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Research Article

LC-MS/MS-based proteomics and metabolomics of HCT-116 colorectal cancer cells: A potential anticancer activity of atorvastatin

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Abstract

Colorectal cancer (CRC) is the third most prevalent tumor in men, the second most common in women, and the fourth leading cause of mortality worldwide. Statins reduce cholesterol levels by hampering the function of 3-hydroxy-3-methyl-glutaryl-CoA reductase enzymes in cholesterol synthesis. Strains have shown anticancer effects against CRC. However, statins' anticancer mechanism is yet unknown. Liquid chromatography–mass spectrometry (LC–MS)-based untargeted metabolomics and proteomics were employed to study statins' effects on CRC using the CRC cell line HCT-116. These approaches were utilized to identify potential underlying metabolic pathways and proteins altered in atorvastatin (a statin)-treated HCT-116 cells. Compared to the control, atorvastatin significantly altered numerous metabolites in HCT-116 cells, including a reduction in the levels of decanoylcarnitine and octanoyl-L-carnitine and in the biosynthesis and metabolism of amino acids like alanine and citrate cycle. Proteomic study showed that atorvastatin-treated HCT-116 cells expressed 127 proteins differently from controls. Novel findings were among them, such as centromere-associated protein E, cytochrome c oxidase subunit 6A1 mitochondrial, and hyaluronan synthase 1. The findings indicate that atorvastatin may have potential anticancer characteristics and highlight the essential role metabolomics and proteomics to understand complex metabolic pathways and proteins relevant to cancer and develop novel treatment targets.

Keywords

colorectal cancer, statin, atorvastatin, proteomics, metabolomics, LC-MS

Introduction

Colorectal cancer (CRC) is considered the third most prevalent cancer among both genders (WHO 2024). It represents approximately 10% of all cancers diagnosed annually (WHO 2024).

Environmental and genetic factors have been described to play a significant role in disease development. It appears that positive family history had a part in about 10–20% of all colorectal patients' cancers, with risk varying based on the number and the level of relatives affected and the range of age diagnosed with CRC (Sung et al. 2005). CRC can be categorized as inherited, sporadic, and familial based on the original mutation (Mármol et al. 2017). Mutations in particular genes can also mediate the initiation of CRC; it can occur in oncogenes, tumor suppressor genes, and genes relevant to DNA repair systems (Kheirelseid et al. 2013).

Despite the excessive research and advances in the treatment modalities of CRC, including radiation, surgical excision, chemotherapy, and molecular targeted therapeutics, CRC is the second most common cause of cancer-related death and one of the most prevalent malignancies linked with significant morbidity and mortality rates (Dekker et al. 2019). The primary reasons for this include the lack of effective screening programs and treatment approaches, alongside rising incidence rates and growing resistance to drugs (Xi and Xu 2021; Dahabiyeh et al. 2023a).

Statins are 3- hydroxy- 3- methyl- glutaryl- CoA (HMG-CoA) inhibitors that are primarily utilized to treat hypercholesterolemia and to reduce cerebrovascular morbidity and mortality (Dobrzycka et al. 2020). The first synthetic statin that reached clinical trials was atorvastatin, a lipophilic statin (Roth 2002). Different studies have demonstrated the antitumor properties of statins in a variety of tumor types (Min et al. 2022; Takada et al. 2022). In recent years, multifaceted novel actions for statins have been reported, such as anti-inflammatory, anti-proliferative, pro-apoptotic, and anti-invasive using several models of studies (Dulak and Józkowicz 2005; Walther et al. 2016; Yokohama et al. 2016). Statins' effectiveness as anticancer medicines has been assessed in both monotherapy and combination treatment with chemotherapeutic medications already used in several clinical trials (Fatehi Hassanabad 2019; Zaky et al. 2023). In addition, integrating both experimental and computational studies has demonstrated the anti-metastatic effects of statins on metastatic solid tumors (Buccioli et al. 2024). However, obtaining comprehensive molecular signatures of statins in cancer cells is still necessary to elucidate the exact molecular mechanism of action behind its anticancer effect. Moreover, further investigations into the molecular mechanisms influencing statin sensitivity in various cancer types are still required to move toward personalized treatment and identify biomarkers of statin-sensitive tumors.

Metabolites and proteins are the downstream biochemical products of the genome and significant determinants of cellular phenotype (Qiu et al. 2023). Disturbances in the level of metabolites and proteins in CRC cells were found associated with the pathophysiology of the disease (Hanahan and Weinberg 2011). Metabolomics is an advanced, robust analytical method for examining a class of small molecules (less than 1500 Da) in cells, biofluids, tissues, and organisms like amino acids, lipids, and carbohydrates (Dahabiyeh and Nimer 2023). Untargeted metabolomics aims to provide a broad overview of metabolism, helping to uncover metabolic abnormalities that could elucidate disease mechanisms and reveal potential diagnostic and therapeutic markers (Dahabiyeh et al. 2023b). Multiple studies have utilized metabolomics to explore the anticancer effects of statins but not atorvastatin (McGregor et al. 2020; Fernandes Silva et al. 2022; Warita et al. 2023).

Proteomics is a systematic analysis of proteins expressed by a genome (Tyers and Mann 2003). It is considered a powerful approach for unraveling the molecular basis of disease and identifying novel therapeutic strategies (Nimer et al. 2023). Proteomics was utilized to assess the anticancer effects of statins in several tumor cell lines related to esophageal squamous cell carcinoma (ESCC), pancreatic, and CRC (Lee et al. 2014; Yuan et al. 2019; Dorsch et al. 2021).

Omics integration has the potential to find new biomarkers for monitoring the effectiveness of treatment and find new targets for therapy, leading to a better understanding of CRC via molecular phenotyping (Zhan et al. 2018; Zafari et al. 2023). Accordingly, potential anti-cancer activity of atorvastatin has been explored on CRC cell line (HCT-116) using LC-MS-based proteomics and metabolomics approaches.

Materials and methods

Drug preparation

Atorvastatin calcium salt trihydrate was purchased as 25 mg lyophilized powder from Santa Cruz Biotechnology, USA, and stored at -20 °C until used. The 8 mg of drug was dissolved in 661 µL of dimethyl sulfoxide (DMSO) (Santa Cruz Biotechnology, USA) to prepare a stock solution of 10 mM. Then the drug solution was filtered by a 0.2 filter paper aliquot and kept at -20 °C until utilized in experiments.

Cell culture

CRC cell line HCT-116 were acquired from the American Type Culture Collection (Rockville, USA) and were grown in Roswell Park Memorial Institute medium (RPMI-1640) with L-glutamine (Euro clone/Italy). The media was enriched with 10% fetal bovine serum (FBS), 100 U/mL penicillin, and 0.1 mg/mL streptomycin (Euroclone, Italy). The cells were kept at 37 °C in a humidified incubator (Euroclone, Italy) with 95% air and 5% CO_2. For the process of subculturing, the cells were rinsed with phosphate-buffered saline (PBS) that did not include calcium

and magnesium ions. The cells were then dislocated using trypsin EDTA (Euro clone, Italy). The dislocated cells were spun in a centrifuge, then resuspended and quantified using a hemocytometer.

Cell viability measurement

HCT-116 cells were seeded at a concentration of 1.0×10^4 cells/well in 96-well plates in 10% FBS RPMI-1640 media, and cells were permitted to adhere over 16 h. After being incubated at 37 °C with 5% CO_2 for 24 h, a range of concentrations were used to treat the cells (0, 5, 10, 20, 30, 40, and 80 μM) of atorvastatin in triplicates, while control cells were exposed to vehicle. Plates were then incubated for 48 h, after which the estimation of cell viability was done by the MTT (3-[4,5-Dimethylthiazol-2-yl]-diphenyltetrazolium Bromide) viability test (HIMEDIA, India). In brief, control and media with treatment were substituted with fresh media, and 100 μL of 0.50 mg/mL stock MTT solution was added to each well. Cells were then kept for 3 h at 37 °C in a humidified incubator.

Following the completion of the incubation time, media were discarded, DMSO was used to dissolve the formazan crystals (100 μL/well for 96-well plates), and the plates were weakly stirred for 10 minutes. The optical density was quantified at 490 nm utilizing a microplate reader (Biotek Epoch, USA).

The average of three separate experiments is shown in the results.

Cell line preparation for omics study

HCT-116 cells were seeded at 1.0×10^6 cells/well in 6-well plates (Euro clone, Italy) in 10% FBS RPMI-1640 media and permitted to adhere over 16 h. Once 80 to 90% confluence was reached, the cells in each well were treated with 10.0 μM atorvastatin and were subjected to vehicle control and treatment conditions for 24 h. Afterward, cells were detached by adding 400 μL of EDTA trypsin in each well. These detached cells were then gathered in 1.5 mL Eppendorf tubes, centrifugated, washed three times, and finally the supernatants were discarded. Seven replicates from treated and control cells were used for the metabolomics study, and three replicates for the proteomics study. Cellular metabolite extraction was performed as detailed previously (Dahabiyeh et al. 2021; Dahabiyeh et al. 2022).

Cell lysis and protein digestion

Cells were dissolved in RIPA buffer (150 mM NaCl, 1% TritonX-100, 0.5% sodium deoxycholate, 0.1% SDS, 50 mM Tris, pH 8.0) and protease phosphatase inhibitors. Cells were homogenized with a sonicator (30 sec × 3 times with a 30-sec interval), and centrifugation was used to eliminate cell debris. Protein concentration was estimated by BCA assay (Pierce), and for each replicate, a uniform amount (10 μg) of protein was precipitated on carboxyl beads as explained earlier (Batth et al. 2019).

Dissolving the precipitated proteins on beads in 50 mM ammonium bicarbonate, reducing and alkylating them, and then digesting them with a 1:50 (w:w) ratio of trypsin (Promega) at 37 °C overnight to complete the digestion process. The tryptic peptides were moved to a fresh tube, acidified, and buffer salts were removed utilizing custom-made C18 stage tips.

LC-MS/MS analysis

Untargeted metabolomics

The dried cell extracts were dissolved in 90 μL methanol:H_2O (2:1, *v/v*), vortexed, and then the samples were subjected to centrifugation at 13,000 *g* at 4 °C for 10 minutes. The upper 60 μL was moved into HPLC vials, and the LC-MS and LC-MS/MS metabolic profiling proceeded as reported previously (Al-Saafin et al. 2023; Hijazi et al. 2023). In brief, the Dionex U3000 UHPLC system coupled with the hybrid quadrupole-orbitrap mass spectrometer (QExactive) (Thermo Fisher Scientific, USA) was utilized to profile the metabolites in the extracted samples. Metabolites were separated on a 4.6 × 150 mm, 5 μm ZIC-*p*HILIC column (Merck Sequant, UK) and the mobile phases were made up of 20 mM ammonium carbonate (A) and acetonitrile (B) (Fisher Scientific, Loughborough, UK) in 15 minutes.

The analysis was conducted using a flow rate of 300 μL/min with gradient elution. The elution process began with 20% A and gradually grew to 95% over a period of 8 minutes. It then returned to its initial 20% A concentration within 2 minutes. Following this, the column was allowed to re-equilibrate at a flow rate of 400 μL/min for 4 minutes before returning to the original flow rate of 300 μL/min in 1 minute. Positive and negative electrospray ionization modes (ESI+, ESI-) and MS/MS spectra were acquired using data-dependent MS/MS (ddMS/MS). The data were obtained using full scan mode with a resolution of 70,000, covering the mass range from m/z 70 to 1050. A standard mixture of in-house 266 standards in a final concentration of 20 μM was also co-analyzed with the samples to aid metabolite identification.

Proteomics

The nanoElute nanoflow ultrahigh-pressure LC system (Bruker Daltonics, Germany) was connected to the timsTOF fleX mass spectrometer, and a CaptiveSpray nanoelectrospray ion source was utilized for the LC-MS/MS analysis.

A capillary C18 column (25 cm length, 75 μm inner diameter, 1.6 μm particle size, 120 Å pore size; IonOpticks, Fitzroy, VIC, AUS) was loaded with 200 ng of peptide digest.

The peptides were separated at 55 °C over a 90-minute gradient with a flow rate of 400 nL/min, using mobile phase A (0.1% formic acid) and mobile phase B (0.1% formic acid in acetonitrile). A gradual increase from 2% to 17% MPB was used over a duration of 57 minutes,

followed by a step from 17% to 25% MPB from 57 to 78 minutes, 25–35% MPB from 78 to 91 minutes, 37–85% MPB from 91 to 94 minutes, and completed with a wash at 85% MPB for another 7 minutes.

A PASEF mode was used for the operation of the timsTOF fleX. Within the range of m/z 100 to 1700, mass spectra for both parent and daughter ions were obtained. The resolution of ion mobility was adjusted to 0.60–1.60 V·s/cm with a ramp duration of 100 ms. Using a duty cycle of nearly 100%, 10 PASEF MS/MS scans were conducted for each cycle for data-dependent acquisition. In order to eliminate the low m/z of monovalent ions for PASEF precursor selection, polygon filtering was implemented in the m/z and ion mobility area. Precursors with an intensity of 20,000 units were subjected to an active exclusion period of 0.4 minutes. The collisional energy was incrementally increased in a stepwise manner, with each step being dependent on the ion mobility.

Data processing and statistical analysis

Untargeted metabolomics

LC-MS and LC-MS/MS datasets of the analyzed samples were pre-processed utilizing Compound Discoverer 3.1 SP1 (Thermo Fisher Scientific, USA) for peak selection, peak alignment, adduct deconvolution, and identifying corresponding peaks. Metabolite identification was achieved by aligning the masses of the measured peaks with the exact mass of metabolites in the Human BioCyc database, comparing the discovered peaks' RT to that of the legitimate standards that were co-analyzed, and/or ddMS/MS fragmentation profiles of the detected peaks with the *mz*Cloud MS/MS spectral database (Thermo Fisher Scientific, USA). Confidence in the metabolites' identification was allocated levels from 1 to 4 (L1-L4) in line with what the chemical analysis working group has recommended and in accordance with the Metabolomics Standards Initiative (MSI) (Sumner et al. 2007). For the present investigation, metabolites that have a very low degree of identification were not considered. Only the metabolites having a high degree of assurance in identification (i.e., L1 and L2) were comprised in this study. The mass characteristics (retention time (RT), m/z) and their quantities after normalization were transferred to Simca 14 (Sartorius Stedim Data Analytics AB, Sweden) for the purpose of multivariate analysis. The imported datasets were scaled according to the Pareto principle and mean-centered. Principal component analysis (PCA), partial least squares-discriminant analysis (PLS-DA), and orthogonal partial least squares-discriminant analysis (OPLS-DA) were utilized to describe the changes amongst the research groups. The goodness of the created models was assessed by monitoring the fitness of the model (R^2X) for PCA, (R^2Y) for PLS-DA and OPLS-DA, and predictive ability (Q^2) values. Models that yield large R^2X and R^2Y (approximately 1) and Q^2 values of ~ 0.5 refer to a robust mode (Worley and Powers 2013). In addition, the OPLS-DA was validated using a permutation test with 100 permutations. Mass ions are behind the distinction between control and treated HCT-116 cancer cell line. The variable importance in projection (VIP) scores from the created OPLS-DA model was utilized to identify the HCT-116 cancer cell line. A VIP score greater than 1.0 is deemed significant for the model (Yin et al. 2009). For univariate analysis, the mass features with their normalized peak areas were processed using MetaboAnalyst 5.0 (Pang et al. 2021). Each dataset was Pareto-scaled and normalized to the median of the whole sample. Statistically significant changes in metabolites between the treated and untreated groups of HCT116 cells were determined utilizing an unpaired two-tailed Student's t-test.

Accounting for the multiple testing problem, the obtained *p*-values were adjusted utilizing the false discovery rate (FDR), where the metabolites of p-value < 0.05 were deemed significant. The metabolites that had an adjusted p-value of < 0.05 in univariate analysis and a VIP (Variable Importance in Projection) score of more than 1.0 in multivariate analysis were identified as probable changed metabolites.

For proteomics

The LC-MS/MS raw data were analyzed using MaxQuant 2.0.1.0 software to identify proteins and perform label-free quantification. The following parameters were set: Methionine oxidation and protein N-acetylation were designated as variable modifications, whereas carbamidomethyl (C) was assigned as a fixed modification. The error windows for the first search and main search are 20 and 6 ppm, respectively. Two miscleavages were permitted when trypsin without the proline restriction enzyme choice was employed. We utilized a threshold of one for minimal unique peptides and an FDR of 1% for protein and peptide identification. A human database, UniProt, was utilized. The FDR rate was determined using reversed sequence generation. The proteinGroup.txt file generated by MaxQuant was imported into the Perseus software (1.6.14.0). Identifications of probable contaminants and inverted sequences were excluded, and the intensities were normalized and translated to a logarithmic scale (log10). Proteins were detected in a minimum of two out of three repeated experiments in at least one set of samples that underwent further examination. The noise values from each sample's normal distribution were utilized to replace all values with zero intensity. A two-sample student's t-test and LFQ intensity measurements were employed to compare protein abundances (p-value < 0.05, S0: 0.1).

Results

Anti-proliferative activity of atorvastatin

The anti-proliferative effect of different atorvastatin concentrations against the HCT-116 cell line is demonstrated in Fig. 1. The IC_{50} value (the concentration of drug that reached 50% cell viability for HCT-116 cells) was 12.0 μM.

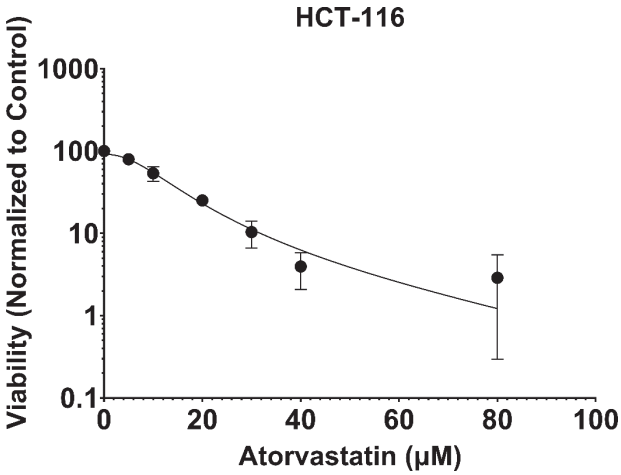

Figure 1. Dose-response curves of atorvastatin against HCT116 cell line. Each concentration point represents the average and SEM of six experimental replicates.

Metabolic profiling of atorvastatin-treated HCT-116 cells using LC-MS

LC-MS metabolic profiling of the cellular extracts of atorvastatin-treated and untreated HCT-116 cells (control) detected 1511 mass ion features of which 257 metabolites were putatively identified.

PCA (R^2X = 0.80, Q^2 = 0.32, Fig. 2a) showed partial separation, whereas PLS-DA (R^2Y = 0.96, Q^2 = 0.70, Fig. 2b) and OPLS-DA (R^2Y = 0.99, Q^2 = 0.80, Fig. 2c) showed a complete separation and clustering of atorvastatin-treated HCT-116 cells compared to control. OPLS-DA was selected for further analysis as it showed a robust cross-validation and permutation test compared to PLS-DA (Suppl. material 1: fig. S1). A total of 445 features were found accountable for the class separation in OPLS-DA (VIP > 1), of which 103 features were identified, including amino acids (e.g., aspartate, glutamine, histidine), 2-oxoglutarate, purine nucleotide, adenine, and adenosine.

In univariate analysis, 58 metabolites were found to be significantly disrupted (adjusted *p*-value < 0.05). A volcano plot applying an adjusted *p*-value < 0.05 and fold change (FC) thresholds of 1.5 showed that 16 and 7 metabolites were down- and upregulated in HCT-116 treated cells versus control, respectively (Fig. 3a). Of these metabolites, 3-hydroxy-3-methylglutaric acid and malate were upregulated, whereas decanoyl-carnitine, octanoyl-L-carnitine, and palmitoyl-L-carnitine were downregulated. A heatmap of the top altered metabolites is presented in (Fig. 3b). Among the upregulated metabolites were malate, 2-hydroxyglutarate, and 3-furoic acid, while decanoyl-carnitine, octanoyl-L-carnitine, histidine, and aspartate were downregulated (Fig. 3b).

A total of 58 metabolites having VIP > 1 and adjusted *p*-value < 0.05 were deemed significantly changed metabolites for further analysis. Pathway analysis showed that citrate cycle, alanine, aspartate, histidine, tryptophan, and phenylalanine metabolism are the most significantly changed pathways in atorvastatin-treated HCT-116 cells compared with controls (Fig. 4).

Fig. 5 shows box-and-whisker plots of a selected set of significantly altered metabolites (decanoyl-carnitine, octanoyl-L-carnitine, palmitoyl-L-carnitine, and aspartate) atorvastatin treated HCT-116 compared to control.

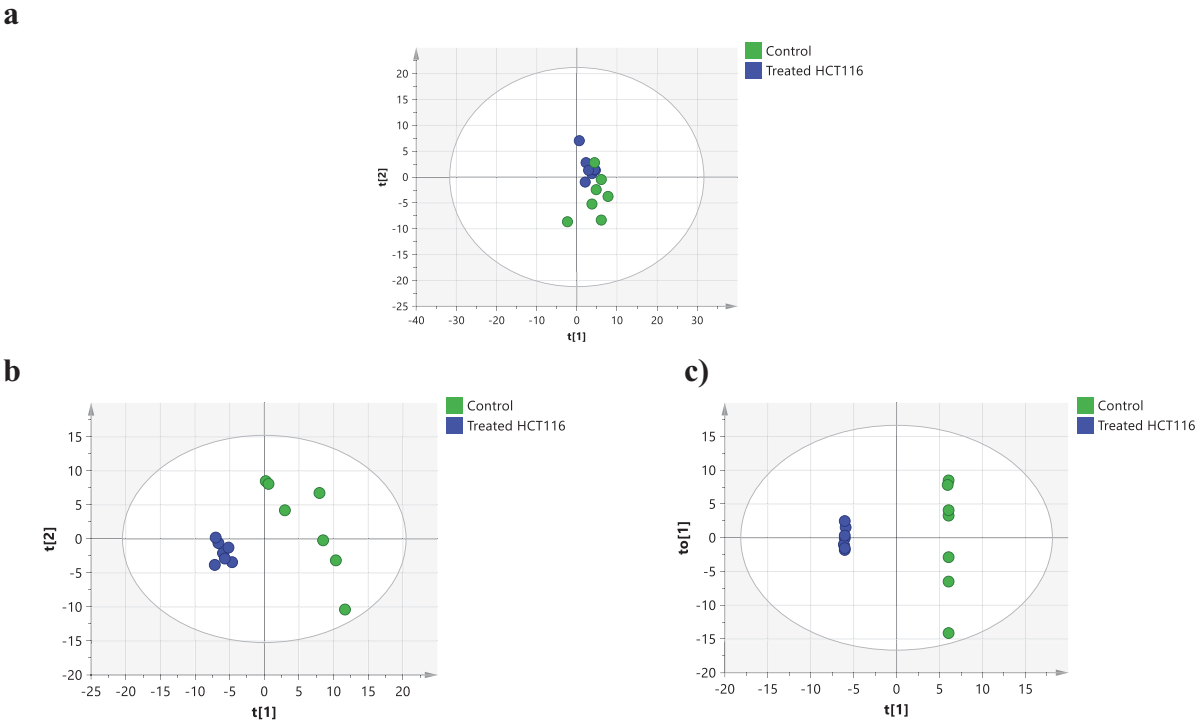

Figure 2. Scores plots of the metabolic profile of HCT-116 samples treated with atorvastatin (blue, n = 7) and untreated control (green, n = 7). **a.** PCA (R^2X = 0.80, Q^2 = 0.32), **b.** PLS-DA (R^2X = 0.47, R^2Y = 0.96, Q^2 = 0.70), and **c.** OPLS-DA (R^2X = 0.68, R^2Y = 0.99, Q^2 = 0.80)

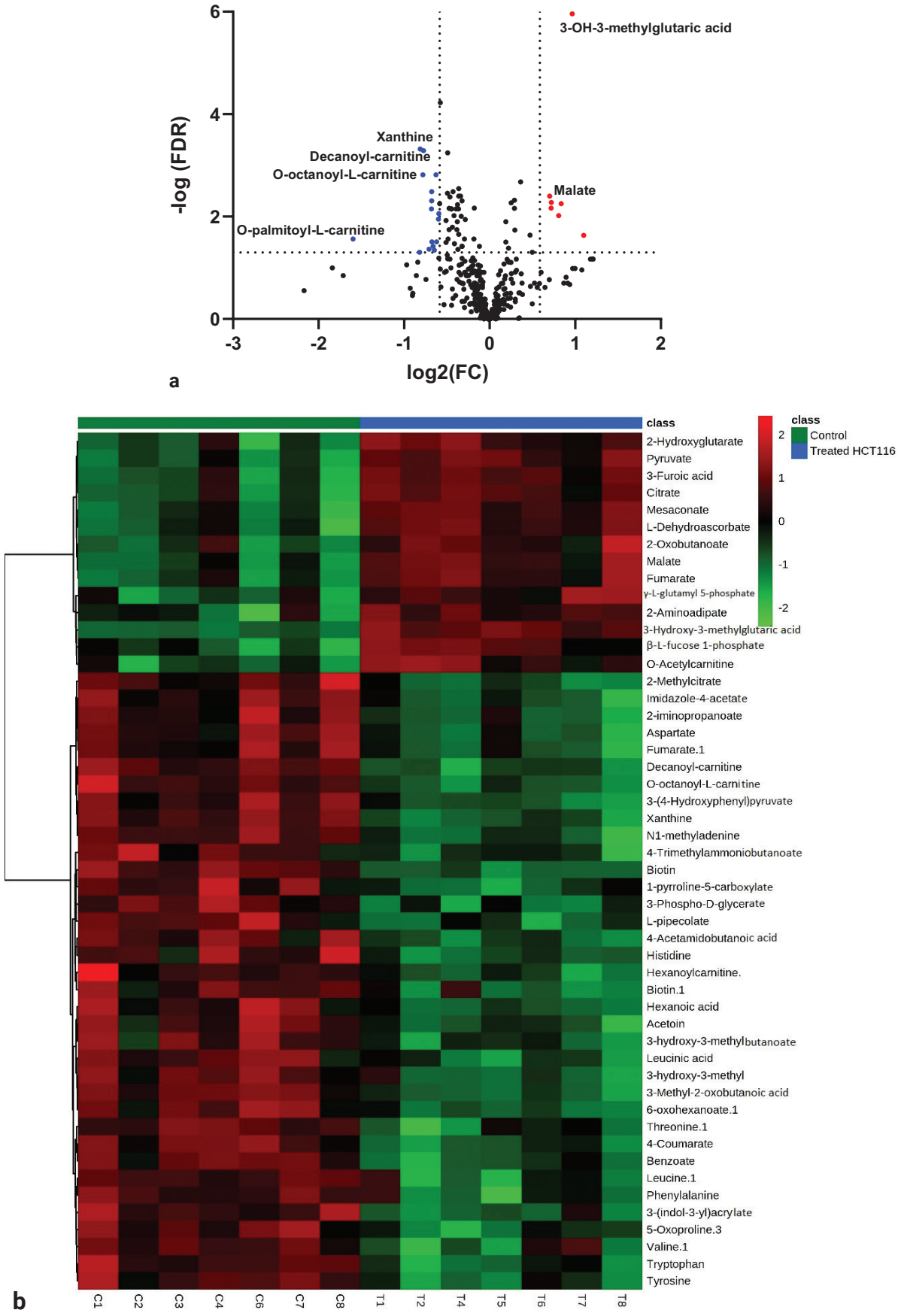

Figure 3. a. Volcano plots showing metabolites that were upregulated (red) and downregulated (blue) in HCT116 cells treated with atorvastatin, utilizing FDR thresholds of less than 0.05 and FC thresholds of 1.5; **b.** Heatmap of the level of the top 50 changed metabolites in atorvastatin-treated HCT-116 cells.

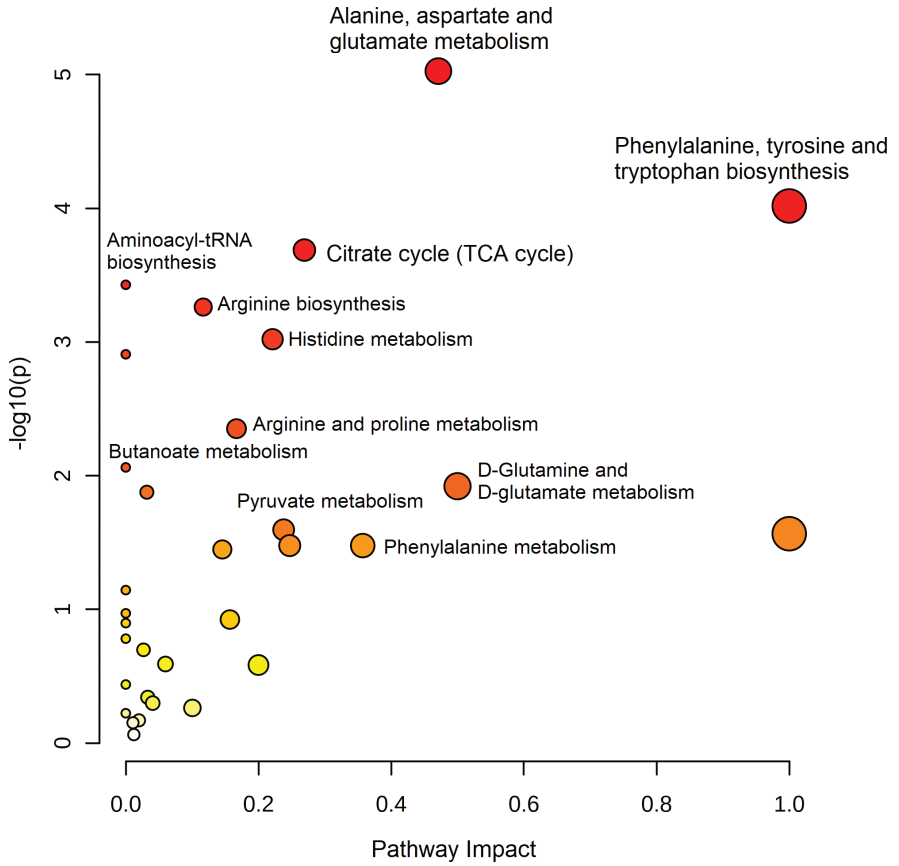

Figure 4. Overview of the metabolic pathways with the highest impact in atorvastatin-treated HCT-116 cells compared to control. The dot color and size correspond to the *p*-value and the pathway impact value, respectively.

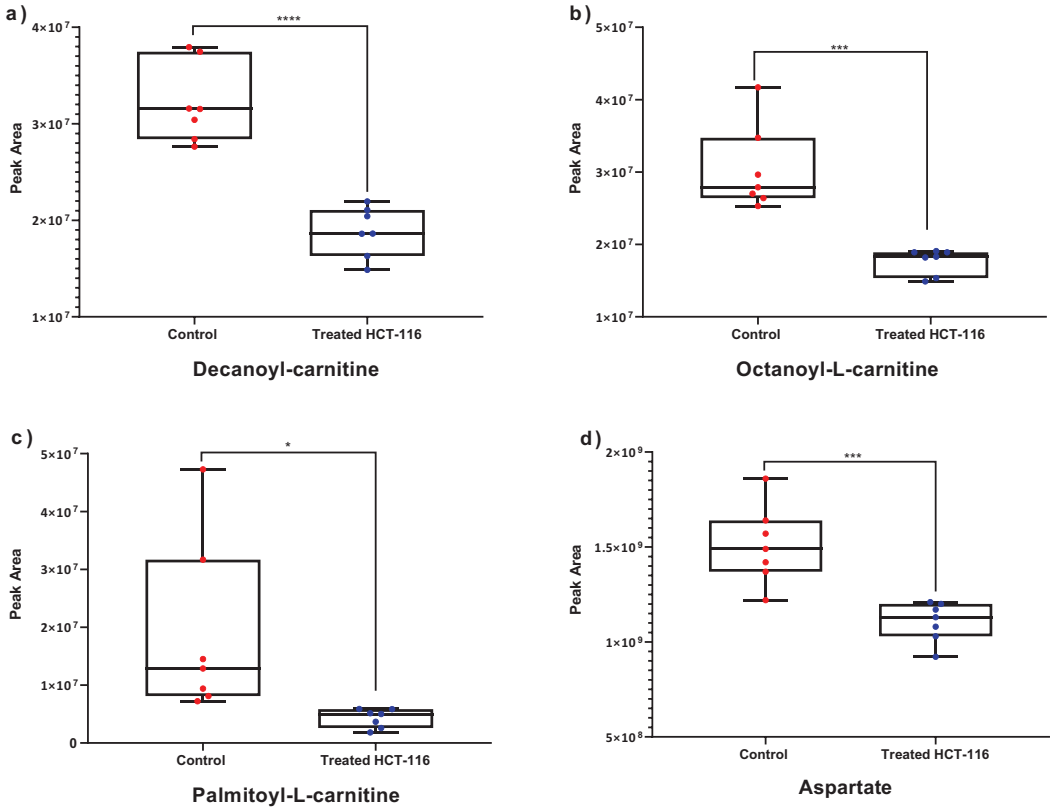

Figure 5. Box-and-whisker plots of potential biomarkers of atorvastatin-treated HCT-116 cells. *, ***, **** refer to p-value <0.05, <0.001, and <0.0001, respectively.

Proteomic analysis of atorvastatin-treated HCT116 cells using LC-MS/MS

A total of more than 5000 proteins were identified and quantified in HCT-116 cells. Among them, 127 proteins that had a mean expression FC ≥ 1.5 or ≤ 0.67 and a *p*-value of < 0.05 were categorized as significantly differentially expressed (DEPs) between the two groups (40 upregulated and 87 downregulated) (Suppl. material 1: tables S1, S2). The most upregulated proteins identified in HCT-116 cells treated with atorvastatin against untreated HCT-116 cells were 3-hydroxy-3-methylglutaryl-coenzyme A reductase, centromere-associated protein E, rho-related GTP-binding protein RhoB, charged multivesicular body protein 1a, and growth/differentiation factor 15 (GDF-15). In contrast, cytochrome c oxidase subunit 6A1, mitochondrial hyaluronan synthase 1, metallothionein-1X, phosphatidylinositol 4-phosphate 5-kinase type-1 alpha, and stearoyl-CoA desaturase 5 were the most downregulated proteins.

Fig. 6 shows a volcano plot of all measured proteins from the data set of HCT-116 cells treated with atorvastatin against untreated cells, showing the correlation between each protein's log2 ratio and statistical significance (–log *p*-value). Forty proteins were shown in the plot (Fig. 6) as red dots indicating up-regulation with a change of more than 1.5-fold and $p < 0.05$. Conversely, 87 proteins were downregulated and showed less than a 0.67-fold change at $p < 0.05$; these proteins are also shown in Fig. 6 as red dots.

Using the PANTHER GO classification method, DEPs were categorized based on the eukaryotic orthologous group (KOG) and gene ontology analysis (GO) at the molecular function and the biological process, respectively (Fig. 7a, b, respectively) (Mi et al. 2019). Molecular function regulator, catalytic activity, and binding were the three most significant functional clusters (Fig. 7a). The majority of the annotated proteins were related to cellular processes and metabolic processes, according to GO analysis (Fig. 7b).

Discussion

Statins are among the drugs that have been shown to possess anticancer activity in many preclinical and clinical studies (Chen et al. 2017; Jiang et al. 2021; Majidi et al. 2021). Nevertheless, studies on the molecular mechanism of action as anticancer in different cancer types are still under-studied. To our knowledge, this study is the first of its kind aimed at examining the anticancer impact of atorvastatin on colorectal cancer cell line (HCT-116) using two different omics approaches, proteomics and metabolomics.

In total, 127 proteins were significantly differentially expressed in atorvastatin-treated HCT-116 cells compared to control, in which 40 proteins were found upregulated, while 87 proteins were downregulated. In addition, 257 metabolites were identified in the metabolic profiles of the samples; 67 of them were found to be significantly altered.

In the untargeted metabolomics, we observed down-regulation of acylcarnitines: decanoyl-carnitine, octanoyl-L-carnitine, and palmitoyl-L-carnitine, which are intermediates formed during the transport of fatty acids into the mitochondria for beta-oxidation (Longo et al. 2016). Since cancer cells proliferate quickly, they require significant energy as well as fatty acids (FA) to make cell membranes. FA oxidation may have an essential role in the development of CRC. Thus, it would appear that stopping this mechanism in CRC cells might be one way to stop the disease from spreading (Mozolewska et al. 2020).

Among the significantly altered pathways between atorvastatin-treated HCT-116 cells and untreated cells were aminoacyl-tRNA biosynthesis, alanine, aspartate, and glutamate metabolism.

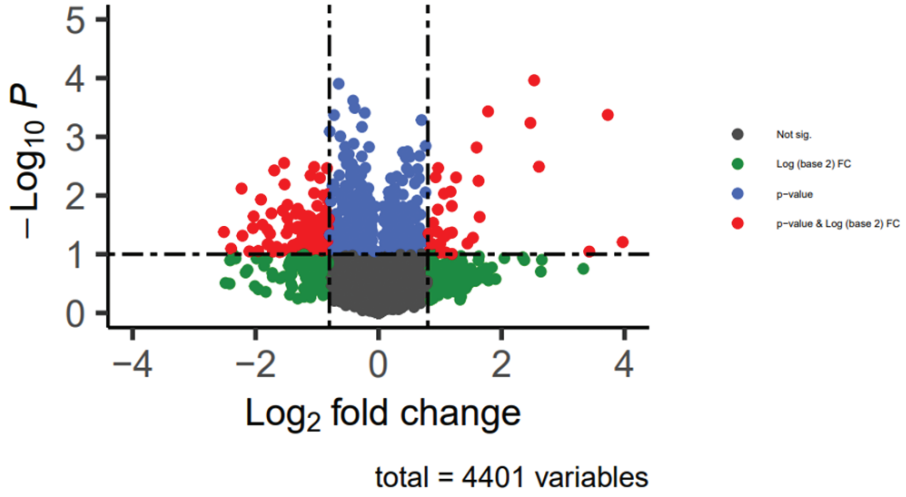

Figure 6. Volcano plot showing the protein abundance changes between HCT-116 cells treated with atorvastatin and untreated cells. The points in the upper right (|fold change| ratio > 1.5) and upper left (|fold change| ratio < 0.67) sections with $P < 0.05$ display up- and down-regulated proteins, respectively, highlighted in red. Statistically, up- and down-regulated proteins without significant biological alterations (|fold change| > 0.67 and < 1.5) are shown in blue, and proteins with no significant alterations between the treated and untreated cells are depicted in grey and green.

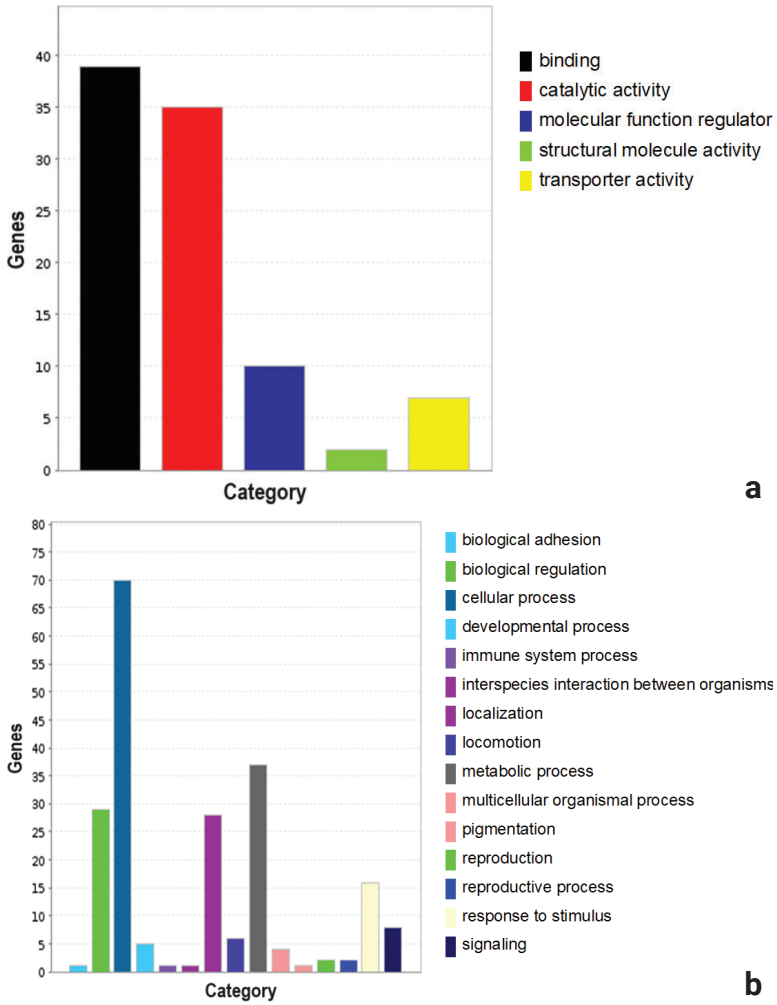

Figure 7. a. Functional classification. Gene Ontology graphs show the molecular function of significantly differentially expressed proteins. The horizontal axis shows the categories of the eukaryotic orthologous group (KOG), and the vertical axis shows the number of genes. **b.** Bar graphs show the gene ontology (GO) distribution of differentially expressed proteins based on major biological process categories. The horizontal axis shows the GO categories, and the vertical axis shows the number of genes.

Tumorigenesis and progression can be facilitated by dysregulated aminoacyl-tRNA production, which is necessary for protein synthesis. Atorvastatin downregulated these pathways compared to control and hence possesses anticancer activity as they are crucial for cell growth. In this process, some amino acids are linked to their matching tRNAs by enzymes called aminoacyl-tRNA synthetases (AARS). Numerous malignancies have been linked to dysregulation of AARS expression, making it an attractive target for the development of novel therapeutics for the treatment of cancers (Lukarska and Palencia 2020; Sung et al. 2022). The metabolism of alanine, aspartate, and glutamate is a vital energy source for the growth and survival of tumor cells (Liu et al. 2019). Thus, targeting these metabolic pathways and biosynthesis of amino acids holds promise as a therapeutic approach for disrupting malignant cell proliferation and survival (Choi and Park 2018).

Regarding proteomics' results, the most prominent functional clusters, including the three main categories in binding, catalytic activity, and molecular function regulator, suggest that the statin may target multiple cellular processes that are critical for cancer cell survival and proliferation.

Among the most upregulated proteins identified in treated HCT-116 cells compared with untreated cells was centromere-associated protein E (CENP-E), which is a mitotic spindle motor protein that belongs to the kinesin superfamily and has an ATPase domain in its motor region. Several studies showed that the overexpression of CENP-E might be involved in the chromosomal instability that characterizes cancer cells (Liang et al. 2017; Liang et al. 2024). One possible treatment approach to stop cancer cells from proliferating incorrectly is to inhibit CENP-E. In HCC1954 breast cancer-cultured cell lines and tumor xenografts, Wood KW et al. demonstrated that the decrease in the CENP-E motor activity resulted in the failure of metaphase chromosomal alignment and halted cell division in the mitotic stage, indicating a strong binding of CENP-E (Wood et al. 2010). Moreover, anticancer drugs such as GSK923295 act as strong CENP-E inhibitors and were specifically investigated in clinical trials (Chung et al. 2012; Lock et al. 2012). For recruitment at kinetochores, CENP-E prenylation, where a lipid covalent addition at the COOH terminus is necessary (El-Arabey et al. 2018). Our result showed that CENP-E was elevated.

Atorvastatin may impact the post-translation process of CENP-E prenylation (Ashar et al. 2000); alternatively, the protein may not function at all or may be impacted by different signaling pathways that require more studies.

We identified a novel protein, GDF-15, which was upregulated in atorvastatin-treated HCT-116 cells compared with untreated cells. GDF15 is a member of the transforming growth factor β (TGFβ) superfamily (Modi et al. 2019). According to earlier studies, GDF15 is increased with concurrent use of many anticancer medications, including NSAIDs, PPARγ ligands, doxorubicin, carboplatin, and genistein (Yang et al. 2003; Wang et al. 2013). Cekanova et al. showed that overexpression of GDF15 protein in nonsteroidal anti-inflammatory drug-activated gene-1 (NAG-1) transgenic mice induced apoptosis by activating caspase 3/7. In addition, it inhibited the formation of lung tumors that were produced and inflammation by downregulating the p38 mitogen-activated protein kinase (MAPK) signaling pathway (Cekanova et al. 2009). Upon reviewing the literature, it appears that no previous studies have examined the impact of statins on GDF-15 protein. Nonetheless, as previously described, atorvastatin may serve an indirect inhibitory function via repressing protein kinase B (AKT) and MAPK signaling pathways that are associated with cell growth and survival (Fliedner et al. 2014; Nakashima et al. 2018). In this study we found downregulation of COX6A1 and HAS1 in HCT-116 treated cells compared to controls, and that can be considered as novel molecular mechanisms of statins as anticancer drugs. COX6A1, a key component of the mitochondrial electron transport chain, acts as an anti-apoptotic factor. In a previous study, Luo et al. (2021) demonstrated that gefitinib, an epidermal growth factor receptor tyrosine kinase (EGFR-TK) inhibitor, inhibited mitochondrial respiratory chain complex IV activity, causing apoptosis and liver damage (Luo et al. 2021). Moreover, according to Fujiwara et al., statins increase caspases-3/-9 activation, induce the expression of Bcl-2 interacting mediator of cell death (Bim), halt the cell cycle at the G1 phase, and reduce the mitochondrial membrane potential ($\Delta\psi m$) by inhibiting the Ras/ERK and Ras/mTOR pathways (Fujiwara et al. 2017).

Hence, it can be proposed that statins could disrupt the mitochondrial respiratory chain complexes in cancer cells. However, further investigations are needed to confirm this hypothesis.

One of the three isoenzymes in charge of cellular hyaluronan synthesis by normal keratinocytes is called hyaluronan synthase 1 (HAS1) (Siiskonen et al. 2015). HAS1 produces anti-adhesive HA, which facilitates tumor cells' metastasis (McAtee et al. 2014). Additionally, as the cancer cell enters the lymphatic or circulatory systems, HAS1 shields it from destruction (Nikitovic et al. 2015). According to Kramer et al. (2010), bladder cancer's capacity for metastasis was increased by HAS1 expression (Kramer et al. 2011). Angiogenesis, invasion, and tumor formation all depend on HAS1 (Patel et al. 2002). Thus, our novel finding of downregulated HSA1 expression following statin treatment further supports the hypothesis that statins possess anticancer properties. This is consistent with previous findings that atorvastatin inhibits cell adhesion and invasion, emphasizing its anti-metastatic effect (Jones et al. 2017; Shaghaghi et al. 2022).

Conclusion

In conclusion, atorvastatin demonstrated an impact effect on the proteome and metabolome of the HCT-116 cell line. Atorvastatin altered 127 proteins involved in the mechanism of CRC cell proliferation, adherence, metastasis, and apoptosis. Forty proteins of them have been upregulated, whereas 87 were downregulated. Moreover, 67 metabolites were significantly altered in treated HCT-116 compared with untreated. Novel proteins, such as CENP-E, GDF-15, COX 6A1, and HSA1, were associated with statin's molecular mechanisms. Moreover, the significantly altered metabolites were involved in citrate cycle, alanine, aspartate, histidine, tryptophan, and phenylalanine metabolism. While our novel metabolomics and proteomics findings provide promising evidence for the anticancer effects of statins in CRC mainly, further validation using multiple cell lines and validation methods is essential to elucidate the molecular mechanism of statins as anticancer drugs and to prove the clinical potential of this therapeutic approach.

Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statements

The authors declared that no clinical trials were used in the present study.

The authors declared that no experiments on humans or human tissues were performed for the present study.

The authors declared that no informed consent was obtained from the humans, donors or donors' representatives participating in the study.

The authors declared that no experiments on animals were performed for the present study.

Use of commercially available immortalised human and animal cell lines: HCT-116 was acquired from the American Type Culture Collection (Rockville, USA)

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Author contributions

Refat Nimer: Writing – original draft, Supervision, Resources, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. Hiba Nazazleh: Writing – review & editing, Methodology, Investigation. Belal Al-Husein: Writing – review & editing, Methodology, Investigation, Formal analysis. Salah Abdelrazig: Writing – review & editing,

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Data availability

All of the data that support the findings of this study are available in the main text or Supplementary Information.

References

Al-Saafin BA, Al-Bakri AG, Abdelrazig S, Dahabiyeh LA (2023) Investigating the effect of the probiotic Lactobacillus plantarum and the prebiotic fructooligosaccharides on Pseudomonas aeruginosa metabolome, virulence factors and biofilm formation as potential quorum sensing inhibitors. Microbial Pathogenesis 177: 106057. https://doi.org/10.1016/j.micpath.2023.106057

Ashar HR, James L, Gray K, Carr D, Black S, Armstrong L, Bishop WR, Kirschmeier P (2000) Farnesyl transferase inhibitors block the farnesylation of CENP-E and CENP-F and alter the association of CENP-E with the microtubules. Journal of Biological Chemistry 275: 30451–30457. https://doi.org/10.1074/jbc.M003469200

Batth TS, Tollenaere MX, Rüther P, Gonzalez-Franquesa A, Prabhakar BS, Bekker-Jensen S, Deshmukh AS, Olsen JV (2019) Protein Aggregation Capture on Microparticles Enables Multipurpose Proteomics Sample Preparation. Molecular & Cellular Proteomics 18: 1027–1035. https://doi.org/10.1074/mcp.TIR118.001270

Buccioli G, Testa C, Jacchetti E, Pinoli P, Carelli S, Ceri S, Raimondi MT (2024) The molecular basis of the anticancer effect of statins. Scientific Reports 14: 20298. https://doi.org/10.1038/s41598-024-71240-6

Cekanova M, Lee SH, Donnell RL, Sukhthankar M, Eling TE, Fischer SM, Baek SJ (2009) Nonsteroidal anti-inflammatory drug-activated gene-1 expression inhibits urethane-induced pulmonary tumorigenesis in transgenic mice. Cancer Prevention Research (Phila) 2: 450–458. https://doi.org/10.1158/1940-6207.Capr-09-0057

Chen B, Zhang M, Xing D, Feng Y (2017) Atorvastatin enhances radiosensitivity in hypoxia-induced prostate cancer cells related with HIF-1α inhibition. Bioscience reports 37: BSR20170340. https://doi.org/10.1042/BSR20170340

Choi YK, Park KG (2018) Targeting Glutamine Metabolism for Cancer Treatment. Biomol Ther (Seoul) 26: 19–28. https://doi.org/10.4062/biomolther.2017.178

Chung V, Heath EI, Schelman WR, Johnson BM, Kirby LC, Lynch KM, Botbyl JD, Lampkin TA, Holen KD (2012) First-time-in-human study of GSK923295, a novel antimitotic inhibitor of centromere-associated protein E (CENP-E), in patients with refractory cancer. Cancer chemotherapy and pharmacology 69: 733–741. https://doi.org/10.1007/s00280-011-1756-z

Dahabiyeh LA, Hourani W, Darwish W, Hudaib F, Abu-Irmaileh B, Deb PK, Venugopala KN, Mohanlall V, Abu-Dahab R, Semreen MH, Bustanji Y (2022) Molecular and metabolic alterations of 2,3-dihydroquinazolin-4(1*H*)-one derivatives in prostate cancer cell lines. Scientific Reports 12(1): 21599. https://doi.org/10.1038/s41598-022-26148-4

Dahabiyeh LA, Hudaib F, Hourani W, Darwish W, Abu-Irmaileh B, Deb PK, Venugopala KN, Mohanlall V, Chandrashekharappa S, Abu-Dahab R, Semreen MH, Bustanji Y (2023a) Mass spectrometry-based metabolomics approach and in vitro assays revealed promising role of 2,3-dihydroquinazolin-4(1H)-one derivatives against colorectal cancer cell lines. European Journal of Pharmaceutical Sciences 182: 106378. https://doi.org/10.1016/j.ejps.2023.106378

Dahabiyeh LA, Mahmoud NN, Al-Natour MA, Safo L, Kim DH, Khalil EA, Abu-Dahab R (2021) Phospholipid-Gold Nanorods Induce Energy Crisis in MCF-7 Cells: Cytotoxicity Evaluation Using LC-MS-Based Metabolomics Approach. Biomolecules 11(3): 364. https://doi.org/10.3390/biom11030364

Dahabiyeh LA, Nimer RM (2023) Metabolomics: A Pipeline for Biomarker Discovery in Genetic Diseases. In: Abdel Rahman AM (Ed.) Clinical Metabolomics Applications in Genetic Diseases. Springer Nature Singapore, Singapore, 43–69. https://doi.org/10.1007/978-981-99-5162-8_3

Dahabiyeh LA, Nimer RM, Sumaily KM, Alabdaljabar MS, Jacob M, Sabi EM, Hussein MH, Abdel Rahman A (2023b) Metabolomics profiling distinctively identified end-stage renal disease patients from chronic kidney disease patients. Scientific Reports 13: 6161. https://doi.org/10.1038/s41598-023-33377-8

Dekker E, Tanis PJ, Vleugels JLA, Kasi PM, Wallace MB (2019) Colorectal cancer. The Lancet 394: 1467–1480. https://doi.org/10.1016/S0140-6736(19)32319-0

Dobrzycka M, Spychalski P, Łachiński AJ, Kobiela P, Jędrusik P, Kobiela J (2020) Statins and colorectal cancer–a systematic review. Experimental and Clinical Endocrinology & Diabetes 128: 255–262. https://doi.org/10.1055/a-0668-5692

Dorsch M, Kowalczyk M, Planque M, Heilmann G, Urban S, Dujardin P, Forster J, Ueffing K, Nothdurft S, Oeck S (2021) Statins affect cancer cell plasticity with distinct consequences for tumor progression and metastasis. Cell Reports 37(8): 110056. https://doi.org/10.1016/j.celrep.2021.110056

Dulak J, Józkowicz A (2005) Anti-angiogenic and anti-inflammatory effects of statins: relevance to anti-cancer therapy. Current Cancer Drug Targets 5: 579–594. https://doi.org/10.2174/156800905774932824

El-Arabey AA, Salama SA, Abd-Allah AR (2018) CENP-E as a target for cancer therapy: Where are we now? Life Sciences 208: 192–200. https://doi.org/10.1016/j.lfs.2018.07.037

Fatehi Hassanabad A (2019) Current perspectives on statins as potential anti-cancer therapeutics: clinical outcomes and underlying molecular mechanisms. Transl Lung Cancer Res 8: 692–699. https://doi.org/10.21037/tlcr.2019.09.08

Fernandes Silva L, Ravi R, Vangipurapu J, Laakso M (2022) Metabolite Signature of Simvastatin Treatment Involves Multiple Metabolic Pathways. Metabolites 12(8): 753. https://doi.org/10.3390/metabo12080753

Fliedner SM, Engel T, Lendvai NK, Shankavaram U, Nölting S, Wesley R, Elkahloun AG, Ungefroren H, Oldoerp A, Lampert G (2014) Anti-Cancer Potential of MAPK Pathway Inhibition in Paragangliomas–Effect of Different Statins on Mouse Pheochromocytoma Cells. PLoS ONE 9: e97712. https://doi.org/10.1371/journal.pone.0097712

Fujiwara D, Tsubaki M, Takeda T, Tomonari Y, Koumoto Y-i, Sakaguchi K, Nishida S (2017) Statins induce apoptosis through inhibition of Ras signaling pathways and enhancement of Bim and p27 expression in human hematopoietic tumor cells. Tumor Biology 39: 1010428317734947. https://doi.org/10.1177/1010428317734947

Hanahan D, Weinberg RA (2011) Hallmarks of cancer: the next generation. Cell 144: 646–674. https://doi.org/10.1016/j.cell.2011.02.013

Hijazi DM, Dahabiyeh LA, Abdelrazig S, Alqudah DA, Al-Bakri AG (2023) Micafungin effect on Pseudomonas aeruginosa metabolome, virulence and biofilm: potential quorum sensing inhibitor. AMB Express 13: 20. https://doi.org/10.1186/s13568-023-01523-0

Jiang W, Hu J-W, He X-R, Jin W-L, He X-Y (2021) Statins: a repurposed drug to fight cancer. Journal of Experimental & Clinical Cancer Research 40: 241. https://doi.org/10.1186/s13046-021-02041-2

Jones HM, Fang Z, Sun W, Clark LH, Stine JE, Tran A-Q, Sullivan SA, Gilliam TP, Zhou C, Bae-Jump VL (2017) Atorvastatin exhibits anti-tumorigenic and anti-metastatic effects in ovarian cancer in vitro. American Journal of Cancer Research 7: 2478.

Kheirelseid EA, Miller N, Kerin MJ (2013) Molecular biology of colorectal cancer: Review of the Literature 3: 72–80. https://doi.org/10.4236/ajmb.2013.32010

Kramer MW, Escudero DO, Lokeshwar SD, Golshani R, Ekwenna OO, Acosta K, Merseburger AS, Soloway M, Lokeshwar VB (2011) Association of hyaluronic acid family members (HAS1, HAS2, and HYAL-1) with bladder cancer diagnosis and prognosis. Cancer 117: 1197–1209. https://doi.org/10.1002/cncr.25565

Lee SJ, Lee I, Lee J, Park C, Kang WK (2014) Statins, 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors, potentiate the anti-angiogenic effects of bevacizumab by suppressing angiopoietin2, BiP, and Hsp90α in human colorectal cancer. British Journal of Cancer 111: 497–505. https://doi.org/10.1038/bjc.2014.283

Liang J, Tian C, Liu L, Zeng X, Zhang Y (2024) Targeting CENP-E augments immunotherapy in non-small cell lung cancer via stabilizing PD-L1. International Immunopharmacology 126: 111294. https://doi.org/10.1016/j.intimp.2023.111294

Liang Y, Ahmed M, Guo H, Soares F, Hua JT, Gao S, Lu C, Poon C, Han W, Langstein J (2017) LSD1-mediated epigenetic reprogramming drives CENPE expression and prostate cancer progression. Cancer Research 77: 5479–5490. https://doi.org/10.1158/0008-5472.CAN-17-0496

Liu W, Wang Q, Chang J (2019) Global metabolomic profiling of trastuzumab resistant gastric cancer cells reveals major metabolic pathways and metabolic signatures based on UHPLC-Q exactive-MS/MS. RSC Advances 9: 41192–41208. https://doi.org/10.1039/c9ra06607a

Lock RB, Carol H, Morton CL, Keir ST, Reynolds CP, Kang MH, Maris JM, Wozniak AW, Gorlick R, Kolb EA (2012) Initial testing of the CENP- E inhibitor GSK923295A by the pediatric preclinical testing program. Pediatric Blood & Cancer 58: 916–923. https://doi.org/10.1002/pbc.23176

Longo N, Frigeni M, Pasquali M (2016) Carnitine transport and fatty acid oxidation. Biochimica et Biophysica Acta (BBA) 1863: 2422–2435. https://doi.org/10.1016/j.bbamcr.2016.01.023

Lukarska M, Palencia A (2020) Chapter Eleven - Aminoacyl-tRNA synthetases as drug targets. In: Ribas de Pouplana L, Kaguni LS (Eds) The Enzymes. Academic Press, 321–350. https://doi.org/10.1016/bs.enz.2020.07.001

Luo P, Yan H, Du J, Chen X, Shao J, Zhang Y, Xu Z, Jin Y, Lin N, Yang B, He Q (2021) PLK1 (polo like kinase 1)-dependent autophagy facilitates gefitinib-induced hepatotoxicity by degrading COX6A1 (cytochrome c oxidase subunit 6A1). Autophagy 17: 3221–3237. https://doi.org/10.1080/15548627.2020.1851492

Majidi A, Na R, Jordan SJ, De Fazio A, Webb PM, Group OS (2021) Statin use and survival following a diagnosis of ovarian cancer: A prospective observational study. International Journal of Cancer 148: 1608–1615. https://doi.org/10.1002/ijc.33333

Mármol I, Sánchez-de-Diego C, Pradilla Dieste A, Cerrada E, Rodriguez Yoldi MJ (2017) Colorectal Carcinoma: A General Overview and Future Perspectives in Colorectal Cancer. International Journal of Molecular Sciences 18(1): 197. https://doi.org/10.3390/ijms18010197

McAtee CO, Barycki JJ, Simpson MA (2014) Emerging roles for hyaluronidase in cancer metastasis and therapy. Advances in Cancer Research 123: 1–34. https://doi.org/10.1016/b978-0-12-800092-2.00001-0.

McGregor GH, Campbell AD, Fey SK, Tumanov S, Sumpton D, Blanco GR, Mackay G, Nixon C, Vazquez A, Sansom OJ (2020) Targeting the metabolic response to statin-mediated oxidative stress produces a synergistic antitumor response. Cancer Research 80: 175–188. https://doi.org/10.1158/0008-5472.CAN-19-0644

Mi H, Muruganujan A, Ebert D, Huang X, Thomas PD (2019) PANTHER version 14: more genomes, a new PANTHER GO-slim and improvements in enrichment analysis tools. Nucleic Acids Research 47: D419-D426. https://doi.org/10.1093/nar/gky1038

Min Y, Liu Z, Wei Z, Li R, Jin J, Zhang Y, Peng X (2022) Association between Statin Use and Survival in Cancer Patients with Brain Metastasis: Retrospective Analysis from the Chinese Population. Pharmaceuticals 15: 1474. https://doi.org/10.3390/ph15121474

Modi A, Dwivedi S, Roy D, Khokhar M, Purohit P, Vishnoi J, Pareek P, Sharma S, Sharma P, Misra S (2019) Growth differentiation factor 15 and its role in carcinogenesis: an update. Growth Factors 37: 190–207. https://doi.org/10.1080/08977194.2019.1685988

Mozolewska P, Duzowska K, Pakiet A, Mika A, Śledziński T (2020) Inhibitors of fatty acid synthesis and oxidation as potential anticancer agents in colorectal cancer treatment. Anticancer Research 40: 4843–4856. https://doi.org/10.21873/anticanres.14487

Nakashima Y, Miyagi-Shiohira C, Noguchi H, Omasa T (2018) Atorvastatin Inhibits the HIF1α-PPAR Axis, Which Is Essential for Maintaining the Function of Human Induced Pluripotent Stem Cells. Molecular Therapy 26: 1715–1734. https://doi.org/10.1016/j.ymthe.2018.06.005

Nikitovic D, Tzardi M, Berdiaki A, Tsatsakis A, Tzanakakis GN (2015) Cancer microenvironment and inflammation: role of hyaluronan. Frontiers in Immunology 6: 169. https://doi.org/10.3389/fimmu.2015.00169

Nimer RM, Alfaqih MA, Shehabat ER, Mujammami M, Abdel Rahman AM (2023) Label-free quantitative proteomics analysis for type 2 diabetes mellitus early diagnostic marker discovery using data-independent acquisition mass spectrometry (DIA-MS). Scientific Reports 13: 20880. https://doi.org/10.1038/s41598-023-48185-3

Pang Z, Chong J, Zhou G, de Lima Morais DA, Chang L, Barrette M, Gauthier C, Jacques P-É, Li S, Xia J (2021) MetaboAnalyst 5.0: narrowing the gap between raw spectra and functional insights. Nucleic Acids Research 49: W388-W396. https://doi.org/10.1093/nar/gkab382

Patel S, Turner PR, Stubberfield C, Barry E, Rohlff CR, Stamps A, McKenzie E, Young K, Tyson K, Terrett J, Box G, Eccles S, Page MJ (2002) Hyaluronidase gene profiling and role of hyal-1 overexpression in an orthotopic model of prostate cancer. International Journal of Cancer 97: 416–424. https://doi.org/10.1002/ijc.1638

Qiu S, Cai Y, Yao H, Lin C, Xie Y, Tang S, Zhang A (2023) Small molecule metabolites: discovery of biomarkers and therapeutic targets. Signal Transduction and Targeted Therapy 8: 132. https://doi.org/10.1038/s41392-023-01399-3

Roth BD (2002) 1 The discovery and development of atorvastatin, a potent novel hypolipidemic agent. Progress in medicinal chemistry 40: 1–22. https://doi.org/10.1016/S0079-6468(08)70080-8

Shaghaghi Z, Alvandi M, Farzipour S, Dehbanpour MR, Nosrati S (2022) A review of effects of atorvastatin in cancer therapy. Medical Oncology 40: 27. https://doi.org/10.1007/s12032-022-01892-9

Siiskonen H, Oikari S, Pasonen-Seppänen S, Rilla K (2015) Hyaluronan synthase 1: a mysterious enzyme with unexpected functions. Frontiers in Immunology 6: 43. https://doi.org/10.3389/fimmu.2015.00043

Sumner LW, Amberg A, Barrett D, Beale MH, Beger R, Daykin CA, Fan TW-M, Fiehn O, Goodacre R, Griffin JL (2007) Proposed minimum reporting standards for chemical analysis: chemical analysis working group (CAWG) metabolomics standards initiative (MSI). Metabolomics 3: 211–221. https://doi.org/10.1007/s11306-007-0082-2

Sung JJY, Lau JYW, Goh KL, Leung WK (2005) Increasing incidence of colorectal cancer in Asia: implications for screening. The Lancet Oncology 6: 871–876. https://doi.org/10.1016/S1470-2045(05)70422-8

Sung Y, Yoon I, Han JM, Kim S (2022) Functional and pathologic association of aminoacyl-tRNA synthetases with cancer. Experimental & Molecular Medicine 54: 553–566. https://doi.org/10.1038/s12276-022-00765-5

Takada K, Shimokawa M, Takamori S, Shimamatsu S, Hirai F, Tagawa T, Okamoto T, Hamatake M, Tsuchiya-Kawano Y, Otsubo K (2022) A propensity score-matched analysis of the impact of statin therapy on the outcomes of patients with non-small-cell lung cancer receiving anti-PD-1 monotherapy: a multicenter retrospective study. BMC Cancer 22: 503. https://doi.org/10.1186/s12885-022-09385-8

Tyers M, Mann M (2003) From genomics to proteomics. Nature 422: 193–197. https://doi.org/10.1038/nature01510

Walther U, Emmrich K, Ramer R, Mittag N, Hinz B (2016) Lovastatin lactone elicits human lung cancer cell apoptosis via a COX-2/PPARγ-dependent pathway. Oncotarget 7: 10345. https://doi.org/10.18632/oncotarget.7213

Wang X, Baek SJ, Eling TE (2013) The diverse roles of nonsteroidal anti-inflammatory drug activated gene (NAG-1/GDF15) in cancer. Biochemical Pharmacology 85: 597–606. https://doi.org/10.1016/j.bcp.2012.11.025

Warita T, Irie N, Zhou Y, Tashiro J, Sugiura A, Oltvai ZN, Warita K (2023) Alterations in the omics profiles in mevalonate pathway-inhibited cancer cells. Life Sciences 312: 121249. https://doi.org/10.1016/j.lfs.2022.121249

Wood KW, Lad L, Luo L, Qian X, Knight SD, Nevins N, Brejc K, Sutton D, Gilmartin AG, Chua PR, Desai R, Schauer SP, McNulty DE, Annan RS, Belmont LD, Garcia C, Lee Y, Diamond MA, Faucette LF, Giardiniere M, Zhang S, Sun CM, Vidal JD, Lichtsteiner S, Cornwell WD, Greshock JD, Wooster RF, Finer JT, Copeland RA, Huang PS, Morgans DJ, Jr., Dhanak D, Bergnes G, Sakowicz R, Jackson JR (2010) Antitumor activity of an allosteric inhibitor of centromere-associated protein-E. Proceedings of the National Academy of Sciences (PNAS) 107: 5839–5844. https://doi.org/10.1073/pnas.0915068107

Worley B, Powers R (2013) Multivariate analysis in metabolomics. Current Metabolomics 1: 92–107. https://doi.org/10.2174/2213235X11301010092

Xi Y, Xu P (2021) Global colorectal cancer burden in 2020 and projections to 2040. Translational Oncology 14: 101174. https://doi.org/10.1016/j.tranon.2021.101174

Yang H, Filipovic Z, Brown D, Breit SN, Vassilev LT (2003) Macrophage inhibitory cytokine-1: a novel biomarker for p53 pathway activation. Molecular Cancer Therapeutics 2: 1023–1029.

Yin P, Wan D, Zhao C, Chen J, Zhao X, Wang W, Lu X, Yang S, Gu J, Xu G (2009) A metabonomic study of hepatitis B-induced liver cirrhosis and hepatocellular carcinoma by using RP-LC and HILIC coupled with mass spectrometry. Molecular BioSystems 5: 868–876. https://doi.org/10.1039/B820224A

Yokohama K, Fukunishi S, Ii M, Nakamura K, Ohama H, Tsuchimoto Y, Asai A, Tsuda Y, Higuchi K (2016) Rosuvastatin as a potential preventive drug for the development of hepatocellular carcinoma associated with non-alcoholic fatty liver disease in mice. International Journal of Molecular Medicine 38: 1499–1506. https://doi.org/10.3892/ijmm.2016.2766

Yuan Q, Dong CD, Ge Y, Chen X, Li Z, Li X, Lu Q, Peng F, Wu X, Zhao J, Liu K (2019) Proteome and phosphoproteome reveal mechanisms of action of atorvastatin against esophageal squamous cell carcinoma. Aging (Albany NY) 11: 9530–9543. https://doi.org/10.18632/aging.102402

Zafari N, Bathaei P, Velayati M, Khojasteh-Leylakoohi F, Khazaei M, Fiuji H, Nassiri M, Hassanian SM, Ferns GA, Nazari E, Avan A (2023) Integrated analysis of multi-omics data for the discovery of biomarkers and therapeutic targets for colorectal cancer. Computers in Biology and Medicine 155: 106639. https://doi.org/10.1016/j.compbiomed.2023.106639

Zaky MY, Fan C, Zhang H, Sun X-F (2023) Unraveling the anticancer potential of statins: Mechanisms and clinical significance. Cancers 15: 4787. https://doi.org/10.3390/cancers15194787

Zhan X, Long Y, Lu M (2018) Exploration of variations in proteome and metabolome for predictive diagnostics and personalized treatment algorithms: Innovative approach and examples for potential clinical application. Journal of Proteomics 188: 30–40. https://doi.org/10.1016/j.jprot.2017.08.020

Supplementary material 1

Additional information

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Data type: docx

Explanation note: **fig. S1:** Permutation test for the validation of the OPLS-DA model generated from the binary comparison between statin treated HCT116 group and control (untreated) group. Q2 (blue squares) and R2Y (green circles). Values from the permuted analysis models (left- side) should be less than the initial generated model values (right- corner). **table S1:** List of 40 upregulated proteins in HCT-116-treated with atorvastatin compared to untreated cells. **table S2:** List of 87 downregulated proteins in HCT-116-treated with atorvastatin compared to untreated cells.

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