Ribosome biogenesis mediates antitumor activity of flavopiridol in CD44+/CD24- breast cancer stem cells

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Abstract. Flavopiridol is a synthetically produced flavonoid that potently inhibits the proliferation of human tumor cell lines. Flavopiridol exerts strong antitumor activity via several mechanisms, including the induction of cell cycle arrest and apoptosis, and the modulation of transcriptional regulation. The aim of the present study was to determine the effect of flavopiridol on a subpopulation of cluster of differentiation (CD)44+/CD24- human breast cancer MCF7 stem cells. The CD44+/CD24- cells were isolated from the MCF7 cell line by fluorescence-activated cell sorting and treated with 100, 300, 500, 750 and 1,000 nM flavopiridol for 24, 48 and 72 h. Cell viability and proliferation assays were performed to determine the inhibitory effect of flavopiridol. Gene expression profiling was analyzed using Illumina Human HT-12 v4 Expression BeadChip microarray. According to the results, the half maximal inhibitory concentration (IC_{50}) value of flavopiridol was 500 nM in monolayer cells. Flavopiridol induced growth inhibition and cytotoxicity in breast cancer stem cells (BCSCs) at the IC_{50} dose. The present study revealed several differentially regulated genes between flavopiridol-treated and untreated cells. The result of the pathway analysis revealed that flavopiridol serves an important role in translation, the ribosome biogenesis pathway, oxidative phosphorylation, the electron transport chain pathway, carbon metabolism and cell cycle. A notable result from the present study is that ribosome-associated gene expression is significantly affected by flavopiridol treatment. The data of the present study indicate that flavopiridol exhibits antitumor activity against CD44+/CD24- MCF7 BCSCs through different mechanisms, mainly by inhibiting translation and the ribosome biogenesis pathway, and could be an effective chemotherapeutic molecule to target and kill BCSCs.

Introduction

Breast cancer is a common type of malignancy in the world and a major cause of mortality in females between 30 and 59 years of age (1). Breast cancer is a heterogeneous disease in terms of histology, pathology, and genetic and molecular profiles (2). Despite diagnostic and therapeutic advances, breast cancer patients still often exhibit relapse or metastasis subsequent to therapy (3).

Tumors are morphologically heterogeneous, composed of undifferentiated and differentiated cells (4). Cancer stem cells (CSCs) have been identified as a subpopulation within the tumor possessing the ability to self-renew and differentiate into non-tumorigenic cell populations that constitute the bulk of the tumor (5). CSCs have been associated with tumor initiation, therapy resistance and tumor recurrence. CSCs are a major problem for cancer therapy, and the elimination of CSCs is required for an effective treatment (6). The presence of CSC population in breast cancer has been demonstrated in several studies (7,8). Breast cancer stem cells (BCSCs) were first isolated by Al-Hajj *et al* (9) in 2003 from the pleural effusions of a patient. Specific cell surface markers and biomarkers are used to identify and isolate BCSCs. The adhesion molecule cluster of differentiation (CD) 44 is a multifunctional cell surface transmembrane glycoprotein that serves a role in cell adhesion, proliferation, differentiation, motility and migration (10). In breast cancer, CD44+/CD24- expression was demonstrated as prospective phenotype to isolate BCSCs. Al-Hajj *et al* (9) reported that breast cancer cells exhibiting an increased expression of CD44+/CD24- were able to form tumors when injected into immunodeficient mice.

Cyclin-dependent kinases (CDKs) serve an essential role in the control of the cell cycle, and are associated with

cytoskeletal dynamics, epigenetic regulation, controlling stem cell self-renewal, regulating metabolism, cell migration, regulation of transcription, DNA damage, and genomic and chromosomal instability (11). The dysregulation of CDK expression contributes to the loss of normal cell cycle control, which leads to the formation and progression of cancer (12). Therefore, the inhibition of CDKs by small-molecule CDK inhibitors may be an effective treatment of cancer. The dysregulation of cyclin D and the CDK pathway in cancer cells may inhibit senescence and promote cellular proliferation (13). By using various different mechanisms, malignant cells may increase cyclin D-dependent activity. The cyclin D-CDK4/6-retinoblastoma pathway controls the cell cycle restriction point, and is commonly dysregulated in breast cancer, making it a possible target for anticancer therapy (14).

Flavopiridol is a semisynthetic flavonoid that was the first CDK inhibitor used in clinical trials (15). Flavopiridol exhibits an antitumor effect against a variety of tumor types, including several solid tumors, through cytostatic activity, and induces cell cycle arrest and apoptosis (16). This flavonoid is a promising anticancer drug that is undergoing phase I and II clinical trials for chronic myeloid leukemia and pancreatic cancer (17,18). Our previous study demonstrated that flavopiridol induced growth inhibition and apoptosis in CD133+/CD44+ prostate CSCs (19).

BCSCs have been proposed to be responsible for numerous properties of breast cancer such as resistance, metastatic properties and recurrence (20). Conventional anticancer therapies may kill the majority of the cancer cells, but CSCs are not affected by these therapies (21). For a more effective treatment of breast cancer, it may be necessary to target CSCs. Genome-wide gene expression profiling based on microarray analysis is a powerful tool to elucidate the possible mechanisms of cancer drugs. The present study aimed to investigate the cytotoxic effects and underlying mechanism of action of flavopiridol against human breast CSCs.

Materials and methods

Cell culture conditions and reagents. Human breast cancer MCF7 cells were obtained from Interlab Cell Line Collection (Genova, Italy) and were grown in monolayer cell culture in RPMI 1640 culture medium (Lonza Group AG, Basel, Switzerland) containing 10% heat-inactivated fetal bovine serum (Gibco; Thermo Fisher Scientific, Inc., Waltham, MA, USA), 1% penicillin and 1% streptomycin (Sigma-Aldrich; Merck KGaA, Darmstadt, Germany). The cells were cultured in 25-cm² polystyrene flasks (Corning Life Sciences, Corning, NY, USA) and incubated for 48 h at 37°C in a humidified atmosphere of 5% CO_2. Flavopiridol (Sigma-Aldrich; Merck KGaA) was prepared as 10 mM stock solution in dimethyl sulfoxide (DMSO), and the final volume of DMSO did not exceed 0.1% of the total incubation volume and was not cytotoxic to the tumor cells at these concentrations (data not shown).

Fluorescence-activated cell sorting (FACS). To sort the CSCs subpopulations in the human breast cancer MCF7 cell line, the antibodies of expressed surface markers CD44+/CD24-, anti-CD44 conjugated to fluorescein isothiocyanate (10 μl/10⁶ cell; FITC; cat. no. 555478; BD Biosciences, Franklin Lakes, NJ, USA) and anti-CD24 conjugated to phycoerythrin (10 μl/10⁶ cell; PE; clone 32D12; Miltenyi Biotec GmbH, Bergisch Gladbach, Germany) were used. The MCF7 cells were seeded and grown to 80% confluence. The cells were detached using a non-enzymatic cell dissociation solution (Sigma-Aldrich; Merck KGaA) and resuspended in RPMI 1640 culture medium. A total of ~5x10⁴ cells were incubated with anti-CD44-fluorescein isothiocyanate (FITC; clone G44 26; BD Biosciences, Franklin Lakes, NJ, USA) and anti-CD24-phycoerythrin (PE; clone 32D12; Miltenyi Biotec GmbH, Bergisch Gladbach, Germany) in FACS stain buffer (cat. no. 554657; BD Pharmingen, Franklin Lakes, NJ, USA) for 15 min at 4°C. After 15 min, the cells were washed with the above FACS wash buffer and resuspended in FACS stain buffer (cat. no 554657, BD Pharmingen, Franklin Lakes, NJ, USA) to a density of 10⁷ cells/ml. The cells were sorted into a CD44+/CD24- population (sorted cells) using a FACSAria flow cytometer (BD Biosciences).

Analysis of cell viability. The viability of the cells following treatment was determined using the Muse® Count & Viability kit (Muse Cell Analyzer; EMD Millipore, Billerica, MA, USA) according to the protocol of the manufacturer. The cells were seeded in triplicate in 6-well plates at a density of 1x10⁴ cells/well. Subsequent to a 24-h incubation, the cells were exposed to 500, 750 and 1,000 nM flavopiridol. The plates were then incubated at 37°C in a 5% CO_2 incubator for 24, 48 and 72 h. Subsequent to incubation, all cells were collected and diluted with PBS. In total, 50 μl of the cell suspension was then added to 450 μl Muse® Count & Viability reagent (dilution, 10X), incubated for 5 min at room temperature and analyzed using the Muse Cell Analyzer. Data were presented as proportional viability (%) by comparing the treated group with the untreated cells.

RNA isolation and microarray analysis. The BCSCs were treated with a dose of flavopiridol equivalent to its half maximal inhibitory concentration (IC_{50}). Total RNA was extracted from the treated and untreated cells using the RNeasy Mini kit (Qiagen, Inc., Valencia, CA, USA) according to the protocol of the manufacturer. Biotin-labeled RNA samples for hybridization on Illumina Human HT-12 v4 Expression BeadChip (Illumina, Inc., San Diego, CA, USA) were prepared according to the recommended sample labeling procedure of Illumina, Inc. A total of 250 ng total RNA was used for cDNA synthesis, followed by an amplification/labeling step to synthesize biotin-labeled cRNA. The quality of the cRNA was controlled using the Agilent 2100 Bioanalyzer (Agilent Technologies, Inc., Santa Clara, CA, USA). Hybridization was performed at 58°C in GEX-HCB buffer (Illumina, Inc.) at a concentration of 150 ng cRNA/μl. The BeadChips were subsequently washed, blocked and conjugated with cyanine 3-streptavidin (Thermo Fisher Scientific, Inc.). The microarrays were scanned in the iScan System (Illumina, Inc.). The obtained amplification data (fold-changes in the quantification cycle values of all the genes) were processed in Agilent GeneSpring Data Analysing Software (Agilent Technologies, Inc.) and >2 fold-change was used for filtering criteria.

Statistical analysis. The statistical software package SPSS version 20.0 for Windows (IBM Corp., Armonk, NY USA)
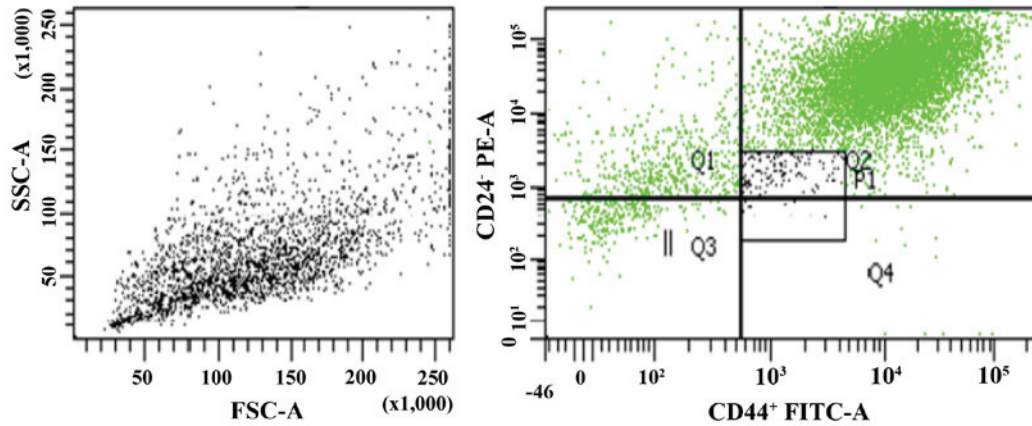
Figure 1. Flow cytometry analysis of CD44+/CD24- subpopulations in MCF7 cell lines. CD44+/CD24- populations are presented in P1. CD, cluster of differentiation; SSC-A, side scatter area; FSC-A, forward scatter area; FITC-A, fluorescein isothiocyanate area; PE-A, phycoerythrin area.

was used for all statistical analysis. All experiments were performed independently three times. Statistical analysis was tested by one-way analysis of variance, followed by Tukey's or Dunnett's post hoc tests. All data are presented as mean ± standard deviation from 3 independent experiments. P<0.05 was considered to indicate a statistically significant difference.

Results

Sorting breast cancer MCF7 cells and purity of the CD44+/CD24- sorted subpopulations. Human breast cancer MCF7 cells were separated with FACS, yielding a CD44+/CD24- population (Fig. 1). The present study obtained MCF7 CSC and non-CSC subpopulations. According to the results, the mean percentage of MCF7 CSCs and non-CSCs were 1.6 and 98.4%, respectively. The purity of the CSCs samples was tested with anti-CD44 and anti-CD24 antibodies. The sorting rate analysis and purity of the cells were evaluated sequentially, and the rate was 96.7±5.4% for the sorted cells. To confirm the flow cytometry analyses, the cells were re-evaluated following sorting, and the analyses were repeated subsequent to one passage. The results revealed that the cell purity following sorting was >90%.

Increasing cytotoxicity of CD44+/CD24- BCSCs with flavopiridol. Cytotoxicity assays were performed to determine the therapeutic effect of flavopiridol. MCF7 CSCs were exposed to 100-1,000 nM flavopiridol for 24, 48 and 72 h, and the percentage of viable cells in the samples was determined by a cell viability assay. Flavopiridol reduced the cell viability of CSCs in a time- and concentration-dependent manner (Fig. 2A-C). According to the data, there were no significant decreases in cell viability at the low doses (100 and 300 nM) of flavopiridol treatment for 24 h compared with that of the untreated cells (P=0.642). After 48 h of treatment, flavopiridol significantly reduced the cell viability of BCSCs at 500, 750 and 1,000 nM compared with that of the untreated cells (P=0.000). After 72 h of treatment with flavopiridol, the IC$_{50}$ was calculated as 500 nM.

Microarray analysis for the identification of differentially expressed genes in MCF7 CD44+/CD24- cells treated with flavopiridol. To analyze the molecular mechanisms underlying the anticancer effect of flavopiridol in BCSCs, the MCF7 CD44+/CD24- cells were treated with 500 nM flavopiridol for 72 h. To identify flavopiridol-regulated genes and determine the possible mechanism underlying the differential role of flavopiridol on the growth of MCF7 CD44+/CD24- cells, global gene expression profiling was undertaken following treatment with flavopiridol using the Illumina Human HT-12 v4 Expression BeadChip. According to the results of microarray analysis, 65 genes were identified as significantly affected subsequent to treatment with flavopiridol, since the expression of 57 genes decreased and the expression of 8 genes increased compared with that in untreated cells at 72 h (Table I).

To investigate the mechanism involved in the flavopiridol-induced antiproliferative effect on MCF7 CD44+/CD24- CSCs, pathway analysis was performed using the WikiPathways database (www.wikipathways.org). Specifically, these pathways are involved in the translation pathway, ribosome biogenesis, oxidative phosphorylation, the electron transport chain pathway, carbon metabolism, mammary gland development, protein modification and the cell cycle (Fig. 3A and B).

Discussion

BCSCs have been identified as subpopulations of cells within breast tumors that possess tumor-initiating potential in addition to the ability to self-renew and differentiate into a diverse range of progeny cells that make up the tumor (22) These cells are resistant to traditional therapies against cancer, including chemotherapy and radiation therapy (5). Although treatments associated with cancer therapy kill the majority of tumor cells, CSCs are not killed (23). Therefore, a more effective strategy for the treatment of breast cancer may target CSCs. The present study investigated the effect and underlying mechanism of flavopiridol on BCSCs with respect to antitumor properties. The results demonstrated that flavopiridol dose-dependently induced the growth inhibition of BCSCs.

To isolate populations of BCSCs within tumors, the phenotypic definition of a CSC must first be established. CSCs have been identified using cell surface markers in the
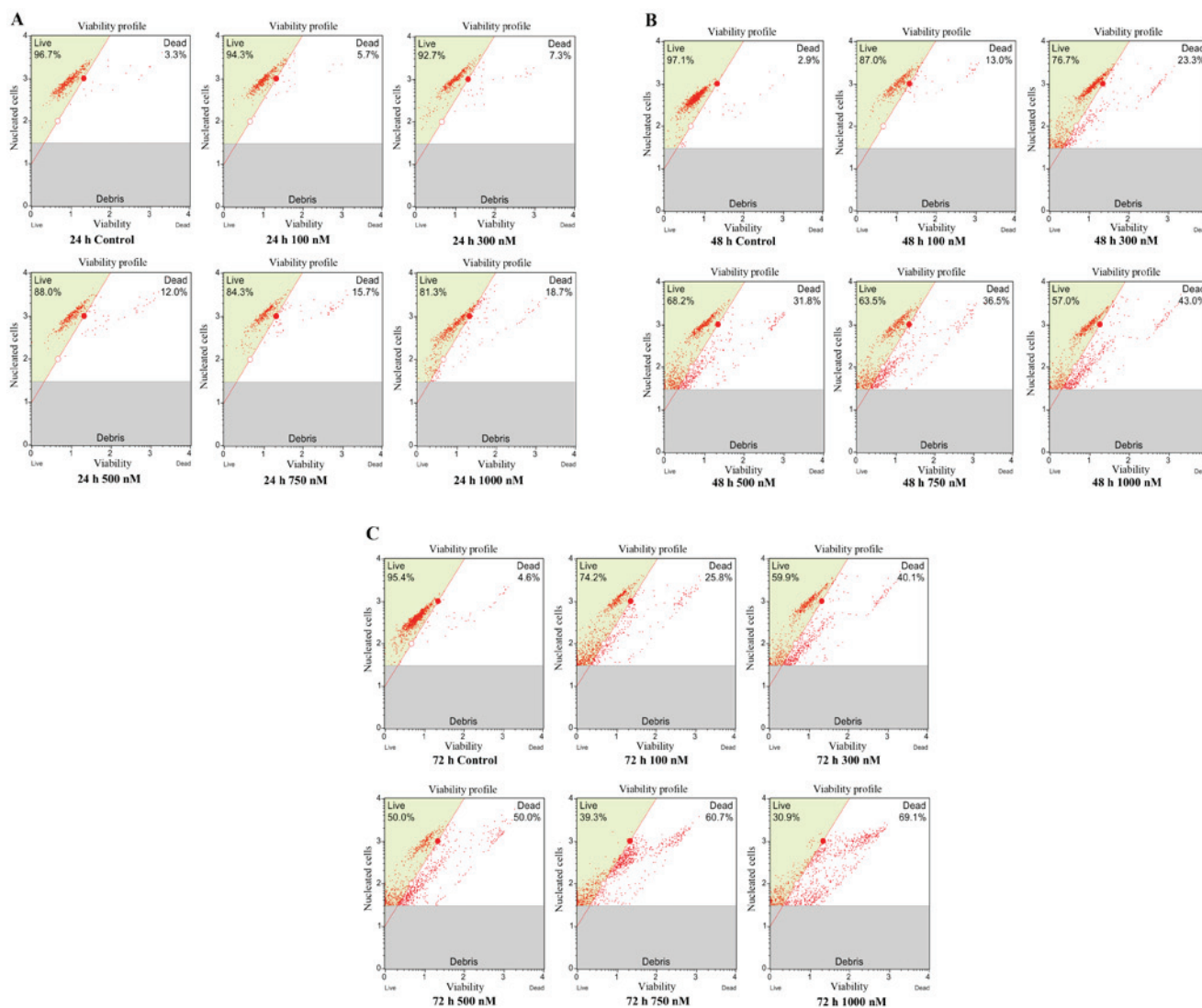
Figure 2. Representative cell viability profile of CD44^{+}/CD24^{-} breast cancer stem cells non-treated or treated with 100, 300, 500, 750 and 1,000 nM flavopiridol subsequent to (A) 24, (B) 48 and (C) 72 h of incubation. Each concentration was studied as three replicates.

majority of cancer types. The present study isolated BCSCs based on the CD44^{+}/CD24^{-} phenotype from the breast cancer MCF7 cell line. Al-Hajj *et al* (9) revealed that breast cancer tumorigenic cells exhibit a CD44^{+}/CD24^{-}/low phenotype. Several studies have used the CD44^{+}/CD24[-]and/or the aldehyde dehydrogenase (ALDH)[+] phenotype for BCSC isolation (9,24). Ginestier *et al* (25) isolated stem-like cells from primary breast xenografts using CD44^{+}/CD24^{-} and ALDH activity, revealing that these cells displayed the greatest tumor-initiating capacity, generating tumors in non-obese diabetic/severe combined immunodeficiency mice from as little as 20 cells.

Cyclins and CDK inhibitors are involved in cell morphogenesis, adhesion, migration, DNA repair, transcription, cytoskeleton dynamics and cell motility. Flavopiridol is the first CDK inhibitor that exhibits an antitumor effect against a variety of tumor types in several solid tumors (26) The results of the present study revealed that flavopiridol reduced the level of cell viability of BCSCs in a dose- and time-dependent manner, and that flavopiridol appears to possess multiple targets within tumor cells. The number of publications involving the

effect of flavopiridol on CSC is quite limited. Soner *et al* (19) demonstrated that flavopiridol induced growth inhibition and apoptosis by the upregulation of p53 and caspases 3 and 8 in CD133^{+}/CD44^{+} prostrate CSCs.

The translation and ribosome biogenesis pathways serve important roles in numerous cellular processes and are more active in cancer cells compared with those in normal cells. The inhibition of translation and ribosome biogenesis have been reported to be associated with alterations in the cell cycle and the regulation of cell growth (27). The present study demonstrated that flavopiridol induced the downregulation of translation and ribosome biogenesis genes in CSCs. According to previous studies, flavopiridol induced G1/S-phase cell cycle arrest (28,29). The mechanism of flavopiridol on the cell cycle may be associated with ribosome biogenesis. Cancer cells have been suggested to exhibit a higher rate of ribosome biogenesis compared with that in normal cells. Changes of proto-oncogenes and tumor-suppressor genes activate the mechanisms that stimulate cell growth and proliferation, and initiate certain pathways that enhance ribosome biogenesis (30,31). Derenzini *et al* (32) demonstrated that the inhibition of

Table I. Changes in the expression of upregulated and downregulated genes following treatment with flavopiridol.

A, Translation pathway and ribosome biogenesis pathway

Probe ID	Symbol	Fold-change[a]	Regulation	Definition
4920193	RPL27A	-2.7026234	Down	RPL27a
6060356	RPL13A	-2.1516730	Down	RPL13a
3360228	RPS20	-2.0229893	Down	RPS20
5290082	RPLP1	-2.1037197	Down	RiP, large, P1
1710369	RPL3	-2.5006313	Down	RPL3, transcript variant 2
620754	RPS5	-2.3889322	Down	RPS5
7040095	RPL17	-2.0128388	Down	RPL17, transcript variant 2
990273	RPL37A	-2.2212677	Down	RP L37a
3060477	RPL8	-2.4217634	Down	RPL8, transcript variant 2
3610241	RPL19	-3.2778310	Down	RPL19
3800332	RPS25	-2.3831854	Down	RPS25
5260682	RPS14	-2.5092149	Down	RPS14, transcript variant 2
5220037	RPS2	-3.9913297	Down	RPS2
6960181	RPS12	-2.9727620	Down	RPS12
7510482	RPS4X	-2.1651378	Down	RPS4, X-linked
20021	RPS15	-2.4493800	Down	RPS15
5560349	RPS11	-2.6253710	Down	RRPS11
5890730	RPS26L	-2.8872151	Down	Predicted: *Homo sapiens* 40S RPS26-like
510195	RPL27	-2.4229383	Down	RPL27
840647	RPL36	-2.7411752	Down	RPL36, transcript variant 1
4250445	RPL4	-2.0928760	Down	RPL4
6250097	RPS9	-2.3448272	Down	RPS9
1410537	RPSA	-2.1424713	Down	RPSA, transcript variant 1
6270546	RPS6	-2.3560820	Down	RPS6
6590377	RPS26	-2.1171474	Down	RPS26
4250445	RPL4	-2.0012200	Down	RPL4
3610309	LOC653881	-2.6014566	Down	Predicted: Similar to RPL3
2490450	LOC91561	-2.2661705	Down	Predicted: Similar to RPS2, transcript variant 3
3440670	LOC402251	-2.3879724	Down	Predicted: Similar to eukaryotic translation elongation factor 1 α 2
4060446	LOC649150	-3.2092447	Down	Predicted: Similar to eukaryotic translation elongation factor 1 α 2
1440398	LOC644511	-2.2366867	Down	Predicted: Similar to RPL13a, transcript variant 1
1570491	LOC648000	-2.3058624	Down	Predicted: Similar to 60S RPL7, transcript variant 1
2320494	LOC653314	-3.1144562	Down	*Homo sapiens* similar to RPL19
6280021	LOC441876	-2.8330393	Down	Predicted: Similar to 40S RPS16,
870593	LOC285053	-2.3860030	Down	Predicted: Similar to RPL18a, transcript variant 1
5720747	LOC441775	-2.6068625	Down	Predicted: Similar to 60S RPL18
2190546	LOC388654	-2.2629400	Down	Predicted: Similar to laminin receptor 1 (RPSA)
5720747	LOC441775	-2.6068625	Down	Predicted: Similar to 60S RPL18
6330373	EEF1B2	-2.3026142	Down	Eukaryotic translation elongation factor 1 β 2, transcript variant 1
3850121	EEF1A1	-3.1777650	Down	Eukaryotic translation elongation factor 1 α 1

B, Oxidative phosphorylation and electron transport chain pathway

3850110	COX6A1	-2.1455740	Down	Cytochrome *c* oxidase subunit Vıa polypeptide 1
4490259	COX8A	-2.9969997	Down	Cytochrome *c* oxidase subunit 8A (ubiquitous)

C, Carbon metabolism

2760358	NME1-2	-2.3299380	Down	NME1-NME2 readthrough
1940360	TPI1	-2.8388138	Down	Triosephosphate isomerase 1

Table I. Continued.

Probe ID	Symbol	Fold-change[a]	Regulation	Definition
6590253	ALDOA	-2.1273860	Down	ALDOA
6520128	GPX4	-2.1577030	Down	Glutathione peroxidase

D, Mammary gland development pathway

5860138	RIPK4	-2.1919790	Down	Receptor-interacting serine-threonine kinase 4

E, G protein-mediated signaling pathway via Gα12/Gα13 family

2850402	PFN1	-2.4898353	Down	Profilin 1

F, Tumor necrosis factor-mediated signaling pathway

670673	BCL2L1	-2.0002713	Down	BCL2-like 1, nuclear gene encoding mitochondrial

G, Signaling pathway pertinent to immunity

1980594	FTHL8	-3.2119188	Down	Ferritin, heavy polypeptide-like 8
2970431	FTHL7	-4.2565985	Down	FTHL7

H, Toll-like receptor signaling pathway

3840154	SPP1	-3.0712519	Down	SPP1, transcript variant 1

I, Signaling by TGF-β receptor complex

1430239	UBC	-2.8056865	Down	UBC

J, Regulatory and cell adhesion signaling pathways

5570132	ACTB	-3.4210854	Down	Actin, β

K, NRF2 pathway

4920767	FTL	-3.1391878	Down	Ferritin, light polypeptide

L, Folate-alcohol and cancer pathway

6510754	ALDH1A1	-2.9203625	Down	Aldehyde dehydrogenase 1 familyer A1

M, Cell adhesion signaling pathway

610437	CD24	2.9155455	Up	CD24 molecule

N, Calcium/calcium-mediated signaling pathway

7100711	CALM2	2.7985630	Up	Calmodulin 2 (phosphorylase kinase, delta)

O, Amino acid metabolism

450161	FAHD1	2.1592160	Up	Fumarylacetoacetate hydrolase domain containing 1

Table I. Continued.

P, Protein modification pathway

Probe ID	Symbol	Fold-change[a]	Regulation	Definition
4590110	SEPT9	2.2080840	Up	Septin 9

Q, Cell cycle

Probe ID	Symbol	Fold-change[a]	Regulation	Definition
870491	BUB3	2.1889267	Up	BUB3 budding uninhibited by benzimidazoles 3

R, Regulation of actin cytoskeleton

Probe ID	Symbol	Fold-change[a]	Regulation	Definition
2760292	PPP1CC	2.6673288	Up	Protein phosphatase 1, catalytic subunit

S, Vasopressin-regulated water reabsorption

Probe ID	Symbol	Fold-change[a]	Regulation	Definition
4230520	DNCL1	2.0563870	Up	Dynein, cytoplasmic, light polypeptide 1

T, Transport pathway

Probe ID	Symbol	Fold-change[a]	Regulation	Definition
1740136	SLC38A2	2.1263490	Up	Solute carrier family 38, member 2

[a]>2 fold-change was considered to be significant ($P<0.05$). NME, nucleoside diphosphate kinase; ALDOA, aldolase A, fructose-bisphosphate; BCL-2, B-cell lymphoma 2; RP, ribosomal protein; FTHL7, ferritin, heavy polypeptide-like 7; SPP1, secreted phosphoprotein 1; UBC, ubiquitin C; CD, cluster of differentiation; TGF, transforming growth factor; ACTB, actin, beta; FTL, ferritin, light polypeptide; ALDH1A1, aldehyde dehydrogenase 1 family, member A1; CD24, CD24 molecule; CALM2, calmodulin 2; FAHD1, fumarylacetoacetate hydrolase domain containing 1; BUB3, BUB3 budding uninhibited by benzimidazoles 3; PPP1CC, protein phosphatase 1, catalytic subunit; DNCL1, dynein, cytoplasmic, light polypeptide 1; SLC38A2, solute carrier family 38, member 2.
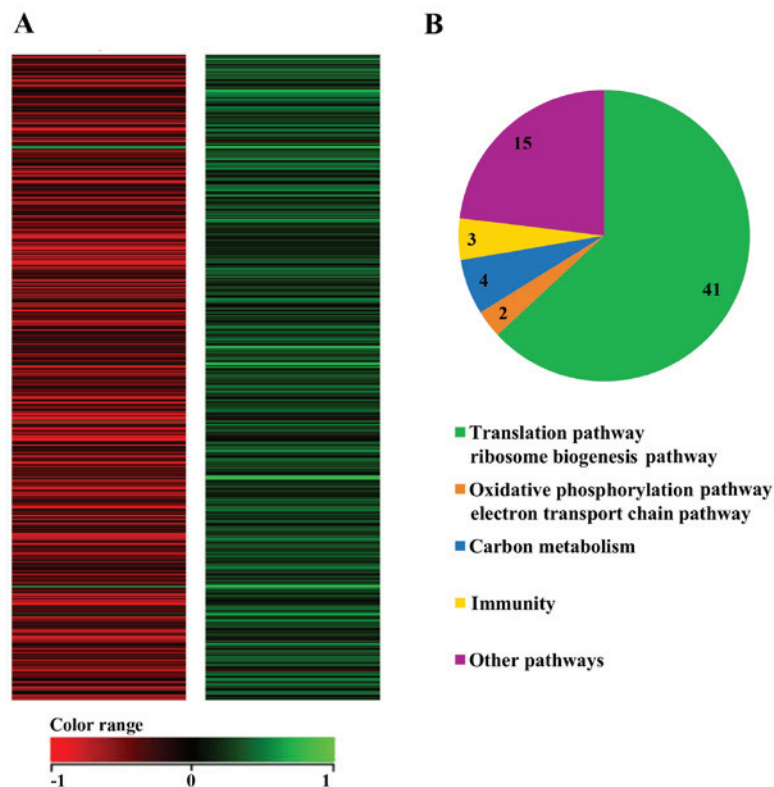

Figure 3. (A) Heat map showing the normalized expression of differentially regulated genes, filtering criteria >2-fold change in flavopiridol-treated and untreated MCF7 CD44+/CD24- cancer stem cells. Red color indicates high expression, while green color indicates expression. (B) Pie chart representing the proportion of genes associated with various pathways. CD, cluster of differentiation.

ribosomal RNA synthesis caused an accelerated or delayed G1/S-phase progression in rat hepatoma cells.

The present study demonstrated the effects of flavopiridol on BCSCs, suggesting that flavopiridol induced growth inhibition in CD44+/CD24- BCSCs and inhibited the translation and ribosome biogenesis pathways. Flavopiridol is one of the most promising chemotherapy drug candidates for the treatment of cancer. However, the data on the effects of flavopiridol on cancer remain limited. An increased understanding of the mechanisms responsible for the effects of the drug is required to improve novel therapeutic strategies for breast cancer. Combination drug therapies targeting CSCs may be an effective method to prevent relapse and resistance in cancer therapies.

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