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13. ABSTRACT (Maximum 200 Words)

Prostate cancer (PCa) is a leading cause of death in men in the United States (1). If detected early, when the tumor is still confined to the prostate, there are a number of treatment regimens that lead to good prognosis. However, if the tumor escapes the prostate prior to diagnosis, patient prognosis is poor. The objective of the proposed study was to identify serine hydrolases that are aberrantly regulated in PCa, and that contribute to progression of the disease. To accomplish this objective we undertook a unique approach, called activity-based protein profiling. In this method, a chemical probe comprised of a "warhead" and a detection reagent, are used to covalently tag the active site of an enzyme. Using this strategy we have identified a number of novel serine hydrolases that are expressed in prostate cancer. Of special importance is an enzyme called fatty acid synthase, which contains a serine hydrolase domain. We have identified lead inhibitors of this domain of fatty acid synthase, and demonstrated that one of these inhibitors is able to block the growth of tumors in mouse models of prostate cancer.

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Table of Contents

Introduction

Prostate cancer (PCa) is a leading cause of death in men in the United States (1). If detected early, when the tumor is still confined to the prostate, there are a number of treatment regimens that lead to good prognosis. However, if the tumor escapes the prostate prior to diagnosis, patient prognosis is poor. The objective of the proposed study was to identify serine hydrolases that are aberrantly regulated in PCa, and that contribute to progression of the disease. We used a novel proteomics strategy called activity-based protein profiling (2) to identify enzymes that exhibit aberrant activity in prostate cancer. One of the enzymes we identified is fatty acid synthase. Fatty acid synthase is the sole enzyme responsible for the cellular synthesis of fatty acids. This enzyme has been previously linked to tumor growth (3, 4), so screen were established to identify novel antagonists of this enzyme. Surprisingly, one of the more potent antagonists that was identified is tetrahydrolipstatin, also called orlistat. This compound is approved by the FDA for treating obesity. Further work showed that orlistat selectively inhibits tumor cell growth and induces tumor cell apoptosis. More significantly, orlistat slows the growth of prostate tumors in mouse models of prostate cancer.

Body

The overall goal of the study is to identify enzymes that are involved in the progression of prostate cancer. Having completed this objective we have moved forward in an attempt to identify antagonists of these enzymes and test their anti-tumor activity.

We have profiled serine hydrolase activity in the mouse TRAMP model, and in a panel of human prostate cancer cell lines. One serine hydrolase whose activity is consistently up-regulated is fatty acid synthase (FAS). This large enzyme consist of six enzymatic pockets and an acyl carrier protein. Together these pockets synthesize palmitate, the primary cellular precursor for all fatty acids. The last enzymatic pocket of fatty acid synthase is a thioesterase, which has the serine hydrolase architecture, and which is hit by the activity-based probe.

In an effort to clarify the functional role that FAS may have in prostate cancer, we screened for inhibitors of this enzyme. Surprisingly, we identified orlistat as an inhibitor of FAS. Orlistat (tetrahydrolipstatin) is and FDA-approved drug that is approved for treating obesity (Xenical). Its stated molecular target is pancreatic lipase, not FAS.

Our studies also show that inhibition of FAS by orlistat will induce cell cycle arrest thereby halting tumor cell proliferation. These effects are selective for tumor cells over normal cells and are mediated through a G1/S cell cycle arrest. Orlistat also selectively induces tumor-cell apoptosis via a caspase-dependent pathway.

Most significantly, we have discovered that orlistat is able to slow the growth of human xenograft prostate tumors growing in nude mice. A daily dose of 175mgs/kg of orlistat delivered by i.p. injection slowed the growth of PC-3 tumors by more than 60%.

Key Research Accomplishments (2002)

- An initial screen for inhibitors of serine hydrolases in prostate cancer lines has been completed. A surprising finding of this screen was the identification of orlistat (an FDA approved drug) as an inhibitor of fatty acid synthase.
- The effects of orlistat on normal and neoplastic prostate cancer cells has been tested, revealing that orlistat inhibits fatty acid synthesis by tumor cells
- By virtue of this activity, orlistat induces a G1/S cell cycle arrest, and in several instances will apoptosis. These effects are selective for tumor cells; orlistat has no effect on normal prostate epithelial cells, nor on normal human fibroblasts.
- Orlistat is able to block prostate tumor growth *in vivo*. Administration of orlistat to mice bearing human prostate xenografts blocks tumor growth. This observation validates FAS as an anti-tumor target, and also puts orlistat forward as a lead molecule for drug development.

Reportable Outcomes

This study has led to the submission of one manuscript, with another in preparation. One patent application has been filed. Two NIH applications have been submitted based on the results we have obtained. .

The submitted manuscript is entitled "Orlistat: A Novel Inhibitor of Fatty Acid Synthase and Potential Anti-tumor Agent" is found in the Appendix.

The patent application that was partially supported by this work is entitled "Inhibition of fatty acid synthase by beta-lactones and other compounds for inhibition of cellular proliferation" was provided in last years report.

Conclusions

The primary conclusions of the study thus far are that:

1) The thioesterase domain of fatty acid synthase is a relevant therapeutic target in prostate cancer

2) The FDA-approved drug orlistat (tetrahydrolipstatin) is a novel inhibitor of the fatty acid synthase thioesterase, and is able to induce G1/S cell cycle arrest and apoptosis in prostate cancer cells.

3) **Fatty acid synthase is a valid target for anti-tumor therapy in prostate cancer, and orlistat is an excellent lead molecule for the initiation of drug development.**

To capitalize on these outcomes, during the final funding year of our work we plan to:

1) Continue studies to determine the minimum effective dose of orlistat for anti-tumor effect in vivo.

2) Expand tests of the anti-tumor effect of orlistat into other mouse models of PCa, including the TRAMP mouse model.

3) Define the mechanism by which inhibition of FAS by orlistat arrests the cell cycle at G1/S and induces tumor cell apoptosis.

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Orlistat is a Novel Inhibitor of Fatty Acid Synthase and a Potential Anti-tumor Agent

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Summary

One of the fundamental principles of pharmacology is that most drugs have side effects. Although considerable attention is paid to detrimental side effects, drugs can also have beneficial side effects. Given the time and expense of drug development, it would be particularly exciting if a systematic method could be applied to reveal all activities, including the unappreciated actions, of a potential drug. The present study takes the first step along this path. An activity-based proteomics strategy was used to simultaneously identify targets and screen for their inhibitors in prostate cancer. Orlistat, an FDA approved drug used for treating obesity was included in this screen. Surprisingly, we find a new molecular target and a potential new application for orlistat. Orlistat is a novel inhibitor of fatty acid synthase, an enzyme strongly linked to tumor progression. By virtue of this inhibition, orlistat can induce tumor cell apoptosis, suggesting it may have applications in treating cancer.

Introduction

Most drugs have side effects. These range in magnitude from simple nuisances to life threatening complications. Even though focus is placed on negative side effects, beneficial side effects are also observed. Unfortunately, the unanticipated effects of a drug are often revealed in the later stages of development, or even after the drug has been approved for use. Given the time and expense of drug development, it would be particularly exciting if all activities of a compound could be revealed at the outset of its development. With such information, care could be taken to minimize detrimental side effects, and testing of the drug could be expanded to other indications should its activity profile warrant.

The ability to perform all encompassing screens of a drugs activity may be on the horizon. The near completion of the human genome sequence makes the blueprint of biology a known. Ongoing research seeks to convert this blueprint into a functional map, such that the biological function of every gene is known. In principle then, one might predict the effects of a drug by knowing all of its targets. Recent emphasis on global profiling strategies, including gene expression profiling and proteomics, drive this type of thinking (1). Yet these profiling technologies measure abundance, not function, and they fall short of making it possible to screen drugs against a plethora of targets.

Recent work in the area of chemical biology points the way toward direct profiling of protein activity, offering a possible solution to the hurdle. Two groups have created chemical probes that react at the active site of multiple enzymes of a given class. Liu et al synthesized a probe containing fluorophosphonate as the warhead and biotin as the reporter, and then used this probe to reveal the serine hydrolase activity profile in biological samples (2). Greenbaum et al. showed that the cysteine proteinases profile could be visualized with probes containing reactive epoxides (3). Because activity-based probes bind at an enzyme's active site, a direct measure of the level of active enzyme can be obtained. Consequently, it becomes possible to use straightforward competition assays to screen for inhibitors of all enzymes within a family.

Methods

Activity Profiling of serine hydrolases.

LNCaP, DU-145 and PC-3 cell lines (ATCC) were maintained in RPMI 1640 (Irvine Scientific) supplemented with 10% fetal bovine serum at 37°C in 5% CO_2. The PrEC cell line (Clonetics) was maintained in defined media supplied by Clonetics. Each cell line was maintained in 150-mm tissue culture dishes. To generated protein lysates, cells were washed with ice-cold phosphate buffered saline (PBS) and harvested by scraping with a cell lifter into cold PBS. Cells were collected by centrifugation, resuspended in 50 mM Tris-Cl, pH 8.0, and then lysed by sonication as described previously (2,4). Soluble and insoluble cell fractions were separated by ultracentrifugation for one hour at 64,000 rpm at 4°C. Protein concentrations were determined by BCA assay (Pierce).

Activity profiling was performed with fp-PEG-TAMRA using previously described methods (2,4). Briefly, soluble fractions (40 µl; 1 mg/ml) were treated with 2µM fp-PEG-TAMRA for one hour at ambient temperature. Reactions were stopped by the addition of Laemmli buffer and boiling. Non-specific reaction of the probe was determined with a duplicate sample boiled for ten minutes prior to labeling with fp-PEG-TAMRA. The labeled samples were resolved by 10% SDS-PAGE and visualized by scanning with a Hitachi flatbed scanner at 605 nm.

Serine hydrolase activity in whole cells was measured with a membrane permeable probe, fp-Bodipy. Following addition of orlistat, the probe was added to cells (final concentration of 2µM) and the reaction was allowed to proceed to completion (1 hr). Cells were lysed by the addition of Laemmli sample buffer, boiled and samples were resolved on SDS-PAGE and visualized by scanning with a Hitachi flatbed scanner at 605 nm.

Inhibition of serine hydrolase activity β-lactones.

Ebelactone A and B stocks were made in DMSO. Orlistat was solublized in EtOH. Cell lysates were generated at 1mg/ml as described above. Samples (40 µg) were incubated

with inhibitors for twenty minutes, and fp-PEG-TAMRA was added and reacted for an additional 30 min.

Identification of labeled serine hydrolases.

To identify serine hydrolases a fluorophosphonate probe linked to biotin was used (2,4). Cell lysates were pre-adsorbed to avidin-agarose to reduce non-specific binding of proteins during the purification. Cell lysates were labeled with fp-PEG-biotin (5 μM) for one hour at room temperature. Protein was separated from unincorporated fp-PEG-biotin by gel filtration on Nap 25 columns. SDS was added to the elutate to a concentration of 0.5% and the sample was denatured by boiling. Samples were diluted with 50 mM Tris, pH 7.5 and 150 mM NaCl and incubated with Avidin-agarose for 1 hr at room temperature. The agarose beads were washed eight times with 50 mM Tris, pH 7.5, 150 mM NaCl containing 1% Tween 20. Labeled protein was eluted with Laemmli buffer containing 1% SDS. Protein was resolved by 10% SDS-PAGE and detected by silver staining. Specific bands were extracted and subjected to in-gel digestion by trypsin and peptide mass fingerprinting with MALDI-TOF as described previously. (5,6)

Expression of the recombinant thioesterase domain of fatty acid synthase.

The portion of the FAS gene(gi:21618359) encoding the thioesterase domain was amplified by PCR using the following primers; 5' ATG ACG CCC AAG GAG GAT GGT CTG GCC CAG CAG (corresponds to nucleotides 6727-6756) and 3' GCC CTC CCG CAC GCT CAC GCG TGG CT (corresponds to nucleotides 7625-7650). The recombinant thioesterase domain was cloned into pTrcHis (InVitrogen) and expressed in E. coli. The recombinant protein corresponds to residues 2202 to 2509 of FAS. The thioesterase was purified by Ni-affinity chromatography and analyzed for activity and inhibition by orlistat using methods described above.

Detection of fatty acid synthase by western blot.

PC-3 cells (5×10^4), treated with orlistat, were boiled in Laemmli buffer, resolved by SDS-PAGE and transferred to nitrocellulose. The membrane was blocked with non-fat milk and probed with an anti-FAS mAb (Pharmingen). Binding was visualized with an

HRP-conjugated rabbit anti-mouse IgG (BioRad) followed by chemiluminescent detection with the Western Lighting Chemiluminescence Reagent (Perkin-Elmer).

Inhibition of fatty acid synthesis by orlistat.

Cellular fatty acid synthesis was measured by the incorporation of [^{14}C]-acetate (7,8). Cells (2.5 x 10^4 cells/well in 24-well plates) were washed twice with PBS and incubated in defined serum-free medium containing 300 μg/ml BSA and insulin, transferin, and selenium as supplements. Media was added to the cells in the presence or absence of orlistat. Cells were incubated with orlistat for up to two hours prior to the addition of 1 μCi of [^{14}C]-acetate. Cells were incubated with [^{14}C]-acetate for 2 hr, at which time media was removed, cells were washed with PBS/EDTA and trypsinized. Cell pellets were washed twice more with PBS and fatty acids were extracted with chloroform-methanol (1:1) for 30 minutes. The extract was dried under N_2 and extracted with water-saturated butanol. Butanol was evaporated under N_2 and labeled fatty acids were detected by scintillation counting.

Effects of Orlistat on cell death. Cells were plated in 96 well tissue culture plates in complete media. After 24 hours, the cells were exposed to Orlistat (Roche) for an additional 24 hrs. Apoptosis was measured with the Cell Death Detection ELISA (Roche), which was performed according to the manufacturer's protocol. As an independent assessment of apoptosis, the amount of cleaved PARP was measured in cells following treatment with orlistat. Cells were cultured with the Orlistat (25 μM) or EtOH for 72 hrs, or with Staurosporine (1 μM) for 5 hours. At each time point total cell extracts were generated by addition of 1x SDS sample buffer. Samples were subjected to Western analysis using antibodies against the cleaved form of poly (ADP-ribose) polymerase (PARP, Cell Signaling). Western blotting was performed according to protocols established by the manufacturer of the anti-PARP antibody

Results and Discussion

An activity-based profiling effort was coupled to a simultaneous screen for antagonists of serine hydrolases in prostate cancer cells. Serine hydrolases were revealed with an activity-based probe comprised of a fluorophosphonate (fp) warhead linked to the TAMRA fluorophore (fp-TAMRA) (9). Primary cultures of normal prostate epithelial cells (PrEC) were compared to three prostate cancer cell lines, LNCaP, DU-145 and PC-3. Cell lysates were reacted with fp-TAMRA and then resolved on SDS-PAGE (Figure 1, lanes 1-8). In each case approximately fifteen different hydrolases are detected as fluorescent bands on SDS gels. The pattern of serine hydrolase expression is generally similar among the cell lines, with two significant distinctions. A band of 62 kDa was active in the normal PrECs, but absent in all tumor lines. Peptide mass fingerprinting showed this enzyme to be carboxylesterase-2. Conversely, a hydrolase with a mass of approximately 270 kDa is expressed in all of the tumor lines, but is absent in normal PrECs. Peptide mass fingerprinting with mass spectrometry showed this band to be fatty acid synthase (FAS), an observation that was confirmed by immunoprecipitating the complex between fp-TAMRA and FAS (data not shown).

FAS is the only eukaryotic enzyme capable of synthesizing palmitate, the precursor for the majority of cellular fatty acids (10). FAS has a unique structure and mode of action. The enzyme contains six separate enzymatic pockets and an acyl carrier protein. FAS condenses acetyl co-A and malonyl co-A, ultimately generating the sixteen carbon polyunsaturated fatty acid palmitate. Palmitate remains covalently attached to the acyl carrier protein of the enzyme until it is liberated by the final enzymatic pocket on the enzyme, the intrinsic thioesterase. This thioesterase is the sole serine hydrolase within FAS and is the target of the fp-TAMRA probe (see below).

FAS is up-regulated in a wide range of tumors. Its function has been strongly linked to tumor cell proliferation (11), making it an attractive therapeutic target. A functional connection between FAS and tumor cell proliferation was originally suggested by work with the fungal product cerulenin and its synthetic derivative c75. These compounds inhibit the first enzymatic domain within FAS, the ketoacyl synthase domain (12,13).

Despite their anti-tumor activity, there are questions as to the selectivity of these agents for FAS. For example, several reports indicate that the effects of cerulenin could be attributed to its inhibition of an uncharacterized palmitoyl transferase activity (14-16).

We capitalized on the fact that fp-TAMRA reacts with the active site of the thioesterase domain of FAS to screen for alternative inhibitors of this enzyme. Three derivatives of natural products, each containing a β-lactone moiety, were tested for the ability to block activity-based labeling of FAS. The β-lactone can undergo nucleophilic attack on the carbonyl carbon of the lactone ring by the active site serine of the esterase, yielding a covalent adduct between enzyme and inhibitor (17). All three compounds inhibit the thioesterase of FAS, but only tetrahydrolipstatin is selective for FAS in tumor cells. Tetrahydroliptstatin, also known as orlistat, is a drug that is approved and widely used for weight management in obese patients (18). The effectiveness of orlistat in obesity is conferred by the drugs ability to inhibit pancreatic lipase in the gastrointestinal tract, thereby preventing uptake of dietary fat. The inhibition of FAS by orlistat has never been reported, and is not believed to be relevant to its mode of action in weight loss.

To characterize the effects of orlistat on FAS in intact cells, we measured the ability of the compound to inhibit the activity of FAS in whole cells. PC-3 cells were treated with a range of orlistat and the level of FAS thioesterase function was measured with a membrane permeable activity-based probe, fp-Bodipy. Orlistat caused a concentration-dependent inhibition of labeling of FAS by fp-Bodipy (Fig. 2A), indicating that orlistat inhibits FAS in intact cells. Orlistat had no effect on the abundance of FAS, which was measured from the same treated samples by Western blot (Fig. 2B). The effects of orlistat on cellular fatty acid synthesis were gauged by measuring the incorporation of [^{14}C]-acetate into fatty acids. Saturating levels of orlistat (30 μM) reduced cellular fatty acid synthesis by ~75% within 30 min (Fig. 2C). Moreover, orlistat was able to block fp-TAMRA labeling of the active site serine of the recombinant thioesterase domain of FAS (Fig. 2D). Because orlistat is a tight-binding irreversible inhibitor of FAS, we cannot define a precise affinity for the compound. However, our observations on the inhibition of the FAS thioesterase domain in cell lysates, and purified from (not shown), suggest

that orlistat's apparent K_i for FAS is near 100 nM. When treating whole cells however, higher concentrations of the compound were necessary to achieve nearly complete inhibition of the enzyme (Fig. 2A). Orlistat has similar effects on inhibition of the FAS thioesterase domain, and on fatty acid synthesis in other prostate cancer lines, as well as in colon and breast cancer cell lines (not shown).

The effects of orlistat on cell growth and survival were examined. Orlistat induced a pronounced anti-proliferative effect in the PC-3 cell line, and exhibited a slight effect on the androgen-dependent LNCaP cells. The compound had little effect on the DU-145 cells or the normal PrEC cells (Figure 3). Orlistat also induced tumor cell apoptosis, but the sensitivity of the individual lines to the compound was somewhat different (Fig 4A). When cell death was assessed at 24hr by measuring DNA fragmentation (Roche Cell Death ELISA), the PC-3 and LNCaP cells exhibited substantial levels of cell death. At this time point however, only a modest effect was observed on death of the DU145 cells. Orlistat was without effect on death of human foreskin fibroblasts or normal PrECs. This finding is consistent with the fact that other reported inhibitors of FAS have limited effects on normal cells (11). Prolonged exposure of each of the tumor cell lines to orlistat resulted in cleavage of poly ADP-ribose polymerase (PARP), another marker of apoptosis (Figure 4B). In this and other apoptosis assays (e.g., staining of annexin on the cell surface), we have yet to observe significant effects of orlistat (1-25 uM) on normal cells, including fibroblasts, normal PsECs or normal mammary epithelial cells (not shown).

Altogether, the findings presented here, and results from our larger survey of a number of tumor and normal cell lines (not shown), lead to the following conclusions; 1) Orlistat is a novel inhibitor of the thioesterase function of FAS. By virtue of this property orlistat can block cellular fatty acid synthesis; 2) Orlistat has little effect on growth or survival of normal cells, but can inhibit proliferation, and induce apoptosis, in tumor cells; 3) Tumor cells have varying levels of sensitivity to orlistat. Since all of the tumor lines we have tested express FAS, the distinctions in response to orlistat appear to be independent of the expression of FAS, or the level of inhibition of FAS by orlistat.

In view of these newly discovered properties of orlistat, the drugs potential as an anti-tumor agent should be considered. The minimal effects of orlistat on normal cells suggest that the compound could have therapeutic index sufficient for anti-tumor therapy. Orlistat also represents an alternative to cerulenin or c75, which inhibit the ketoacyl synthase domain of the enzyme. There are however, issues that remain to be addressed prior to testing or orlistat. First, it is not clear why subsets of tumor cells respond to orlistat far better than others. Since we have obtained similar results with hormone-dependent and independent breast cancer cell lines, we suspect that sensitivity to orlistat may relate to hormone dependence. Differences in the Akt survival pathway must also be considered, but deriving a mechanistic basis for such conclusions requires further study. Second, in its approved formulation, orlistat is administered orally. Because of its extremely low oral bioavailability, the effects of the drug are largely confined to the gastrointestinal tract, where it inactivates pancreatic lipase (18). Therefore, the formulation and route of delivery would have to be changed to treat tumors of the breast, prostate etc.. Nevertheless, the lack of overt toxicity of this compound when administered by i.v. injection (Roche NDA), speaks to the feasibility of such an approach. One cannot exclude the possibility that the oral formulation of orlistat could be useful in treating tumors of the GI tract, like colon cancer. We have found orlistat to block FAS and to induce apoptosis in a number of colon cancer lines (forthcoming report), so treating patients at high risk for colon cancer in a prophylactic manner could be considered.

Finally, the identification of orlistat's ability to inhibit the thioesterase domain of FAS was made possible by the application of activity-based profiling. The analysis we performed involved a screen of just three β-lactones against a little over 35 different serine hydrolases. Even within this small test set, a novel target and indication was identified for an approved drug. It is reasonable to believe that a high throughput version of such a screen could drive decision making in drug development. Information from such screens could lead to the identification of more selective leads much earlier in development. As in the case of this report, such an analysis might also point toward unanticipated targets and indications for other drugs.

Figure Legends

Figure 1. Activity profiling of normal and neoplastic prostate epithelial cells. Lysates were generated from primary cultures of normal prostatic epithelial cells (PrEC), and from three prostate tumor cell lines (LNCaP, DU-145, and PC-3). Lysates were incubated with fp-PEG-TAMRA for one hour at room temperature. Non-specific labeling with the activity probe was measured in samples that were denatured by boiling (lanes marked +). Samples were resolved by 10 % SDS-PAGE and visualized at 605 nm using a Hitachi flat bed gel scanner (lanes 1-8). The effect of three β-lactones on the activity-labeling of serine hydrolases from PC-3 cells was assessed in a similar manner. Prior to incubation with fp-PEG-TAMRA, lysates were pre-incubated with ebelactone A (lane 10), ebelactone B (lane 11) or orlistat (lane 12). Following labeling with fp-PEG-TAMRA the reactions were halted and enzyme activity visualized as described above.

Figure 2. Effect of orlistat on cellular activity of FAS.

(A) FAS activity in intact PC-3 cell was measured with a cell-permiable activity probe, fp-Bodipy. PC-3 cells were first incubated with a range of orlistat. Then the remaining FAS activity was assessed by adding fp-Bodipy to the cells. Cell lysates were separated on SDS-PAGE and visualizing active serine hydrolases as described in Methods.

(B) The effect of orlistat on the level of FAS protein was measured using the same cell lysates as (A) by Western blot.

(C) The effect of orlistat on cellular fatty acid synthesis was gauged by measuring the incorporation of [^{14}C]-acetate into fatty acids as described in Methods.

(D) The ability of orlistat to block labeling of the recombinant thioesterase domain of FAS was measured by pre-incubating the thioesterase with a range of orlistat and subsequently with the activity probe fp-TAMRA. Samples were run on SDG-PAGE and signal was visualized as described.

Figure 3. Orlistat inhibits tumor cell proliferation.

The effect of orlistat on cell proliferation was measured by the incorporation of BrdU. PC3 (●), LNCap (■), DU145 (▲), and PrEC cells (♦) were seeded as sub-confluent mono-layers into microtiter plates. Cells were exposed to orlistat for 32 hrs, then a BrdU labeling solution was added to the wells. BrdU incorporation was measured according to the manufacturers protocol (Roche Cell Proliferation ELISA). Proliferation is expressed as a percentage of proliferation in untreated cells.

Figure 4. Orlistat induces tumor cell death.

(A). PC3, LNCaP, DU145, normal prostate epithelial cells (PrEC), and normal foreskin fibroblasts cells were exposed to Orlistat (12.5μM) for 48 hrs. Tumor cell death was measured with the Cell Death Detection ELISA (Roche), which measures DNA fragments within immuno-captured nucleosomes. DNA fragmentation was assessed by measuring A_{405}-A_{490}

(B). LNCaP, DU145, and PC3 cells were cultured with orlistat (Or,12.5 uM) for 72 hrs, with Staurosporine (St) as a positive control for induction of cell death, or cultured in media without any stimulus (C) . Following incubation, cell extracts were generated and subjected to western analysis using antibodies selective for the cleaved form of PARP.

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Figure 1

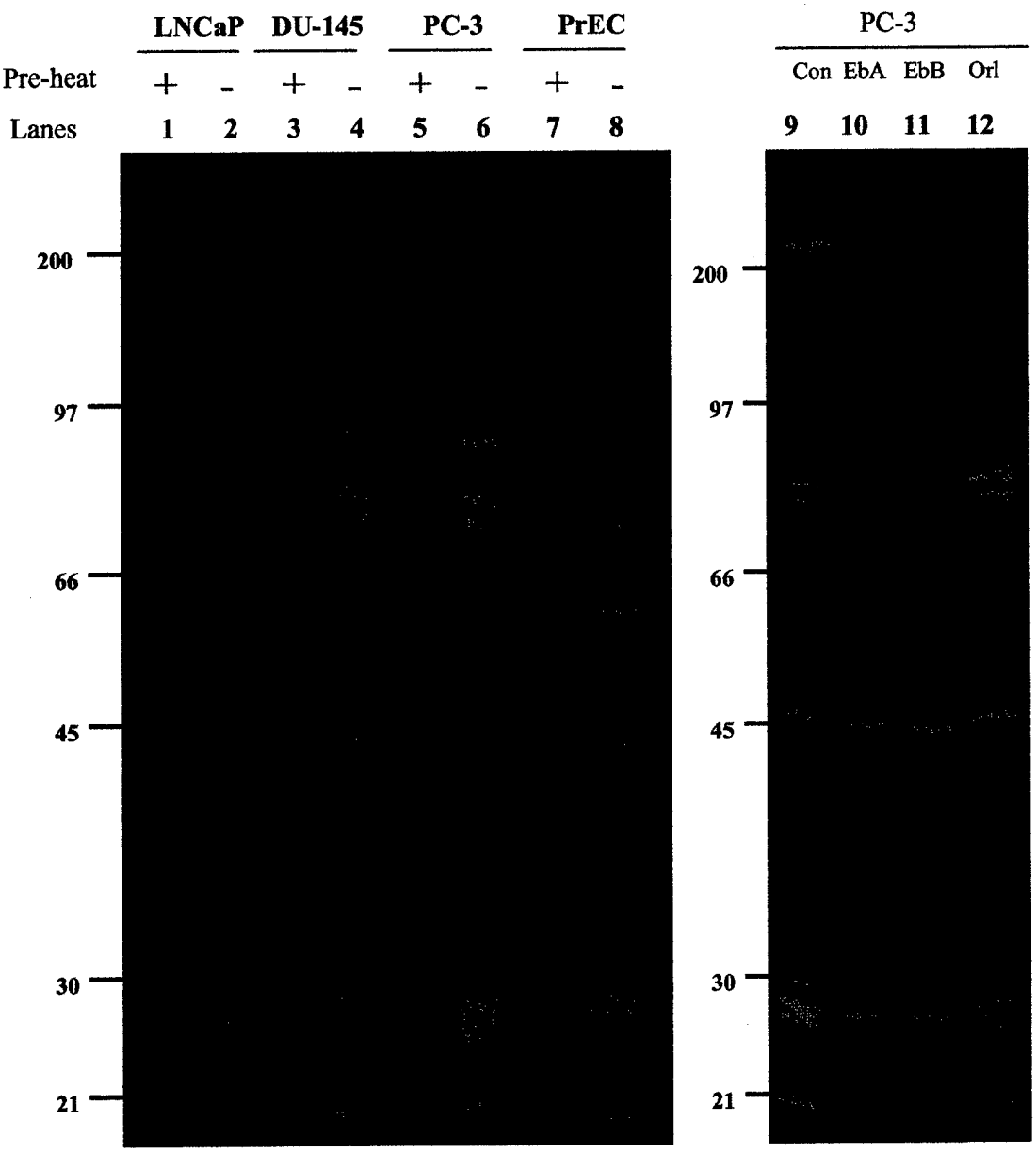
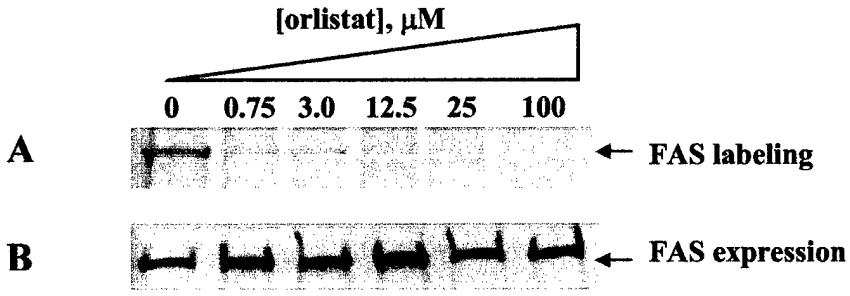
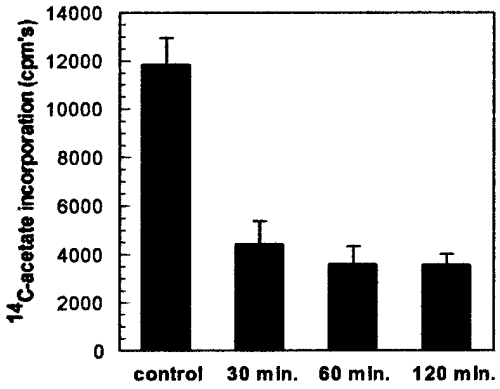

LNCaP
DU-145
PC-3
PrEC
PC-3
Pre-heat
+ - + - + - + -
Con EbA EbB Orl
Lanes
1 2 3 4 5 6 7 8
9 10 11 12
200
97
66
45
30
21
200
97
66
45
30
21

Figure 2


[orlistat], μM
0
0.75
3.0
12.5
25
100
A
FAS labeling
B
FAS expression

C


14C-acetate incorporation (cpm's)
14000
12000
10000
8000
6000
4000
2000
0
control
30 min.
60 min.
120 min.

D

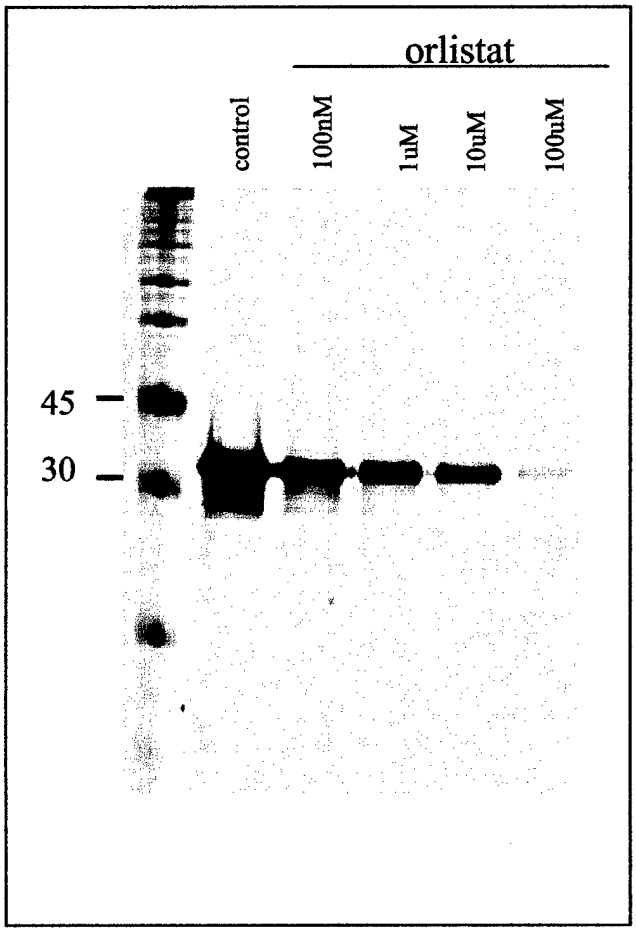

orlistat
control
100nM
1uM
10uM
100uM
45
30

Figure 3

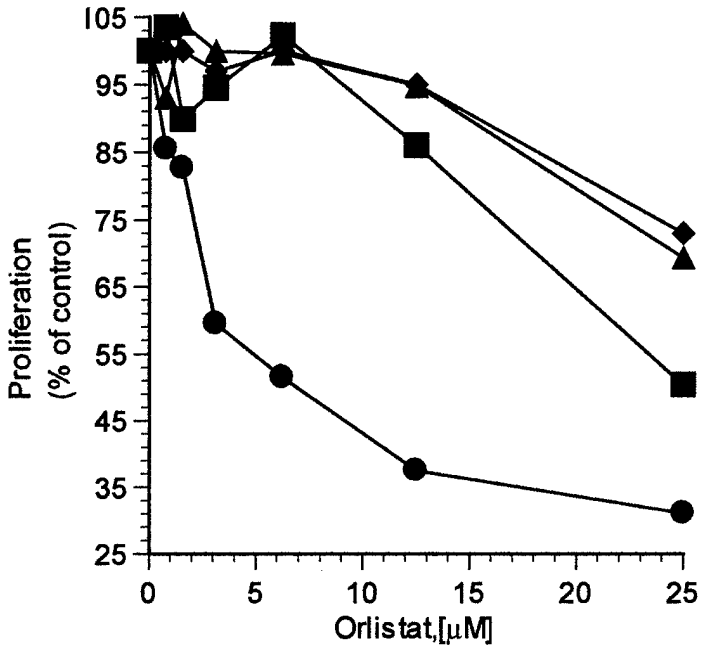
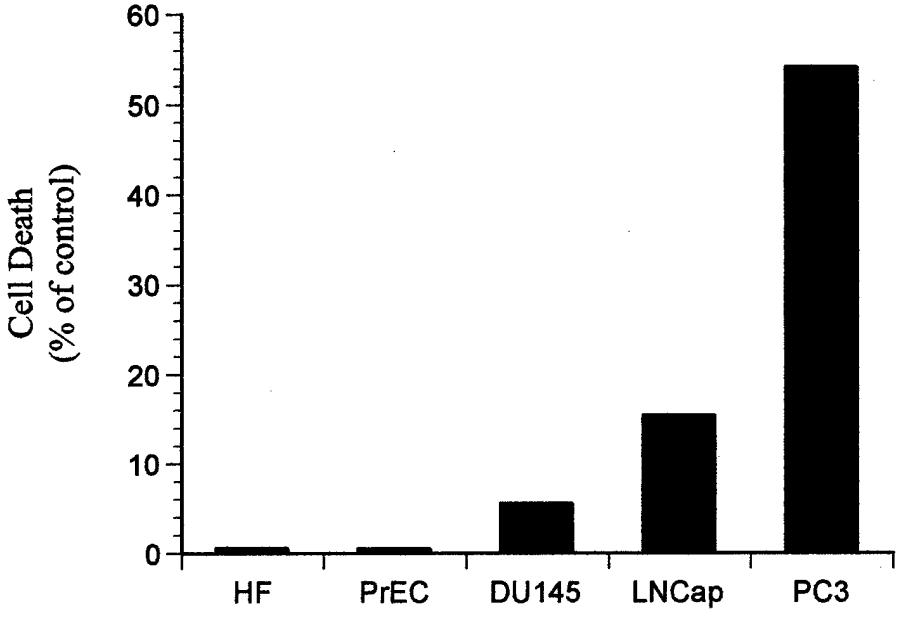
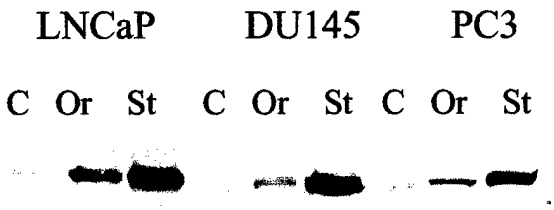

105
95
85
75
65
55
45
35
25
Proliferation
(% of control)
0
5
10
15
20
25
Orlistat,[μM]

Figure 4

A.


Cell Death
(% of control)
60
50
40
30
20
10
0
HF
PrEC
DU145
LNCap
PC3

B.


LNCaP
DU145
PC3
C Or St C Or St C Or St