Transcriptional Regulation of Chemokine Expression in Ovarian Cancer

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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 |
|-----------------|-----------------------|--------------|-----------|
| CCL2            | Monocyte chemotactic protein 1 (MCP-1) | Tumor biopsies, serum and ascites | Negus et al., 1995 [18] Milliken et al., 2002 [19] |
| CCL5            | RANTES                | Tumor ascites, plasma and peritoneal fluid | Milliken et al., 2002 [19] Negus et al., 1997 [20] |
| CCL11           | Eotaxin               | Primary ovarian cancer cells obtained from ascites | Leviña et al., 2009 [21] Nolen et al., 2010 [22] |
| CCL25           | Thymus expressed chemokine (TECK) | Tumor tissue | Singh et al., 2011 [23] |
| CCL28           | Mucosae-associated epithelial chemokine (MEC) | Tumor tissue | Facciabene et al., 2011 [24] |
| CXCL1           | Growth-regulated protein α (GRO-α) | Plasma and tumor ascites | Lee et al., 2006 [25] Yang et al., 2006 [26] |
| CXCL2           | Growth-regulated protein β (GRO-β) | Ovarian cancer cell lines | Son et al., 2007 [27] Kavandi et al., 2012 [28] |
| CXCL8           | Interleukin 8 (IL-8)  | Tumor tissue, ascites, serum and cyst fluid | Lee et al., 1996 [29] Xu et al., 1999 [30] |
| CXCL12          | Stromal cell-derived factor (SDF-1) | Tumor biopsies, tissues and ascites | Zou et al., 2001 [31] Scotton et al., 2002 [32] |
| CXCL16          | Transmembrane chemokine CXCL16 | Epithelial ovarian carcinoma tissue | Guo et al., 2011 [33] Gooden et al., 2014 [34] |
| CX3CL1          | Fractalkine           | Epithelial ovarian carcinoma tissue | Gaudin et al., 2011 [35] |
| XCL1/2          | Lymphotactin          | Tumor ascites and ovarian cancer cell lines | Kim et al., 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 proteosomal 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 NFκB recruitment to the IL-8 promoter [64,65]. In addition, hypoxia induces a direct binding of Hif-1α to the hypoxia-response element (HRE) located next to the NFκB 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 NFκB to IL-8 promoter in ovarian cancer cells [70]. In addition, in endothelial cells, lactate was shown to activate the NFκB-dependent IL-8 transcription by inducing degradation of IκBα [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 β-hydroxybutyrate (βOHB) is an endogenous and specific inhibitor of HDACs, and that administration of exogenous βOHB increases histone acetylation, correlating with changes in transcription. Since HDACs regulate chemokine transcription by both deacetylating histones and p65 NFκB [53–58], it will be important to analyze whether βOHB 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 NFκB-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 NFκB 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 NFκB-dependent genes. The responsible mechanisms involve a gene specific and IKKβ-dependent recruitment of S536 phosphorylated p65 NFκB to IL-8 and CCL2 promoters, suggesting that anti-inflammatory therapy targeting IKKβ 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 Alggen 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 | CTTACG    |
| GATA   | -88/-82 | CTTATC     | PEA3    | -1644/-1636 | AAACATCC  |
| C/EBP  | -112/-106 | TTGTCCT | GR      | -1790/-1782 | TTGTCCT   |
| ELF    | -143/-130 | CTACTTCTGGAA | AR    | -1789/-1781 | TGGTCCTCT |
| Hif-1 * | -127/-122 | CACAG | FOXP3   | -1959/-1950 | AAACATTCTT |
| AP-1 * | -139/-131 | TTCTCGGAA | C/EBP  | -1980/-1973 | TTGGACA   |
| STAT1-3 * | -139/-131 | TTCTCGGAA | Pbx-1   | -2132/-2120 | AGCATGACTGGA |
| C-Ets1 | -140/-133 | CTTCCCTG | FOXO-3  | -2184/-2176 | CTTATTTA  |
| NF-AT  | -181/-172 | GGAAGAGT | CUTL-1  | -2309/-2303 | ATTGGT    |
| E47    | -239/-232 | GTCTGGG   | PR      | -2358/-2351 | GAACACT   |
| RP58   | -256/-245 | GTTCACATCTG | Smad3 | -2521/-2511 | GAGGCGAGCA |
| HNF-1  | -654/-646 | TAATATT    | ERα     | -2570/-2562 | CTGACCTC  |
| TMF    | -708/-701 | TATAACA    | c-Jun   | -2580/-2574 | CATGGG    |
| HNF-3  | -742/-735 | CTATTTA    | NFκB *  | -2600/-2591 | GGAATTCC  |
| AP-2   | -747/-741 | GCAGGC     | ZDX/BCL6 | -2632/-2621 | GGGACCTC  |
| c-Jun  | -942/-935 | TGACTTA    | E47     | -2678/-2671 | ATCTGG    |
| HMG1   | -1042/-1035 | GGAATT  | ETF     | -2717/-2708 | CACAGCC    |
| IRF-3  | -1089/-1082 | GCTTCC  | GATA     | -2902/-2893 | CTTTATCT  |
| BTEB3  | -1287/-1278 | AGGAGGAGG | PU-1     | -3041/-3031 | TTACTTCTC |
| NF-Y   | -1315/-1307 | ATTTGGCA | YY1      | -3264/-3257 | AAAATGG   |
| USF-2b | -1447/-1439 | GTCATTGG | 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, NFκB; 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 | AATTCC | FOXP3  | -791/-782 | CAACATTTT |
| MBP-1  | -78/-68 | GGGAATTTCC | MZF-1 | -810/-803 | CAGGGGA |
| NFkB*  | -79/-68 | CGGAATTTCC | TGF1  | -870/-862 | TGACAACC |
| CDP*   | -97/-87 | GGGATCGATC | C/EBP | -980/-974 | TTGCAC |
| E47    | -90/-83 | ATCTGGA | YY-1   | -1061/-1054 | TAAATGG |
| E2F-1  | -126/-119 | GGGCGGGG | e-Ets  | -1076/-1069 | CAGGAAG |
| SP3    | -128/-119 | GGGCGGGGG | AR    | -1394/-1386 | TGGTCTT |
| SP-1*  | -130/-121 | GGGGGGGGG | c-Jun  | -1491/-1483 | TGACTCAT |
| R2     | -137/-131 | TCCACC | Pax     | -1909/-1902 | CTTTGAC |
| LF-A1  | -247/-240 | TGGGGCA | ERα    | -2057/-2050 | TGGGTCAA |
| AP-2*  | -279/-273 | GCAGGCC | NF-Y    | -2060/-2052 | ATGGGTCT |
| AREB6  | -296/-288 | CAGGTGGT | LEF-1  | -2807/-2799 | CTTTGTTG |
| Smad3  | -563/-553 | TTCACAGACA | HNF-1 | -2966/-2958 | TAAATTT |
| PR     | -602/-595 | GAACATT | RAR    | -3102/-3094 | ATGCCCCA |
| GR     | -605/-596 | GCAGAACAT | NHp-1 | -3103/-3096 | TGACCTT |
| TMF    | -739/-732 | TGTTATA | PEA3   | -3110/-3102 | GGATGTAT |
| GATA   | -767/-761 | GATAAG | ATF    | -3452/-3443 | TGACGTTAA |

* 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 |
|--------|------|----------|--------|------|----------|
| NFkB*  | -76/-67 | GGGATTTCC | BTEB3  | -862/-853 | AAGCGGAGT |
| CREB   | -83/-74 | CGGACGCTCA | NF-Y   | -970/-962 | GAACCAT |
| ATF-2  | -83/-74 | CGGACGCTCA | HMG I  | -999/-992 | AATTTC |
| HLF    | -104/-95 | GTTACGCAA | IRF    | -999/-992 | AATTTC |
| E2F-1  | -111/-104 | GGCAGGA | NF-AT  | -1001/-992 | AAAATTTTC |
| NF-1   | -113/-108 | TTTGCC | CUTL1  | -1085/-1079 | ATTGA |
| LF-A1  | -139/-132 | CCGGGCA | FOXP3  | -1115/-1106 | CTTAATTT |
| GATA   | -192/-184 | GTTATCT | PR A   | -1257/-1250 | GAAC |
| AP2α   | -198/-192 | GCAGGC | C/EBP  | -1367/-1360 | TGAGCA |
| STAT3* | -218/-210 | TTGGGGAA | MZF1   | -1380/-1373 | CAGGGGA |
| ERα    | -241/-233 | CTTGACCA | HNF-1  | -1440/-1432 | ATATTAC |
| PEA3   | -276/-268 | GGATGTAG | TMF    | -1880/-1873 | TATAA |
| Elk-1   | -296/-292 | GAAG | E47    | -1830/-1823 | TTCTGG |
| STAT3* | -318/-310 | GGGATCGATC | Nkx2  | -1827/-1820 | CTGGAGG |
| p53    | -339/-332 | CTTGCCCC | HNF-1  | -2153/-2146 | TAAATGG |
| AhR    | -418/-410 | GGCTGCGT | YY1    | -2153/-2146 | TAAATGG |
| c-Jun  | -437/-430 | TGACACA | HSF1   | -2409/-2401 | ATTCTAGG |
| c-Fos  | -451/-443 | TGCGCTAC | ETF    | -2505/-2496 | GGGGCTG |
| c-Ets  | -473/-467 | CAGGAAG | AP3    | -2636/-2629 | GAGTTAG |
| USF-1  | -508/-499 | ACACGTGAT | Smad3 | -3112/-3102 | CAGTCAGA |
| AREB6  | -574/-566 | AACACCTG | LEF-1  | -3101/-3093 | CAACAA |
| FOXJ2  | -621/-611 | AAAATAAAC | TCF-1  | -3102/-3093 | AACAAAG |
| AR     | -673/-665 | TGTTCCA | GR     | -3256/-3247 | ACAGAAC |

* 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     |
|----------|----------|--------------|---------|----------|--------------|
| NFκB *   | -80/-70  | GGAATTTC    | E47     | -859/-852| ATCTGGCA     |
| PU-1     | -83/-73  | GGAATTTCTCC | PR      | -868/-861| ACTCTTTCC    |
| NRF *    | -88/-77  | ATTCCCTGTA  | HSFI    | -867/868 | CTTGGAAT     |
| C/EBP *  | -94/-87  | TTGCAAA     | IRF     | -973/-964| TTCCCCATTA    |
| MZF-1    | -112/-105| GAGGGA      | RAR     | -1068/-1061| AGAGGTC   |
| EBF      | -118/-107| TGCCCTGAGGG | ERα     | -1067/-1060| GAGGTC     |
| C/EBP *  | -119/-112| TTGCACA     | p53     | -1258/-1251| CTTGCC       |
| AP-1 *   | -129/-121| TGACTCAG    | FOXP3   | -1304/-1295| AAAATGAAG   |
| c-Ets    | -141/-132| TAGGAAGTC   | RelA    | -1367/-1357| GCCATTCCCC   |
| Elk-1    | -139/-134| GGAAG       | YY1     | -1372/-1365| AAAATGG     |
| LEF-1    | -187/-179| GATCAAAG    | Smad3   | -1403/-1393| GAAAACAGACA  |
| Hif-1 *  | -234/-229| GTGCCG      | Nkx1    | -1457/-1450| CTTCAAG     |
| GRα      | -335/-327| TTGTCTTA    | AP2α    | -1473/-1467| CCAAGC      |
| AREB6    | -328/-320| AACACCTG    | TCF1    | -1663/-1654| ACAACAAAAG  |
| AR       | -334/-326| TTGTTCTAA   | NF-AT   | -1687/-1677| CTAATTTC     |
| NF       | -424/-416| ATTTGCTC    | HMGI    | -1685/-1677| AATTTC      |
| AP3      | -535/-528| TAAATC      | HLF     | -1695/-1686| TTGTGTAAC    |
| HNF-3    | -606/-599| TAAATGT     | CUTL1   | -1858/1852 | TTGGT       |
| FOXO3    | -651/-641| CTATATCTA   | PEA3    | -2174/-2166| GACATCC      |
| GATA     | -651/-644| CTTATCTAT   | HOX11   | -2200/-2193| CGTTAGG      |
| c-Myb    | -792/-784| CAACTGCCC   | RARγ    | -2225/-2217| GGCTCACCA    |
| C/EBP    | -798/-792| TTGCTC      | AIRE    | -2555/-2545| ATGTTATCAT   |
| GR       | -847/-838| CTGTTCCTCT  | 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.
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.
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].

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 IkBa, 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 (GGAATTTC) 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 IkBα [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 NFκB 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.

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