Oncologie

Tech Science Press

DOI: 10.32604/oncologie.2022.025419

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ARTICLE

Research on Clinical Effectiveness of Aspirin for Treating Breast Cancer and Cell Protein Biomarkers on Aspirin Treatment in Drug-Resistant Estrogen Receptor-Positive Breast Cancer Cells

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Received: 12 July 2022 Accepted: 09 September 2022

ABSTRACT

Background: Aspirin (ASA) has been reported to have an antitumor effect but the role of ASA in the prevention and treatment of breast cancer (BC) is still controversial. This study aimed to identify clinical effectiveness of ASA in the treatment of BC and explore the antitumor target proteins of ASA that may be involved in overcoming tamoxifen resistance in estrogen receptor (ER)-positive BC cells. **Materials and Methods:** Randomized controlled trials (RCTs) of ASA in the treatment of BC were queried from the databases, including PubMed, Web of Science, Cochrane Library, WanFang, and Chinese National Knowledge Infrastructure. According to the quality standard recommended in the Newcastle-Ottawa Scale (NOS), the outcome indexes were analyzed by RevMan 5.3 and Stata 12.0 software. Cell culture experiments were performed to explore the effect of tamoxifen combined with ASA on the proliferation of ER-positive BC cell lines MCF-7 and MCF-7/TAM. Cell cytotoxicity was determined by the 3-(4, 5-di-2-yl)-2, 5-ditetrazolium bromide (MTT) assay. A quantitative proteomic analysis was conducted between the control and experimental groups to identify differentially expressed proteins (DEPs). Subsequently, Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses were used for bioinformatic analysis of DEPs. The protein expression in patients of ER-positive BC was analyzed by immunochemistry (IHC). **Results:** Nine RCTs including 162,381 patients were selected for this study. The meta-analysis revealed that daily ASA use, as compared with its non-use, was associated with a decreased risk of BC death: relative risk (RR) = 0.83%, 95% CI [0.73, 0.94], Z = 2.89, $P = 0.004$ ($P < 0.05$). Cell culture experiments showed that tamoxifen combined with ASA can drastically inhibit the cell growth of MCF-7 and MCF-7/TAM cells more than the administration of tamoxifen alone ($P < 0.05$). Fifty-seven DEPs were up-regulated, while eighty-five DEPs were down-regulated in MCF-7/TAM cells after the ASA combination treatment. Several GO terms were significantly enriched, such as neutrophil degranulation, retinal metabolic process, sterol biosynthetic process, and prostaglandin metabolic process. KEGG pathway enrichment analysis also verified three associated pathways including metabolic pathways, chemical carcinogenesis-reactive oxygen species, and biosynthesis of amino acids. In ER-positive BC patients with Ki67 > 20%, the positive expression of one significantly DEP MYC was much higher than in BC patients with Ki67 ≤ 20% (40.91% *vs.* 12.50%, $P < 0.05$). There was no significant difference in MYC protein expression among the other subgroups ($P > 0.05$). **Conclusions:** Our results show that ASA has a clinical value in the treatment of BC. ASA could overcome tamoxifen resistance in ER-positive BC cells through some key proteins, which may be potential therapeutic targets for patients with tamoxifen resistance.

KEYWORDS

Breast cancer; meta-analysis; aspirin; estrogen receptor; drug-resistant; proteomics

1 Introduction

Around 12% of all cancer-related deaths worldwide occur due to breast cancer (BC), which is the leading cause of cancer-related death among women [1]. Depending on the clinical tumor subtypes of BC, therapeutic backbones include surgery, radiation therapy, endocrine therapy (ET), chemotherapy, and anti-HER2 targeting [2]. The most common type of BC is estrogen receptor (ER) positive, which accounts for about 60% of all BC cases [3]. These tumors are initially dependent on the steroid hormone estrogen acting through the ER. ET is a common treatment for ER-positive BC, which decreases the risk of recurrence and improves progression-free survival in a subset of patients with ER-positive BC. Most commonly, tamoxifen is used to treat BC for a 5-year treatment period at least because of its inhibitory effects on the transcriptional activity of ER [4]. Despite its initial therapeutic efficacy, resistance to tamoxifen limits its long-term benefit for patients with BC, which remains an important and challenging clinical problem [5,6]. Nearly 30% of patients with BC receiving tamoxifen eventually experience disease relapse, and secondary resistance to tamoxifen during treatment can lead tumor metastasis and death [7]. Molecular mechanisms of resistance to tamoxifen have not yet been determined. Some mechanisms of tamoxifen resistance are complex and include ER mutations [8], the metabolic pathway of tamoxifen [9], activation of alternative oncogenic signaling, and deregulation of oncogenic proteins [10]. As a result, there is a great need for more comprehensive research on novel treatment options to overcome the resistance of tamoxifen in ER-positive BC.

In recent years, several studies have demonstrated that aspirin (ASA) has anticancer effects in solid tumors, such as colorectal cancer, prostate cancer, and endometrial cancer [11–13]. However, previous studies of ASA in patients have produced controversial results. Some studies have shown that ASA can reduce the incidence of BC, while other studies were of the opposite opinion. The mechanism of the association between the anticancer effects and ASA is complex [14]. Multiple observational studies have reported an improved BC survival with regular ASA use [15,16]. As ASA has anti-tumor effects, we found it to play a novel role in tamoxifen resistance in ER-positive BC cells and proposed possible mechanisms in our previous study. Additionally, ASA combined with tamoxifen can inhibit cell cycle progression by downregulating cyclin D1, thus overcoming tamoxifen resistance [17]. In tamoxifen-resistant ER-positive BC cell lines, ASA might also inhibit the upstream regulators of MYC and cyclin D1 proteins, such as mTOR and NF-κB signaling pathways. These proteins and factors could upregulate MYC and cyclin D1 protein levels excessively, which is necessary for the survival of cancer cells. However, information regarding changes in proteins associated with tamoxifen resistance in ER-positive BC cells treated with the ASA and tamoxifen combination remains sparse.

Proteomics can help to identify and analyze a whole set of proteins in biological systems systematically and quantitatively. Proteomic studies can interpret not only changes in protein expression levels, but also posttranslational modifications that are essential for the regulation of protein functions [18,19]. In this study, based on evidence showing that ASA may contribute to the breakthrough of tamoxifen resistance, we further analysed tamoxifen-resistant MCF-7/TAM cells and identified altered protein expressions concerning ASA treatment using proteomic analysis techniques. We leveraged the data to better understand the effects of ASA treatment in overcoming tamoxifen resistance in BC.

2 Materials and Methods

2.1 Study Design and Research Sample

This study was designed according to the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines for literature search, study design, and data analysis. We identified eligible researches published up to 2021 on the treatment of BC with ASA from databases, including PubMed, Web of Science, Cochrane Library, Wanfang, and Chinese National Knowledge Infrastructure. Two experienced librarians helped develop a comprehensive search strategy including free text and MeSH terms. A number of search terms were used such as "breast cancer", "breast neoplasms", "ASA", and "non-steroidal anti-inflammatory drugs (NSAIDs)". Inclusion criteria included: (1) Published literature on aspirin in the treatment of BC; (2) The number of cases in each group exceeded 60 cases; (3) Follow-up data are available; (4) The data are detailed and reliable, and the results are clearly expressed. The relative risk (RR) and 95% confidence interval can be extracted from the relevant data. Exclusion criteria included: (1) Review, conference abstract and non-original literature; (2) Cannot provide data for statistical analysis; (3) Repeatability test; (4) Cell culture methods or animal studies; (5) Lack of follow-up data.

2.2 Study Quality Assessment

The design and quality of all studies were assessed using the Newcastle-Ottawa Scale (NOS). Two researchers independently read and evaluated the literature according to NOS scoring criteria, including patient selection, comparability, and study results. Studies with scores >5 were included in the follow-up studies.

2.3 Chemicals and Reagents

4-hydroxy-tamoxifen (4-OHT) and ASA (Sigma-Aldrich, St. Louis, MO, USA) were dissolved in dimethyl sulphoxide (DMSO) (Sigma-Aldrich, St. Louis, MO, USA). Sodium deoxycholate, dithiothreitol, ammonium bicarbonate, and iodoacetamide were procured from Sigma (Sigma-Aldrich, St. Louis, MO, USA). Tris-(2-carboxyethyl) phosphine was procured from Thermo Scientific (Thermo Fisher Scientific, Waltham, MA, USA). Modified sequencing grade trypsin was procured from Promega (Promega Corporation, Wisconsin, USA). High-performance liquid chromatography-mass spectrometry (HPLC/MS) grade solvents were used to prepare all mobile phases and solutions, including water, acetonitrile, methanol, and formic acid (Thermo Fisher Scientific, Waltham, MA, USA). Anti-ER antibody (clone SP1), anti-PR antibody (clone 1E2), anti-HER2 antibody (clone 4B5), and anti-MYC antibody (clone Y69) (Roche, Switzerland) were used in accordance with the manufacturer's instructions.

2.4 Cell Culture and Cell Viability Analysis

The human BC cell lines MCF-7 and MCF-7/TAM (tamoxifen-resistant) were cultured in Dulbecco's Modified Eagle's Medium (DMEM) (Gibco, USA) supplemented with 10% fetal bovine serum (FBS), 100 U/ml penicillin and 100 μg/ml streptomycin (Gibco, USA) in a 5% CO_2 humidified incubator at 37°C. Cells were grown in 96 well culture plates and treated with both compounds and 4-OHT alone. The cytotoxicity of ASA and 4-OHT was evaluated by MTT assay according to the manufacturer's instructions (Sigma-Aldrich, St. Louis, MO, USA). Each experiment was carried out three times. Optical density (OD) values at 450 nm wavelength were determined by enzyme-labeled instruments (Bio-Rad, USA).

2.5 Methods of Proteomics Experiments

2.5.1 Sample Preparation

Seeding of MCF-7 and MCF-7/TAM cells in 10 cm^2 flasks was performed, and the cells were placed in a humidified incubator at 37°C and 5% CO_2. At 70% confluency, treatments with 2 mM ASA and 3 μM 4-OHT (experimental group) or 4-OHT alone (control group) were carried out. Following six days of incubation, the cells in two groups were washed thrice with pre-cooled phosphate buffer saline (PBS) (Gibco, USA) and centrifuged to obtain cell pellets.

2.5.2 Protein Extraction

A total of 1×10^7 cells were lysed with 200 μL radioimmunoprecipitation assay (RIPA) lysis buffer (Thermo Fisher Scientific, Waltham, MA, USA) for 30 min at 4°C, and the debris was removed by centrifugation at 12,000 g for 30 min. Subsequently, the supernatant was collected and the protein concentration was determined by bicinchoninic acid protein assay (Thermo Fisher Scientific, Waltham, MA, USA).

2.5.3 Trypsin Digestion

Protein samples were reduced with a final concentration of 10 mM dithiothreitol at 25°C for 30 min on a shaking plate at 1,200 rpm. Afterward, samples were alkylated with 20 mM iodoacetamide (IAA) and incubated at 25°C for 30 min in the dark. Trichloroacetic acid (100%, 1/10 vol) was added to the samples, followed by incubation at 4°C for 30 min. The samples were washed with pre-chilled acetone and centrifuged at 16,000 g for 15 min at 4°C. This step was performed twice and the pellet was allowed to dry for 10 min. Finally, trypsin in 100 μL of 100 mM Tris/HCL (pH = 8.5) was added to each sample. The protein-to-enzyme ratio was 50:1. Samples were incubated overnight at 37°C to release peptides. Two microliters of 100% formic acid was added to the samples, followed by vortexing and centrifugation at 10,000 g for 30 s. Samples were desalted using an Oasis HLB extraction cartridge (Waters Corporation, Milford, MA, USA) according to the manufacturer's instructions. After drying with a SpeedVac vacuum concentrator (Thermo Fisher Scientific, Waltham, MA, USA), peptides were dissolved in 0.1% formic acid for liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis.

2.5.4 LC-MS/MS Analysis

For proteomics analysis, the samples were allowed to dissolve in 0.1% formic acid and then analyzed on a commercial C18 column (Acclaim PepMap RSLC, 75 μm × 50 cm, Thermo Scientific, Waltham, MA, USA) using an Ultra3000 UHPLC connected to Exploris 480 mass spectrometer (Thermo Scientific, Waltham, MA, USA). Peptides were separated at 300 nl/min for 105 min using a linear gradient of acetonitrile (ACN) 5% to 45% with 0.1% formic acid. The MS acquisition was set to the data-dependent acquisition (DDA) mode, including a full MS survey scan from m/z 350 to m/z 1,200 at a resolution of 60,000 full width at half maximum (FWHM) (at m/z 200) with accudenz gradient centrifugation (AGC) set to 5E6 (maximum injection time of 50 ms), followed by 20 MS/MS scans at a resolution of 15,000 FWHM with AGC set to 2E5 (maximum injection time of 100 ms). A total of twenty of the most intense precursors with an isolation width of m/z 1.6 were selected for fragmentation via high energy collision dissociation (HCD) with 30 normalized collision energy (NCE). An exclusion time of 60 s was set for dynamic exclusion.

2.5.5 Protein Identification and Quantification

In the UniProt human database, the SEQUEST searching engine was used to search MS/MS spectra in proteome discoverer (PD) 2.4 software. The search criteria were as follows: complete trypsin specificity was required; cleavages of up to two deletions were allowed; oxidation (M) and acetylation (N terms of protein) were set as the variable modification and carbamidomethyl (C) was set as fixed modification; precursor ion quality of tolerance was set to 10 ppm and used in orbitrap quality analyzer all MS; MS2 spectra of quality of fragment ions tolerance was set to 20 mmu. The percolator provided of PD was used to calculate peptide false discovery rate. A search in the reverse decoy database was performed to identify false findings based on peptide profile match. Peptides belonging only to a particular group of proteins were considered unique. The false discovery rate for protein identification was also set to 0.01. Label-free quantification was performed only for proteins with two or more unique peptide matches. Protein ratio was calculated as all belong to a protein peptide hit the median. Quantitative accuracy expressed protein ratio variability.

2.5.6 Study Population, Samples and Immunohistochemistry (IHC)

Patients with luminal-like invasive BC were included in the study who also had complete data on specimens analysis-based scores for ER, progesterone receptor (PR), human epidermal growth factor

receptor 2 (HER2), Ki67, and MYC were included in the study. Formalin-fixed and paraffin-embedded (FFPE) tumor specimens were examined by IHC, anti-ER antibody, anti-PR antibody, anti-HER2 antibody, and anti-MYC antibody were used for the IHC analysis according to the manufacturer's instructions. ER/PR-positivity was assessed as ≥1% of tumor cells in the tissue sample. HER2 was evaluated 0 or +1 as negative and +3 as positive. When the IHC test score was 2+, the gene amplification status was determined using fluorescence in situ hybridization (FISH), and MYC positivity was defined as ≥10% cells showing brown color. Ki67 index of ≤20% was considered low risk while >20% was considered high risk.

2.5.7 Bioinformatics and Statistical Analyses

Data were extracted from each observation in both the experimental and control groups. Review Manager 5.3 was utilized to assess the RR and 95% confidence interval (CI). Stata 12.0 software was used to conduct Begg's test. A funnel plot and Begg's test were used to visually evaluate the publication bias. A Principal component analysis (PCA) score plot and hierarchical clustering (Pearson correlation) based heat map were generated using PD 2.4 software. GO enrichment analysis and KEGG pathway enrichment analysis were performed using the DAVID web service (https://david.ncifcrf.gov/home.jsp) with the DEPs revealed by using the provided proteome data. SPSS 23.0 was utilized to detect the data, and $P < 0.05$ was considered statistically significant.

3 Results

3.1 Study Features

All studies were queried from domestic and foreign databases and nine studies were selected based on established standards [20–28] (Fig. 1). A total of 59,934 patients were enrolled in the ASA experimental group, while 102,447 patients were enrolled in the control group. ASA was administered at a low dose (75–150 mg/d), and and the follow-up time was in the range of 41.3–134 months. The NOS scores of nine screened studies were from 7 to 9. This suggests that the studies included in this research are of good quality and can be used for meta-analysis (Table 1).

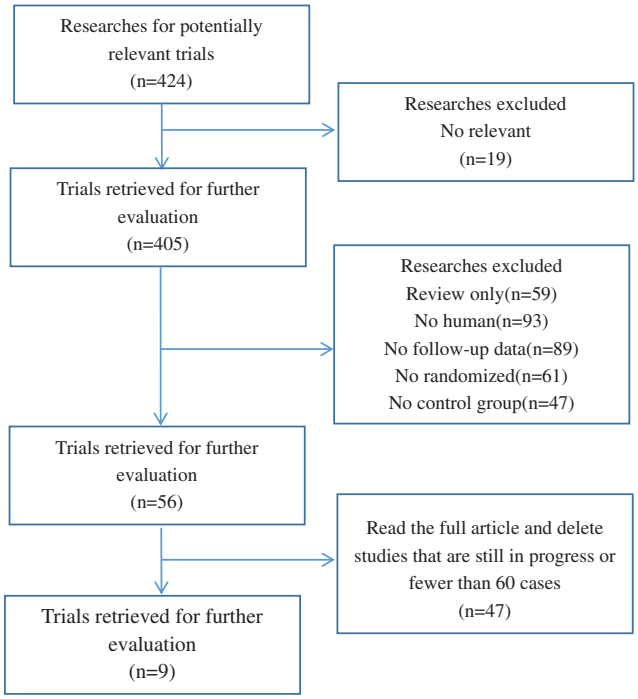


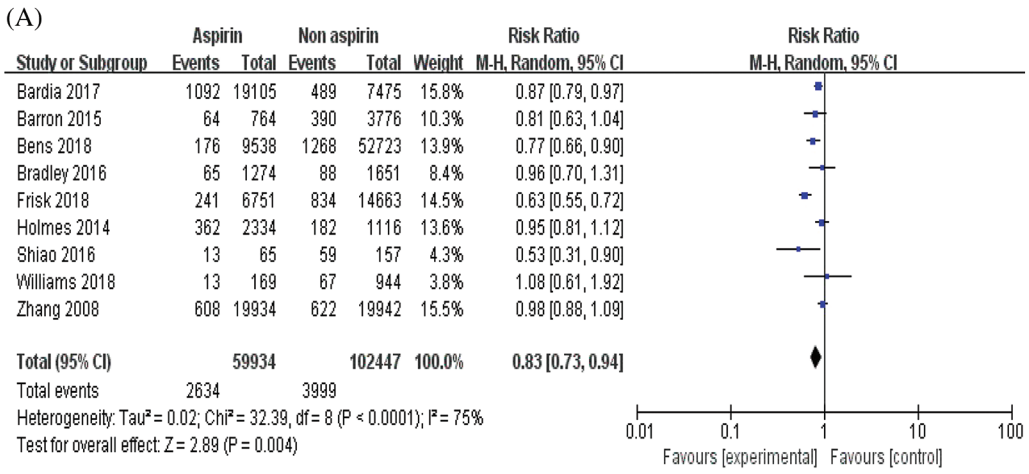
Figure 1: Flowchart of the selection of the clinical trials

Table 1: Summary of studies NOS score

Source	Published year	Study population selection score	Comparability rating	Study outcome rating	NOS score
Bardia et al. [20]	2017	3	2	2	7
Barron et al. [21]	2015	4	2	3	9
Frisk et al. [22]	2018	3	2	2	7
Bens et al. [23]	2018	3	2	3	8
Holmes et al. [24]	2014	4	2	2	8
Bradley et al. [25]	2016	4	2	3	9
Shiao et al. [26]	2016	3	2	3	8
Williams et al. [27]	2018	3	2	3	8
Zhang et al. [28]	2008	3	2	3	8

3.2 Association between ASA use and Prognosis in Patients with BC

The combined analysis of the data of nine included studies showed that there was large heterogeneity among the studies ($P < 0.0001$, $I^2 = 75\%$). Therefore, the random effects model was adopted, with the combined effect size of RR being 0.83%, 95% CI [0.73, 0.94], $Z = 2.89$, $P = 0.004$ ($P < 0.05$). The difference was statistically significant. According to the forest plot (Fig. 2a), the survival rate of patients with BC was higher within the ASA group and the risk of death was less than that in the non-ASA group, thus suggesting that ASA helps improve the prognosis of patients with BC. For publication bias assessment, a funnel plot was constructed for all included studies (Fig. 2b). The results showed that the funnel plot was obviously symmetrical. The result of Begg's test for publication bias was not statistically significant ($P > 0.05$), indicating that the results were less affected by publication bias and the conclusion was reliable.



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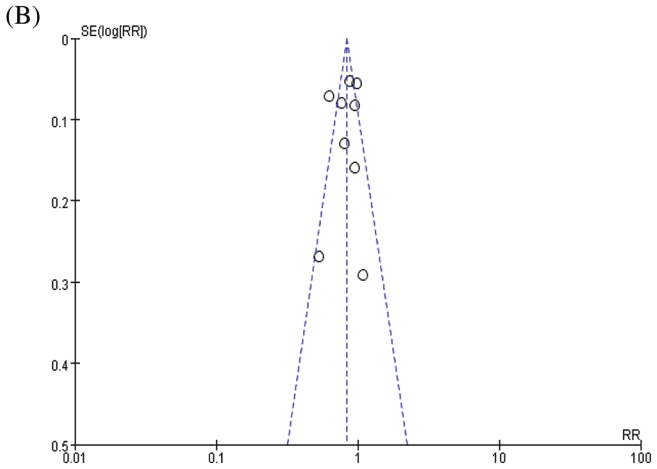


Figure 2: Meta-analyses of the included studies examining aspirin and overall survival in BC. (A) forest plot. (B) funnel plot

3.3 Effects of ASA Combined with 4-OHT on the Proliferation of MCF-7 and MCF-7/TAM Cells

MCF-7 and MCF-7/TAM cells were exposed to 4-OHT (3 μM) alone and 4-OHT (3 μM) combined with ASA (2 mM) for six days. After treatment, cell viability was determined by an MTT assay after treatment. The inhibitory effects of 4-OHT alone on MCF-7 and MCF-7/TAM cells were significantly different. Cell viability exhibited no significant decrease in MCF-7/TAM cells treated with 4-OHT alone. In contrast, cell viability of MCF-7/TAM cells in the 4-OHT combined with ASA groups $(63.93 \pm 5.98)\%$ was significantly lower than in the 4-OHT alone groups $(104.30 \pm 5.67)\%$ ($P < 0.05$) (Fig. 3).

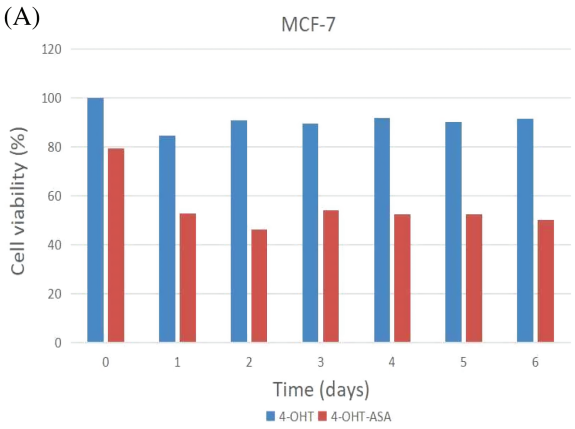


Figure 3: (Continued)

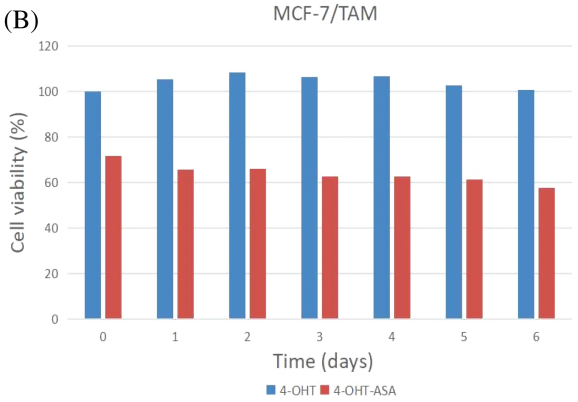
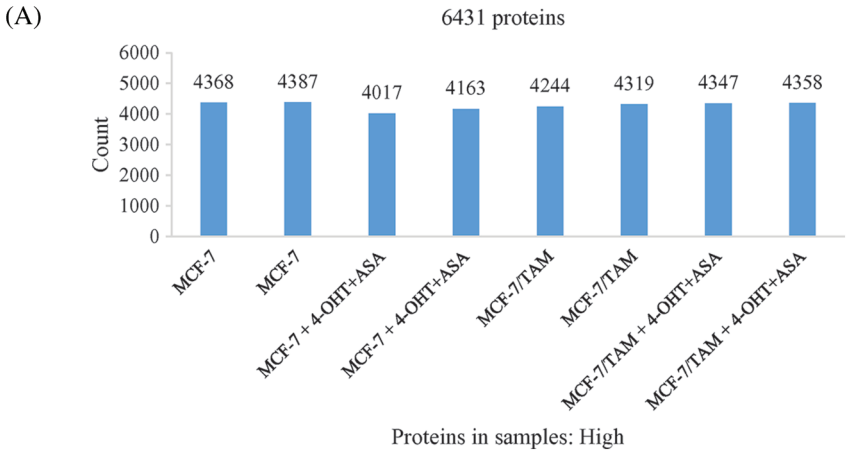


Figure 3: 4-OHT (3 μM) can inhibit cell growth in MCF-7 cells only and 4-OHT (3 μM) in combination with ASA (2 mM) can dramatically inhibit cell growth both in MCF-7 and MCF-7/TAM cells. (A) MCF-7 cells were treated with 4-OHT (3 μM) only and 4-OHT (3 μM) in combination with ASA (2 mM) for 0, 1, 2, 3, 4, 5, and 6 days. (B) MCF-7/TAM cells were treated with 4-OHT (3 μM) only and 4-OHT (3 μM) in combination with ASA (2 mM) for 0, 1, 2, 3, 4, 5, and 6 days

3.4 Overview of the Proteomic Results

To investigate the cell biological changes and mechanisms of ASA combined with 4-OHT, proteomic analysis was performed to find the difference in protein expression of MCF-7 and MCF-7/TAM cells before and after the ASA and 4-OHT combination treatment. 6,431 intracellular proteins were recognized with high reliability in all eight cell samples from LC-MS/MS (Fig. 4a). After filtration (the criteria were: found in all samples, FDR < 0.01 and unique peptides ≥ 2), 3,847 proteins were used to search for searching dysregulated proteins. PCA and hierarchical clustering of protein expression changes (Fig. 4b) showed a clear separation among each group.



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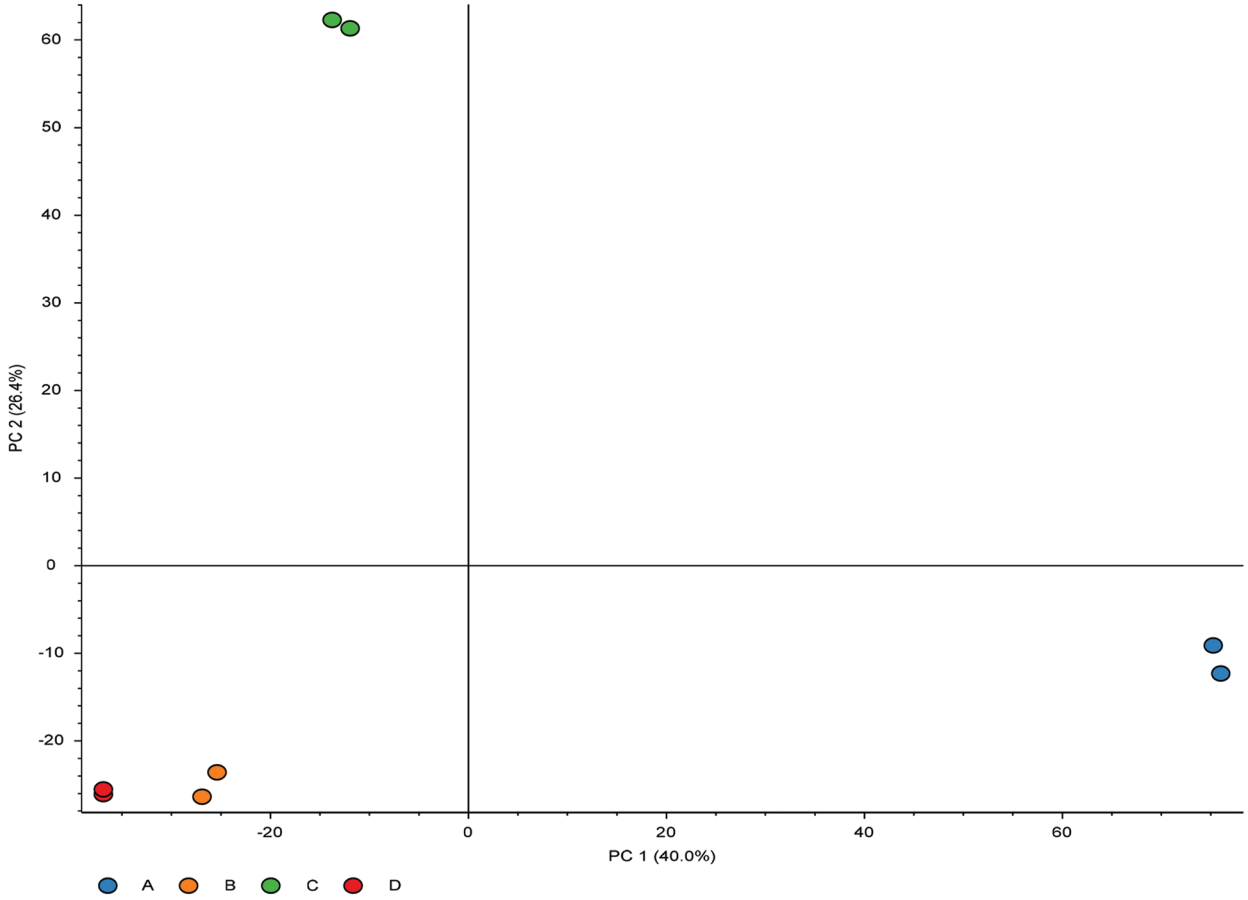
(B)



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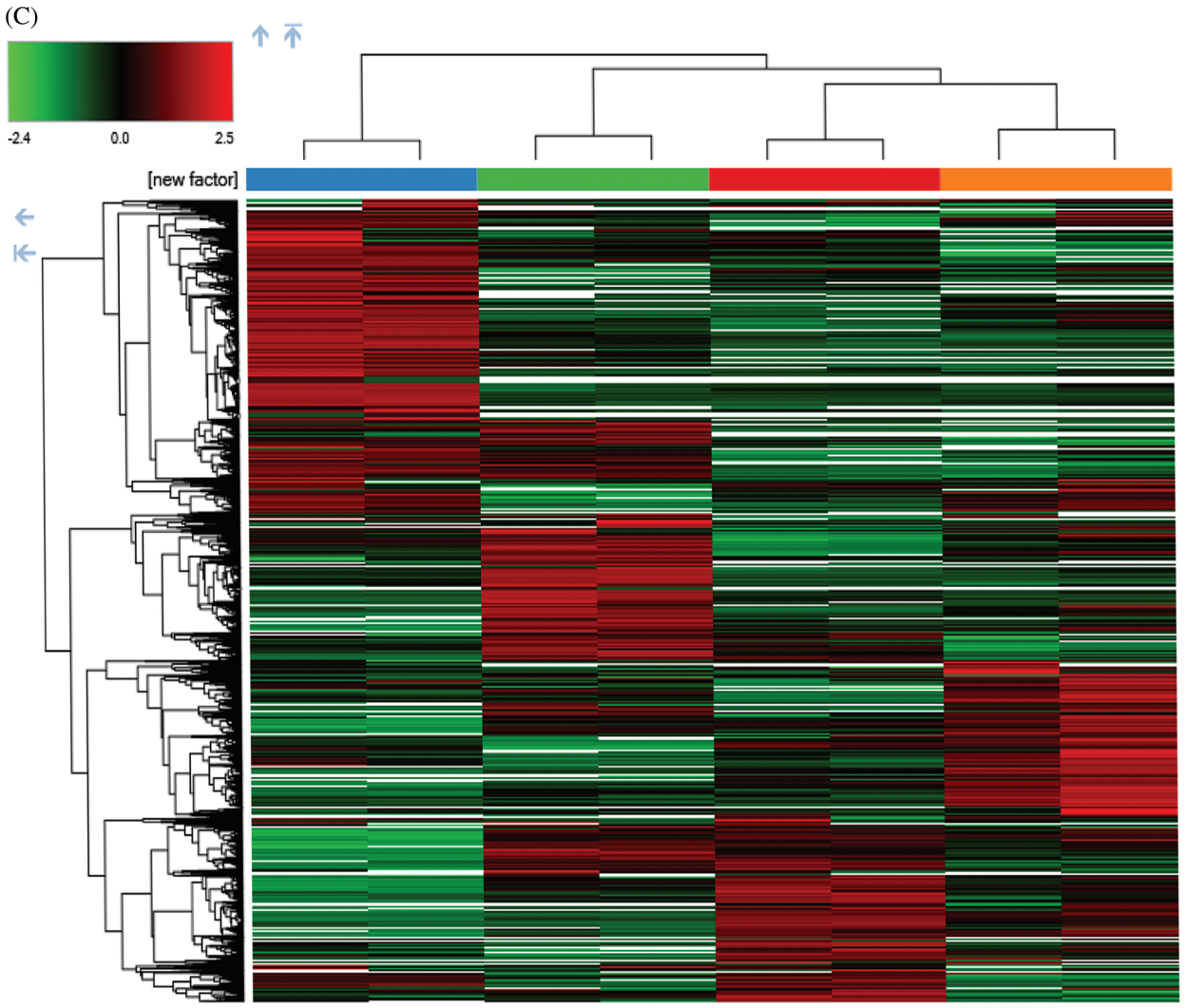


Figure 4: Overview of the proteomic results. (A) Proteins identified with high confidence in each cell sample. (B) Score plot of the PCA representing the distribution of samples in terms of protein expression profiles. (C) Hierarchical clustering heatmap representing the expression profiles of each sample. Sample groups are marked by colors. Blue represent MCF-7 cell samples; orange represent MCF-7/TAM cell samples; green represent MCF-7 cell samples in treatment of ASA and 4-OHT combination; red represent MCF-7/TAM cell samples in treatment of ASA and 4-OHT combination

3.5 Differentially Expressed Proteins (DEPs) of MCF-7/TAM Cells after Treatment with ASA and 4-OHT Combination

To identify the biological impact of ASA combined with 4-OHT on tamoxifen-resistant cells, we compared the proteomes of MCF-7/TAM cells before and after the ASA and 4-OHT combination treatment. A total of 57 DEPs were up-regulated after the combination treatment, while 85 DEPs were down-regulated (Fold change >2 or <0.5 and adjusted $P < 0.05$) (Fig. 5 and Table 2). GO analyses were then performed on these DEPs and the top-ranked GO terms were demonstrated in terms of biological process (Fig. 6a), cellular subtype (Fig. 6b), and molecular function (Fig. 6c). Several GO terms were

significantly enriched, such as neutrophil degranulation, retinal metabolic process, sterol biosynthetic process, and prostaglandin metabolic process. KEGG pathway enrichment analysis also verified three associated pathways including metabolic pathways, chemical carcinogenesis-reactive oxygen species, and biosynthesis of amino acids (Fig. 6d).

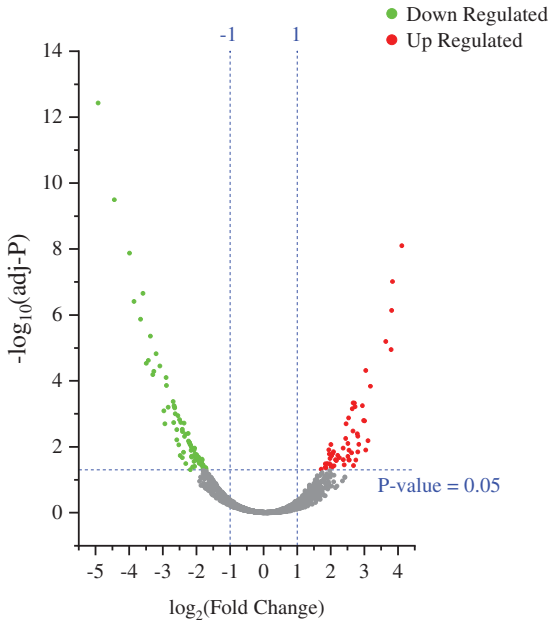
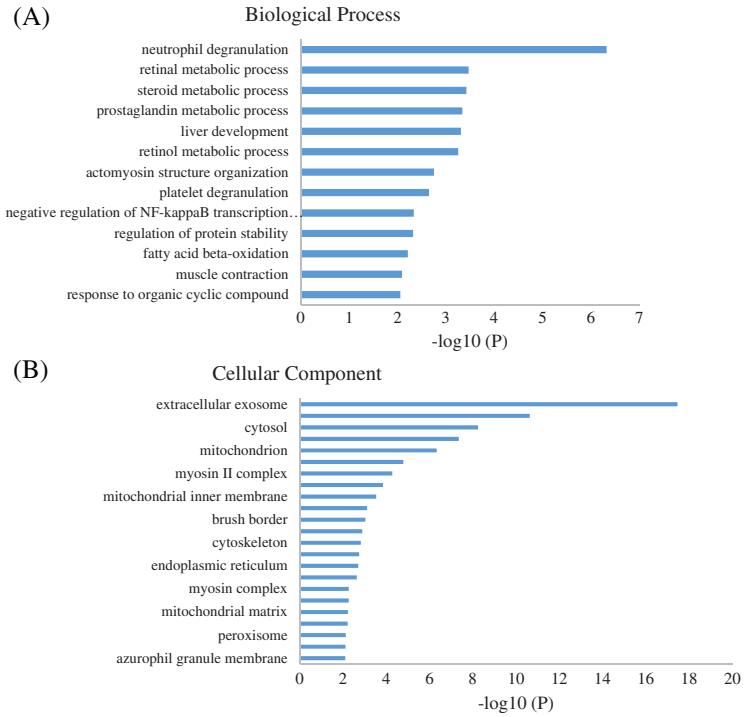


Figure 5: Volcano plot exhibit significantly DEPs



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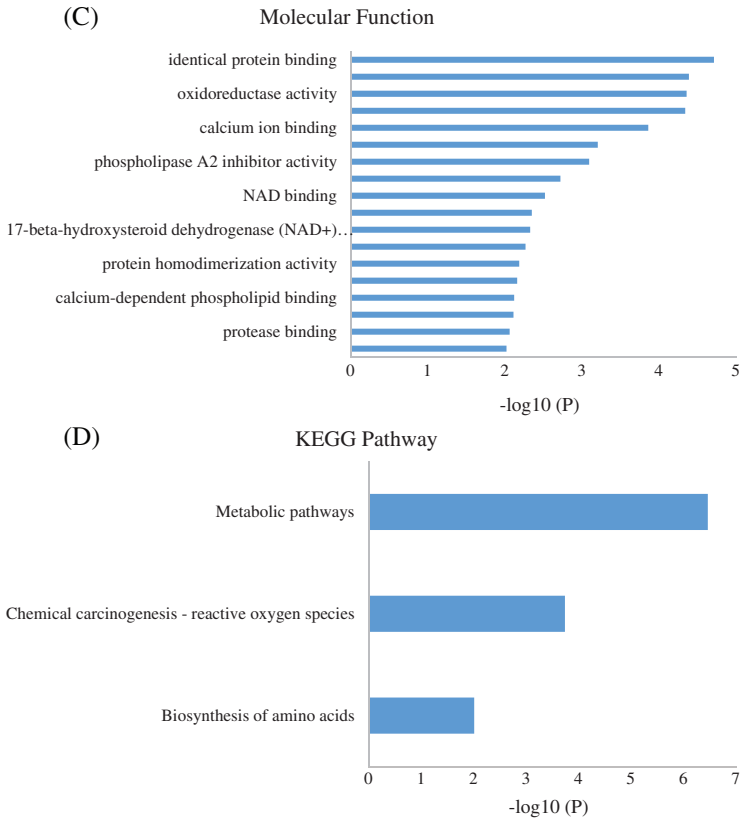

Figure 6: GO enrichment result of the DEPs of MCF-7/TAM Cells after Treatment with ASA and 4-OHT combination in terms of (A) biological process, (B) cellular component, and (C) molecular function ($P < 0.01$). (D) KEGG enrichment pathway result ($P < 0.01$)

Table 2: DEPs of MCF-7/TAM cells after and before treatment with ASA and 4-OHT combination

Protein annotation	Gene name	Fold change	Adjusted *P*	Class
Calmodulin-like protein 5	CALML5	0.033	<0.00001	Down regulated
Galectin-3	LGALS3	0.046	<0.00001	Down regulated
Cytosolic purine 5'-nucleotidase	NT5C2	0.063	<0.00001	Down regulated
Myristoylated alanine-rich C-kinase substrate	MARCKS	0.069	<0.00001	Down regulated
Beta-arrestin-1	ARRB1	0.079	<0.00001	Down regulated
Aldo-keto reductase family 1 member C3	AKR1C3	0.083	<0.00001	Down regulated
Hermansky-Pudlak syndrome 6 protein	HPS6	0.089	<0.00001	Down regulated
OCIA domain-containing protein 2	OCIAD2	0.093	<0.00001	Down regulated
Thymosin beta-4	TMSB4X	0.097	<0.00001	Down regulated
Death-associated protein 1	DAP	0.102	<0.00001	Down regulated
Lysophospholipase D GDPD3	GDPD3	0.104	<0.00001	Down regulated
Cystatin-B	CSTB	0.109	<0.00001	Down regulated

(Continued)

Table 2 (continued)

Protein annotation	Gene name	Fold change	Adjusted *P*	Class
Isoform 2 of Gelsolin	GSN	0.118	<0.00001	Down regulated
Integrator complex subunit 6	INTS6	0.128	0.00081	Down regulated
MICOS complex subunit MIC13	MICOS13	0.131	0.00199	Down regulated
SH3 domain-binding glutamic acid-rich-like protein	SH3BGRL	0.134	<0.00001	Down regulated
Latexin	LXN	0.135	0.00014	Down regulated
Retinoic acid-induced protein 3	GPRC5A	0.140	0.00063	Down regulated
Probable tRNA N6-adenosine threonylcarbamoyltransferase	OSGEP	0.155	0.00042	Down regulated
Prostaglandin E synthase	PTGES	0.157	0.00184	Down regulated
Isoform 6 of Myosin-14	MYH14	0.158	0.00057	Down regulated
major vault protein	MVP	0.159	0.00062	Down regulated
Aldo-keto reductase family 1 member C2	AKR1C2	0.160	0.00066	Down regulated
Septin-8	SEPTIN8	0.162	0.00100	Down regulated
Phospholipid phosphatase 1	PLPP1	0.167	0.00300	Down regulated
ETS domain-containing transcription factor ERF	ERF	0.167	0.00612	Down regulated
Aldehyde dehydrogenase family 1 member A3	ALDH1A3	0.171	0.00115	Down regulated
Pterin-4-alpha-carbinolamine dehydratase 2	PCBD2	0.174	0.00871	Down regulated
kynureninase	KYNU	0.177	0.00151	Down regulated
HLA class I histocompatibility antigen, alpha chain E	HLA-E	0.179	0.01857	Down regulated
Isocitrate dehydrogenase [NADP] cytoplasmic	IDH1	0.181	0.00183	Down regulated
Collagen alpha-1(XII) chain	COL12A1	0.187	0.00297	Down regulated
Utrophin	UTRN	0.187	0.02154	Down regulated
dehydrogenase/reductase sdr family member 4	DHRS4	0.188	0.00346	Down regulated
Frataxin, mitochondrial	FXN	0.192	0.01470	Down regulated
Pyridoxal kinase	HEL-S-1a; PDXK	0.194	0.00192	Down regulated
Canalicular multispecific organic anion transporter 2	ABCC3	0.196	0.00487	Down regulated
Protein S100-A10	S100A10	0.201	0.00411	Down regulated
Phosphatidylinositol 4-phosphate 3-kinase C2 domain-containing subunit beta	PIK3C2B	0.201	0.03222	Down regulated
3-ketoacyl-CoA thiolase, peroxisomal	ACAA1	0.211	0.00395	Down regulated
Protein MYC	MYC	0.213	0.00670	Down regulated
PDZ and LIM domain protein 1	PDLIM1	0.216	0.00745	Down regulated
Pre-B-cell leukemia transcription factor-interacting protein 1	PBXIP1	0.217	0.00745	Down regulated

(Continued)

Table 2 (continued)

Protein annotation	Gene name	Fold change	Adjusted *P*	Class
Myosin-9	MYH9	0.220	0.00841	Down regulated
centrin-2	CETN2	0.221	0.04955	Down regulated
UDP-glucose 6-dehydrogenase	UGDH	0.222	0.00904	Down regulated
PDZ and LIM domain protein 5	PDLIM5	0.224	0.01298	Down regulated
annexin A3	ANXA3	0.226	0.01035	Down regulated
dedicator of cytokinesis protein 5	DOCK5	0.227	0.01937	Down regulated
Mothers against decapentaplegic homolog 2	LOC101348656; LOC111142395; Smad2; SMAD2	0.227	0.02069	Down regulated
Rab GTPase-activating protein 1	RABGAP1	0.232	0.04096	Down regulated
Coronin-1A	CORO1A	0.238	0.02766	Down regulated
Ras-related protein Rab-32	RAB32	0.239	0.01147	Down regulated
DnaJ homolog subfamily C member 5	DNAJC5	0.239	0.01264	Down regulated
Cytochrome P450 1B1	CYP1B1	0.241	0.01096	Down regulated
RAD50-interacting protein 1	RINT1	0.241	0.03940	Down regulated
15-hydroxyprostaglandin dehydrogenase [NAD (+)]	HPGD	0.243	0.01669	Down regulated
Isoform 11 of Synaptotagmin-like protein 2	SYTL2	0.245	0.01947	Down regulated
2'-deoxynucleoside 5'-phosphate N-hydrolase 1	C6orf108; DNPH1	0.245	0.02142	Down regulated
Myosin light polypeptide 6	MYL6	0.248	0.01906	Down regulated
Band 4.1-like protein 1	EPB41L1	0.251	0.01735	Down regulated
saccharopine dehydrogenase-like oxidoreductase	SCCPDH	0.251	0.01783	Down regulated
Endophilin-B2	SH3GLB2	0.252	0.02069	Down regulated
Succinate–CoA ligase [ADP-forming] subunit beta, mitochondrial	SUCLA2	0.256	0.02524	Down regulated
Myosin regulatory light chain 12B	MYL12B	0.261	0.02654	Down regulated
Protein SGT1 homolog	SUGT1	0.262	0.02059	Down regulated
Annexin A2	ANXA2	0.265	0.02895	Down regulated
Short-chain specific acyl-CoA dehydrogenase, mitochondrial	ACADS	0.265	0.03173	Down regulated
Tropomyosin alpha-4 chain	TPM4	0.266	0.02950	Down regulated
CD63 antigen	CD63	0.267	0.02450	Down regulated
Calpastatin	CAST	0.267	0.03053	Down regulated
cytochrome P450 1A1	CYP1A1	0.271	0.02582	Down regulated
Calcium-binding protein 39	CAB39	0.271	0.02837	Down regulated
PDZ domain-containing protein GIPC1	GIPC1	0.274	0.03251	Down regulated

(Continued)

Table 2 (continued)

Protein annotation	Gene name	Fold change	Adjusted *P*	Class
Pyridoxine-5'-phosphate oxidase	PNPO	0.277	0.02874	Down regulated
Transmembrane 9 superfamily member 2	TM9SF2	0.278	0.02867	Down regulated
Leukocyte elastase inhibitor	SERPINB1	0.280	0.03702	Down regulated
radixin	RDX	0.281	0.02384	Down regulated
Annexin A9	ANXA9	0.282	0.02532	Down regulated
Protein TFG	TFG	0.286	0.03499	Down regulated
6-phosphogluconate dehydrogenase, decarboxylating	PGD	0.287	0.04689	Down regulated
Isoform 2 of Apoptosis-associated speck-like protein containing a CARD	PYCARD	0.289	0.03765	Down regulated
Kunitz-type protease inhibitor 2	SPINT2	0.303	0.04310	Down regulated
bifunctional 3'-phosphoadenosine 5'-phosphosulfate synthase 2	PAPSS2	0.305	0.04529	Down regulated
xaa-Pro dipeptidase	PEPD	0.305	0.04867	Down regulated
Cytochrome c oxidase subunit 5A, mitochondrial	COX5A	3.288	0.04780	Up regulated
Plastin-3	PLS3	3.575	0.04114	Up regulated
Acetyl-CoA acetyltransferase, mitochondrial	ACAT1	3.587	0.03298	Up regulated
60S ribosomal protein L18a	RPL18A	3.621	0.04336	Up regulated
4F2 cell-surface antigen heavy chain	SLC3A2	3.720	0.03228	Up regulated
Asparagine synthetase [glutamine-hydrolyzing]	ASNS	3.845	0.01246	Up regulated
AFG3-like protein 2	AFG3L2	3.886	0.01646	Up regulated
Sorbitol dehydrogenase	SORD	3.924	0.03284	Up regulated
Pyrroline-5-carboxylate reductase 1, mitochondrial	PYCR1	3.940	0.02282	Up regulated
Keratin, type II cytoskeletal 80	KRT80	4.009	0.00858	Up regulated
Serine/threonine-protein kinase VRK1	VRK1	4.052	0.04063	Up regulated
Deoxyuridine 5'-triphosphate nucleotidohydrolase, mitochondrial	DUT	4.067	0.03752	Up regulated
Procollagen-lysine, 2-oxoglutarate 5-dioxygenase 3	PLOD3	4.068	0.01878	Up regulated
annexin A6	ANXA6	4.175	0.01572	Up regulated
NADH dehydrogenase [ubiquinone] iron-sulfur protein 8, mitochondrial	NDUFS8	4.178	0.04021	Up regulated
large neutral amino acids transporter small subunit 1	SLC7A5	4.243	0.01413	Up regulated
dehydrogenase/reductase SDR family member 2, mitochondrial	DHRS2	4.270	0.03765	Up regulated
Adenylate kinase 4, mitochondrial	AK4	4.430	0.02456	Up regulated

(Continued)

Table 2 (continued)

Protein annotation	Gene name	Fold change	Adjusted *P*	Class
methionine synthase	MTR	4.487	0.02572	Up regulated
Nucleoporin p58/p45	NUP58	4.494	0.02573	Up regulated
Fatty acyl-CoA reductase 1	FAR1	4.620	0.01814	Up regulated
Ethanolaminephosphotransferase 1	SELENOI	4.786	0.02142	Up regulated
protein S100-A14	S100A14	5.169	0.01092	Up regulated
Serine/threonine-protein kinase MRCK gamma	CDC42BPG	5.185	0.02472	Up regulated
apolipoprotein L2	APOL2	5.274	0.03524	Up regulated
60S ribosomal protein L35a	RPL35A	5.445	0.00557	Up regulated
Nucleobindin-2	NUCB2	5.497	0.00201	Up regulated
lysosomal Pro-X carboxypeptidase	PRCP	5.693	0.00791	Up regulated
Zinc transporter SLC39A7	SLC39A7	5.703	0.01961	Up regulated
Argininosuccinate synthase	ASS1	5.778	0.00133	Up regulated
Oxysterol-binding protein-related protein 3	OSBPL3	5.811	0.02508	Up regulated
NADH dehydrogenase [ubiquinone] 1 subunit C2	NDUFC2	5.834	0.01271	Up regulated
60S ribosomal protein L22-like 1	RPL22L1	5.865	0.02533	Up regulated
neutral amino acid transporter A	SLC1A4	6.168	0.01528	Up regulated
ADP/ATP translocase 1	SLC25A4	6.234	0.00071	Up regulated
Astrocytic phosphoprotein PEA-15	PEA15	6.348	0.00333	Up regulated
Solute carrier family 2, facilitated glucose transporter member 1	SLC2A1	6.359	0.00047	Up regulated
Isoform 2B of GTPase KRas	KRAS	6.393	0.03693	Up regulated
Galectin-3-binding protein	LGALS3BP	6.512	0.00047	Up regulated
Clusterin	CLU	6.610	0.00061	Up regulated
Sodium-and chloride-dependent taurine transporter	SLC6A6	6.721	0.02539	Up regulated
Inactive glutathione hydrolase 2	GGT2	6.917	0.00404	Up regulated
THO complex subunit 6 homolog	THOC6	6.982	0.00478	Up regulated
Titin	TTN	6.999	0.01441	Up regulated
Syntaxin-18	STX18	7.128	0.00838	Up regulated
Microsomal glutathione S-transferase 3	MGST3	7.687	0.00057	Up regulated
Cytochrome c oxidase assembly factor 3 homolog, mitochondrial	COA3	7.892	0.00160	Up regulated
ER lumen protein-retaining receptor 1	KDELR1	8.023	0.00163	Up regulated
Polyhomeotic-like protein 3	PHC3	8.241	0.01251	Up regulated
2'-5'-oligoadenylate synthase 2	OAS2	8.248	<0.00001	Up regulated
peroxisomal multifunctional enzyme type 2	HSD17B4	8.617	0.00650	Up regulated
	OXCT1	9.063	0.00015	Up regulated

(Continued)

Table 2 (continued)

Protein annotation	Gene name	Fold change	Adjusted *P*	Class
Succinyl-CoA: 3-ketoacid coenzyme A transferase 1, mitochondrial				
Ubiquitin/ISG15-conjugating enzyme E2 L6	UBE2L6	12.466	<0.00001	Up regulated
NADH-ubiquinone oxidoreductase chain 2	MT-ND2	13.926	<0.00001	Up regulated
Protein S100-A16	S100A16	14.091	<0.00001	Up regulated
Creatine kinase U-type, mitochondrial	CKMT1A; CKMT1B	14.299	<0.00001	Up regulated
Reticulocalbin-1	RCN1	17.306	<0.00001	Up regulated

3.6 Clinicopathological Characteristics of Patients

The proteomic analyses of MCF-7/TAM cells showed that MYC protein might be one of the most significantly therapeutic target protein for ASA combined with tamoxifen. Our previous work had demonstrated that MYC protein could enhance tamoxifen resistance *in vitro* experiment, we further analyzed the expression of MYC in tumor specimens of patients beginning or continuing long-term tamoxifen treatment. Between October 2019 to March 2022, sixty-eight patients were enrolled in Peking University Shenzhen Hospital. Data from patients within the an age range of 25–86 years (median age: 50.02 ± 13.58 years) were enrolled for this analysis. The pathological types were all invasive BC with ER positive. HER2 was positive in 14 (20.59%) of the 68 cases. There were 23 cases in clinical stage I (71.43%), 41 cases in clinical stage II (60.29%), three cases in clinical stage III (4.41%), and one case in clinical stage IV (1.48%). Also, 18 patients were luminal A (26.47%), 15 patients were luminal B HER2-positive (22.06%), and 35 patients were luminal B HER2-negative (51.47%). Of the 68 luminal-like invasive BC cases, 21 (30.88%) were positive for MYC protein expression, while 47 (69.12%) were negative for MYC protein (Table 3 and Fig. 7).

Table 3: Clinicopathological data and MYC protein expression in patients

Characteristics	N	Percentage (%)
Age		
≤35 year	8	11.76
>35 year	60	88.24
Menopausal status		
Premenopausal	40	58.82
postmenopause	28	41.18
Tumor category		
T1	34	50.0
T2	32	47.06
T3	2	2.94
Lymph node status		
N0	48	70.59
N1	17	25.0

(Continued)

Table 3 (continued)

Characteristics	N	Percentage (%)
N2	1	1.47
N3	2	2.94
HER2		
positive	14	20.59
negative	54	79.41
Ki67		
≤20%	24	35.29
>20%	44	64.71
Histologic grading		
I	8	11.76
II	38	55.88
III	22	32.36
Vessel carcinoma embolus		
positive	14	20.59
negative	54	79.41
Nerve invasion		
positive	9	13.24
negative	59	86.76
Clinical stages		
I	23	33.82
II	41	60.29
III	3	4.41
IV	1	1.48
Molecular subtyping		
luminal A	18	26.47
luminal B HER2 positive	15	22.06
luminal B HER2 negative	35	51.47
MYC		
positive	21	30.88
negative	47	69.12

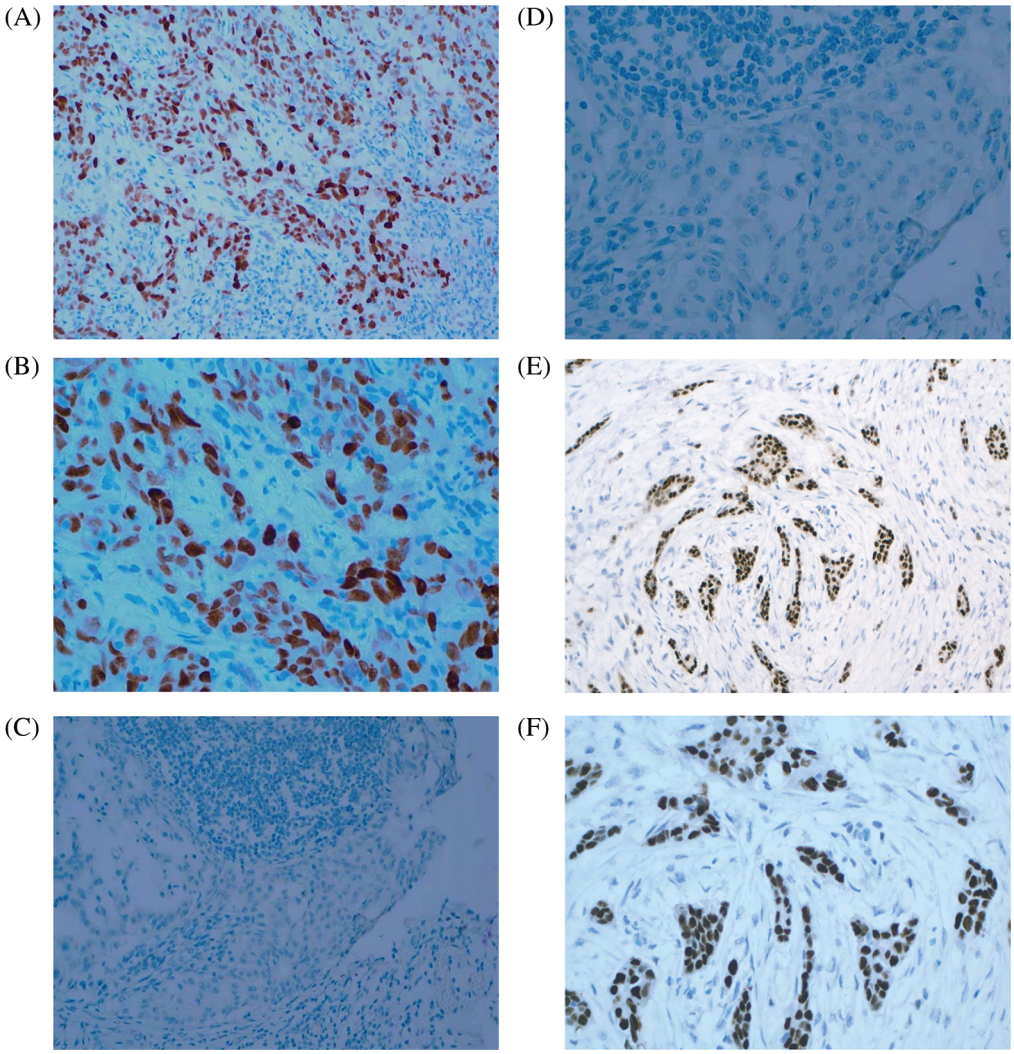

Figure 7: MYC and ER expression in BC tissues. (A) MYC positive (×200 magnification). (B) MYC positive (×400 magnification). (C) MYC negative (×200 magnification). (D) MYC negative (×400 magnification). (E) ER positive (×200 magnification). (F) ER positive (×400 magnification)

3.7 Associations between MYC and Clinical Markers

The MYC protein was expressed in different types of ER-positive BC tissues, with the highest expression in luminal B HER2-negative (57.14%), followed by luminal B HER2-positive BC (33.33%); whereas the lowest expression was in luminal A BC (9.53%). In patients with BC having Ki67 > 20% (40.91%), the proportion of MYC-positive cases was significantly higher ($P < 0.05$) than that of those having Ki67 ≤ 20% (12.50%). However, among the other subgroups, there was no significant difference in MYC protein expression ($P > 0.05$), as shown in Table 4.

Table 4: MYC expression in different clinicopathological types of ER-positive BC patients

Characteristics	MYC positive (N)	MYC negative (N)	$\chi 2$	*P*
Age				
≤35 year	3	5	0.17	0.67
>35year	18	42		
Menopausal status				
Premenopausal	12	27	0.01	0.98
postmenopause	9	20		
Tumor category				
T1	8	26	1.86	0.39
T2	12	20		
T3	1	1		
Lymph node status				
N0	12	36	4.20	0.24
N1	7	10		
N2	1	0		
N3	1	1		
HER2				
positive	7	8	2.25	0.13
negative	14	39		
Ki67				
≤20%	3	21	5.87	0.015
>20%	18	26		
Histologic grading				
I	2	7	3.86	0.15
II	12	15		
III	7	25		
Vessel carcinoma embolus				
positive	7	9	1.62	0.20
negative	14	38		
Nerve invasion				
positive	5	6	1.31	0.25
negative	16	41		
Clinical stages				
I	7	16	7.48	0.06
II	11	30		

(Continued)

Table 4 (continued)

Characteristics	MYC positive (N)	MYC negative (N)	$\chi 2$	*P*
III	3	0		
IV	0	1		
Molecular subtyping				
luminal A	2	16	5.99	0.05
luminal B HER2 positive	7	8		
luminal B HER2 negative	12	23		

4 Discussion

In recent years, the continuous emergence of anti-cancer studies on ASA has enabled the traditional drug ASA to have important new functions. As a result, the potential application of ASA in colorectal cancer, BC, and other solid tumors has been developed [29,30]. Inflammation in the tumor microenvironment is one of the factors that promote tumor growth. A large number of epidemiological evidence suggests that ASA, which suppresses inflammation, reduces the risk of cancer [31]. ASA exerts its anti-inflammatory effects mainly by inhibiting cyclooxygenase (COX), which is a key enzyme in prostaglandin (PG) biosynthesis from arachidonic acid (AA). The two major isoforms of COX (COX-1 and COX-2) catalyze the conversion of AA to prostaglandins, which are metabolized into different prostaglandins by tissue-specific synthetase. ASA inhibits COX-1 and COX-2 activity, the latter of which is thought to be an important factor in the transition of inflammation to tumorigenesis. Another important effect of ASA is antiplatelet agglutination, which improves blood circulation by antagonizing platelet agglutination. A recent study found that ASA inhibits platelet aggregation, preventing cancer cells from taking advantage of platelet adhesion properties to combine with other cells, thus reducing the ability of tumors to metastasize. The clinical results of ASA in BC remain controversial. In some studies, low dose of ASA was associated with a reduced risk of BC, especially for ER-positive and HER2-negative subtypes, without an association with ER-negative BC [32,33]. This study included nine high-quality clinical trials. Due to the high heterogeneity of the studies, the conclusions are controversial. For instance, Zhang et al. [28] and Holmes et al. [24] thought ASA does not affect BC, whereas other researchers, such as Shiao et al. [26] made the opposite conclusion. The meta-analysis found that ASA could reduce the death rate of patients and improve the prognosis of patients compared with the non-ASA group. These results indicated that ASA had certain clinical effect on improving the prognosis of BC patients. Further experiments *in vitro* showed that ASA with 4-OHT could significantly inhibit the proliferation of tamoxifen resistance cells. Studies on the reversal of drug resistance of other tumors by ASA showed that cisplatin plus ASA significantly reduced the survival rate of cisplatin-resistant tumor cells, and ASA could inhibit the expression of tumor cell-related proteins, such as ALDH1, CD44, CD133, and P53 [34,35]. ASA reduced the growth and invasion of pancreatic ductal adenocarcinoma and significantly enhanced the therapeutic effect of gemcitabine. These findings were confirmed in tissue samples from patients who had taken ASA or not before surgery [36]. Therefore, combined with the previous experimental results, this study further explored the changes in cellular proteins related to the tumor-sensitizing effect of ASA to tamoxifen treatment.

Proteomic analysis is important in the comprehensive study of human biology, and the biological processes involved in complex diseases, including tumors, can be identified by combining proteomic and bioinformatics tools [37,38]. Systems biology tools for data analysis are often used to interpret results to define the biological significance of differentially abundant proteins. Quantitative proteomics technology

combined with bioinformatics was used to identify tamoxifen resistance-associated proteins. According to the proteomic profiling of the BC cell lines understudied, 60 proteins were up-regulated and 34 proteins were down-regulated in MCF-7/TAM cells. To further analyze the effect and related mechanism of ASA on tamoxifen resistance in BC, the proteins differentially expressed after ASA and 4-OHT combined treatment were further identified from the dysregulated proteins described above. It was found that the expression of proteins, including CALML5, ARRB1, ACAA1, OCIAD2, DNAJC5, PLPP1, and MYC, were upregulated in MCF-7/TAM cells and depressed after the ASA and 4-OHT combined treatment. In our previous research, the MYC gene and protein levels in tamoxifen resistant cells were knocked down using shRNA gene interference technology, which also increased the sensitivity of resistant BC cells to tamoxifen again [17]. The high expression of the MYC protein also brings a high risk to patients with BC, and the MYC protein has been reported as a tumor marker of BC [39]. Based on the Kaplan-Meier Plotter database, researchers conducted survival correlation analysis on 3,951 patients with BC and the results showed that the MYC protein was positively correlated with the survival risk of BC. MYC is also involved in the process of endocrine resistance in BC. Compared with non-endocrine drug-resistant cells, it was found that the MYC protein was overexpressed in all drug-resistant cell lines and that inhibition of the MYC protein resulted in differential blocking of the non-estrogen-dependent proliferation of drug-resistant cells [40]. Among the patients enrolled in this study, the MYC protein was expressed in the highest expression ratio in luminal B HER2 negative patients, while patients with luminal B HER2 negative were the largest proportion of patients receiving endocrine therapy. Therefore, the occurrence of drug resistance should be monitored in long-term endocrine therapy. MYC protein is significantly associated with tumor histological type. MYC protein expression level was higher in medullary carcinoma and lower in lobular carcinoma [41]. We have further analyzed the difference in MYC protein expression among BC tissues with different clinicopathological indicators and found that the positive proportion of the MYC protein was higher in BC tissues with high Ki67 expression. Among the BC tissues with positive MYC protein expression, the proportion of Ki67 > 20% was as high as 85.71%, which was much higher than that of the MYC protein negative group, thus indicating that the MYC protein expression was closely related to tumor cell proliferation. Salicylic acid is the main metabolite of ASA, and it could reduce the MYC protein levels in human colon cancer cells [42]. Human platelets enhance colon cancer cell proliferation by upregulating and activating the MYC protein, and the upregulation of the MYC protein and proliferation of cancer cells are both reversed by ASA against the platelet concentration, thus suggesting that ASA inhibition of platelets may affect cancer cell proliferation by regulating the MYC protein [43].

In this study, here we also found that some proteins as ASS1, OXCT1, RCN1, DUT, and GGT2 were downregulated in MCF-7/TAM cells and recovered after ASA and 4-OHT combined treatment. ASS1 is a key enzyme in arginine biosynthesis, and its abundance is reduced in many tumors. In a random sample of 149 BCs, ASS1 was low or undetected in more than 60% of the patients independent molecular subtype [44]. In former researches, ASS1 had been reported as a tumor suppressor, and a lack of ASS1 in cancer was found to induce arginine auxotrophy [45–47]. In this study, the combination of ASA and 4-OHT increased the expression level of ASS1. Genetic manipulation studies of ASS1 had confirmed that ASS1 expression inhibited fibroblast proliferation, migration, and invasion. OXCT1 was also recovered after ASA and 4-OHT combined treatment in MCF-7/TAM cells. Epigenetic silencing of OXCT1 in ovarian cancer was confirmed to be associated with cisplatin resistance. Overexpression of OXCT1 restored sensitivity to cisplatin chemotherapy, suggesting that OXCT1 is a resistant repressor in cancer [48].

In conclusion, our data provide the necessary evidence that ASA may have a certain effect on improving the prognosis of BC patients, and tamoxifen resistant of ER-positive BC cells can be reversed through ASA and 4-OHT combined treatment. Several proteins were downregulated or activated involved in ASA and 4-

OHT combined treatment, which might be identified as potential targets for the treatment of ER-positive BC with tamoxifen resistance. However, the limitations of this study may be the limited data from animal intervention studies and the long-term follow-up of participants, and further research is needed in the future.

Ethical Approval and Informed Consent Statement: Written informed consents were obtained from all participants, and this study was permitted by the Ethics Committee of Peking University Shenzhen Hospital (No. 2021-037. Approval Date: 2021.03.04).

Author Contributions: All authors contributed to the study design. Y.L. conceptualized and designed this study. J.C. and M.L. performed the cell culture, sample preparation experiments and immunohistochemistry. J.C. and R.P. performed the meta-analysis, proteomic experiments and analyzed the data. J.C. and R.P. wrote the draft of the manuscript. M.L. and Y.L. revised the manuscript. All author have read and approved the final manuscript.

Availability of Data and Materials: The dataset supporting the conclusions of this article is included within this article and is available from the corresponding author upon request.

Funding Statement: This work was supported by Shenzhen Key Medical Discipline Construction Fund (No. SZXK017), Shenzhen High-Level Hospital Construction Fund.

Conflicts of Interest: The authors declare that they have no conflicts of interest to report regarding the present study.

References

1. Leon-Ferre, R. A., Polley, M. Y., Liu, H., Gilbert, J. A., Cafourek, V. et al. (2018). Impact of histopathology, tumor-infiltrating lymphocytes, and adjuvant chemotherapy on prognosis of triple-negative breast cancer. *Breast Cancer Research and Treatment, 167(1),* 89–99. DOI 10.1007/s10549-017-4499-7.
2. Harbeck, N., Gnant, M. (2017). Breast cancer. *Lancet, 389(10074),* 1134–1150. DOI 10.1016/S0140-6736(16)31891-8.
3. Li, Y., Kong, X., Xuan, L., Wang, Z., Huang, Y. H. (2021). Prolactin and endocrine therapy resistance in breast cancer: The next potential hope for breast cancer treatment. *Journal of Cellular and Molecular Medicine, 25(22),* 10327–10348. DOI 10.1111/jcmm.16946.
4. Paganini-Hill, A., Clark, L. J. (2000). Preliminary assessment of cognitive function in breast cancer patients treated with tamoxifen. *Breast Cancer Research and Treatment, 64(2),* 165–176. DOI 10.1023/A:1006426132338.
5. Araki, K., Miyoshi, Y. (2018). Mechanism of resistance to endocrine therapy in breast cancer: The important role of PI3K/Akt/mTOR in estrogen receptor-positive, HER2-negative breast cancer. *Breast Cancer, 25(4),* 392–401. DOI 10.1007/s12282-017-0812-x.
6. Caffa, I., Spagnolo, V., Vernieri, C., Valdemarin, F., Becherini, P. et al. (2020). Fasting-mimicking diet and hormone therapy induce breast cancer regression. *Nature, 583(7817),* 620–624. DOI 10.1038/s41586-020-2502-7.
7. Bekele, R. T., Venkatraman, G., Liu, R. Z., Tang, X., Mi, S. et al. (2016). Oxidative stress contributes to the tamoxifen-induced killing of breast cancer cells: Implications for tamoxifen therapy and resistance. *Scientific Reports, 6,* 21164. DOI 10.1038/srep21164.
8. Toy, W., Weir, H., Razavi, P., Lawson, M., Goeppert, A. U. et al. (2017). Activating ESR1 mutations differentially affect the efficacy of ER antagonists. *Cancer Discovery, 7(3),* 277–287. DOI 10.1158/2159-8290.CD-15-1523.
9. Cronin-Fenton, D. P., Damkier, P. (2018). Tamoxifen and CYP2D6: A controversy in pharmacogenetics. *Advances in Pharmacology, 83,* 65–91. DOI 10.1016/bs.apha.2018.03.001.
10. Tryfonidis, K., Zardavas, D., Katzenellenbogen, B. S., Piccart, M. (2016). Endocrine treatment in breast cancer: Cure, resistance and beyond. *Cancer Treatment Reviews, 50,* 68–81. DOI 10.1016/j.ctrv.2016.08.008.
11. Burn, J., Sheth, H., Elliott, F., Reed, L., Macrae, F. et al. (2020). Cancer prevention with aspirin in hereditary colorectal cancer (Lynch syndrome), 10-year follow-up and registry-based 20-year data in the CAPP2 study: A

double-blind, randomised, placebo-controlled trial. *Lancet, 395(10240),* 1855–1863. DOI 10.1016/S0140-6736 (20)30366-4.

12. Assayag, J., Pollak, M. N., Azoulay, L. (2015). The use of aspirin and the risk of mortality in patients with prostate cancer. *The Journal of Urology, 193(4),* 1220–1225. DOI 10.1016/j.juro.2014.11.018.
13. Matsuo, K., Cahoon, S. S., Yoshihara, K., Shida, M., Kakuda, M. et al. (2016). Association of low-dose aspirin and survival of women with endometrial cancer. *Obstetrics and Gynecology, 128(1),* 127–137. DOI 10.1097/ AOG.0000000000001491.
14. Xie, S., Wang, Y., Huang, Y., Yang, B. (2021). Mechanisms of the antiangiogenic effects of aspirin in cancer. *European Journal of Pharmacology, 898,* 173989. DOI 10.1016/j.ejphar.2021.173989.
15. Chen, W. Y., Holmes, M. D. (2017). Role of aspirin in breast cancer survival. *Current Oncology Reports, 19(7),* 48. DOI 10.1007/s11912-017-0605-6.
16. Ma, S., Guo, C., Sun, C., Han, T., Zhang, H. et al. (2021). Aspirin use and risk of breast cancer: A meta-analysis of observational studies from 1989 to 2019. *Clinical Breast Cancer, 21(6),* 552–565. DOI 10.1016/j. clbc.2021.02.005.
17. Cheng, R., Liu, Y. J., Cui, J. W., Yang, M., Liu, X. L. et al. (2017). Aspirin regulation of MYC and cyclind1 proteins to overcome tamoxifen resistance in estrogen receptor-positive breast cancer cells. *Oncotarget, 8(18),* 30252–30264. DOI 10.18632/oncotarget.16325.
18. Wang, D., Eraslan, B., Wieland, T., Hallström, B., Hopf, T. et al. (2019). A deep proteome and transcriptome abundance atlas of 29 healthy human tissues. *Molecular Systems Biology, 15(2),* e8503. DOI 10.15252/ msb.20188503.
19. Martínez-Rodríguez, F., Limones-González, J. E., Mendoza-Almanza, B., Esparza-Ibarra, E. L., Gallegos-Flores, P. I. et al. (2021). Understanding cervical cancer through proteomics. *Cells, 10(8),* 1854. DOI 10.3390/ cells10081854.
20. Bardia, A., Keenan, T. E., Ebbert, J. O., Lazovich, D., Wang, A. H. et al. (2016). Personalizing aspirin use for targeted breast cancer chemoprevention in postmenopausal women. *Mayo Clinic Proceedings, 91(1),* 71–80. DOI 10.1016/j.mayocp.2015.10.018.
21. Barron, T. I., Murphy, L. M., Brown, C., Bennett, K., Visvanathan, K. et al. (2015). *De novo* post-diagnosis aspirin use and mortality in women with stage I-III breast cancer. *Cancer Epidemiology, Biomarkers & Prevention, 24(6),* 898–904. DOI 10.1158/1055-9965.EPI-14-1415.
22. Frisk, G., Ekberg, S., Lidbrink, E., Eloranta, S., Sund, M. et al. (2018). No association between low-dose aspirin use and breast cancer outcomes overall: A Swedish population-based study. *Breast Cancer Research, 20(1),* 142. DOI 10.1186/s13058-018-1065-0.
23. Bens, A., Friis, S., Dehlendorff, C., Jensen, M. B., Ejlertsen, B. et al. (2018). Low-dose aspirin use and risk of contralateral breast cancer: A danish nationwide cohort study. *Preventive Medicine, 116,* 186–193. DOI 10.1016/j.ypmed.2018.09.015.
24. Holmes, M. D., Olsson, H., Pawitan, Y., Holm, J., Lundholm, C. et al. (2014). Aspirin intake and breast cancer survival-A nation-wide study using prospectively recorded data in Sweden. *BMC Cancer, 14,* 391. DOI 10.1186/1471-2407-14-391.
25. Bradley, M. C., Black, A., Freedman, A. N., Barron, T. I. (2016). Prediagnostic aspirin use and mortality in women with stage I to III breast cancer: A cohort study in the prostate, lung, colorectal, and ovarian cancer screening trial. *Cancer, 122(13),* 2067–2075. DOI 10.1002/cncr.30004.
26. Shiao, J., Thomas, K. M., Rahimi, A. S., Rao, R., Yan, J. et al. (2017). Aspirin/antiplatelet agent use improves disease-free survival and reduces the risk of distant metastases in stage II and III triple-negative breast cancer patients. *Breast Cancer Research and Treatment, 161(3),* 463–471. DOI 10.1007/s10549-016-4081-8.
27. Williams, A. D., Li, Y. R., So, A., Steel, L., Carrigan, E. et al. (2018). The impact of aspirin use on breast cancer subtype and clinical course. *The Journal of Surgical Research, 230,* 71–79. DOI 10.1016/j.jss.2018.04.040.

28. Zhang, S. M., Cook, N. R., Manson, J. E., Lee, I. M., Buring, J. E. (2008). Low-dose aspirin and breast cancer risk: Results by tumour characteristics from a randomised trial. *British Journal of Cancer, 98(5),* 989–991. DOI 10.1038/sj.bjc.6604240.
29. Yang, L., Lv, Z., Xia, W., Zhang, W., Xin, Y. et al. (2018). The effect of aspirin on circulating tumor cells in metastatic colorectal and breast cancer patients: A phase II trial study. *Clinical & Translational Oncology, 20(7),* 912–921. DOI 10.1007/s12094-017-1806-z.
30. Coyle, C., Cafferty, F. H., Rowley, S., MacKenzie, M., Berkman, L. et al. (2016). ADD-ASPIRIN: A phase III, double-blind, placebo controlled, randomised trial assessing the effects of aspirin on disease recurrence and survival after primary therapy in common non-metastatic solid tumours. *Contemporary Clinical Trials, 51,* 56–64. DOI 10.1016/j.cct.2016.10.004.
31. Gilligan, M. M., Gartung, A., Sulciner, M. L., Norris, P. C., Sukhatme, V. P. et al. (2019). Aspirin-triggered proresolving mediators stimulate resolution in cancer. *Proceedings of the National Academy of Sciences, 116(13),* 6292–6297. DOI 10.1073/pnas.1804000116.
32. Clarke, C. A., Canchola, A. J., Moy, L. M., Neuhausen, S. L., Chung, N. T. et al. (2017). Regular and low-dose aspirin, other non-steroidal anti-inflammatory medications and prospective risk of HER2-defined breast cancer: The california teachers study. *Breast Cancer Research, 19(1),* 52. DOI 10.1186/s13058-017-0840-7.
33. Bardia, A., Olson, J. E., Vachon, C. M., Lazovich, D., Vierkant, R. A. et al. (2011). Effect of aspirin and other NSAIDs on postmenopausal breast cancer incidence by hormone receptor status: Results from a prospective cohort study. *Breast Cancer Research and Treatment, 126(1),* 149–155. DOI 10.1007/s10549-010-1074-x.
34. Zhao, M., Wang, T., Hui, Z. (2020). Aspirin overcomes cisplatin resistance in lung cancer by inhibiting cancer cell stemness. *Thoracic Cancer, 11(11),* 3117–3125. DOI 10.1111/1759-7714.13619.
35. Guo, J., Zhu, Y., Yu, L., Li, Y., Guo, J. et al. (2021). Aspirin inhibits tumor progression and enhances cisplatin sensitivity in epithelial ovarian cancer. *PeerJ, 9,* e11591. DOI 10.7717/peerj.11591.
36. Zhang, Y., Liu, L., Fan, P., Bauer, N., Gladkich, J. et al. (2015). Aspirin counteracts cancer stem cell features, desmoplasia and gemcitabine resistance in pancreatic cancer. *Oncotarget, 6(12),* 9999–10015. DOI 10.18632/oncotarget.3171.
37. Aslam, B., Basit, M., Nisar, M. A., Khurshid, M., Rasool, M. H. (2017). Proteomics: Technologies and their applications. *Journal of Chromatographic Science, 55(2),* 182–196. DOI 10.1093/chromsci/bmw167.
38. Nusinow, D. P., Szpyt, J., Ghandi, M., Rose, C. M., McDonald, E. R. et al. (2020). Quantitative proteomics of the cancer cell line encyclopedia. *Cell, 180(2),* 387–402.e16. DOI 10.1016/j.cell.2019.12.023.
39. Constantinou, C., Papadopoulos, S., Karyda, E., Alexopoulos, A., Agnanti, N. et al. (2018). Expression and clinical significance of claudin-7, PDL-1, PTEN, c-kit, c-met, MYC, ALK, CK5/6, CK17, p53, EGFR, ki67, p63 in triple-negative breast cancer-A single centre prospective observational study. *In Vivo, 32(2),* 303–311.
40. Sengupta, S., Biarnes, M. C., Jordan, V. C. (2014). Cyclin dependent kinase-9 mediated transcriptional de-regulation of cMYC as a critical determinant of endocrine-therapy resistance in breast cancers. *Breast Cancer Research and Treatment, 143(1),* 113–124. DOI 10.1007/s10549-013-2789-2.
41. Green, A. R., Aleskandarany, M. A., Agarwal, D., Elsheikh, S., Nolan, C. C. et al. (2016). MYC functions are specific in biological subtypes of breast cancer and confers resistance to endocrine therapy in luminal tumours. *British Journal of Cancer, 114(8),* 917–928.
42. Ai, G., Dachineni, R., Muley, P., Tummala, H., Bhat, G. J. (2016). Aspirin and salicylic acid decrease MYC expression in cancer cells: A potential role in chemoprevention. *Tumour Biology, 37(2),* 1727–1738.
43. Mitrugno, A., Sylman, J. L., Ngo, A. T., Pang, J., Sears, R. C. et al. (2017). Aspirin therapy reduces the ability of platelets to promote colon and pancreatic cancer cell proliferation: Implications for the oncoprotein MYC. *American Journal of Physiology. Cell Physiology, 312(2),* C176–C189.
44. Qiu, F., Chen, Y. R., Liu, X., Chu, C. Y., Shen, L. J. et al. (2014). Arginine starvation impairs mitochondrial respiratory function in ASS1-deficient breast cancer cells. *Science Signaling, 7(319),* ra31.
45. Li, J. M., Yang, D. C., Oldham, J., Linderholm, A., Zhang, J. et al. (2021). Therapeutic targeting of argininosuccinate synthase 1 (ASS1)-deficient pulmonary fibrosis. *Molecular Therapy, 29(4),* 1487–1500.

46. Trott, J. F., Hwang, V. J., Ishimaru, T., Chmiel, K. J., Zhou, J. X. et al. (2018). Arginine reprogramming in ADPKD results in arginine-dependent cystogenesis. *American Journal of Physiology. Renal Physiology, 315(6),* F1855–F1868.
47. Patil, M. D., Bhaumik, J., Babykutty, S., Banerjee, U. C., Fukumura, D. (2016). Arginine dependence of tumor cells: Targeting a chink in cancer's armor. *Oncogene, 35(38),* 4957–4972.
48. Yang, S. D., Ahn, S. H., Kim, J. I. (2018). 3-Oxoacid CoA transferase 1 as a therapeutic target gene for cisplatin-resistant ovarian cancer. *Oncology Letters, 15(2),* 2611–2618.