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14. ABSTRACT

Our overall objective in the current application is to establish the viability of a new strategy of treating late stage PCa through therapeutic targeting of cholesterol metabolism in vivo, using combination of cutting edge spectroscopic imaging and other technologies, including biochemistry assays and preclinical testing. The innovation of this study is that it targets altered cholesterol metabolism, an understudied field of cancer research. Our central hypothesis is that abrogating cholesterol esterification events will result in an effective strategy for treating late stage PCa. This hypothesis will be tested by first validating the presence of altered cholesterol metabolism in human prostate cancer patient specimens. We will then evaluate the therapeutic benefit of CE depletion in appropriate animal models of PCa, and elucidate pathways linking cholesterol metabolism with cancer aggressiveness. An interdisciplinary research team has been assembled, with expertise in spectroscopic imaging & nanomedicine (Dr. J. X. Cheng, PI), biochemistry (Dr. X. Liu, co-PI), and prostate cancer biology (Dr. T. Ratliff, co-PI).

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Lay Abstract:

Since the introduction of prostate specific antigen screening, prostate cancer has become the most widely diagnosed non-skin cancer in men in the United States (220,800 cases estimated in 2015). While often diagnosed in clinically localized stages, PCa remains the second leading cause of cancer-related mortality in American men with over 27,540 projected deaths in 2015. For men with advanced prostate cancer, androgen deprivation therapy in the form of bilateral orchiectomy or pharmacologic castration is an accepted standard therapy. Despite initial disease control, androgen deprivation therapy alone is non-curative and the subsequent development of castration-resistant prostate cancer (CRPC) occurs in the lifespan of almost all men who do not succumb to non-cancer deaths. For men with metastatic CRPC, docetaxel was approved in 2004 as the first-line cytotoxic chemotherapy owing to a modest increase in overall survival compared to mitoxantrone. Since 2010, there has been a tremendous increase in treatment options available for metastatic CRPC patients, including novel anti-androgen therapy with abiraterone and others. Nevertheless, the effectiveness of current therapies is palliative with an improvement in overall survival of 2-5 months compared to placebo. Therefore, a critical need exists to develop novel therapeutic strategies for advanced prostate cancer.

Cancer cells adopt metabolic pathways that differ from their normal counterparts by high rates of glycolysis and biosynthesis of essential macromolecules to fuel rapid growth. Among dysregulated metabolic pathways, altered lipid metabolism is increasingly recognized as a signature of cancer cells. Enabled by label-free coherent Raman scattering microscopy, our laboratory has performed the first quantitative analysis of lipogenesis at single cell level in human patient cancerous tissues. Our imaging data revealed an aberrant cholesterol ester accumulation in high-grade prostate cancer and metastases, but not in normal prostate or prostatitis. Cholesterol is an essential biomolecule that plays important roles in the maintenance of membrane structure, signal transduction, and provision of precursor to hormone synthesis. While cholesterol accumulation is known to be a hallmark of atherosclerosis, its exact role in cancer progression remains elusive. Our unexpected finding of cholesterol ester accumulation in advanced human prostate cancer triggered us to ask whether such cholesterol ester accumulation could become a potential target for prostate cancer treatment. Our pilot study has indeed showed that pharmacological inhibition of cholesterol ester accumulation significantly suppressed prostate cancer aggressiveness without affecting normal cell viability. Based on these appealing data, we hypothesize that abrogating cholesterol ester accumulation will result in an effective strategy for treating advanced prostate cancer. This hypothesis will be tested through two specific aims. First, we will develop a clinically viable strategy of cholesterol depletion and evaluate its therapeutic effect on tumor growth in appropriate animal models of prostate cancer. Second, to understand how such treatment strategy benefits prostate cancer, we will elucidate the mechanism by which cholesterol ester accumulation contributes to prostate cancer aggressiveness.

At the completion of this project, it is our expectation that we will have provided strong evidences to support the concept that inhibition of cholesterol ester accumulation is a viable and potentially attractive therapeutic intervention strategy to treat advanced prostate cancer. Notably, several small molecule inhibitors of cholesterol accumulation, e.g. avasimibe, have gone through clinical trials to treat atherosclerosis but failed due to the lack of effectiveness. Our proposed study will demonstrate a novel use of existing drugs to treat advanced prostate cancer, and it is anticipated that preclinical studies and/or clinical trials will follow shortly after the completion of this project. Ultimately, the adoption of such strategy will substantially improve the clinical outcome for metastatic prostate cancer patients that are resistant to hormone therapy. Our deeper mechanistic study will contribute to the understanding of dysregulated cholesterol metabolism in advanced prostate cancer, which in turn provides the biological foundation of targeting cholesterol accumulation for treatment of metastatic prostate cancer.

Table of Contents

Page

1. INTRODUCTION:

Our *overall objective in the current application* is to establish the viability of a new strategy of treating late stage PCa through therapeutic targeting of cholesterol metabolism *in vivo*, using combination of cutting edge spectroscopic imaging and other technologies, including biochemistry assays and preclinical testing. *The innovation of this study* is that it targets altered cholesterol metabolism, an understudied field of cancer research. Our *central hypothesis* is that abrogating cholesterol esterification will result in an effective strategy for treating late stage PCa. This hypothesis will be tested by first validating the presence of altered cholesterol metabolism in human prostate cancer patient specimens. We will then evaluate the therapeutic benefit of CE depletion in appropriate animal models of PCa, and elucidate pathways linking cholesterol metabolism with cancer aggressiveness. An interdisciplinary research team has been assembled, with expertise in spectroscopic imaging & nanomedicine (Dr. J. X. Cheng, PI), biochemistry (Dr. X. Liu, co-PI), and prostate cancer biology (Dr. T. Ratliff, co-PI).

2. KEYWORDS:

Prostate cancer, lipid droplet, metabolism, cholesterol, cholesteryl ester, Raman spectroscopy

3. ACCOMPLISHMENTS:

a. What were the major goals of the project?

The two major goals of this project are (1) Develop a clinically viable cholesteryl ester depletion strategy to suppress the proliferation of late-stage prostate cancer *in vivo*; (2) Determine the relative contribution of altered cholesterol metabolism to prostate cancer aggressiveness.

b. What was accomplished under these goals?

We have accomplished all the tasks stated in the approved SOW. The major results are summarized below.

Task 1: Determine bioavailability of avasimibe after systemic administration of avasimibe micelles.

During year 1 of this project, we have submitted the animal protocol to Purdue University Animal Care Committee (PACUC) and to DoD Animal Care and Use Review Office for review. The protocol has been approved by PACUC as well as by the DoD Animal care and Use Review Office. Upon this approval, we have performed the following experiments as we proposed.

First, we have determined the maximum tolerated dosage (MTD) of avasimibe by tail vein injection of various doses (40, 20, 15, 10, 6 mg/kg) to Balb/c mouse (n=6 for each dose). Avasimibe was loaded in avasimin with 10% loading efficacy. Phosphate buffer (PBS) injection was used as a control. **Finding**: We did not observe weight loss in any of these doses. The body weight data of mice treated with 15, 10, 6 mg/kg avasimibe was shown in **Figure 1.1** below. These data indicate that avasimibe is a relatively non-toxic drug.

Avasimin (avasimibe) (mg/kg)	Death # / Total #
200 (20)[a]	0/1
100 (10)[a]	0/1
75 (7.5)[b]	0/4
50 (5)[b]	0/4
30 (6)[b]	0/4

a. One i.v. injection
b. Four total i.v. injections, one every 4 days

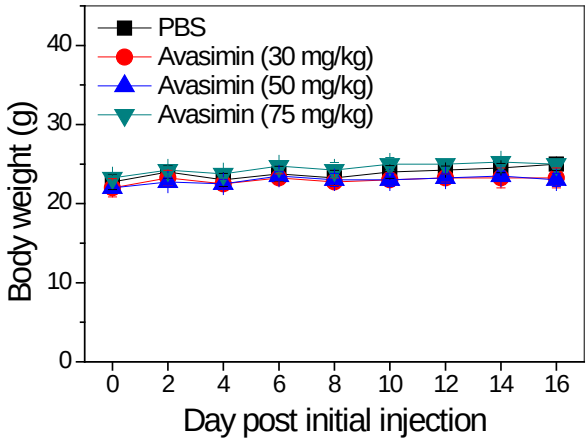

Figure 1.1. Characterization of in vivo safety of high-does avasimibe.

Second, we have determined the blood-bioavailability upon intravenous injections of the avasimibe/albumin formulation at the dose of 75mg/kg (containing 7.5 mg/kg avasimibe) to Balb/c mouse (n=3). The blood concentration of avasimibe at 7 different time points (2 min, 15 min, 30 min, 60 min, 2 h, 3 h, and 6 h post injection) was measured by LC-MS at Purdue Bindley Bioscience Facility. Data are shown in **Figure 1.2**. **Finding**: Area under the curve (AUC) of Avasimibe/ albumin formulation by intravenous administration was 136.36μg.h/mL. As control, Avasimibe was administered orally at two different doses, 15mg/kg and 100mg/kg. The AUC for the 15mg/kg, and 100mg/kg oral doses was 14.92μg.h/mL, and 49.9μg.h/mL respectively. These results are an indication that avasimibe/albumin formulation significantly increases avasimibe bioavailability.

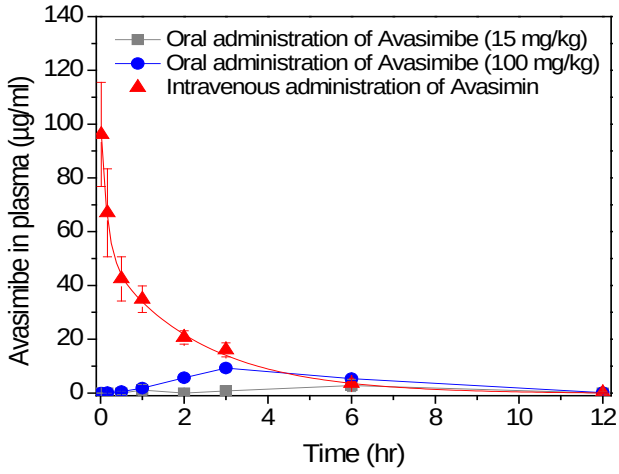

Figure 1.2. Blood bioavailability of avasimibe following intravenous administration of avasimin (75 mg/kg, containing 7.5 mg/kg avasimibe) or oral administration of avasimibe (15 mg/kg) (n=3). The data were fitted with two-compartment model ($y = A_1 e^{(-x/t1)} + A_2 e^{(-x/t2)} + y_0$).

Third, we determined the bio-distribution of avasimibe in a prostate cancer xenograft mouse model (n=3, nude mice). To establish the human prostate cancer xenograft mouse model, PC-3 cells were used. The concentration of avasimibe was measured by LC-MS analysis of tissue homogenates. Data are shown in **Figure 1.3**. **Finding**: After intravenous administration of avasimibe/ albumin formulation, the concentration of avasimibe in the tumor was determined to be 34 μM, which is more than 4-fold higher than the IC_{50} of the drug in PC3 cells (7.3 μM). In contrast, there was no drug detected in tumor after oral administration of avasimibe, whereas most of the orally administered drug was detected in the liver, intestines and feces.

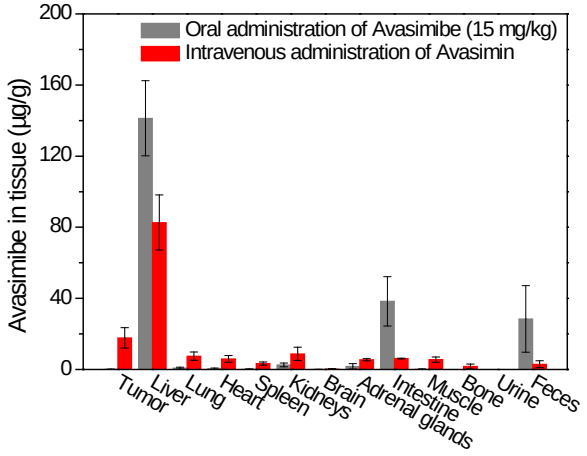

Figure 1.3. Tissue distribution of avasimibe at 2 hr after intravenous injection of avasimin (75 mg/kg, containing 7.5 mg/kg avasimibe) or oral administration of avasimibe (15 mg/kg) (n=3 for each group). Data are expressed as mean ± SD.

Task 2: Determine avasimibe's anti-cancer effect and organ toxicity using a xenograft mouse model of prostate cancer.

We determined the anti-cancer effect of the avasimibe/albumin formulation in prostate cancer xenograft mouse model. As control, PBS was intravenously injected at the same interval. Tumor volume and body weight were monitored daily. Survival rate was determined over a 10-week period. **Finding:** When compared to the control group, avasimibe/ albumin formulation significantly reduced tumor growth (**Figure 2.1 left column** and **Figure 2.2**) with no obvious body weight change (**Figure 2.3**). An increase in survival rate was also evident with the avasimibe/albumin treatment (**Figure 2.1 right column**).

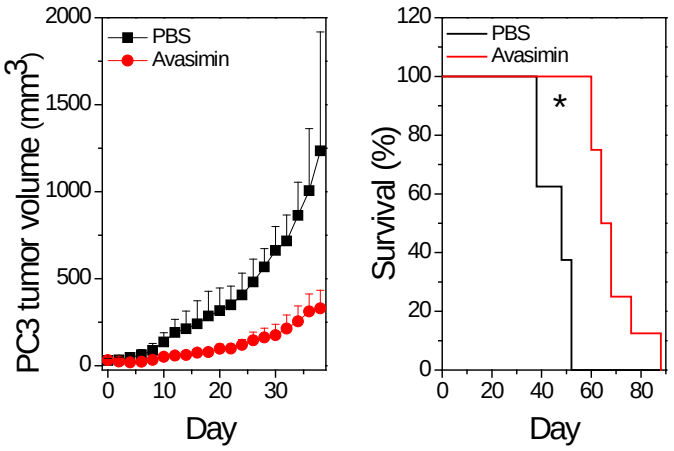

Figure 2.1. Anti-tumor effects of avasimin for PC3 tumor xenograft. Tumor volume (left) and survival rate (right) of PC3 tumor xenograft mice (n=8 for each group). The mice received avasimin (75 mg/kg, containing 7.5 mg/kg avasimibe) or PBS by intravenous injection daily for the first 5 days, following by intravenous injection once every 4 days. Day was counted after post initial treatment. *$P < 0.005$ (log-rank test).

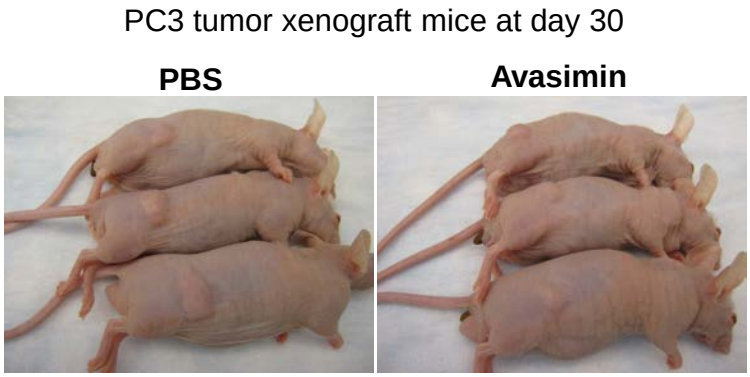

Figure 2.2. Comparison of tumor volume between PBS-treated and avasimin-treated groups. Photographs of PC3 tumor xenograft mice (representatively, n=3) at 30 days post treatment.

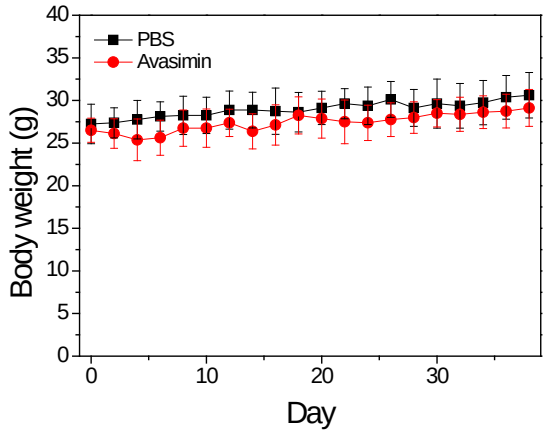

Figure 2.3. No body weight changes in mice treated with avasimin. PC3 tumor xenograft mice (n=8 for each group) were received with avasimin (75 mg/kg, containing 7.5 mg/kg avasimibe) and PBS by intravenous injection daily for the first 5 days, followed by intravenous injection once every 4 days.

Task 3: Determine the consequence of avasimibe administration on arachidonic acid (AA) and cholesteryl ester levels in prostate cancer cells.

Our liquid chromatography-mass spectrometry analysis of PC-3 cell lysates revealed that ACAT inhibition by avasimibe or ACAT-1 knockdown by shRNA significantly reduced the level of AA in PC-3 cells **(Figure 3.1)**

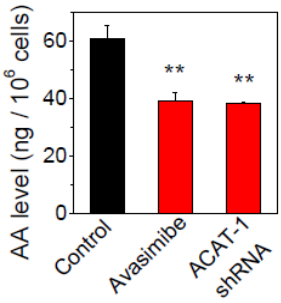

Figure 3.1. AA levels in PC-3 cells treated with avasimibe or ACAT-1 shRNA (n = 3). Avasimibe treatment: 7.5 μM, 2 day; ACAT-1 shRNA: 3 day transfection.

We further studied the lipid levels in the PC3 tumor model. SRL imaging showed a large amount of lipid accumulation in the tumor tissues (**Figure 3.2**). Avasimin treatment distinctly reduced the amount of LDs for the PC3 tumors. Raman spectra from LDs in the tumor tissues demonstrated the cholesterol ring vibration band at 702 cm^{-1} (**Green bar in Figure 3.3**). The CE level (25±5 %) in LDs of PC3 tumor tissue was smaller than that of cultured PC3 cells (74±15 %) (**Figure 3.3**). After repeated avasimin treatment, CE level (%) for PC3 tumors significantly reduced. The increase (more than 30 %) in free cholesterol levels for the tumor treated with avasimin was also determined (**Figure 3.4**).

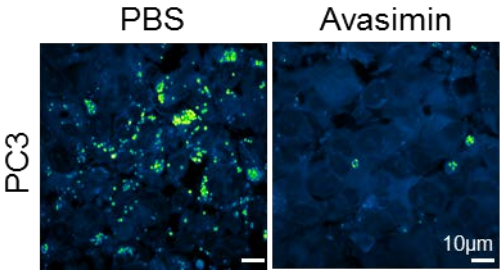

Figure 3.2. SRL images of PC3 tumor from xenograft mouse model.

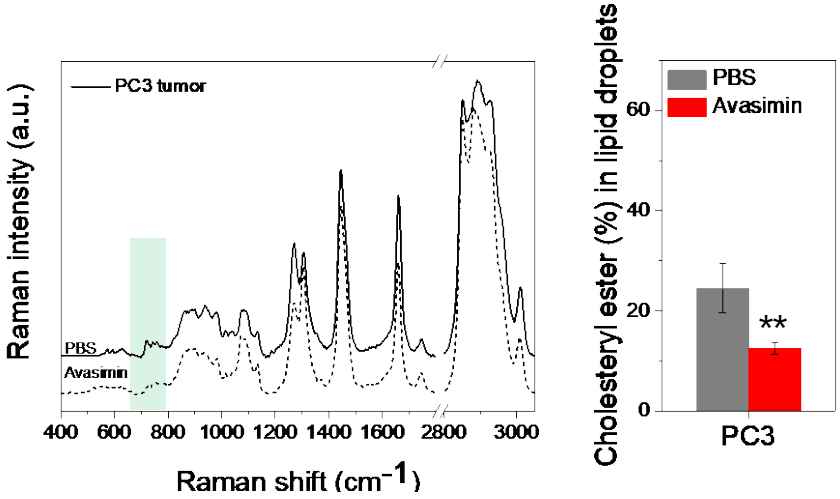

Figure 3.3. Raman spectra of lipid droplets in the tissues shown in 3.2. Green bar indicates the Raman band of cholesterol at 702 cm^{-1}. Solid and dashed lines represent groups treated with PBS and avasimin, respectively. Quantification of cholesteryl ester molar percentage (%) in lipid droplets based on the Raman spectra (n = 5 - 10).

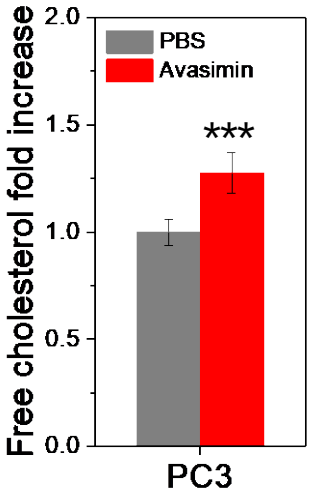

Figure 3.4. Free cholesterol increase in the tumor tissues (n = 4).

Task 4: Establish a solid correlation between cholesteryl ester accumulation / depletion and the potential of tumor cell migration *in vitro*.

In order to evaluate the potential of tumor cell migration, we performed standard transwell assays in prostate cancer cells. Cholesteryl ester depletion by avasimibe, Sandoz, or ACAT-1 knockdown using shRNA suppressed migration and invasion capabilities of PC-3 cells (**Figure 4.1**). On the other hand, when the cholesteryl ester accumulation is promoted by PTEN knockdown using shRNA in DU145 cells, we observed greater capabilities of migration and invasion compared to the cholesteryl ester-poor PTEN wild-type DU145 cells (**Figure 4.2**).

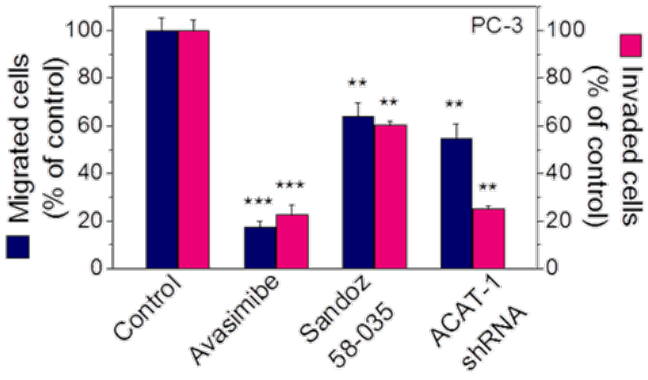

Figure 4.1. Quantification of migrated and invaded PC-3 cells pre-treated with avasimibe, Sandoz 58-035, or ACAT-1 shRNA (n = 3). Avasimibe treatment: 5 µM, 2 days; Sandoz 58-035 treatment: 10 µM, 2 days; ACAT-1 shRNA: 2 day transfection.

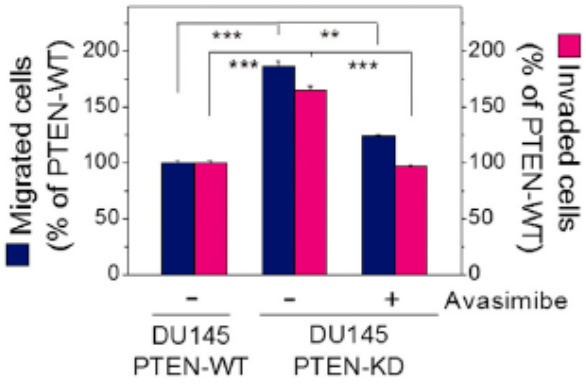

Figure 4.2. Quantification of migrated and invaded DU145 cells with PTEN wild-type or PTEN knockdown using shRNA (n = 3). Avasimibe treatment: 5 µM for 1 day.

We further compared migration/invasion capabilities of two LNCaP cell lines, LNCaP-LP (low passage) and LNCaP-HP (high passage). LNCaP-HP cells were derived upon continuous passage from LNCaP-LP until the passage number was over 60. Compared to cholesteryl ester poor LNCaP-LP cells, cholesteryl ester-rich LNCaP-HP cells showed greater migration and invasion capabilities (**Figure 4.3**).

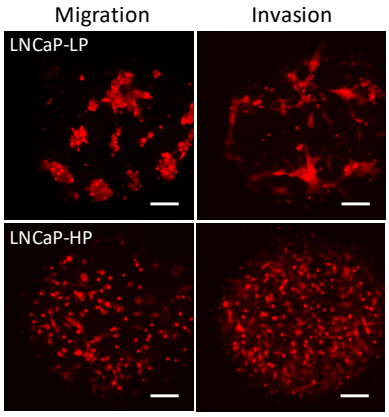

Figure 4.3. Representative images of migration and invasion of LNCaP-LP and LNCaP-HP cells. Scale bar: 50 μm.

To summarize, we established a solid correlation between cholesteryl ester accumulation / depletion and the potential of tumor cell migration in vitro. Cholesteryl ester accumulation promotes prostate cancer migration and invasion potentials in multiple prostate cancer cell lines. When cholesteryl ester is depleted either by ACAT-1 inhibitors or genetic manipulations, cancer cells showed reduced migration and invasion capabilities (**Table 4.1**).

Table 4.1. Effect of cholesteryl ester accumulation on prostate cancer cell migration potential.

	PC-3	PC-3 + ACAT-1 inhibitors	PC-3 + ACAT-1 KD	DU145 PTEN WT	DU145 PTEN KD	LNCaP-LP	LNCaP-HP
CE level	High	Low	Low	Low	High	Low	High
Migration / invasion potential	High	Low	Low	Low	High	Low	High

Task 5: Elucidate the pathway linking CE accumulation and PCa cell aggressiveness.

We performed gene expression analysis using RT^2 Profiler PCR array, in which we looked at expression levels of 84 human prostate cancer related genes after inhibiting cholesteryl ester storage in PC3 cells. Our results revealed that multiple negative regulators of metastasis were upregulated after avasimibe treatment in PC3 cells (**Figure 5.1**).

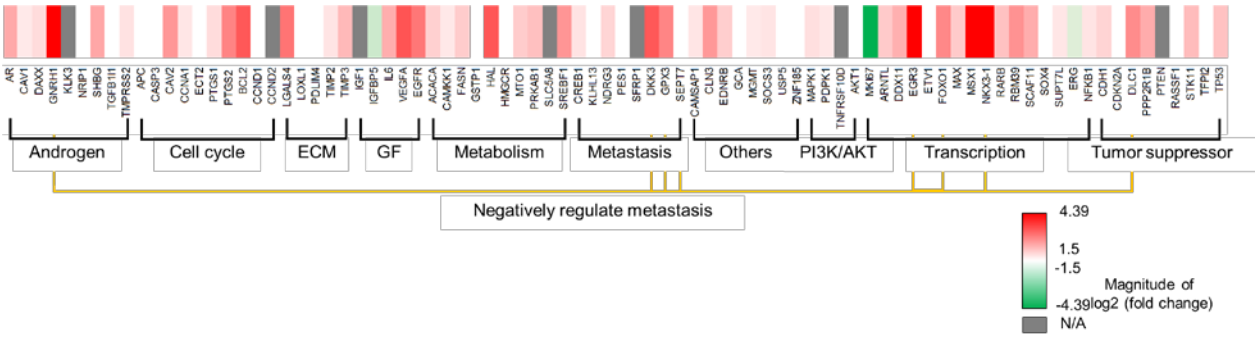

Figure 5.1 Changes of 84 human prostate cancer related genes after ACAT-1 inhibition by avasimibe in PC3 cells. PC3 cells were treated with 5 μM avasimibe for 3 days.

Among these upregulated genes, DKK3 is a negative regulator of Wnt/β-catenin pathway, a major pathway associated with metastasis in prostate cancer. Immunoblotting of β-catenin further confirmed that ACAT-1 inhibition by avasimibe or ACAT-1 knockdown by shRNA significantly downregulated Wnt/β-catenin pathway (**Figure 5.2**). As an independent evidence, immunofluorescent staining of β-catenin showed decrease in the nuclear localized β-catenin after avasimibe treatment, indicating inactivation of Wnt/β-catenin pathway (**Figure 5.3**).

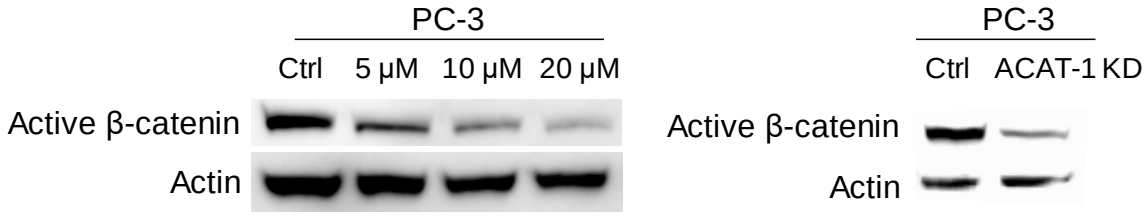

Figure 5.2. Immunoblotting of β-catenin in PC-3 cells with avasimibe or ACAT-1 shRNA. Avasimibe were treated with the indicated concentration for 3 days. ACAT-1 knockdown PC-3 cell line with stable ACAT-1 knockdown was generated by transducing with ACAT-1 shRNA containing lentivirus.

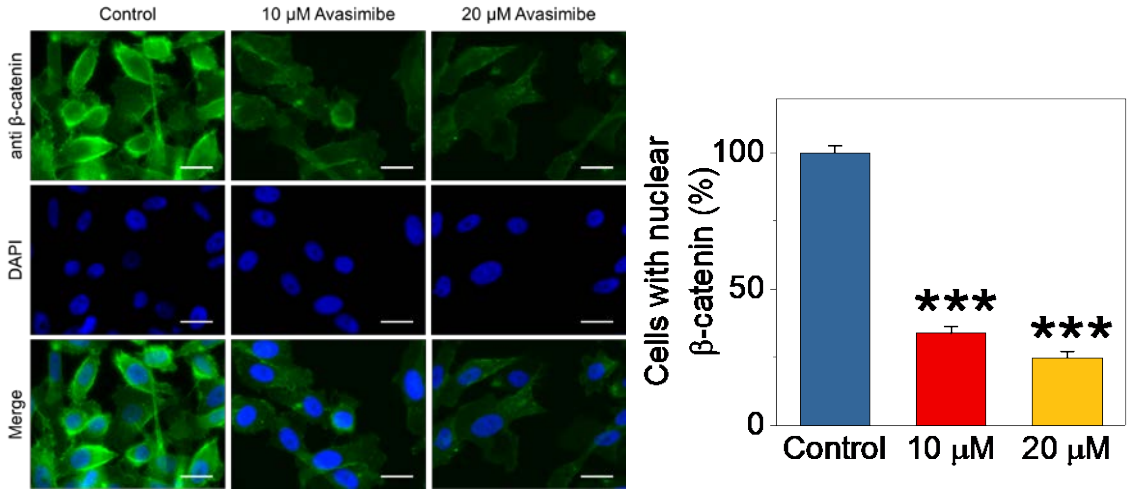

Figure 5.3. Immunofluorescent staining of β-catenin in PC-3 cells with avasimibe. Avasimibe were treated with the indicated concentration for 3 days.

We further studied whether downregulation of β-catenin by inhibition of ACAT-1 is found in other cholesteryl ester-rich prostate cancer cells. We measured β-catenin levels in LNCaP-HP and DU145 PTEN-KD cells with avasimibe treatment. From immunoblotting of β-catenin, we found that β-catenin pathway is also downregulated upon avasimibe treatment (**Figure 5.4**).

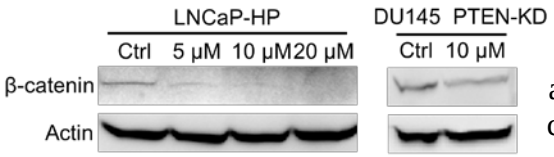

Figure 5.4. Immunoblotting of β-catenin in LNCaP-HP and DU145 PTEN-KD cells with avasimibe. Avasimibe were treated with the indicated concentration for 3 days.

Because β-catenin activation is through Wnt secretion and translocation to membrane, we measured Wnt localization in PCa cells after avasimibe treatment. From immunofluorescent staining of Wnt3a, we found that membrane bound Wnt3a is reduced significantly (**Figure 5.5**). We further measured the level of secreted Wnt3a after avasimibe treatment. From immunoblotting of Wnt3a, we found that intracellular Wnt3a protein level is increased, whereas medium Wnt3a protein level is reduced after avasimibe treatment (**Figure 5.6**). These results indicate that Wnt3a secretion is inhibited by avasimibe treatment.

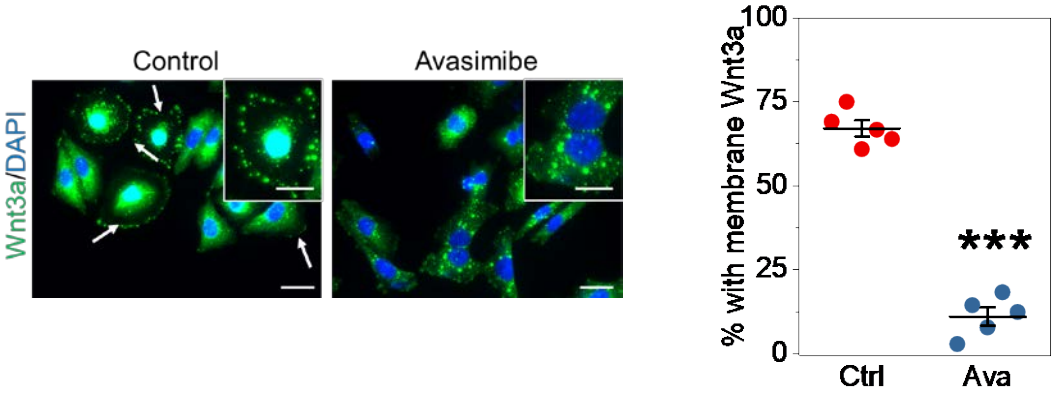

Figure 5.5. Immunofluorescent staining of Wnt3a in DU145 PTEN-KD cells with avasimibe. Cells were treated with 10 μM Avasimibe for 2 days.

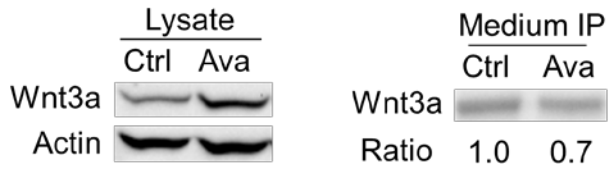

Figure 5.6. Immunoblotting of Wnt3a in DU145 PTEN-KD cells with avasimibe. Cells were treated with 10 μM Avasimibe for 2 days. Medium Wnt3a was immunoprecipitated and normalized by precipitated protein amount.

To test whether Wnt/β-catenin is an essential pathway that links CE accumulation to aggressiveness of PCa, we performed a migration rescue experiment with Wnt3a. CE depletion

by avasimibe significantly suppressed migration capability of PCa. When Wnt3a was supplemented into the medium, migration capability of the cells was rescued significantly (**Figure 5.7**), although not to the full extent (~86% recovery). These results indicate that CE depletion suppresses PCa aggressiveness largely through Wnt/β-catenin pathway.

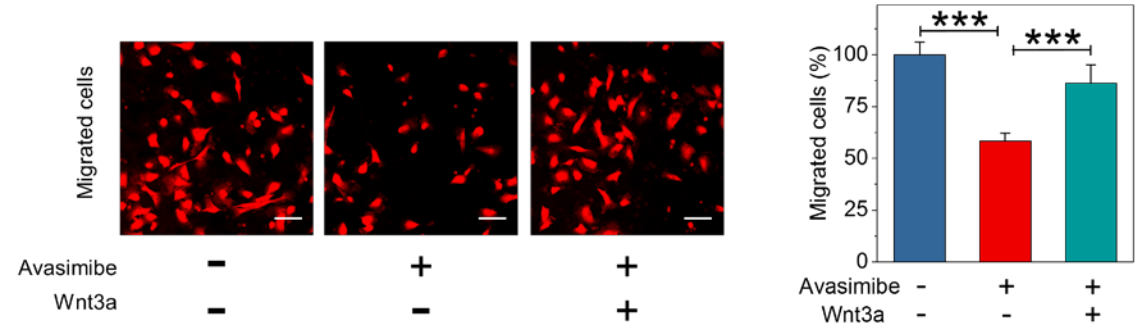

Figure 5.7. Migration of cells pre-treated with avasimibe (10 μM, 2 days) and subsequent Wnt3a supplement (100 ng/mL).

In Wnt/β-catenin signaling pathway, lipid modification of Wnt is a prerequisite for Wnt activation. During the acylation process, fatty acids serve as substrates for this reaction. Considering the crosstalk between regulation of cholesterol and fatty acid metabolisms, we hypothesized that CE depletion limits availability of fatty acid substrates for Wnt acylation. To test our hypothesis, the levels of free fatty acids in prostate cancer were analyzed using LC/MS. Monounsaturated fatty acid (C14:1, C16:1, C18:1) synthesis or uptake in CE-depleted cells was reduced significantly (**Figure 5.8**). These results support our hypothesis that CE depletion reduces free fatty acid availability in prostate cancer.

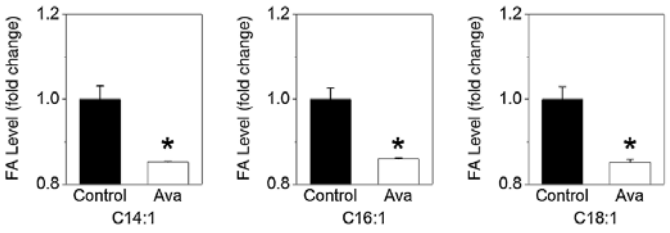

Figure 5.8 LC-MS measurement of monounsaturated fatty acids from lipids extracted from PC-3. Fatty acid levels were normalized by cell number in each group. Error bars, SEM (n = 3). *, P < 0.05.

On the basis of our data, we propose that blocking cholesterol esterification creates imbalance in cancer lipid metabolism that is important for modulating the activities of Wnt/β-catenin pathway (**Figure 5.9**). Under normal condition, excess cholesterol in prostate cancer prostate cancer cells is esterified and stored in lipid droplets by ACAT, and high level of lipid synthesis and/or uptake provides sustained, ample supply of fatty acids (i.e., palmitic acid and palmitoleic acid) for acylation of Wnt protein. Lipid-modified Wnt is secreted and bound to membrane to exert its functions, such as promoting cell migration and invasion. By inhibition of cholesterol esterification, expression and cleavage of Sterol Regulatory Element-binding Protein-1 (SREBP-1) decreased. Consequently, the unavailability of fatty acids inhibited the Wnt acylation and secretion. Unable to bind to the membrane, the inactivation of Wnt/β-catenin signaling suppressed prostate cancer migration and metastasis.

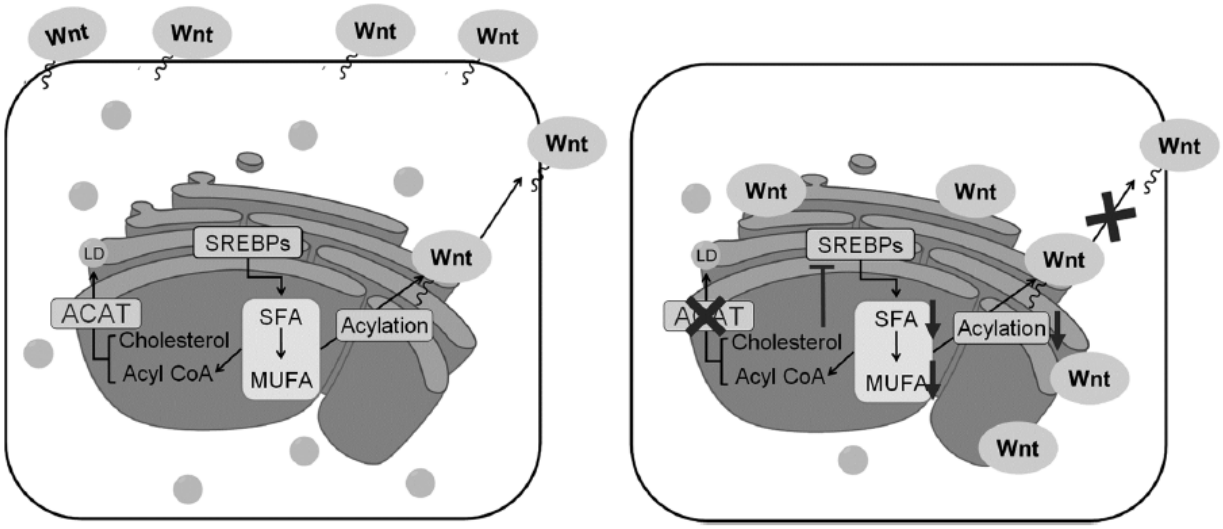

Figure 5.9 Schematic showing the molecular mechanism. SFA, saturated fatty acid; MUFA, unsaturated fatty acid; LD, lipid droplet.

Task 6: Determine the role of cholesteryl ester accumulation/depletion in metastasis in a prostate orthotopic model.

We established a prostate cancer orthotopic mouse model in collaboration with Purdue University Center for Cancer Research. The picture below shows the growth of tumor at the site of prostate at 1 month after cell implantation (**Figure 6.1**). Cancer metastasis was observed from week 4 post-transplantation of PC-3M cells into the prostate of NSG mice (**Figure 6.1**).

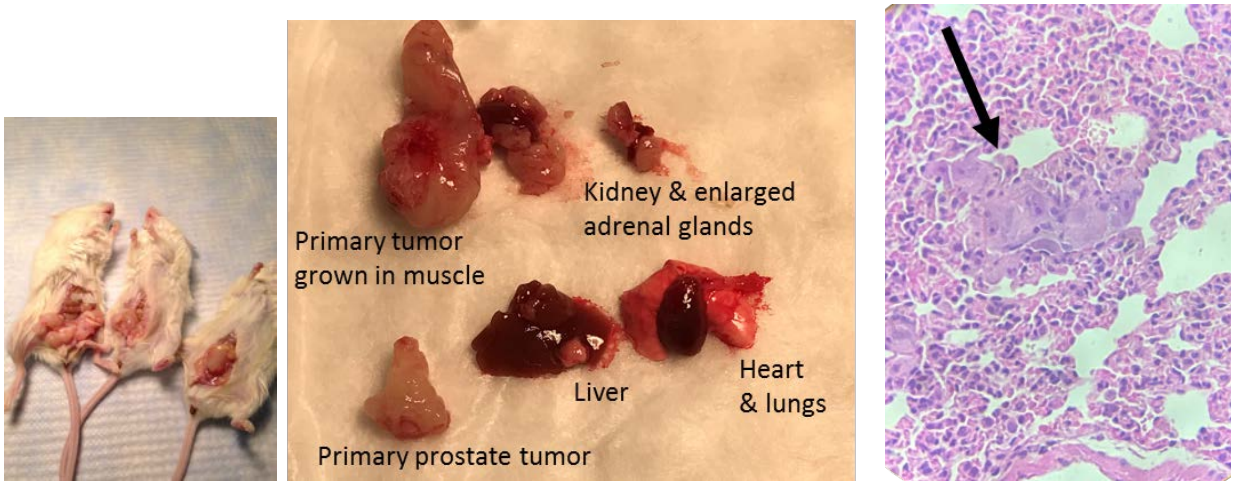

Figure 6.1. Pictures of primary prostate tumor and metastatic tumor on multiple organs. Histological examination of H&E stained tissues verified the development of metastatic tumors.

After establishing the orthotopic model, we treated the mice bearing prostate tumor with Avasimin, a systemically injectable nanoformulation of avasimibe. Avasimin was injected daily (75 mg/kg, containing 7.5 mg/kg avasimibe) via intraperitoneal injection from 10-day post

transplantation. Sterile PBS was used in the vehicle group. Body weight and the primary tumor growth estimation via palpation were recorded twice a week. The tissues were collected at 5-week post implantation to evaluate primary prostate tumor size and lung metastasis (**Figure 6.2**).

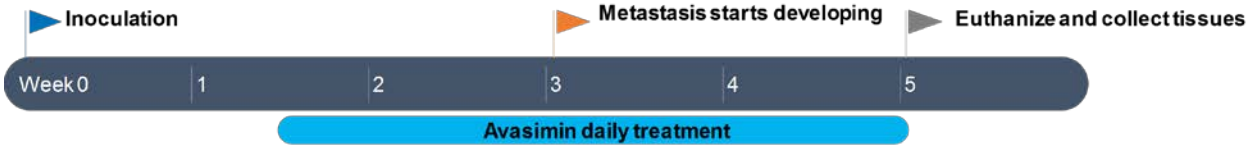

Figure 6.2. Schematic of PCa orthotopic model study.

Avasimin treatment reduced the growth rate of primary tumors significantly, and inhibited the tumor size by ~1.4-fold at the end of 25-day treatment (**Figure 6.3**). Prostate tissues were stained with human mitochondria antibody, which has a high specificity to human cells. Primary prostate tumors were invasive in the control mice, whereas Avasimin treated group showed more confined primary prostate tumor (**Figure 6.4**). Ki-67 staining of the adjacent tissue sections showed lower expression of Ki-67 in Avasimin-treated group compared to control group, indicating that Avasimin slowed proliferation of primary tumor (**Figure 6.4**).

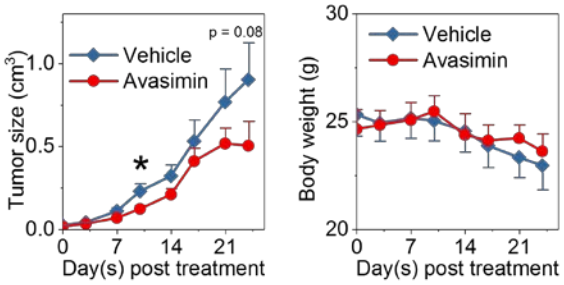

Figure 6.3. Left, primary prostate tumor growth curve estimated by palpation (n = 7 for vehicle; n = 6 for Avasimin). Right, body weight of the mice over 25-day treatments.

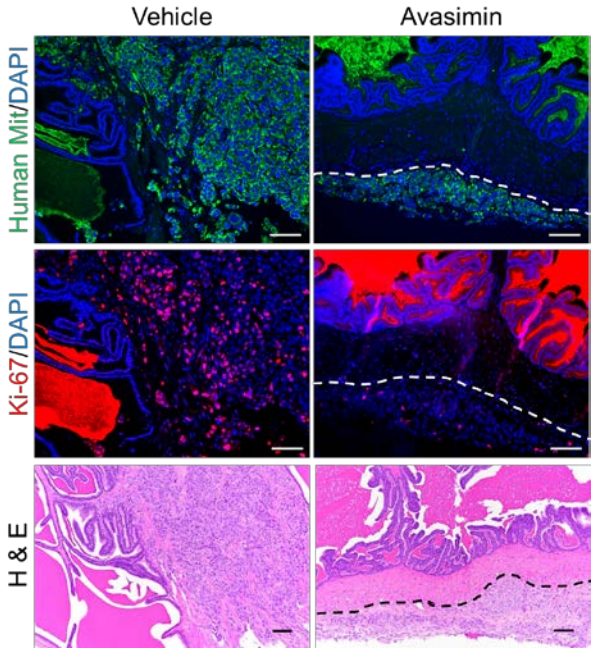

Figure 6.4. Immunofluorescent chemistry (IFC) and H&E staining of primary prostate tumor tissues harvested at the end of the study. Dashed lines indicate clear tumor margins in the vehicle group. Scale bar: 100 µm.

To assess metastasis, lung tissues were stained with the human mitochondria antibody (**Figure 6.5**) and the number of metastatic clusters were counted. Distinct metastasis was defined by a clearly defined cluster of 5 or more cells. The metastatic clusters were counted from the whole

lung sections and 3 – 5 lobes of lung were sectioned and counted for each mouse. Avasimin treatment reduced the number of metastatic clusters significantly (~50% reduction) (**Figure 6.5**). Ki-67 staining of the adjacent tissue sections showed lower level of Ki-67 in Avasimin-treated group compared to control group, which supports that Avasimin reduced proliferation of metastatic tumors (**Figure 6.6**).

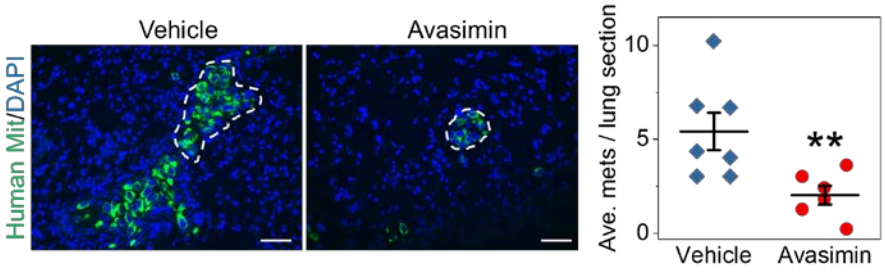

Figure 6.5. IFC staining of lung tissues harvested at the end of the study with distinct metastatic clusters indicated. Scale bar: 50 µm. Quantification of metastatic clusters in lung tissues.

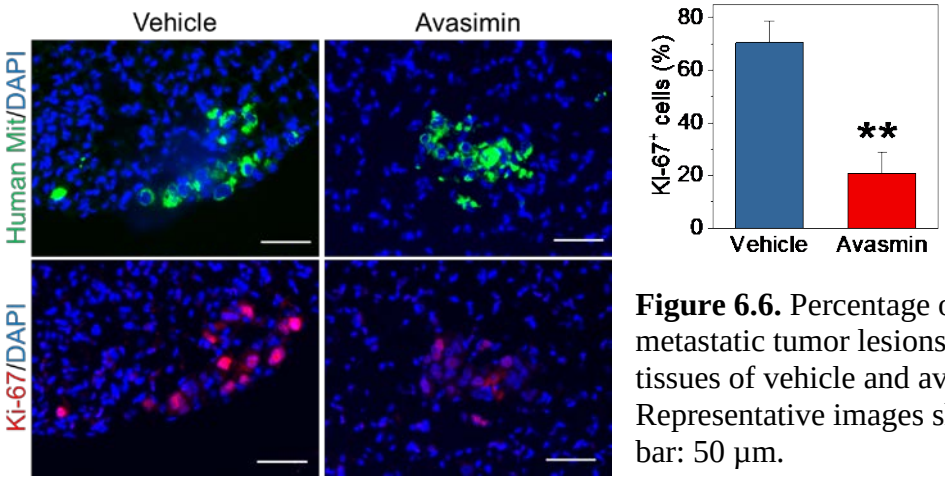

Figure 6.6. Percentage of Ki-67+ cells in metastatic tumor lesions harvested from lung tissues of vehicle and avasimin-treated mice. Representative images shown on the left. Scale bar: 50 µm.

c. **What opportunities for training and professional development has the project provided?**

In total, two postdoctoral fellows (Junjie Li, Jack Li), three PhD students (Seung Young Lee, Hyeon Jeong Lee, Renee E Wenig), and two undergraduate students (Jien Nee Tai, Rui Liu) worked on this project. Seung Young Lee has graduate and is now an assistant professor at University of Illinois at Chicago. Junjie Li co-founded a company Resarci Therapeutic LLC to repurpose avasimibe for cancer treatment. Renee E Wenig has graduated and is now a postdoc at NorthShore University HealthSystem. Hyeon Jeong Lee has graduated and is now a postdoc at Boston University. Rui Liu has graduated and is now in a graduate program at Boston University. Jien Nee Tai has graduated and is now a research associate at Chugai Pharmabody.

d. **How were the results disseminated to communities of interest?**

The results were disseminated to communities of interest through a few invited presentations:

Aug 2015, “Spectroscopic imaging of living systems: emerging platform for biology and medicine”, Eli Lilly, Indianapolis

May 26-28, 2015, Targeting Cancer Metabolism Conference, “Spectroscopic imaging of cancer metabolism at single cell level”, Boston, MA

April 2, 2015, Purdue Center for Cancer Research, “In vivo spectroscopic imaging: emerging platform for biology and medicine”, Purdue University.

09-09-2016, “Lipid metabolism: from single cell biology to in vivo diagnosis”, Big Ten Cancer Research Consortium Summit, Indianapolis, IN.

06-29-2016, “Molecular spectroscopic imaging towards precision medicine”, Cancer Moonshot, Purdue University.

02-28-2018, “Cholesteryl Ester Accumulation Drives Prostate Cancer Aggressiveness and Metastasis”, BU-BMC Cancer Center Retreat, Boston University.

e. **What do you plan to do during the next reporting period to accomplish the goals?**

Nothing to Report.

4. **IMPACT:**

a. **What was the impact on the development of the principal discipline(s) of the project?**

Nothing to report.

b. **What was the impact on other disciplines?**

Nothing to report.

c. **What was the impact on technology transfer?**

US9164084 B2 “A method for determining aggressiveness of a cancer and treatment thereof” Filed 10/20/2015. This IP is based on our finding of cholesterol ester storage in aggressive cancer.

d. **What was the impact on society beyond science and technology?**

Nothing to report.

5. **CHANGES/PROBLEMS:**

a. **Changes in approach and reasons for change**

Nothing to report.

b. **Actual or anticipated problems or delays and actions or plans to resolve them**

Nothing to report.

c. **Changes that had a significant impact on expenditures**

Nothing to report.

d. **Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents**

Nothing to report.

e. **Significant changes in use or care of human subjects**

Nothing to report.

f. **Significant changes in use or care of vertebrate animals.**

Nothing to report.

g. **Significant changes in use of biohazards and/or select agents**

Nothing to report.

6. **PRODUCTS:**

a. **Publications, conference papers, and presentations**
Report only the major publication(s) resulting from the work under this award.

i. **Journal publications.**

Seung-Young Lee, Junjie Li, Jien-Nee Tai, Timothy L. Ratliff, Kinam Park, Ji-Xin Cheng*, "Avasimibe encapsulated in human serum albumin blocks cholesterol esterification for selective cancer treatment", ACS Nano, 2015, 3: 2420-2432.

Acknowledgement of DoD support (yes).

Hyeon Jeong Lee, Jie Li, Renee E Vickman, Junjie Li, Rui Liu, Abigail C Durkes, Bennett D Elzey, Shuhua Yue, Xiaoqi Liu, Timothy L Ratliff, Ji-Xin Cheng. "Cholesterol Esterification Inhibition Suppresses Prostate Cancer Metastasis by Impairing the Wnt/β-catenin Pathway." Molecular Cancer Research, 2018, 16:974-985.

Acknowledgement of DoD support (yes).

ii. **Books or other non-periodical, one-time publications.**

Nothing to report.

iii. **Other publications, conference papers, and presentations.**

Nothing to report.

b. **Website(s) or other Internet site(s)**
Nothing to report.

c. **Technologies or techniques**
Nothing to report.

d. **Inventions, patent applications, and/or licenses**

Based on the albumin formulation of avasimibe, a non-provisional patent was filed through Purdue University, filing date: Sept 10, 2015, application No. 14/850,941

"Cholesteryl Ester-Depleting Nanomedicine for Nontoxic Cancer Chemotherapy", PRF 66947

e. **Other Products**
Nothing to report.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

a. **What individuals have worked on the project?**

Name: Professor Ji-Xin Cheng
Project role: PI
Research Identifier: NA
Nearest person month worked: 1 month
Contribution to project: Dr. Cheng guided the entire project. He had weekly meetings with the students and organized bio-monthly meetings for the entire team.

Name: Professor Xiaoqi Liu
Project role: co-investigator
Research Identifier: NA
Nearest person month worked: 0.5 month
Contribution to project: Experimental design during lunch meetings and regular bio-monthly meetings.

Name: Professor Tim Ratliff
Project role: co-investigator
Research Identifier: NA

Nearest person month worked: 0.5 month
Contribution to project: Experimental design during lunch meetings and regular bio-monthly meetings.

Name: Hyeon Jeong Lee
Project role: graduate student
Research Identifier: NA
Nearest person month worked: 8 months
Contribution to project: Ms. Lee obtained data showing the avasimibe treatment effected reduced the rate of tumor migration and invasion in vitro and in vivo. She further found that avasimibe treatment impacted the activity of the Wnt/beta-catenin pathway.

Name: Renee Wenig
Project role: graduate student
Research Identifier: NA
Nearest person month worked: 1 month
Contribution to project: Ms. Wenig helped Ms. Lee in the study of the Wnt/beta-catenin pathway.

Name: Rui Liu
Project role: undergraduate student
Research Identifier: NA
Nearest person month worked: 1 month
Contribution to project: Ms. Liu helped Ms. Lee in the biochemistry experiments and animal studies. She also maintained the cell culture.

Name: Dr. Junjie Li
Project role: postdoctoral fellow
Research Identifier: NA
Nearest person month worked: 1 month
Contribution to project: Mr. Li helped Ms. Lee in performing the tumor migration assay.

Name: Dr. Jack Li
Project role: postdoctoral fellow
Research Identifier: NA
Nearest person month worked: 1 month
Contribution to project: Dr. Li helped Ms. Lee in performing the western blotting assays and immunofluorescence imaging.

b. **Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?**

Nothing to report.

c. **What other organizations were involved as partners?**

Nothing to report.

8. **SPECIAL REPORTING REQUIREMENTS**

a. **COLLABORATIVE AWARDS: NA**

b. **QUAD CHARTS: NA**

9. **APPENDICES:**

Publications:

ACS Nano, 2015, 3: 2420-2432

Molecular Cancer Research, 2018, 16:974-985