8-13-2018

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Recommended Citation

Ignacio RM, Lee ES, Wilson AJ, Beeghly-Fadiel A, Whalen MM, Son DS. Chemokine Network and Overall Survival in TP53 Wild-Type and Mutant Ovarian Cancer. Immune Netw. 2018 Aug;18(4):e29. https://doi.org/10.4110/in.2018.18.e29

Immune Netw. 2018 Aug;18(4):e29
https://doi.org/10.4110/in.2018.18.e29
pISSN 1598-2629·eISSN 2092-6685

IMMUNE NETWORK

Brief Communication

Chemokine Network and Overall Survival in *TP53* Wild-Type and Mutant Ovarian Cancer

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Received: Jun 16, 2018
Revised: Aug 7, 2018
Accepted: Aug 8, 2018

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Conflict of Interest
The authors declare no potential conflicts of interest.

Abbreviations
CI, confidence interval; EOC, epithelial ovarian cancer; GEO, Gene Expression Omnibus; HGSOC, high-grade serous ovarian cancer; HR, hazard ratio; LGSOC, low-grade serous ovarian cancer; NCBI, National Center for Biotechnology Information; OC, ovarian cancer; OS, overall survival; PFS, progression-free survival; RFS, recurrence-free survival; TCGA, The Cancer Genome Atlas; *TP53*, tumor suppressor protein p53; *TP53*m, tumor

ABSTRACT

Ovarian cancer (OC) has the highest mortality rate among gynecological malignancies. Because chemokine network is involved in OC progression, we evaluated associations between chemokine expression and survival in tumor suppressor protein p53 (*TP53*) wild-type (*TP53*WT) and mutant (*TP53*m) OC datasets. *TP53* was highly mutated in OC compared to other cancer types. Among OC subtypes, CXCL14 was predominantly expressed in clear cell OC, and CCL15 and CCL20 in mucinous OC. *TP53*WT endometrioid OC highly expressed CXCL14 compared to *TP53*m, showing better progression-free survival but no difference in overall survival (OS). *TP53*m serous OC highly expressed CCL8, CCL20, CXCL10 and CXCL11 compared to *TP53*WT. CXCL12 and CCL21 were associated with poor OS in *TP53*WT serous OC. CXCR2 was associated with poor OS in *TP53*m serous OC, while CXCL9, CCL5, CXCR4, CXCL11, and CXCL13 were associated with better OS. Taken together, specific chemokine signatures may differentially influence OS in *TP53*WT and *TP53*m OC.

Keywords: Chemokines; Ovarian cancer; Overall survival; *TP53*

INTRODUCTION

Ovarian cancer (OC) is the fifth leading cause of cancer deaths among women with 22,240 new cases and 14,070 deaths estimated in the US in 2018 (1). OC alone accounts for 5% of cancer deaths, which is due to the fact that early-stage OC is asymptomatic, thus when diagnosed it has frequently already spread throughout the abdominal cavity (2). Although the 5-year survival rate of OC is 92% for women diagnosed at an early-localized stage, it has low survival rate, only 17% to 28% for those with advanced-diseased stage (3). OC has been classified based on cell of origin, such as epithelial ovarian cancer (EOC) which arises in the epithelium and accounts for up to 90% of cases, and non-EOCs from germ cells for 3% and sex cord-stromal cells for 2% (4). Furthermore, EOC is histologically subdivided into 4 subtypes such as serous (up to 70%), endometrioid (10%), mucinous (6%), and clear cell (6%) (4,5) that differ in their epidemiologic, genetic changes, tumor markers, and response

suppressor protein p53 mutant; *TP53*WT, tumor suppressor protein p53 wild-type

Author Contributions

Conceptualization: Son DS, Lee ES, Beeghly-Fadiel A, Whalen MM; Data curation: Son DS, Ignacio RMC, Wilson AJ, Beeghly-Fadiel A;Formal analysis: Son DS, Ignacio RMC, Wilson AJ, Beeghly-Fadiel A;Funding acquisition: Son DS, Lee ES, Wilson AJ, Beeghly-Fadiel A, Whalen MM; Investigation: Son DS, Ignacio RMC, Lee ES, Wilson AJ, Beeghly-Fadiel A; Methodology: Son DS, Ignacio RMC, Beeghly-Fadiel A; Supervision: Son DS; Validation: Son DS, Beeghly-Fadiel A; Visualization: Son DS; Writing - original draft: Son DS, Ignacio RMC; Writing - review & editing: Son DS, Ignacio RMC, Lee ES, Wilson AJ, Beeghly-Fadiel A, Whalen MM.

to therapy (6). In particular, high-grade serous ovarian cancer (HGSOC) is highly-aggressive, is diagnosed at advanced-stage, and has a poor prognosis (7). A frequent molecular alteration that accounts to over 95% of cases of HGSOC is the mutation in the tumor suppressor protein p53 (*TP53*) (8,9). In contrast, low-grade serous ovarian cancer (LGSOC) as well as other subtypes, such as mucinous, endometrioid and clear cells frequently do not carry *TP53* mutations (10,11).

To increase the survival rate of advanced OC, we need to identify molecular drivers that can serve as prognostic markers. Chemokines and their receptors have recently been receiving attention due to their possible involvement in OC progression and metastasis (12,13). Chemokines, chemotactic cytokines interacting with G-protein-coupled receptors, contribute to cell proliferation, inflammation, metastasis, and tumorigenesis (14-16). *TP53* mutations play a significant role in shifting the effects of inflammation toward oncogenic outcomes, making cancer cells more aggressive in response to inflammatory cytokines (17). We have shown that the frequent mutation of *TP53* in OC enhances proinflammatory chemokines, leading to inflammatory tumor microenvironment (18). In addition, *TP53* inactivation during carcinogenesis affects immune surveillance by interfering with chemokine expression (19).

To date, there have been no prior reports on patient survival based on *TP53* status and chemokines in OC. Here, we investigated the chemokine network in different subtypes of human EOC, focusing on whether there is association in *TP53* status and chemokine network and how this correlates with OC survival.

MATERIALS AND METHODS

Data analysis

Data analysis was performed on publicly available microarray data-sets that were deposited in the National Center for Biotechnology Information (NCBI) Gene Expression Omnibus (GEO) (http://www.ncbi.nlm.nih.gov/geo/) database under accession numbers GSE6008 and GSE63885. For GSE6008, RNA expression data were from 99 individual OC cases (37 endometrioid, 41 serous, 13 mucinous, and 8 clear cell carcinomas) and 4 individual normal ovarian samples. GSE63885 is gene expression data from 101 OC surgical samples, including 71 serous carcinomas, for which somatic *TP53* gene mutation status was available.

We utilized Gitools 2.3.1 (http://www.gitools.org) based on Oracle Java 7, an open-source tool to perform Genomic Data Analysis and Visualization as interactive heat-maps (20). Kaplan-Meier plotter database (http://kmplot.com/analysis/index.php?p=service&cancer=ovar) was utilized to evaluate progression-free survival (PFS) and overall survival (OS) using proportional hazards regression to estimate hazard ratios (HRs) and 95% confidence intervals (CIs) based on gene expression profile of chemokines among 1,656 OC patients from GEO and The Cancer Genome Atlas (TCGA); chemokines were specified with probe sets (Affymetrix HG-U133A, HG-U133A 2.0, and HG-U133 Plus 2.0 microarrays) (21). *TP53* alteration frequency profile across many types of cancers was acquired from cBioPortal (www.cbioportal.org) (22,23); studies with <25% of altered samples were not included. Data on expression levels were analyzed using Student's *t*-test and 1-way ANOVA as appropriate. If statistical significance ($p \leq 0.05$) was indicated by ANOVA, then data were further analyzed using Tukey's pairwise comparisons to detect specific group differences.

RESULTS AND DISCUSSION

TP53 is the most frequently altered gene in many types of malignancies, with mutations in at least 50% of human cancers. First, we checked the alteration frequency in *TP53* across different types of cancers. *TP53* mutation was common across many malignancies, while deletion, amplification and multiple alterations in *TP53* occurred in only a few types of cancers (**Fig. 1**). OC in the published TCGA dataset, which contains only HGSOC (9), had the highest percentage of *TP53* mutation (**Fig. 1**). Even all of *de novo* HGSOC are supposed to contain *TP53* somatic mutations or deletions, except for the rare HGSOC that develop from a low-grade serous tumor precursor (24).

OC is associated with chronic inflammation (25). Previously, we reported that loss or mutation of *TP53* may promote tumor progression by enhancing proinflammatory chemokines in OC (18). Chemokines and their receptors are differentially expressed in tumors, influencing cancer progression. We then evaluated whether the different subtypes of EOC would show different patterns of chemokine expression profiles based on publicly available GEO data (GSE6008). Clear cell OC has high CXCL14 expression, while mucinous OC had high CCL15 and CCL20 expression (**Fig. 2**). Functional roles of these chemokines in clear cell and mucinous OC are largely unknown. On the other hand, normal ovarian samples highly expressed CCL18 and CXCL12 compared to other EOC subtypes (**Fig. 2**). Consistently, normal ovarian stroma expressed a higher level of CXCL12 compared to EOC (26). Although serum CCL18 was elevated in patients with EOC (27), patients with higher CCL18 in tumor samples had better OS in OC (HR=0.88; 95% CI=0.77–1.00; n=1,656) based on our analysis. Data of *TP53* mutation in clear cell and mucinous OC subtypes are not available for statistical analysis because of limited data. Next, we classified endometrioid EOC into *TP53* wild-type (*TP53*WT) and *TP53* mutant (*TP53*m) and assessed chemokine signatures. CXCL14 was highly expressed in *TP53*WT endometrioid OC compared to *TP53*m (**Fig. 3A**). Because of limited data to identify *TP53*WT and *TP53*m endometrioid OC patients for OS measurement, we used total endometrioid OC to investigate OS and PFS based on the expression levels of CXCL14. Patients with high expression of CXCL14 has better PFS (HR=0.22; 95% CI=0.08–0.63) but unchanged OS in endometrioid OC (**Fig. 3B**). CXCL14 protein level is positively correlated to OS of breast cancer patients (28). Moreover, CXCL14 acts as a tumor suppressor gene that is epigenetically silenced during lung tumorigenesis, and the re-expression of CXCL14 leads to increased cell death and reduced growth of lung tumor xenografts (29). CXCL14 is a potent angiostatic chemokine which prevents chemokine- and growth factor-induced angiogenesis (30). High expression levels of CXCL14 in clear cell and endometrioid OC may be one reason why these subtypes have better prognosis than serous OC. Interestingly, CXCL14 was reported to be expressed in normal tissue, such as the epithelia (31), and OC stroma (26).

We determined the chemokine signature in *TP53*WT and *TP53*m serous EOC subtype from GEO dataset (GSE63885). Chemokines, such as CCL8, CCL20, CXCL10 and CXCL11, are highly expressed in *TP53*m compared to *TP53*WT serous OC (**Fig. 4**). Our previous study showed that CCL20 is expressed dominantly in CXCR2-driven OC, leading to shorter survival, greater tumor spread in the peritoneal cavity, and larger tumor weights in xenograft OC models (26). Chemokines can exert pro- and anti-tumorigenic effects, depending upon the context (32). CXCL10 is thought to impair angiogenesis and has anti-tumor actions (33,34). Increased expression of CXCL10 had also been linked with advanced human malignancies such as melanoma (35) and OC (36). The chemokine network is involved in the metastasis of *TP53*WT and *TP53*m malignancies. The mutant TP53 protein enhances the

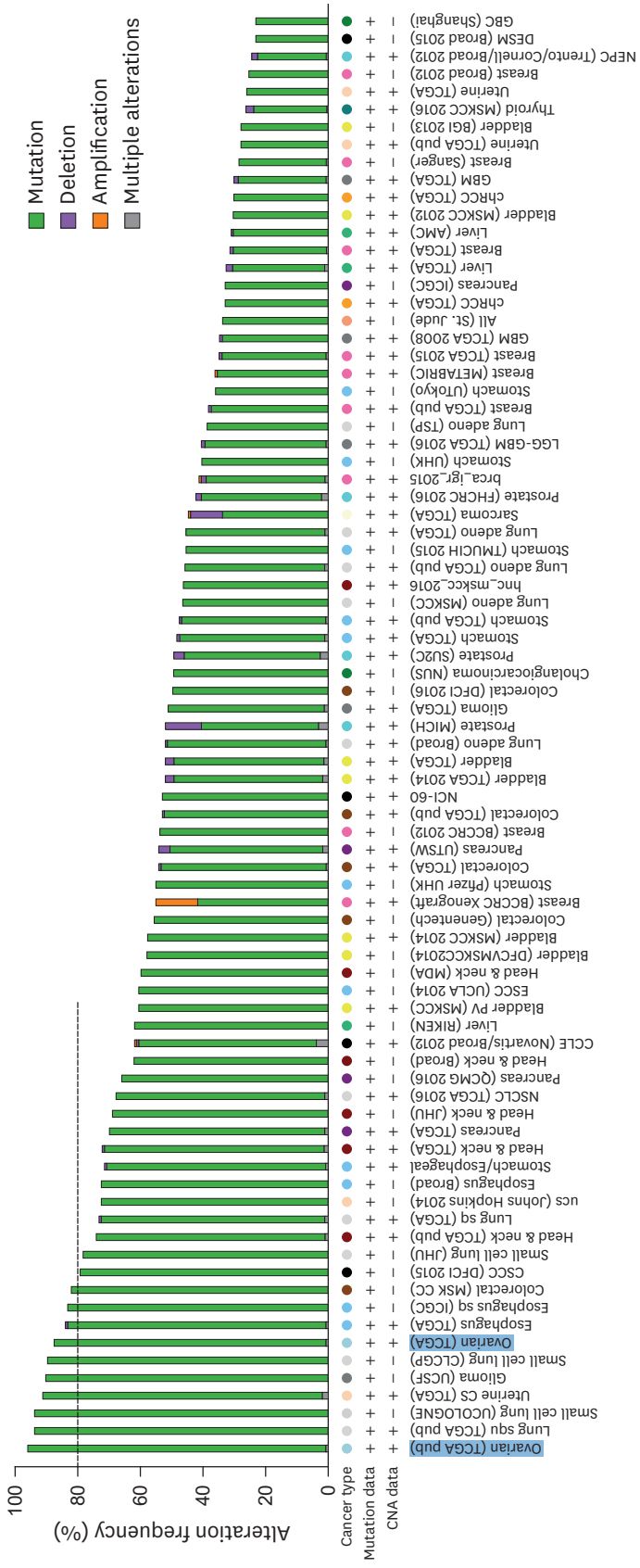

Figure 1. Analysis of alteration frequency (%) in *TP53* across many cancer types. The 150 studies deposited in cBioPortal (www.cbioportal.org) were assessed to analyze the *TP53*% alteration frequency profile. The 68 studies with <25% alteration frequency were filtered out to create this graph. Green, blue, red and gray bars indicate mutation, deletion, amplification and multiple alterations in *TP53*, respectively. The dot line indicates 80%. Blue highlighting indicates OC data from TCGA.

https://doi.org/10.4110/in.2018.18.e29

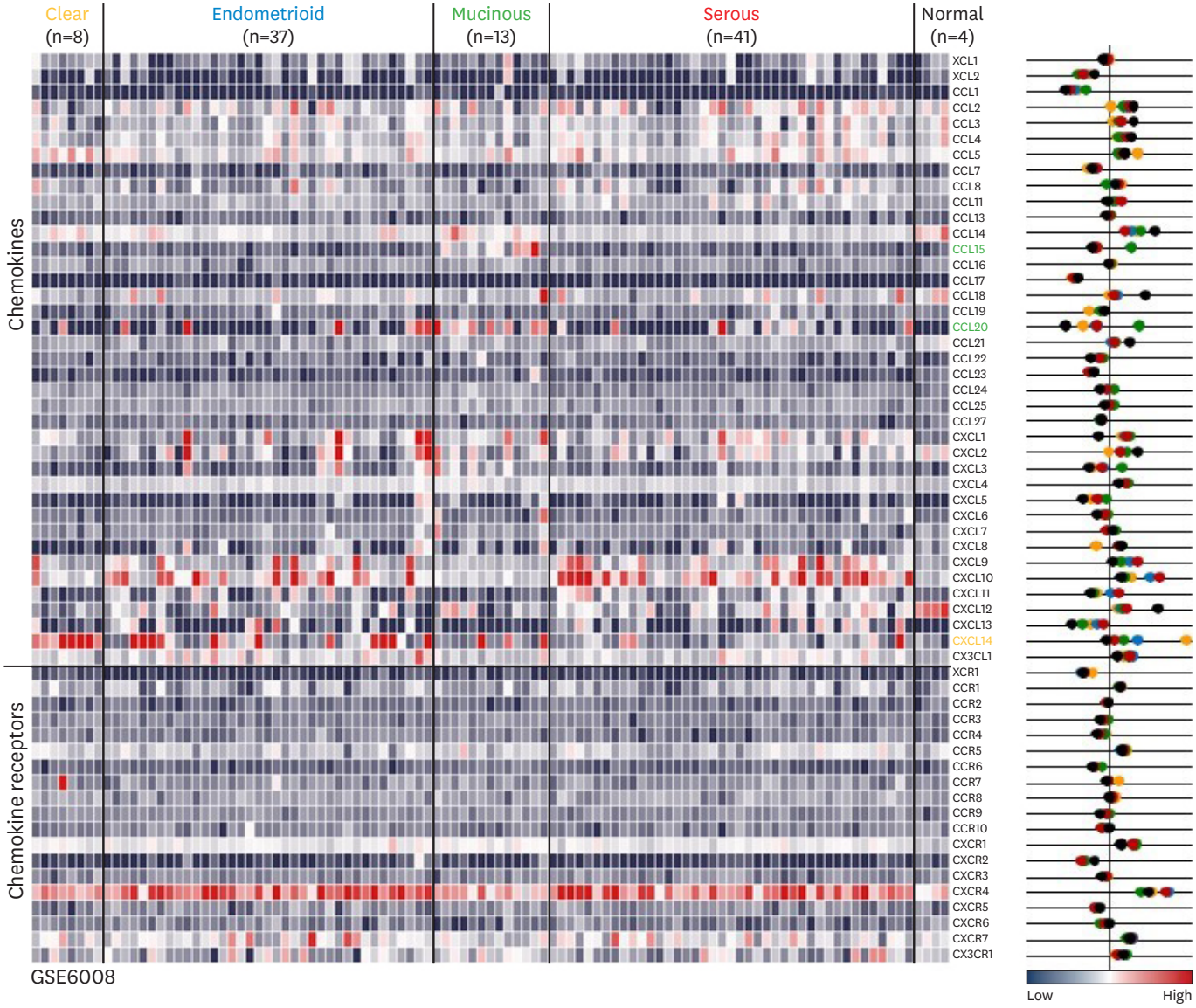

Figure 2. Chemokine and chemokine receptor signatures in EOC subtypes. Heatmap of chemokine expression profiles in EOC subtypes, including clear cell (n=8, yellow), endometrioid (n=37, blue), mucinous (n=13, green), and serous (n=41, red), and normal ovarian samples (n=4, black) from the NCBI GEO (http://www.ncbi.nlm.nih.gov/geo/) database (GSE6008) using Gitools 2.3.1. Bold yellow, green and black letters indicate dominant chemokines in clear cell, mucinous and normal samples, respectively. The right panel indicates statistical analysis of chemokine expression intensities using ANOVA and Tukey's pairwise comparisons.

secretion of CXC chemokines via the NF-κB pathway, leading to increase cell migration (37). Particularly, CXCL1 and CXCL8, implicated in cancer invasiveness and angiogenesis, are downregulated in *TP53*WT-transfected OC (18). In addition, increased expression levels of CXCL5, CXCL8, and CXCL12 were found in *TP53*m cells compared to *TP53*WT cells (37). In several malignancies such as lung, melanoma and breast cancer, knockdown of the mutant TP53 protein led to reduced chemokine levels and cell migration (37).

Finally, we utilized datasets available in the Kaplan-Meier plotter database to evaluate OS based on chemokine signatures in *TP53*WT and *TP53*m serous OC. **Tables 1** and **2** show HRs for OS based on chemokine and chemokine receptor signatures in *TP53*WT and *TP53*m serous OC, respectively. High expression levels of CXCL12 (HR=1.84; 95% CI=1.05–3.22) and CCL21 (HR=1.76; 95%

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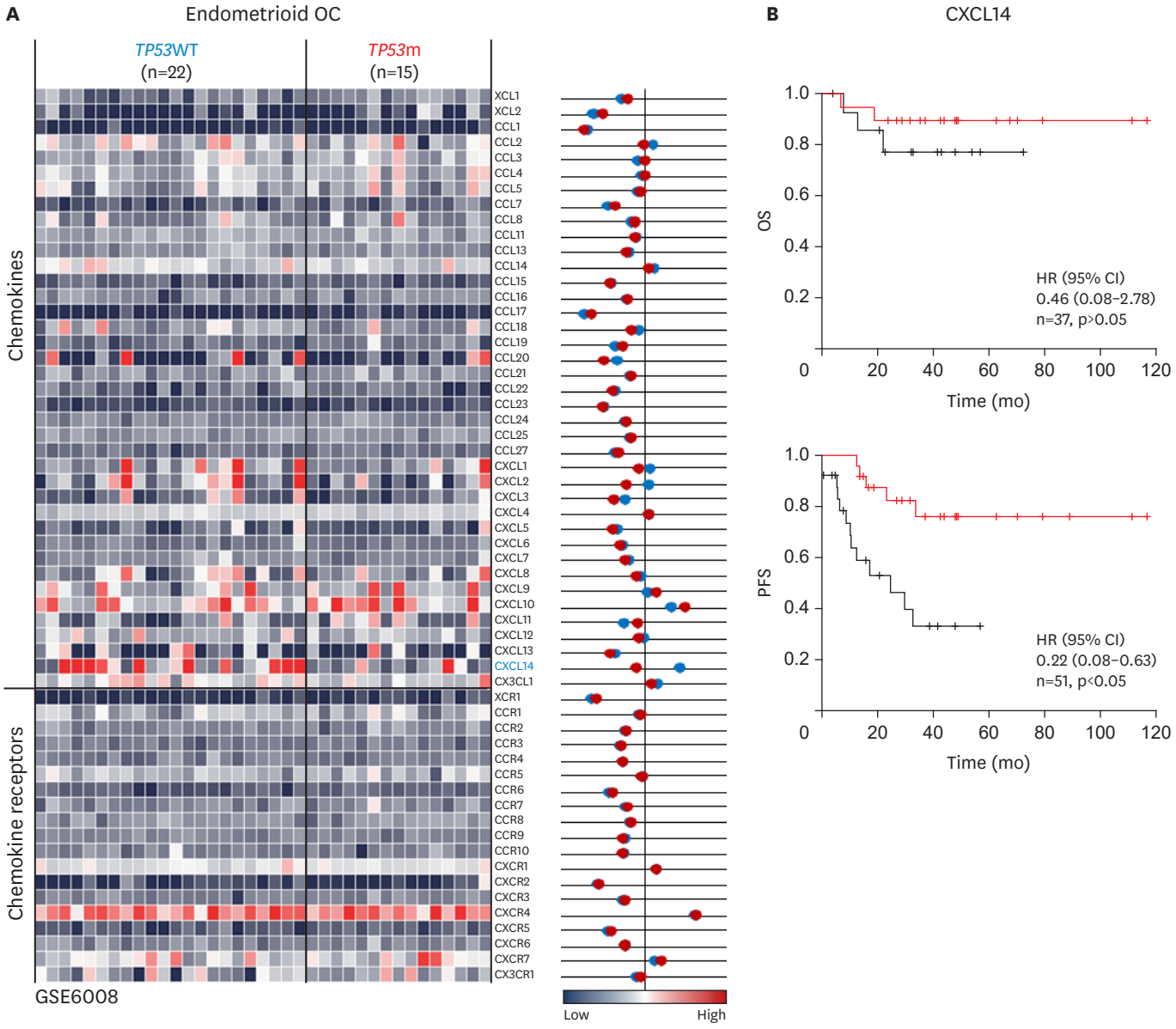

Figure 3. Chemokine and chemokine receptor signatures in *TP53*WT and *TP53*m endometrioid OC. (A) Heatmap of chemokine and chemokine receptor expression profiles in *TP53*WT (n=22) and *TP53*m (n=15) endometrioid OC from NCBI GEO (http://www.ncbi.nlm.nih.gov/geo/) database (GSE6008) using Gitools 2.3.1. Blue letter indicates the dominant chemokine in *TP53*WT endometrioid OC. Right panel indicates statistical analysis of chemokine expression intensities using Student's *t*-test. (B) Kaplan-Meier plots for OS and PFS based on expression levels of CXCL14 in endometrioid OC patients from GEO and TCGA (probes; Affymetrix HG-U133A, HG-U133A 2.0, and HG-U133 Plus 2.0 microarrays). Black and red letters indicate low and high expression of CXCL14, respectively.

CI=1.01–3.06) were associated with poor OS in *TP53*WT serous OC (**Fig. 5A and B**). In a similar study, EOC patients with highly expressed CXCL12 had poor survival compared to patients with lower levels (13). High expression of CXCR2 (HR=1.34; 95% CI=1.06–1.69) was associated with poor OS in *TP53*m serous OC, while high expressions of CXCL9 (HR=0.78; 95% CI=0.62–0.98), CCL5 (HR=0.77; 95% CI=0.61–0.98), CXCR4 (HR=0.75; 95% CI=0.59–0.94), CXCL11 (HR=0.72; 95% CI=0.57–0.91), and CXCL13 (HR=0.64; 95% CI=0.51–0.81) were associated with better OS (**Fig. 5A and C**). CXCR2 was correlated with poor OS and recurrence-free survival (RFS) in non-metastatic patients with non-clear cell renal carcinoma patients (38). In addition, analysis of 12 studies with a total of 2,461 cancer patients including laryngeal squamous cell carcinoma, lung

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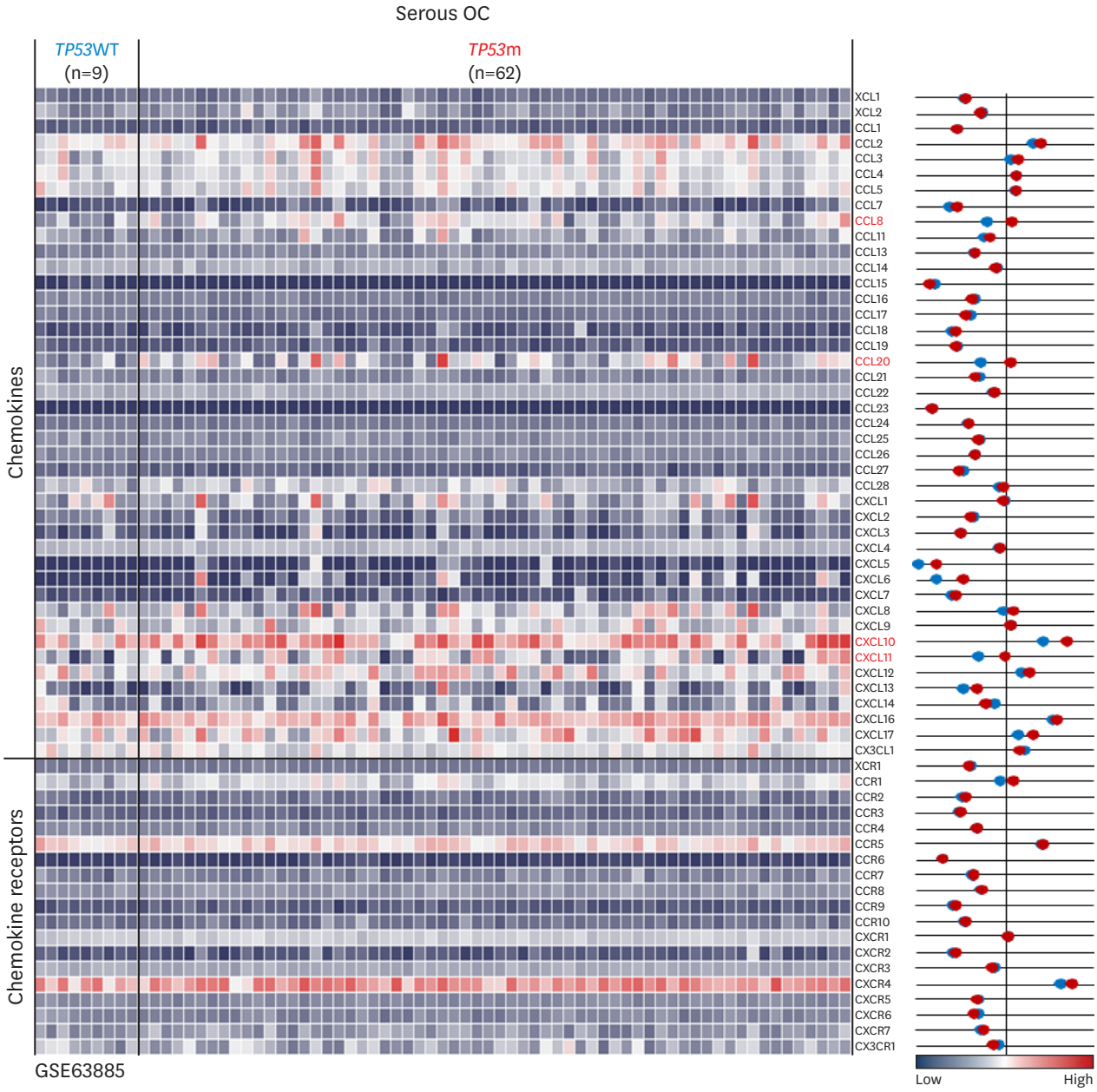

Figure 4. Chemokine and chemokine receptor signatures in *TP53*WT and *TP53*m serous OC. (A) Heatmap of chemokine and chemokine receptor expression profiles in *TP53*WT (n=9) and *TP53*m (n=62) serous EOC from NCBI GEO (http://www.ncbi.nlm.nih.gov/geo/) database (GSE63885) using Gitools 2.3.1. Red letters indicate dominant chemokines in *TP53*m serous OC. Right panel indicates statistical analysis of chemokine expression intensities using Student's *t*-test.

cancer and gastric cancer indicated that high expression level of CXCR2 was associated with poor OS and RFS (39). Overexpression of CXCR2 was associated with poor OS and disease-free survival of patients with HGSOC (40). These studies suggest poorer OS in *TP53*m serous OC with high levels of CXCR2 (**Table 2**, **Fig. 5A and C**). CXCL9 and CCL5 released by IL18-stimulated natural killer cells aid in the recruitment of effector T cells by promoting type I response against cancer (41-43). Consistently, high expression levels of CXCL9 and CCL5 in *TP53*m have better OS (**Table 1**, **Fig. 5A and C**). EOC patients with high expression of CXCL12 and low expression of CXCR4 showed worse survival (13). Accordingly, high expression of CXCL12 in *TP53*WT serous OC has worse OS, while high levels of CXCR4 in *TP53*m serous OC has better OS (**Tables 1** and **2**, **Fig. 5**).

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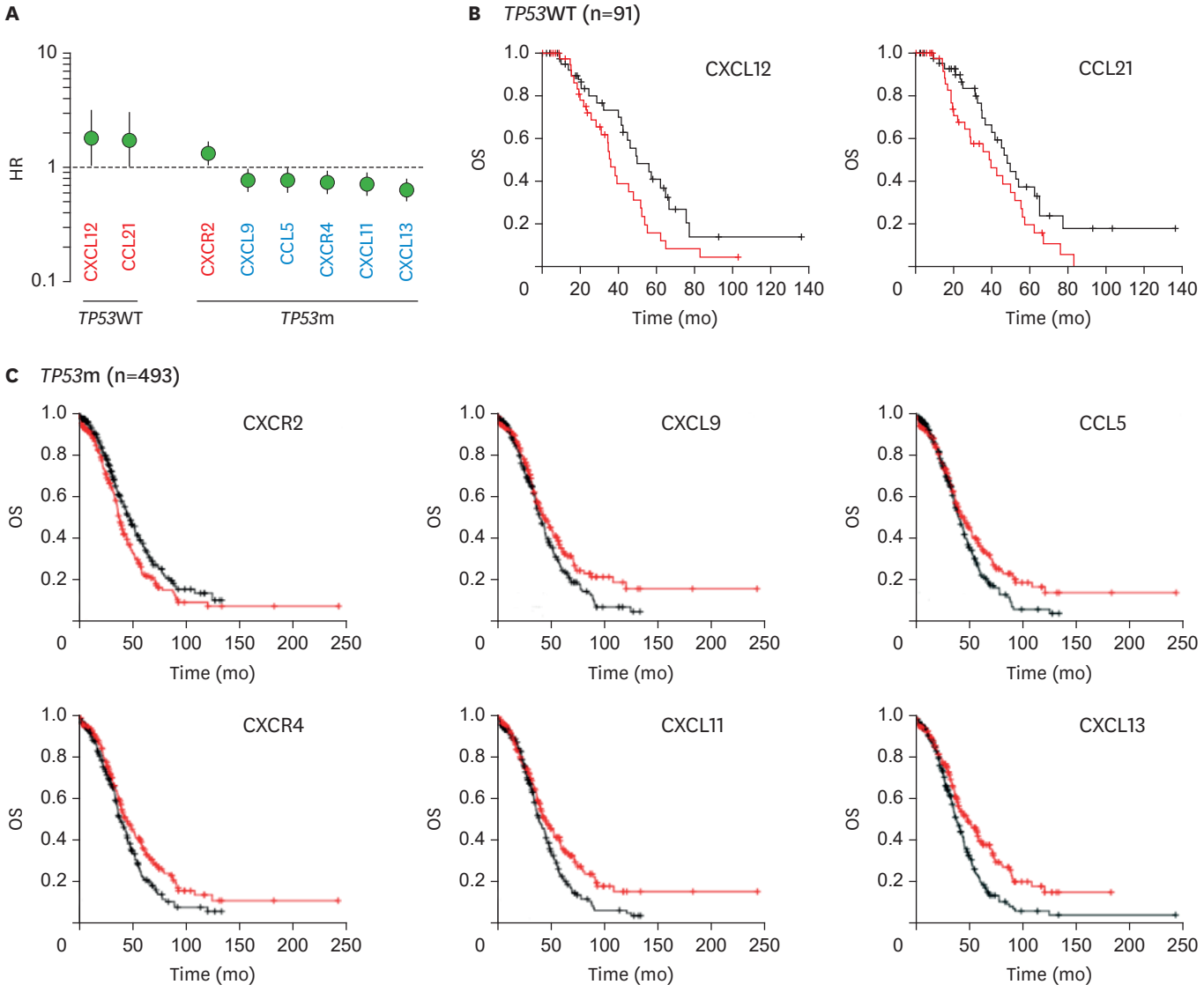

Figure 5. Chemokine and chemokine receptor signatures associated with OS in *TP53*WT and *TP53*m serous OC. (A) HR of chemokines and chemokine receptors that were either positively or negatively related with OS in *TP53*WT and *TP53*m serous OC using GEO and TCGA datasets available in the Kaplan-Meier plotter database (http://kmplot.com/analysis/index.php?p=service&cancer=ovar). (B) Kaplan-Meier OS for CXCL12 and CCL21 in *TP53*WT serous OC (n=91). (C) Kaplan-Meier OS for CXCR2, CXCL9, CCL5, CXCR4, CXCL11 and CXCL13 in *TP53*m serous OC (n=493). Blue and red letters indicate low and high expression of chemokines and chemokine receptors, respectively.

The present study has several limitations. Less common OC subtypes such as clear cell, mucinous and endometrioid OC had limited data available. Also, we could not perform OS analysis for *TP53*WT and *TP53*m endometrioid OC because of limited data number. Even OS analysis for serous OC, a representative OC, has unbalanced number between *TP53*WT (n=91) and *TP53*m (n=493) serous OC because of more frequent mutation of OC compared to other cancer types (**Fig. 1**). We cannot exclude the possibility that these *TP53*WT serous OC most likely represent LGSOC (24), since published TCGA data encompassing only HGSOC show that above 95% of tumors are *TP53*m (9). Therefore, differences in chemokine signatures between, and survival of patients with *TP53*WT and *TP53*m serous OC may be a reflection of different subtype. Further studies and additional data will help to overcome these limitations.

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Table 1. HRs for OS based on expression levels of chemokines in serous OC

Chemokines	ID	*TP53* mutation			*TP53*WT		
		HR	95% CI	No. of cases	HR	95% CI	No. of cases
XCL1	206365_at 206366_x_at	1.01	0.80–1.27	493	0.87	0.50–1.53	91
XCL2	214567_s_at	0.86	0.69–1.09	493	0.71	0.40–1.25	91
CCL1	207533_at	1.09	0.86–1.37	493	1.14	0.66–1.98	91
CCL2	216598_s_at	0.87	0.69–1.10	493	0.71	0.40–1.26	91
CCL3	205114_s_at	0.94	0.75–1.19	493	0.90	0.52–1.57	91
CCL4	204103_at	0.90	0.71–1.13	493	1.17	0.67–2.03	91
CCL5	204655_at 1405_i_at	**0.77**	0.61–0.98	493	1.24	0.71–2.16	91
CCL7	208075_s_at	0.89	0.71–1.12	493	0.75	0.43–1.32	91
CCL8	214038_at	0.79	0.63–1.00	493	0.76	0.43–1.36	91
CCL11	210133_at 206407_s_at	0.97	0.77–1.22	493	0.92	0.53–1.62	91
CCL13	216714_at	0.92	0.73–1.16	493	0.86	0.49–1.50	91
CCL14	205392_s_at	0.89	0.70–1.12	493	1.38	0.79–2.41	91
CCL15	210390_s_at	1.11	0.88–1.40	493	0.93	0.53–1.62	91
CCL16	207354_at	1.22	0.97–1.53	493	0.82	0.47–1.43	91
CCL17	207900_at 209924_at	1.24	0.98–1.56	493	0.78	0.45–1.35	91
CCL18	32128_at	0.87	0.69–1.10	493	0.81	0.47–1.42	91
CCL19	210072_at	0.93	0.74–1.17	493	1.42	0.81–2.49	91
CCL20	205476_at	0.86	0.68–1.08	493	1.14	0.65–1.98	91
CCL21	204606_at	0.99	0.79–1.25	493	**1.76**	1.01–3.06	91
CCL22	207861_at 210548_at	1.08	0.86–1.37	493	0.95	0.54–1.65	91
CCL23	210549_s_at	1.08	0.86–1.36	493	1.28	0.74–2.23	91
CCL24	221463_at	1.00	0.79–1.25	493	1.56	0.89–2.75	91
CCL25	206988_at	1.12	0.89–1.41	493	1.23	0.71–2.13	91
CCL26	223710_at	0.98	0.66–1.47	111			16
CCL27	207955_at	0.96	0.76–1.21	493	0.72	0.41–1.27	91
CCL28	238750_at 224240_s_at	1.04	0.70–1.56	111			16
CXCL1	204470_at	0.83	0.66–1.05	493	0.97	0.56–1.68	91
CXCL2	209774_x_at	0.86	0.68–1.08	493	1.20	0.69–2.08	91
CXCL3	207850_at	0.95	0.76–1.20	493	1.32	0.76–2.31	91
CXCL4	206390_x_at	1.02	0.81–1.28	493	1.57	0.89–2.74	91
CXCL5	214974_x_at 207852_at 215101_s_at	0.97	0.77–1.23	493	1.29	0.73–2.27	91
CXCL6	206336_at	0.85	0.67–1.07	493	1.12	0.64–1.97	91
CXCL7	214146_s_at	1.10	0.87–1.38	493	1.32	0.76–2.30	91
CXCL8	202859_x_at 211506_s_at	0.98	0.78–1.24	493	0.85	0.49–1.48	91
CXCL9	203915_at	**0.78**	0.62–0.98	493	1.12	0.64–1.98	91
CXCL10	204533_at	0.80	0.63–1.01	493	0.66	0.36–1.20	91
CXCL11	211122_s_at 210163_at	**0.72**	0.57–0.91	493	0.69	0.39–1.21	91
CXCL12	203666_at 209687_at	1.05	0.84–1.33	493	**1.84**	1.05–3.22	91
CXCL13	205242_at	**0.64**	0.51–0.81	493	0.94	0.53–1.67	91
CXCL14	218002_s_at	1.16	0.92–1.46	493	1.14	0.66–1.98	91
CXCL16	223454_at	0.82	0.55–1.22	111			16
CXCL17	226960_at	0.99	0.66–1.47	111			16
CX3CL1	823_at 203687_at	1.13	0.90–1.43	493	0.94	0.54–1.63	91

Bold HR: $p<0.05$ increase or decrease.

https://doi.org/10.4110/in.2018.18.e29

Table 2. HRs for OS based on expression levels chemokine receptors in serous OC

Chemokine receptor	ID	*TP53* mutation			*TP53*WT		
		HR	95% CI	No. of cases	HR	95% CI	No. of cases
XCR1	221468_at	0.99	0.78–1.25	493	1.05	0.60–1.83	91
CCR1	205099_s_at 205098_at	0.92	0.73–1.16	493	1.03	0.59–1.80	91
CCR2	206978_at 207794_at	0.94	0.75–1.19	493	1.56	0.90–2.72	91
CCR3	208304_at	1.14	0.90–1.43	493	0.83	0.47–1.45	91
CCR4	208376_at 217970_s_at 220671_at	1.15	0.91–1.45	493	0.84	0.48–1.48	91
CCR5	206991_s_at	0.98	0.78–1.23	493	0.92	0.53–1.60	91
CCR6	206983_at	1.25	0.99–1.58	493	1.13	0.65–1.96	91
CCR7	206337_at	1.00	0.79–1.25	493	1.05	0.61–1.83	91
CCR8	208059_at	1.03	0.82–1.30	493	0.94	0.54–1.63	91
CCR9	206887_at 207445_s_at	1.13	0.90–1.43	493	1.04	0.58–1.87	91
CCR10	220565_at	1.07	0.85–1.35	493	1.17	0.68–2.04	91
CXCR1	207094_at	0.92	0.73–1.16	493	1.23	0.70–2.14	91
CXCR2	207008_at	**1.34**	1.06–1.69	493	0.82	0.47–1.44	91
CXCR3	207681_at 217119_s_at	1.20	0.95–1.51	493	0.80	0.46–1.40	91
CXCR4	209201_x_at 211919_s_at 217028_at	**0.75**	0.59–0.94	493	0.92	0.53–1.62	91
CXCR5	206126_at 216734_s_at	1.00	0.80–1.26	493	0.96	0.55–1.68	91
CXCR6	206974_at 211469_s_at	0.89	0.71–1.12	493	1.09	0.63–1.90	91
CXCR7	212977_at	0.98	0.78–1.23	493	0.88	0.50–1.53	91
CX3CR1	205898_at	1.05	0.83–1.32	493	1.66	0.95–2.90	91

Bold HR: p<0.05 increase or decrease.

In conclusion, a distinct chemokine signature may be seen in *TP53*WT and *TP53*m OC and be associated with better or worse OS, suggesting possible utility as a biomarker for OC prognosis. In particular, CXCR2 has worse OS in *TP53*m serous OC, the most representative OC, and may be a potential target for OC therapy.

ACKNOWLEDGEMENTS

This research was supported, in whole or in part, by National Institutes of Health as the following grants: R01ES024756 (E.L.), NIAID SC1AI089073 (D.S.), NCI SC1CA200519 (D.S.) and U54CA163069 (D.S., A.B., M.M.W.). Its contents are solely the responsibility of the authors and do not necessarily represent the official views of NIH. In addition, the authors gratefully acknowledge the members and supporters of the Vanderbilt Ovarian Cancer Alliance (VOCAL).

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