Cancer Cell International

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Repurposing pitavastatin and atorvastatin to overcome chemoresistance of metastatic colorectal cancer under high glucose conditions

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Abstract

Background Colorectal cancer (CRC) poses a significant clinical challenge because of drug resistance, which can adversely impact patient outcomes. Recent research has shown that abnormalities within the tumor microenvironment, especially hyperglycemia, play a crucial role in promoting metastasis and chemoresistance, and thereby determine the overall prognosis of patients with advanced CRC.

Methods This study employs data mining and consensus molecular subtype (CMS) techniques to identify pitavastatin and atorvastatin as potential agents for targeting high glucose-induced drug resistance in advanced CRC cells. CRC cells maintained under either low or high glucose conditions were established and utilized to assess the cytotoxic effects of pitavastatin and atorvastatin, both with and without 5-fluorouracil (5-FU). CRC 3D spheroids cultured were also included to demonstrate the anti-drug resistance of pitavastatin and atorvastatin.

Results A bioinformatics analysis identified pitavastatin and atorvastatin as promising drug candidates. The CMS4 CRC cell line SW480 (SW480-HG) was established and cultured under high glucose conditions to simulate hyperglycemia-induced drug resistance and metastasis in CRC patients. Pitavastatin and atorvastatin could inhibit cell proliferation and 3D spheroid formation of CMS4 CRC cells under high glucose conditions. In addition, both pitavastatin and atorvastatin can synergistically promote the 5-FU-mediated cytotoxic effect and inhibit the growth of 5-FU-resistant CRC cells. Mechanistically, pitavastatin and atorvastatin can induce apoptosis and synergistically

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promote the 5-FU-mediated cytotoxic effect by activating autophagy, as well as the PERK/ATF4/CHOP signaling pathway while decreasing YAP expression.

Conclusion This study highlights the biomarker-guided precision medicine strategy for drug repurposing. Pitavastatin and atorvastatin could be used to assist in the treatment of advanced CRC, particularly with CMS4 subtype CRC patients who also suffer from hyperglycemia. Pitavastatin, with an achievable dosage used for clinical interventions, is highly recommended for a novel CRC therapeutic strategy.

Keywords Colorectal cancer, Hyperglycemia, Drug resistance, Consensus molecular subtype, Pitavastatin, Atorvastatin

Background

Colorectal cancer (CRC) ranks as the fourth most common cancer and is the leading global cause of cancer-related death [1]. Despite the introduction of nationwide screening programs and higher rates of colonoscopy assessments, which have stabilized CRC incidence in developed countries, there has been a noticeable increase in CRC cases among individuals younger than 50 years [2, 3]. The standard treatment for advanced CRC involves 5-fluorouracil (5-FU) and oxaliplatin-based chemotherapy [4]. However, chemotherapy resistance remains a significant challenge for CRC treatment, with a five-year survival rate for stage IV cancer of only 12% [5]. Furthermore, the microenvironment that surrounds cancer cells plays a crucial role in shaping responses to drug treatments. Diabetes mellitus (DM) is one of the most prevalent comorbidities for CRC patients. Approximately one in eleven adults is estimated to have DM [6], and epidemiological studies have indicated that both DM and prediabetes are linked to an increased risk of developing CRC [7–9]. Notably, hyperglycemia in DM is known to induce the upregulation of insulin and insulin-like growth factors, thereby promoting cancer cell metastasis and chemoresistance, which impacts the overall prognosis of CRC patients [10].

Currently, CRC is no longer categorized as a single disease entity due to the presence of various genetic alterations with different responses to standard treatments. Therefore, treatments should be targeted according to a sophisticated CRC classification based on molecular features [11]. The consensus molecular subtype (CMS) of CRC was proposed in 2015 to resolve inconsistencies among different gene expression-based classification systems, and this resulted in CRC cases being predominantly classified into four distinct CMS groups [12]. To capture CMS characteristics within specific settings, Sveen et al. developed a CMS classifier for CRC cells. Tests of this classifier found that its concordance ranged from 85 to 92%, indicating robust performance [13]. Among the four CMS groups, patients with CMS4 were found to exhibit the worst overall and relapse-free survival rates, as well as high levels of stromal infiltration, angiogenesis, and invasiveness [14]. Moreover, CMS4 accounts for 26% of all early-stage CRC cases at diagnosis, and the ratio increases to 40% in stage IV disease [15]. Overall, CMS4 CRC tumors exhibit a limited response to standard treatments, highlighting the urgent need to explore alternative therapeutic strategies. Given that considering both genetic and environmental factors is crucial in this endeavor, there is an unmet clinical need for new drugs capable of targeting metastatic CMS4 CRC tumors.

Statins, 3-hydroxy-3-methylglutaryl coenzyme-A (HMG-CoA) reductase inhibitors, which are commonly known as cholesterol-lowering drugs, have garnered significant recent attention due to the potential roles they may play beyond lipid regulation [16]. For example, recent evidence suggests that statins may possess anticancer properties since they influence various aspects of cancer development and progression. The anticancer effects of statins are mediated by multiple mechanisms, including cell cycle blockades, the induction of apoptosis, anti-inflammatory effects, and metastasis inhibition [18]. Accumulated studies over the past decade have consistently demonstrated the beneficial impact of statins on clinical outcomes for various cancers, including colorectal, gastric, breast, lung, liver, and kidney cancers [19, 20]. However, despite promising preclinical and epidemiological data, conflicting results regarding the potential anticancer effects of statins have emerged from both clinical and observational studies, primarily due to variations in cohort diversity and follow-up study design [21–23]. Therefore, assessing the subtype-specific effects of statins may help translate them to clinical practice for preventing or treating CRC.

In this study, our results showed that pitavastatin and atorvastatin could induce apoptosis and effectively reverse 5-FU resistance under high glucose conditions in the CMS4 cells. These results hold significant promise and may serve as a guide for future clinical trials aimed at repurposing pitavastatin and atorvastatin for the treatment of CRC patients with the metastatic CMS4 group, particularly those suffering from hyperglycemia.

Methods

Dependency map (DepMap) portal

The DepMap portal (https://depmap.org/portal/) [24] serves as a comprehensive repository for multiomics databases since it incorporates datasets from the Profiling Relative Inhibition Simultaneously in Mixtures (PRISM) and Cancer Cell Line Encyclopedia (CCLE) databases. The PRISM database contains drug sensitivity screening data for 4,686 compounds found in 750 cells. An overall PRISM score reflects the drug sensitivity of each compound in each cell in the PRISM database and is expressed as the $\log_2$ fold change in the proliferation rates of treated and untreated cells. It is a powerful tool for exploring the potential of repurposing non-oncology drugs for cancer treatment. Overall, these publicly accessible datasets facilitate rigorous bioinformatic and statistical analysis. We conducted Mann-Whitney U tests to systematically investigate non-oncological compounds and assess their potential for targeting metastatic and CMS4 CRC cells.

Predicting the mechanism of action of Statins through CLUE database

Connectivity scores and differentially expressed gene (DEG) data can be accessed from CLUE (https://clue.io/) to investigate the mechanisms of action of pitavastatin and atorvastatin. Connectivity scores quantify the degree of similarity between compound spectra or genetic perturbation spectra using pattern-matching algorithms. This information is valuable as it may offer initial insights into the biological effects of statins. The DEGs identified were then analyzed using ConsensusPathDB (CPDB, http://cpdb.molgen.mpg.de/) to further reveal which specific pathways are affected by statins.

Cell culture and chemical

The human CRC cells lines, including SW480-vehicle (i.e., Vector alone control) and SW480-ATG5 KO (i.e., an ATG5 knockout), were acquired from Professor Hsiao-Sheng Liu's laboratory at Kaohsiung Medical University, Kaohsiung, Taiwan [25]. These cells were maintained in Dulbecco's Modified Eagle's Medium (DMEM) with 5 mM glucose condition. To assess the effects of hyperglycemia on CRC cells, SW480 cells were cultured in DMEM medium with either 5 mM (Gibco, cat. 12100046) or 25 mM (Gibco, cat. 31600034) glucose for 4 weeks. This resulted in low glucose-adapted SW480 cells (SW480-LG) to mimic normoglycemic conditions and high glucose-adapted SW480 cells (SW480-HG) to simulate hyperglycemic conditions. The culture medium with 5 mM glucose mimics the normal physiological level of glucose in human serum (100 mg/dL), while the medium with 25 mM glucose simulates the serum of patients with severe diabetic hyperglycemia. The other human colorectal adenocarcinoma cell line, DLD-1, and its' 5-FU-resistant subline (referred to as DLD-1R) were established as described previously [26]. DLD-1 and DLD-1R cells were cultured in Roswell Park Memorial Institute (RPMI) 1640 Medium (Gibco, cat. 31800022). In brief, DLD-1R cells were trained in a medium with gradually increasing doses of 5-FU until reaching a final concentration of 20 μM. DLD-1R cells were constantly cultured in a 5-FU-containing (20 μM) medium to maintain their resistance property. All media were supplemented with 10% fetal bovine serum (FBS, HyClone) and 1% antibiotic-antimycotic solution (Penicillin, streptomycin, and amphotericin B Solution) (Biological Industries). The cells were cultured in a 37 °C incubator containing 5% CO_2 and passaged using 0.5% Trypsin EDTA Solution C (Biological Industries) every 3 days. Pitavastatin (cat. HY-B0144) and atorvastatin (cat. HY-B0589) were purchased from Master of Bioactive Molecules (MedChem Express, USA).

Cell viability assay

The cell viability assay refers to the previously reported method [27]. The SW480-LG, SW480-HG, DLD-1, and DLD-1R cells were cultured at a density of 3,000 cells per well in a 96-well plate. Following a one-day incubation period for cell adhesion, cells were treated with 5-FU (1.25, 2.5, 5, 10, and 20 μM) or pitavastatin and atorvastatin (1.25, 2.5, 5, and 10 μM). After 24 to 72 h of treatment, living cells were fixed with 10% trichloroacetic acid (TCA, Sigma, cat. SI-T6399-250G) for 1 h. The cells were then stained with 0.4% Sulforhodamine B (SRB) sodium salt dye (Sigma, cat. S1402) for 1 h and washed with 1% acetic acid (J.T baker, cat. JT-9508-03) to remove the unstained dye. After three times wash, 10 mM Tris-base (Amresco, cat. CPT-0826) solution was added to solubilize the protein-bound dye, and the optical density was measured at 510 nm with a multimode microplate reader (Infinite 200 PRO).

Migration assay

The migration assay refers to the previously reported method [28]. In brief, SW480-LG and SW480-HG cells were seeded at a density of 2×10^5 cells/well in the upper chambers and were incubated with serum-free medium, while medium with 10% FBS as attractants were added in the lower chambers. Pitavastatin (0.63 and 1.25 μM) and atorvastatin (1.25 and 2.5 μM) were added simultaneously in both upper and lower chambers, respectively, followed by incubation at 37 °C for 24 h. After incubation, the non-migrating cells on the upper side of the membrane were removed with a cotton bud, and the migrated cells in the lower chamber were fixed with a 4% formaldehyde solution and stained with crystal violet.

The areas of stained cells were measured with Image J software.

Western blotting

CRC cells were seeded at a density of 5×10^5 in a 6 cm dish. Following treatment with pitavastatin (1.25, 2.5, 5 μM) and atorvastatin (2.5, 5, 10 μM) for 48 h, the cell lysates were analyzed by Western blotting referring to the previously reported method [29]. The primary antibodies ATG5, p62, LC3B, PERK, p-PERK (T982), ATF4, CHOP, cleaved-Caspase3, cleaved PARP, Bax, p-YAP (S127), Snail, IRS1, ZO-1, YAP-1 antibodies were used for detection, and GAPDH was used as the internal control. The signaling from secondary antibodies was detected by adding horseradish peroxidase (HRP) substrate peroxide solution/luminol reagents (ImmobilonTM Western Chemiluminescent Substrate, Millipore; mixed at a 1:1 ratio) and detected using a chemiluminescence system (Fuji LAS-4000 Fujifilm). The images were analyzed with Image J software. Additional information for antibodies is shown in Additional File 1: Table S1.

Colony formation assay

The colony formation assay was based on the previously reported method [30]. The SW480-LG and SW480-HG cells were seeded at a density of 500 cells per well in a 6-well plate. Following a one-day incubation period for cell adhesion, the culture medium containing the drugs was refreshed every three days. To evaluate the effects of anti-colony formation, various concentrations of 5-FU (0.63, 1.25, 2.5, 5, and 10 μM), along with pitavastatin (0.16, 0.31, 0.63, 1.25, and 2.5 μM) and atorvastatin (0.63, 1.25, 2.5, 5, and 10 μM) were employed, respectively. In the combination treatment, 5-FU (1.25 μM) was administered in conjunction with pitavastatin (0.63 and 1.25 μM) or atorvastatin (1.25 and 2.5 μM). After 9 days, the cells were stained with crystal violet for 1 h to visualize single colonies. The cells were washed with phosphate-buffered saline (PBS) (Amresco, cat. K813-500ML) and fixed with 4% formaldehyde solution (Sigma, cat. 252549) for 15 min. The residual crystal violet dye was removed by rinsing with tap water. One colony was defined as containing more than 20 cells, and the numbers of the colonies were counted with Image J software.

Synergistic effect analysis

Synergistic effect analysis was performed as per a previously reported method [31]. Cell viability was determined using the SRB assay. SW480-LG and SW480-HG cells were seeded at 3,000 cells per well in a 96-well plate. Following a one-day incubation period for cell adhesion, 5-FU (1.25 and 2.5 μM) was co-administered with pitavastatin (1.25 and 2.5 μM) or atorvastatin (1.25 and 2.5 μM) and incubated for 48 h. Next, CompuSyn software (http://www.combosyn.com/) was used to assess the synergistic effect of 5-FU and pitavastatin/atorvastatin in inducing cytotoxic death in SW480 cells, following a previously described method [32, 33]. The combination index (CI) defines synergism ($CI < 1$), an additive effect ($CI = 1$), and antagonism ($CI > 1$).

3D spheroid cultured assays

SW480-LG and SW480-HG cells were each seeded at a density of 1,000 cells per 50 μL of the kit gel mixture according to the manual instructions of the ACD 3D culture kit (GEcoll Biomedical Co., Ltd., Taiwan) (a scaffold-based system) [34]. Next, 50 μL of the resulting cell-gel mixture was applied to a 24-well plate. After a 5-minute reaction on ice and a subsequent 15-minute reaction with a specialized buffer that facilitates gel cross-linking, the gel becomes both stable and flexible. For DLD-1 and DLD-1R cells, cells were seeded at a density of 1,000 cells per well onto the R^3CE 24-Well 3D culture Plate (Acrocyte Therapeutics Inc. Taiwan) (a scaffold-free system) [35]. Following a 7-day incubation period to facilitate spheroid formation, 5-FU (1.25, 2.5, 5, and 10 μM), pitavastatin (1.25, 2.5, 5, and 10 μM), and atorvastatin (1.25, 2.5, 5, and 10 μM) were added to both experimental setups for an additional duration of 7 days.

Immunofluorescence (IF) staining and confocal microscopy

For IF staining, spheroids were first fixed with 4% formaldehyde for 1 h and then washed twice with PBS at room temperature. Fixed spheroids were then blocked with 3% bovine serum albumin (BSA) at 4 °C overnight before being stained with CD44 (i.e., Alexa Fluor™ 488), YAP1 (labeled with Alexa Fluor™ 488), Alexa Fluor™ 568 Phalloidin, and Hoechst 33,342. IF staining images were then obtained using a Zeiss LSM900 confocal microscope at a magnification of 400X.

Statistical analysis

Mann-Whitney U tests were used to evaluate the statistical significance of differences in the mean sensitivity to compounds listed in the PRISM database on primary and metastatic cancer, as well as on CMS4 and other CMS cells. Subsequent statistical analyses were conducted using Student's *t*-tests. All statistical analysis results are presented as mean ± standard error of the mean (SEM), and statistical significance is represented using different symbols. Specifically, the number of symbols corresponds to the significance level: one symbol indicates $p < 0.05$, two symbols indicate $p < 0.01$, and three symbols indicate $p < 0.001$. All experiments were repeated at least three times.

Results

Pitavastatin and Atorvastatin are identified as putative treatments for metastatic CMS4 CRC

The discordance observed among various gene expression-based classification systems can lead to inconsistent outcomes for clinical CRC trials, and we therefore postulated that a more nuanced classification of CRC based on gene expression profiles could yield valuable insight. As illustrated in the flowchart (Fig. 1A), we aimed to accelerated pharmacogenetic pairing for this study. This was achieved by making use of comprehensive gene expression and drug susceptibility data accessible

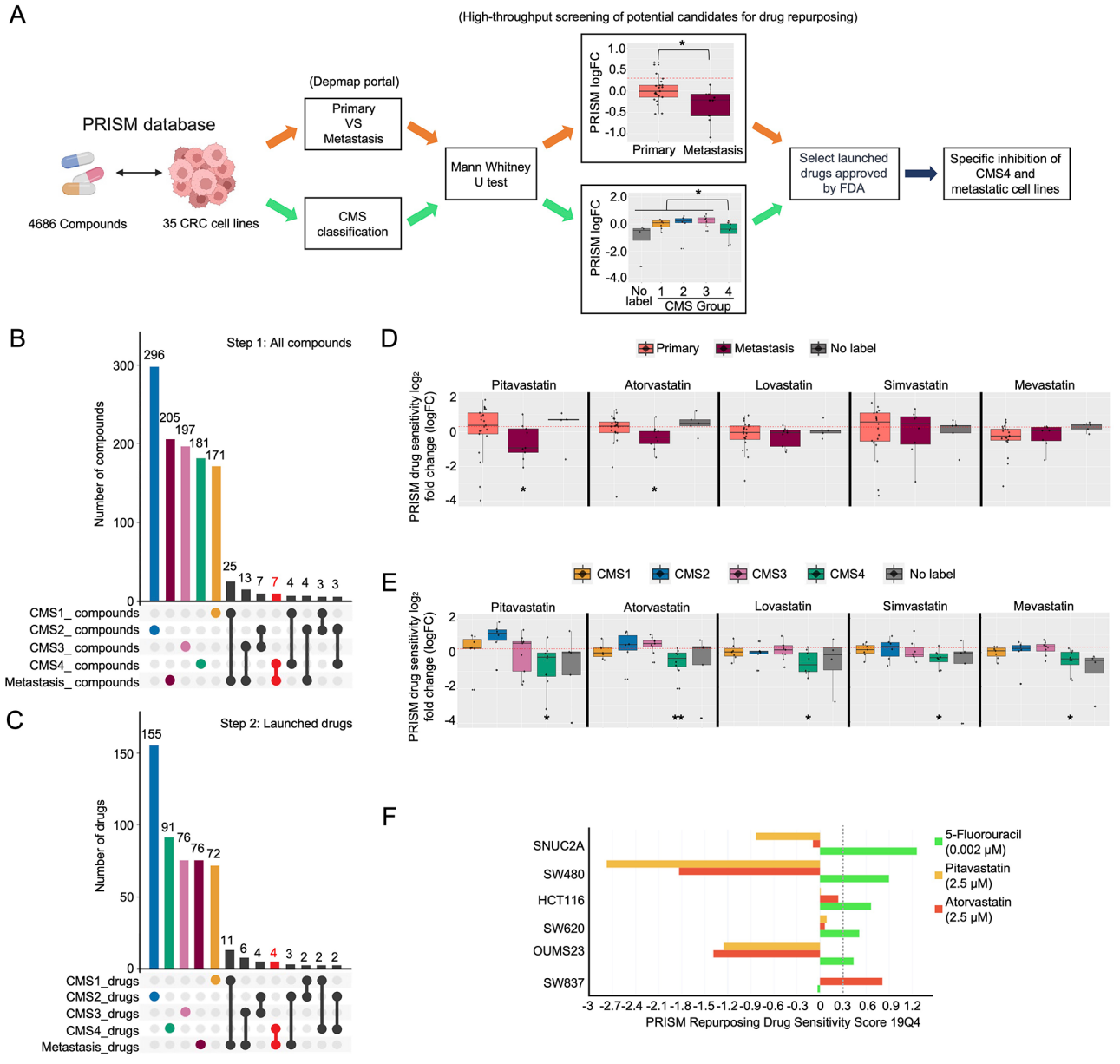

Fig. 1 Pitavastatin and atorvastatin are identified as putative treatments for metastatic CMS4 CRC. (**A**) PRISM screening, a high-throughput DNA-barcoding technique, is used to analyze the cell viability of 35 CRC cells treated with 4,686 compounds. PRISM drug sensitivity represents the $\log_2$-fold change in cell proliferation rate following drug treatment compared with an untreated group. Mann-Whitney U tests ($p < 0.05$) were used to identify specifically sensitive drugs in metastatic CRC cells and CMS-specific groups. (**B**-**C**) The numbers of compounds and on-market drugs at the intersection of drug candidates representing five groups (i.e., CMS1-4 specific drugs and metastasis-specific drugs). The horizontal bar represents the set size (i.e., the total number of drugs identified as candidates from each of the five groups). In contrast, the vertical bar represents the size of the intersection (i.e., the number of drugs included in each intersecting set). (**D**-**E**) Illustrations of the specific sensitivity of pitavastatin, atorvastatin, lovastatin, simvastatin, and mevastatin against metastatic (*Metastatic cells compared to primary cells) and CMS4 cells (* CMS4 cells compared to other CMS cells). (**F**) Analysis of the PRISM database used to compare the sensitivity of CRC cells to pitavastatin, atorvastatin, and 5-FU. There was no pitavastatin sensitivity data for SW837 cells found in the database. The cut-off value of drug sensitivity was smaller than 0.3. One symbol represents $p < 0.05$; two symbols indicate $p < 0.01$

through the DepMap portal [24], which functions as a repository for cellular multiomics datasets. The PRISM database was used to conduct high-throughput screening of drugs that may be suitable for repurposing [36]. Additionally, the sensitivity of primary and metastatic CRC cells was also compared. Furthermore, these cells were subdivided into four CMS groups based on the results of analysis conducted using the CMS caller R package [14, 37]. This refined CMS classification of cells plays a crucial role in effectively stratifying drugs. This extensive screening process led to the identification of a subset of the drugs that may have specific inhibitory effects on CMS4 and metastatic CRC cells among all those found in the database. Our results demonstrated that seven compounds highlighted in the red bar at the intersection, namely SU-11,274, DMNB, eltanolone, atorvastatin, cefixime, pitavastatin, and bivalirudin, exhibited effectiveness against both CMS4 and metastatic cells (Fig. 1B). However, further screening revealed that only four drugs, including atorvastatin, cefixime, pitavastatin, and bivalirudin, have been approved by the U.S. Food and Drug Administration (FDA) for safe drug repurposing (Fig. 1C). Next, the sensitivity of other statin drugs, including lovastatin, simvastatin, and mevastatin, in both primary and metastatic cells was further investigated. Additionally, the sensitivity of these drugs in different CMS CRC groups were also evaluated. These results identified only two statins, i.e., pitavastatin and atorvastatin, that could affect both CMS4 and metastatic CRC cells (Fig. 1D-E and Additional File 2: Table S2). We also queried the PRISM database regarding the effects of metformin, the most used antidiabetic drug, on CMS4 and metastasis CRC cells; however, metformin failed to show any specific anti-CMS4 (Additional File 3: Figure S1A) or anti-metastasis cytotoxicity (Additional File 3: Figure S1B). Taken together, these results indicated that pitavastatin and atorvastatin are potential candidates for repurposing to treat metastatic CMS4 CRC cells.

Currently, 5-FU-based chemotherapy remains the most common treatment for patients with metastatic CRC [38]. However, the response rate of 5-FU in combination with other anticancer drugs is only 40-50% due to chemoresistance [39]. Moreover, the presence of hyperglycemia further increases the resistance of metastatic CRC to 5-FU [40]. Therefore, the development of strategies to overcome 5-FU resistance in metastatic CRC remains urgent, especially for patients impacted by hyperglycemia. Since statins are commonly used drugs for patients with diabetes, the effects of specific statins, such as pitavastatin and atorvastatin, on 5-FU resistance were examined under different glucose conditions. Initially, sensitivity data for 5-FU (0.002 μM), pitavastatin (2.5 μM), and atorvastatin (2.5 μM) treatment of CMS4 CRC cells were extracted from the PRISM database to assess candidate cells (Fig. 1F). The results indicated that SNUC2A, SW480, and OUMS23 cells were sensitive to pitavastatin and atorvastatin, indicating these two statin drugs might have the potential to overcome 5-FU resistance. Moreover, the SW480 cells exhibited the highest sensitivity to pitavastatin and atorvastatin. Consequently, the SW480 cells were selected for subsequent experiments.

Pitavastatin and Atorvastatin can overcome high glucose-induced drug resistance and synergistically promote 5-FU-mediated cytotoxicity

Existing evidence shows that hyperglycemia has a substantial impact on the incidence, chemotherapy resistance, and prognosis of CRC, as well as on the outcomes of both localized and metastatic CRC patients [41]. To further examine this phenomenon, we sought to replicate the hyperglycemic microenvironment in vitro. To do so, the SW480 cells were first cultured under normoglycemic conditions (i.e., low glucose medium) that mirrored the normal physiological glucose levels found in human serum to generate SW480-LG cells. In parallel, SW480 cells were cultured under hyperglycemic conditions (i.e., high glucose medium), thereby approximating the serum glucose levels observed in patients with severe diabetic hyperglycemia, to generate SW480-HG cells (Additional File 3: Figure S2). Subsequent Western blotting analysis revealed that SW480-HG cells exhibited altered expression of the glucose metabolism-related marker IRS1 and Yes-associated protein (YAP1) (Additional File 3: Figure S3A-S3B). YAP1 has been implicated in enhancing tolerance to ER stress, thereby facilitating evasion of apoptosis [42]. Additionally, the expression of Snail was up-regulated under high-glucose conditions, indicating an increased metastatic potential in response to high glucose levels (Additional File 3: Figure S3C).

Consistent with predictions from the PRISM Database, SW480-LG cells demonstrated greater sensitivity to pitavastatin, and atorvastatin as compared to 5-FU. However, under high glucose conditions, SW480-HG cells exhibited resistance to 5-FU while retaining sensitivity to both pitavastatin and atorvastatin, even after 72 h of treatment (Table 1 and Additional File 3: Figure S4A-S4C). This phenomenon was also evident by colony formation assays, in which SW480-HG cells showed higher resistance to 5-FU (Fig. 2A and Additional File 3: Figure S5A) but retained sensitivity to increasing doses of pitavastatin and atorvastatin (Fig. 2B-C and Additional File 3: Figure S5B-S5C). However, pitavastatin and atorvastatin were unable to inhibit the viability of HT29 cells, which were identified as CMS3 CRC cells, raising the possibility that the cytotoxic effects of pitavastatin and atorvastatin might be influenced by the genetic background of specific cells (Additional File 3: Figure

Table 1 Summary of IC_{50} values for 5-FU, Pitavastatin, and Atorvastatin in SW480-LG and SW480-HG cells

Drug		5-FU (μM)		Pitavastatin (μM)		Atorvastatin (μM)	
Cell		SW480-LG	SW480-HG	SW480-LG	SW480-HG	SW480-LG	SW480-HG
Time	24 h	110.6 ± 2.0	240.7 ± 2.4*	6.3 ± 2.7	24.9 ± 1.3**	28.8 ± 1.4	93.4 ± 2.0*
	48 h	24.0 ± 1.4	87.8 ± 1.9*	1.8 ± 0.9	3.5 ± 1.0	4.7 ± 1.5	9.1 ± 2.3
	72 h	8.6 ± 2.0	18.1 ± 3.2***	0.4 ± 0.2	0.6 ± 0.2	1.6 ± 0.3	3.3 ± 0.9

Data represent the mean ± SEM ($N = 3$). (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ vs. SW480-LG cells)

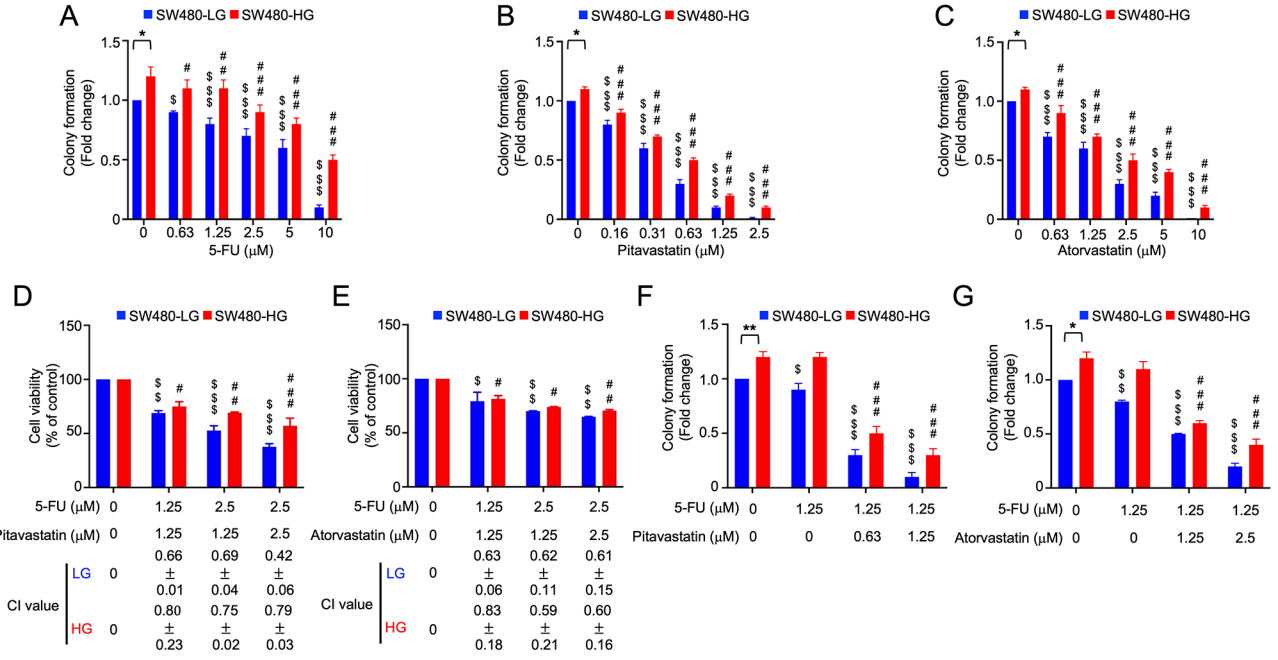

Fig. 2 Pitavastatin and atorvastatin can overcome high glucose-induced drug resistance and synergistically promote 5-FU-mediated cytotoxicity. (**A**-**C**) Colony formation assay of SW480-LG and SW480-HG following treatment with 5-FU, pitavastatin, or atorvastatin for nine days. (**D**-**E**) The combination index (CI) of SW480-LG and SW480-HG cells treated with 5-FU in combination with pitavastatin or atorvastatin. CI defines synergism (CI < 1), an additive effect (CI = 1), and antagonism (CI > 1). (**F**-**G**) Colony formation assay of SW480-LG and SW480-HG following treatment with a combination of 5-FU and pitavastatin or atorvastatin. Data represent the mean ± SEM ($N = 3$). $: Denotes a comparison with the SW480-LG control; #: Indicates a comparison with the SW480-HG control; The number of symbols corresponds to the significance level: one symbol indicates $p < 0.05$; two symbols indicate $p < 0.01$; and three symbols indicates $p < 0.001$. LG: Low glucose; HG: High glucose

S6A-S6B and Additional File 4: Table S3). Further investigation into the cytotoxicity of lovastatin, a recognized CMS4-targeting drug, showed that although lovastatin could gradually inhibit the growth of SW480-LG cells, it did not impede the viability of SW480-HG cells, which further evidenced that pitavastatin and atorvastatin have the potential to overcome hyperglycemia-mediated drug resistance (Additional File 3: Figure S7A-S7C and Additional File 5: Table S4).

We further conducted SRB assays to assess whether pitavastatin and atorvastatin could enhance the cytotoxicity of chemotherapeutic agents, such as 5-FU. Co-treatment of 5-FU with either pitavastatin or atorvastatin at constant ratios (i.e., 1:1 and 1:2) was performed to analyze the drug combination effects. Additionally, we calculated the combination index (CI) was calculated to assess the synergy between the drugs. The synergistic cytotoxic effects in combinations of 5-FU and pitavastatin (Fig. 2D) or 5-FU and atorvastatin (Fig. 2E) in both SW480-LG and -HG cells, were evidenced by CI values of less than 1 (Fig. 2D-E). Further colony formation assays yielded similar results. Thus, compared to 5-FU treatment alone, combined treatment of 5-FU plus pitavastatin (Fig. 2F and Additional File 3: Figure S8A) or 5-FU plus atorvastatin (Fig. 2G and Additional File 3: Figure S8B) further decreased colony formation in both SW480-LG and -HG cells.

Although SW480-HG cells exhibited resistance to 5-FU (1.25 μM) compared to SW480-LG cells, the addition of different doses of pitavastatin (0.63 or 1.25 μM) or atorvastatin (1.25 or 2.5 μM) restored the cytotoxicity of 5-FU (Fig. 2F-G and Additional File 3: Figure S8A-S8B). These observations align with our bioinformatics predictions suggested that pitavastatin and atorvastatin can effectively overcome 5-FU resistance induced by high glucose conditions.

Pitavastatin and Atorvastatin can inhibit the migration ability and spheroid formation stimulated by high glucose

To investigate the impact of pitavastatin and atorvastatin on high glucose-stimulated migration ability, we performed a transwell assay. As anticipated, SW480-HG cells demonstrated increased migration ability compared to SW480-LG cells, indicating that SW480-HG cells exhibited metastatic behavior under high glucose conditions (Fig. 3A). However, both pitavastatin and atorvastatin could still inhibit cell migration under high glucose

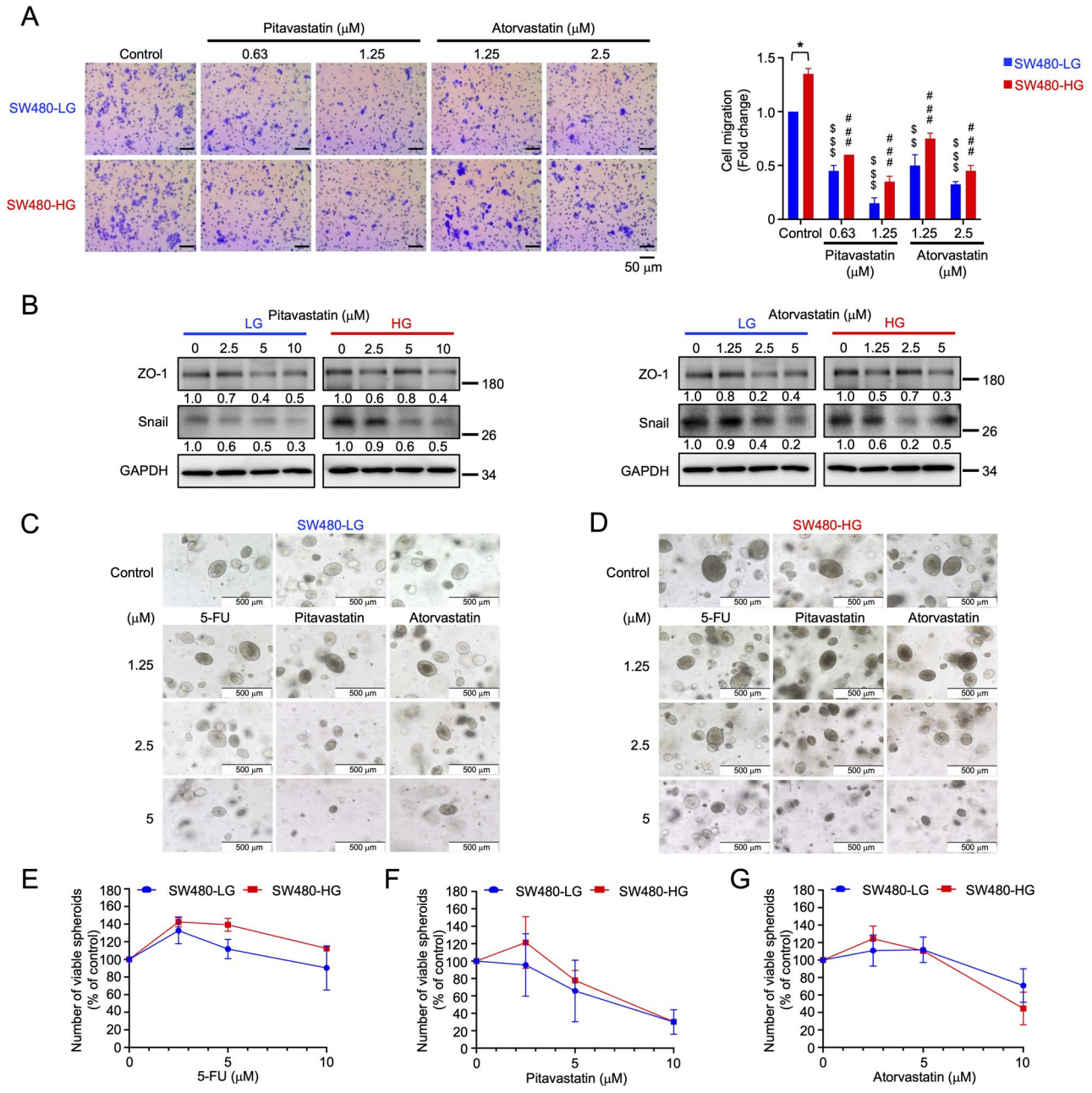

Fig. 3 Pitavastatin and atorvastatin can inhibit the migration ability and spheroid formation stimulated by high glucose. (**A**) A transwell assay shows decreased cell migration following treatment with pitavastatin or atorvastatin for 24 h in SW480-LG and SW480-HG cells. Representative images are shown. An untreated SW480-LG condition serves as a baseline for comparison. (**B**) Following a 48-hour treatment with pitavastatin and atorvastatin, both statins demonstrated an ability to influence the expression of ZO-1 and Snail proteins. (**C**-**D**) SW480-LG and SW480-HG cells were cultured using an ACD 3D culture system at a density of 1000 cells per well. Images were acquired using an Olympus IX83 inverted microscope with a 10X objective. The scale bar represents 500 μm. (**E**-**G**) Effects of 5-FU, pitavastatin, and atorvastatin on spheroid formation by SW480-LG and SW480-HG cells. Data represent mean ± SEM ($N = 3$). $: Indicates a comparison with the SW480-LG control; #: Indicates a comparison with the SW480-HG control; The number of symbols corresponds to the significance level: one symbol represents $p < 0.05$; two symbols indicate $p < 0.01$; and three symbols denote $p < 0.001$. LG: Low glucose; HG: High glucose

conditions (Fig. 3A). Interestingly, Western blotting revealed that both pitavastatin and atorvastatin treatment reduced ZO-1 and Snail protein levels under both low- and high-glucose conditions (Fig. 3B). Collectively, these findings underscored the potential of pitavastatin and atorvastatin to suppress potentiated metastatic regulation under high glucose conditions.

The emergence of 5-FU chemotherapy resistance induced by hyperglycemia presents a significant obstacle to treat CRC patients [43]. To better replicate the physiological conditions of tissues in vivo and gain deeper insights into cellular processes such as proliferation, differentiation, and drug response, the potential efficacy of pitavastatin and atorvastatin was subsequently evaluated using a 3D spheroid cultured assay. To do so, SW480-LG and SW480-HG cells were treated with 5-FU, pitavastatin, and atorvastatin, respectively. Our results showed that SW480-HG cells displayed a greater inclination for spheroid formation than SW480-LG cells (Fig. 3D vs. 3 C, upper panel). Additionally, prolonged (i.e., 7 days) administration of pitavastatin and atorvastatin significantly reduced spheroid cell viability in both SW480-LG and SW480-HG cells when compared with 5-FU control treatment (Fig. 3E-G). Subsequently, the cytotoxic effects of 5-FU in combination with pitavastatin or atorvastatin in SW480-HG cells were investigated and observed that the combined treatment also further influenced spheroid formation (Additional File 3: Figure S9). Consequently, pitavastatin and atorvastatin were shown to restore sensitivity to 5-FU and alter spheroid formation, potentially impacting tumor growth and metastasis in vivo.

Pitavastatin and Atorvastatin affect intrinsic 5-FU resistance in CRC cells

Next, the potential impact of pitavastatin and atorvastatin on intrinsic 5-FU-resistant DLD-1R cells were investigated. These cells differed in that DLD-1R cells exhibit greater resistance to 5-FU than DLD-1 cells (Table 2 & Additional File 1: Figure S10A). Although initial resistance to pitavastatin and atorvastatin was observed 24 h after treatment, these cells exhibited sensitivity at 48 and 72 h, particularly to pitavastatin (Table 2 & Additional File 1: Figure S10B-S10C). Similarly, comparable results were observed in the colony formation assay, where DLD-1R cells demonstrated greater resistance to 5-FU than DLD-1 cells (Fig. 4A & Additional File 1: Figure S11A). Both DLD-1 and DLD-1R cells retained sensitivity to pitavastatin and atorvastatin in a dosage-dependent manner (Fig. 4B-C & Additional File 1: Figure S11B-S11C). Since there is increasing evidence of a link between 5-FU resistance and cancer stemness, i.e., a subpopulation of cells within tumors characterized by self-renewal and tumor-initiating properties [44, 45]. A 3D spheroid formation assay was therefore used to examine the effects of pitavastatin and atorvastatin on the intrinsic 5-FU resistance of CRC cells. In the Cyto3D™ live-death experiment, the combined treatment of 5-FU with pitavastatin or atorvastatin increased the DLD-1 spheroid death (Red signal) (Fig. 4D). In addition, DLD-1R cells exhibited greater spheroid formation than DLD-1 cells (Fig. 4E vs. 4 F, upper panel). Furthermore, pitavastatin and atorvastatin had a more pronounced effect on spheroid size than the 5-FU treatment alone, which was accompanied by cell fragmentation, cell volume reduction, and irregular cell edges (Fig. 4E-F). Additionally, in the spheroid cell viability assay, it was observed that compared to treatment with 5-FU alone, pitavastatin and atorvastatin significantly reduced the survival rate of DLD-1R cells (Fig. 4G-I). Interestingly, both pitavastatin and atorvastatin effectively downregulated the expression of cancer stem-like cell marker, such as CD44 in DLD-1 and DLD-1R spheroid cells, with pitavastatin treatment showing particularly notable suppression (Fig. 4J-K). Taken together, these findings suggested that targeting CD44 expression in CRC cells, particularly those intrinsically resistant to 5-FU, can disrupt spheroid formation, thereby offering a promising therapeutic strategy for treating intrinsic 5-FU-resistant CRC cells.

Underlying mechanisms driving the effects of Pitavastatin and Atorvastatin on CRC cells

To attain a comprehensive understanding of the pathway alterations involved in CRC, differentially expressed gene (DEG) signatures for pitavastatin or atorvastatin-treated CRC cells were extracted from the CLUE database. Subsequently, ConsensusPathDB (CPDB) was utilized to predict the specific pathways involved in pitavastatin or atorvastatin-triggered events. Pitavastatin and atorvastatin showed similar mechanisms of action and both impact on CRC development (Fig. 5A-B). In addition, pitavastatin and atorvastatin also affected apoptosis, autophagy, the TGF-β signaling pathway, hippo signaling,

Table 2 Summary of IC_{50} values for 5-FU, Pitavastatin, and Atorvastatin in DLD-1 and DLD-1R cells

Drug		5-FU (μM)		Pitavastatin (μM)		Atorvastatin (μM)	
Cell		DLD-1	DLD-1R	DLD-1	DLD-1R	DLD-1	DLD-1R
Time	24 h	408.7 ± 2.6	1010 ± 3.0*	110.5 ± 2.0	42.1 ± 1.6	64.2 ± 1.8	59.7 ± 1.8
	48 h	4.1 ± 0.8	154 ± 2.2**	6.3 ± 3.2	5.8 ± 3.7	8.1 ± 2.4	7.3 ± 1.5
	72 h	1.8 ± 0.8	176 ± 2.3***	3.7 ± 0.7	3.1 ± 0.5	5.4 ± 0.5	5.5 ± 0.7

Data represent the mean ± SEM ($N = 3$). (*$p < 0.05$, **$p < 0.05$, ***$p < 0.001$ vs. DLD-1R cells)

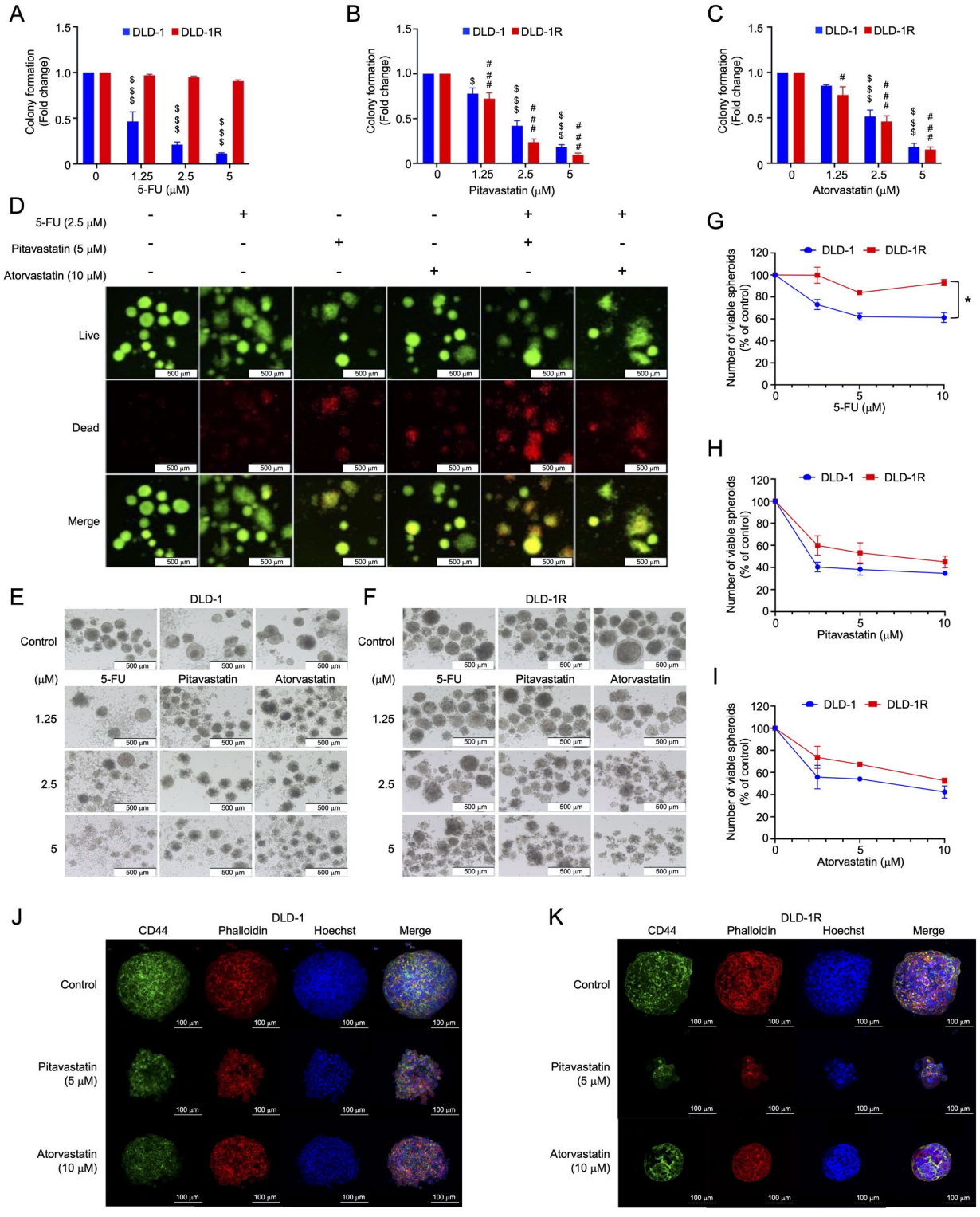

Fig. 4 (See legend on next page.)

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Fig. 4 Pitavastatin and atorvastatin affect intrinsic 5-FU resistance in CRC cells. (**A**-**C**) Colony formation assay of DLD-1 and DLD-1R cells following treatment with 5-FU, pitavastatin, or atorvastatin for nine days. (**D**) The Cyto3D™ dead-live assay following cotreatment with 5-FU and pitavastatin or atorvastatin in DLD-1 spheroids. Live-dead imaging of DLD-1 spheroids was conducted using the Cyto3D Live-Dead assay. Bright-field and fluorescence images were acquired using an Olympus IX83 inverted microscope with a 10X objective. The scale bar represents 500 µm. (**E**-**F**) DLD-1 and DLD-1R were seeded onto R^3CE plates at 1000 cells per well density. 5-FU, pitavastatin, and atorvastatin were added to the culture medium at the indicated concentrations for 7 days. Images were acquired using an Olympus IX83 inverted microscope with a 10X objective. The scale bar represents 500 µm. (**G**-**I**) Effects of 5-FU, pitavastatin, and atorvastatin on spheroid formation by DLD-1 and DLD-1R cells. (**J**-**K**) Drug-treated DLD-1 and DLD-1R spheroids were stained with CD44 (labeled with Alexa Fluor™ 488), Alexa Fluor™ 568 Phalloidin, and/or Hoechst 33,342. IF staining images were acquired using a Zeiss LSM900 confocal microscope with a 20X objective. The scale bar represents 100 µm. Data represent mean ± SEM ($N = 3$). DLD-1R: DLD-1 5-FU resistance

epithelial-mesenchymal transition (EMT), and so on (Summarized in Additional File 6: Table S5).

Autophagy plays a pivotal role in promoting cancer cell death by inducing the degradation and recycling of cellular components, which ultimately leads to cell death [46]. Moreover, there is compelling evidence indicating that statins exert a cytotoxic effect on cancer cells by augmenting the formation of autophagosomes, which consequently triggers apoptosis [47]. Our results revealed that treatment with pitavastatin and atorvastatin significantly increased the expression of autophagy-related markers, including p62 and LC3B II, indicating the modulation of autophagy (Fig. 5C). Furthermore, the mechanism through which statins induce ER stress and activate UPR leading to cell death requires further elucidation [48]. There are differing perspectives on how YAP regulates ER stress, and the relationship between YAP and UPR is complex. YAP plays a crucial role in UPR activity and ER expansion to relieve ER stress, and the PERK kinase-eIF2α axis is associated with YAP activation during the adaptation phase of the UPR. However, prolonged ER stress-induced Hippo signaling triggers a negative feedback loop involving the assembly of the GADD34/PP1 complex, thereby inhibiting YAP, and promoting apoptosis [42]. Our results demonstrated that treatment with pitavastatin and atorvastatin significantly increased the expression of p-PERK, PERK, ATF4, and CHOP in both SW480-LG and -HG cells. In addition, YAP1 expression was reduced, whereas the phosphorylated-YAP level was increased, potentially promoting apoptosis (Fig. 5C). Again, our IF results also found that treatment with pitavastatin and atorvastatin resulted in reduced YAP1 expression in DLD-1R cells (Additional File 1: Figure S12). Moreover, the observable upregulation of apoptosis-related markers—including cleaved PARP and Bax, as well as cleaved Caspase-3—in response to pitavastatin and atorvastatin treatment, which subsequently led to apoptosis in both SW480-LG and -HG cells (Fig. 5D). Taken together, our experimental results indicated that pitavastatin or atorvastatin treatment may impair autophagy flux, trigger ER stress and activation of the PERK/ATF4/CHOP signaling pathway, as well as modulation of YAP1 expression, and therefore to the induction of apoptosis.

Pitavastatin and Atorvastatin induce apoptosis partly by stimulating autophagy and inducing ER stress

To further elucidate the underlying mechanism responsible for pitavastatin and atorvastatin-induced apoptosis, the PI3K inhibitor 3-MA (an early autophagy inhibitor) (Fig. 6A), ATG5 KO SW480 cells (autophagy-deficient cells) (Fig. 6B), and the selective PERK inhibitor GSK2606414 (Fig. 6C) were employed. Co-treatment with 3-MA could reduce pitavastatin- and atorvastatin-induced LC3B II expression and PERK/ATF4/CHOP protein levels, indicating these statins could induce autophagy and further affect ER stress/UPR signaling (Fig. 6A). Pitavastatin and atorvastatin treatment also induced the expression of PERK/ATF4/CHOP in parental SW480 cells, while it was slightly decreased in ATG5 KO SW480 cells, again indicating these statins can induce an autophagy-dependent ER stress/UPR signaling (Fig. 6B). Although the co-treatment with 3-MA did not inhibit pitavastatin or atorvastatin-induced cleaved PARP and cleaved caspase 3, their slightly decrease in ATG5 KO cells may indicate the partial role of autophagy in pitavastatin- or atorvastatin-induced apoptosis. Next, co-treatment with GSK2606414 slightly affected pitavastatin- and atorvastatin-induced LC3B II expression in SW480-LG and SW480-HG cells, while significantly inhibiting the expression of ER stress/UPR markers such as ATF4 and CHOP (Fig. 6C). Downregulation of ATF4 and CHOP appeared to slightly inhibit the apoptosis of SW480-LG cells, while significantly inhibiting the apoptosis of SW480-HG cells, as evidenced by the reduction of cleaved PARP. Additionally, upregulation of YAP1 in GSK2606414 co-treated cells (Fig. 6C), thereby endowing the cells with anti-apoptotic potential. Taken together, these results indicated that ER stress/UPR signaling plays a role in pitavastatin and atorvastatin-induced apoptosis, particularly under high glucose conditions.

Discussion

By using data mining techniques and CMS classification, this study identified pitavastatin and atorvastatin as potential anti-CMS4 as well as anti-metastasis CRC drugs. As expected, pitavastatin and atorvastatin not only can inhibit the survival and metastatic potential of CMS4 cells but also can influence 5-FU-resistant cells and spheroid formation, particularly under high glucose

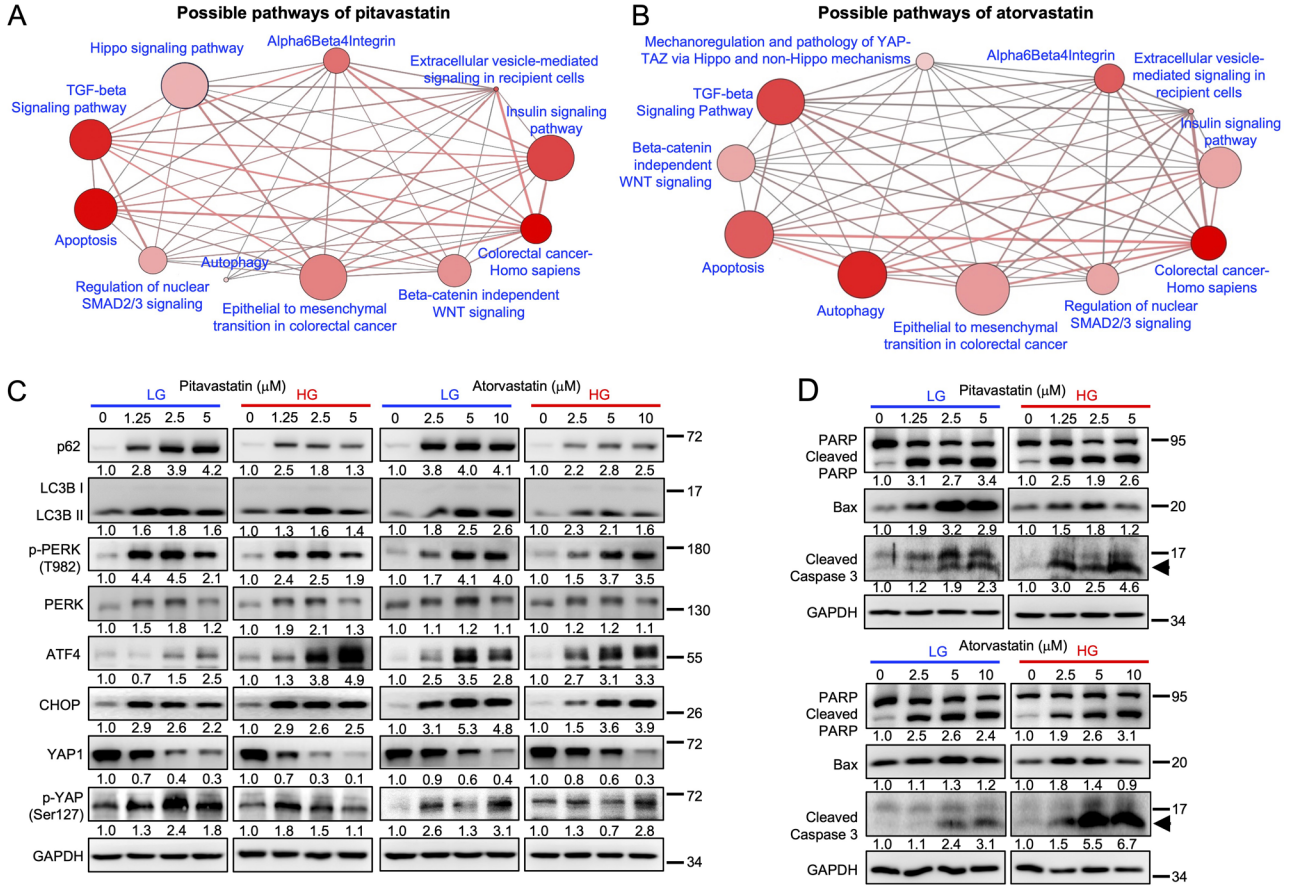

Fig. 5 Underlying mechanisms driving the effects of pitavastatin and atorvastatin on CRC cells. The network illustrates the connections between pathways associated with pitavastatin (**A**) and atorvastatin (**B**). The size of each dot denotes a pathway gene set, while lines between two dots represent connections between two pathways. Finally, the line width represents the strength of the relationship. (**C**-**D**) Western blotting data indicates that treatment with pitavastatin and atorvastatin for 48 h significantly influences the expression of markers associated with autophagy, ER, and apoptosis in both SW480-LG and SW480-HG cells. LG: Low glucose; HG: High glucose

conditions. Pitavastatin and atorvastatin treatment modulated autophagy and followed ER stress/UPR signaling, which may ultimately lead to apoptosis or other forms of cell death in CRC cells. Additionally, both pitavastatin and atorvastatin decreased the expression of YAP1, contributing to cell apoptosis. Consequently, we provide evidence to repurpose pitavastatin and atorvastatin, which originally served as primary lipid-lowering drugs, for treating patients with metastatic CRC, which may also benefit patients suffering from hyperglycemia.

Examining gene expression data sourced from large drug sensitivity databases can be used to provide information on drug repurposing strategies to combat specific forms of cancer. These databases have been widely used to explore how genetic alterations in cancer cells affect their response to drugs. However, varying degrees of genomic heterogeneity exist among cells, even among those of the same cancer type. It is therefore difficult to identify possible drug candidates that depend only on single mutant genes. However, by incorporating a more comprehensive classification of molecular paradigms for each cancer type in the search phase (e.g., CMS in CRC), we may be able to predict which drugs efficiently and precisely can be repurposed for target-based cancer treatment.

The CMS procedure helps to integrate gene expression-based classifications of CRC to facilitate clinical translation [12]. CMS has been proven to be a prognostic factor and has been gradually applied in numerous clinical trials [15]. In this study, we focused on CMS4 CRC since it is a common form of advanced-stage disease and exhibits the worst prognosis. In general, CMS4 CRC is resistant to the current anti-EGFR agent cetuximab in both chemorefractory and chemo-naïve settings [49, 50]. Moreover, no benefit of oxaliplatin treatment against CMS4 CRC has yet been reported [51]. The limited affected treatment for CMS4 CRC encouraged us to identify candidate drugs that are also capable of being repurposed for treating metastatic CMS4 CRC.

In addition to genetic heterogeneity, differences in the CRC microenvironment, including hyperglycemia, also play a pivotal role in influencing responses to drug

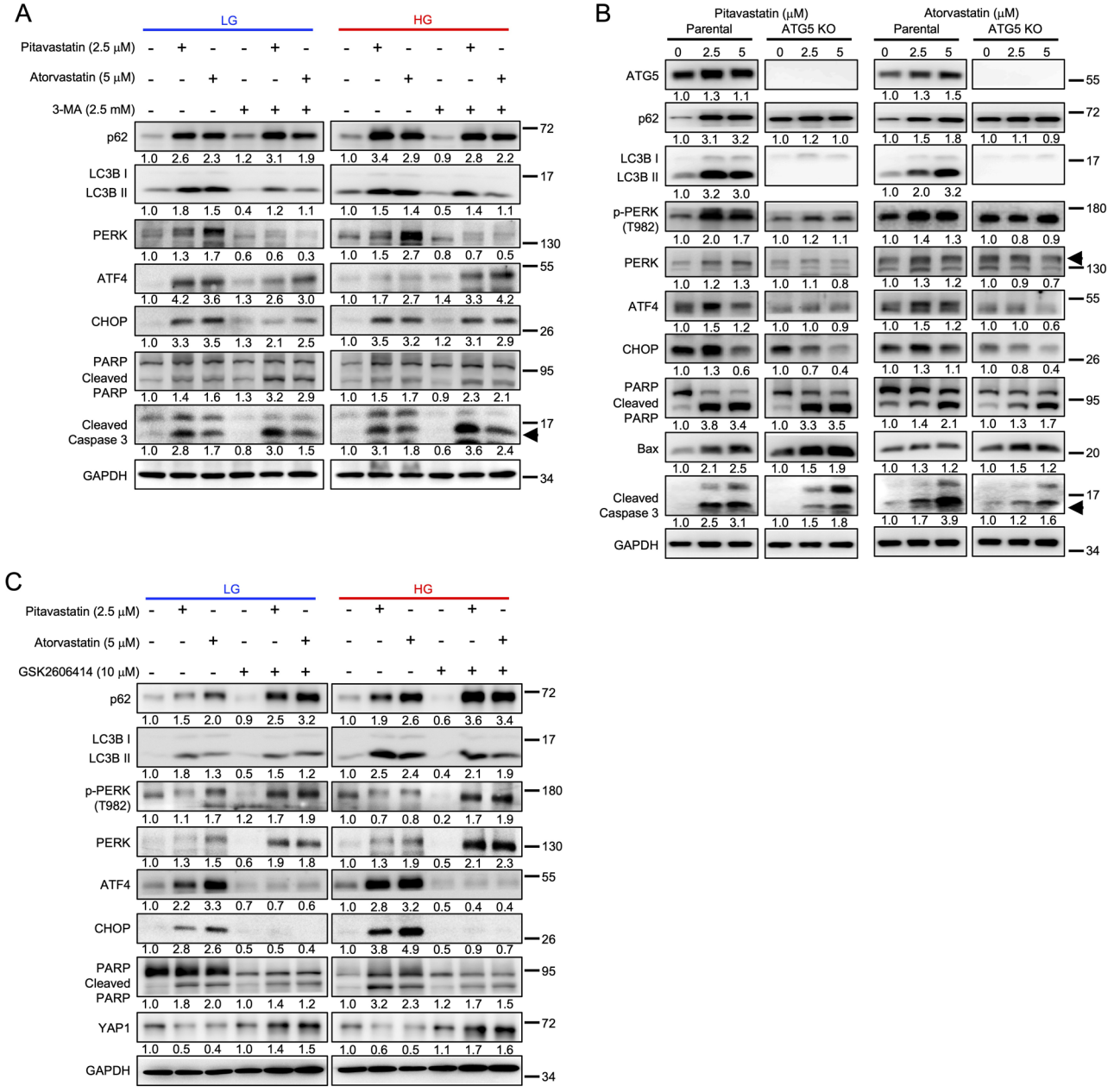

Fig. 6 Pitavastatin and atorvastatin induce apoptosis partly by stimulating autophagy and inducing ER stress. Western blotting data show the impact of various inhibitors on the expression of markers associated with autophagy, ER, and apoptosis. (**A**) SW480-LG and SW480-HG cells were co-treated with 3-MA and pitavastatin or atorvastatin. (**B**) Both SW480-vehicle and SW480-ATG5 KO cells were treated with pitavastatin and atorvastatin. (**C**) GSK2606414 was used in a cotreatment with pitavastatin or atorvastatin on SW480-LG and SW480-HG cells. LG: Low glucose; HG: High glucose

treatments. For example, Ma et al. reported that a high glucose environment attenuated 5-FU-mediated growth inhibition in CRC cells by decreasing cell death and increasing DNA replication [52]. In another study, Ikemura et al. revealed that the efficacies of 5-FU and oxaliplatin were limited in a CRC mouse model with induced hyperglycemia, the decreased overall survival was the result of chemoresistance [53]. DM also has been found to have a negative impact on CRC prognosis. Several meta-analyses have identified an association between DM and increased all-cause mortality and worse disease-free survival in patients with advanced CRC [54–59]. In Taiwan, Yang et al. further reported that high blood glucose levels could affect both overall and disease-free survival in patients with stage III CRC who were also receiving adjuvant 5-FU and oxaliplatin-based chemotherapy [40]. Similar observations can be found in the present study. For example, SW480-HG cells were more resistant to 5-FU than SW480-LG cells. It is therefore critical to develop novel strategies to overcome chemoresistance in advanced CRC by considering both genetic and environmental factors.

One of the main findings of the present study is that pitavastatin and atorvastatin, but not other statins, have the potential to treat metastatic CMS4 CRC in patients with comorbid hyperglycemia. However, based on genetic heterogeneity, the results of preclinical and clinical trials have shown that treatments involving statins combined with chemotherapy drugs may not always be effective for treating CRC. Therefore, prospective studies stratified by biomarkers are required to evaluate the efficacy of statins when combined with conventional chemotherapy (Additional File 7: Table S6). Moreover, a systematic review of randomized controlled trials did not confirm the efficacy of statins in treating patients with solid malignant tumors, including CRC [60]. Our analysis also indicated that inappropriate selection of statin drugs may result in no significant efficacy in CRC patients. (Additional File 3: Figure S13). The data from this study suggest that statins were specifically effective against CMS4 cancers. Moreover, other studies have observed that statin use is associated with a lower incidence of left-sided colon cancers and rectal cancers, which are common sites of CMS4 cancers [61–64].

Although lipid-lowering effects may be similar among statins, their potential to treat cancer may differ significantly. Statins can be classified as hydrophilic or lipophilic, with each group having a different overall tissue distribution. For example, hydrophilic statins (e.g., pravastatin, rosuvastatin, and fluvastatin) are mainly located in the liver. In contrast, lipophilic statins (e.g., simvastatin, mevastatin, lovastatin, pitavastatin, and atorvastatin) readily diffuse across cell membranes and are distributed throughout many body tissues [65, 66]. Thus, another meta-analysis found that lipophilic, but not hydrophilic, statins could significantly reduce the risk of CRC [62]. Moreover, rosuvastatin, pitavastatin, and atorvastatin can be chemically synthesized to possess a fluorophenyl group that can form an additional linkage to HMG-CoA reductase, which exhibits a more potent inhibition [67, 68]. In addition to the different pharmacokinetics of different statins, their mechanisms on CRC cells may also vary significantly. For example, a subgroup analysis of the TOHO lipid intervention trial using pitavastatin (TOHO-LIP) showed that the anticancer effect of pitavastatin may be drug-specific [69].

We further categorized pitavastatin, atorvastatin, lovastatin, simvastatin, mevastatin, and pravastatin into three classes based on their sensitivity for treating CRC cells. Class I statins (i.e., pitavastatin and atorvastatin) were sensitive against metastatic CRC cells, whereas class II statins (i.e., lovastatin, simvastatin, and mevastatin) were sensitive only to primary CRC cells. The one class III statin (pravastatin) identified was not effective in treating CRC cells. By comparing the gene signatures of six different statins in the CLUE database, we found class I and II statins show some degrees of similarities of DEGs, while little similarity can be found as compared to class III statins (Data not shown). Taken together, these results show that the significant cytotoxicity of pitavastatin and atorvastatin against CRC is related not only to their specific pharmacokinetics but also that these statins generate unique changes in gene expression relative to other statins.

Since hypercholesteremia is one of the most common comorbidities in DM patients, statins are frequently prescribed for this patient population [70]. Despite the similar potency of all statins in reducing serum cholesterol levels, they may show significant differences in their ability to treat CMS4 CRC cells and overcome 5-FU drug resistance. This may be related to dosage as well as their mechanisms of action against CRC. For example, in this study, the dosage of pitavastatin to inhibit SW480 was significantly lower than that of atorvastatin. However, the equivalent dosage between pitavastatin and atorvastatin when used to reduce CRC cell viability was similar to that used to generate a cholesterol-lowering effect. Although strict control of serum glucose levels in

patients with CRC and DM may improve their prognosis, elevated postprandial serum glucose levels may exist even with normal fasting glucose and glycated hemoglobin (HbA1c) levels. Moreover, direct administration of the blood-glucose-lowering agent metformin is unable to kill CMS4 CRC cells [71]. Therefore, the proper selection of specific statins (i.e., pitavastatin or atorvastatin) to treat hypercholesteremia in patients with both metastatic CRC and DM may help reduce the potential resistance to chemotherapy and improve cancer-specific survival.

Next, pitavastatin and atorvastatin-treated CRC gene signatures were extracted from CLUE database and utilized CPDB analysis to predict the specific pathways involved in their triggering events. Our findings confirm that these statins impact CRC development via similar pathways (Fig. 5A and B). Prior research indicates that statins induce cancer cell death by triggering autophagy and apoptosis [47]. Intriguingly, our results demonstrate that pitavastatin and atorvastatin indeed stimulate autophagy, as evidenced by elevated levels of p62 and LC3B II, along with increased apoptosis-related markers such as cleaved PARP, Bax, and cleaved Caspase-3 (Fig. 5C and D). However, co-treatment with 3-MA, which affects autophagy, did not apparently inhibit pitavastatin and atorvastatin-induced apoptosis-related markers, such as cleaved PARP and cleaved caspase 3 (Fig. 6A). Since these statin drugs-induced apoptosis-related markers slightly decreased in ATG5 KO SW480 cells, indicating modulating autophagy may still partly result in statin drugs-induced apoptosis (Fig. 6B). The robust upregulation of PERK/ATF4/CHOP expression under pitavastatin or atorvastatin treatment, indicating the induction of ER stress/UPR signaling. The administration of GSK2606414 (PERKi) decreased the cleavage of PARP, especially under high glucose conditions, indicating ER stress/UPR signaling played a crucial role in pitavastatin or atorvastatin-induced apoptosis (Fig. 6C). Additionally, since YAP serves as a critical regulator promoting cell survival under endoplasmic reticulum stress [42], pitavastatin or atorvastatin-induced increase of phosphorylated YAP, which promote its cytosolic retention and degradation by ubiquitin-proteasome system, may at least, partly, confer pitavastatin or atorvastatin-induced apoptosis (Fig. 5C). Interestingly, administration of GSK2606414 reversed pitavastatin and atorvastatin-induced ATF4/CHOP signaling as well as the decrease of YAP (Fig. 6C). The increase of YAP expression under PERK inhibition may partly result from the decrease of phosphorylated YAP. Recently, YAP also has been demonstrated to degrade via ER stress-mediated ER-associated degradation (ERAD) [72], it will be of interest to investigate whether YAP or its interplay with ER stress signaling plays a crucial role in pitavastatin or atorvastatin-induced apoptosis. The upregulation of YAP has been found to possess an anti-apoptotic effect in many cells, however, it also has been found to promote apoptosis and other forms of cell death, such as ferroptosis and pyroptosis, in some cell types [73]. Other ER stress inhibitors will be included to validate the role of ER stress/UPR signaling and YAP expression in pitavastatin and atorvastatin-mediated cell death. Whether other forms of cell death mechanisms were involved in pitavastatin, and atorvastatin-induced cell death still needs further investigation.

From a clinical standpoint, it's worth noting that the doses of pitavastatin and atorvastatin utilized in our cell culture experiments greatly exceeded typical clinical standards. This could potentially impede the translation of our research findings into practical clinical applications. For instance, the minimum dose of atorvastatin employed in our cell culture experiments was 2.5 μM, equivalent to a clinical dose of 1396 μg/L. This dosage is approximately 70 times higher than the current clinical standard of 20 μg/L [74]. Similarly, the lowest dose of pitavastatin used in our cell culture experiments was 1.25 μM, translating to a clinical dose of 1101 μg/L. This is approximately 5 times higher than the current clinical dose of 200 μg/L [75]. This discriminates the utilities of pitavastatin from atorvastatin in clinical practice. To enhance the feasibility of clinical therapeutic interventions for CRC, it may be beneficial to reduce the need for administering a single high dose by extending the duration of treatment. This approach could potentially maintain efficacy while minimizing the risk of adverse effects associated with high doses.

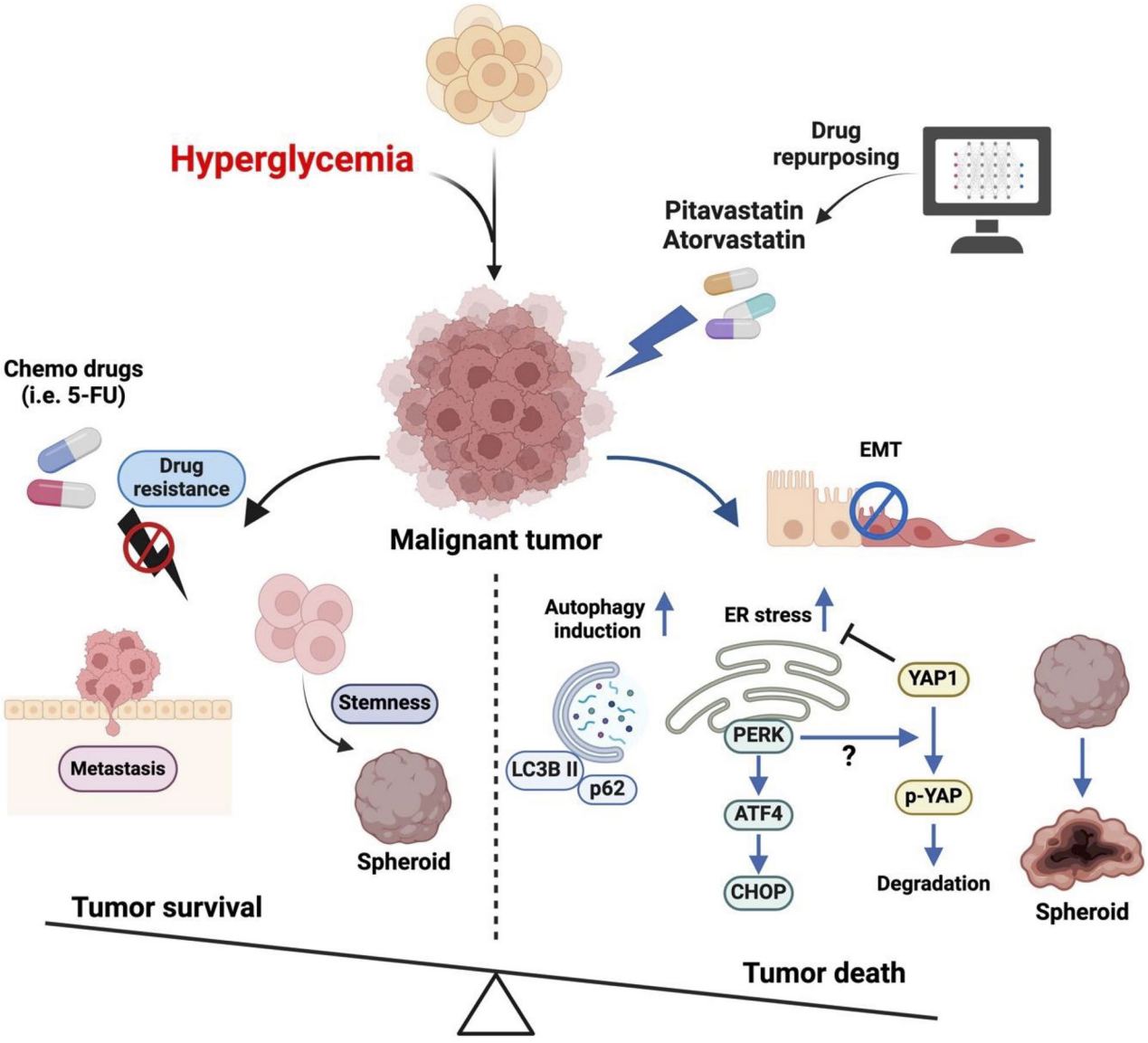

Fig. 7 Schematic representation of the mechanism of action of pitavastatin and atorvastatin. This illustration was created using BioRender.com

Conclusions

Our study underscores the detrimental effects of high glucose conditions on the metastasis and chemoresistance of CMS4 CRC cells. By integrating gene expression-based cancer classification with extensive drug sensitivity data under varying glucose conditions, we repurpose pitavastatin and atorvastatin as anti-CMS4 CRC drugs, particularly effective under high glucose conditions. Pitavastatin and atorvastatin exhibited significant efficacy in inhibiting cell migration and spheroid formation in highly metastatic CMS4 CRC under high glucose conditions, achieved through induction of autophagy and activation of ER stress/UPR, coupled with inhibition of YAP expression, leading to apoptosis (Fig. 7). Overall, this study highlights the utility of a gene expression-based cancer cell classification system for high-throughput prediction of cancer therapeutic drug repurposing. These findings may inform future clinical trials aimed at repurposing pitavastatin, which dosage might be achievable in clinical settings, to treat patients with metastatic CMS4 CRC, particularly under hyperglycemic conditions.

Abbreviations

5-FU	5-fluorouracil
BSA	Bovine serum albumin
CCLE	Cancer Cell Line Encyclopedia
CI	Combination index
CMAP	Connectivity Map
CMS	Consensus molecular subtype
CPDB	ConsensusPathDB
CRC	Colorectal cancer
CSC	Cancer stem-like cell
DEGs	Differential expression genes
DepMap	Dependency Map
DM	Diabetes mellitus
DMEM	Dulbecco's Modified Eagle's Medium
ERAD	ER-associated degradation
EMT	Epithelial-mesenchymal transition
ER	Endoplasmic reticulum
FBS	Fetal bovine serum
FDA	Food and Drug Administration
HbA1c	Glycated hemoglobin
HG	High glucose
HMG-CoA	3-hydroxy-3-methylglutaryl coenzyme-A
HMGCR	3-hydroxy-3-methylglutaryl-coenzyme-A reductase
HRP	Horseradish peroxidase
IF	Immunofluorescence
KO	Knockout
LG	Low glucose
MedChem Express	Master of Bioactive Molecules
PBS	Phosphate-buffered saline
PRISM	Profiling Relative Inhibition Simultaneously in Mixtures
R^3CE	Rapid, Reproducible, Rare Cell 3D Expansion
RPMI	Roswell Park Memorial Institute
SEM	Standard error of the mean
SRB	Sulforhodamine B
TCA	Trichloroacetic acid
TGF-β	Transforming growth factor-β
TOHO-LIP	TOHO lipid intervention trial using pitavastatin
UPR	Unfolded protein response
YAP	Yes-related protein

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12935-025-03712-2.

Supplementary Material 1

Supplementary Material 2

Supplementary Material 3

Supplementary Material 4

Supplementary Material 5

Supplementary Material 6

Supplementary Material 7

Acknowledgements

We acknowledge the generous gift of SW480-vehicle and SW480-ATG5 KO cells from Professor Hsiao-Sheng Liu at Kaohsiung Medical University, Kaohsiung, Taiwan. Additionally, we gratefully acknowledge the generous gift of DLD-1 and DLD-1R cells from Professor Yi-Shiou Chiou, also at Kaohsiung Medical University, Kaohsiung, Taiwan. We extend our gratitude to Yu-Chieh Chu and Chih-Wei Wu from Taipei First Girls High School, Taipei, Taiwan, for their assistance with the experiments.

Author contributions

Research Supervision: T-S.C., J-M.L., W-M.C., and C-Y. F. H.; Conceptualization and Study Design: P-C.L., M.T-B.N., S-Y.C., T-S.C., J-M.L., and C-Y. F. H.; Experimental Work: P-C.L., Y-T.L., Y-T.H., S-Y.C., T-Y.H., Y-C.C., and C-W.W.; Intellectual Contributions: T-H.N., T-H.T., H-T.H., M-F.L., and W-M.C.; Manuscript Writing: P-C.L., M.T-B.N.,T-S.C., W-M.C., J-M.L and C-Y. F. H. All authors have read and approved the final manuscript.

Funding

This work was supported by grants from the Integrated Research Plan of the National Science and Technology Council (MOST111-2320-B-A49-036) to Chi-Ying F Huang, MOST 111-2320-B-030-008 and 109-2320-B-030-008-MY3 to Jin-Mei Lai, NSTC 113-2321-B-A49-024 to Peter Mu-Hsin Chang, and MOST111-2320-B-039-019-MY3 to Ming-Fen Lee. Additional support was supported by grants from Fu Jen Catholic University Hospital (PL202108010V) to Jin-Mei Lai, Department of Health, Taipei City Government (11201-62-016) and Taipei City Hospital (TPCH-113-45) to Wei-Ming Cheng. The Ministry of Education, Higher Education SPROUT Project for the Cancer and Immunology Research Center (112W31101, 113W031101), and the Chong Hin Loon Memorial Cancer and Biotherapy Research Center, National Yang Ming Chiao Tung University, Taipei, Taiwan to Chi-Ying F Huang. Funding was also provided by Melissa Lee Cancer Foundation (MLCF_V113_A11302) to Peter Mu-Hsin Chang, and by China Medical University, Taiwan (CMU113-MF-48 and CMU111-MF-44) to Ming-Fen Lee.

Data availability

No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

All authors have participated in the study and consented to publication.

Competing interests

The authors declare no competing interests.

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Received: 4 April 2024 / Accepted: 22 February 2025
Published online: 06 March 2025

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