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Autocrine activation of JAK2 by IL-11 promotes platinum drug resistance

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Conflicts of Interest

No potential conflicts of interest.

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Abstract

Antineoplastic platinum agents are used in first-line treatment of ovarian cancer, but treatment failure frequently results from platinum drug resistance. Emerging observations suggest a role of reactive oxygen species (ROS) in the resistance of cancer drugs including platinum drugs. However, the molecular link between ROS and cellular survival pathway is poorly understood. Using quantitative high throughput combinational screen (qHTCS) and genomic sequencing, we show that in platinum resistant ovarian cancer elevated ROS levels sustain high level of IL-11 by stimulating FRA1-mediated IL-11 expression and increased IL-11 causes resistance to platinum drugs by constitutively activating JAK2-STAT5 via an autocrine mechanism. Inhibition of JAK2 by LY2784544 or IL-11 by anti-IL-11 antibody overcomes the platinum resistance *in vitro* or *in vivo*. Significantly, clinic studies also confirm the activated IL-11-JAK2 pathway in platinum-resistant ovarian cancer patients, which highly correlates with poor prognosis. These findings not only identify a novel ROS-IL-11-JAK2-mediated platinum resistance mechanism but also provide a new strategy for using LY2784544 or IL-11-mediated immunotherapy to treat platinum-resistant ovarian cancer.

Keywords

JAK2; IL-11; platinum drug; cisplatin resistance; ovarian cancer

Introduction

Ovarian cancer is the fifth leading cause of cancer-related death among women and the deadliest gynecological cancer in the United States ³¹. The current standard treatment for ovarian cancer consists of surgery followed by platinum drug based chemotherapy ^{15, 21}. Platinum drugs act by entering the nucleus of the cell and forming covalent adducts with DNA, thus decreasing cell viability ⁷. In addition to nuclear DNA damage, cisplatin can induce reactive oxygen species (ROS) response that can significantly enhance the cytotoxic effect ^{5, 20}. Up to 80% of patients with ovarian cancers initially respond to cisplatin-based chemotherapy and achieve remission ^{1, 4}. However, cancer relapse occurs in about 80% of patients, and the relapsed ovarian cancer patients are more resistant to platinum-based therapy ¹¹. Studies in the past characterized a plethora of mechanisms for cisplatin resistance, including reduced intracellular cisplatin accumulation, increases in metabolic inactivation of cisplatin, repair of cisplatin-induced DNA damage, and inactivation of p53 ^{10, 36}. Emerging observations also indicated a role of ROS in cisplatin resistance ³⁴. However, the detailed molecular mechanism of how ROS contributes to platinum drug resistance by regulating cell survival pathway remains largely unknown.

Janus kinase 2 (JAK2) is a member of the Janus kinase family, which contains JAK1, JAK2, JAK3 and tyrosine kinase 2 (TYK2). JAKs are recruited to cellular membrane and activated by cytokine-activated receptors ²⁸. Activated JAKs phosphorylate and activate signal transducer and activator of transcription (STAT) factors, which then translocate to nucleus to

regulate the expression of genes involving in cell proliferation and apoptosis²⁹. Interleukin-11 (IL-11) is a member of the GP130 family that includes IL-6, IL-27, IL-31, OSM and LIF³. IL-11 binds to the transmembrane receptor IL-11 R α , causing activation of JAK2 and hence the downstream STAT pathway^{2,9}. However, the role of IL-11 in the response of cancer cells to chemotherapy remains largely unknown.

In this study, for the first time, we provided an unbiased high throughput analysis to study platinum drug resistance in ovarian cancer by combined use of quantitative high-throughput combinational screening (qHTCS) and genomic sequencing techniques. The advantage of this approach is that it not only identifies novel drug combinations to treat drug resistance cancers but also discovers novel drug resistant mechanisms that cannot be identified by each technique alone. Indeed, we identified LY2784544, a JAK2 inhibitor under phase II clinical trial for myeloproliferative neoplasms¹⁹, to be an effective drug to efficiently resensitize platinum drug resistant ovarian cancer *in vitro* and *in vivo*. Detailed mechanistic analyses indicated that in platinum-resistant ovarian cancer cells ROS promotes FRA1-dependent expression of IL-11, resulting in constitutive activation of JAK2-STAT5 pathway and resistance to platinum drugs. Most importantly, this novel mechanism is supported by our clinic findings: JAK2 activation correlates with high levels of IL-11 in the most tested platinum resistant ovarian tumors and the activated IL-11-JAK2 pathway is correlated with a low overall survival rate of ovarian cancer patients receiving platinum drug-based chemotherapy.

Results

Establishment of platinum resistant ovarian cancer cells

The objective of this study is to conduct both qHTCS and genomic sequencing using platinum drug resistant ovarian cancer cells to discover new mechanisms of resistance as well as novel drug combinations that can overcome platinum drug resistance (Fig. 1A). To accomplish this objective, we first established cisplatin resistant ovarian cancer cells using procedures as described previously and in methods¹³. Specifically, starting with two ovarian cancer cell lines (SKOV3 and IGROV1), we obtained sub-cell lines, called SKOV3 CR and IGROV1 CR, that were able to grow in the medium containing a high concentration of cisplatin (Fig. 1B). Subsequent cell viability analyses revealed that the IC₅₀ of cisplatin for SKOV3 CR was increased by 5-fold and IC₅₀ of cisplatin for IGROV1 CR by 3-fold (Fig. 1B). SKOV3 CR cells were also resistant to carboplatin (Supplementary Fig. 1A), which is also used to treat ovarian cancer patients clinically²⁵. Apoptosis analyses indicated that cisplatin induced more apoptosis in SKOV3 cells than in SKOV3 CR cells (Supplementary Fig. 1B and 1C). Significantly, *in vivo* studies indicated that SKOV3 CR tumor was more resistant to cisplatin than SKOV3 tumor through anti-apoptosis (Supplementary Fig. 1D).

We next measured the DNA crosslinking in both cells post cisplatin treatment using a modified comet assay and found that there was no difference in the extent of cisplatin-DNA crosslinking between SKOV3 and SKOV3 CR cells²³ (Supplementary Fig. 1E). Thus, SKOV3 CR cells remain susceptible to cisplatin-induced DNA damage and it is unlikely that there are defects in cisplatin uptake.

Identification of LY2784544 that overcomes cisplatin resistance via a qHTCS

We employed these cells in a qHTCS to identify novel drug combinations to re-sensitize the cells to cisplatin. To this end, we screened a total of 6,016 compounds from multiple compound libraries including NPC (NCGC Pharmaceutical Collection), MIPE (Mechanism Interrogation Plate) and LOPAC (The Library of Pharmacologically Active Compounds). Two rounds of qHTCS screen were performed (Supplementary Fig. 2A). From the first screen, we identified a total of 112 compounds that efficiently inhibited the proliferation of SKOV3 CR cells (Supplementary Fig. 2B). To further identify compounds that have synergy with cisplatin, these 112 compounds were screened at 11 different concentrations in combination with vehicle, 1 μ M cisplatin or 20 μ M cisplatin (Fig. 1C and Supplementary Fig. 2A). Through this second screen, thirteen compounds that improved the cytotoxicity of cisplatin in SKOV3 CR cells were identified (Supplementary Table S1). In the next screen, cisplatin was tested at 11 different concentrations from 0.046 to 47 μ M in combinations with these thirteen compounds (Supplementary Fig. 2A). Three compounds, LY2784544 (JAK2 inhibitor), MLN4924 (NEDD8 inhibitor) and NSC319726 (p53 mutant reactivator), were found to have significant reciprocal synergy with cisplatin on SKOV3 CR cells (Fig. 1C).

Among the three compounds, LY2784544 has better cisplatin IC₅₀ shift ability than MLN4924 or NSC319726, which significantly increased the sensitivity of SKOV3 CR cells to cisplatin to a degree similar to SKOV3 cells (Supplementary Fig. 2C, left panel). Cisplatin sensitized SKOV3 CR cells to LY2784544 reciprocally (Supplementary Fig. 2C, right panel). The synergistic effects of MLN4924 or NSC319726 on cisplatin resistant ovarian cancer cells have been reported independently by other groups^{13, 38}, validating the use of this qHTCS to identify compounds overcoming cisplatin resistance. In subsequent studies, we mainly focused on investigating the mechanism by which LY2784544 overcomes cisplatin resistance of ovarian cancer cells.

JAK2 inhibition synergizes with platinum drugs in platinum resistant ovarian cancer cells

We conducted multiple analyses to confirm the synergistic effects of LY2784544 with cisplatin in cisplatin resistant SKOV3 CR cells. First, we measured cell proliferation using sulforhodamine B (SRB) assay(and calculated the synergy between LY2784544 and cisplatin using Combenefit⁸, and found excellent synergy in SKOV3 CR cells compared to that in SKOV3 cells (Fig. 1D and Supplementary Fig. 3A). We also calculated the combination index (CI), which quantitatively measures the interaction between two drugs (synergism: CI < 1; additive effect: CI = 1; and antagonism CI > 1)⁶. Significantly, the combination of cisplatin and LY2784544 exhibited excellent synergy on SKOV3 CR cells (mean CI = 0.69) and additive effect on SKOV3 cells (mean CI = 0.93) (Supplementary Fig. 3B). Using similar analysis, the combination of cisplatin and LY2784544 also had an excellent synergy (mean CI = 0.71) on IGROV1 CR cells (Fig. 1E).

Given that both SKOV3 CR and IGROV1 CR cells acquired resistance under a cell culture condition, we next examined the synergy of LY2784544 and cisplatin in a paired ovarian cancer cells, PEO1 and PEO4, derived directly from one patient before and after chemotherapy¹⁷. PEO1 cells are platinum sensitive and PEO4 cells are platinum resistant cells. We did find that the IC₅₀ of cisplatin for PEO4 cells was increased by 3-fold compared

to that for PEO1 cells (Fig. 1F). Importantly, the combination of cisplatin and LY2784544 exhibited excellent synergy (mean CI = 0.34) in PEO4 cells (Fig. 1G).

Using a clonogenic assay, we found that SKOV3 CR cells exhibited a higher survival rate compared to SKOV3 cells after a cisplatin treatment. Combinatorial treatment with cisplatin plus LY2784544 significantly reduced the survival of SKOV3 CR compared to cisplatin or LY2784544 treatment alone (Supplementary Fig. 3C). We also examined the synergy of LY2784544 with carboplatin in SKOV3 CR cells. Consistently, the combination of carboplatin and LY2784544 also exhibited an excellent synergistic effect (mean CI = 0.75) on SKOV3 CR cells (Supplementary Fig. 3D).

To confirm the JAK2 inhibitor's effect on synergy with cisplatin, we next tested other two JAK2 inhibitors, TG101348 and TG-46. Similarly, both showed a synergistic effect with cisplatin on SKOV3 CR cells (Supplementary Fig. 3E and S3F). Taken together, our data strongly demonstrate a general synergistic effect of JAK2 inhibitors with platinum drugs on platinum drug resistant ovarian cancer cells.

LY2784544 overcomes cisplatin resistance of ovarian cancer cells by inhibiting JAK2-mediated pathways in vitro and in vivo

We next explored the molecular mechanism by which the JAK2 signaling pathway confers cisplatin resistance in ovarian cancer. We first examined the activation of the JAK2-mediated pathway in three pairs of cisplatin sensitive (SKOV3, IGROV1, PEO1) and resistant (SKOV3 CR, IGROV1 CR, PEO4) cell lines. Compared to the three sensitive cell lines, the JAK2 protein levels were not altered in paired resistant cell lines (Fig. 2A). Strikingly, phosphorylation of JAK2 at Y1007/1008 significantly increased in all resistant cells compared to their sensitive counterparts. Consistent with the activation of JAK2, we found the phosphorylation of STAT5 (Y694), a downstream target of JAK2^{19, 37}, was increased in all three resistant cell lines (Fig. 2A). The downregulation of *JAK2* by shRNA significantly sensitized the SKOV3 CR cells to cisplatin (Fig. 2B and C), confirming that the cisplatin resistance in SKOV3 CR is JAK2-dependent.

We next examined the effects of LY2784544 on the JAK2-STAT5 signaling pathway in both SKOV3 and SKOV3 CR cells. Apparently, LY2784544 significantly reduced the phosphorylation of both JAK2 and STAT5 in both SKOV3 and SKOV3 CR cells (Fig. 2D). Treatment of SKOV3 CR cells with LY2784544 efficiently decreased the activation of JAK2 and STAT5 but did not cause apoptosis as evidenced by a lack of cleaved PARP (Fig. 2E). However, the combination of cisplatin and LY2784544 not only reduced the phosphorylation of JAK2 and STAT5 but also enhanced apoptosis (Fig. 2E and F and Supplementary Fig. 4A).

To determine whether LY2784544 can sensitize SKOV3 CR cells to cisplatin *in vivo*, we subcutaneously implanted SKOV3 CR cells into nude mice to form tumors, followed by various treatments. Although SKOV3 CR tumors were resistant to cisplatin or LY2784544, the combinatorial treatment with cisplatin and LY2784544 significantly not only reduced tumor growth (Fig. 2G), but also improved survival of the mice with SKOV3 CR tumors (Fig. 2H). To evaluate the efficacy of this combinational treatment and whether this *in vivo*

tumor suppression was mediated through inhibition of JAK2, we collected SKOV3 CR xenograft tumor samples four days after full dosage treatment. Immunohistochemistry (IHC) analyses indicated that LY2784544 treatment efficiently reduced the phosphorylation of JAK2 and the treatment with LY2784544 plus cisplatin significantly induced apoptosis in the tumor as indicated by TUNEL staining (Fig. 2I–K). To evaluate the toxicity of treatment of cisplatin plus LY2784544 in mice, we carefully monitored the weight of the mice throughout the treatments and found that there was no significant reduction in body weight during the whole treatment period (Supplementary Fig. 4B). No obvious histopathological alterations and lesions were found in liver and kidney of the treated animals (Supplementary Fig. 4C).

The LY2784544 was reported as a selective JAK2 inhibitor¹⁹. To confirm the selectivity of LY2784544, we conducted an *in vitro* kinase profiling to exam the interaction of LY2784544 with more than 450 human kinases. The results showed that LY2784544 exhibited stronger binding to JAK2 than JAK1 and TYK2 (Supplementary Fig. 4D), indicating the high selectivity of LY2784544 towards JAK2.

Identification of IL-11 as a key factor that regulates JAK2 activity in vitro and in vivo

We next investigated how JAK2 is activated in cisplatin resistant ovarian cancer cells. Given that JAK2 could be constitutively activated by mutations including the V617F mutation^{12, 16, 18, 22, 30}, we first sequenced and compared the *JAK2* gene in SKOV3 and SKOV3 CR cells (Supplementary Fig. 5A). However, we did not find any mutations in the *JAK2* gene in SKOV3 CR cells.

Since cytokine-activated receptors can regulate the activation of JAK2^{3, 24, 26, 35}, we hypothesized that secreted factors in the medium of SKOV3 CR cells might cause, in an autocrine fashion, activation of JAK2, resulting in resistance to cisplatin. To test this possibility, we collected conditioned medium from SKOV3 CR cells and then used the medium to culture SKOV3 cells. Strikingly, SKOV3 cells cultured in the conditioned medium from SKOV3 CR displayed an increased resistance to cisplatin compared to cells cultured in the conditioned medium from SKOV3 (Fig. 3A), indicating that secreted factors from SKOV3 CR cells caused the cisplatin resistance of SKOV3 cells. Strikingly, SKOV3 cells cultured in the conditioned medium from SKOV3 CR cells activated JAK2 and STAT5 (Fig. 3B).

To identify the cytokine factors in the conditioned medium from SKOV3 CR cells that active JAK2, we screened the cytokine levels in the conditioned medium of SKOV3, SKOV3 CR, PEO1, and PEO4 cells using a cytokine array. The result indicated significantly elevated levels of four cytokines (Eotaxin-2, Eotaxin-3, IL-11, and uPAR) in the medium of both SKOV3 CR and PEO4 cells compared with their parental cells (Fig. 3C and D). Furthermore, we also examined and compared gene expression profiles between SKOV3 and SKOV3 CR cells. There was a total of 1085 up-regulated genes and 1686 down-regulated genes in SKOV3 CR compared to SKOV3 cells (Fig. 3E). Comparing the genes in the cytokine pathways, we found that a total of 74 genes are either up- or down-regulated (more than 2-fold change) in SKOV3 CR cells (Fig. 3F). We then compared these four cytokine genes with 30 up-regulated cytokine genes identified by genomic sequencing as well as 155

genes involving in KEGG JAK/STAT signaling pathway (Pathway: map04630), and found that IL-11 is the only up-regulated cytokine that is most likely to cause activation of JAK pathway in platinum drug resistant ovarian cancer cells (Fig. 3G). We therefore analyzed and compared IL-11 mRNA levels by qPCR in sensitive and resistant cells and found that IL-11 mRNA levels increased significantly in all three resistant cell lines (Fig. 3H). Consistently, the amounts of secreted IL-11 proteins in the medium of all three resistant cells were significantly higher than that in the medium of their sensitive counterparts (Fig. 3I). To rule out the possibility that JAK2-STAT5 activation in the resistant cells might be due to the overexpression of IL-11 receptors, we next examined the expression of subunits of IL-11 receptor, including IL-11R α , IL-6R α , EPOR and G-CSFR, in SKOV3 and SKOV3 CR cells, and found that the levels of each protein did not increase in SKOV3 CR compared to SKOV3 cells (Supplementary Fig. 5B).

Given that both IL-11 mRNA and IL-11 protein are increased in the resistant cells, we hypothesized that IL-11 might also be up-regulated in cisplatin resistant ovarian tumors *in vivo*. To test this hypothesis, we examined the levels of IL-11 protein in SKOV3 and SKOV3 CR tumors by IHC and found that IL-11, as well as levels of phosphorylation of JAK2, were significantly increased in SKOV3 CR xenograft tumor cells (Supplementary Fig. 5C and 5D). Taken together, our results indicate that IL-11 levels are up-regulated in cisplatin resistant cancer cells *in vitro* and *in vivo*.

Autocrine activation of IL-11 promotes cisplatin resistance by regulating JAK2-STAT5 activation in ovarian cancer cells

The above findings suggested that IL-11 might be a critical factor contributing to cisplatin resistance by stimulating JAK2 activity in ovarian cancer cells. Therefore, we first tested whether IL-11 can stimulate the activation of JAK2 in SKOV3 cells. Indeed, recombinant IL-11 treatment increased the phosphorylation of JAK2 and STAT5 (Fig. 4A). To support the notion that IL-11 contributes to cisplatin resistance in ovarian cancer cells, we found that treatment of SKOV3 with recombinant IL-11 induced resistance to cisplatin (Fig. 4B). When IL-11 neutralization antibody was added to the culture medium of SKOV3 CR and IGROV1 CR, we observed reduced phosphorylation of JAK2 and STAT5 (Fig. 4C), and increased the sensitivity of the cells to cisplatin (Fig. 4D, upper panel, SKOV3 CR; lower panel, IGROV1 CR). Thus, secreted IL-11 acts as an autocrine factor to stimulate the activation of the JAK2-STAT5 pathway, thereby inducing cisplatin resistance in these cells.

To further test the relationship between IL-11 and cisplatin resistance in ovarian cancer cells, we depleted *IL-11* by shRNA in SKOV3 CR cells and found that downregulation of *IL-11* not only decreased the IL-11 secretion (Fig. 4E) but also reduced the activation of JAK2 and STAT5 (Fig. 4F). More significantly, depletion of IL-11 re-sensitized SKOV3 CR cells to cisplatin (Fig. 4G). Addition of recombinant IL-11 to the medium restored the cisplatin resistance of SKOV3 CR cells with downregulated IL-11 by shRNA (Fig. 4H), indicating that reduced resistance by IL-11 depletion is not due to the shRNA off-target effect. To further confirm that IL-11-induced cisplatin resistance is JAK2-dependent, we downregulated JAK2 by siRNA in SKOV3 cells with recombinant IL-11 for four hours and then cisplatin treatment for five days (Fig. 4I). Although the addition of recombinant IL-11

could induce resistance to cisplatin in cells containing JAK2, IL-11 no longer induced cisplatin resistance in the cells with downregulated JAK2 (Fig. 4I). These findings indicate that JAK2 signaling is a major mechanism involved in IL-11-induced cisplatin resistance in SKOV3 cells.

Cisplatin induces IL-11-mediated autocrine activation of JAK2-STAT5 *in vitro* and *in vivo*

We next explored the molecular mechanism of how ovarian cancer cells acquire resistance to cisplatin during cisplatin treatment, which can explain how ovarian cancer patients develop resistance to platinum drugs after receiving platinum-based therapy.

Given that the elevated IL-11 stimulates JAK2 activation to induce cisplatin resistance, it is highly possible that cisplatin may result in increased expression and secretion of IL-11 as a compensatory surviving mechanism. To avoid cell death, we treated cells with cisplatin less than 50% of IC₅₀. Indeed, the small dosage of cisplatin treatment increased secretion of IL-11 in SKOV3 cells, and furthermore, IL-11 secretion appeared to be dependent on cisplatin doses and treatment time (Fig. 5A). *IL-11* mRNA levels were also increased in cells following cisplatin treatment (Fig. 5B), indicating that elevated secretion of IL-11 is due to up-regulated expression of *IL-11* gene. As expected, JAK2 and STAT5 were activated after cisplatin treatment (Fig. 5C).

To further test whether ovarian cancer cells have a similar response to cisplatin *in vivo*, we treated SKOV3 xenograft tumor with various doses of cisplatin for 2 weeks (Fig. 5D). Four days after the last treatment, blood and tumor samples were collected for analyses of IL-11 levels. Significantly, cisplatin treatment dramatically increased human IL-11 levels in the serum of mice (Fig. 5E). IHC analysis indicated a significant increase of IL-11 as well as pJAK2 levels in SKOV3 tumors (Fig. 5F). Interestingly, the levels of both IL-11 and pJAK2 in the tumors were dependent on cisplatin doses: the higher the cisplatin dose the higher the levels of IL-11 and pJAK2 (Fig. 5G). Thus, cisplatin treatment induces the activation of the IL-11-JAK2 pathway in tumor cells *in vivo* as well. These results provide *in vivo* and *in vitro* evidence that may explain how ovarian cancer patients develop IL-11-JAK2-mediated platinum drug resistance.

ROS induces autocrine activation of IL-11 by promoting expression of FOSL1 (FRA1)

The next question is how IL-11 is activated in platinum drug resistant ovarian cancer cells. Since DNA damage induces ROS generation^{14, 33}, we next investigated whether ROS is involved in up-regulation of IL-11. To this end, we first compared ROS level between SKOV3 and SKOV3 CR cells. Strikingly, the basal ROS level in SKOV3 CR cells was significantly higher than that in SKOV3 cells (Fig. 6A). To investigate if ROS in SKOV3 CR cells regulate IL-11 expression, we treated SKOV3 CR cells with a ROS inhibitor YCG063 and found that inhibition of ROS significantly reduced IL-11 secretion as well as JAK2 and STAT5 phosphorylation (Fig. 6B–D and Supplementary Fig. 6A), indicating that ROS is critical for the secretion of IL-11 in cisplatin resistant cells. Next, we treated SKOV3 cells with cisplatin, YCG063 or in combination. Cisplatin treatment alone remarkably activated ROS production while YCG063 suppressed it in SKOV3 cells (Fig. 6E and Supplementary

Fig. 6B). Furthermore, inhibition of ROS in SKOV3 cells suppressed the cisplatin-induced IL-11 secretion (Fig. 6F), phosphorylation of JAK2 and STAT5 (Fig. 6G).

Having found that the ROS regulates IL-11 secretion, we then decided to identify critical ROS responsive genes that regulate *IL-11* expression. To achieve this goal, we first conducted pathway analyses using PathwayNet and identified a total of 20 transcription factors that likely regulate *IL-11*²⁷ (Fig. 6H). By comparing these candidate genes with the genes identified from RNA-Seq analyses, we found that the *FOSL1* is the only gene that is significantly up-regulated by more than 2-fold in SKOV3 CR cells (Fig. 6H). Further analyses indicated that the levels of *FOSL1*-encoded protein, FRA1, and phosphorylated (Ser265) FRA1 were increased in SKOV3 CR cells compared to SKOV3 cells (Fig. 6I). Depletion of FRA1 by *FOSL1* siRNA significantly reduced IL-11 secretion in SKOV3 CR cells (Fig. 6J and K), indicating that FRA1 is critical for IL-11 expression. We next tested whether ROS is required for activation or expression of FRA1 by treating SKOV3 CR cells with YGC063. Significantly, inhibition of ROS reduced the expression of FRA1 as well as its phosphorylation (Fig. 6L), indicating that the ROS signaling is required for the activation of FRA1.

Clinical evidence of activated IL-11-JAK2 pathway in platinum drug resistant ovarian cancer patients

To investigate and confirm the role of the IL-11-JAK2 pathway in platinum drug resistance of ovarian cancer patients, we first compared the IL-11 mRNA levels from patient samples of a total of 23 platinum sensitive patients and 16 resistant patients (Supplementary Table S2). We found that IL-11 levels in the platinum drug-resistant group are higher than that in sensitive group (Fig. 7A). Significantly, the group of patients with higher mRNA levels of IL-11 exhibited worse prognosis in terms of PFS and OS than the group with low mRNA *IL11* level (Supplementary Fig. 7A). We also examined and compared the serum IL-11 levels from a total of 21 platinum sensitive patients and 16 resistant patients (Supplementary Table S3). We found that the platinum drug-resistant group had a mean level of 120.2 pg/ml of serum IL-11, which is significantly higher than the platinum drug sensitive group (22.8 pg/ml of IL-11) (Fig. 7B). Also, the group of patients with higher serum levels of IL-11 (> 40 pg/ml) exhibited worse prognosis in terms of PFS and OS (Fig. 7C) than the group with low serum IL-11 level (< 40 pg/ml), indicating an inverse correlation between the serum levels of IL-11 and the survival rate of ovarian patients following platinum drug-based therapy. Out of these 37 patients, we identified one patient with both sensitive and resistant samples. The resistant tumors exhibited higher levels of IL-11 and p-JAK2 expression (Fig. 7D and Supplementary Table S4).

We also evaluated the correlation of the IL-11-JAK2 pathway in the samples from the same patient before platinum drug treatment and after tumor recurrence. We identified a total of seven patients who have received platinum drug based chemotherapy and had a recurrence 11–41 months post treatment (Supplementary Table S4). IHC staining indicated that IL-11 levels were increased in four of seven (57.1%) patients (Fig. 7E and Supplementary Fig. 7B). Strikingly, there was a significant correlation between IL-11 and pJAK2 levels in all tested primary and recurrent patient tumor samples (Fig. 7F).

To further investigate the role of JAK2 activation in the regulation of platinum drug resistance in ovarian cancer, we next examined the correlation of the JAK2 activity with ovarian patient survival rate using large scaled ovarian cancer data bases. Since JAK2 phosphorylation but not proteins levels are up-regulated in platinum resistant cells, we therefore investigated the correlation of the JAK2 pathway with cisplatin resistance by analyzing and comparing the JAK2-regulated functional pathways and gene expression profiles obtained from genomic sequencing. Specifically, we first analyzed the genes that are functionally linked with JAK2 by using PathwayNet and identified a total of 500 genes linked to JAK2 activity. Analyzing gene expression from RNA-Seq data, we identified a total of 1085 genes that are up-regulated at least 2-fold in SKOV3 CR compared to SKOV3 cells (Fig. 3E). Comparing these two sets of genes, we found a total of 22 overlapped genes (Fig. 7G), which we named as JAK2 signature genes. Given that JAK2 activity is up-regulated in cisplatin resistant cells, we hypothesized that the levels of these JAK2 signature genes should inversely correlate to survival rate. We analyzed and compared JAK2 signature genes from 1816 patients found in 15 datasets using a Kaplan-Meier plotter³². Indeed, patients with platinum drug treatment history exhibited a worse 5-year PFS and OS when JAK2 signature genes expression levels in their tumors were higher (Fig. 7H). Thus, a higher activity of the JAK2 pathway is highly correlated with worse ovarian cancer patient survival following platinum drug-based therapy.

Discussion

In this study, we have demonstrated that qHTCS is an unbiased and quick method to study platinum drug resistance in ovarian cancer. The advantage of using such an approach is that it not only quickly identifies novel drug combinations in an unbiased manner but also the most likely drug target proteins and signal transduction pathways, especially those with post-transcriptional modification such as this JAK activation-mediated pathway. Specifically, we have successfully identified that the elevated ROS production is responsible for activation of the IL-11-mediated autocrine mechanism that activates JAK-2-STAT5 signal transduction pathway to endow the ovarian cancer cells with cisplatin resistance (Supplementary Fig. 7C, left panel). Furthermore, targeting JAK2 or IL-11 can re-sensitize platinum drug resistant ovarian cancer cells to the platinum drug (Supplementary Fig. 7C, right panel). Importantly, this novel molecular mechanism of platinum resistance has been confirmed by clinical studies. Thus, tumors with elevated IL-11 production and JAK2 activity in patients are more likely to exhibit cisplatin resistance and patients with such tumors have a poor prognosis following platinum-based therapy.

LY2784544 is currently investigated in clinical Phase 2 trial for its potential to treat myeloproliferative neoplasms (NCT01594723). Our studies indicate that LY2784544 can efficiently sensitize platinum resistant ovarian cancer cells to cisplatin both in *vitro* and in animals due to its ability to inhibit JAK2, thereby blocking the IL-11 autocrine mechanism for platinum resistance. Therefore, the use of specific JAK2 inhibitors, such as LY2784544, is likely to lead to a promising, more efficient combinatorial chemotherapy with platinum drugs as the first-line treatment for ovarian cancers or to treat relapsed ovarian cancer patients after platinum-based chemotherapy. Nevertheless, future clinical trials are needed to

evaluate the safety and efficacy of the combination of platinum drugs and LY2784544 or other JAK2 inhibitors for ovarian cancer treatment.

The discovery of an IL-11 autocrine mechanism that activates JAK2 and signals transduction to induce platinum drug resistance in ovarian cancer cells is exciting because it explains why ovarian cancer patients with high serum and tumor levels of IL-11 exhibit poor prognosis compared to those with low serum and tumor IL-11 levels. It is striking that elevated ROS levels are required to sustain high levels of IL-11 via regulating the expression of FRA1 (Fig. 6L). The ROS signaling can cause elevated expression of survival factors such as BCL-2 and MCL1 and decrease levels of cell death factors³⁴. However, the detailed molecular mechanism of how ROS regulates these factors is unknown. Thus, for the first time our studies provide a novel mechanism by which ROS promotes cell survival through stimulating FRA1-mediated expression of IL-11 in ovarian cancer cells to promote, in an autocrine fashion, their resistance to platinum drugs

Our clinical findings indicate that platinum drug treatment of patients does increase the serum levels of IL-11, which is consistent with our findings in animals. Thus, this study suggests a new strategy to increase the efficacy of platinum-based therapy for ovarian cancer and may be for other cancer types – that is, to develop and use agents, such as humanized monoclonal antibodies against IL-11 and/or IL-11R α , to sensitize platinum resistant ovarian cancer to platinum drugs again. However, since cisplatin treatment stimulates the expression of IL-11 in tumor cells, there is more reason to consider the combined use of a platinum drug with an agent that targets IL-11 or IL-11R α to prevent cancer cells from acquiring resistance to the platinum drugs during treatment. Given that we can monitor the serum IL-11 level in patients, measurement of serum IL-11 level also provides us with a useful biomarker to evaluate the potential resistance of patients to platinum drugs. However, this hypothesis needs to be carefully evaluated using a large cohort of ovarian cancer patients in future studies.

Materials and Methods

Cell lines and resistance cell line establishment

The human ovarian cancer cell line SKOV3 was obtained from the American Type Culture Collection (ATCC). PEO1, PEO4, and PEA1 were from Sigma-Aldrich. IGROV1 was a kind gift from Wei Zheng. SKOV3 cells were cultured in McCoy's 5A Medium supplemented with 10% fetal bovine serum (FBS). A2780, IGROV1, PEO1, PEO4, and PEA1 cells were cultured in RPMI-1640 supplemented with 10% FBS. The cisplatin resistant cell line SKOV3 CR and IGROV1 CR were generated by treated with cisplatin for six cycles (4 hr cisplatin treatment, followed by release to cisplatin free medium for three weeks). Only early-passage (< 10 passages) resistant cells were used for the study. All cells were cultured at 37 °C in a humidified incubator containing 5% CO₂.

Quantitative high-throughput combinational screening (qHTCS)

Compound screening experiments were performed as previously described³².

Cell viability assay

Cell viability was determined by a sulforhodamine B (SRB) assay as described previously³⁷.

Animal experiments

The detailed procedure of animal experiments is provided in Supplementary data.

Cytokine array

Cytokine arrays on cell culture medium were performed using the Human Cytokine Antibody Array Kit (Abcam, 120 Targets) according to the manufacturer's protocol. Single intensity was analyzed using ImageJ (NIH) software. The results were then normalized using internal controls, and the relative protein levels were determined across four biological replicates.

ELISA

The cell-free culture medium, mouse serum, and patient serum were analyzed for IL-11 levels using a human IL-11 ELISA kit (R&D Systems) according to the manufacturer's instructions. The absorbance was read at 450 nm using a microplate reader (BioTek). The data were normalized to the cell number.

Reactive oxygen species (ROS) detection

ROS was detected with a live cell-permeable, fluorophore CellROX Orange reagent (Thermo Fisher Scientific) according to the manufacturer's instructions. After treatment, cells were incubated with CellROX Orange reagent and Hoechst (Thermo Fisher Scientific) at 37 °C for 30 min, followed by washing twice with prewarmed PBS. Cells were imaged using a Nikon Eclipse 80i microscope. ROS intensity was analyzed using ImageJ (NIH) software.

Patients

The detailed information on human patients is provided in Supplementary data.

Statistical analysis

Each value reported represents the mean $\pm$ standard error of the mean (SEM) or mean $\pm$ standard deviation (SD) as noted in the figure legends. A Student's t-test was used to analyze differences in tumor volumes between two groups. For comparisons between multiple groups, a one-way ANOVA or two-way analysis was used with a Bonferroni post-test. For Kaplan Meier survival analysis, a Log-rank (Mantel-Cox) test was used to compare each of the arms. All statistical tests were two-sided. Differences were considered statistically significant at a P value of less than 0.05. All statistical analyses were performed with GraphPad Prism, software version 7.00.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1. Identification of LY2784544 that re-sensitize platinum-resistant ovarian cancer cells to platinum drugs by qHTCS

(A) Schematics illustration of the combination of qHTCS and genomic sequencing for the identification of potent compounds to overcome platinum resistant as well as for the identification of novel cisplatin resistant mechanisms. Alphabetical letters indicate potential targeting pathways.

(B) Viability of SKOV3 and SKOV3 CR cells (left), IGROV1 and IGROV1 CR (right) treated with increasing concentrations of cisplatin for 5 days. The IC_{50} was indicated.

(C) Heatmap: enrichment of SKOV3 CR for a strong response to specific drug categories (rows) combine with cisplatin. Drug-category-response scores are based on IC_{50} (μM). The three compounds with reciprocal synergy with cisplatin are indicated.

(D) Synergy matrix (bottom) and surface plots (top) showing the synergy between cisplatin and LY2784544 in SKOV3 and SKOV3 CR cells ($n = 3$).

(E) The synergistic effects of LY2784544 and cisplatin on IGROV1 CR cells. CI values are presented below the bars.

(F) Viability of PEO1 and PEO4 cells treated with increasing concentrations of cisplatin for 5 days. The IC_{50} was indicated.

(G) The synergistic effects of LY2784544 and cisplatin on PEO4 cells. The CI values are presented below the bars.

In all panels of this figure, data are represented as mean $\pm$ SD from three independent experiments performed in triplicate.

Figure 2. LY2784544 targets JAK2 signaling in platinum resistant ovarian cancer cell *in vitro* and *in vivo*

(A) Phosphorylation of JAK2 and STAT5 in parental and platinum resistant ovarian cancer cells.

(B) JAK2 knockdown by shRNA decreases phosphorylation of JAK2 and STAT5 in puromycin-selected SKOV3 CR cells.

(C) Viability of SKOV3 CR cells treated with increasing concentrations of cisplatin for 5 days after JAK2 knockdown. Data are represented as mean $\pm$ SD from three independent experiments performed in triplicate. ***p < 0.001 by two-way ANOVA.

(D) LY2784544 down-regulates JAK2-STAT5 signaling in SKOV3 CR cells. Cells were treated with the indicated concentrations of LY2784544 for 48 hr.

(E) Immunoblotting for the indicated proteins in SKOV3 CR cells treated with vehicle, 3 μ M LY2784544, 5 μ M cisplatin, or the combination (Combo) for 48 hr.

(F) Apoptosis percentage of SKOV3 CR cells treated with vehicle, 3 μ M LY2784544, 5 μ M cisplatin, or the combination (Combo) for 48 hr and analyzed for Annexin V and Propidium Iodide staining by flow cytometry. Data are represented as mean $\pm$ SD from three independent experiments performed in triplicate. **p < 0.01, ***p < 0.001, n.s. means not significant by one-way ANOVA.

(G) Growth curves of SKOV3 CR xenograft tumors treated with vehicle, LY2784544 (15 mg/kg/day intraperitoneally), cisplatin (8 mg/kg/week intraperitoneally), or LY2784544 plus

cisplatin (Combo) for 2 weeks. Data are represented as means $\pm$ SEM, $n = 6$ mice/group. *** $p < 0.001$ by two-way ANOVA. A photograph of the representative tumor from mice in each treatment arm is shown. Ruler scale is in cm.

(H) Kaplan-Meier survival curves of SKOV3 CR tumor-bearing mice treated with cisplatin and/or LY2784544 as in (G). The endpoint was scored when a tumor reached 1.5 cm or when a mouse showed any sign of distress. ($n = 6$, * $p < 0.05$, ** $p < 0.01$ by log-rank (Mantel-Cox) test).

(I) Representative hematoxylin and eosin (H&E), IHC and TUNEL staining of tumor samples from mice treated with cisplatin and/or LY2784544, tumors were collected 4 days after the last treatment. Scale bar, 100 μ m for H&E and pJAK2 IHC, 50 μ m for TUNEL.

(J) Quantification of IHC staining for pJAK2 in tumors treated as in (I). $n = 5$ mice/group. Data are represented as means $\pm$ SEM. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, n.s. means not significant by one-way ANOVA.

(K) Quantification of apoptotic cells in tumors treated as in (I). Data are represented as mean $\pm$ SD. $n = 5$ mice/group. *** $p < 0.001$, n.s. means not significant by one-way ANOVA.

Figure 3. IL-11 regulates JAK2 activity and cisplatin resistance in ovarian cancer cells

(A) Viability of SKOV3 cells treated with increasing concentrations of cisplatin for 5 days after pretreatment for 2 days with the indicated amount of conditioned medium from SKOV3 or SKOV3 CR cells. Data are represented as mean $\pm$ SD from three independent experiments performed in triplicate. CM, conditioned medium. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ by Student's t-test.

(B) Phosphorylation of JAK2 and STAT5 in SKOV3 cells after pretreatment for 2 days with the indicated amount of conditioned medium from SKOV3 or SKOV3 CR cells. CM, conditioned medium.

(C and D) Representative image (C) and quantification (D) of cytokine arrays of the expression levels of the indicated cytokines detected in the culture medium of paired cisplatin-sensitive (SKOV3, PEO1) and resistant (SKOV3 CR, PEO4) ovarian cancer cells. Data are represented as mean $\pm$ SD. Numbers above the symbols show the fold change of cytokines in paired cisplatin-sensitive and resistant ovarian cancer cells.

(E) Volcano plot showing up-regulated and down-regulated genes in SKOV3 CR compared with SKOV3. A change is considered significant if the p -value is < 0.05 and the change is > 2 -fold. Genes with very significant p -values are shown in the figure with p -value = 1×10^{-5} . The bright area in the plot indicates the high density of genes.

(F) Hierarchical clustering of selected cytokine genes reveals a significant 2-fold change in SKOV3 CR compared with SKOV3. Colors in the heatmap represent the gene expression levels after z-score normalization across different samples.

(G) Venn diagram shows the overlap of genes in the KEGG JAK/STAT signaling pathway (Pathway: map04630), genes identified by genomic sequencing of SKOV3/SKOV3 CR cells and genes identified by the cytokine array.

(H and I) Bar graphs showing the levels of mRNA (h) and secreted IL-11 (i) and in ovarian cancer parental cells (SKOV3, IGROV1, PEO1) and cisplatin resistant cells (SKOV3 CR, IGROV1 CR, PEO4). Levels of *IL-11* mRNA and IL-11 protein were measured by RT-qPCR and ELISA respectively and are shown as means $\pm$ SD from three independent experiments performed in triplicate. ** $p < 0.01$ by Student's t-test.

Figure 4. IL-11-mediated autocrine mechanism promotes cisplatin resistance via regulating JAK2-STAT5 activation in ovarian cancer cells

(A) Immunoblotting of phosphorylated JAK2 and STAT5 in SKOV3 cells treated with 10 ng/mL of IL-11 for 4 hr.

(B) Viability of SKOV3 cells incubated with 10 ng/mL of IL-11 for 4 hr and then treated with increasing concentrations of cisplatin for 5 days. Data are represented as mean $\pm$ SD from three independent experiments performed in triplicate. ** $p < 0.01$, *** $p < 0.001$ by Student's t-test.

(C) Immunoblotting of phosphorylated JAK2 and STAT5 in SKOV3 CR and IGROV1 CR cells treated with control mouse IgG or neutralizing anti-IL-11 antibody for 4 hr.

(D) Viability of SKOV3 CR (top) and IGROV1 CR (bottom) cells incubated with a control mouse IgG or an IL-11 neutralizing antibody for 4 hr and then treated with increasing concentrations of cisplatin for 5 days. Data are represented as mean $\pm$ SD from three independent experiments performed in triplicate. ** $p < 0.01$, *** $p < 0.001$ by Student's t-test.

(E) The levels of IL-11 secretion in SKOV3 CR cells transfected with IL11 shRNA and selected with puromycin. *** $p < 0.001$ by one-way ANOVA.

(F) Immunoblotting of phosphorylated JAK2 and STAT5 in SKOV3 CR cells transfected with IL11 shRNA and selected with puromycin.

(G) Viability of SKOV3 CR cells treated with increasing concentrations of cisplatin for 5 days after IL-11 knockdown with IL11 shRNA. Data are represented as mean $\pm$ SD from three independent experiments performed in triplicate. *** $p < 0.001$ by two-way ANOVA.

(H) Recombinant IL-11 reversed the effect of IL11 shRNA on the sensitivity of SKOV3 CR cells to cisplatin. SKOV3 CR shIL-11 cells were incubated with IL-11 (17 pg/ml for SKOV3

CR shIL11-1; 47 pg/ml for SKOV3 CR shIL11-2; calculated from 5e) for 4 hr and then treated with 1 μ M of cisplatin for 5 days. Data are represented as mean $\pm$ SD from three independent experiments performed in triplicate. ** $p < 0.01$, *** $p < 0.001$ by one-way ANOVA.

(I) Knockdown JAK2 by siRNA block IL-11 mediated resistance of SKOV3 cells to cisplatin. SKOV3 cells were transfected with JAK2 siRNA for 24hr and then incubated with recombinant IL-11 for 4 hr, followed by increasing concentrations of cisplatin treatment for 5 days. *** $p < 0.001$ by two-way ANOVA.

Figure 5. Cisplatin treatment induces IL-11 secretion and JAK2-STAT5 activation *in vitro* and *in vivo*

(A) IL-11 levels measured by ELISA in the medium of SKOV3 cells treated with cisplatin of various dosages for 4 days (left) and 1 μ M for various times (right) as indicated. Data are represented as mean $\pm$ SD from three independent experiments performed in triplicate. **p < 0.01, ***p < 0.001 by one-way ANOVA.

(B) *IL11* mRNA were measured by qPCR in SKOV3 cells treated with cisplatin of various dosages for 4 days (left) and 1 μ M for various times (right) as indicated. Data are represented as mean $\pm$ SD from three independent experiments performed in triplicate. **p < 0.01, ***p < 0.001 by one-way ANOVA.

(C) Levels of phosphorylated JAK2 and STAT5 in SKOV3 cells treated with cisplatin of various dosages for 4 days (left) and 1 μ M for various times (right) as indicated.

(D) A schematic presentation of the experimental set-up.

(E) Serum IL-11 levels measured by ELISA in mice bearing SKOV3 xenograft tumors and treated with vehicle or cisplatin (2 – 6 mg/kg/twice a week intraperitoneally for 2 weeks) as in (D). Data are represented as mean $\pm$ SD. n = 3 mice/group. ***p < 0.001 by one-way ANOVA.

(F) Representative H&E and IHC images of SKOV3 xenograft tumors treated as in (D).

(G) Quantification of IHC staining for pJAK2 and IL-11 in tumors as in (F). Data are represented as means $\pm$ SEM. *p < 0.05, **p < 0.01, ***p < 0.001, n.s. means not significant by one-way ANOVA.

Figure 6. ROS induces IL-11 expression by promoting expression of *FOSL1* (FRA1)

(A) Representative images (left) and quantification (right) of reactive oxygen species (ROS) production in SKOV3 and SKOV3 CR cells. Data are represented as means $\pm$ SD from three independent experiments. ** $p < 0.01$ by Student's t-test. Scale bar, 100 μ m.

(B) Quantification of reactive oxygen species (ROS) production in SKOV3 CR cells after treated with the ROS inhibitor YCG063 20 μ M for 24 hr. Data are represented as means $\pm$ SD from three independent experiments. *** $p < 0.001$ by Student's t-test.

(C) IL-11 levels measured by ELISA in the medium of SKOV3 CR cells treated with YCG063 20 μ M for 24 hr. Data are represented as mean $\pm$ SD from three independent experiments performed in triplicate. *** $p < 0.001$ by Student's t-test.

(D) Immunoblotting of phosphorylated JAK2 and STAT5 in SKOV3 CR cells treated with YCG063 (20 μ M) for 24 hr.

(E) Quantification of reactive oxygen species (ROS) production in SKOV3 cells after treated with the ROS inhibitor YCG063 (20 μ M), cisplatin (1 μ M), or both for 24 hr. Data are represented as means $\pm$ SD from three independent experiments. ** $p < 0.01$, *** $p < 0.001$, n.s. means not significant by one-way ANOVA.

(F) IL-11 levels measured by ELISA in SKOV3 cells treated with YCG063 (20 μ M), cisplatin (1 μ M) or both for 24 hr. Data are represented as mean $\pm$ SD from three independent experiments performed in triplicate. *** $p < 0.001$ by one-way ANOVA.

(G) Immunoblotting of phosphorylated JAK2 and STAT5 in SKOV3 CR cells treated with YCG063 (20 μ M), cisplatin (1 μ M) or both for 24 hr.

(H) Heatmap of transcription factors that can potentially regulate *IL11* in SKOV3 and SKOV3 CR. Colors in the heat-map represent the gene expression levels after z-score normalization across different samples. Fold change bar on the right shows that *FOSL1* is the most significant up-regulated gene.

(I) Total and phosphorylated levels of FRA1 in SKOV3 and SKOV3 CR cells.

(J) Depletion of *FOSL1* by siRNA decreases the phosphorylated and total FRA1 protein levels in SKOV3 CR cells

(K) IL-11 levels measured by ELISA in the medium of SKOV3 CR cells transfected with *FOSL1* siRNA for 48 hr. Data are represented as mean $\pm$ SD from three independent experiments performed in triplicate. *** $p < 0.001$ by Student's t-test.

(L) Immunoblotting of phosphorylated and total FRA1 protein in SKOV3 CR cells treated with YCG063 20 μ M for 24 hr.

Figure 7. Clinical evidence of activated IL-11-JAK2 pathway in platinum drug resistant ovarian cancer patients

(A) Comparison of the mRNA IL-11 levels measured by qPCR in platinum sensitive ($n = 23$) and resistant ($n = 16$) ovarian cancer patients. * $p < 0.05$ by unpaired student t-test. Sen, sensitive cases. Res, resistant cases.

(B) Comparison of the serum IL-11 levels measured by ELISA in platinum sensitive ($n = 21$) and resistant ($n = 16$) ovarian cancer patients. ** $p < 0.01$ by unpaired student t-test. Sen, sensitive cases. Res, resistant cases.

(C) Kaplan–Meier survival curves showing 5-year PFS rate (left panel) and OS rate (right panel) of 37 ovarian cancer patients as shown in (d), who were stratified by serum IL-11 levels (40 pg/ml); log-rank (Mantel-Cox), P values and HRs are shown.

(D) Representative IHC image (left panel) and quantification (right panel) of the cisplatin sensitive and resistant tumor samples from the same patient (P1).

(E) IHC quantification of IL-11 and p-JAK2 in platinum sensitive and recurrent tumor samples from seven patients (P2–P8). Data are represented as mean $\pm$ SD.

(F) Scatter plots showing a correlation between IL-11 and pJAK2 levels in both primary and recurrent patient tumors (P2–P8).

(G) Venn diagram indicating the overlap of genes in the JAK2 functional gene set and up-regulated genes set in SKOV3 CR compared with that in SKOV3 cells (left panel). The heatmap (right panel) shows 22 overlapped genes (JAK signature gene set). Colors in the

heatmap represent the gene expression levels after z-score normalization across different samples.

(H) Kaplan–Meier analyses of 5-year PFS rate (left panel) and OS rates (right panel) based on clinical and molecular data for ovarian cancer patients (n = 502 for PFS, n = 478 for OS). The patients were stratified by the expression levels in their tumors of the JAK2 signature genes. Medium survival, log-rank (Mantel-Cox) p values and HRs are shown.