Fatostatin Inhibits Cancer Cell Proliferation by Affecting Mitotic Microtubule Spindle Assembly and Cell Division

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Running title: Fatostatin Inhibits Cell Division

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ABSTRACT

The sterol regulatory element binding protein (SREBP) transcription factors have become attractive targets for pharmacological inhibition in the treatment of metabolic diseases and cancer. SREBPs are critical for the production and metabolism of lipids and cholesterol, which are essential for cellular homeostasis and cell proliferation. Fatostatin was recently discovered as a specific inhibitor of SCAP (SREBP cleavage-activating protein), which is required for SREBP activation. Fatostatin possesses antitumor properties including the inhibition of cancer cell proliferation, invasion and migration, and it arrests cancer cells in G2/M phase. Although Fatostatin has been viewed as an antitumor agent due to its inhibition of SREBP and its effect on lipid metabolism, we show that Fatostatin’s anticancer properties can also be attributed to its inhibition of cell division. We analyzed the effect of SREBP activity inhibitors including Fatostatin, PF-429242 and Betulin on the cell cycle and determined that only Fatostatin possessed antimitotic properties. Fatostatin inhibited Tubulin polymerization, arrested cells in mitosis, activated the spindle assembly checkpoint and triggered mitotic catastrophe and reduced cell viability. Thus Fatostatin’s ability to inhibit SREBP activity and cell division could prove beneficial in treating aggressive types of cancers like glioblastomas that have elevated lipid metabolism, fast proliferation rates and often develop resistance to current anticancer therapies.

Cancer cell proliferation relies on the ability of cancer cells to reprogram their metabolic pathways to meet the biosynthetic and bioenergetic demands required for rapid cell divisions (1-3). Consequently, the lipid metabolic pathway, which is critical for lipid homeostasis, is often dysregulated in cancer (4,5). Key regulators of the lipid metabolic pathway include SREBP1a, 1c and 2, which function as transcription factors to control the expression of genes involved in fatty acid and cholesterol synthesis (6). SREBP is synthesized as an inactive precursor that is inserted into the ER membrane and when the cellular lipid levels decline SREBP is transported to the Golgi membrane through a SREBP cleavage-activating protein (SCAP)-dependent process (6,7). The N-terminal transcription factor domain of SREBP is then proteolyzed by two site-specific proteases (S1P and S2P) and released from the Golgi membrane (8,9). This mature form of SREBP is transported to the nucleus where it binds to sterol regulatory elements (SREs) upstream of lipid genes to transactivate their expression (6,10,11).

Due to the importance of SREBP in lipid homeostasis and cancer proliferation, several
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approaches have been taken to inhibit the SREBP maturation pathway (6,12). A screen for modulators of adipogenesis identified Fatostatin, which inhibited SREBP in cell culture and in vivo by binding to SCAP and inhibiting SCAP’s ability to transport SREBP to the Golgi (13,14). Similarly, Betulin, was shown to inhibit SREBP in cell culture and in vivo by binding to SCAP and stimulating the interaction between SCAP and insulin-induced gene (Insig), which inhibited SCAP’s ability to transport SREBP to the Golgi (15). Additionally, a screen for S1P protease inhibitors identified PF-429242, which also inhibited SREBP in cell culture and in vivo (16,17).

Several studies showed that Fatostatin has anticancer properties in cell culture and in vivo mouse models of prostate and brain cancers (18-20). Additionally, Fatostatin arrested cancer cells in G2/M (18), indicating that inhibition of SREBP activity leads to a G2/M arrest and/or that Fatostatin was inhibiting a second target that was critical for G2/M progression. In this study we analyzed the mechanism of Fatostatin’s anticancer activity and determined that Fatostatin not only targets SCAP but also the mitotic microtubule spindle that is critical for cell division.

RESULTS

Fatostatin Induces Spindle Damage and Mitotic Arrest- To explore the mechanism of Fatostatin’s anticancer property, we first verified that Fatostatin was able to induce a G2/M arrest as described previously (18). U87, T98G, MDA-MB-453 and Jurkat T-cells were treated with DMSO or Fatostatin (5µM) for 24 or 48 hours, stained with propidium iodide and the percentage of cells in G2/M was quantified by FACS. Indeed, Fatostatin arrested all cell lines in G2/M (Fig. 1A). However, cell lines that overexpressed active forms of hSREBP1 or hSREBP2 (devoid of the C-terminal regulatory domains and constitutively transactivate SREBP target genes independent of SCAP) were still sensitive to Fatostatin and arrested in G2/M (Fig. 1, B and C). This indicated that the Fatostatin induced G2/M arrest was independent of SCAP inhibition and SREBP maturation. Next we verified Fatostatin’s anticancer properties. HeLa cells were treated with increasing concentrations of Fatostatin for 48 hours and cell viability was measured using the CellTiter-Glo cell viability assay. Fatostatin increased cell death in a dose-dependent manner (Fatostatin IC₅₀=2.11µM, IC₉₀=6.36µM) (Fig. 1D). Herein all experiments were carried out with 2.11µM Fatostatin, 10µM Betulin, 10µM PF-429242, 100nM Taxol or 330nM Nocodazole, unless otherwise noted. Next we asked if Fatostatin was specifically targeting cancer cells or all types of cells. Cancer (HeLa, SH-SY5Y, U2OS) and normal (RPE, MEFs) cells were treated with Fatostatin and the percent viability was measured after 60 hours. Interestingly, Fatostatin reduced the viability of cancer cells (~10-20% cell viability) more than normal cells (~50-62% cell viability) (Fig. 1E).

Next we verified that Fatostatin was able to inhibit SREBP activity by analyzing the gene expression levels of SREBP target lipid genes (FASN, SCD1, HMGCR and HMGCS) and non-SREBP genes (GAPDH and RPL32) in HeLa cells that had been treated with DMSO, Fatostatin, Betulin or PF-429242 for 24 hours. Indeed, Fatostatin was able to inhibit the expression of SREBP target genes similar to Betulin and PF-429242 (Fig. 1F). Next, we probed the nature of the Fatostatin induced G2/M arrest. Cancer (HeLa, SH-SY5Y, U2OS) and normal (RPE, MEFs) cells were treated with DMSO, Fatostatin, Betulin, PF-429242 or Taxol for 20 hours. Cells were stained for DNA and α-tubulin and imaged by immunofluorescence (IF) microscopy. DMSO, Betulin and PF-429242 treated cells displayed a normal interphase cell morphology (data not shown) and a normal mitosis with a bipolar spindle and chromosomes properly aligned at the metaphase plate (Fig. 2, A-C). However, Fatostatin treated cells arrested in mitosis with aberrant spindles (fragmented and multipolar) (Fig. 2, D and E). This arrest was different from the mitotic arrest induced by Taxol, which arrests cells in prometaphase with stabilized microtubule asters (Fig. 2F). Quantification of this data showed that Fatostatin treated mitotic cells had defective spindles, similar to Taxol, which was more apparent in cancer cells (~80-90% defective spindles) than in normal cells (~50-58% defective spindles) (Fig. 2G). In contrast, PF-429242 and Betulin had no effect on the percentage of mitotic cells with defective spindles in cancer or normal cell lines (Fig. 2G). These results indicated that unlike other SREBP activity
inhibitors, only Fatostatin exhibited antimitotic properties.

To verify Fatostatin’s antimitotic activity, cancer (HeLa, SH-SY5Y, U2OS) and normal (RPE, MEFs) cells were treated with DMSO, Fatostatin, Betulin, PF-429242 or Taxol for 20 hours. Cells were fixed and stained for DNA, α-tubulin and PH3 (phosphorylated Ser10 on histone H3, a biochemical marker of mitotic cells) and imaged by IF microscopy. Fatostatin treatment led to an increase in PH3 positive cells, similar to Taxol, which was more apparent in cancer cells (~75-85% PH3+ cells) than in normal cells (~45-48% PH3+ cells) (Fig. 2, H and I). In contrast, PF-429242 and Betulin had no effect on the percentage of PH3 positive cells (Fig. 2I). These results indicated that unlike other SREBP activity inhibitors, Fatostatin was arresting cells in mitosis. To further confirm this, HeLa cells were synchronized in G1/S and released into DMSO, Fatostatin, PF-429242 or Taxol. Protein extracts were prepared every 2 hours for 24 hours and immunoblotted for markers of mitosis. Consistently, Fatostatin arrested cells in mitosis with high PH3 and Cyclin B levels, similar to Taxol, compared to DMSO and PF-429242 that had no effect (Fig. 2J).

Fatostatin Destabilizes the Mitotic Microtubule Spindle- Defective multipolar spindles can arise through multiple mechanisms, including centrosome over-duplication and pericentriolar material (PCM) fragmentation (23,24). To probe the nature of the Fatostatin induced defective spindles, we analyzed the number of centrosomes (centriole pairs) and the status of the PCM in DMSO, Fatostatin, Betulin, PF-429242 or Taxol treated HeLa cells. Cells were fixed and stained for DNA, α-tubulin and Centrin (centriole marker) or Pericentrin (PCM marker) and imaged by IF microscopy. Fatostatin treated mitotic cells had more than two pericentriolar foci, while only few Taxol, DMSO, Betulin and PF-429242 treated cells had ≥2 pericentriolar foci (% ≥2 Pericentrin foci for Fatostatin=85±4.8, p<.0001; Taxol=11±2.2, p<.0001; Betulin=5.7±1.7, NS; PF-429242=6.3±2.5, NS; compared to DMSO=4.70±1.2) (Fig. 3, A and B). Whereas all cells had a similar number of centriole pairs (Fig. 3, C and D). These results indicated that the increased PCM fragmentation in Fatostatin treated cells could be generating multipolar spindles. However, cells treated with microtubule destabilizers like Vinblastine appear to generate multipolar spindles through spindle destabilization (25). Consistent with this, Fatostatin treated cells also displayed defects in kinetochore-microtubule attachment, where lagging chromosomes had unattached kinetochores (Fig. 3, E and F). Therefore we asked if Fatostatin was targeting the microtubule spindle. First, we performed in vitro Tubulin polymerization reactions in the presence of DMSO, Taxol, Nocodazole and Fatostatin. Whereas Taxol promoted Tubulin polymerization, Nocodazole and Fatostatin inhibited Tubulin polymerization compared to DMSO (Fig. 3G). Additionally, treatment of HeLa cells with increasing concentrations of Fatostatin led to a concentration-dependent decrease in polymerized microtubules (Fig. 3H). These results indicated that Fatostatin was directly targeting Tubulin leading to Tubulin depolymerization, spindle fragmentation and the generation of multipolar spindles with PCM centers. To probe the mechanism by which Fatostatin was inhibiting Tubulin polymerization we asked if Fatostatin was able to dock favorably into the Colchicine or Vinblastine binding sites on β-Tubulin, both Tubulin-destabilizing sites. Fatostatin docked favorably into the Colchicine-binding site, with a docking score 80% that of Colchicine, and showed several interactions including an arene-cation interaction between methyl-benzene and Lys352 and lipophilic interactions between the propylpyridine scaffold and Leu242, Val238, Leu248, Leu255 (Fig. 3, I and J). Therefore Fatostatin was likely binding to the Colchicine-binding site of β-Tubulin to inhibit Tubulin polymerization.

Fatostatin Activates the Spindle Assembly Checkpoint- To further understand the nature of the Fatostatin induced mitotic arrest we analyzed whether Fatostatin activated the spindle assembly checkpoint to arrest cells in mitosis. HeLa cells were treated with DMSO or Fatostatin for 20 hours and stained for DNA, α-tubulin and AurB/INCENP/Survivin [components of the chromosome passenger complex (CPC)], or BubR1 [component of the mitotic checkpoint complex (MCC)] and imaged by IF microscopy. All Fatostatin treated mitotic cells had AurB/INCENP/Survivin and BubR1 staining at...
the kinetochores, compared to only a few metaphase DMSO treated cells (Fig. 4, A-D). These results indicated that Fatostatin activated the spindle assembly checkpoint to arrest cells in mitosis.

**Fatostatin Induces Mitotic Catastrophe**

Next, we sought to understand the cellular consequences of arresting cells in mitosis with Fatostatin. HCT116-GFP-H2B cells were synchronized in G1/S and released into DMSO, Fatostatin, Betulin, PF-429242 or Taxol and cells were imaged by time-lapse microscopy 6 hours post release (Fig. 4E and supplemental Movies S1-S5). Fatostatin treated cells arrested in mitosis, failed to divide and underwent mitotic catastrophe (cell death during mitosis or failed cell division followed by cell death), similar to Taxol, while DMSO, Betulin and PF-429242 treated cells were able to divide normally (Fig. 4F). To determine if the Fatostatin induced mitotic catastrophe was caspase dependent, we analyzed the activity of Caspase 3/7 in cells treated with DMSO, Fatostatin, PF-429242 or Taxol for 24 hours. Fatostatin induced Caspase 3/7 activation similar to Taxol, while DMSO and PF-429242 had no effect (Fig. 4G). Next we sought to determine if Fatostatin’s effect on mitosis could occur in an acute temporal manner. HCT116-GFP-H2B cells were released from a G1/S arrest and 6 hours post release cells were treated with Fatostatin or Taxol and imaged just before mitotic entry. Both Fatostatin and Taxol arrested cells in mitosis and induced mitotic catastrophe as shown previously (Fig. 4H and supplemental Movies S6 and S7). This indicated that Fatostatin’s induced mitotic arrest was independent of its inhibition of SREBP maturation, consistent with our previous data showing that cells overexpressing active forms of hSREBP1 or hSREBP2 were still sensitive to Fatostatin and arrested in G2/M (Fig. 1, B and C). Interestingly washing out the Fatostatin from Fatostatin arrested cells allowed cells to continue through mitosis and divide (Fig. 4I and supplemental Movie S8), indicating that it was a reversible inhibitor. Together, these results indicate that independent of its inhibition of SREBP maturation and expression of lipid metabolism target genes, Fatostatin arrests cells in mitosis, which leads to Caspase 3/7 activation, mitotic catastrophe and reduced cell viability.

**DISCUSSION**

*In vivo* Fatostatin is very well tolerated, it inhibits lipid metabolism through its inhibition of SREBP maturation, and has great antitumor activity (13,18-20). Here we explored the mechanism of Fatostatin’s antitumor activity and determined that in addition to inhibiting lipid metabolism, Fatostatin also inhibits Tubulin polymerization, which perturbs mitotic spindle assembly and leads to mitotic catastrophe. Therefore we have uncovered a new targeting mechanism for Fatostatin that explains its effective antitumor activity. Additionally, Fatostatin’s ability to inhibit lipid metabolism and cell division, could be particularly useful for targeting aggressive types of cancers that reprogram their metabolic pathways and undergo rapid cell divisions like glioblastomas, where Fatostatin has already been shown to be effective at reducing tumor burden *in vivo* (20). These findings may motivate future studies aimed at targeting more than one critical pathway that cancer cells rely on to proliferate and the development of Fatostatin for treating cancer.

**EXPERIMENTAL PROCEDURES**

**Cell Culture**- HeLa, SH-SY5Y and RPE cells were grown in DMEM/F12 and U2OS and HCT116 in McCoy’s 5A (GIBCO:11320-033, 16600-082); U87 and T98G in IMDM, MDA-MB-453 and MEFs in DMEM, and Jurkat T-cells in RPMI1640 (Fisher:12440053, 11965092, 11875093) with 10% FBS and Penicillin-Streptomycin (Fisher:15140122) in 5% CO₂ at 37°C.

**Fixed and Live-cell Imaging**- Was carried out as described previously (21,26). For quantifying spindle and mitotic defects, 100 cells from 3 independent experiments were counted and the data are presented as the average ± standard deviation. For time-lapse microscopy, HCT116-GFP-H2B cells were arrested in G1/S with 2mM Thymidine (Sigma:T9250) for 18 hours, washed 3 times with PBS and twice with complete media and 1) released into fresh media with or without the indicated drugs, or 2) released into the cell cycle and drugs were added 6 hours post-release. For Fatostatin reversibility tests, cells were treated with Fatostatin for 16 hours, washed, released and imaged. Maximum intensity projection images were converted to AVI movies. Each frame
represents a five or ten-minute interval as indicated.

**Compound Potency-** Cells were treated with increasing concentrations of Fatostatin (0-20µM) for 48 hours and subjected to the CellTiter-Glo assay (Promega:G7570) as described previously (22), data is presented in relative light units (RLU). Collaborative drug discovery software (CDD Inc.) was used for generating IC<sub>50</sub> and IC<sub>90</sub> values.

**Caspase Assay-** Cells were treated with drugs for 24 hours and the Caspase-Glo 3/7 assay (Promega:G8090) was used to measure Caspase 3/7 activity. Plates were read with a Tecan M1000 plate reader and the data was presented in RLU=s.

**Tubulin Polymerization Reactions-** Were carried out as described previously (22) with DMSO or 3µM Nocodazole, Fatostatin, or Taxol. Data is presented in relative fluorescence units (RFU).

**Antibodies-** ACA (Cortex Biochem:CS1058); Phospho-S10-H3 (Millipore:06570); α-tubulin (Serotec:mca77g); Cyclin B (Santa Cruz:mca-245); Survivin (Zymed:37-2000); INCENP, Pericentrin (Abcam:ab12183, ab4448); AurB (BD Biosciences:611082); and BubR1 was a gift from Hongtao Yu. FITC and CY3 Secondary antibodies were from Jackson Immuno Research.

**Compounds-** DMSO, Taxol, Fatostatin, PF-429242, Betulin, Hoechst 33342 and Nocodazole (Sigma:D4540, T7402, F8932, SML0667, B9757, B2261 and M1404).

**FACS-** Cells were treated with 5µM Fatostatin for 24 hours (U87 cells were treated for 48 hours), stained with propidium iodide (PI) for 20 minutes and analyzed on a LSRII flow cytometer (BD Biosciences) with FlowJo software. For SREBP overexpression experiments, U87 cells were infected with lentivirus expressing truncated hSREBP1a (aa 1-490), hSREBP2 (aa 1-484) or empty vector (pLJM1) as described previously (20).

**RT-qPCR-** Cells were treated for 24 hours with the indicated drugs and collected for gene expression analysis. RNA preparation procedure and qPCR primers were described previously (20).

**Molecular Modeling-** Was performed using MOE software as described previously (27). Fatostatin, Colchicine, Vinblastine and β-Tubulin structures were from PubChem and PDB databases (PDB ID:1Z2B) respectively.

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**Conflict of interest-** The authors declare that they have no conflicts of interest with the contents of this article.

**Author contributions-** AAG, KC, SH, CN, CK, KJW, YL, SJB and JZT performed experiments, discussed results and wrote the paper.
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FOOTNOTES
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†The abbreviations used are: Fato, Fatostatin; Bet, Betulin; IC₅₀, half maximal inhibitory concentration.
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FIGURE LEGENDS

FIGURE 1. Fatostatin inhibits SREBP maturation and arrests cancer cells in G2/M. (A-B) U87, T98G, MDA-MB-453 and Jurkat T-cells treated with DMSO or 5µM Fatostatin for 24 hours (U87 cells were treated for 48 hours). Cells were stained with propidium iodide and their cell cycles analyzed by FACS. (B) Same as in (A), except that U87 cells were infected with lentivirus expressing truncated hSREBP1a (aa 1-490), hSREBP2 (aa 1-484) or an empty vector (pLJM1). (C) A fraction of cells in (B) were collected and analyzed for SREBP target lipid gene expression (FASN and HMGC5S1) by RT-qPCR. Data is presented as relative normalized mRNA levels to the 36B4 control gene. (D) HeLa cells were treated with increasing concentrations of Fatostatin for 48 hours. Cell viability was measured and IC50s and IC90s were generated. RLU- relative light units. (E) Cancer (HeLa, SH-SY5Y, U2OS) and normal (RPE, MEFs) cells were treated with DMSO, 2.11µM Fatostatin, 10µM Betulin or 10µM PF-429242 for 60 hours and cell viability was measured as in (D). (F) HeLa cells were treated as in (E) for 24 hours and gene expression levels of SREBP target lipid genes and non-SREBP genes were quantified by RT-qPCR. Data is presented as relative normalized mRNA levels to the 36B4 control gene.

FIGURE 2. Fatostatin induces spindle damage and mitotic arrest. (A-F) Immunofluorescence microscopy of HeLa cells treated with DMSO (A), 10µM PF-429242 (B), 10µM Betulin (C), 2.11µM Fatostatin (D and E) or 100nM Taxol (F) for 20 hours. Cells were stained for DNA and Tubulin. Scale bar=5µm. (G) Quantification of the % DMSO, PF-429242, Betulin, Fatostatin or Taxol treated mitotic cells with defective spindles in cancer (HeLa, SH-SY5Y, U2OS) and normal (RPE, MEFs) cells. Data represent average ± standard deviation of three independent experiments, 100 cells counted for each. (H) HeLa cells treated with 2.11µM Fatostatin for 20 hours and stained for DNA, Tubulin and phospho-Ser10 Histone H3. Scale bar=5µm. (I) Quantification of the % DMSO, PF-429242, Betulin, Fatostatin or Taxol treated cells that stained positive for PH3 in cancer (HeLa, SH-SY5Y, U2OS) and normal (RPE, MEFs) cells. Data represent average ± standard deviation of three independent experiments, 100 cells counted for each. (J) HeLa cells were arrested in G1/S and released into DMSO, 10µM PF-429242, 2.11µM Fatostatin or 100nM Taxol. Cell extracts were prepared at the indicated time points post release and immunoblotted for Cyclin B (degraded during mitosis), PH3 (mitotic marker) and GAPDH (loading control).

FIGURE 3. Fatostatin inhibits Tubulin polymerization and perturbs the microtubule spindle. (A and C) Immunofluorescence microscopy of HeLa cells treated with DMSO, 10µM PF-429242, 10µM Betulin, 2.11µM Fatostatin or 100nM Taxol for 20 hours and stained for DNA, Tubulin and Pericentrin (A) or Centrin (C). Scale bar=5µm. (B and D) Quantification of the % DMSO, PF-429242, Betulin, Fatostatin or Taxol treated mitotic cells that have >2 Pericentrin foci (B) or >2 pairs of centrioles (D). Data represent average ± standard deviation of three independent experiments, 100 cells counted for each. (E) HeLa cells were arrested in G1/S and released into DMSO, 10µM PF-429242, 2.11µM Fatostatin or 100nM Taxol. Centrin (E). (F) Zoom-in view of a Fatostatin treated cell in (E), see yellow box, showing unattached kinetochores (white arrows). (G) Summary of Tubulin polymerization reactions carried out with 3µM Nocodazole, 3µM Fatostatin, 3µM Taxol or DMSO. Polymerization was monitored for 100 minutes and relative fluorescence units (RFU) were quantified over time. (H) HeLa cells were treated with increasing concentrations of Fatostatin (0-20µM) and stained for DNA and Tubulin. Scale bar=5µm. (I-J) Docking of Fatostatin into the β-Tubulin Colchicine-binding site (PDB ID: 1Z2B) in stick figure representation (I) and highlighting the Fatostatin molecular interactions including an arene-cation interaction between methyl-benzene and Lys352 and lipophilic interactions between the propylpyridine scaffold and Leu242, Val238, Leu248 and Leu255 (J).

FIGURE 4. Fatostatin induces SAC activation and mitotic catastrophe. (A-D) Immunofluorescence microscopy of HeLa cells treated with DMSO or Fatostatin for 20 hours and stained for DNA, Tubulin and AurB (A), INCENP (B), Survivin (C) or BubR1 (D). Scale bar=5µm. (E) HCT116-GFP-H2B cells were arrested in G1/S and released into media with DMSO, 10µM PF-429242, 10µM Betulin, 2.11µM
Fatostatin or 100nM Taxol. Cells were imaged every 10 minutes using time-lapse microscopy and snapshots of representative cell divisions are shown. Time is in minutes. (F) Quantification of the % cells undergoing normal or defective (multipolar) cell divisions, or cell death (during mitosis or after a failed mitosis) that were treated with drugs indicated in (E). Data represent the average ± standard deviation of three independent experiments, 40 cells counted for each. (G) HeLa cells were treated with DMSO, 2.11μM Fatostatin, 10μM PF-429242 or 100nM Taxol for 24 hours and Caspase 3/7 activity was monitored. Data represent the average Caspase 3/7 activity in relative light units (RLU) ± the standard deviation of three independent experiments. (H) Same as in (E), except that 2.11μM Fatostatin or 100nM Taxol was added 6 hours post G1/S release, just prior to mitotic entry, and cells were imaged every 5 minutes. (I) Same as in (E), except that cells were arrested in mitosis with 2.11μM Fatostatin for 16 hours, and released into fresh media before imaging. See also supplemental Movies S1-S8.
Fatostatin Inhibits Cell Division

FIGURES

FIGURE 1

A

B

C

D

E

F

IC_{50} (uM) = 2.11
IC_{90} (uM) = 6.36

% Cell Viability

Relative Norm. mRNA

SREBP target lipid genes
Fatostatin Inhibits Cell Division

FIGURE 2

A. DNA α-Tub Merge
B. PF-429242
C. Betulin
D. Fatostatin
E. Fatostatin
F. Taxol

G. % mitotic cells with defective spindles

H. DNA PH3 α-Tub Merge

I. % cells PH3+

J. Thymidine released HeLa cells

| Hours | DMSO | PF-429242 | Fatostatin | Taxol |
|-------|------|-----------|------------|-------|
| 0     | 2    | 4         | 6          | 8     |
| 10    | 12   | 14        | 24         |       |
|       |      |           |            |       |

Cyclin B
PH3
GAPDH
Fatostatin Inhibits Cell Division

**FIGURE 3**

A. DNA, Pericentrin, α-Tub, Merge

B. **B**  

***p<.0001  

* p<.05  

***p<.001  

* p<.05  

NS  

% mitotic cells with >2 Pericentrin foci

D. % mitotic cells with >2 pairs of centrioles

**F**  

Merged zoom-in

**H**  

Fluorescence (RFU)

DNA, α-Tub, Merge

**J**  

Fatostatin (μM)
Fatostatin Inhibits Cell Division

FIGURE 4

A

Table: DNA, AurB, α-Tub, Merge

Prometaphase
Metaphase
Anaphase
Prometaphase

DMSO
Fatostatin

B

Table: DNA, INCENP, α-Tub, Merge

Fatostatin

C

Table: DNA, Survivin, α-Tub, Merge

Fatostatin

D

Table: DNA, BubR1, α-Tub, Merge

Fatostatin

E

Table: Plate cells, Release, Image

Plate cells
Release
Image

DMSO
PF-42 9242
Betulin
Fatostatin
Taxol

F

Graph: % cells

DMSO
PF
Bet
Fato
Taxol

G

Graph: Caspase 3/7 activity (RLU)

DMSO
Fato
PF
Taxol

H

Table: Plate cells, Drug

Plate cells
Drug

Fatostatin
Taxol

I

Table: Plate cells, Release/Remove Fato

Plate cells
Release/Remove Fato
Fatostatin

Fato
Image

DMSO
40
50
60
430

13
Fatostatin inhibits cancer cell proliferation by affecting mitotic microtubule spindle assembly and cell division
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