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TITLE: Targeting Fatty Acid Synthase Gene for Prostate Cancer Therapy

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# Targeting Fatty Acid Synthase Gene for Prostate Cancer Therapy

## Abstract
Fatty acid synthase (FAS) is significantly over-expressed in prostate tumor cells and inhibition of FAS results in apoptosis, suggesting that FAS is an ideal target for drug development. The overarching hypothesis of this project is that a specific inhibitor for FAS dimerization will block the function of this enzyme and cause apoptosis of the tumor cell. Our specific aims are (i) to characterize the apoptotic pathway induced by FAS inhibition, and (ii) to identify small chemical compounds that specifically inhibit dimer formation of FAS enzyme. During the last four months (Apr. 1-July 31, 2008) we mainly focused our effort on the second specific aim and screened a compound library provided by Developmental Therapeutics Program of NCI. We also screened a library of natural products that particularly focused on marine lives and found that one of the natural products in the library showed strong activity of inhibiting FAS. We also found that the purified compound, Cacalol, significantly blocked the activity and expression of FAS. Our results suggest that this compound has a potential utility for the treatment of prostate cancer.

## Subject Terms
None Listed.
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Introduction

Prostate cancer is the second most common malignancy in adult males as well as the second leading cause of cancer-related deaths in the United States. However, virtually no treatment option is available for patients at an advanced stage due to the fact that none of the existing chemotherapeutic agents are effective to treat this type of cancer. Therefore, we essentially need a new approach to define specific target molecules with well-defined clinical rationale so that we will have a better chance of developing a more effective therapeutic agent. Fatty acid synthase (FAS) is significantly over-expressed in prostate tumor cells and inhibition of FAS results in apoptosis, suggesting that FAS is an ideal target for drug development. Although the detailed mechanism of apoptosis induced by FAS inhibition is yet to be elucidated, our preliminary data indicate that (i) FAS is over-expressed in human prostate cancer and this high-expression correlated with poor survival of patients, (ii) specific inhibition of FAS expression by siRNA significantly increased ceramide, and (iii) the high level of ceramide resulted in induction of pro-apoptotic genes, BNIP3, DAPK2 and TRAIL followed by apoptosis. In order to identify an effective inhibitor of FAS, we have also developed a novel high-throughput screening system to block dimerization of FAS enzyme in the hope that screened drugs can be used for the treatment of prostate cancer patients. We plan to test the hypothesis that a specific inhibitor for FAS dimerization will block the function of this enzyme and cause apoptosis of the tumor cell. Our specific aims are (i) to characterize the apoptotic pathway induced by FAS inhibition, and (ii) to identify small chemical compounds that specifically inhibit dimer formation of FAS enzyme. Our effort for the last 4 months (Apr. 1 –July 31) has been mainly focused on the second specific aim.

Body

Our working hypothesis for Specific aim 1 is that apoptosis induced by FAS inhibition is caused by the suppression of CPT1 followed by accumulation of ceramide which in turn activates pro-apoptotic genes, BNIP3, TRAIL and DAPK2. We found that inhibition of FAS by chemical inhibitor or shRNA resulted in accumulation of ceramide followed by apoptosis and that three pro-apoptotic genes, BNIP3, TRAIL and DAP kinase 2, were significantly up-regulated in response to the FAS inhibition (1-3). These lines of evidence strongly support our hypothesis that apoptosis induced by the FAS inhibition is caused by suppression of CPT1 followed by accumulation of ceramide which in turn activates pro-apoptotic genes, BNIP3, TRAIL and DAPK2. We are also currently performing immunohistochemical analysis to examine the level of CPT1, FAS, BNIP3, TRAIL and DAP kinase 2 in clinical specimens from prostate cancer patients. The results of these experiments will provide us with critical information to understand the mechanism of apoptosis induced by FAS inhibition.
In Specific aim 2, we hypothesize that a specific inhibitor for FAS dimerization will block the function of this enzyme and cause apoptosis of the tumor cell. For this purpose, we are currently screening the compound library provided by Developmental Therapeutics Program of NCI/NIH. This program has more than 200,000 compounds that are available to us for screening. We are also screening a library of natural products (obtained from Marine Biology Institute in Japan (Kamaishi city)) that is particularly focused on marine lives. We found that one of the natural products in the library showed strong activity of inhibiting FAS. This product inhibited both the activity and expression of FAS. The activity was purified by TLC followed by HPLC. We also found that this compound is identical with Cacalol. Our results of in vivo experiment using a xenograft mouse model indicate that Cacalol has strong inhibitory activity to tumor growth. These results suggest that this compound has a potential utility for the treatment of prostate cancer. We are currently examining the exact mechanism of FAS inhibition by Cacalol by analyzing inhibitory kinetics of Cacalol using purified FAS enzyme.

**Key Research Accomplishments**

1. We found that inhibition of FAS can cause apoptosis through the up-regulation of pro-apoptotic genes, BNIP3, TRAIL and DAP kinase

2. Our preliminary data indicate that the expression of FAS and BNIP3 is inversely correlated in prostate cancer.

3. We found that Cacalol significantly inhibits both activity and expression of FAS.

**Reportable Outcomes**

At this point, we do not have any reportable outcomes mainly due to the limited time period (Apr. 1-July 31).

**Conclusion**

Our results strongly support the hypothesis that apoptosis induced by FAS inhibition is caused by suppression of CPT1 followed by accumulation of ceramide which in turn activates pro-apoptotic genes, BNIP3, TRAIL and DAPK2. Further analysis of this pathway may reveal a more detailed mechanism of apoptosis induced by FAS inhibition which may lead to the identification of a better target to block the FAS pathway. We indeed found a compound called Cacalol which significantly inhibits the FAS activity. This compound may have potential utility for the treatment of prostate cancer.

“**So what**”
The most significant finding during this period is the discovery of Cacalol as an inhibitor of FAS enzyme and this compound indeed blocks tumor growth in vivo. Although it needs further analysis for the detailed mechanism of inhibition, Cacalol may serve as a lead compound to identify a potent anti-cancer drug which can be tested for a clinical trial in the future.

References


Appendices

N/A