Molecular Response to PARP1 Inhibition in Ovarian Cancer Cells as Determined by Mass Spectrometry Based Proteomics

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Abstract

Poly (ADP)-ribose polymerase (PARP) inhibitors have entered routine clinical practice for the treatment of high-grade serous ovarian cancer (HGSOC), yet the molecular mechanisms underlying treatment response to PARP1 inhibition (PARP1i) are not fully understood. Here, we used unbiased mass spectrometry based proteomics with data-driven protein network analysis to systematically characterize how HGSOC cells respond to PARP1i treatment. We found that PARP1i leads to pronounced proteomic changes in a diverse set of cellular processes in HGSOC cancer cells, which we confirmed in an independent perturbation dataset. We interpret decreases in the levels of the pro-proliferative transcription factors SP1 and β-catenin and in growth factor signaling as reflecting the anti-proliferative effect of PARP1i; and the strong activation of pro-survival processes NF-κB signaling and lipid metabolism as PARPi-induced adaptive resistance mechanisms. Based on these observations, we nominate several protein targets for combined therapeutic inhibition with PARP1. Our study improves the current understanding of PARP1 function, highlights the potential that the anti-tumor efficacy of PARP1i may not only rely on DNA damage repair mechanisms and informs on the rational design of PARP1i combination therapies in ovarian cancer.

Introduction

High-grade serous ovarian cancer (HGSOC) is the most common and most aggressive subtype of ovarian cancer, responsible for the majority of ovarian cancer deaths (1). A crucial step forward for the treatment of HGSOC patients was the introduction of molecular inhibitors that target poly (adenosine diphosphate (ADP)-ribose) polymerase (PARP) enzymes (2). PARP enzymes play central roles in the DNA damage response and their inhibition has demonstrated significant improved progression-free survival in HGSOC patients that lack homologous recombination (HR) capacity through mutations in *BRCA1* or *BRCA2* genes (3,4). It has become standard of care to treat patients with a germline or somatic *BRCA1/2* mutation with PARP inhibitors (PARPi) maintenance after completion of adjuvant treatment. PARPi are also used in the recurrent setting, especially in patients that had not been treated with PARPi. Other indications for treatment with PARPi include patients with mutations in other DNA repair genes (e.g., *BRIP1*, *PALB2*, *FANCD2*) (5).

Several models exist to explain the mechanisms of PARPi action, which is still not fully understood (6). Originally, it was widely believed that the therapeutic benefit of PARPi is primarily due to unrepaired single-stranded DNA breaks, which are converted to irreparable toxic DNA double-strand breaks (DSB) during replication in HR-deficient cells and lead to cell death (7). Other models suggest that due to the diverse roles of PARP proteins in the DNA repair response, PARPi-induced deficiencies in other DNA repair pathways may also contribute to tumor sensitivity to PARPi (8). A more recent model is the 'trapping' of PARP proteins at DNA damage sites during DNA replication, leading to replication collapse, the accumulation of unresolved DSBs and eventually to cell death (9).

Beyond its primary roles in DNA repair, the PARP1 protein, which is responsible for over 80% of PARP enzyme-related activities (6), has also been shown to directly bind and co-activate oncogenic transcription factors that promote cell proliferation, growth and differentiation in different human cancer models (10). For example, in the context of colorectal cancer, it was demonstrated that PARP1 binds transcription factor TCF4 and interacts in a complex with TCF4 and β-catenin contributing to early colorectal carcinogenesis (11). In osteosarcoma tumor cells, PARP1 has been found to co-activate oncogenic transcription factor B-MYB (12). Similarly, in a model of human skin cancer and chronic myelogenous leukemia, it was shown that PARP1 positively regulates the activity of activator protein-1 and hypoxia inducible factor 1 alpha, thereby promoting tumor cell survival and tumor growth (13). Also, several studies showed that PARP1 directly interacts with transcription factor nuclear factor kappa B (NF-κB) and functions as its transcriptional co-activator for κB-dependent gene expression (14). However, although additional proteins have been reported to interact with PARP1, little is known about their relevance for the anti-tumor activity or the emergence of resistance to PARP1i in the context of HGSOC (6,7). Indeed, despite their clinical efficacy in HGSOC with deficiencies in HR, PARPi have also been found to be effective in HGSOC patients regardless of *BRCA* status or HR deficiency (3,4) and the FDA and EMA have recently broadened the approval of PARPi as maintenance monotherapy for ovarian cancer patients independently of *BRCA* or HR status (15). An improved understanding of the cellular mechanisms underlying PARPi treatment response is thus needed to allow more accurate treatment decisions involving PARPi therapies as well as to overcome often seen PARPi resistance (16).

Here, we set out to comprehensively assess the cellular response PARP1i in HGSOC cells using quantitative mass spectrometry (MS)-based proteomics and data-driven protein network analysis. To this end, we systematically analyzed the proteomic response profile following PARP1i treatment to 1) explore PARP1's role in cellular processes beyond the DNA damage response in HGSOC cells, 2) identify potential markers of PARP1i sensitivity or resistance and 3) determine candidate targets for PARP1i combination therapies.

Results

PARP1i perturbation profiling in Ovsaho ovarian cancer cells using MS-based proteomics

Protein response profiling allows to monitor and capture cellular behavior in response to drug perturbation providing information about molecular processes underlying treatment response that could contribute to sensitivity or resistance (17,18). Thus, to gain insight into molecular processes induced by PARP1i in HGSOC, we treated HGSOC Ovsaho cells with the selective PARP1 protein inhibitor AG-14361 and measured the protein response profile by unbiased MS based-proteomics. We selected Ovsaho cells, which are a well-defined preclinical model of HGSOC reflecting the genomic and proteomic features of HGSOC patient tumors (19,20) (Figure 1A). In addition, we used AG-14361, a potent PARP inhibitor, that selectively inhibit the PARP1 protein (Ki < 5nM) (Material and Methods). We treated Ovsaho cells at inhibitor's half-maximal inhibitory concentration (IC50) to inhibit cell proliferation by 50% after 72 hrs as determined by dose-response curves. The treatment time was set to 72hrs to measure the adaptive effect of the cells on PARP1 inhibition (PARP1i). Ovsaho cells were treated with increasing concentrations of PARP1 inhibitor AG-14361 and cell viability was examined for 72 hrs by counting cells using live-imaging (Material and Methods). Treatment with PARP1 inhibitor resulted in a concentration-

dependent decrease of cell numbers with IC50 value at 20 μM after 72 hrs (Figure 1B). Next, Ovsaho cells were treated at inhibitor IC50 concentration (20μM) or DMSO as control in three biological replicates and samples were collected after 72 hrs for MS-based proteomics (Figure 1C).

Figure 1

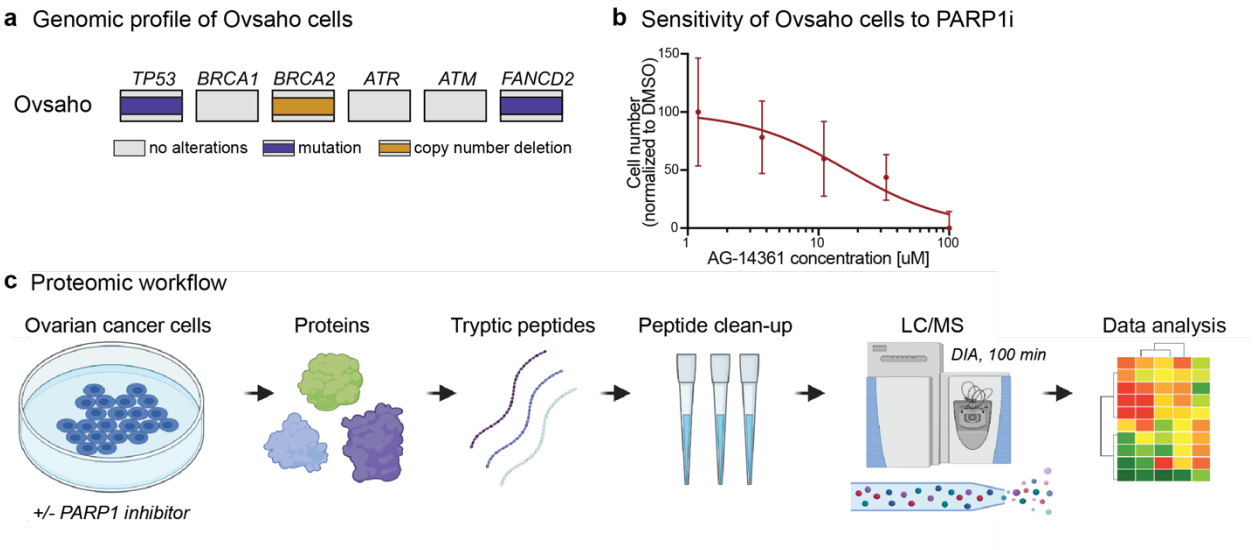


Figure 1. Measuring the sensitivity to PARP1 inhibition in Ovsaho cells and drug response profiling using LC/MS-MS-based proteomics. (a) Genomic profile of Ovsaho cells with HR-associated genes. Ovsaho cells have a mutation in the *TP53* and *FANCD2* gene and a copy number deletion of *BRCA2* gene. (b) Dose-response curve based on cell number count after 72hr. PARP1 inhibitor AG-14361 treated Ovsaho cells versus control treatment. DMSO was used as control vehicle. Data represent three independent experiments and error bars represent standard derivation of technical replicates with a total number of experiments = 9. (c) Schematic view of the LC/MS-MS workflow. PARP1i-treated Ovsaho ovarian cancer cells were prepared for LC/MS-MS. Following protein extraction and tryptic digest, proteins were separated and measured in single runs using a quadrupole Orbitrap mass spectrometer. Label-free protein quantification was performed using the Spectronaut software environment. (LC-MS) Liquid Chromatography with tandem mass spectrometry. Figure 1C was created with BioRender.com.

We employed a data-independent acquisition (DIA) method combined with label-free based quantification (Material and Methods), resulting in ~5,000 quantified protein groups per single replicate measurement (Figure 2A). Biological replicates of both PARP1i-treated and control cells correlated well with median Pearson correlation coefficients of 0.91 and 0.95, respectively

(SFigure 1A). We evaluated replicate reproducibility by calculating the coefficient of variation (CV) between biological replicates. The results of the analysis revealed a median CV of less than 20% between biological replicate protein measurements confirming good experimental reproducibility between replicates (SFigure 1B).

Identification of PARP1i-responsive proteins

Based on our reproducible mass spectrometric measurements, we next analyzed the proteomic alterations after PARP1i treatment. We first identified which proteins were significantly changed upon PARP1i treatment by comparing the protein expression levels values of PARP1i-treated samples to control samples. We filtered for proteins with a quantitative difference of at least 1.41-fold (log2ratio (PARP1i/DMSO) > 0.5 or < -0.5). Statistical significance of the protein expression level change was determined by statistical testing using an unpaired t-test with a p-value of < 0.05 providing statistical evidence that there is less than 5% probability that the change in expression is due to natural random variation. In addition, we applied Benjamini-Hochberg (BH) correction for multiple hypothesis testing to control the false discovery rate (FDR) at a cutoff of < 0.2 (Material and Methods).

Overall, we identified 93 significantly regulated proteins after PARP1i treatment relative to control treatment of which 54 were increased, and 39 proteins decreased (Figure 2B, STable1). Of note, these included several proteins with well-known associations to PARP1 in ovarian cancer. For example, inhibition of PARP1 affected the expression of proteins involved in DNA repair mechanisms including DNA repair scaffold protein XCCR1 (X-ray cross complementing protein

1) (log2ratio (PARP1i/DMSO) = -1.037, p-value = 0.01) and DNA damage regulator TTI2 (Tel two-interacting protein 2) (log2ratio (PARP1i/DMSO) = 0.69, p-value = 0.01).

Figure 2

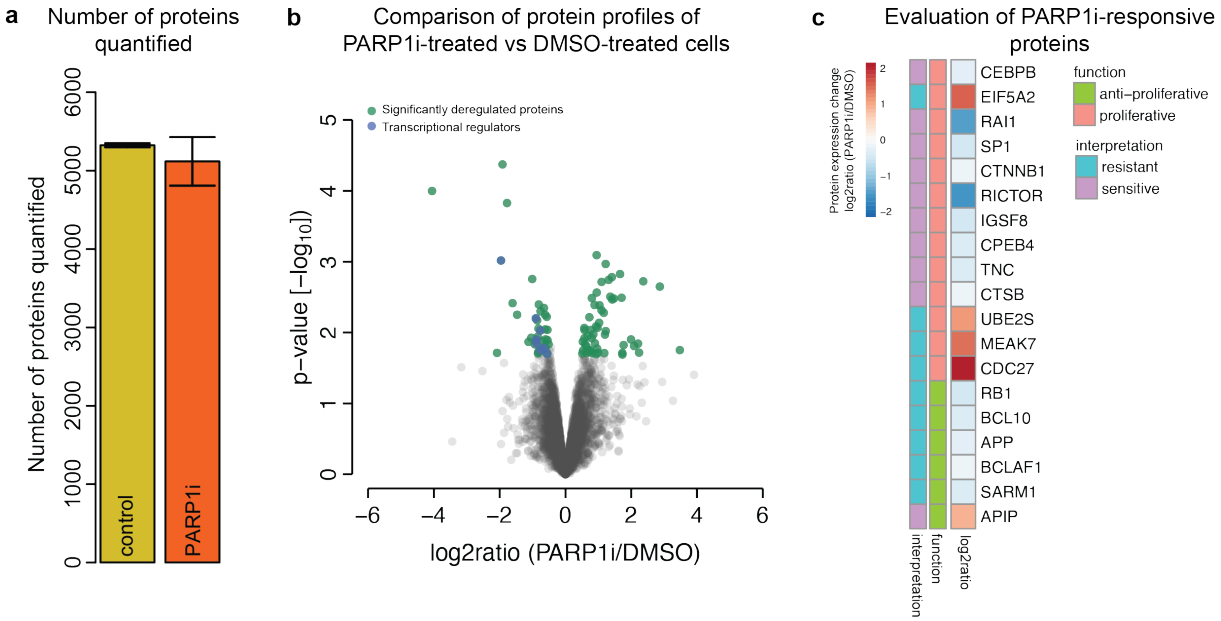


Figure 2. PARP1 inhibitor induced protein response profile in Ovsaho cells. (a) Number of protein groups detected by MS for each perturbation condition, three biological replicates per condition. Numbers are reported as mean of the three biological replicates and error bars show standard deviation for each condition. (b) Identification of PARP1i induced proteins whose expression is significantly changed compared to control DMSO treatment. Volcano plot of statistical significance against log2 protein expression change between PARP1i-treated versus control cells after 72hrs. In green are significantly expressed proteins with log2ratio (PARP1i/DMSO) ≤ -0.5 or ≥ 0.5 with p-value ≤ 0.05 (FDR < 0.2) and in blue proteins with transcriptional activity (STable1). (c) PARP1i-responsive proteins involved in pro-proliferative or anti-proliferative processes and possible interpretation for their relevance to sensitivity and resistance to PARP1i based on their proliferative function and protein expression change level upon PARP1i treatment relative to control (DMSO) treatment. If a pro-proliferative protein (or a protein with pro-proliferative functions) was upregulated upon PARPi treatment, it was considered as marker of resistance to PARPi; if it was downregulated, it was considered sensitive to PARPi. The reverse holds for the anti-proliferative proteins.

We also identified proteins that are not part of the DNA repair response and to our knowledge have not yet been reported in the context of PARPi-treated HGSOC cells (STable1). Most notably, we found that treatment with PARP1i significantly altered the expression level of proteins with transcription- and translation-regulatory activities, including transcription factors SP1 (specificity

protein 1), RB1 (retinoblastoma protein 1), CPEB4 (cytoplasmic polyadenylation element binding protein 4), CEBPB (CCAAT enhancer binding protein beta), RAI1 (retinoic acid-induced gene 1), β-catenin and BCL10 (B cell lymphoma 10) (Figure 2B, C, STable1). Since these proteins are well known to be involved in diverse cellular processes by regulating the transcription of a variety of genes (21–27), we next assessed their potential relevance to PARP1i-induced anti-tumor effects or resistance mechanisms.

Individual PARP1i-responsive proteins as markers of PARP1i sensitivity and resistance

To analyze whether our identified PARP1i-responsive proteins are associated with treatment sensitivity or resistance, we inferred their anti- and pro-proliferative properties based on a review of the literature research and assessed their protein expression level change upon PARP1i. Our analysis revealed several proteins that have been reported to promote cell proliferation (STable1). Moreover, we found that the majority of these proteins were significantly downregulated upon PARP1i treatment. For example, we found a marked decrease of transcription factors β-catenin (log2ratio (PARP1i/DMSO) = -0.56; p-value = 0.01) and SP1 (log2ratio (PARP1i/DMSO) = -0.88; p-value = 0.01) (Figure 2C), which induce the expression of genes involved in the promotion of cell proliferation such as *c-myc* and *CCND1* (21,25). The broad PARP1i-induced downregulation of pro-proliferative transcription factors could thus possibly contribute to PARP1i-induced cytotoxicity.

We also identified proteins that could contribute to adaptive survival mechanisms in response to PARP1i (Figure 2C). For example, we found that tumor suppressor RB1 was strongly downregulated (log2ratio (PARP1i/DMSO) = -0.88; p-value = 0.01), whereas candidate oncogene

eIF5A2 (eukaryotic translation initiation factor 5A isoform 2) was strongly upregulated (log2ratio (PARP1i/DMSO) = 1.411; p-value = 0.001). Similarly, cell cycle promoting factors ubiquitin-conjugating enzyme E2S (UBE2S, (log2ratio (PARP1i/DMSO) = 0.932; p-value = 0.0081) and CDC27 (cell division cycle 27; log2ratio (PARP1i/DMSO) = 1.99; p-value = 0.012) showed a marked increase of protein expression levels upon PARP1i. In sum, these observations show that HGSOC Ovsaho cancer cells reprogram a diverse array of proteins in response to PARP1i treatment, of which some could plausibly contribute to PARP1i-induced cytotoxicity or resistance due to their reported anti- and pro-proliferative properties.

Identification of functional protein networks induced by PARP1i

Understanding the impact of a drug on the protein network can provide further insight into the context in which the drug target works. Therefore, we next aimed to identify the cellular context of PARP1i-responsive proteins. To this end, we performed protein network analysis using the data-driven computational framework NetboxR, which identifies functional protein network modules independent and across the boundaries of pre-defined protein lists by integrating protein network interactions with a clustering algorithm. This data-driven approach thus allows the discovery of *de novo* functional protein network modules and overcomes the issue of the occurrence of proteins in more than one of the curated protein/gene sets (28,29) (Material and Methods). We annotated identified protein network modules with pathway process labels by assigning module protein members to curated pathways based on the Reactome database using the pathway enrichment analysis tool g:profiler with an adjusted (adj.) p-value < 0.05 threshold (30).

Figure 3

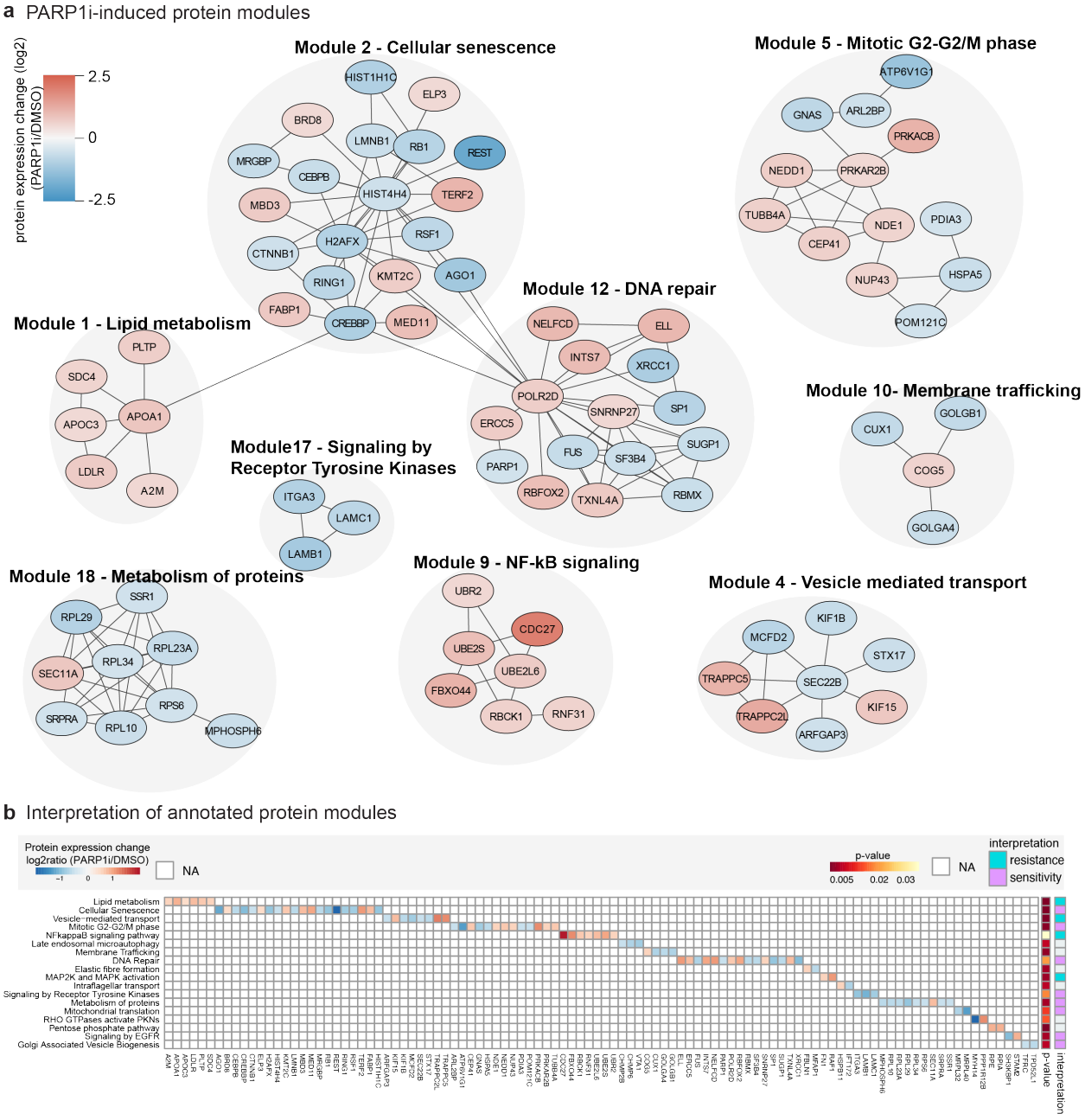


Figure 3. Data-driven exploration of protein signaling network modules in response to PARP1i. Proteins were grouped into functional modules using Netbox algorithm (FDR corrected p-value ≤ 0.05), which combines prior-knowledge of protein network interactions with a clustering algorithm to identify functional protein modules across the boundaries of curated and pre-defined lists of proteins (28,29) (Material and Methods). As a next step, protein modules were characterized by assigning module protein members to pathways based on the Reactome database using g:profiler analysis (adj. p-value ≤ 0.05) (STable2). (a) Depicted are annotated protein modules with > 3 protein members. Nodes represent proteins and are colored based on protein expression change (log2ratio PARP1i/DMSO). Edges represent protein

interactions. (b) Heatmap showing annotated protein modules and protein expression changes of corresponding protein members (STable2). Based on their function and protein expression changes, annotated protein modules were evaluated to be associated with PARP1i-induced sensitivity or resistance (STable2).

Our data-driven protein network analysis revealed 23 functional protein network modules comprising 117 proteins that significantly respond to PARP1i (FDR-corrected p-value < 0.05) (STable2). Figure 3A shows the effects of PARP1i treatment on major protein networks. In this figure, each node represents a protein and edges represent protein interactions. Our analysis revealed several processes that have previously been reported to be associated with PARPi treatment in ovarian cancer cells, confirming our approach's ability to identify molecular processes underlying PARP1i treatment responses. For example, we found protein modules whose proteins were significantly over-represented in cellular senescence (adj. p-value = 1.025×10^{-4}), DNA repair (adj. p-value = 1.882×10^{-2}) and G2-G2/M cell cycle phase (adj. p-value = 7.014×10^{-4}) (Figure 3A, B; STable2) (31,32). In addition, we identified one protein module annotated as MAPK activation (adj. p-value = 2.247×10^{-3}) (STable2). MAPK activation has previously been identified as PARPi resistance mechanism and has been reported as a strong candidate for PARP1i combination therapy (33). Consistent with our results on the individual protein level, our protein network analysis also revealed PARP1i-responsive processes with reported pro-proliferative properties that have not yet been reported in association with PARP1 in HGSOC cells, such as NF-κB signaling (adj. p-value = 3.456×10^{-2}), EGFR signaling (adj. p-value = 3.421×10^{-3}) and lipid metabolism (adj. p-value = 1.197×10^{-6}) (Figure 3A, B; STable2). We therefore next asked whether these processes could likely contribute to PARP1i-induced cytotoxicity or resistance.

Molecular processes associated with PARP1i cytotoxicity or resistance

Based on the function of identified protein network modules in proliferative processes and the protein expression change of module members, we evaluated whether PARP1i-responsive processes could promote cytotoxicity or resistance. Most notable, we found that the inhibition of PARP1 leads to an overall decreased activity of cell growth-inducing signaling pathways, including tyrosine kinase receptor signaling (adj. p-value = 1.680×10^{-2}) and EGFR signaling (adj. p-value = 3.421×10^{-3}), suggesting that these processes could contribute to PARP1i-induced cytotoxicity (Figure 3B).

We also discovered cellular networks that are indicative of adaptive response mechanisms to PARP1i. In particular, we noted a large cluster of nodes related to NF-κB signaling (adj. p-value 3.456×10^{-2}) and lipid metabolism (adj. p-value 1.197×10^{-6}) with all protein members being highly expressed in response to PARP1i (Figure 3A, B). NF-κB is a pivotal factor in regulating immune responses and inflammation and has been implicated in tumor growth. In ovarian cancer, it was found that the activation of NF-κB promotes tumor cell proliferation (34). Moreover, activated NF-κB signaling has been observed in response to diverse anti-cancer therapeutic agents in several pre-clinical studies and its inhibition has been reported to enhance apoptosis and to re-sensitize drug resistant tumors potentiating the anti-tumor efficacy of anti-cancer therapies (35,36). Furthermore, a more recent study reported high levels of NF-κB activity in PARPi resistant human ovarian cancer cell line UWB1.289, which derived from a tumor of papillary serous histology. The authors demonstrated that inhibition of NF-κB with inhibitor bortezomib increased the sensitivity to PARPi olaparib in PARPi-resistant UWB1.289 cells (37). Similarly, increased lipid metabolism is involved in chemotherapy resistance and other small agents such as VEGF neutralizing antibody

bevacizumab. The inhibition of lipid metabolism enzymes has been shown to decrease tumor growth (38). Based on these observations, we interpret the increased protein expression levels of proteins involved in NF-κB signaling and lipid metabolism in response to PARP1i treatment as adaptive mechanism of cells to survive, while reduced growth factor signaling and reduced mitochondrial translation might contribute to cytotoxicity.

Confirmation of PARP1i-induced cellular processes in other HGSOC cell lines

We next validated our findings using independent perturbation datasets involving different HGSOC cell lines and PARPi olaparib and rucaparib. To this end, we employed the recently published L1000 dataset from the LINCS (library of integrated network-based cellular signatures) data portal. The L1000 is a gene expression assay in which the mRNA expression profiles of specific 978 landmark genes are measured in response to drug perturbations (39). From the dataset, we found three cell lines TYKNU, COV644 and RMUGS that closely match patient HGSOC tumors (19) and were treated with pan PARP1/2 inhibitors olaparib and rucaparib. By applying data-driven protein network analysis on strongly drug responsive genes (log2ratio (inhibitor/control) > 0.5 or < -0.5) (Material and Methods) and annotating identified functional modules with pathway labels using pathway enrichment (Material and Methods), we re-captured almost all previously identified annotated protein networks across tested cell lines (FDR corrected p-value < 0.05), including tyrosine kinase signaling, mitochondrial translation, NF-κB signaling and lipid metabolism (Figure 4 A,B: STable3). Moreover, correlation analysis of the overall protein module activity based on overlapping annotated protein network modules between L1000 and our proteome profiling (Material and Methods), revealed a positive correlation of olaparib-induced module activity with PARP1i-induced module activity (Pearson correlation coefficient R

= 0.045; rucaparib Pearson correlation coefficient R = -0.52; Material and Methods, STable3) with olaparib-treated cell line RMUGS showing the highest correlation in activity (R = 0.85, p-value = 0.0052; Figure 4 C).

Figure 4

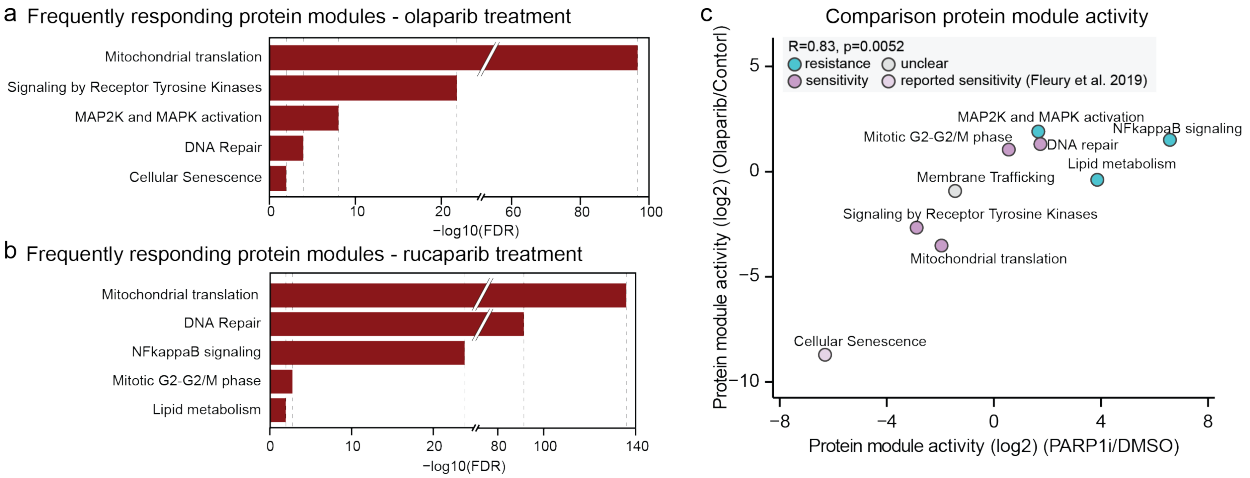


Figure 4. Confirmation of PARP1i-induced protein networks in HGSOC cell lines. Protein network and pathway analysis were performed on independent L1000 transcriptome dataset for HGSOC cell lines RMUGS, COV644 and TYKNU treated with pan-PARPi olaparib and rucaparib as described previously (FDR-corrected p-value < 0.05; Material and Methods, STable3). (a, b) Barplots represents top 5 frequently responding annotated protein modules (adj. p-value < 0.05) across all cell lines (STable3). (c) Correlation analysis of the overall protein module activity based on overlapping annotated modules between L1000 olaparib-treated RMUGS cell line and proteome profiling. Activity was calculated by integrating protein expression changes in modules (38) (Material and Methods).

In sum, these results are in agreement with our findings and highlight the function of PARP1i in a wide range of biological processes with tumor growth promoting properties in HGSOC.

Nomination of candidate PARPi combination therapy targets

Strong protein signals in response to drug perturbations provide a strong basis for the rational design of combination therapies (17,18). Based on our observations on protein expression and

functional protein networks, we report four promising targets as candidates for future PARPi combination studies (Table 1).

Table1. Nomination of candidate PARPi combination therapies based on identified molecular processes and existing small molecule drugs with clinical relevance

Molecular process	Target	Drug combination with PARPi
Lipid metabolism	FASN	PARPi + TVB-2640
NF-kB signaling	IκBα	PARPi + Bortezomib
Cell proliferation	eIF5A-2	PARPi + N(1)-guanyl-1,7,-diamineoheptane (GC7)
Mitotic exit	UBE2S/APC/C	PARPi + proTAME

For example, we propose follow-up PARPi combination experiments with molecular agents targeting UBE2S. UBE2S showed a marked increase upon PARP1i in our protein profiling dataset and has been reported to induce mitotic exit (40). Since mitotic exit by slippage has been found as typical response of *TP53*-mutant cancer cells to resist drug-induced mitotic cell death and blocking mitotic exit has already shown to successfully reduce tumor growth in paclitaxel-treated ovarian cancer models (41), we speculate that additional blocking of mitotic exit by means of e.g. UBE2S depletion, Cdc20 inhibition or APC/C inhibitor proTAME could enhance anti-tumor effects of PARPi in HGSOC. We also propose to target components of the NF-κB signaling pathway or lipid metabolism in combination with PARPi. As discussed above, both processes were highly activated following PARP1i and are recognized as emerging targets for pharmacological interventions for the treatment of cancer (38). Moreover, a first clinical phase I study with the small molecule inhibitor TVB-2640 targeting fatty acid synthase FASN demonstrated antitumor activity in combination with chemotherapy in ovarian cancer patients (42). In conclusion, we nominate four drugs to inhibit aforementioned targets in combination with PARPi in future preclinical investigations. These drugs have already shown their anti-tumor potential as single agent or in combination treatment in a variety of pre-clinical and clinical studies (38,41,43,44).

Discussion

It is widely believed that the anti-tumor efficacy of PARPi is due to synthetic lethality between PARPi and defects in HR deficient cancer cells (7). However, PARPi have also demonstrated therapeutic benefits in HGSOC patients regardless of HR status (15). In addition, several studies report that the PARP1 protein is implicated in cell growth and cell proliferation by modulating the activity of transcription factors (10), which leads to the notion that PARPi might also confer anti-tumor effects beyond the DNA repair mechanism and indicate a broader utility for PARPi in the treatment of cancer patients (6).

Our proteomics data support this view. In agreement with previous studies (10), we find that PARP1i blocks protein expression of many pro-proliferative proteins; in particular, transcriptional activators, which plausibly contribute to PARP1i-induced cytotoxicity in our HGSOC model system. However, the roles of PARP1 as transcriptional co-activator or repressor of transcription factors are known to be complex, gene and context-specific (45). Further studies are thus warranted to precisely delineate the relationship of PARP1 with individual pro-proliferative transcription factors and the effect on the expression of target genes and contribution to cell proliferation in HGSOC cells.

PARPi offers a significant clinical benefit, but most patients develop resistance (46). Most of the mechanisms of PARPi resistance identified involve the restoration of stalled replication fork protection, as well as reactivation of the HR pathway based on genetic and epigenetic changes in the tumor (47). Frequently reported is for example the restoration of functional BRCA1/2 through secondary mutations, BRCA1 promoter methylation or the rewiring of the DNA damage response

network (16). Therefore, multiple strategies to overcome PARPi resistance are designed to selectively disrupt HR in cancer cells, e.g. via combined inhibition of PARP and CDK1 (48), and thus to re-sensitize the cells to PARPi (47). Our unbiased proteomics approach, identified other potential resistance mechanisms that could be exploited therapeutically, including PARPi-induced activation of NF-κB signaling and lipid metabolism. Previous studies already reported promising anti-tumor effects by the combined inhibition of PARP1/2 and NF-κB signaling (37). Interestingly, targeting lipid metabolism is also effective in platinum-resistant ovarian cancer cells (49).

Our findings are derived from proteomics response profiles in one *BRCA2*-mutated HGSOC cell line and we validated our findings in three non-HR or *BRCA1/2*-mutated HGSOC cell lines through an independent drug perturbation dataset based on mRNA expression profiles. This provides strong evidence that identified and confirmed PARP1-induced processes are independent of the type of DNA damage repair mechanisms in HGSOC. However, further experiments are needed to determine if our observations can be generalized for other HR deficient HGSOC cells, non-HR mutated HGSOC cells or perhaps also other cancer types. Future follow-up studies should thus expand the cell-line panel as well as include preclinical mouse models and clinical samples to establish the potential clinical relevance of the findings.

This work improves the current understanding of PARP1 function in HGSOC cells and sheds light onto mechanisms and biomarkers of response to PARP1i treatment, which may be of relevance for the development of PARPi combination therapies in HGSOC.

Materials and Methods

Cell culture experiments. Ovsaho cells were obtained from JCRB (Japanese Collection of Research Bioresources Cell Bank) cell bank (NIBIOHN, Cell No. JCRB 1046) and grown in MCDB105/199 medium with 10% FBS and substituted with 1% Penicillin-Streptomycin. All cells were free of Mycoplasma and their identity was verified by whole exome sequencing at the Broad Institute. For dose-response curves, cells were treated for 72 hrs with DMSO or the indicated doses of the PARP1 inhibitor AG-14361 (Selleckchem, #S2178). Cell viability was measured using the software in a live-imaging IncuCyte station by counting live cells. Live cells were labeled by adding the Incucyte NucLight Rapid Red (NRR) Reagent (Essen Bioscience, #4717, 1:4000) to the medium. Datapoints of three biological experiments and three technical replicates (total n=9) were merged and fitted using a non-linear log(inhibitor) versus normalized response with variable slope for dose-response curves using Prism (GraphPad).

Liquid chromatography-mass spectrometry (LC-MS) measurements. Tryptic digestion of proteins from samples were followed by a nanoflow LC/MS-MS analysis of peptides using the quadrupole Orbitrap mass spectrometer (Q Exactive HF-X, Thermo Fisher Scientific, Bremen, Germany) (Kelstrup et al. 2017) coupled to an EASYnLC 1200 ultra-high-pressure system (Thermo Fisher Scientific) through a nano-electrospray ion source. Briefly, about 300 ng peptides were loaded on a 50-cm HPLC-column with a 75-µm inner diameter (New Objective, USA; in-house packed with ReproSil-Pur C18-AQ 1.9-μm silica beads; Dr. Maisch GmbH, Germany). Peptides were separated using a linear gradient from 5% to 60% solvent B (80% ACN; 0.1% formic acid) in solvent A (0.1% formic acid). Total gradient length was 100 min and the column temperature was maintained at 60 °C with an in-house made column oven. The. The MS was

operated in a data-independent acquisition (DIA) mode. The DIA method consisted of one MS1 scan (m/z 350 to 1,650, resolution 60,000, maximum ion injection time 60 ms, AGC target 3E6) and 32 DIA segments with varying isolation widths ranging from 39.7 Th to 562.8 Th inversely proportional to the *m/z* distribution of tryptic peptides and with an overlap of 1 Th. MS2 scan resolution was set to 30,000, maximum injection time to 54 ms and the automated gain control (AGC) target to 3E6. A stepped collision energy of 25, 27.5 and 30 was used and the default MS2 charge state was 2. Data was acquired in profile mode.

MS data analysis. DIA raw files were analyzed with Spectronaut Pulsar X software (Biognosys, version 12.0.20491.20) using the default settings for targeted data extraction. We used a project specific OVSAHO cell line proteome dataset as spectral library comprising 7,720 protein groups (103,554 precursors). The false discovery rates at the precursor and protein levels were < 1% with the 'mutated' decoy model. Prior to data analysis, data was filtered by 'No Decoy' and 'Quantification Data Filtering'.

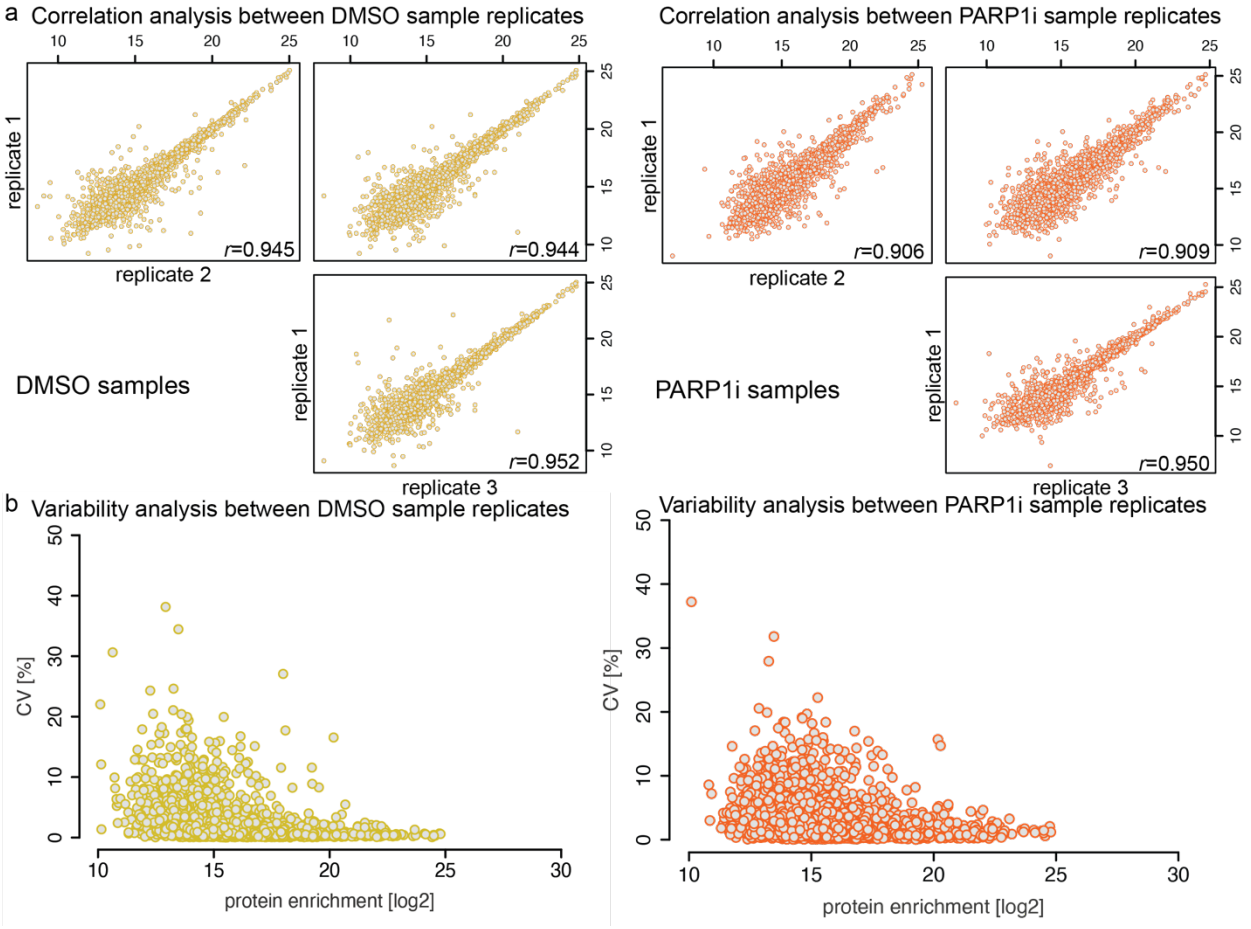
Proteomics data analysis and computational modeling. Data analysis was done in the R software environment (Version 3.6.2). Variance between samples was tested using F-test. Statistical significance was assessed using moderated un-paired t-test comparing inhibitor-treated samples with control samples (DMSO was used as control). The p-values were corrected for an FDR of < 0.2 by the Benjamini–Hochberg method. Results were filtered to have both a significant FDR corrected p-value and a minimum log2(expression ratio) of 0.5. The NetboxR algorithm (28,29) was used to identify functional protein modules with FDR corrected p-value ≤ 0.05. For protein network analysis we used all proteins based on their variance level by selecting for proteins

whose log2(expression ratio) was > 0.5 or <-0.5 and greater than technical noise, as defined by standard deviation of biological replicates. Networks were inferred based on the Reactome protein network (version 8), proteins are represented as nodes and interactions as edges. Statistical significance of discovered modules was assessed using the global network null model and local network null model with a p-value of ≤ 0.05. Linker p-value cut off of 0.05 was used to identify linker proteins (STable2). Derived network was illustrated using Cytoscape and Adobe Illustrator software. Based on the Reactome dataset, pathway enrichment analysis was performed using g:profiler p-value ≤ 0.05 (30). Activity scores of annotated protein modules were calculated as the sum of all expression change values in modules (17).

L1000 dataset. The publicly available mRNA expression profiles of ovarian cancer cells from the L1000 Dataset -small molecule perturbagens- LINCS Phase 1 (50) (Todd Golub, Aravind Subramanian: L1000 Dataset -small molecule perturbagens- LINCS Phase 1, 2014, LINCS (collection), http://identifiers.org/lincs.data/LDG-1188, was retrieved on May 26, 2020 and data level 4 was used.

Supplementary Figure

SFigure 1



SFigure 1. (a) Pearson correlation coefficients (*r*) were calculated for biological replicates (n=3) of samples. (b) Quantification of sample comparability determined by coefficient of variation (CV) of replicates.

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Author's contributions: AF designed the study, collected the samples, analyzed and interpreted the proteomics data and wrote the manuscript. FC provided advice for the design of the study, acquired and analyzed the mass spectrometry data and critically revised the paper. LC carried out the dose-response experiments, analyzed live-cell imaging data, prepared the samples for MS measurements and edited the manuscript. CYS participated in the design of the study, analyzed the WES data and edited the paper. MM and CS designed and supervised the study and critically revised the paper. All authors have approved the final version.

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