Targeting tumor-macrophage interaction via the Notch2-Jag1 axis reverses tumor resistance to paclitaxel

Fazhi Yu

University of Science and Technology of China

Qin Zhou

University of Science and Technology of China

Ying Zhou

University of Science and Technology of China

Yijia Xie

University of Science and Technology of China

Peng Zhang

University of Science and Technology of China

Wei He

The University of Texas MD Anderson Cancer Center

Weiqiang Yu

University of Science and Technology of China

Yuwei Wu

University of Science and Technology of China

Aoxing Cheng

University of Science and Technology of China

Hanyuan Liu

University of Science and Technology of China

Cheng Sun

University of Science and Technology of China

Qingfa Wu

University of Science and Technology of China

Kaiguang Zhang

University of Science and Technology of China

Xiaopeng Ma

University of Science and Technology of China

Jue Shi

Hong Kong Baptist University

Jing Guo

University of Science and Technology of China

Zhenye Yang (✉ zhenye@ustc.edu.cn)

University of Science and Technology of China <https://orcid.org/0000-0001-7797-5891>

Article

Keywords: tumor-macrophage interaction, Notch2 signaling, chemoresistance, taxol

Posted Date: July 6th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-638003/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Targeting tumor-macrophage interaction via the Notch2-Jag1 axis reverses tumor resistance to paclitaxel

Fazhi Yu^{1, 2, #}, Qin Zhou^{2, #}, Ying Zhou^{1, 3, #}, Yijia Xie², Peng Zhang², Wei He⁴, Weiqiang Yu², Yuwei Wu², Aoxing Cheng², Hanyuan Liu¹, Cheng Sun^{1, 2}, Qingfa Wu², Kaiguang Zhang¹, Xiaopeng Ma¹, Jue Shi^{5, *}, Jing Guo^{1, *}, Zhenye Yang^{1, 2, *}

Affiliations:

¹ The First affiliated hospital of USTC, Division of Life Sciences and Medicine, University of Science and Technology of China, Hefei, China.

² Hefei National Laboratory for Physical Sciences at Microscale, Division of Life Sciences and Medicine, University of Science and Technology of China, Hefei, China.

³ Department of Gynecology, Affiliated Provincial Hospital of Anhui Medical University, Hefei, China.

⁴ Department of Epigenetics and Molecular Carcinogenesis, The University of Texas MD Anderson Cancer Center, Smithville, TX 78957, USA

⁵ Center for Quantitative Systems Biology, Department of Physics and Department of Biology, Hong Kong Baptist University, Hong Kong, China

These authors contributed equally to this work

* Correspondence: zhenye@ustc.edu.cn; jguo2013@ustc.edu.cn; jshi@hkbu.edu.hk

Abstract

Taxanes are widely used in chemotherapy, but intrinsic and acquired resistance limit the clinical outcomes. Studies showed tumor interaction with suppressive macrophages plays a key role in taxane resistance, yet therapeutic strategies that deplete or repolarize macrophages are challenging. Here we uncovered a novel tumor-macrophage interaction via Notch2-Jag1 justacrine signaling that can be targeted to sensitize paclitaxel response without affecting the broad macrophage functions. Using translome profiling, we identified Notch2 upregulation during taxol-induced prolonged mitosis. Notch2 was subsequently activated in the post-mitotic G1 phase by Jag1 expressed on neighboring macrophages, which promoted tumor cell survival by upregulating p38 and anti-apoptotic proteins. Notch2 also upregulated cytokines that further recruited Jag1-expressing macrophages. By targeting this Notch2-Jag1 interaction with a pan-Notch inhibitor, RO4929097, taxol resistance was significantly attenuated in multiple mouse tumor models. Our results point to combining Notch inhibitor with taxane as an effective strategy to selectively disrupt tumor-macrophage interaction underlying chemoresistance.

Keywords: tumor-macrophage interaction, Notch2 signaling, chemoresistance, taxol

Introduction

Despite the development of targeted drugs and immunotherapy, classic chemotherapeutics, such as DNA damaging drugs, anti-mitotics and anti-metabolites, remain the main treatment modality for most cancer patients. Responses to cytotoxic anticancer drugs are known to vary significantly between disease types and even patients with the same disease, and much effort has been devoted to developing combinatorial strategies that can sensitize tumor response to chemotherapy for resistant tumor types¹⁻³. The recent advancements in immuno-oncology have elucidated multi-level tumor-immune cell interactions that confer chemoresistance, providing novel therapeutic angles to pursue alternative combinatorial strategies to improve the effectiveness of chemotherapy. One of the key immune cell types known to confer chemoresistance is the suppressive, tumor-associated macrophages (TAMs)⁴⁻⁶. TAMs can promote resistance to drug-induced tumor cell death, e.g., by secreting growth factors and inhibiting cell death signaling in tumor cells, suppressing anti-tumor T cell immunity and/or stimulating tumor vascularization. So far therapeutic approaches targeting pro-tumor TAMs to reverse chemoresistance include blocking colony-stimulating factor-1 (CSF-1)/CSF-1R or chemokine (C-C motif) receptor 2 (CCR2) to inhibit TAM infiltration to tumor⁷⁻⁹, neutralizing IL-4/IL-4R to repolarize TAMs away from the pro-tumor M2 phenotype¹⁰, and using a CD40 agonist to promote macrophage activation¹¹. Although these combinatorial strategies were found to be effective in sensitizing chemotherapeutic responses in multiple mouse tumor models and early clinical trials, they may incur

considerable risk of toxicity and side effects in essential organs, as the treatments directly deplete macrophage or perturb key macrophage functions.

In this study we aim to explore and develop a more tumor-selective therapeutic strategy that specifically disrupts chemoresistance-associated tumor-macrophage interactions without compromising the systemic macrophage functions. We chose to investigate chemoresistance to paclitaxel, as a number of *in vivo* studies have demonstrated that tumor-infiltrated macrophages play a crucial role in conferring resistance to taxol-induced growth arrest and cell death, and depleting/repolarizing macrophages can effectively reverse the resistance^{8, 12-14}. Taxanes, i.e., paclitaxel and its derivatives, are commonly used to treat various cancers, in particular solid tumors. Taxanes bind to the β subunit of tubulin dimer and block microtubule dynamics^{15, 16}. This results in aberrant spindles and erroneous MT-kinetochore attachments during mitosis, leading to activation of the spindle assembly checkpoint (SAC) and prolonged mitotic arrest. Taxanes are thus called anti-mitotic drugs. Taxane-treated cells either die in the mitotic arrest or slip out of mitosis without division to an abnormal G1 state, from which the cells may die, arrest or progress through cell cycle^{17-20 21-24}. Similar response phenotypes were also observed in mouse tumor models²⁵. Various combinatorial strategies have been proposed to sensitize cell death response to paclitaxel²⁶, such as inhibiting pro-apoptotic protein^{27, 28}. Whether such combinations indeed can improve patient response are yet to be determined. And we are still in need of alternative avenues to develop therapeutic approaches to combat taxane resistance.

To uncover novel tumor-macrophage interactions specific to taxol resistance, in particular via direct cell-cell contact, we combined transcriptome profiling of taxol-induced mitotic cells and co-culture analysis of cancer cells and macrophages. We found that Notch2 receptor in cancer cells was selectively upregulated during taxol-induced prolonged mitosis. Interaction of Notch2 with Jag1 expressed on the neighboring macrophages then activated juxtacrine signaling in the post-mitotic G1 state, which promoted resistance to taxol-induced cell death by upregulating pro-survival p38 signaling and anti-apoptotic proteins. Activation of Notch2 signaling via tumor-macrophage interaction also upregulated cytokines that stimulated recruitment of Jag1-expressing, suppressive macrophages, further enhancing taxol resistance. Our findings thus suggested Notch2 is a viable combinatorial target to attenuate tumor-macrophage interaction and taxol resistance. Indeed combining a pan-Notch inhibitor, RO4929097, with paclitaxel, was able to effectively suppress tumor growth in multiple mouse tumor models that were resistant to paclitaxel treatment alone, illustrating that Notch2 inhibitor is an effective combinatorial drug candidate for selectively targeting tumor-macrophage interaction to reverse taxol resistance.

Results

Taxol-induced Notch2 upregulation is associated with chemoresistance

To identify molecular mediator(s) for contact-dependent tumor-macrophage interaction that contributes to taxol resistance, we first conducted translome profiling of upregulated membrane proteins/receptors in taxol-resistant cancer cells during drug-induced prolonged mitotic arrest. We hypothesized that as prolonged mitosis is the primary phenotype of taxol, cellular alternations occurring during the mitotic arrest likely play crucial roles in engendering chemoresistance. And since transcription is largely silenced during mitosis, upregulation of pro-tumor molecular mediators probably occurs at the post-transcriptional level, such as translation. To perform the mitotic translome analysis, mitotic cells were harvested by shaking-off from untreated and Taxol-treated HeLa cells and subjected for ribosome profiling (Fig. 1a). More than nine thousands mRNAs were identified to be translated during normal mitosis, and 89% of these genes were also found in two previously published datasets, in which the translational landscape of mitosis was analyzed also by ribosome profiling^{29,30}, validating that our data are well in line with results from other studies (Supplementary Fig. 1a). From the genes identified to be upregulated during prolonged mitosis (Fig. 1a), cell membrane receptors were picked out and compared with those identified from two other available translome analysis of prolonged mitosis using ribosome profiling²⁹ or proteomics³¹. The Venn diagram showed that Notch2 is the only upregulated membrane receptor identified by all three studies (Fig. 1b).

Notch2 is one of the four Notch signaling receptors that are frequently dis-regulated in cancers and are involved in multiple oncogenic phenotypes, including drug resistance via juxtacrine signaling at cell-cell contact^{32, 33}. We first validated Notch2 was upregulated at the protein level during mitotic arrest. Similar to cyclin B, which is known to be actively translated during prolonged mitosis³⁴, the level of Notch2 protein significantly increased during Taxol-induced prolonged mitosis (Fig. 1c), while the mRNA level did not change (Supplementary Fig. 1b). The mRNA of Notch2 was not increased until six hours after mitotic exit, while the protein level of Notch2 was already at a high level when cells entered G1 phase (Supplementary Fig. 1c). The increase of Notch2 protein was abolished by cycloheximide (Fig. 1c), indicating that Notch2 was upregulated at the protein synthesis level. We also confirmed the upregulation of Notch2 protein in two other taxol-resistant cancer cell lines, the ovarian cancer line OVCAR8 and the lung cancer line A549 (Fig. 1d). In addition, we found Notch2 upregulation can be induced by other anti-mitotic compounds, such as nocodazole, STLC (Eg5 inhibitor) and BI2536 (Plk1 inhibitor) (Supplementary Fig. 1d). Overall our results showed that Notch2 is actively translated and upregulated during prolonged mitosis.

We next evaluated the clinical relevance of Notch2 level in patients that were treated with taxanes. Paired primary tissues and recurrent surgical tissues from ovarian cancer patients were subject to immunochemical staining (Fig. 1e, f). The IHC score (Supplementary Fig. 1e, defined by expression levels, detailed in methods) revealed a significant correlation between Notch2 level and tumor recurrence in these patients after

paclitaxel/platinum treatment (Fig. 1g). For patients that relapsed after adjuvant therapy with paclitaxel, progression-free survival rate is substantially lower for those with tumors showing high expression of Notch2 (Fig. 1h). Such correlation was not observed for patients without taxol-based adjuvant therapy, indicating that Notch2 expression is particularly associated with tumor recurrence after taxol treatment. In addition, analysis of ovarian cancer patient data from the TCGA database confirmed that the overall survival rates are low for high Notch2 expression, but not for other notch receptors, in the taxol treatment cohorts (Fig. 1i, Supplementary Fig. 1f). These clinical patient samples suggested that Notch2 level and the extent of Notch2 signaling are closely correlated with taxol resistance in the clinical context.

Notch2 upregulation confers resistance to post-mitotic taxol-induced cell death

To examine whether Notch2 is essential for cancer cell survival under paclitaxel treatment, we depleted Notch2 by RNAi knockdown or CRISPR-Cas9 knockout and measured cell death response to paclitaxel. Live cell imaging analyses showed that Notch2 knock-down cells without taxol treatment exhibited normal mitotic progression and no increase in cell death (Supplementary Fig. 2a). Upon taxol treatment, Notch2 knockdown significantly enhanced the extent of cell death in the post-mitotic G1 phase and accelerated the time to death from mitotic exit (Fig. 2a, b and Supplementary Fig. 2b). Similar results were obtained in Notch2 knockout cells by CRISPR-Cas9 (Supplementary Fig. 2c-e). We also examined apoptosis induction in detail for the distinct

cell cycle phases, i.e., before mitosis, during mitosis and after mitosis (Fig. 2c). While the level of Hes1, a known Notch2 target gene, was significantly reduced after Notch2 depletion, the levels of cleaved-PARP and cleaved-caspase-3, two apoptotic markers, did not appear until cells exited mitosis and entered into the G1 phase (Fig. 2d). This suggested that Notch2 promotes anti-apoptotic signaling for taxol-treated cells specifically in the post-mitotic G1 phase. Furthermore, Notch2 depletion reduced the IC50 of taxol, but not that of carboplatin, for both A549 cells (Fig. 2e) and OVCAR8 cells (Supplementary Fig. 2f), indicating Notch2 upregulation specifically promotes taxol resistance.

We next evaluated the functional involvement of Notch2 in a xenograft tumor model. Consistent with the cell culture results, depletion of Notch2 did not affect tumor growth in the untreated mice, but significantly attenuated the tumor growth (Fig. 2f and Supplementary Fig. 2g) and increased active caspase-3 level in the taxol-treated mice (Supplementary Fig. 2h), confirming that Notch2 is involved in promoting taxol resistance *in vivo*. Moreover, overexpression of the active intracellular domain of Notch2 (NICD2) downregulated the active caspase-3 level (Fig. 2g) and promoted tumor growth in the taxol-treated mice (Fig. 2h). These *in vivo* results illustrated that upregulated Notch2 is required for tumor resistance to paclitaxel treatment.

Notch2 signaling is activated by Jag1 expressed on the neighboring macrophages

Notch and the downstream signaling cascades are activated by binding to the cognate

ligands expressed on neighboring cells. Well-known Notch ligands include Jag1, Jag2, Dll1, Dll3 and Dll4³³. Based on analysis of published database of the five notch ligands, we found Jag1 exhibited the highest expression in macrophages isolated from ascites of both human patients (Fig. 3a) and tumor-bearing mice (Fig. 3b). We also detected high level of Jag1 in the CD68 and F4/80 positive macrophages from the ascites of ovarian cancer patients (Fig. 3c) and ID8 mouse model (Fig. 3d). Moreover, analysis of the TCGA dataset showed strong correlation between Notch2 level and the levels of M2-type macrophage infiltration in multiple cancer types (Supplementary Fig. 3a, b), and that Jag1 exhibited the highest expression in ovarian and lung cancer (Supplementary Fig. 3c, d). These data all pointed to Jag1 expressed on macrophages as the potential activator of the pro-tumor Notch2 signaling in cancer cells.

To investigate the Jag1 involvement, we first used recombinant Jag1 that was pre-coated on coverslip to activate Notch2. We found it indeed resulted in nuclear accumulation of Notch2 (Supplementary Fig. 3e) and Hes1 expression (Supplementary Fig. 3f). Further live cell imaging analysis showed that pre-coated FC-r Jag1, but not FC, reduced cell death in the post-mitotic G1 phase under paclitaxel treatment (Fig. 3e and Supplementary Fig. 3g). And this pro-survival function of Jag1 was abolished in Notch2 knockout cells (Fig. 3e). To further confirm the role of Jag1-expressing macrophages in the observed taxol resistance, a co-culture assay of OVCAR8 cells with M2-type macrophages derived from THP-1, a human leukemic monocyte cell line, was performed (Fig. 3f). While M2-type macrophage that derived from THP-1 promoted the survival of

cancer cells, they failed to do so after Notch2 depletion in the cancer cells (Fig. 3f, Supplementary Fig. 3h). Resistance to taxol-induced cell death was observed in OVCAR8 cells in co-culture with the wildtype macrophages, but not the Jag1-depleted macrophages (Fig. 3g and Supplementary Fig. 3i). Together with the Notch2 results discussed above, our data pointed to that tumor-macrophage interaction via the Notch2-Jag1 juxtacrine signaling in the tumor microenvironment mediates resistance to taxol-induced cancer cell death.

Notch2 upregulation promotes post-mitotic cancer cell survival by activating p38 signaling

To further pinpoint how activation of Notch2-Jag1 signaling confers taxol resistance at the molecular and cellular level, we investigated the downstream signaling pathways of Notch2 using transcriptome analysis of Notch2-depleted cells. Principal component analysis of the transcriptome data from knockdown OVCAR8 cells using two different Notch2 shRNA constructs gave consistent results (Fig. 4a and supplementary Fig. 4a). PI3K-AKT signaling and MAPK signaling were found to be the major downstream pro-survival pathways that mediated the Notch2 effect (Fig. 4b, c). In particular, the key p38 MAP kinase was confirmed to be downregulated both at the mRNA (Fig. 4d and supplementary Fig. 4b) and protein levels (supplementary Fig. 4c) in the Notch2 knockdown cells. While depletion of Notch2 promoted taxol-induced cell death, we found overexpression of p38 fully rescued the pro-death phenotype of Notch2 depletion,

indicating that p38 is likely involved in the pro-survival activity of Notch2 (Fig. 4e). Moreover, p38 inhibition by SB203580 significantly increased cell death of OVCAR8, HeyA8 and A549 cells (Fig. 4f and supplementary Fig. 4d, e), further pointing to the involvement of p38 in mediating the taxol resistance. In terms of the direct apoptotic regulators, levels of the anti-apoptotic protein *MCL1* and *BCL2* were reduced upon Notch2 depletion, suggesting that Notch2 promotes post-mitotic cell survival partly via upregulating the expression of MCL1 and BCL2 in the taxol-treated cells (Fig. 4g, h). Overexpression of p38 in Notch2-depleted cells rescued the levels of MCL1 and BCL2, again indicating p38 is the key downstream mediator of Notch2 activity, e.g., in upregulation anti-apoptotic proteins (Fig. 4h). In addition, the levels of Notch2 and p38 α were correlated in patient samples of ovarian and lung cancers from the TCGA dataset (Supplementary Fig. 4f). These data together revealed that Notch2 promotes resistance to taxol-induced cell death via upregulating the p38-MCL1 signaling cascade.

CPEB mediated poly-A elongation promotes Notch2 translation in prolonged mitosis

Among the four Notch receptors, Notch2 is the only one upregulated during prolonged mitosis (Fig. 5a), despite that both Notch2 and Notch3 are expressed in ovarian and lung cancers according to the TCGA datasets (Supplementary Fig. 5a). To explore the origin of differential translation of distinct Notch receptors in prolonged mitosis, we examined the characteristics of Notch mRNA in comparison with the mRNA of cyclin B, a highly translated protein in mitosis. Cyclin B mRNA has multiple

cytoplasmic polyadenylation element (CPE) and polyadenylation hexanucleotide (PH) elements. It has been reported that CPEB (cytoplasmic polyadenylation element binding protein) and CPSF (cleavage and polyadenylation specificity factor) promote the polyadenylation and translation of mRNA that contain sequences of CPE and PH³⁵. Similar to cyclin B, there are eight CPE and five PH elements within the 3' UTR of Notch2 mRNA (Fig. 5b, c). In contrast, few CPE or PH sequences were found in the mRNA of the other three Notch receptors (Fig. 5b). CPEB RIP assay demonstrated that similar to cyclin B, whose mRNA is known to bind CPEB, mRNA of Notch2 bound CPEB1 proteins in mitotic cells (Fig. 5d), suggesting possible polyadenylation of Notch2 mRNA during prolonged mitosis. In addition, CPEB inhibition with cordycepin, an inhibitor of CPEB-dependent polyadenylation, significantly attenuated Notch2 upregulation (Fig. 5e), illustrating that CPEB-directed polyadenylation promoted the synthesis of Notch2 during mitotic arrest. Consistent with the role of Notch2 in promoting post-mitotic cell survival, CPEB inhibition that reduced Notch2 level and activity enhanced the extent of post-mitotic death of taxol-treated A549 (Fig. 5f) and OVCAR8 cells (Supplementary Fig. 5b). These results thus suggested that CPEB-mediated polyadenylation of Notch2 mRNA and subsequent protein synthesis led to Notch2 upregulation during prolonged mitotic arrest.

Notch2 upregulation stimulates the recruitment of pro-tumor macrophages

In addition to signaling kinases and anti-apoptotic proteins, cytokines, such as CSF1

and IL-1 β , were also downregulated upon Notch2 knockdown based on the RNA-Seq analysis (Fig. 4b, c). We subsequently confirmed, by qPCR and ELISA, that paclitaxel treatment induced expressions of CSF1 and IL-1 β , and Notch2 depletion markedly reduced their expressions (Fig. 6a, b, Supplementary Fig. 6a, b). CSF1 and IL-1 β are known to recruit pro-tumor M2 macrophages^{8, 36}, implicating a possible immunomodulatory role of Notch2 activation in augmenting pro-tumor macrophages and the pro-survival tumor-macrophage interactions. To measure the effect of Notch2 activation in recruiting macrophages, conditioned medium from control and Notch2-depleted OVCAR8 cells were collected and their effects on the migration of THP-1 were measured by a trans-well assay (Fig. 6c). Compared to medium from the taxol-treated wildtype cells, medium from the Notch2-depleted cells showed significantly less effect in promoting THP-1 migration (Fig. 6c). Addition of recombinant CSF1 and IL-1 β to the medium from Notch2-depleted cells resulted in an extent of THP-1 migration similar to that induced by wildtype medium, indicating that Notch2 signaling stimulates macrophage recruitment via upregulating cytokines (Fig. 6d). Moreover, mouse xenograft tumor model showed that Notch2 knockdown significantly attenuated macrophage infiltration into the tumor under paclitaxel treatment (Fig. 6e), confirming the role of Notch2 in promoting the recruitment of macrophages to the tumor microenvironment. Analyses of clinical data from the TCGA datasets also showed strong correlation between Notch2 level and the levels of CSF1 and IL-1 β (Fig. 6f, Supplementary Fig. 6c). Our data thus demonstrated that Notch2 promotes a pro-tumor immune microenvironment by

recruiting M2-type macrophages, further enhancing the chemoresistance-associated tumor-macrophage interactions.

Notch2 inhibitor sensitizes tumor response to paclitaxel in multiple mouse tumor models

As Notch2 upregulation and Notch2-Jag1 signaling drive the resistance to taxol-induced cell death and Notch2 knockdown largely abrogates this chemoresistance in various cancer cell types that we investigated (Fig. 2), combining Notch2 inhibition and paclitaxel is likely an effective strategy to combat taxol resistance and attenuate tumor growth. Gamma-secretase inhibitor, RO4929097, has been used as a pan-Notch inhibitory agent and demonstrated good anti-tumor efficacy in multiple clinical trials³⁷⁻³⁹. We thus tested the combinatorial effect of RO4929097 and paclitaxel in both culture cancer cell lines and mouse tumor models. Notch2 inhibition by RO4929097 was confirmed by the reduced expression of the Notch2 target gene, Hes1 (Supplementary Fig. 7a).

Combination of RO4929097 with paclitaxel, but not carboplatin, enhanced the growth arrest of OVCAR8 cells as compared to chemotherapy alone (Supplementary Fig. 7b).

Cell viability assay further confirmed that RO4929097 sensitized the cell death response of OVCAR8 cells to paclitaxel at the clinically relevant concentrations (Supplementary Fig. 7c), with cleaved Caspase-3, an apoptotic marker, significantly elevated in these cells (Supplementary Fig. 7d). The combinatorial treatment of RO4929097 plus paclitaxel also resulted in enhanced growth inhibition in mouse xenograft tumor models

of OVCAR8 (Fig. 7a), as compared to paclitaxel or RO4929097 treatment alone. We also noted that the number of infiltrated macrophages in taxol-treated tumor was significantly reduced with RO4929097 (Fig. 7b). Similar to the cell culture data, RO4929097 did not further suppress tumor growth when combined with carboplatin (Supplementary Fig. 7f). The effect of combinatorial treatment was also evaluated with a syngenic model (ID8 intraperitoneal transplantation). Compared to paclitaxel treatment alone, RO4929097 plus paclitaxel showed much more significant attenuation of tumor growth (Fig. 7c) as well as reduction of M2-type macrophages in the tumor (Fig. 7d, e and supplementary Fig. 7g), illustrating that blocking notch signaling damps the recruitment of pro-tumor macrophages under taxol treatment.

To further test the efficacy of combinatorial treatment of RO4929097 and paclitaxel in a clinically relevant model, a patient-derived xenograft (PDX) model for ovarian cancer was generated (Fig. 7f). The tumor cells were from a patient with recurrent tumor after adjuvant chemotherapy with paclitaxel/lobaplatin (Supplementary Fig. 7h), and the PDX was validated by staining multiple ovarian cancer markers (Supplementary Fig. 7i). Upon combinatorial treatment of paclitaxel and RO4929097, robust synergistic effect in suppressing tumor growth was observed in the PDX tumor (Fig. 7g, h). Of note, the combinatorial treatment also significantly inhibited the level of activated p38 and Mcl1 in the PDX model (Fig. 7i), agreeing with results from cell culture analysis that showed Notch signaling upregulated p38 signaling and anti-apoptotic proteins under taxol treatment.

Discussion

Chemoresistance is a major challenge for cancer treatment and mechanistic understanding of the molecular and cellular determinants underlying both intrinsic and acquired resistance is key to develop new combinatorial strategies to improve the clinical efficacy of chemotherapeutics for a broad patient population. By investigating contact-dependent tumor-macrophage interaction that contributes to tumor resistance to paclitaxel, we identified a novel signaling mechanism via Notch2-Jag1 interaction between tumor cells and neighboring macrophages that is druggable to sensitize tumor response to paclitaxel. The newly proposed therapeutic approach of Notch2 inhibition is possibly more tumor selective and can effectively disrupt pro-survival tumor-macrophage interaction without broadly affecting normal macrophage functions in innate immune defenses. Moreover, we found Notch2 inhibition can reverse paclitaxel resistance by attenuating pro-survival signaling in tumor cells as well as damping the immuno-suppressive tumor microenvironment, i.e., by inhibiting infiltration of M2-type macrophages. Such effect on blocking both intrinsic and extrinsic pro-tumor factors makes Notch2 an attractive combinatorial target to combat paclitaxel resistance.

On the mechanism of chemoresistance, we found the membrane receptor Notch2 is synthesized and upregulated during prolonged mitotic arrest induced by paclitaxel. Shortly after the cells exit mitosis and enter the next G1 phase, Notch2 is activated by its cognate activating ligand, Jag1, expressed on the neighboring macrophages. Cleaved intracellular Notch2 then translocates to the nucleus and activates the transcription of a

series of target genes. The downstream network activated by Notch2 includes both intracellular signaling that upregulates anti-apoptotic proteins and extracellular signaling via cytokine production that reshapes the tumor microenvironment (Fig. 7j). For the intracellular signaling, p38 is a key hub to coordinate the stress response and anti-apoptotic signaling. Follow-up study is needed to further elucidate the detailed regulation of the Notch2-p38 signaling cascade. The extracellular signaling contributes to resistance to taxol-induced cell death through a positive feedback on tumor-macrophage interaction. On the one hand, Notch2 increases the production of cytokines that recruit M2-type macrophages known to mediate chemoresistance. The tumor-infiltrated macrophages, on the other hand, further enhances Notch2 activation via the Jag1 ligand expressed on their surface, therefore forming a positive feedback loop to promote Notch2-mediated pro-survival signaling (Fig. 7j). As the transcription of Notch2 only starts a few hours after cells exit the prolonged mitosis (Supplementary Fig. 1c), the advantage of Notch2 upregulation at the translational level during mitotic arrest could be that it renders a much faster response from the pro-survival pathways, as soon as the cells exit the prolonged mitosis and form contacts with the neighboring macrophages expressing Jag1. The rapid activation of Notch2-mediated pro-survival signaling may protect cancer cells from apoptosis that is initiated during the prolonged mitotic arrest.

Compounds that target γ -secretase can be used as pan-notch inhibitors and have been tested either alone or in combination with taxanes in a number of clinical trials for different cancer types^{37, 40, 41}. In a recently reported phase I clinical trial, RO4929097, a γ -

secretase inhibitor, was combined with neoadjuvant paclitaxel and carboplatin to treat patients with triple negative breast cancer. 36% of the patients had pathologic complete response³⁹. As Notch inhibition with paclitaxel, but not carboplatin, showed synergistic effects (Fig. 7), the combinatorial drug action of Notch inhibitor is likely mitosis dependent. Moreover, based on study of the clinical samples (Fig. 1), we found Notch2 can also be used as a prognostic biomarker for taxane sensitivity at least for ovarian and lung cancers. The increased expression of Notch2 in relapse tumor also suggests a correlation between Notch2 and taxane resistance. To further develop Notch2 therapeutics, biologic antibody against Notch2 may render higher specificity than small-molecule inhibitors to enhance taxane efficacy for patients with Notch2-positive tumors.

Methods

Cell lines and cell culture

All cell lines were obtained from the sources indicated in the Key Resources Table.

HeLa, A549, OVCAR8, ID8, HEK293T, and HeyA8 were cultured in DMEM

(Invitrogen/Thermo Fisher Scientific, MA, USA) supplemented with 10% fetal bovine

serum (FBS) (Gibco, Grand Island, NY, USA) and 1% penicillin/streptomycin (P/S)

(Beyotime Biotechnology, Jiangsu, China) at 37°C with 5% CO₂. THP-1 cells were

cultured in RPMI-1640 (Invitrogen/Thermo Fisher Scientific, MA, USA) supplemented

with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin. Cells were tested

regularly for mycoplasma.

Live cell imaging

For live cell imaging, 2×10^4 cells were seed into 8 well-Sliders (#80826, ibidi) and

maintained at 37°C overnight in DMEM with 10% fetal bovine serum. The medium was

changed to phenol red-free L-15 medium (Invitrogen/Thermo Fisher Scientific, MA,

USA) with 10% FBS and 1% P/S, and drugs or DMSO were added before the start of the

imaging experiments. Time-lapse images were acquired at 5 min intervals with a 20×

objective lens mounted on an Eclipse Ti microscope (Nikon, Tokyo, Japan). Time of

mitosis and interphase was determined morphologically (cells from rounding up to

flattening defined as mitosis, after flattening defined as interphase). Moreover, initiation

of death was defined by the characteristic morphological changes of blebbing and cell

lysis.

Cell synchronization

HeLa, A549, and OVCAR8 cells were synchronized by double thymidine block (2 mM, Sigma). Specifically, cells were plated in 10 cm dishes one day before treatment. Then 2 mM thymidine was added to the dishes to arrest the cells for 19 hours (hrs). After washing the dishes three times with PBS, cells were released into fresh culture medium for 9 hrs, which was followed by a second thymidine block for 16 hrs. Then the dishes were washed three times with PBS to release the cells to complete DMEM again. 9 hrs later, DMSO and 5 nM paclitaxel were added to each group, respectively. The mitotic cells were collected by shake-off. And the mitotic cells from DMSO and taxol treatment were defined as mitosis (M) and prolonged mitosis (pM) cells, respectively.

For A549 synchronization in Fig. 2d, A549 cells were plated in 10 cm dishes one day before treatment, followed by adding 100 nM taxol to the medium. 24 hrs later, mitotic cells were collected by shake-off. The cells that remained attached to the dishes were defined as interphase cells. Moreover, part of the mitotic cells was re-plated to a 35 mm dish for 12 hrs, and these cells were defined as Next G1 cells.

Ribosome profiling and mRNA sample preparation

For ribosome profiling, HeLa cells were synchronized by double thymidine block (2 mM, Sigma). Mitotic (M) and prolonged mitotic (pM) HeLa cells were lysed in lysis buffer

(20 mM Tris pH 7.5, 150 mM KCl, 5 mM MgCl₂, 1 mM dithiothreitol, 8% glycerol) supplemented with 0.5% Triton X-100, 30 U/ml Turbo DNase (Ambion, Life Technologies, CA, United States) and 100 µg/ml cycloheximide (Sigma Aldrich, MO, United States). Ribosome protected fragments were then isolated and sequenced as previously described ⁴². The resulting mRNAs were modestly fragmented by partial hydrolysis in bicarbonate buffer so that the average size of fragments was ~80 bp. The fragmented mRNAs were separated by denaturing PAGE and fragments of 26–34 nt were selected. The sequencing libraries were prepared and sequenced as previously described ⁴².

Western blot

Cells were seeded to cell culture dishes 24 hrs prior to treatment. To detect apoptotic markers (Caspase-3 and PARP), the culture media were centrifuged at 1200 rpm for 5 mins to collect the apoptotic cells. The cells that remained attached to the dish were washed using PBS three times before adding lysis buffer. Both apoptotic and surviving cells were lysed using cell lysis buffer (50mM Tris HCl, pH 7.4, with 250mM NaCl, 1 mM EDTA, 50mM NaF, and 0.5% Triton X-100, together with protease inhibitors), and mixed together. For other experiments, the culture media were aspirated and the cells in the dishes were washed with PBS for three times before adding lysis buffer. Cells were lysed for 30 mins on ice before centrifuging at 12000 rpm for 15 mins at 4°C. The supernatants of the samples were collected. The protein concentration of each sample was

determined using Bradford Kit (Sangon Biotech, Shanghai, China). For immunoblotting, proteins were resolved by SDS-PAGE and transferred onto polyvinylidene difluoride (PVDF) membranes (Millipore). Immunoblots were developed in Western Lightning Chemiluminescence Reagent Plus (Advansta, Menlo Park, CA, USA).

Cell viability assay

Approximately 5000 cells per well were seeded in 96-well plates 24 hrs prior to the addition of drugs and treated with the indicated drug concentrations for 72 hrs. Cell viability was measured using the CellTiter-Glo Luminescent Viability Assay kit (Promega) according to the manufacturer's instructions. Dose response curves were generated, and IC50s were calculated using Graphpad Prism. All samples were analyzed in triplicate, and standard deviations were from 2-3 independent experiments.

Colony formation assay

For colony formation assays, approximately 20000 cells per well were seeded into 12-well plates and treated with various concentrations of drugs for 72 hrs. Cells were then fixed in 4% formaldehyde for 30 mins and stained with 0.1% (w/v) crystal violet solution.

CRISPR-Cas9 gene targeting

One pair of guides against the exon-30 sequence of Human Notch2 was designed using

the guide design tool found at: (<https://zlab.bio/guide-design-resources/>) with the input of Human Notch2 exon-30 sequence. Guide sequences were cloned into the pSpCas9 (BB)-2A-Puro vector that was previously described ⁴³. Lipo3000 was used to transfect the cells, and puromycin was used to select the transfected cells. Cells were selected for five days after transfection, then plated for single colonies. Multiple candidate clones were picked and tested for gene disruption by western blot. The ones in which Notch2 protein expression was downregulated were select for genomic extraction and PCR. The PCR fragment was send to SANGON for sequencing to confirm that Notch2 exon-30 was disrupted. The CRISPR-guide sequences and vector information were listed in the Key Resources Table.

Lentivirus production and infection

For packaging lentiviral particles in HEK293T cells, 5×10^5 cells were seed in a 60 mm dish in complete DMEM culture medium one day before transfection. On the day of transfection, 18 μ l PEI was pipet directly into 182 μ l serum and antibiotics-free DMEM medium without contacting with the walls of the Eppendorf tube, then vortexed for one second to mix the transfection medium and incubated for 5 mins at room temperature. 3 μ g of lentiviral construct as well as 1.5 μ g of psPAX2 and pMD2.G were added to the transfection medium above, then vortexed for one second to mix the contents and incubated for at least 15 mins to allow the complex to form. The medium from HEK293T cells was removed and 3 ml fresh complete medium was added. Then the transfection

medium was added to the cells in a drop-wise manner. The dishes were swirled to ensure transfection mixture distribution over the entire plate surface. The virus was harvested 48 hrs after transfection and filtered through non-pyrogenic filters with a pore size of 0.45 mm (Merck Millipore, Billerica, MA, USA).

For infection, cells were seeded at $1-3 \times 10^5$ cells per well in a 35 mm dish. 24 hrs later, diluted lentivirus and 8 $\mu\text{g/ml}$ polybrene (Sigma-Aldrich, St.Louis, MO, USA) were added to the cells. 24 hrs after infection (48 hrs for THP1), the medium with viral particles was removed. The cells were washed with PBS twice and complete culture medium was added to the targeting cells. For stable cell selection, puromycin (1 $\mu\text{g/ml}$) was added 48 hrs post-infection.

RNA Immunoprecipitation (RIP)

RIP was carried out as previously described⁴⁴. Briefly, HeLa cells in prolonged mitosis were crosslinked in a UV cross-linker (UVP) at 120 mJ cm^{-2} strength for 2 mins. And then cells were harvested in ice-cold RIPA buffer (50 mM Tris-HCl, pH 8.0), 150 mM NaCl, 5 mM EDTA, 1% NP-40 and 1% SDS) and sonicated for 5 mins with a Sonics Vibra-Cell with a 3-second on and 6-second off procedure. The cell suspension was centrifuged at $13,000 \times g$ for 20 mins at 4°C , and the supernatant was collected. Antibody or IgG (as control) was added and incubated for 4 hrs at 4°C with Protein G Dynabeads (Life Technologies) for antigen coupling. Then suspension of sample was added and allowed to bind for at least 4 hrs at 4°C . The protein antibody-bead complexes were

washed two times with RIPA buffer and two times with RIPA buffer supplemented with 500 mmol/L NaCl. One-fifth of the beads after the last wash was heated 100°C for 10 mins in SDS-loading buffer and then saved for western blotting. The remaining protein antibody-bead complexes were digested with proteinase K at 55°C for 30 mins, followed by extraction with Trizol Reagent (Takara, Dalian, China) to obtain RNA.

RNA-Seq and KEGG pathway enrichment analysis

RNA was extracted from OVCAR8 cells using Trizol Reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol. Subsequently, total RNA was quantified using a Nano Drop and Agilent 2100 bioanalyzer (Thermo Fisher Scientific, MA, USA). The Beijing Genomics Institute (BGI, Shenzhen, China) performed the RNA integrity checking, library preparation and sequencing. Oligo (dT)-attached magnetic beads were used to purify mRNA. The purified mRNA was fragmented into small pieces with fragment buffer at appropriate temperature. Then cDNA was generated according to the manufacturer's protocol by BGI. The cDNA product was validated on the Agilent Technologies 2100 bioanalyzer for quality control. The final library was amplified with phi29 to make DNA nanoball (DNB) which had more than 300 copies of one molecule. DNBs were loaded into the patterned nanoarray and single end 50 bases reads were generated on BGISEQ500 platform (BGI-Shenzhen, China).

Sequence reads were aligned to the human reference genome with GRCh38.p11 (hg38).

Differential Expression Genes (DEGs) were analyzed using the DESeq2 R package.

KEEG pathway Enrichment Analysis was performed using KOBAS (<http://kobas.cbi.pku.edu.cn/kobas3/genelist/>). The input gene lists were generated from the overlapping of differentially downregulated genes in two comparisons based on adjusted p value < 0.05 and log2 Fold Change < -0.5 (Notch2-sh1 compared to NTC, Notch2-sh2 compared to NTC), which consists of 522 significantly downregulated genes in total. Cut-off values of FDR < 0.05 were used to select the top enriched pathways. Original data are available in the NCBI Gene Expression Omnibus (GEO) under the accession number GSE158569.

Gene expression analysis by qRT-PCR

qRT-PCR was conducted as previously described⁴⁵. Briefly, total RNA was extracted from cells using Trizol Reagent (Invitrogen, Carlsbad, CA, USA) following the manufacturer's protocol. Extracted RNA samples were quantified using Nanodrop (Life Technologies, Carlsbad, CA, USA). cDNA was prepared from 1 µg RNA using Oligo (dT) and Superscript III reverse transcriptase according to the manufacturer's protocol (Vazyme, China). Quantitative PCR was performed using SYBR® Premix (Vazyme, China). Data were collected using a PIKOREAL 96 real-time PCR system (Thermo Scientific, MA, USA). All reactions were performed in triplicate.

The primers used in this study are listed in Key Resources Table. The primers used in this study were designed basing on PrimerBank or OriGene (OriGene Technologies, Inc.) and were blasted to confirm the specificity using NCBI primer blast. The primers were

synthesized by SANGON (Shanghai, China).

Soluble Jag1 ligand immobilization and cell stimulation

The recombinant rat Jag1-Fc fusion chimera, goat anti-Human IgG FC antibody and recombinant human IgG1-FC (R&D Systems, Minneapolis, MN) were dissolved in PBS at a final concentration of 10 µg/mL. Soluble Jag1 ligand and the control for Jag1-human IgG-Fc immobilization was performed according to previous study^{46, 47}. Briefly 2 µg or 0.5 µg goat anti-Human IgG FC antibody were added to 12-well plate or 8 well-Sliders per well and incubate for 2 hrs at room temperature. The solution was then aspirated and washed with PBS three times. Then 1 µg or 0.25 µg recombinant rat Jag1-Fc fusion chimera and recombinant human IgG1-FC were added to the coated 12-well plate or 8 well-Sliders respectively, and incubated at 4°C for 20 hrs. The solution was aspirated and washed with PBS before plating about 1×10^5 cells or 1×10^4 cells per well.

ELISA

Cells were seeded in a 60 mm dish with 3 ml complete culture medium 24 hrs prior to treatment. To determine IL-1 β and CSF1 levels in the cell culture medium, following the indicated treatments for 72 hrs, conditioned media were centrifuged at 1000 x g for 10 mins and supernatants were collected to determine the cytokine levels. Cytokine levels were measured using ELISA kits (ABclonal) according to the manufacturer's instructions. Cell numbers were counted to normalize the total level of the cytokines.

Monocyte differentiation and co-culture assays

M2-polarized THP-1 macrophages were generated as described previously⁴⁸. Briefly, 2×10^6 THP-1 cells were seeded into a 35 mm dish and treated with 320 nM PMA for 6 hrs, then cultured with PMA plus 20 ng/ml IL-4 and 20 ng/ml IL-13 for another 18 hrs. To generate M1-polarized THP-1 macrophages, THP-1 cells were treated with 320 nM PMA for 6 hrs and then cultured with PMA plus 100 ng/ml LPS for 18 hrs.

For co-culture assay, OVCAR8-GFP stable cells were seeded with M2 macrophages (ratio 1:1) into 6-well plates 24 hrs prior to adding taxol. The density of the OVCAR8-GFP cells was captured using Olympus microscope equipment with a GFP channel every day for 3 days. Moreover, the confluence of OVCAR8-GFP cells was measured using image J (Analyze>Analyze particles).

In vivo mouse tumor models

All animals were maintained in the house under specific pathogen-free conditions with unrestricted access to food and water for the duration of the experiments. All mouse experiments were approved by The Ethics Committee of the University of Science and Technology of China (2019-N (A) -059). Balb/c mice (athymic nu/nu, 6-8 weeks old, SLAC Laboratory Animal) were subcutaneously injected with 5×10^5 A549 cells or OVCAR8 cells. For Notch2 knockdown cell injection, A549 cells that stably express Notch2-shRNA were injected. And for NICD2 overexpression cells, A549 cells that

stably express NICD2 were injected. For experiments with Notch inhibitor, A549 and OVCAR8 cells were subcutaneously injected into the mice. The mice were randomly divided into different groups when the size of the tumor reached 100-150 mm³. Paclitaxel (10 mg/kg) and carboplatin (20 mg/kg) were administered by intraperitoneal (i.p.) injection every two days. RO4929097 (3 mg/kg) were administered orally every two days. RO4929097 was dissolved as previously described. Tumor growth was recorded by blind measurement of two perpendicular diameters of the tumors and tumor size was calculated using the formula $\frac{4}{3} \times (\text{width}/2)^2 \times (\text{length}/2)$. Tumors were harvested at the experimental endpoint and tumor proliferation was determined by Ki-67 staining. For all animal studies, animals were randomly chosen.

For the murine ID8 model, 8-10 weeks-old female C57BL/6 mice (SLAC Laboratory Animal) were injected intraperitoneal (i.p) with 5×10^6 ID8-luciferase cells.

Bioluminescence images in vivo were carried out using IVIS-Spectrum system (Caliper Life Sciences, USA). The mice were randomly divided into different groups four weeks after ID8 injection, and were gave paclitaxel and RO4929097 using the same concentrations and methods as OVCAR8 and A549 cells carrying mice. Ascites fluid was collected for flow cytometry analysis one weeks later. Six weeks after tumor inoculation, ascites fluid was collected for Immunofluorescence.

For Patient-Derived Xenograft (PDX) Model, fresh tissues from patients with histologically confirmed serous ovarian cancer were collected at the time of debulking surgery at the first affiliated hospital of University of Science and Technology of China

(USTC). All surgically resected tumors were collected after written patient consent and following the institutional review board-approved protocols of USTC. Patient heterotransplant number was used to protect patient identity. Nonnecrotic adjacent malignant tissue was procured by clinical staff with specialized training in gross dissection. The tumor tissue was minced on ice. Approximately 0.3 to 0.5 cm³ of tumor slurry was mixed with 1:1 Matrigel (Corning) before being injected into at least three female NOD-SCID mice by intraperitoneal. Moribund mice with tumors were sacrificed, and to maintain each tumorgraft model as the low passage, tumor from the initial founder mouse/mice was expanded with a single passage into 10+ mice to generate sufficient tumor volume for banking and future experiments. If the founder tumorgraft volume was insufficient, a preexpansion amplification of tumor volume may be necessary for a small cohort of mice. Cryopreserved tumors were minced and stored 1:1 in freezing media (90% FBS and 10% dimethyl sulfoxide) overnight at -80°C and then in liquid nitrogen indefinitely.

Flow cytometry analysis (FACS)

Ascites was collected and incubated in Red Blood Cell Lysis Buffer (Beyotime Biotechnology) for 10-15 mins at 4°C to lyse red blood cells. Cells were washed and counted using automated cell counter (Countstar). 1×10^6 cells were blocked with FcR Blocking Reagent (BioLegend) and stained with cell-surface markers including CD45, CD11b, F4/80, and CD206 (BioLegend). Flow cytometry acquisition was performed on

CytoFLEX (BD Biosciences), and data were analyzed using CytExpert software.

Chemotaxis assay

For cell migration, THP-1 cells were seeded (10^5 cells/ 100 μ l DMEM containing 0.1% BSA) onto the top chamber of transwell filters (3- μ m; Corning). Filters were placed in a 24-well plate that contains conditioned medium isolated from the vehicle- or taxol-treated (5 nM) OVCAR8 cell lines. 24 hrs following incubation, cells in the lower chamber were isolated and counted. Samples were run in triplicates for each group.

Immunofluorescence

Immunofluorescence was conducted as previously described⁴⁹. For cell lines, cells were plated onto 22×22 mm coverslips 24 hrs prior to treatment. Cells were washed with warmed PHEM (60mM PIPES (pH 6.8), 25mM HEPES, 10 mM EGTA and 2 mM $MgCl_2$) and fixed in 4% formaldehyde for 15 mins at room temperature. For tumor tissues, the tissues were fixed in 4% formaldehyde for 14 hrs at 4°C prior to dehydrating by 30% sucrose for 24 hrs at 4°C. Then the tissues were embedded into OTC gel and stored at -80°C before dissection for slides. For human or mouse ascites, fresh ascites were centrifuged at 2000 rpm for 5 mins at 4°C to collect cells and spread on Poly-L-Lysine coated slides. About 10 mins later, when the ascites on slides were dried, cells were fixed in 4% formaldehyde for 15 mins at room temperature. Then cells or tissues were extracted by 0.2% Triton X-100 in PHEM, blocked with 1% bovine serum albumin

in TBST for 30 mins, incubated with primary antibody for 2 hrs at room temperature, washed with TBST three times and incubated with secondary antibodies for an additional 1 hr at room temperature. DNA was stained with 4, 6-diamidino-2-phenylindole (DAPI) for about 3 mins. Coverslips were mounted using ProLong antifade (Sigma). Images were acquired on a DeltaVision microscope (GE Healthcare, Buckinghamshire, UK) with a 60× or 20× objective lens, with optical sections acquired 0.3-0.5 μm apart in the z-axis, and were shown as maximal intensity projections.

Histology and Immunohistochemistry (IHC)

Tissue specimens from mice were fixed in 10% buffered formalin for 24 hrs and stored in 70% ethanol until paraffin embedding. 5 mm sections were stained with hematoxylin and eosin (HE) or used for immunohistochemical studies.

Human OV tissue samples were obtained from The First Affiliated Hospital of USTC. All samples were obtained with informed consent, and the study was approved by the Ethics Committee of USTC (2019-N (H)-212), as well as the principles expressed in the Declaration of Helsinki. Patients' characteristics are shown in Table 1.

Immunohistochemistry was performed on formalin-fixed, paraffin-embedded mouse and human tissue sections using a biotin-avidin method as described before⁴⁵. Sections were developed with DAB and counterstained with hematoxylin. Images were taken using a Nikon Ti microscope equipped with a 20× lens. Quantification of Notch2 IHC chromogen intensity was performed as previously described⁵⁰. Briefly, Staining intensity

(0, 1, 2, 3) and percentage of positive cells among cancer (0-25% recorded as 1, 25-50% as 2, 50-75% as 3 and >75% as 4) were evaluated by a pathologist in USTC. The final immunoreactive scores were calculated by multiplying the two numbers as described before⁵⁰.

TCGA and GEO data analysis

Clinical data and RNA expression data with normalized RSEM values of the non-small cell lung cancer datasets (TCGA-LUAD and TCGA-LUSC) and the serous ovarian cancer dataset (TCGA-OV) were retrieved from FireBrowse (<http://firebrowse.org>). 288 lung cancer patients and 343 ovarian cancer patients had associated comprehensive follow-up records, RNA expression data and records of clinical treatment.

Notch receptors and its ligands expression levels were compared using RSEM values. For the survival curves, TCGA-OV and TCGA-LUAD patients were divided into two groups: patients that were treated with taxane-based therapy, including paclitaxel and docetaxel, and those that did not receive taxane-based treatment, but received other types of therapies. The curated clinical information was recorded as previous work⁵¹. Kaplan-Meier survival curves were drawn with the survminer R package (<https://CRAN.R-project.org/package=survminer>). Patients were stratified along with the best-cutoff value.

GSE40484⁵² and GSE126080⁵³ were downloaded from NCBI-GEO datasets (<https://www.ncbi.nlm.nih.gov/geo/>). Expression profiling of macrophages sorted from ovarian cancer ascites of mice and human were analyzed by microarray. Expression

levels of Notch receptors and ligands were extracted and analyzed using R package and Graphpad.

Tumor-infiltrating immune cell analysis

TIMER2.0 (<http://timer.cistrome.org/>), an online tool, was used to analyze tumor-infiltrating macrophage cells in TCGA-OV and TCGA-LUAD patients⁵⁴. The results obtained from the traditional method-CIBESORT-ABS were extracted.

Statistical analysis

All the data analyses were performed with GraphPad Prism statistical software. p value was determined by two-way ANOVA for tumor growth, or Log-rank test for survival, or two-tailed t-tests for other analyses. A value of $p < 0.05$ was considered statistically significant.

Data and code availability

Ribosome profiling and RNA sequencing data generated during this study are available at the NCBI-GEO database with the project accession ID GEO: GSE158569. Previously published expression datasets used in this study are available through GEO under GEO: GSE40484 and GSE126080. The Cancer Genome Atlas (TCGA) data were downloaded from Firebrowse (<http://firebrowse.org/>).

Acknowledgments

We thank all members in the Yang lab for discussion. This work is supported by: the "Strategic Priority Research Program" of the Chinese Academy of Sciences, Grant No. XDB19000000 (ZY); National Science Foundation of China 31701178, 31970670 (JG); National Science Foundation of China 31771498, 92057104 (ZY); National Science Foundation of China 32000492 (FY); National Science Foundation of China 32000528 (AC); The Collaborative Innovation Program of Hefei Science Center, CAS 2019HSC-CIP011 (ZY); The Hefei National Laboratory for Physical Sciences at the Microscale (KF2020009) (ZY); The Open Project of CAS Key Laboratory of Innate Immunity and Chronic Disease (ZY); The Fundamental Research Funds for the Central Universities (ZY, KZ, YP, AC and FY).

Conflict of Interest

The authors declare that they have no conflict of interest.

Author contributions

FY, JS, JG, and ZY conceived and designed the study. FY, QZ and YX conducted the ribosome profiling assay. YJ performed time lapse microscopy. QW, WY and FY conducted data analysis of sequencing data. AC, TX and JS performed enzymatic activity measurement. FY, HL and CS performed PDX experiments. FY, PZ and AC conducted plasmids construction and western analysis. WY and YW did the experiment. YZ, XM,

WH and KZ did the clinical data analysis. AC did the FRET and biosensor assay. FY and QZ performed statistical analysis. FY, JS, JG and ZY performed overall data interpretation and wrote the paper.

References

1. Dagogo-Jack, I. & Shaw, A.T. Tumour heterogeneity and resistance to cancer therapies. *Nat Rev Clin Oncol* **15**, 81-94 (2018).
2. Vasan, N., Baselga, J. & Hyman, D.M. A view on drug resistance in cancer. *Nature* **575**, 299-309 (2019).
3. Leary, M., Heerboth, S., Lapinska, K. & Sarkar, S. Sensitization of Drug Resistant Cancer Cells: A Matter of Combination Therapy. *Cancers (Basel)* **10** (2018).
4. Ruffell, B. & Coussens, L.M. Macrophages and therapeutic resistance in cancer. *Cancer Cell* **27**, 462-472 (2015).
5. Mantovani, A. & Allavena, P. The interaction of anticancer therapies with tumor-associated macrophages. *J Exp Med* **212**, 435-445 (2015).
6. Larionova, I. *et al.* Interaction of tumor-associated macrophages and cancer chemotherapy. *Oncoimmunology* **8**, 1596004 (2019).
7. Paulus, P., Stanley, E.R., Schafer, R., Abraham, D. & Aharinejad, S. Colony-stimulating factor-1 antibody reverses chemoresistance in human MCF-7 breast cancer xenografts. *Cancer Res* **66**, 4349-4356 (2006).
8. DeNardo, D.G. *et al.* Leukocyte complexity predicts breast cancer survival and functionally regulates response to chemotherapy. *Cancer Discov* **1**, 54-67 (2011).
9. Mitchem, J.B. *et al.* Targeting tumor-infiltrating macrophages decreases tumor-initiating cells, relieves immunosuppression, and improves chemotherapeutic responses. *Cancer Res* **73**, 1128-1141 (2013).
10. Linde, N. *et al.* Vascular endothelial growth factor-induced skin carcinogenesis depends on recruitment and alternative activation of macrophages. *J Pathol* **227**, 17-28 (2012).
11. Beatty, G.L. *et al.* A phase I study of an agonist CD40 monoclonal antibody (CP-870,893) in combination with gemcitabine in patients with advanced pancreatic ductal adenocarcinoma. *Clin Cancer Res* **19**, 6286-6295 (2013).
12. Affara, N.I. *et al.* B cells regulate macrophage phenotype and response to chemotherapy in squamous carcinomas. *Cancer Cell* **25**, 809-821 (2014).
13. Hughes, R. *et al.* Perivascular M2 Macrophages Stimulate Tumor Relapse after Chemotherapy. *Cancer Res* **75**, 3479-3491 (2015).
14. Shree, T. *et al.* Macrophages and cathepsin proteases blunt chemotherapeutic response in breast cancer. *Genes Dev* **25**, 2465-2479 (2011).
15. Nogales, E., Wolf, S.G., Khan, I.A., Luduena, R.F. & Downing, K.H. Structure of tubulin at 6.5 Å and location of the taxol-binding site. *Nature* **375**, 424-427 (1995).
16. Jordan, M.A. & Wilson, L. Microtubules as a target for anticancer drugs. *Nat Rev Cancer* **4**, 253-265 (2004).
17. Rieder, C.L. & Maiato, H. Stuck in division or passing through: what happens when cells cannot satisfy the spindle assembly checkpoint. *Dev Cell* **7**, 637-651 (2004).
18. Gascoigne, K.E. & Taylor, S.S. How do anti-mitotic drugs kill cancer cells? *J Cell Sci* **122**, 2579-2585 (2009).

19. Weaver, B.A. How Taxol/paclitaxel kills cancer cells. *Mol Biol Cell* **25**, 2677-2681 (2014).
20. Janssen, A. & Medema, R.H. Mitosis as an anti-cancer target. *Oncogene* **30**, 2799-2809 (2011).
21. Shi, J., Orth, J.D. & Mitchison, T. Cell type variation in responses to antimitotic drugs that target microtubules and kinesin-5. *Cancer Res* **68**, 3269-3276 (2008).
22. Gascoigne, K.E. & Taylor, S.S. Cancer cells display profound intra- and interline variation following prolonged exposure to antimitotic drugs. *Cancer Cell* **14**, 111-122 (2008).
23. Yang, Z., Kenny, A.E., Brito, D.A. & Rieder, C.L. Cells satisfy the mitotic checkpoint in Taxol, and do so faster in concentrations that stabilize syntelic attachments. *J Cell Biol* **186**, 675-684 (2009).
24. Brito, D.A. & Rieder, C.L. The ability to survive mitosis in the presence of microtubule poisons differs significantly between human nontransformed (RPE-1) and cancer (U2OS, HeLa) cells. *Cell Motil Cytoskeleton* **66**, 437-447 (2009).
25. Orth, J.D. *et al.* Analysis of mitosis and antimitotic drug responses in tumors by in vivo microscopy and single-cell pharmacodynamics. *Cancer Res* **71**, 4608-4616 (2011).
26. Shi, J. & Mitchison, T.J. Cell death response to anti-mitotic drug treatment in cell culture, mouse tumor model and the clinic. *Endocr Relat Cancer* **24**, T83-T96 (2017).
27. Shi, J., Zhou, Y., Huang, H.C. & Mitchison, T.J. Navitoclax (ABT-263) accelerates apoptosis during drug-induced mitotic arrest by antagonizing Bcl-xL. *Cancer Res* **71**, 4518-4526 (2011).
28. Tan, N. *et al.* Navitoclax enhances the efficacy of taxanes in non-small cell lung cancer models. *Clin Cancer Res* **17**, 1394-1404 (2011).
29. Park, J.E., Yi, H., Kim, Y., Chang, H. & Kim, V.N. Regulation of Poly(A) Tail and Translation during the Somatic Cell Cycle. *Mol Cell* **62**, 462-471 (2016).
30. Tanenbaum, M.E., Stern-Ginossar, N., Weissman, J.S. & Vale, R.D. Regulation of mRNA translation during mitosis. *Elife* **4** (2015).
31. Ozlu, N. *et al.* Quantitative comparison of a human cancer cell surface proteome between interphase and mitosis. *EMBO J* **34**, 251-265 (2015).
32. Mollen, E.W.J. *et al.* Moving Breast Cancer Therapy up a Notch. *Front Oncol* **8**, 518 (2018).
33. Meurette, O. & Mehlen, P. Notch Signaling in the Tumor Microenvironment. *Cancer Cell* **34**, 536-548 (2018).
34. Domenech, E. *et al.* AMPK and PFKFB3 mediate glycolysis and survival in response to mitophagy during mitotic arrest. *Nat Cell Biol* **17**, 1304-1316 (2015).
35. Novoa, I., Gallego, J., Ferreira, P.G. & Mendez, R. Mitotic cell-cycle progression is regulated by CPEB1 and CPEB4-dependent translational control. *Nat Cell Biol* **12**, 447-456 (2010).
36. Voloshin, T. *et al.* Blocking IL1beta Pathway Following Paclitaxel Chemotherapy Slightly Inhibits Primary Tumor Growth but Promotes Spontaneous Metastasis. *Mol Cancer Ther* **14**, 1385-1394 (2015).
37. Diaz-Padilla, I. *et al.* A phase II study of single-agent RO4929097, a gamma-secretase inhibitor of Notch signaling, in patients with recurrent platinum-resistant epithelial ovarian cancer: A study of the Princess Margaret, Chicago and California phase II consortia. *Gynecol Oncol* **137**, 216-222 (2015).
38. Strosberg, J.R. *et al.* A phase II study of RO4929097 in metastatic colorectal cancer. *Eur J Cancer* **48**, 997-1003 (2012).

39. Sardesai, S. *et al.* A phase I study of an oral selective gamma secretase (GS) inhibitor RO4929097 in combination with neoadjuvant paclitaxel and carboplatin in triple negative breast cancer. *Invest New Drugs* **38**, 1400-1410 (2020).
40. Cui, D. *et al.* Notch Pathway Inhibition Using PF-03084014, a gamma-Secretase Inhibitor (GSI), Enhances the Antitumor Effect of Docetaxel in Prostate Cancer. *Clin Cancer Res* **21**, 4619-4629 (2015).
41. Takebe, N., Nguyen, D. & Yang, S.X. Targeting notch signaling pathway in cancer: clinical development advances and challenges. *Pharmacol Ther* **141**, 140-149 (2014).
42. Ingolia, N.T., Lareau, L.F. & Weissman, J.S.J.C. Ribosome profiling of mouse embryonic stem cells reveals the complexity and dynamics of mammalian proteomes. **147**, 789-802 (2011).
43. Ran, F.A. *et al.* Genome engineering using the CRISPR-Cas9 system. *Nat Protoc* **8**, 2281-2308 (2013).
44. Li, Z. *et al.* Exon-intron circular RNAs regulate transcription in the nucleus. *Nat Struct Mol Biol* **22**, 256-264 (2015).
45. Yin, T. *et al.* Aurora A Inhibition Eliminates Myeloid Cell-Mediated Immunosuppression and Enhances the Efficacy of Anti-PD-L1 Therapy in Breast Cancer. *Cancer Res* **79**, 3431-3444 (2019).
46. Rosati, E. *et al.* Constitutively activated Notch signaling is involved in survival and apoptosis resistance of B-CLL cells. *Blood* **113**, 856-865 (2009).
47. Nandagopal, N. *et al.* Dynamic Ligand Discrimination in the Notch Signaling Pathway. *Cell* **172**, 869-880 e819 (2018).
48. Shen, Q. *et al.* Notch Shapes the Innate Immunophenotype in Breast Cancer. *Cancer Discov* **7**, 1320-1335 (2017).
49. Yu, F. *et al.* Aurora-A promotes the establishment of spindle assembly checkpoint by priming the Haspin-Aurora-B feedback loop in late G2 phase. *Cell Discov* **3**, 16049 (2017).
50. Li, Z. *et al.* Loss of the FAT1 Tumor Suppressor Promotes Resistance to CDK4/6 Inhibitors via the Hippo Pathway. *Cancer Cell* **34**, 893-905 e898 (2018).
51. Zierhut, C. *et al.* The Cytoplasmic DNA Sensor cGAS Promotes Mitotic Cell Death. *Cell* **178**, 302-315 e323 (2019).
52. Segura, E. *et al.* Human inflammatory dendritic cells induce Th17 cell differentiation. **38**, 336-348 (2013).
53. Goossens, P. *et al.* Membrane cholesterol efflux drives tumor-associated macrophage reprogramming and tumor progression. **29**, 1376-1389. e1374 (2019).
54. Li, T. *et al.* TIMER2.0 for analysis of tumor-infiltrating immune cells. *Nucleic Acids Res* **48**, W509-W514 (2020).

Figure legends

Figure 1. Notch2 receptor is upregulated by taxol treatment and associated with chemoresistance.

a, The diagram showed the ribosome profiling process (left) and the Volcano-plot of mRNA expressions of prolonged mitotic (pM) versus mitotic (M) cell (right). Significantly upregulated genes ($\log_2(\text{Foldchange}) > 1.8$ and $p\text{Value} < 0.05$) (purple circle) and downregulated genes ($\log_2(\text{Foldchange}) < -1.8$ and $p\text{Value} < 0.05$) (dark gray circle) were presented. Genes defined as non-significantly differentially expressed are denoted in black.

b, Venn diagram showing the differentially expressed genes (DEGs) identified by ribosome profiling conducted in this study and Park, 2016²⁹, and the genes expressed higher on membrane of mitotic cells than interphase cells from results by Özlü, 2015³¹.

c, Western blot analysis of Notch2 and cyclin B1 (CCNB1) in HeLa cells in mitosis (M) and prolonged mitosis (pM) induced by taxol (left). Cells in prolonged mitosis were further treated with DMSO or 10 μM Cycloheximide (CHX) for 4 hours. Quantification results are shown on the right and error bars are SEM.

d, Western blot analysis of Notch2 in OVCAR8 and A549 cells in mitosis (M) and prolonged mitosis (pM) (bottom). Quantification results are shown at the top.

e, The criteria for the collection of patient samples.

f, g, Representative Notch2 staining in matching images of tumor specimens from ovarian cancer patients obtained pre (primary)- and post (recurrent)-taxane therapy (f). The IHC score of Notch2 was quantified (g). Scale bars are 200 μm .

h, Kaplan-Meier survival analysis of ovarian cancer patients. Patients were divided according to the IHC score of Notch2 (cutoff: median) in tumor tissues of pre (primary)- and post (recurrent)-taxane therapy, respectively.

i, Survival curves of ovarian cancer patients from the TCGA database stratified according to treatment (taxanes versus others) and expression levels of Notch2 (cutoff: auto-select best cutoff).

Data were averaged from at least three replicates for each sample and/or treatment condition. p values were determined by two-tailed student's t test (ns, not significant; **p < 0.01, ***p < 0.001).

Figure 2. Notch2 upregulation confers resistance to post-mitotic taxol-induced cell death.

a. Cell fate profiles of A549 cells with Notch2 knock-down (sh) and non-target control (NTC) stable cells. A549 cells were imaged by time-lapse microscopy. T = 0 indicates the start of the imaging experiments. Each horizontal bar represents a single cell, with the colors indicating distinct cell behaviors and fates. At least 80 cells were analyzed per condition (left). And western blot analysis of the knock-down efficiency of Notch2-shRNA were shown on the right.

b. Quantification of the percentage of cell death in the post-mitotic G1 phase based on three independent experiments of (a).

c, d. Immunoblots of apoptosis-related factors in A549 cells after Notch2 knock-down.

A549 cells were synchronized as schematic diagram depicted (**c**). Representative results (**d**, left), and quantification of the apoptosis-related factors were shown (**d**, right).

e. Taxol and carboplatin sensitivity of A549 cells with Notch2 knock-down. Cells were transduced with Notch2 shRNA-containing plasmids followed by taxol, carboplatin or vehicle treatment.

f. Representative dissected tumors (left) and tumor weight (right) of A549-xenograft mice. A549 cells with Notch2 knock-down (sh) or Non-target control (NTC) were injected and 10 mg/kg paclitaxel was administered by intraperitoneal injection when tumor volume reached about 100 mm³.

g. Western blot analysis of NICD2, Hes1, active Caspase-3 and loading control in A549 cells with overexpression of the activated intracellular domain of Notch2 (NICD2).

h. Representative dissected tumors (left) and tumor weight (right) of xenograft mice with control A549 cells or A549 cells over-expressing the activated intracellular domain of Notch2 (NICD2). 10 mg/kg paclitaxel was administered by intraperitoneal injection when tumor volume reached about 100mm³.

Data are shown as mean $\pm$ SD, obtained from at least three experimental replicates. p values were determined by two-tailed Student's t test (ns, not significant; *p < 0.05, **p < 0.01, ***p < 0.001).

Figure 3. Notch2 signaling is activated by Jag1 expressed on the neighboring macrophages.

a. Analysis of mRNA levels of Notch receptor ligands in macrophages from ovarian patients' ascites. The database GSE40484 downloaded from GEO was used.

b. Analysis of mRNA levels of Notch receptor ligands in macrophages from ID8 cells of mouse ascites. The database GSE126080 downloaded from GEO.

c. Immunofluorescence of Jag1 expression on macrophage in ovarian cancer patients' ascites. Representative images (left) and quantification of relative intensity of Jag1 are shown (right). Three patients' ascites were analyzed.

d. Immunofluorescence of Jag1 expression on macrophages from ascites of ID8 tumor bearing mice. Representative images were shown (left). The scale bars are 10 μ m. Quantification of relative intensity of JAG1 are shown (right).

e. Cell fate profiles of A549 cells with Notch2 KO in comparison with WT upon interacting with Fc or Jag-1 Fc. A549 cells were treated with Fc or Jag-1 Fc for 15 hrs before taxol treatment and then imaged by time-lapse microscopy under taxol for 72 hrs. T = 0 indicates when imaging started. Each horizontal bar represents a single cell, with the colors indicating distinct cell behaviors and fates. At least 80 cells were analyzed per condition. The percentage of cell death in the post-mitotic G1 phase was qualified based on three independent experiments (right).

f. Co-culture assay of OVCAR8 cells with THP-1-derived M2-macrophage (M2 ϕ). The cartoon shows the experimental set-up (left). Quantification of the area covered by GFP+

cell is shown (right).

g. Co-culture assay of OVCAR8 cells with THP-1-derived M2 macrophage (M2 ϕ). THP-1 cells were transduced with two different shRNA clones of Jag1 or vector control, then induced to M2 macrophage M2 (M2 ϕ) and co-cultured with OVCAR8-GFP cells in the presence of DMSO or paclitaxel. Quantification of the area covered by GFP+ cell is shown (Right).

Data are shown as mean $\pm$ SD, obtained from three experimental replicates. p values were determined by two-tailed Student's t test (ns, not significant; *p < 0.05, **p < 0.01, ***p < 0.001).

Figure 4. p38 is required for Notch2-mediated post-mitotic cell survival upon taxol treatment.

a. Principal-component analysis (PCA) of gene expression for parental and Notch2 knock-down OVCAR8 cells. Two different Notch2 shRNA clones were used.

b. Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis of DEGs in RNA-Seq data (Notch2 knock-down vs Non-target control (NTC)).

c. Heatmap showing log₂ (Fold-change) value of DEGs in PI3K-Akt and MAPK signaling pathway in KEGG enrichment.

d. RT-PCR detection of p38 α mRNA levels in OVCAR8 cells after Notch2 knock-down. Cells treated with DMSO and 10 nM taxol for 48 hrs.

e. Cell viability of OVCAR8 cells with Notch2 knock-down and transfected with p38 α or

vector in the presence of 10 nM taxol.

f. Cell viability of OVCAR8 cells in the presence of DMSO, 10 nM taxol, 20 μ M SB203580 or taxol plus SB203580.

g. RT-PCR detection of *MCL1* and *BCL1* mRNA levels in OVCAR8 cells after Notch2 knock-down. Cells were treated with DMSO or 10 nM taxol for 48 hrs.

h. Western blot analysis of p38 α , phospho-p38 and apoptosis-related factors in OVCAR8 cells with Notch2 knock-down alone (left) and OVCAR8 cells with Notch2 knock-down plus p38 α or vector. OVCAR8 cells were treated with DMSO or 10 nM taxol for 48 hrs. The quantified western blot results are shown on the right.

Data are shown as mean $\pm$ SD, obtained from three experimental replicates. p values were determined by two-tailed Student's t test (ns, not significant; *p < 0.05, **p < 0.01, ***p < 0.001).

Figure 5. CPEB-mediated poly-A elongation promotes Notch2 translation in prolonged mitosis.

a. Western blot analysis of Notch receptors in HeLa cells in mitosis (M) and prolonged mitosis (pM) cells.

b. Characteristics of the Notch receptors and cyclin B1 transcripts' 3'-UTR. The length and number of cytoplasmic polyadenylation elements (CPE) and polyadenylation hexanucleotide (PH) are presented.

c. Schematic representation of the number and position of CPE elements, polyadenylation

hexanucleotide (PH) and polyadenylation signals in the 3'-UTR of the Human Notch2 transcript.

d. RT-PCR detection of mRNA levels of the indicated genes after immunoprecipitation of CPEB1 in HeLa cells in prolonged mitosis (left). The CPEB1 IP efficiency were confirmed using western blot (right).

e. Western blot analysis of Notch2 and cyclin B1 (CCNB1) in HeLa cells in prolonged mitosis (pM) (left). Cells in prolonged mitosis were treated with DMSO or 10 μ M Cordycepin (Cord) for 4 hrs before collection. Quantified results are shown (Right).

f. Cell fate profiles of A549 cells treated by DMSO, 10 nM taxol, 10 μ M Cordycepin (Cord) or taxol plus cordycepin. Cells were imaged by time-lapse microscopy under continuous drug treatment for 72 hrs. T = 0 indicates the start of live cell imaging. Each horizontal bar represents a single cell, with the colors indicating distinct cell behaviors and fates. At least 80 cells were analyzed per condition.

Data are shown as mean $\pm$ SD, obtained from three experimental replicates. p values were determined by two-tailed Student's t test (ns, not significant; *p < 0.05, **p < 0.01).

Figure 6. Notch2 upregulation stimulates the recruitment of pro-tumor macrophages.

a. RT-PCR detection of CSF1 and IL1B mRNA levels in OVCAR8 cells after Notch2 knockdown. Cells were treated with DMSO or 10 nM taxol for 48 hrs.

b. ELISA-based quantification of the secreted CSF1 and IL1 β in the medium of OVCAR8 cells after Notch2 knockdown. Cells were treated with DMSO or 10 nM taxol for 48 hrs

before culture supernatant was collection.

c, d. Chemotactic migration of THP-1 cells in response to conditioned medium from OVCAR8 cells treated with either vehicle or 10 nM taxol for 48 hrs were evaluated by Boyden chamber assay. Flow diagram of the experiment are shown in (c). And the number of migrated cells were quantified in (d).

e. Representative images of tumor sections from A549-xenograft mice stained with F4/80 (red) and DAPI (blue) (left). Quantification of the percentage of F4/80⁺ macrophage cells are shown (right). Error bars are SEM. Scale bars are 20 μ m.

f. Pearson correlation analysis of Notch2 versus CSF1 mRNA expression in ovarian cancer using the TCGA database.

Data are shown as mean $\pm$ SD, obtained from three experimental replicates. p values were determined by two-tailed Student's t test (ns, not significant; *p < 0.05, **p < 0.01, ***p < 0.001).

Figure 7. Notch2 inhibitor sensitizes tumor response to paclitaxel in multiple mouse tumor models.

a. Tumor growth of mice carrying OVCAR8 cell xenografts treated with RO4929097 and paclitaxel either alone or in combination. Representative tumors (left) and tumor weight (right) for each group are shown. Paclitaxel and RO4929097 were administered by intraperitoneal injection and orally, respectively, when tumor volume reached ~ 100 mm³.

b. Representative images of tumor sections from OVCAR8-xenografts stained with F4/80

(red) and DAPI (blue) (left). Quantification of the percentage of F4/80⁺ macrophage cell are shown (right). Scale bars are 20 μ m.

c. Representative images of mice carrying ID8-luciferase cells three weeks after treatment (left) and the corresponding tumor growth curves (right). Paclitaxel and RO4929097 were administered respectively by intraperitoneal injection and orally, four weeks after ID8 injection.

d, e. Representative images of flow cytometry results (d) and percentage of macrophage infiltration (e) in ascites from ID8-carrying mice after one week treatment.

f. Schematic diagram depicting ovarian cancer PDX model derivation.

g, h. Effect of RO4929097, paclitaxel and their combination on tumor growth of ovarian cancer PDX mice. Representative tumors (g) and tumor volume (h) for each group are shown. Paclitaxel and RO4929097 were administered by intraperitoneal injection and orally, respectively, when tumor volume reached about 100 mm³.

i. Western blot analysis of Notch signaling effect on p38 and apoptosis-related factors in tumor tissues from PDX mice.

j. Proposed working model for Notch2 translation during prolonged mitosis and the Notch2-Jag1 juxtacrine signaling between tumor and macrophages that confers taxol resistance in the post-mitotic tumor cells.

Data are shown as mean $\pm$ SD, obtained from three experimental replicates. p values were determined by two-tailed Student's t test (ns, not significant; *p < 0.05, **p < 0.01, ***p < 0.001).

Supplementary Information

Seven figures and two tables are supplemented with the main text.

Fig.S1. Notch2 receptor is upregulated by taxol treatment and associated with chemoresistance.

a. Venn diagram showing the overlapping genes protected by ribosome during mitosis in this study, Park 2016, and Tanenbaum 2015.

b. RT-PCR analysis of cyclin B1 (CCNB1) and Notch2 mRNA levels in HeLa cells in mitosis (M) and prolonged mitosis (pM).

c. HeLa cells in mitosis (M) and prolonged mitosis (pM) induced by 0.5 mM Nocodazole were harvested by shake-off and re-plated in culture dish with fresh medium for various hours before being collected for mRNA and protein analysis. RT-PCR and western blotting were conducted to analyze the mRNA expression and protein level of Notch2.

d. Western blot analysis of Notch2 in HeLa cells in mitosis (M) and prolonged mitosis (pM). Cells in prolonged mitosis were induced by 5 nM taxol, 0.5 μ M Nocodazole (Noc), 5 μ M STLC and 100 nM BI2536, respectively.

e. Representative IHC staining images of tumor specimens from ovarian cancer patients. Staining intensity of 0 (negative), 1 (weak), 2 (moderate), and 3 (strong) of Notch2 are shown.

Data are shown as mean $\pm$ SD, obtained from three experimental replicates. p values were determined by two-tailed Student's t test (ns, not significant; *p < 0.05, **p < 0.01).

Fig. S2. Notch2 upregulation confers resistance to post-mitotic taxol-induced cell death.

a. Cell fate profiles of A549 cells imaged by time-lapse microscopy. A549 cells with Notch2 knockdown (sh) or non-target control (NTC) were treated with DMSO. T = 0 indicates the start of live cell imaging. Each horizontal bar represents a single cell, with the colors indicating distinct cell behaviors and fates. At least 80 cells were analyzed per condition.

b. Quantification of the time from mitotic exit to cell death for cells dying in the post-mitotic G1 phase in Figure 2a.

c. Western blot analysis of the knockout efficiency of Notch2.

d. Cell fate profiles of Notch2 knockout or WT A549 cells imaged by time-lapse microscopy. A549 cells were treated with DMSO or 100 nM taxol. T = 0 indicates the start of live cell imaging. Each horizontal bar represents a single cell, with the colors indicating distinct cell behaviors and fates. At least 80 cells were analyzed per condition.

e. Summary of the percentage of cell death in the post-mitotic G1 phase (left) and the time from mitotic exit to cell death (right), based on three independent experiments.

f. Taxol and carboplatin sensitivity of OVCAR8 cells with Notch2 knockdown.

g. Representative images of tumor sections of A549 cells with Notch2 knockdown stained for Ki67 (red) and DAPI (blue) (left) and quantification of the percentage of Ki67⁺ cells (right).

h. Western blot analysis of the apoptosis-related factors in tumor tissues from A549 cells

with Notch2 knockdown or NTC.

Data are shown as mean $\pm$ SD, obtained from at least three experimental replicates. p values were determined by two-tailed Student's t test (ns, not significant; *p < 0.05, **p < 0.01, ***p < 0.001).

Fig. S3. Notch2 signaling is activated by Jag1 expressed on the neighboring macrophages.

a. Correlation of Notch2 expression with the infiltration level of different types of macrophages was estimated by CIBERSORT-ABS in TIMER2.0 in ovarian cancer using the TCGA database.

b. Correlation of Notch2 expression with the infiltration level of different types of macrophages were estimated by CIBERSORT-ABS in TIMER2.0 in lung adenocarcinoma of NSCLC using TCGA database.

c, d. Analysis of mRNA levels of Notch ligands in ovarian cancer (c) and lung adenocarcinoma of NSCLC (d) using TCGA data.

e. Immunofluorescence detection of Notch2 receptor in A549 cells. A549 cells were plated on Fc or Jag-1 Fc for 15 hrs, and Notch2 was analyzed using immunofluorescence (red). DAPI was used to visualize cell nuclei. Scale bar is 10 μ m. Quantification of NICD2 fluorescence intensity in cell nucleus using relative fluorescence units are shown (right).

f. Western blot analysis of Notch signaling activity by Hes1 expression in A549 cells plated on Fc or Jag-1 Fc for 15 hrs or 48 hrs.

g. Cell fate profiles of A549 cells plated on Fc or Jag-1 Fc coated dishes. A549 cells were plated 15 hrs before imaging by time-lapse microscopy for 72 hrs. T = 0 indicates the start of live cell imaging. Each horizontal bar represents a single cell, with the colors indicating distinct cell behaviors and fates. At least 80 cells were analyzed per condition.

h. Representative images of OVCAR8-GFP cells in co-culture with THP-1-derived M2 macrophages (M2 ϕ).

i. Western blot analysis of the knockdown efficiency of Jag1 in THP-1 cells.

Data are shown as mean $\pm$ SD, obtained from three experimental replicates. p values were determined by two-tailed Student's t test (ns, not significant; *p < 0.05, **p < 0.01, ***p < 0.001).

Fig. S4. p38 is required for Notch2-mediated post-mitotic survival after taxol treatment.

a. Heatmap illustrates differentially expressed genes (DEGs) of OVCAR8 cells transfected with Notch2-shRNA or Non-target control (NTC) vector.

b. RT-PCR detection of the DEGs mRNA levels in OVCAR8 cells after Notch2 knockdown.

c. Western blot analysis of Notch2, p38 α , phospho-p38 and Actin (loading control) in HeyA8 and A549 cells after Notch2 knockdown. Cancer cells were treated with 10 nM and 100 nM Taxol, respectively, for 48 hrs.

d. Cell viability of HeyA8 and A549 cells in the presence of DMSO, taxol (20 nM and 100

nM, respectively), 20 μ M SB203580 or taxol plus SB203580.

e. Colony-formation assays of HeyA8, A549 and OVCAR8 cells treated with DMSO, taxol (20 nM, 100 nM and 10 nM, respectively), 20 μ M SB203580 or taxol plus SB203580.

f. Pearson correlation analysis of Notch2 versus p38 α mRNA expression in ovarian cancer and lung adenocarcinoma of NSCLC using TCGA database. p values were determined by Log-rank (Mantel-Cox) test.

Data are shown as mean $\pm$ SD, obtained from three experimental replicates. p values were determined by two-tailed Student's t test (*p < 0.05, **p < 0.01, ***p < 0.001).

Fig. S5. CPEB-mediated poly-A elongation promotes Notch2 translation in prolonged mitosis

a. Analysis of Notch receptors at mRNA level in ovarian cancer and lung adenocarcinoma of NSCLC using TCGA data.

b. Cell fate profiles of OVCAR8 cells treated with DMSO, 10 nM taxol, 10 μ M Cordycepin (Cord) or taxol plus cordycepin. Cells were imaged by time-lapse microscopy under continuous drug treatment for 72 hrs. T = 0 indicates the start of live cell imaging. Each horizontal bar represents a single cell, with the colors indicating distinct cell behaviors and fates. At least 80 cells were analyzed per condition.

Fig. S6. Notch2 upregulation stimulates the recruitment of pro-tumor macrophages.

a. RT-PCR analysis of *CSF1* and *IL1 β* mRNA levels in A549 cells after Notch2

knockdown. Cells were treated with DMSO or 100 nM taxol for 48 hrs.

b. RT-PCR analysis of *CSF1* and *IL1 β* mRNA levels in A549 cells of Notch2-WT or KO transfected with vector or NICD2 expression construct. Cells were treated with 100 nM taxol for 48 hrs.

c. Pearson correlation between Notch receptors and CSF1 and IL1B mRNA expression in ovarian cancer and lung adenocarcinoma of NSCLC in TCGA database.

Data are shown as mean $\pm$ SD, obtained from three experimental replicates. p values were determined by two-tailed Student's t test (ns, not significant; *p < 0.05, **p < 0.01, ***p < 0.001).

Fig. S7. Notch2 inhibitor sensitizes tumor response to paclitaxel in multiple mouse tumor models.

a. Western blot analysis of Notch signaling activity by Hes1 expression in OVCAR8 cells treated with different concentration of RO4929097.

b. Colony formation assay of OVCAR8 cells treated with DMSO, 10 nM taxol or 100 nM carboplatin with or without 0.5 μ M RO4929097 (RO, pan-Notch inhibitor).

c. Cell viability of OVCAR8 cells in the presence of DMSO or 10 nM taxol with or without 0.5 μ M RO4929097.

d. Western blot analysis of apoptosis-related factor in OVCAR8 cells treated with DMSO, 10 nM taxol with or without 0.5 μ M RO4929097 for 48 hrs.

e. Tumor growth of mice carrying A549-xenografts treated with RO4929097 and paclitaxel.

Representative tumors (left) and tumor volume (middle) for each group are shown.

f. Tumor growth of mice carrying OVCAR8-xenografts treated with RO4929097 and carboplatin (Car). Representative mice (left) and tumor volume (right) for each group are shown.

g. The gating strategy for flow cytometry analysis shown in Fig.7d.

h. Information of the ovarian cancer patient whose tumor tissue was used to establish the PDX mouse model.

i. Immunohistochemistry (IHC) detection of ovarian cancer markers in tissues from the patient and PDX model. HE, hematoxylin and eosin.

Error bars are SEM. p values were determined by two-tailed Student's t test (ns, not significant; *p < 0.05, **p < 0.01, ***p < 0.001).

Table S1. Patients' information.

Table S2. Reagent and Resource.

Figure 1

Supplementary Figure 1

Figure 2

Supplementary Figure 2

Figure 3

Supplementary Figure 3

Figure 4

Supplementary Figure 4

Figure 5

Supplementary Figure 5

Figure 6

Supplementary Figure 6

a

b

c

Figure 7

Supplementary Figure 7

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [TableS1.Patientsinformation.pdf](#)
- [TableS2.ReagentandResource.pdf](#)