recruitment to the IL-8 promoter [64,65]. In addition, hypoxia induces a direct binding of Hif-1 $\alpha$  to the hypoxia-response element (HRE) located next to the NF $\kappa$ B binding site in human IL-8 promoter, resulting in the increased IL-8 expression [66].

One of the consequences of Hif-1 activation is the increased expression of glycolytic genes, resulting in increased aerobic glycolysis, glucose consumption, and lactic acid production (Warburg effect) [67–69]. The high rate of glucose consumption and lactic acid production contributes to the acidification of the tumor environment and cancer progression. Xu *et al.* showed that acidic pH increases the IL-8 transcription by enhancing the binding of AP-1 and NF $\kappa$ B to IL-8 promoter in ovarian cancer cells [70]. In addition, in endothelial cells, lactate was shown to activate the NF $\kappa$ B-dependent IL-8 transcription by inducing degradation of I $\kappa$ B $\alpha$  [71]. The role of lactate and other metabolites of the glycolytic pathway in the regulation of pro-angiogenic chemokine expression in ovarian cancer cells is yet to be investigated, especially since recent studies have indicated high levels of aerobic glycolysis and lactate production in ovarian tumors [72,73].

While hyperglycemia and obesity are thought to be contributing factors to cancer development and progression, caloric restriction has been associated with reduced cancer incidence [74–77]. During reduced calorie intake or exercise, the body switches to obtaining energy from fatty acid oxidation, which results in ketone bodies production. Intriguingly, the recent study by Shimazu *et al.* [78] has demonstrated that the ketone body  $\beta$ -hydroxybutyrate ( $\beta$ OHB) is an endogenous and specific inhibitor of HDACs, and that administration of exogenous  $\beta$ OHB increases histone acetylation, correlating with changes in transcription. Since HDACs regulate chemokine transcription by both deacetylating histones and p65 NF $\kappa$ B [53–58], it will be important to analyze whether  $\beta$ OHB and other HDAC inhibitors regulate chemokine expression in ovarian cancer cells, and whether this is modulated by the metabolic state.

### 2.3. Chemokine Modulation by Chemotherapeutic Interventions

There is growing evidence that the increased chemokine expression by tumor cells modulates not only cancer development but also cancer responsiveness and resistance to chemotherapy [79]. A major contributor to the acquired chemoresistance of ovarian cancer cells is the increased expression of NF $\kappa$ B-dependent chemokines that is induced by the platinum-based drugs carboplatin and cisplatin, and by the mitotic inhibitors docetaxel and paclitaxel [29,80–83]. The mechanisms responsible for the increased IL-8 expression induced by paclitaxel in ovarian cancer cells involve increased expression of toll-like receptors (TLRs) and increased p65 NF $\kappa$ B binding to IL-8 promoter [80,83].

Bortezomib (BZ) is the first FDA approved proteasome inhibitor, which has shown a limited effectiveness in ovarian cancer treatment as a single agent [84–87]. However, BZ has been considered in combination with cisplatin, since BZ prevents the cisplatin-induced degradation of cisplatin influx transporter, resulting in enhanced cisplatin uptake and tumor cell killing [88,89]. We have recently shown that BZ increases expression of IL-8 and CCL2 in ovarian cancer cells, while it does not affect expression of other NF $\kappa$ B-dependent genes. The responsible mechanisms involve a gene specific and IKK $\beta$ -dependent recruitment of S536 phosphorylated p65 NF $\kappa$ B to IL-8 and CCL2 promoters, suggesting that anti-inflammatory therapy targeting IKK $\beta$  might increase the BZ effectiveness in ovarian cancer treatment [41]. Since approximately 50% of women diagnosed with ovarian cancer die from

chemoresistant metastatic disease, understanding the molecular mechanisms by which chemotherapeutic interventions increase the chemokine expression in ovarian cancer cells should lead to the development of more effective combination strategies.

### 3. Chemokine Transcriptional Regulation in Ovarian Cancer Cells

Chemokines listed in Table 1 have all been identified in ovarian cancer cells and tissues. Various online databases can be used to assess putative transcription factor binding sites. For this review, we have obtained chemokine promoter sequences from the NCBI database and used the Algen promoter-mapping program to search for the transcription factor binding sites [90,91]. All found putative binding sites are listed in Tables 2–5; the binding sites that have been experimentally confirmed are highlighted in bold and labeled with an asterisk. Below, we limit discussion of the transcriptional mechanisms only to the chemokines that have been experimentally confirmed in ovarian cancer cells. While the first insights into the chemokine transcriptional regulation were obtained by using *in vitro* electrophoretic mobility shift assays (EMSA) or overexpression experiments, chromatin immunoprecipitations (ChIP) generally provides a more realistic picture about the transcription factor binding to endogenous promoter sequences in living cells.

**Table 2.** List of putative transcription factor binding sites in human CCL2 promoter.

Factor	Site	Sequence	Factor	Site	Sequence
SP-1	-54/-44	ACTCCGCCCT	c-Fos	-1465/-1457	CTGACTCC
Nkx-1	-65/-58	CCTCCTG	p53	-1541/-1534	GGGCAGG
Elk-1	-76/-71	GGAAG	HOX-11	-1571/-1564	CCTAACG
GATA	-88/-82	CTTATC	PEA3	-1644/-1636	AAACATCC
C/EBP	-112/-106	TTGCTC	GR	-1790/-1782	TTGTTCTC
ELF	-143/-130	CTACTTCCTGGAA	AR	-1789/-1781	TGTTCTCT
<b>Hif-1 *</b>	-127/-122	CACAG	FOXP3	-1959/-1950	AAACATTTT
<b>AP-1 *</b>	-139/-131	TTCCTGGAA	C/EBP	-1980/-1973	TTGCACA
<b>STAT1-3 *</b>	-139/-131	TTCCTGGAA	Pbx-1	-2132/-2120	AGCATGACTGGA
C-Ets1	-140/-133	CTTCCTG	FOXO-3	-2184/-2176	CTTATTTA
NF-AT	-181/-172	GGAAAAAGT	CUTL-1	-2309/-2303	ATTGGT
E47	-239/-232	GTCTGGG	PR	-2358/-2351	GAACACT
RP58	-256/-245	GTTACACATCTG	Smad3	-2521/-2511	GAGGCAGACA
HNF-1	-654/-646	TAATATTT	ER $\alpha$	-2570/-2562	CTGACCTC
TMF	-708/-701	TATAACA	c-Jun	-2580/-2574	CATGGG
HNF-3	-742/-735	CTATTTA	<b>NF<math>\kappa</math>B *</b>	-2600/-2591	GGAATTTC
AP-2	-747/-741	GCAGGC	ZDX/BCL6	-2632/-2621	GGAACTTC
c-Jun	-942/-935	TGACTTA	E47	-2678/-2671	ATCTGGA
HMG1	-1042/-1035	GGAAATT	ETF	-2717/-2708	CACAGCCCC
IRF-3	-1089/-1082	GCTTTCC	GATA	-2902/-2893	CTTTATCT
BTEB3	-1287/-1278	AGGAGGAGG	PU-1	-3041/-3031	TTACTTCCTC
NF-Y	-1315/-1307	ATTGGGCA	YY1	-3264/-3257	AAAATGG
USF-2b	-1447/-1439	GTCATTTG	RAR	-3429/-3421	ATCTCACC

\* Experimentally confirmed binding sites, Hif-1; Hypoxia inducible factor-1, AP-1; Activator protein-1, STAT1-3; Signal transducer and activator of transcription 1-3, NF $\kappa$ B; Nuclear factor kappa B.

**Table 3.** List of putative transcription factor binding sites in human CXCL1 promoter.

Factor	Site	Sequence	Factor	Site	Sequence
IRF-3	-50/-43	GCTTTCC	Elk-1	-771/-766	GGAAG
HMG I	-75/-68	AATTTCC	FOXP3	-791/-782	CAACATTTT
MBP-1	-78/-68	GGGAATTTCC	MZF-1	-810/-803	CAGGGGA
<b>NFκB</b> *	-79/-68	CGGGAATTTCC	TGIF	-870/-862	TGACAACC
<b>CDP</b> *	-97/-87	GGGATCGATC	C/EBP	-980/-974	TTGCAC
E47	-90/-83	ATCTGGA	YY-1	-1061/-1054	TAAATGG
E2F-1	-126/-119	GGCGGGG	c-Ets	-1076/-1069	CAGGAAG
SP3	-128/-119	GGGGCGGGG	AR	-1394/-1386	TGTTCTCT
<b>SP-1</b> *	-130/-121	GGGGGCGGG	c-Jun	-1491/-1483	TGACTCAT
R2	-137/-131	TCCACC	Pax	-1909/-1902	CCTTGAC
LF-A1	-247/-240	TGGGGCA	ERα	-2057/-2050	TGGGTCAA
<b>AP-2</b> *	-279/-273	GCAGGC	NF-Y	-2060/-2052	ATTGGGTC
AREB6	-296/-288	CAGGTGGT	LEF-1	-2807/-2799	CTTTGTTG
Smad3	-563/-553	TTCACAGACA	HNF-1	-2966/-2958	TAATATTT
PR	-602/-595	GAACATT	RAR	-3102/-3094	ATGCCTTAG
GR	-605/-596	GCAGAACAT	NHP-1	-3103/-3096	TGACCTT
TMF	-739/-732	TGTTATA	PEA3	-3110/-3102	GGATGTAT
GATA	-767/-761	GATAAG	ATF	-3452/-3443	TGACGTAAG

\* Experimentally confirmed binding sites, CDP; CAATT displacement protein, SP-1; Specificity protein 1, AP-2; Activator protein 2.

**Table 4.** List of putative transcription factor binding sites in human CXCL2 promoter.

Factor	Site	Sequence	Factor	Site	Sequence
<b>NFκB</b> *	-76/-67	GGGAATTTCC	BTEB3	-862/-853	AAGCGGAGT
CREB	-83/-74	CGGACGTCA	NF-Y	-970/-962	GAACCAAT
ATF-2	-83/-74	CGGACGTCA	HMG I	-999/-992	AATTTCC
HLF	-104/-95	GTTACGCAA	IRF	-999/-992	AATTTCC
E2F-1	-111/-104	GGCGGGA	NF-AT	-1001/-992	AAAATTTCC
NF-1	-113/-108	TTGGC	CUTL1	-1085/-1079	ATTGAT
LF-A1	-139/-132	CGGGGCA	FOXP3	-1115/-1106	CTTAATTTT
GATA	-192/-184	GGTTATCT	PR A	-1257/-1250	GAACACT
AP2α	-198/-192	GCAGGC	C/EBP	-1367/-1360	TGAGCAA
<b>STAT3</b> *	-218/-210	TTGGGGAA	MZF1	-1380/-1373	CAGGGGA
ERα	-241/-233	CTGACCCA	HNF-1	-1440/-1432	ATATTAAC
PEA3	-276/-268	GGATGTAG	TMF	-1880/-1873	TATAACA
Elk-1	-296/-292	GAAG	E47	-1830/-1823	TTCTGGA
<b>STAT3</b> *	-318/-310	GGGATCGATC	Nkx2	-1827/-1820	CTGGAGG
p53	-339/-332	CTTGCCC	HNF	-2153/-2146	TAAATGG
AhR	-418/-410	GCGTGCGT	YY1	-2153/-2146	TAAATGG
<b>c-Jun</b> *	-437/-430	TGACACA	HSF1	-2409/-2401	ATTCTAGG
c-Fos	-451/-443	TGCGTCAT	ETF	-2505/-2496	GGGGCTGTC
c-Ets	-473/-467	CAGGAAG	AP3	-2636/-2629	GAGTTAG
USF-1	-508/-499	ACACGTGAT	Smad3	-3112/-3102	CAGTCAGACA
AREB6	-574/-566	AACACCTG	LEF-1	-3101/-3093	CAACAAAG
FOXJ2	-621/-611	AAAATAAACA	TCF-1	-3102/-3093	ACAACAAAG
AR	-673/-665	TGTTCCAA	GR	-3256/-3247	ACAGAACAT

\* Experimentally confirmed binding sites, c-Jun; Jun proto-oncogene.

**Table 5.** List of putative transcription factor binding sites in human CXCL8 promoter.

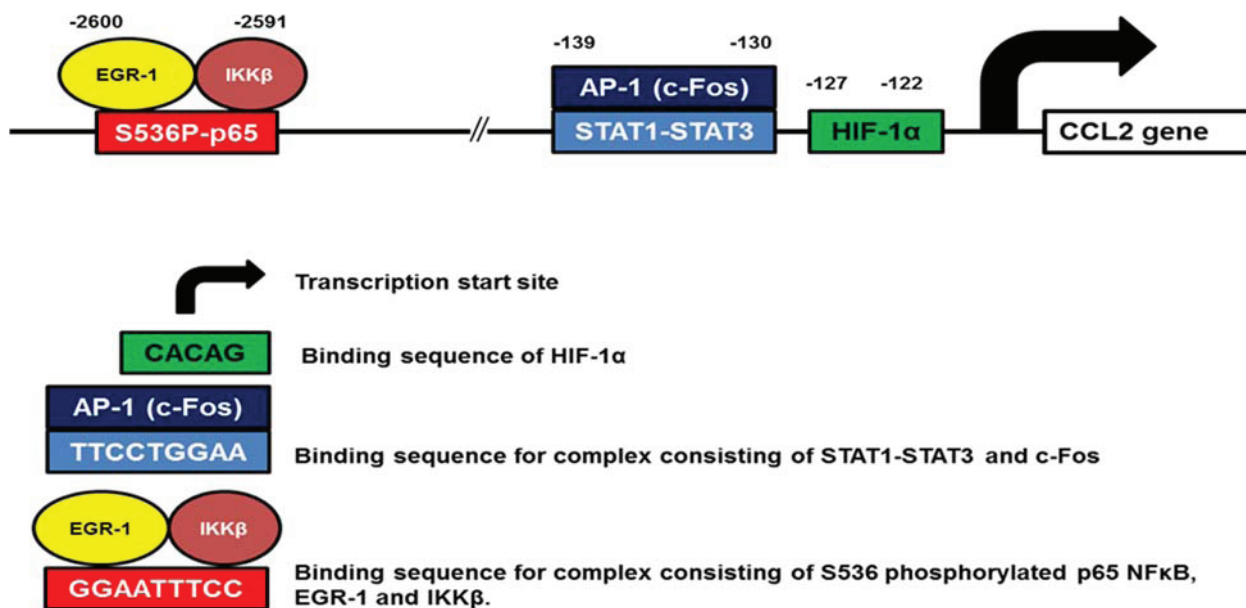
Factor	Site	Sequence	Factor	Site	Sequence
<b>NFκB *</b>	-80/-70	GGAATTTCC	E47	-859/-852	ATCTGGA
PU-1	-83/-73	GGAATTTCTC	PR	-868/-861	ACTCTTC
<b>NRF *</b>	-88/-77	ATTCCTCTGA	HSF1	-867/868	CCTTGAAT
<b>C/EBP *</b>	-94/-87	TTGCAAA	IRF	-973/-964	TTTCCATTA
MZF-1	-112/-105	GAGGGA	RAR	-1068/-1061	AGAGGTC
EBF	-118/-107	TGCCCTGAGGG	ERα	-1067/-1060	GAGGTCA
<b>C/EBP *</b>	-119/-112	TTGCACA	p53	-1258/-1251	CTTGCCC
<b>AP-1 *</b>	-129/-121	TGACTCAG	FOXP3	-1304/-1295	AAAATGAAG
c-Ets	-141/-132	TAGGAAGTC	RelA	-1367/-1357	GGCATTCCCC
Elk-1	-139/-134	GGAAG	YY1	-1372/-1365	AAAATGG
LEF-1	-187/-179	GATCAAAG	Smad3	-1403/-1393	GAAACAGACA
<b>Hif-1 *</b>	-234/-229	GTGCG	Nkx1	-1457/-1450	CCTCAAG
GRα	-335/-327	TTGTTCTA	AP2α	-1473/-1467	CCAGGC
AREB6	-328/-320	AACACCTG	TCF1	-1663/-1654	ACAACAAAG
AR	-334/-326	TGTTCTAA	NF-AT	-1687/-1677	CTAATTTTCC
NF	-424/-416	ATTGGCTC	HMG1	-1685/-1677	AATTTTCC
AP3	-535/-528	TAAATC	HLF	-1695/-1686	TTGTGTAAC
HNF-3	-606/-599	TAAATGT	CUTL1	-1858/1852	TTGGT
FOXO3	-651/-641	CTTATCTA	PEA3	-2174/-2166	GCACATCC
GATA	-651/-644	CTTTATCT	HOX11	-2200/-2193	CGTTAGG
c-Myb	-792/-784	CAACTGCC	RARγ	-2225/-2217	GGCTCACC
C/EBP	-798/-792	TTGCTC	AIRE	-2555/-2545	ATGGTTATCT
GR	-847/-838	CTGTTCTCT	Oct1	-2744/-2733	TCACTTTGCAT

\* Experimentally confirmed binding sites, C/EBP; CCAAT enhancer binding protein, NRF; NFκB repressing factor.

### 3.1. CCL2

CCL2 (MCP-1) is an important determinant of macrophage infiltration in ovarian tumors [92,93]. Although CCL2 has been originally thought to have an inhibitory effect on ovarian cancer progression [94–96], recent studies have indicated that CCL2 increases invasion of ovarian cancer cells and resistance to chemotherapy [97,98]. The putative transcription factor binding sites identified in human CCL2 promoter are listed in Table 2. Experimental studies demonstrated binding of NFκB, STAT1, STAT3, AP-1, and Hif-1α to the CCL2 promoter in OC cells (Figure 1).

Even though the NFκB binding site is located in the distal regulatory region of human CCL2 promoter (Figure 1), several studies have demonstrated p65 NFκB involvement in the regulation of CCL2 expression in OC cells [27,41,99]. In addition, CCL2 expression is regulated by IKKβ-dependent recruitment of the transcription factor EGR-1, and inhibition of IKKβ activity decreases p65 and EGR-1 promoter recruitment and CCL2 expression [41]. Interestingly, the NFκB binding site in human CCL2 promoter has the same nucleotide sequence as the NFκB site in human IL-8/CXCL8 promoter. Curiously, both CCL2 and IL-8 are increased by paclitaxel [83] and bortezomib [41], indicating that the paclitaxel and BZ-induced CCL2 (and IL-8) increase is promoter specific.



**Figure 1.** Schematic illustration of human CCL2 promoter.

Activity of the transcription factors STAT-1 and STAT-3 is also constitutively increased in OC cells, where it promotes cell motility and invasiveness [100]. Phosphorylation of STAT3 at tyrosine residues 705 and 727 increases its transcriptional activity [101]. In OC cells, IL-6 [102] and M-CSF [103] induce phosphorylation and activation of STAT3, and increase the CCL2 expression. In addition to NFκB and STAT transcription factors, studies in other cell types indicated that the CCL2 expression is positively regulated by AP-1 and Hif-1α [104–107].

Though no transcription factors have been reported to be involved in the negative regulation of CCL2 in OC cells, studies involving other cell types have reported negative regulators of CCL2. Specifically, NFκB p50/p50 homodimers, HDAC1, and the transcription factors Nrf2 and SMRT have been suggested to suppress the CCL2 expression in hepatic cells and adipocytes [108–110].

### 3.2. CXCL1

CXCL1 (GRO-α) contributes to ovarian cancer progression by inducing endothelial and epithelial cell proliferation and migration [25,26]. The putative transcription factor binding sites identified in human CXCL1 promoter are listed in Table 3. Experimental studies have demonstrated binding of the transcription factors p65 NFκB, AP-2, CCAAT displacement protein (CDP), and the stimulating protein-1 (SP-1) to the CXCL1 promoter in human cells (Figure 2). In ovarian cancer cells, though, the CXCL1 gene expression was found to be regulated mainly by NFκB pathway, specifically by the p65 DNA binding [25,27,28,111,112].

In addition to the positive regulation by p65 NFκB, AP-2 and SP-1, studies using human melanocytes have indicated that the CXCL1 expression is negatively controlled by the transcriptional repressors CDP and the poly(ADPribose) polymerase-1 (PARP-1) [113,114]. The exact mechanisms of how CDP and PARP-1 inhibit the CXCL1 expression are not fully understood; however, they likely involve displacement of trans-activating factors that bind to CXCL1 promoter, resulting in transcriptional repression.



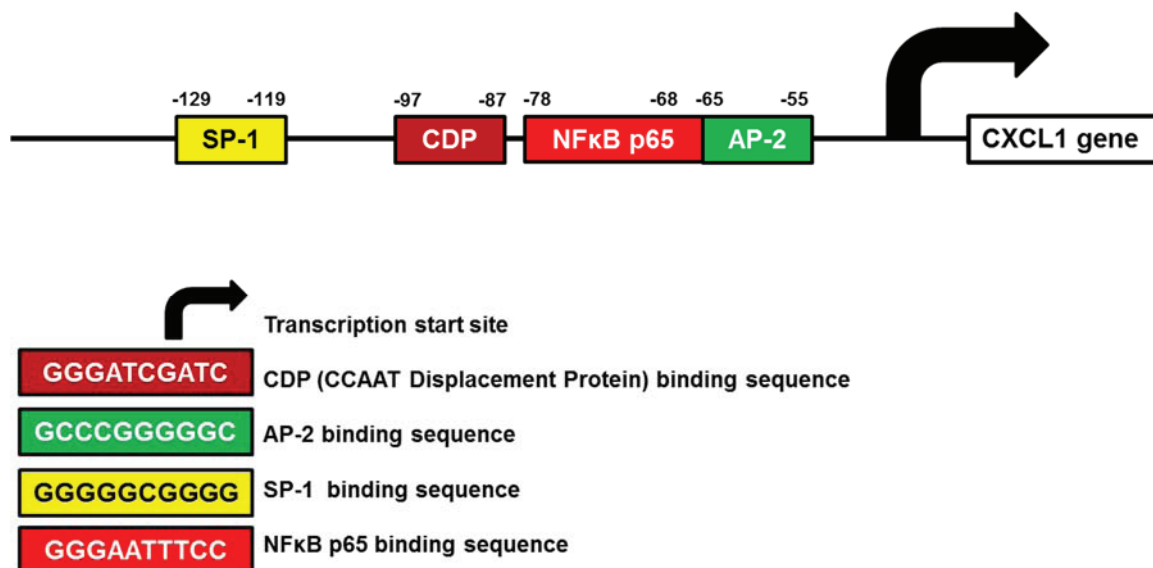


Figure 2. Schematic illustration of human CXCL1 promoter.

### 3.3. CXCL2

The putative transcription factor binding sites identified in human CXCL2 (GRO-β) promoter are listed in Table 4. However, experimental studies have demonstrated only binding of NFκB, AP-1, and STAT3 to human CXCL2 promoter (Figure 3). In ovarian cancer cells, the CXCL2 expression is dependent on IκBα [28] and IKKβ [44]. In addition, the CXCL2 expression in OC cells is induced by TNF, and is inhibited by overexpression of the tumor suppressor p53 [115].

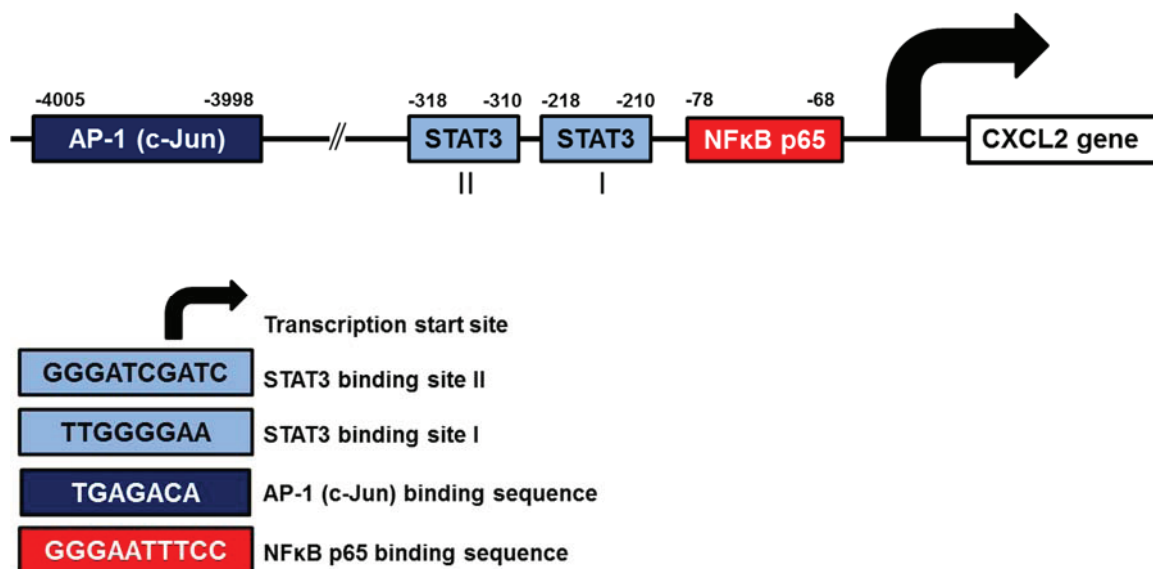
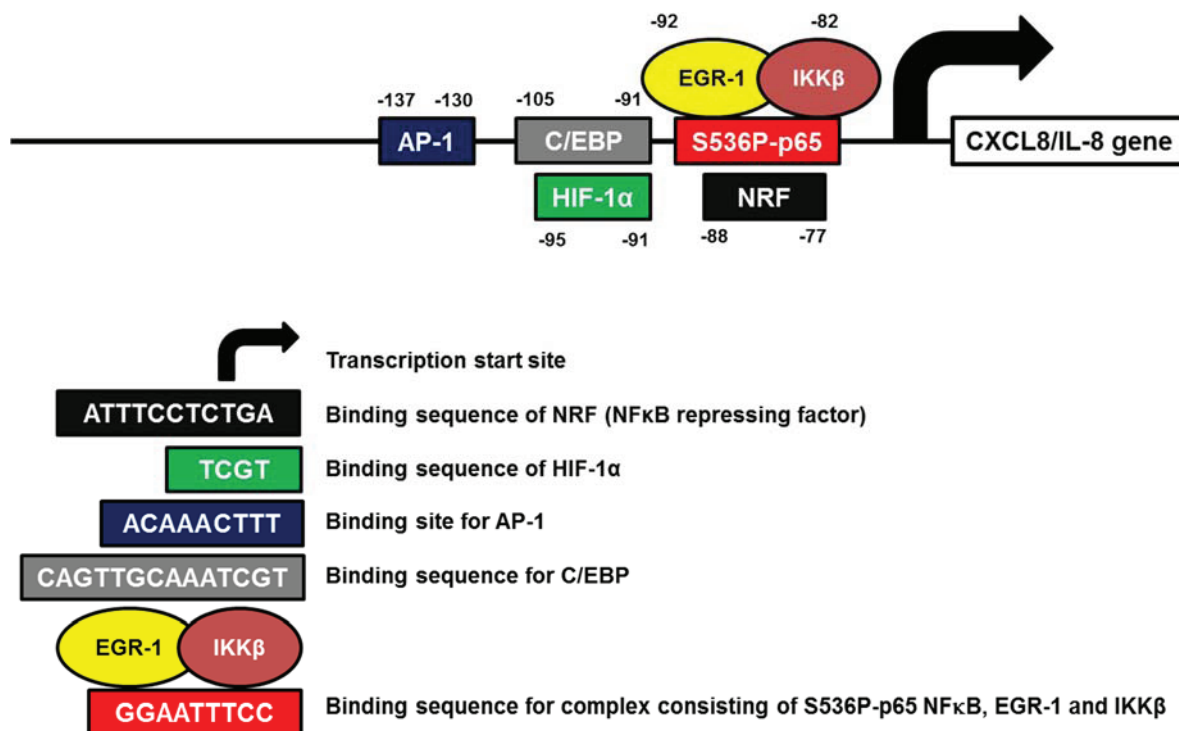


Figure 3. Schematic illustration of human CXCL2 promoter.

### 3.4. CXCL8

CXCL8 (IL-8), an inflammatory chemokine originally discovered as the neutrophil chemoattractant and inducer of leukocyte-mediated inflammation [1–3], contributes to cancer progression through its induction of tumor cell proliferation, migration and angiogenesis [4–9]. The expression levels of IL-8

directly correlate with ovarian cancer progression, and suppression of IL-8 expression inhibits angiogenesis and tumorigenicity of ovarian cancer cells [13,116–118]. A number of studies have identified a minimal region in human IL-8 promoter that spans nucleotides -1 to -140, is necessary for IL-8 transcription, and contains binding sites for NFκB, AP-1, CCAAT enhancer-binding protein beta (C/EBP or NF-IL6), Hif-1, and NFκB-repressing factor (NRF) [119–127]. In addition, the IL-8 transcription in ovarian cancer cells is positively regulated by the transcription factor early growth response-1 (EGR-1) binding to IL-8 promoter, and by enzymes of IKK complex that phosphorylate both IκBα, leading to its cytoplasmic degradation, and p65 NFκB, resulting in its increased transcriptional activity (Figure 4) [41–45].



**Figure 4.** Human CXCL8 promoter with the identified transcription factor binding sites.

NFκB is crucial for the IL-8 expression, and regulates IL-8 in all cell types [128]. The NFκB binding sequence (GGAATTTCC) is located between -80 and -70 of the IL-8 gene [120]. In most cell types, the IL-8 transcription is regulated predominantly by p65 homodimers [37,121,129–131]. Phosphorylation of p65 NFκB on serines 276 and 536 increases its transcriptional activity and interaction with other transcription factor and regulators, and decreases its affinity for nuclear IκBα [129–133]. We have recently shown that in ovarian cancer cells, the IL-8 transcription is regulated by S536-p65 NFκB, IKKβ, and EGR-1, and that proteasome inhibition developed as a strategy to inhibit NFκB-dependent transcription, paradoxically increases the IL-8 expression in ovarian cancer cells by increasing the S536-p65, IKKβ and EGR-1 recruitment to IL-8 promoter [41].

Adjacent to the NFκB site in the IL-8 promoter are C/EBP and Hif-1 binding sites (Figure 4). Even though the direct involvement of C/EBP and Hif-1 in the IL-8 regulation in ovarian cancer cells has yet to be demonstrated, the up-regulation of IL-8 expression by hypoxia in ovarian cancer cells has been well documented [30,134].

Transcription of IL-8 is also regulated by the transcription factor AP-1 that consists of Fos, FosB, Jun, and Jun-B subunits. Activation of AP-1 mediates the increased IL-8 expression in hypoxia, paclitaxel, and lysophosphatidic acid (LPA) treated OC cells [30,80,135]. Interestingly, a recent study has shown that the stress hormones norepinephrine and epinephrine enhance the IL-8 expression by a FosB-dependent mechanism [136]. Table 5 lists all putative transcription factor binding sites identified in the human CXCL8/IL-8 promoter.

Although studies from other cell types have shown that the IL-8 expression is negatively regulated by the NF $\kappa$ B repressing factor NRF, nuclear receptor corepressor (NCoR), the silencing mediator for retinoic acid and thyroid hormone receptor SMRT, and HDACs [54,137–139], the potential involvement of these corepressors in OC cells has yet to be demonstrated. Considering the important role these corepressors play in the IL-8 regulation, it will be important to elucidate their function in ovarian cancer setting.

#### 4. Conclusions and Perspectives

As we continue to improve our understanding of the mechanisms regulating chemokine expression in ovarian cancer cells, our knowledge will contribute to the development of new therapeutic strategies targeting the increased chemokine expression in chemoresistant metastatic ovarian cancer. Several important questions remain to be answered: What are the specific molecular targets and mechanisms responsible for the chemokine expression induced by chemotherapeutic drugs and hypoxia? What is the role of HDACs and other transcriptional repressors in regulating the chemokine expression in ovarian cancer cells? What is the role of the metabolic state of ovarian cancer cells in regulating the chemokine expression? Answers to these questions may open new avenues for therapeutic approaches for treating ovarian cancer.

#### Acknowledgments

We apologize to any scientists whose work could not be cited in this review due to space limitations; Work in the Vancurova lab is supported by grant CA173452 from the National Institutes of Health.

#### Author Contributions

All authors have contributed to the drafting, writing and critical revision of the manuscript, and have approved the final version of the manuscript.

#### Conflicts of Interest

The authors declare no conflicts of interest.

#### References

1. Baggiolini, M.; Walz, A.; Kunkel, S.L. Neutrophil-activating peptide-1/interleukin 8, a novel cytokine that activates neutrophils. *J. Clin. Investig.* **1989**, *84*, 1045–1049.

2. Kunkel, S.L.; Strieter, R.M.; Chensue, S.W.; Basha, M.; Standiford, T.; Ham, J.; Remick, D.G. Tumor necrosis factor-alpha, interleukin-8 and chemotactic cytokines. *Prog. Clin. Biol. Res.* **1990**, *349*, 433–444.
3. Baggiolini, M.; Dewald, B.; Moser, B. Human chemokines: An update. *Annu. Rev. Immunol.* **1997**, *15*, 675–705.
4. Murphy, P.M. Chemokines and the molecular basis of cancer metastasis. *N. Engl. J. Med.* **2001**, *345*, 833–835.
5. Balkwill, F.; Mantovani, A. Inflammation and cancer: Back to Virchow? *Lancet* **2001**, *357*, 539–545.
6. Zlotnik, A. Chemokines and cancer. *Int. J. Cancer* **2006**, *119*, 2026–2029.
7. Mantovani, A.; Allavena, P.; Sica, A.; Balkwill, F. Cancer-related inflammation. *Nature* **2008**, *454*, 436–444.
8. Lazennec, G.; Richmond, A. Chemokines and chemokine receptors: New insights into cancer-related inflammation. *Trends Mol. Med.* **2010**, *16*, 133–144.
9. Rainczuk, A.; Rao, J.; Gathercole, J.; Stephens, A.N. The emerging role of CXC chemokines in epithelial ovarian cancer. *Reproduction* **2012**, *144*, 303–317.
10. Zlotnik, A.; Yoshie, O. Chemokines: A new classification system and their role in immunity. *Immunity* **2000**, *12*, 121–127.
11. Fernandez, E.J.; Lolis, E. Structure, function, and inhibition of chemokines. *Annu. Rev. Pharmacol. Toxicol.* **2002**, *42*, 469–499.
12. Zlotnik, A.; Yoshie, O. The chemokine superfamily revisited. *Immunity* **2012**, *36*, 705–716.
13. Xu, L.; Fidler, I.J. Interleukin 8: An autocrine growth factor for human ovarian cancer. *Oncol. Res.* **2000**, *12*, 97–106.
14. Szlosarek, P.; Balkwill, F. The inflammatory cytokine network of epithelial cancer: Therapeutic implications. *Novartis Found. Symp.* **2004**, *256*, 227–237.
15. Waugh, D.J.; Wilson, C. The interleukin-8 pathway in cancer. *Clin. Cancer Res.* **2008**, *14*, 6735–6741.
16. Sarvaiya, P.J.; Guo, D.; Ulasov, I.; Gabikian, P.; Lesniak, M.S. Chemokines in tumor progression and metastasis. *Oncotarget* **2013**, *4*, 2171–2185.
17. Muralidhar, G.G.; Barbolina, M.V. Chemokine receptors in epithelial ovarian cancer. *Int. J. Mol. Sci.* **2013**, *15*, 361–376.
18. Negus, R.P.; Stamp, G.W.; Relf, M.G.; Burke, F.; Malik, S.T.; Bernasconi, S.; Allavena, P.; Sozzani, S.; Mantovani, A.; Balkwill, F.R. The detection and localization of monocyte chemoattractant protein-1 (MCP-1) in human ovarian cancer. *J. Clin. Investig.* **1995**, *95*, 2391–2396.
19. Milliken, D.; Scotton, C.; Raju, S.; Balkwill, F.; Wilson, J. Analysis of chemokines and chemokine receptor expression in ovarian cancer ascites. *Clin. Cancer Res.* **2002**, *8*, 1108–1114.
20. Negus, R.P.; Stamp, G.W.; Hadley, J.; Balkwill, F.R. Quantitative assessment of the leukocyte infiltrate in ovarian cancer and its relationship to the expression of C-C chemokines. *Am. J. Pathol.* **1997**, *150*, 1723–1734.
21. Levina, V.; Nolen, B.M.; Marrangoni, A.M.; Cheng, P.; Marks, J.R.; Szczepanski, M.J.; Szajnik, M.E.; Gorelik, E.; Lokshin, A.E. Role of eotaxin-1 signaling in ovarian cancer. *Clin. Cancer Res.* **2009**, *15*, 2647–2656.

22. Nolen, B.M.; Lokshin, A.E. Targeting CCL11 in the treatment of ovarian cancer. *Expert Opin. Ther. Target.* **2010**, *14*, 157–167.
23. Singh, R.; Stockard, C.R.; Grizzle, W.E.; Lillard, J.W., Jr.; Singh, S. Expression and histopathological correlation of CCR9 and CCL25 in ovarian cancer. *Int. J. Oncol.* **2011**, *39*, 373–381.
24. Facciabene, A.; Peng, X.; Hagemann, I.S.; Balint, K.; Barchetti, A.; Wang, L.; Gimotty, P.A.; Gilks, C.B.; Lal, P.; Zhang, L. Tumour hypoxia promotes tolerance and angiogenesis via CCL28 and Treg cells. *Nature* **2011**, *475*, 226–230.
25. Lee, Z.; Swaby, R.F.; Liang, Y.; Yu, S.; Liu, S.; Lu, K.H.; Bast, R.C., Jr.; Mills, G.B.; Fang, X. Lysophosphatidic acid is a major regulator of growth-regulated oncogene alpha in ovarian cancer. *Cancer Res.* **2006**, *66*, 2740–2748.
26. Yang, G.; Rosen, D.G.; Zhang, Z.; Bast, R.C., Jr.; Mills, G.B.; Colacino, J.A.; Mercado-Uribe, I.; Liu, J. The chemokine growth-regulated oncogene 1 (Gro-1) links RAS signaling to the senescence of stromal fibroblasts and ovarian tumorigenesis. *Proc. Natl. Acad. Sci. USA* **2006**, *103*, 16472–16477.
27. Son, D.; Parl, A.K.; Rice, V.M.; Khabele, D. Keratinocyte chemoattractant (KC)/human growth-regulated oncogene (GRO) chemokines and pro-inflammatory chemokine networks in mouse and human ovarian epithelial cancer cells. *Cancer Biol. Ther.* **2007**, *6*, 1302–1312.
28. Kavandi, L.; Collier, M.A.; Nguyen, H.; Syed, V. Progesterone and calcitriol attenuate inflammatory cytokines CXCL1 and CXCL2 in ovarian and endometrial cancer cells. *J. Cell Biochem.* **2012**, *113*, 3143–3152.
29. Lee, L.F.; Schuerer-Maly, C.C.; Lofquist, A.K.; van Haaften-Day, C.; Ting, J.P.; White, C.M.; Martin, B.K.; Haskill, J.S. Taxol-dependent transcriptional activation of IL-8 expression in a subset of human ovarian cancer. *Cancer Res.* **1996**, *56*, 1303–1308.
30. Xu, L.; Xie, K.; Mukaida, N.; Matsushima, K.; Fidler, I.J. Hypoxia-induced elevation in interleukin-8 expression by human ovarian carcinoma cells. *Cancer Res.* **1999**, *59*, 5822–5829.
31. Zou, W.; Machelon, V.; Coulomb-L'Hermin, A.; Borvak, J.; Nome, F.; Isaeva, T.; Wei, S.; Krzysiek, R.; Durand-Gasselien, I.; Gordon, A.; *et al.* Stromal-derived factor-1 in human tumors recruits and alters the function of plasmacytoid precursor dendritic cells. *Nat. Med.* **2001**, *7*, 1339–1346.
32. Scotton, C.J.; Wilson, J.L.; Scott, K.; Stamp, G.; Wilbanks, G.D.; Fricker, S.; Bridger, G.; Balkwill, F.R. Multiple actions of the chemokine CXCL12 on epithelial tumor cells in human ovarian cancer. *Cancer Res.* **2002**, *62*, 5930–5938.
33. Guo, L.; Cui, Z.M.; Zhang, J.; Huang, Y. Chemokine axes CXCL12/CXCR4 and CXCL16/CXCR6 correlate with lymph node metastasis in epithelial ovarian carcinoma. *Chin. J. Cancer* **2011**, *30*, 336–343.
34. Gooden, M.J.; Wiersma, V.R.; Boerma, A.; Leffers, N.; Boezen, H.M.; ten Hoor, K.A.; Hollema, H.; Walenkamp, A.M.; Daemen, T.; Nijman, H.W.; *et al.* Elevated serum CXCL16 is an independent predictor of poor survival in ovarian cancer and may reflect pro-metastatic ADAM protease activity. *Br. J. Cancer* **2014**, *110*, 1535–1544.
35. Gaudin, F.; Nasreddine, S.; Donnadiou, A.C.; Emilie, D.; Combadière, C.; Prévot, S.; Machelon, V.; Balabanian, K. Identification of the chemokine CX3CL1 as a new regulator of malignant cell proliferation in epithelial ovarian cancer. *PLOS ONE* **2011**, *6*, e21546.

36. Kim, M.; Rooper, L.; Xie, J.; Rayahin, J.; Burdette, J.E.; Kajdacsy-Balla, A.A.; Barbolina, M.V. The lymphotactin receptor is expressed in epithelial ovarian carcinoma and contributes to cell migration and proliferation. *Mol. Cancer Res.* **2012**, *10*, 1419–1429.
37. Huang, S.; Robinson, J.B.; Deguzman, A.; Bucana, C.D.; Fidler, I.J. Blockade of NF $\kappa$ B signaling inhibits angiogenesis and tumorigenicity of human ovarian cancer cells by suppressing expression of VEGF and IL-8. *Cancer Res.* **2000**, *60*, 5334–5339.
38. Mabuchi, S.; Ohmichi, M.; Nishio, Y.; Hayasaka, T.; Kimura, A.; Ohta, T.; Kawagoe, J.; Takahashi, K.; Yada-Hashimoto, N.; Seino-Noda, H.; *et al.* Inhibition of NF $\kappa$ B increases the efficacy of cisplatin in *in vitro* and *in vivo* ovarian cancer models. *J. Biol. Chem.* **2004**, *279*, 23477–23485.
39. Annunziata, C.M.; Stavnes, H.T.; Kleinberg, L.; Berner, A.; Hernandez, L.F.; Birrer, M.J.; Steinberg, S.M.; Davidson, B.; Kohn, E.C. NF $\kappa$ B transcription factors are coexpressed and convey a poor outcome in ovarian cancer. *Cancer* **2010**, *116*, 3276–3284.
40. Leizer, A.L.; Alvero, A.B.; Fu, H.H.; Holmberg, J.C.; Cheng, Y.; Silasi, D.; Rutherford, T.; Mor, G. Regulation of inflammation by the NF $\kappa$ B pathway in ovarian cancer stem cells. *Am. J. Reprod. Immunol.* **2011**, *65*, 438–447.
41. Singha, B.; Gatla, H.R.; Manna, S.; Chang, T.P.; Sanacora, S.; Poltoratsky, V.; Vancura, A.; Vancurova, I. Proteasome inhibition increases recruitment of I $\kappa$ B kinase  $\beta$  (IKK $\beta$ ), S536P-p65, and transcription factor EGR1 to interleukin-8 (IL-8) promoter, resulting in increased IL-8 production in ovarian cancer cells. *J. Biol. Chem.* **2014**, *289*, 2687–2700.
42. Mabuchi, S.; Ohmichi, M.; Nishio, Y.; Hayasaka, T.; Kimura, A.; Ohta, T.; Kawagoe, J.; Takahashi, K.; Yada-Hashimoto, N.; Seino-Noda, H.; *et al.* Inhibition of inhibitor of NF $\kappa$ B phosphorylation increases the efficacy of paclitaxel in *in vitro* and *in vivo* ovarian cancer models. *Clin. Cancer Res.* **2004**, *10*, 7645–7654.
43. Chen, R.; Alvero, A.; Silasi, D.; Kelly, M.; Fest, S.; Visintin, I.; Leiser, A.; Schwartz, P.; Rutherford, T.; Mor, G. Regulation of IKK $\beta$  by miR-199a affects NF $\kappa$ B activity in ovarian cancer cells. *Oncogene* **2008**, *27*, 4712–4723.
44. Hernandez, L.; Hsu, S.C.; Davidson, B.; Birrer, M.J.; Kohn, E.C.; Annunziata, C.M. Activation of NF $\kappa$ B signaling by inhibitor of NF $\kappa$ B kinase beta increases aggressiveness of ovarian cancer. *Cancer Res.* **2010**, *70*, 4005–4014.
45. Hsu, S.; Kim, M.; Hernandez, L.; Grajales, V.; Noonan, A.; Anver, M.; Davidson, B.; Annunziata, C.M. IKK- $\epsilon$  coordinates invasion and metastasis of ovarian cancer. *Cancer Res.* **2012**, *72*, 5494–5504.
46. Hayden, M.S.; Ghosh, S. Shared principles in NF $\kappa$ B signaling. *Cell* **2008**, *132*, 344–362.
47. Smale, S.T. Dimer-specific regulatory mechanisms within the NF $\kappa$ B family of transcription factors. *Immunol. Rev.* **2012**, *246*, 193–204.
48. Natoli, G. NF $\kappa$ B and chromatin: Ten years on the path from basic mechanisms to candidate drugs. *Immunol. Rev.* **2012**, *246*, 183–92.
49. Strait, K.A.; Warnick, C.T.; Ford, C.D.; Dabbas, B.; Hammond, E.H.; Ilstrup, S.J. Histone deacetylase inhibitors induce G2-checkpoint arrest and apoptosis in cisplatin-resistant ovarian cancer cells associated with overexpression of the Bcl-2-related protein Bad. *Mol. Cancer Ther.* **2005**, *4*, 603–611.

50. Wilson, A.J.; Holson, E.; Wagner, F.; Zhang, Y.; Fass, D.M.; Haggarty, S.J.; Bhaskara, S.; Hiebert, S.W.; Schreiber, S.L.; Khabele, D. The DNA damage mark pH2AX differentiates the cytotoxic effects of small molecule HDAC inhibitors in ovarian cancer cells. *Cancer Biol. Ther.* **2011**, *12*, 484–493.
51. Singh, B.N.; Zhou, H.; Li, J.; Tipton, T.; Wang, B.; Shao, G.; Gilbert, E.N.; Li, Q.; Jiang, S. Preclinical studies on histone deacetylase inhibitors as therapeutic reagents for endometrial and ovarian cancers. *Future Oncol.* **2011**, *7*, 1415–1428.
52. Khabele, D. The therapeutic potential of class I selective histone deacetylase inhibitors in ovarian cancer. *Front. Oncol.* **2014**, doi:10.3389/fonc.2014.00111.
53. Ashburner, B.P.; Westerheide, S.D.; Baldwin, A.S., Jr. The p65 (RelA) subunit of NFκB interacts with the histone deacetylase (HDAC) corepressors HDAC1 and HDAC2 to negatively regulate gene expression. *Mol. Cell Biol.* **2001**, *21*, 7065–7077.
54. Rahman, I.; Gilmour, P.S.; Jimenez, L.A.; MacNee, W. Oxidative stress and TNFα induce histone acetylation and NFκB/AP-1 activation in alveolar epithelial cells: Potential mechanism in gene transcription in lung inflammation. *Mol. Cell Biochem.* **2002**, *234–235*, 239–248.
55. Tomita, K.; Barnes, P.; Adcock, I. The effect of oxidative stress on histone acetylation and IL-8 release. *Biochem. Biophys. Res. Commun.* **2003**, *301*, 572–577.
56. Mayo, M.W.; Denlinger, C.E.; Broad, R.M.; Yeung, F.; Reilly, E.T.; Shi, Y.; Jones, D.R. Ineffectiveness of histone deacetylase inhibitors to induce apoptosis involves the transcriptional activation of NFκB through the Akt pathway. *J. Biol. Chem.* **2003**, *278*, 18980–18989.
57. Yang, S.R.; Chida, A.S.; Bauter, M.R.; Shafiq, N.; Seweryniak, K.; Maggirwar, S.B.; Kilty, I.; Rahman, I. Cigarette smoke induces proinflammatory cytokine release by activation of NFκB and posttranslational modifications of histone deacetylase in macrophages. *Am. J. Physiol. Lung Cell Mol. Physiol.* **2006**, *291*, L46–L57.
58. Ziesche, E.; Kettner-Buhrow, D.; Weber, A.; Wittwer, T.; Jurida, L.; Soelch, J.; Muller, H.; Newel, D.; Kronich, P.; Schneider, H.; *et al.* The coactivator role of histone deacetylase 3 in IL-1-signaling involves deacetylation of p65 NFκB. *Nucl. Acids Res.* **2013**, *41*, 90–109.
59. Kim, K.S.; Sengupta, S.; Berk, M.; Kwak, Y.G.; Escobar, P.F.; Belinson, J.; Mok, S.C.; Xu, Y. Hypoxia enhances lysophosphatidic acid responsiveness in ovarian cancer cells and lysophosphatidic acid induces ovarian tumor metastasis *in vivo*. *Cancer Res.* **2006**, *66*, 7983–7990.
60. Zhong, H.; de Marzo, A.M.; Laughner, E.; Lim, M.; Hilton, D.A.; Zagzag, D.; Buechler, P.; Isaacs, W.B.; Semenza, G.L.; Simons, J.W. Overexpression of hypoxia-inducible factor 1α in common human cancers and their metastases. *Cancer Res.* **1999**, *59*, 5830–5835.
61. Birner, P.; Schindl, M.; Obermair, A.; Breitenecker, G.; Oberhuber, G. Expression of hypoxia-inducible factor 1α in epithelial ovarian tumors: Its impact on prognosis and on response to chemotherapy. *Clin. Cancer Res.* **2001**, *7*, 1661–1668.
62. Braicu, E.I.; Luketina, H.; Richter, R.; Castillo-Tong, D.C.; Lambrechts, S.; Mahner, S.; Concin, N.; Mentze, M.; Zeillinger, R.; Vergote, I. HIF1α is an independent prognostic factor for overall survival in advanced primary epithelial ovarian cancer—A study of the OVCAD Consortium. *Oncol. Targets Ther.* **2014**, *7*, 1563–1569.

63. Kryczek, I.; Lange, A.; Mottram, P.; Alvarez, X.; Cheng, P.; Hogan, M.; Moons, L.; Wei, S.; Zou, L.; Machelon, V.; *et al.* CXCL12 and vascular endothelial growth factor synergistically induce neoangiogenesis in human ovarian cancers. *Cancer Res.* **2005**, *65*, 465–472.
64. Koong, A.C.; Chen, E.Y.; Giaccia, A.J. Hypoxia causes the activation of NF $\kappa$ B through the phosphorylation of I $\kappa$ B $\alpha$  on tyrosine residues. *Cancer Res.* **1994**, *54*, 1425–1430.
65. Culver, C.; Sundqvist, A.; Mudie, S.; Melvin, A.; Xirodimas, D.; Rocha, S. Mechanism of hypoxia-induced NF $\kappa$ B. *Mol. Cell Biol.* **2010**, *30*, 4901–4921.
66. Kim, K.S.; Rajagopal, V.; Gonsalves, C.; Johnson, C.; Kalra, V.K. A novel role of hypoxia-inducible factor in cobalt chloride- and hypoxia-mediated expression of IL-8 chemokine in human endothelial cells. *J. Immunol.* **2006**, *177*, 7211–7224.
67. Kroemer, G.; Pouyssegur, J. Tumor cell metabolism: Cancer’s Achilles’ heel. *Cancer Cell* **2008**, *13*, 472–482.
68. Hsu, P.P.; Sabatini, D.M. Cancer cell metabolism: Warburg and beyond. *Cell* **2008**, *134*, 703–707.
69. Semenza, G.L. HIF-1 mediates metabolic responses to intratumoral hypoxia and oncogenic mutations. *J. Clin. Investig.* **2013**, *123*, 3664–3671.
70. Xu, L.; Fidler, I.J. Acidic pH-induced elevation in interleukin 8 expression by human ovarian carcinoma cells. *Cancer Res.* **2000**, *60*, 4610–4616.
71. Vegran, F.; Boidot, R.; Michiels, C.; Sonveaux, P.; Feron, O. Lactate influx through the endothelial cell monocarboxylate transporter MCT1 supports an NF $\kappa$ B/IL-8 pathway that drives tumor angiogenesis. *Cancer Res.* **2011**, *71*, 2550–2560.
72. Anderson, A.S.; Roberts, P.C.; Frisard, M.I.; McMillan, R.P.; Brown, T.J.; Lawless, M.H.; Hulver, M.W.; Schmelz, E.M. Metabolic changes during ovarian cancer progression as targets for sphingosine treatment. *Exp. Cell Res.* **2013**, *319*, 1431–1442.
73. Caneba, C.; Yang, L.; Baddour, J.; Curtis, R.; Win, J.; Hartig, S.; Marini, J.; Nagrath, D. Nitric oxide is a positive regulator of the Warburg effect in ovarian cancer cells. *Cell Death Dis.* **2014**, *5*, e1302.
74. Kellenberger, L.D.; Bruin, J.E.; Greenaway, J.; Campbell, N.E.; Moorehead, R.A.; Holloway, A.C.; Petrik, J. The role of dysregulated glucose metabolism in epithelial ovarian cancer. *J. Oncol.* **2010**, doi:10.1155/2010/514310.
75. Gallagher, E.J.; LeRoith, D. Diabetes, cancer, and metformin: Connections of metabolism and cell proliferation. *Ann. NY Acad. Sci.* **2011**, *1243*, 54–68.
76. Hursting, S.D.; Dunlap, S.M.; Ford, N.A.; Hursting, M.J.; Lashinger, L.M. Calorie restriction and cancer prevention: A mechanistic perspective. *Cancer Metable* **2013**, doi:10.1186/2049-3002-1-10.
77. Al-Wahab, Z.; Tebbe, C.; Chhina, J.; Dar, S.A.; Morris, R.T.; Ali-Fehmi, R.; Giri, S.; Munkarah, A.R.; Rattan, R. Dietary energy balance modulates ovarian cancer progression and metastasis. *Oncotarget* **2014**, *5*, 6063–6075.
78. Shimazu, T.; Hirsche, M.D.; Newman, J.; He, W.; Shirakawa, K.; le Moan, N.; Grueter, C.A.; Lim, H.; Saunders, L.R.; Stevens, R.D.; *et al.* Suppression of oxidative stress by  $\beta$ -hydroxybutyrate, an endogenous histone deacetylase inhibitor. *Science* **2013**, *339*, 211–214.
79. De Visser, K.E.; Jonkers, J. Towards understanding the role of cancer-associated inflammation in chemoresistance. *Curr. Pharm. Des.* **2009**, *15*, 1844–1853.



80. Lee, L.F.; Haskill, J.S.; Mukaida, N.; Matsushima, K.; Ting, J.P. Identification of tumor-specific paclitaxel (Taxol)-responsive regulatory elements in the interleukin-8 promoter. *Mol. Cell Biol.* **1997**, *17*, 5097–5105.
81. Aghajanian, C. Clinical update: Novel targets in gynecologic malignancies. *Semin. Oncol.* **2004**, *31*, 22–26.
82. Kelly, M.G.; Alvero, A.B.; Chen, R.; Silasi, D.A.; Abrahams, V.M.; Chan, S.; Visintin, I.; Rutherford, T.; Mor, G. TLR-4 signaling promotes tumor growth and paclitaxel chemoresistance in ovarian cancer. *Cancer Res.* **2006**, *66*, 3859–3868.
83. Szajnik, M.; Szczepanski, M.J.; Czystowska, M.; Elishaev, E.; Mandapathil, M.; Nowak-Markwitz, E.; Spaczynski, M.; Whiteside, T.L. TLR4 signaling induced by lipopolysaccharide or paclitaxel regulates tumor survival and chemoresistance in ovarian cancer. *Oncogene* **2009**, *28*, 4353–4363.
84. Frankel, A.; Man, S.; Elliott, P.; Adams, J.; Kerbel, R.S. Lack of multicellular drug resistance observed in human ovarian and prostate carcinoma treated with the proteasome inhibitor PS-341. *Clin. Cancer Res.* **2000**, *6*, 3719–3728.
85. Aghajanian, C.; Dizon, D.S.; Sabbatini, P.; Raizer, J.J.; Dupont, J.; Spriggs, D.R. Phase I trial of bortezomib and carboplatin in recurrent ovarian or primary peritoneal cancer. *J. Clin. Oncol.* **2005**, *23*, 5943–5949.
86. Ramirez, P.T.; Landen C.N., Jr.; Coleman, R.L.; Milam, M.R.; Levenback, C.; Johnston, T.A.; Gershenson, D.M. Phase I trial of the proteasome inhibitor bortezomib in combination with carboplatin in patients with platinum- and taxane-resistant ovarian cancer. *Gynecol. Oncol.* **2008**, *108*, 68–71.
87. Aghajanian, C.; Blessing, J.A.; Darcy, K.M.; Reid, G.; deGeest, K.; Rubin, S.C.; Mannel, R.S.; Rotmensch, J.; Schilder, R.J.; Riordan, W. A phase II evaluation of bortezomib in the treatment of recurrent platinum-sensitive ovarian or primary peritoneal cancer: A Gynecologic Oncology Group study. *Gynecol. Oncol.* **2009**, *115*, 215–220.
88. Jandial, D.D.; Farshchi-Heydari, S.; Larson, C.A.; Elliott, G.I.; Wrasidlo, W.J.; Howell, S.B. Enhanced delivery of cisplatin to intraperitoneal ovarian carcinomas mediated by the effects of bortezomib on the human copper transporter 1. *Clin. Cancer Res.* **2009**, *15*, 553–560.
89. Howell, S.B.; Safaei, R.; Larson, C.A.; Sailor, M.J. Copper transporters and the cellular pharmacology of the platinum-containing cancer drugs. *Mol. Pharmacol.* **2010**, *77*, 887–894.
90. Messeguer, X.; Escudero, R.; Farre, D.; Nunez, O.; Martinez, J.; Alba, M.M. PROMO: Detection of known transcription regulatory elements using species-tailored searches. *Bioinformatics* **2002**, *18*, 333–334.
91. Farre, D.; Roset, R.; Huerta, M.; Adsuara, J.E.; Rosello, L.; Alba, M.M.; Messeguer, X. Identification of patterns in biological sequences at the ALGGEN server: PROMO and MALGEN. *Nucleic Acids Res.* **2003**, *31*, 3651–3653.
92. Negus, R.P.; Turner, L.; Burke, F.; Balkwill, F.R. Hypoxia down-regulates MCP-1 expression: Implications for macrophage distribution in tumors. *J. Leukoc. Biol.* **1998**, *63*, 758–765.
93. Sica, A.; Saccani, A.; Bottazzi, B.; Bernasconi, S.; Allavena, P.; Gaetano, B.; Fei, F.; LaRosa, G.; Scotton, C.; Balkwill, F.; *et al.* Defective expression of the monocyte chemotactic protein-1 receptor CCR2 in macrophages associated with human ovarian carcinoma. *J. Immunol.* **2000**, *164*, 733–738.

94. Wojnarowicz, P.; Gambaro, K.; de Ladurantaye, M.; Quinn, M.C.; Provencher, D.; Mes-Masson, A.M.; Tonin, P.N. Overexpressing the CCL2 chemokine in an epithelial ovarian cancer cell line results in latency of *in vivo* tumorigenicity. *Oncogenesis* **2012**, *1*, e27.
95. Fader, A.N.; Rasool, N.; Vaziri, S.A.; Kozuki, T.; Faber, P.W.; Elson, P.; Biscotti, C.V.; Michener, C.M.; Rose, P.G.; Rojas-Espaillet, L.; *et al.* CCL2 expression in primary ovarian carcinoma is correlated with chemotherapy response and survival outcomes. *Anticancer Res.* **2010**, *12*, 4791–4798.
96. Arnold, J.M.; Huggard, P.R.; Cummings, M.; Ramm, G.A.; Chenevix-Trench, G. Reduced expression of chemokine (C-C motif) ligand-2 (CCL2) in ovarian adenocarcinoma. *Br. J. Cancer* **2005**, *92*, 2024–2031.
97. Moisan, F.; Francisco, E.B.; Brozovic, A.; Duran, G.E.; Wang, Y.C.; Chaturvedi, S.; Seetharam, S.; Snyder, L.A.; Doshi, P.; Sikic, B.I. Enhancement of paclitaxel and carboplatin therapies by CCL2 blockade in ovarian cancers. *Mol. Oncol.* **2014**, *8*, 1231–1239.
98. Furukawa, S.; Soeda, S.; Kiko, Y.; Suzuki, O.; Hashimoto, Y.; Watanabe, T.; Nishiyama, H.; Tasaki, K.; Hojo, H.; Abe, M.; *et al.* MCP-1 promotes invasion and adhesion of human ovarian cancer cells. *Anticancer Res.* **2013**, *33*, 4785–4790.
99. Szlosarek, P.W.; Grimshaw, M.J.; Kulbe, H.; Wilson, J.L.; Wilbanks, G.D.; Burke, F.; Balkwill, F.R. Expression and regulation of tumor necrosis factor alpha in normal and malignant ovarian epithelium. *Mol. Cancer Ther.* **2006**, *5*, 382–390.
100. Silver, D.L.; Naora, H.; Liu, J.; Cheng, W.; Montell, D.J. Activated signal transducer and activator of transcription (STAT) 3: Localization in focal adhesions and function in ovarian cancer cell motility. *Cancer Res.* **2004**, *64*, 3550–3558.
101. Zhang, X.; Guo, A.; Yu, J.; Possemato, A.; Chen, Y.; Zheng, W.; Polakiewicz, R.D.; Kinzler, K.W.; Vogelstein, B.; Velculescu, V.E.; *et al.* Identification of STAT3 as a substrate of receptor protein tyrosine phosphatase T. *Proc. Natl. Acad. Sci. USA* **2007**, *104*, 4060–4064.
102. Coward, J.; Kulbe, H.; Chakravarty, P.; Leader, D.; Vassileva, V.; Leinster, D.A.; Thompson, R.; Schioppa, T.; Nemeth, J.; Vermeulen, J.; *et al.* Interleukin-6 as a therapeutic target in human ovarian cancer. *Clin. Cancer Res.* **2011**, *17*, 6083–6096.
103. Takaishi, K.; Komohara, Y.; Tashiro, H.; Ohtake, H.; Nakagawa, T.; Katabuchi, H.; Takeya, M. Involvement of M2-polarized macrophages in the ascites from advanced epithelial ovarian carcinoma in tumor progression via Stat3 activation. *Cancer Sci.* **2010**, *101*, 2128–2136.
104. Sutcliffe, A.M.; Clarke, D.L.; Bradbury, D.A.; Corbett, L.M.; Patel, J.A.; Knox, A.J. Transcriptional regulation of monocyte chemotactic protein-1 release by endothelin-1 in human airway smooth muscle cells involves NFκB and AP-1. *Br. J. Pharmacol.* **2009**, *157*, 436–450.
105. Chen, I.Y.; Chang, S.C.; Wu, H.Y.; Yu, T.C.; Wei, W.C.; Lin, S.; Chien, C.L.; Chang, M.F. Upregulation of the chemokine (C-C motif) ligand 2 via a severe acute respiratory syndrome coronavirus spike-ACE2 signaling pathway. *J. Virol.* **2010**, *84*, 7703–7712.
106. Dragomir, E.; Manduteanu, I.; Calin, M.; Gan, A.M.; Stan, D.; Koenen, R.R.; Weber, C.; Simionescu, M. High glucose conditions induce upregulation of fractalkine and monocyte chemotactic protein-1 in human smooth muscle cells. *Thromb. Haemost.* **2008**, *100*, 1155–1165.

107. Mojsilovic-Petrovic, J.; Callaghan, D.; Cui, H.; Dean, C.; Stanimirovic, D.B.; Zhang, W. Hypoxia-inducible factor-1 (HIF-1) is involved in the regulation of hypoxia-stimulated expression of monocyte chemoattractant protein-1 (MCP-1/CCL2) and MCP-5 (Ccl12) in astrocytes. *J. Neuroinflamm.* **2007**, doi:10.1186/1742-2094-4-12.
108. Elsharkawy, A.M.; Oakley, F.; Lin, F.; Packham, G.; Mann, D.A.; Mann, J. The NFκB p50:p50:HDAC-1 repressor complex orchestrates transcriptional inhibition of multiple pro-inflammatory genes. *J. Hepatol.* **2010**, *53*, 519–527.
109. Ichihara, S.; Yamada, Y.; Liu, F.; Murohara, T.; Itoh, K.; Yamamoto, M.; Ichihara, G. Ablation of the transcription factor Nrf2 promotes ischemia-induced neovascularization by enhancing the inflammatory response. *Arterioscler. Thromb. Vasc. Biol.* **2010**, *30*, 1553–1561.
110. Toubal, A.; Clement, K.; Fan, R.; Ancel, P.; Pelloux, V.; Rouault, C.; Veyrie, N.; Hartemann, A.; Treuter, E.; Venteclef, N. SMRT-GPS2 corepressor pathway dysregulation coincides with obesity-linked adipocyte inflammation. *J. Clin. Investig.* **2013**, *123*, 362–379.
111. Dong, Y.; Kabir, S.M.; Lee, E.; Son, D. CXCR2-driven ovarian cancer progression involves upregulation of proinflammatory chemokines by potentiating NFκB Activation via EGFR-transactivated Akt signaling. *PLOS ONE* **2013**, *8*, e83789.
112. Son, D.S.; Kabir, S.M.; Dong, Y.; Lee, E.; Adunyah, S.E. Characteristics of chemokine signatures elicited by EGF and TNF in ovarian cancer cells. *J. Inflamm.* **2013**, doi:10.1186/1476-9255-10-25.
113. Nirodi, C.; Hart, J.; Dhawan, P.; Moon, N.S.; Nepveu, A.; Richmond, A. The role of CDP in the negative regulation of CXCL1 gene expression. *J. Biol. Chem.* **2001**, *276*, 26122–26131.
114. Amiri, K.; Ha, H.; Smulson, M.; Richmond, A. Differential regulation of CXC ligand 1 transcription in melanoma cell lines by poly(ADP-ribose) polymerase-1. *Oncogene* **2006**, *25*, 7714–7722.
115. Son, D.; Kabir, S.M.; Dong, Y.; Lee, E.; Adunyah, S.E. Inhibitory effect of tumor suppressor p53 on proinflammatory chemokine expression in ovarian cancer cells by reducing proteasomal degradation of IκB. *PLOS ONE* **2012**, *7*, e51116.
116. Yoneda, J.; Kuniyasu, H.; Crispens, M.A.; Price, J.E.; Bucana, C.D.; Fidler, I.J. Expression of angiogenesis-related genes and progression of human ovarian carcinomas in nude mice. *J. Natl. Cancer Inst.* **1998**, *90*, 447–454.
117. Merritt, W.M.; Lin, Y.G.; Spannuth, W.A.; Fletcher, M.S.; Kamat, A.A.; Han, L.Y.; Landen, C.N.; Jennings, N.; de Geest, K.; Langley, R.R.; *et al.* Effect of interleukin-8 gene silencing with liposome-encapsulated small interfering RNA on ovarian cancer cell growth. *J. Natl. Cancer Inst.* **2008**, *100*, 359–372.
118. Pecot, C.V.; Rupaimoole, R.; Yang, D.; Akbani, R.; Ivan, C.; Lu, C.; Wu, S.; Han, H.; Shah, M.Y.; Rodriguez-Aguayo, C.; *et al.* Tumour angiogenesis regulation by the miR-200 family. *Nat. Commun.* **2013**, doi:10.1038/ncomms3427.
119. Mukaida, N.; Shiroo, M.; Matsushima, K. Genomic structure of the human monocyte-derived neutrophil chemotactic factor IL-8. *J. Immunol.* **1989**, *143*, 1366–1371.
120. Mukaida, N.; Mahe, Y.; Matsushima, K. Cooperative interaction of nuclear factor-kappa B- and cis-regulatory enhancer binding protein-like factor binding elements in activating the interleukin-8 gene by pro-inflammatory cytokines. *J. Biol. Chem.* **1990**, *265*, 21128–21133.

121. Kunsch, C.; Rosen, C.A. NF $\kappa$ B subunit-specific regulation of the interleukin-8 promoter. *Mol. Cell Biol.* **1993**, *13*, 6137–6146.
122. Stein, B.; Baldwin, A.S., Jr. Distinct mechanisms for regulation of the interleukin-8 gene involve synergism and cooperativity between C/EBP and NF $\kappa$ B. *Mol. Cell Biol.* **1993**, *13*, 7191–7198.
123. Matsusaka, T.; Fujikawa, K.; Nishio, Y.; Mukaida, N.; Matsushima, K.; Kishimoto, T.; Akira, S. Transcription factors NF-IL6 and NF $\kappa$ B synergistically activate transcription of the inflammatory cytokines, interleukin 6 and interleukin 8. *Proc. Natl. Acad. Sci. USA* **1993**, *90*, 10193–10197.
124. Kunsch, C.; Lang, R.K.; Rosen, C.A.; Shannon, M.F. Synergistic transcriptional activation of the IL-8 gene by NF $\kappa$ B p65 (RelA) and NF-IL-6. *J. Immunol.* **1994**, *153*, 153–164.
125. Oliveira, I.C.; Mukaida, N.; Matsushima, K.; Vilcek, J. Transcriptional inhibition of the interleukin-8 gene by interferon is mediated by the NF $\kappa$ B site. *Mol. Cell Biol.* **1994**, *14*, 5300–5308.
126. Yasumoto, K.; Okamoto, S.; Mukaida, N.; Murakami, S.; Mai, M.; Matsushima, K. Tumor necrosis factor alpha and interferon gamma synergistically induce interleukin 8 production in a human gastric cancer cell line through acting concurrently on AP-1 and NF $\kappa$ B-like binding sites of the interleukin 8 gene. *J. Biol. Chem.* **1992**, *267*, 22506–22511.
127. Nourbakhsh, M.; Kalble, S.; Dorrie, A.; Hauser, H.; Resch, K.; Kracht, M. The NF $\kappa$ B repressing factor is involved in basal repression and interleukin (IL)-1-induced activation of IL-8 transcription by binding to a conserved NF $\kappa$ B-flanking sequence element. *J. Biol. Chem.* **2001**, *276*, 4501–4508.
128. Hoffmann, E.; Dittrich-Breiholz, O.; Holtmann, H.; Kracht, M. Multiple control of interleukin-8 gene expression. *J. Leukoc. Biol.* **2002**, *72*, 847–855.
129. Sasaki, C.Y.; Barberi, T.J.; Ghosh, P.; Longo, D.L. Phosphorylation of RelA/p65 on serine 536 defines an I $\kappa$ B $\alpha$ -independent NF $\kappa$ B pathway. *J. Biol. Chem.* **2005**, *280*, 34538–34547.
130. Ghosh, C.C.; Ramaswami, S.; Juvekar, A.; Vu, H.Y.; Galdieri, L.; Davidson, D.; Vancurova, I. Gene-specific repression of proinflammatory cytokines in stimulated human macrophages by nuclear I $\kappa$ B $\alpha$ . *J. Immunol.* **2010**, *185*, 3685–3693.
131. Manna, S.; Singha, B.; Phyo, S.A.; Gatla, H.R.; Chang, T.P.; Sanacora, S.; Ramaswami, S.; Vancurova, I. Proteasome inhibition by bortezomib increases IL-8 expression in androgen-independent prostate cancer cells: The role of IKK $\alpha$ . *J. Immunol.* **2013**, *191*, 2837–2846.
132. Buss, H.; Dörrie, A.; Schmitz, M.L.; Hoffmann, E.; Resch, K.; Kracht, M. Constitutive and IL-1-inducible phosphorylation of p65 NF $\kappa$ B at serine 536 is mediated by multiple protein kinases including I $\kappa$ B kinase (IKK)- $\alpha$ , IKK $\beta$ , IKK $\epsilon$ , TRAF family member-associated (TANK)-binding kinase 1 (TBK1), and an unknown kinase and couples p65 to TATA-binding protein associated factor II31-mediated IL-8 transcription. *J. Biol. Chem.* **2004**, *279*, 55633–55643.
133. Moreno, R.; Sobotzik, J.M.; Schultz, C.; Schmitz, M.L. Specification of the NF $\kappa$ B transcriptional response by p65 phosphorylation and TNF-induced nuclear translocation of IKK epsilon. *Nucleic Acids Res.* **2010**, *38*, 6029–6044.
134. Xu, L.; Pathak, P.S.; Fukumura, D. Hypoxia-induced activation of p38 mitogen-activated protein kinase and phosphatidylinositol 3'-kinase signaling pathways contributes to expression of interleukin 8 in human ovarian carcinoma cells. *Clin. Cancer Res.* **2004**, *10*, 701–707.

135. Fang, X.; Yu, S.; Bast, R.C.; Liu, S.; Xu, H.J.; Hu, S.X.; LaPushin, R.; Claret, F.X.; Aggarwal, B.B.; Lu, Y.; *et al.* Mechanisms for lysophosphatidic acid-induced cytokine production in ovarian cancer cells. *J. Biol. Chem.* **2004**, *279*, 9653–9661.
136. Shahzad, M.M.; Arevalo, J.M.; Armaiz-Pena, G.N.; Lu, C.; Stone, R.L.; Moreno-Smith, M.; Nishimura, M.; Lee, J.W.; Jennings, N.B.; Bottsford-Miller, J.; *et al.* Stress effects on FosB- and IL8-driven ovarian cancer growth and metastasis. *J. Biol. Chem.* **2010**, *285*, 35462–35470.
137. Bartels, M.; Schweda, A.T.; Dreikhausen, U.; Frank, R.; Resch, K.; Beil, W.; Nourbakhsh, M. Peptide-mediated disruption of NFκB/NRF interaction inhibits IL-8 gene activation by IL-1 or Helicobacter pylori. *J. Immunol.* **2007**, *179*, 7605–7613.
138. Hoberg, J.E.; Yeung, F.; Mayo, M.W. SMRT derepression by the IκB kinase alpha: A prerequisite to NFκB transcription and survival. *Mol. Cell* **2004**, *16*, 245–255.
139. Nozell, S.; Laver, T.; Patel, K.; Benveniste, E.N. Mechanism of IFN-beta-mediated inhibition of IL-8 gene expression in astrogloma cells. *J. Immunol.* **2006**, *177*, 822–830.

© 2015 by the authors; licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution license (<http://creativecommons.org/licenses/by/4.0/>).