INTERVENTION OF THE MYRISTOYL- PROTEIN BIOSYNTHESIS FOR THE TREATMENT OF PROSTATE CANCER

by

XIAOFEI TAN

(Under the Direction of Houjian Cai)

ABSTRACT

Epidemiological studies about the correlation of dietary fat intake and prostate cancer are very controversial. I hypothesize that metabolism of dietary saturated fatty acids (SFAs) contribute to elevated activity of oncogenic proteins, thereby tumor progression. This thesis analyzes the contribution of dietary SFAs to prostate cancer, and summarizes biosynthetic pathways of how metabolism of SFAs will lead to the elevated levels of myristoyl-proteins. The metabolic processes include that 1) exogenous FAs are transported into cells in a variety of pathways, or endogenous FAs are biosynthesized through *De novo* synthesis or lipid salvage pathways; 2) Biosynthesis of acyl-CoAs catalyzed by Acyl-CoA synthetase Long Chain Family proteins. Finally, a variety of small molecule compounds has been developed to target either the myristoyl-CoA or the Gly-peptide binding sites of N-myristoyltransferase (NMT) to inhibit myristoylation process. The pharmaceutical intervention of NMT activity could be developed as chemotherapeutic approach for the treatment of cancer progression.

INDEX WORDS: Dietary fat, Myristoylation, Fatty Acylation, Src kinase, Fatty acid transporters, N-myristoyltransferase

INTERVENTION OF THE MYRISTOYL- PROTEIN BIOSYNTHESIS FOR THE TREATMENT OF PROSTATE CANCER

by

XIAOFEI TAN

Bachelor of Engineering, University of Georgia, 2017

A Thesis Submitted to the Graduate Faculty of The University of Georgia in Partial Fulfillment

of the Requirements for the Degree

MASTER OF SCIENCE

ATHENS, GEORGIA

2017

INTERVENTION OF THE MYRISTOYL- PROTEIN BIOSYNTHESIS FOR THE TREATMENT OF PROSTATE CANCER

by

XIAOFEI TAN

Major Professor:	Houjian Cai
Committee:	Brian S. Cummings
	Chad Paton

Electronic Version Approved:

Suzanne Barbour

Dean of the Graduate School

The University of Georgia

August 2017

ACKNOWLEDGEMENTS

I would like to thank my devoted advisor, Dr. Cai, without him I would've not finish my master's program and the thesis. His kindness and patience, support and encouragement has been guiding me these years. Your profound inspiration for me not only in academic research, but also in personality and life.

I would like to acknowledge my committee members Dr. Brian S. Cummings and Dr. Chad Paton for their guidance. I am obligated to give my great appreciation to all my lovely lab members, before and now: Qianjin, Sungjin, Yongjie, Lishann, Ru Wen, Omar, Sumit, Faris, Xiaohan, Junyi. I came to the lab as a rookie clueless about pretty much everything. I have been so lucky for having your support, witness, and accompanying in these two wonderful years at the fourth floor of Pharmacy South.

Last but not least, I would like to thank my family. Give my deepest gratitude to my mom and dad for your unconditioned caring and listening. Thank you Yifei for always believing in me, and finally, thank you Rui for showing up in my life and your tireless love.

TABLE OF CONTENTS

LIST OF FIGURES

Page

LIST OF TABLES

Page

LIST OF ABBREVIATIONS

ACP	Acyl carrier protein
AA	Arachidonic acid
ACLY	ATP Citrate Lyase
ACC	Acetyl-CoA carboxylase
ACS	Acyl-CoA synthetase
ACSL	Long-Chain Acyl-CoA Synthetase
BMI	Body mass index
CTD	Control diet
CRPC	Castration-resistant prostate cancer
DHA	Docosahexanoic acid
EPA	Eicosapentanoic acid
ER	Endoplasmic Reticulum
FA	Fatty acid
FABP	Fatty acid binging protein
FAS	Fatty acid synthetase
FATP	Fatty acid transporter protein
HFD	High-fat diet
LPL	Lipoprotein lipase
KD	Knockdown
KO	Knockout
NMT	N-myristoyltransferases
PCa	Prostate cancer
PPAT	Peri-prostatic adipose tissue
PSA	Prostate specific antigen
PLA	Phospholipase A
SFK	Src family kinase
TAG	Triglyceride
WT	Wild type
SREBP	Sterol regulatory element-binding protein

CHAPTER 1

Introduction

1.1 Background of Prostate Cancer and Current Treatments

Prostate cancer is the second leading cause of cancer-related death in men and the most common cancer in men in developed countries [1], accounting for estimated 10.7% of new cancer cases and 4.4% of all cancer deaths in 2016 [2]. The prostate gland lies in front of the rectum and below the bladder[3]. The primary function of the prostate is to produce a secretion which forms a part of seminal fluid. The secretion is essential to the survival and mobility of sperm. Prostate specific antigen (PSA) is produced in the prostate. Both benign and malignant growth of the prostate can put pressure on and destroy the glandular tissue which can lead to an increased leakage of PSA into the bloodstream [4]. Although the value of PSA does not precisely reflect prostate pathological status, a high PSA value in the blood stream is currently the most common indicator of prostate cancer.

Prostate cancer begins with abnormal epithelial proliferation and prostatic intraepithelial neoplasia (Figure 1). Most tumors begin to form in proximity to the outer surfaces of a prostate gland. Some tumors can grow to a volume where they start to obstruct urination, progression to invasive carcinoma and metastases mainly in the skeleton(Figure 2). Aggressive tumors can eventually break the capsule and infiltrate nearby organs. If tumor cells separate and enter the blood stream, prostate cancer may spread to other parts of the body. Prostate cancer progression despite androgen-deprivation therapy, a rise in serum levels of PSA, or appearance of new

metastases. Eventually, the stage called castration-resistant prostate cancer (CRPC) is PCa progression despite androgen-deprivation therapy, with a rise in serum levels of PSA, or appearance of new metastases [5]. 90% of men with CRPC will develop bone metastases which can produce significant morbidity [6]. The exact triggers of prostate primary and metastatic cancer are complex and still under investigation. The incidence of prostate cancer increases exponentially with age [7]. Approximately 80% of men above the age of 70 have prostate cancer. Additionally, diet, family history, lifestyle, and other factors also affect prostate cancer. Many researchers have been demonstrated that high-fat diet accelerates cancer progression [8-11]. Therefore, an investigation of how statured fatty acids, especially myristic acid, play a role in prostate cancer progression is the theme of this thesis.

The median age at diagnosis of carcinoma prostate carcinoma is 66 years [12]. In the United States, the 5-year relative survival rate for men diagnosed from 2007 to 2013 with the local or regional disease was 100%, and the rate for distant disease drops to 29.8%; a 98.6% survival rate was observed for all stages combined [13]. For the low grade of prostate cancer, surgical remove prostate gland and some tissue around it is always the best option to treat prostate carcinoma. Radiation is also as the first treatment for cancer that is still just in the prostate gland. Besides, radiation is applied if the cancer is not removed completely or recurs in the area of the prostate after surgery [14]. For local or regional prostate cancers that have grown outside the prostate gland and into nearby tissues, hormone therapy along with radiation may be used [15]. Chemotherapy is applied if the disease is not get controlled by hormone therapy. Docetaxel, Cabazitaxel, Mitoxantrone, Estramustine are the typical prostate cancer chemo drugs. Enzalutamide is used for the treatment of metastatic, castration-resistant prostate cancer [16].

CRPC is the primary cause of prostate cancer death[17]. The treatment of prostate cancer remains difficult because patients are resistant to current chemotherapeutic agents; therefore, it is important to continue to search for cellular functions that can result in the inhibition of the malignant growth of prostate cells and progression of prostate cancer.

1.2 Difference of dietary contents between Western and Eastern countries is correlated with the risk of prostate cancer

As we consider, the prostate cancer incidence is much higher in Western countries compared with Asian countries (Figure 3). For example, the PC rate in the US is nine times higher than that in Japan[18]. However, an epidemiological study shows that prostate cancer incidence is higher among Japanese immigrants to the United States than that in Japan (Figure 4), suggesting a potential connection of the alteration of lifestyle including the diet with prostate cancer [19].

Western diets contain a high intake of fat and several FAs (Table 1). The standard American diet is about 50% carbohydrate, 15% protein, and 35% fat [20]. The characteristics of a typical American diet can often be described as high in sugar and saturated fats, including foods like nachos, bacon, steak, pizza, burgers, and fries. In contrast, the Asian diet, while varying between the many different countries, can mostly be described as centered around plants and vegetables, rice and grains, seafood, poultry, and fruit. The average Japanese diet is about 60% carbohydrate, 15% protein, and 25% fat [21] which indicates the lower total fat and lower saturated FAs. For example, a traditional Japanese meal includes fish, such as salmon or mackerel, simmered vegetables, miso soup, rice and green tea.

The study showed that consumption of beef meat and high intake of animal fat and dairy products increased PC risk [22]. Fourteen or more vegetables and seven to fourteen fruit servings

per week decreased PC risk by 96% and 98%, respectively [22].The Japanese fish consumption is twice as Americans, which suggests that the Japanese have a higher unsaturated FAs intake than Americans. Most Japanese meals are served with soy, in the form of tofu and edamame, vegetables such as ginger, eggplant, cabbage, broccoli, cauliflower, and kale, or seaweed including nori and wakame. These diet components contain a significantly low amount of dietary fat. As a result, the dietary components might directly lead to a fact that Japanese obesity rate is the lowest in the developed world -- 3% in Japan versus 32% in the US [23].

1.3 The relative risks of prostate cancer imparted by Obesity

The consumption of dietary content reflects obesity rate. Obesity has been well known as a high-risk group for type 2 diabetes and cardiovascular disease [24]. There is a growing consensus that obesity has a significant association with risk of many cancers in observational studies [25], including prostate cancer which accounts for almost one-third of cancer-related deaths. However, there is no increase prostate cancer risk with a higher BMI as listed in Table 2. Many studies have been performed over the past 30 years that investigate the relationship between cancer and obesity by measuring body mass index (BMI). These results show that higher adult BMI is associated with increased risk of prostate, pancreatic, liver ovarian, endometrial, lung, esophagus and colorectal cancer (Table 3). The positive correlation of BMI with cancer risk suggests lipid deposition contributes to tumor initiation and progression. Moreover, obesity is also correlated with the progression of higher grade disease and poorer outcomes of many cancers [26].

Table 2 is a summary of the association between body mass index and prostate cancer risk. The results of studies on the correlation between body fat and prostate cancer risk are controversial

[27-35]. One study showed that obese men had a 47 % increased rate of overall mortality compared to normal weight men (risk ratio = 1.47, 95 % CI 1.03–2.10) [34]. Compared to maintaining a stable weight, a weight loss >5 % or a weight gain >5 % after diagnosis almost doubled the rate of mortality (risk ratio = 1.94, 1.93 respectively) [34]. Moreover, another study showed that obesity reduces the risk of low-grade (Gleason <7) prostate cancer (risk ratio = 0.74; P = 0.001) and increased the risk of high-grade (Gleason ≥7) PCa (risk ratio = 1.28; P-value =0.042) [28]. However, a study showed that for men over 60 years old, prostate cancer risk for obese men (BMI ≥30 kg/m^2) was lower than that in men with a lower BMI (23-24.9 kg/m^2) (risk ratio = 0.52, 95% CI = 0.33, 0.83; P-value <.001) or had a family history of prostate cancer (risk ratio = 0.74, 95% CI: 0.45, 1.19; P-value =0.01) [31]. Additionally, a study showed for white males over 40 years, BMI and prostate cancer death provide a J-shaped relationship (risk ratio: 1.041, 95% CI:1.007, 1.077; P-value =0.04) [30]. Nilsen et al [36] reported no association between body mass index and prostate cancer incidence(risk ratio = 1.2; 95% CI 0.9, 1.6).

One explanation for these observations is that obesity is associated with lower PSA [37] and testosterone[38] levels, which could lead to confounding the detection and analysis of cancers [28]. Another reason may be that obesity is involved in the progression of prostate cancer rather than initiation [39]. In conclusion, obesity could be a risk factor for high-grade PCa.

Obesity increases the incidence of advanced prostate cancer by 14%, increases recurrence after castration by 21%, and increases prostate cancer-specific death by 15% compared to men who are not obese[40]. Molecular biological mechanisms of how obesity is related to prostate cancer are very complex. A study shows that the human peri-prostatic adipose tissue (PPAT) does not accelerate prostate tumor growth. Mice tumor cell proliferation was similar in co-grafted patient- matched PPAT with prostate cancer tissues and grafted prostate cancer tissues. Only

after artificially altering the expression of prostate cancer oncogene or suppressor gene (e.g. TRAMP, Hi-Myc, Pten−/+), does diet-induced obesity promote prostate cancer progression in mice within xenografts derived from PC3 or LNCaP cells [40]. This study suggests that obesity promotes high-grade, aggressive prostate cancer while engrafted moderate-grade prostate cancer tissues were not different between the LFD and HFD mice groups [40]. Moreover, prostate epithelial cells (BPH-1) elongated and were more motile when treated with PPAT conditioned media which indicated that factors secreted from PPAT accelerated tumorigenesis in cultured prostate epithelial cells. Therefore, the general consensus is that obesity is associated with high-grade or aggressive prostate cancer [41], and might have weak or no association with moderate-grade prostate cancers [40].

1.4 High-fat diet and fatty acid in prostate cancer progression

The strong association between national rate of prostate cancer mortality and national consumption of dietary fat indicates that high-fat diet plays a significant role in prostate cancer progression [42]. Epidemiologic studies showed that high-fat consumption as a potential cause of this disease, especially animal fat is directly related to the risk of advanced prostate cancer [43-45]. One evidence is that diet can influence endogenous androgen levels. Androgens especially dihydrotestosterone (DHT) control prostate cells growth [45]. Another evident is that dietary FAs accelerated prostate cell growth by protein acylation. Especially myristoylation is an essential anchor for some oncogenic proteins to localize at the cell membrane, thereby carrying out the molecular functions of these oncogenes in cancer cells [46].

Some studies suggest that the associations of fatty acids depend on prostate cancer severity.

Saturated fat intake increases the risk of advanced prostate cancer (highest vs. lowest quintile HR 1.21) and fatal prostate cancer (HR 1.47) [47]. Specifically, levels of myristic acid in the blood were associated with higher prostate cancer incidence (Table 4), increasing rate ratios almost twofold (odds ratio, 1.93; 95% confidence interval, 1.02–3.64) [48]. Also, higher prostate cancer risk with increased serum palmitic acid level (OR=1.53, 95% CI = 1.07 - 2.20)[49]. However, the results of studies on the association between saturated fatty acid and prostate cancer risk are not constant. Most previous studies have found no association with levels of myristic, palmitic [50, 51]or stearic acids [52]and prostate cancer risk [53-55]. This controversial and gap in knowledge do need further investigation, while I believe that greater prostate cancer risk with elevated saturate fatty acid levels. The positive association between monounsaturated FAs such as palmitoleic acid and oleic acid levels and prostate cancer is relatively consistent with almost all studies report [53]. Lower blood levels of MUFAs in the present study are associated with lower risk (Table 4). Moreover, most studies are in agreement with that eicosapentaenoic acid (20:5, EPA) and docosahexaenoic acid (22:6, DHA) inhibited prostate cancer cell proliferation, whereas the n-6 fatty acids linoleic acid (LA) and arachidonic acid (AA) stimulated proliferation [56]. Whereas an exception has been reported that the modest associations of eicosapentaenoic (OR = 1.14, 95% CI = 1.01 - 1.29) , and docosapentaenoic acid(OR = 1.16, 95% CI = 1.02 - 1.33) with prostate cancer[57]. There were no significant associations between prostate cancer risk and fat from red meat, dairy products, and fish[54].

CHAPTER 2

Biogenesis of Myristoyl-CoA

2.1 Diet as the exogenous source of myristic acid

Typically, 35% of kcals of the consumed diet comes from fat in the US diet, and 95% of dietary fat is triglycerides. The bulk of dietary fat will enter the adipocytes as triglyceride storage. Some dietary fat will be taken up by muscles for energy needs, and some will be taken up by the liver.

Myristic acid is a 14-carbon saturated fatty acid (SFA)[58]. Dietary food is the major source of myristate. Myristic acid exists as glycerol ester, in most animal and vegetable fats and oils. In animal fats, such as meat, eggs, milk, shellfish, and eel, it is present in small quantities [59], about 1.4 g/100 g. Butter and coconut oil are the exception: roughly 8.3 g/100 g in butter and 17 g/100 g in coconut oil. In contrast that the average myristate intake of Japanese is about 1 g/d, American is about 6 g/d [60]. The uptake and metabolism of myristate by cells are more rapidly than palmitate [59]. Moreover, the cholesterol-raising potency of myristic acid is 1.5 times higher than palmitic acid [61]. Our body first removes the glycerol from the fat and gets free fatty acids and monoglyceride by lipases, which are activated hormonally. The hormones, like insulin and adrenaline, bind to the receptor on a cell, causing a signaling process to occur inside the cell that results in the lipases being activated. Large amounts of free FAs circulate in plasma to provide fuel for ATP sources, substrates for more complex lipids or to participate in signaling pathways. FFA's concentration in blood is from about 100 μM to more than 1 mM, and almost all FFA are bound to serum albumin. The pool of circulating free FAs

contains over 40 different FA molecules [62].

2.2 Transportation of exogenous fatty acids

FAs must activate through covalent modification by CoA via fatty acyl-CoA synthetases in order to be further esterified or incorporated into membranes, or oxidized to CO_2 as an energy source, or signaling lipids[63]. Another reason is that fatty acids are toxic for cells since they act like detergents which may denature proteins. Cells take fatty acids and link them to CoA rapidly and keep fatty acid from becoming detergent. The mechanism of cellular long-chain fatty acid uptake is putative [64].

There are five pathways having been reported to transport FAs across the plasma membrane (Figure 5). 1) Fatty acids could dissociate with albumin and cross the plasma membrane by passive diffusion. 2) $FABP_{pm}$ is a peripheral membrane protein which could act alone or together with CD36, facilitate FAs translocate into cells. CD36 is a transmembrane protein, also known as fatty acid translocase (FAT). 3) CD36 itself could also mediate FAs transport cross the lipid bilayer. Once FAs get into the cells, FAs are bound with $FABP_c$. The extracellular albumin and intracellular $FABP_c$ work the same way in the blood circulation and cytoplasm, respectively. 4) Fatty acid transporter protein (FATP) belongs to Acyl-CoA synthetase (ACS) superfamily which can transport VLC-FAs and converts then directly into fatty acyl-CoA. 5) A minority of FAs are thought to be transported by FATP1 and rapidly activated by plasma membrane ACS1 to form acyl-Co esters. A prevalent view is that protein-mediated uptake is dominant at physiological concentrations of FAs and that simple diffusion becomes important at higher, nonphysiological concentrations of FAs [65].

2.2.1 Roles of CD36

CD36 (cluster of differentiation 36), have been found on the surface of many cell types, especially in the heart and skeletal muscle cells. It imports fatty acids inside cells which also has an effect on fatty acid metabolism both under basal conditions and during metabolic challenges [66]. CD36 null mice reduce fatty acid uptake 50% to 80% in heart and 40% to 75% in skeletal muscle. While oxidation-enhancing agent stimulation of fatty acid uptake was also markedly impaired in CD36 KO mice, wild type (WT) mice have 7.5-fold higher FAs uptake than knock out (KO) mice in myocytes; and 6-fold higher in skeletal muscle. Moreover, insulin stimulation of fatty acid uptake was three times less in the muscle of CD36 null mice compared with WT mice (Table 5) [66]. These impair in fatty acid uptake also contributed to altered rates of fatty acid metabolism.

Another study showed that CD36 is amplified in metastasis in most types of tumors and CD36+ cells require fatty acid to promote metastasis [67]. They monitored tumors from wild type and CD36 null mice fed either with a high-fat diet (HFD) or control diet (CTD). HFD promote CD36+ cell metastasis tumors and have no effect on primary tumors. CD36 KO mice almost complete inhibit metastasis tumors and have no effect on primary tumors, either. Moreover, Thrombospondin which is a secreted glycoprotein, TSP-1 and TSP-2 have also been known as the anti-angiogenic molecules [68]. Interestingly, the inhibition of TSP-1 or CD36 inhibits anti-angiogenesis and metastasis [69] and also suppresses the migration of LNCaP cells [70], suggesting that the CD36 is responsible for the TSP-1-induced migration. By binding to CD36, TSP-2 could induce human PCa cells migration as well [71].

2.2.2 Roles of plasma membrane fatty acid binding protein ($FABP_{pm}$)

$FABP_{pm}$ is a 40-43 kDa plasma membrane protein and located at outer leaflet of the phospholipid bilayer. In mammalian tissue, $FABP_{pm}$ transports long-chain saturated and unsaturated fatty acids cross the plasma membrane [72]. Several studies demonstrate that $FABP_{pm}$ inhibition reduced fatty acids uptake by 50% to 75% in adipose, liver, jejune, heart and skeletal-muscle cells which indicated the contribution of $FABP_{pm}$ to the overall fatty acid uptake [64]. Upregulation of $FABP_{pm}$ could increase FA oxidation instead of store as triacylglycerol[73]. However, $FABP_{pm}$ overexpression (+173%) only increases the rate of palmitate by 79%, which suggest FABPpm alone has limited capacity.

FABP1 (also known as liver-FABP)is mainly expressed in the liver and intestine, is the only isoform that binds both FA and fatty acyl-CoA; the other FABP isoforms bind only FA [74]. Although the loss of FABP1 reduces FA binding capacity in the liver, total liver lipid content, including TAG and free FA, is still unchanged (Table 6) [75]. Liver-FABP and intestine-FABP were upregulated (5–9- fold) in DU145 prostate cancer cell line in vitro, whereas adipose-FABP, epidermal-FABP, and heart-FABP were down-regulated (3–20-fold) in cancer cells when compared to normal cells in tissue cultures and biopsy samples [76]. Block the expression of epidermal-FABP induced proliferation in DU 145 cells[76]. It is believed that adipose-FABP is present in normal cells and prostate cancer DU145 cells lack expression of adipose-FABP. Another study showed that induction of adipose-FABP blocked the growth of DU145 cells suggesting its role as a tumor suppressor [77]. Liver-FABP protein was secreted from DU 145 cells, blocking the expression of Liver-FABP resulted in remarkable effects on apoptosis and cell proliferation of prostate cancer cell lines[78].

The heart-type FABP3 is most abundantly expressed in the heart, skeletal muscle, and the brain. FABP3-deficient mice have defective FA oxidation and more reliant on glucose for the energy source in both heart and muscle (Table 6). FABP3 KO mice are extremely cold intolerant [79]. Disruption of the FABP4 (adipose-FABP)gene in mice increases the content of free FA in the cytosol, FABP4 is generally thought to facilitate FA transport between intracellular compartments for storage or export [80].

2.2.3 Roles of fatty acid transport protein (FATP)

FATPs have six isomers each with a tissue-specific distribution (Table 5). Only FATP1 and FATP5 are present on the plasma membrane; others are located in the mitochondria or ER and peroxisomal membranes [81]. FATP1 is highly expressed in brain, heart, skeletal muscle and adipocytes[82]. Transgenic overexpression of FATP1 by 8-fold in the mice heart increased the FA uptake rate by 4-fold[83]. Although in the FATP1 KO mice, the basal rate of FA uptake had no change in the adipocytes and skeletal muscle[84], insulin-stimulated FA uptake was significantly decreased, and the mice were cold intolerant[81]. FATP5 is prominent in hepatocytes. Ablation of FATP5 reduced 50% FA uptake and contained 48% less TAG [85], but almost three times more phosphatidylserine in the liver. FATP5-null mice also show a defective bile acid conjugation and decreased food intake which could protect from diet-induced obesity and non-alcoholic fatty liver disease [86].

2.3 De Novo Synthesis of myristic acid

Acetyl Coenzyme A(acetyl-CoA) is the building block of fatty acids and is converted by pyruvate in the TCA cycle (Figure 6). When the acetyl-CoA in the mitochondria starts to

accumulate, FA de nove synthesis starts with high levels of citrate and ATP. The synthesis of FAs begins with the irreversible carboxylation of acetyl-CoA which generates malonyl-CoA by transferring CO_2 from biotin to the acyl-CoA molecule. The enzyme that catalyzes and commits acetyl groups to FA is acetyl-CoA carboxylase. Fatty acid synthetase (FASN) is a single polypeptide chain and contains seven different catalytic domains that catalyze the assembly of fatty acid chains via fatty acid synthase. In the loading step, acetyl-CoA and malonyl-CoA are attached to acyl carrier protein (ACP). Then, both acetyl-CoA and malonyl-CoA are condensed by fatty acid synthase to from acetoacetyl-ACP. The third step is reduction through which NADPH is oxidized to form hydroxybutyryl ACP. Next is dehydration which removes a hydroxyl group to form a double bond, thereby forming a crotonyl attached to ACP and releasing an H_2O. Then the second reduction step is to form butyryl-ACP, NADPH as a source of e- and H+. The last four steps are repeated six more times with malonyl-ACP to elongate the chain until palmitate is produced.

2.3.1 FA de novo synthesis in cancer

Normal cells rely on complete oxidation in the presence of oxygen as energy support, while most cancer cells rely on anaerobic oxidation and cause lactose accumulation. In healthy adult humans, FA de novo synthesis mainly happens in cells with high lipid metabolisms like adipocytes, hepatocytes, sebaceous glands, and in hormone-sensitive cells like breast, endometrium, prostate, and adrenal cortex [87]. However, overexpression of lipogenic enzyme FASN is also found in aggressive tumors and fetal tissues to proliferate epithelial cells [88] indicates that FA synthesis in cancer cells almost reach embryonic state. The predominant product of enhanced FASN activity is palmitic acid[89]. Moreover, early studies showed that FA

and lipid synthesis rate in tumors at a rate is similar to the liver[90] to meet large lipid needs of a rapidly growing. Blood levels of specific fatty acids could reflect the activity of De novo lipogenesis which has been implicated in prostate cancer [53].

Usually, normal cells uptake FA from exogenous sources, and cancer cells are more dependent on de novo FA synthesis [89]. However, studies show that advanced cancer cells and those cancer cells surviving chemotherapy consume exogenous FA as well. On account of the differences in FA uptake and consumption between normal cells and cancer cells, targeting FA metabolism might be specific and selective for uncontrolled cancer cells[91].

Upregulation of lipogenic enzymes, such as FASN, ATP Citrate Lyase (ACLY) and ACC couple with phenotypic alteration in a variety of tumors and always represent a poor prognosis in cancer patients [92]. Inhibiting ACC expression leads to a major decrease in the cellular palmitic acid and apoptosis induction[93]. FASN or ACLY inhibitors can attenuate prostate cancer cell proliferation only in the absence of lipoproteins. Knockdown or chemical inhibition of ACLY prevents lipid formation by glucose metabolism. This alteration further reduces murine tumor proliferation and stops xenograft tumorigenesis of human cancer cells. Studies have shown that downregulation of FASN concomitantly reduces TG and phospholipid levels thereby inhibiting cell growth and promoting apoptosis in LNCaP cells[94]. Cancer cells have been demonstrated to be selectively killed by small molecule inhibitors of FASN, as most cancer cells have been found to rely on de novo FA synthesis mediated by FASN [87]. The first FASN inhibitor in clinical trials is TVB-2640 [95]. Phase I clinical trial has been involved patients with non-small long cancer, breast cancer, and ovarian cancer, phase II clinical trial will open later this year for HER2-positive breast cancer. The dose-limiting side effects are skin and subcutaneous disorder,

especially hand-foot syndrome, as well as eye toxicity including iritis, corneal edema, and keratitis [96].

Unlike palmitate, myristate is not abundant in our bodies, making it difficult to track the De Novo Synthesis of myristic acid. A study showed that treatment with palmitic acid (C16:0) resulted in a slight increase of the C14:0 CoA and smaller increases of other shorter acyl-CoAs in HepG2 cells [97].The increases are possibly due to degradation by β-oxidation of the synthesized acyl-CoA inside the mitochondria, which yields an acetyl-CoA and a fatty acyl-CoA two carbons shorter after each cycle. Likewise, treatment with C12:0 resulted in increases of the C14:0 CoA by elongation of acyl-CoAs which demonstrates that humans can either shorten longer fatty acids or elongate laurate (12:0) into myristate.

Another study has been performed in African Trypanosomes which causes human sleeping sickness. This parasite demands huge amounts of myristate as a protein anchor for their surface glycoproteins[98]. This study initiated myristate biosynthesis in the cell-free system by adding a combination of butyryl-CoA, NADPH, and [14C] malonyl-CoA as a radiolabel. As a result, [14C] myristoylated protein was expected. Then [3H] caprylate (8:0) and [3H] laurate (12:0) as metabolic precursors were used for fatty acid synthesis and similarly labeled proteins were also observed. In contrast, [^{3}H] palmitate (16: 0) did not label proteins which mean Trypanosomes cannot shorten longer fatty acids by oxidation. The sources of myristate could be an elongation of laurate or caprylate, and de nove synthesis fatty acids stop at myristic acid which provides us a hypothesis that myristate could also be the final product of de nove synthesis in humans.

2.3.2 Upstream regulators (sterol regulatory element binding proteins) of endogenous FA biosynthesis

Sterol regulatory element-binding protein (SREBP-1) is a transcription factor which regulates FA synthesis gene expression. Inhibiting SREBP-1 in cancer cells could reduce the enzyme transcription level of FA synthesis including ACLY, ACC, FAS, SCD-1, and GPAT, thereby reducing cancer cell growth [99]. Higher levels of SREBP-1 and SREBP-2 have been demonstrated in the process of prostate cancer progression to androgen independence [100]. Chemical inhibition of SREBP shows a down-regulation of SREBP-1 and SREBP-2 target genes, while significantly limiting cell proliferation has been reported in multiple cancer cell lines [101].

2.4 Biogenesis of myristic acid from lipid salvage pathways

The major source of myristic acid is De Nove synthesis and Diet. Besides, lipid salvage pathway may slightly contribute to the biogenesis of myristic acid.

2.4.1 Triglyceride lipid droplet

When dietary fats enter the small intestine, lipases break down the FAs, our body will repackage the redundant FAs as triglyceride and storage in lipid droplets (LDs) which are organelles and comprised of a neutral lipid [102]. Triglycerides constantly enter the adipocyte in the fed state via triglyceride rich lipoproteins which could be either chylomicrons or VLDL. Lipoprotein lipase (LPL) breaks down triglycerides and allows them to be taken up by the receptors and fatty acid transporters. Some fatty acids are then restored as triglycerides inside the adipocyte lipid droplets. When FAs are needed, hormone-sensitive lipases break down the triglyceride

producing FAs which are then released into circulation and are able to be oxidized or associated with certain proteins[103].

2.4.2 Phospholipid

A phospholipid is one of the common lipids in our body, and it is the building block of the cell membrane. Phospholipases (PLC, PLD, and PLA) are critical mediators of cell signaling [104]. They can generate many bioactive lipid mediators, such as diacylglycerol, phosphatidic acid, lysophosphatidic acid and arachidonic acid. These lipid mediators can promote tumorigenesis, including proliferation, migration, invasion, and angiogenesis.

Phospholipase A1 (PLA1) hydrolyze phospholipids into saturated FAs and a lysophospholipid by cleaving fatty acyl ester bonds at the sn-1 sites of the glycerol backbone [105]. Then saturated FAs can undergo further post-translational modification or other metabolic pathways (Figure 7). PLA1 has not been well investigated and may relate to cancer. However, many PLA1 enzymes have some neutral lipase activity like hydrolyzing triacylglycerol in the sn-1 cleavage site. Several PLA1 sequences exhibit sequence similarities to the pancreatic, hepatic, and endothelial lipases [106]. Other PLA1 sequences have no similarity to lipase.

2.5 Conversion to myristoyl-CoA by Acyl-CoA synthetases

Acyl-CoA synthetases(ACS) catalyzes the conversion of FA to an acyl-CoA, which is required for exogenous and endogenous FA to enter lipid bioactive pool and participate in multiple metabolic pathways [97]. Between birth and adulthood, ACS activity in rat liver increases sevenfold [107] and long-chain acyl-CoA synthetase (ACSL) activity in mouse heart increases more than tenfold [108]. There are five Long-Chain Acyl-CoA Synthetase isoforms, ACSL1,

ACSL3, ACSL4, ACSL5, and ACSL6. Different isoforms have different substrate specificity or preference for endogenous versus dietary FA [81].

ACSL1 is highly expressed in liver, heart, adipose tissues, and skeletal muscle [109]. Studies showed ACSL1 specifically esterifies 18:0 FA to 18:0-CoA[110]. From birth to adulthood, ACSL1 mRNA in mouse increases 2.5-fold[108]. In liver, ACSL1 upregulates with fasting or high-fat diets and downregulated with high-sucrose diets[111]. In the KO mice, total ACSL activity decreases 50% in liver, also hepatic acyl-CoA content decreases 25–35% [110]. In heart[108] and white or brown adipose [112], KO of ACSL1 decreases 80–90% total ACSL activity, concomitantly with profound decreases in FA oxidation which strongly suggest that ACSL1 plays a primary role in channeling toward β-oxidation. Overexpression ACSL1 in macrophages increases 20:4ω6 metabolism and promotes inflammation and atherosclerosis [113].

ACSL3 is expressed on lipid droplets and Endoplasmic Reticulum(ER) in most tissues [114]. Studies showed ACSL3 specifically contributes to the incorporation of 10:0-CoA, 20:4-CoA and 20:5-CoA. Knockdown ACSL3 diminishes de novo FA synthesis from [14C] acetate into lipid extracts [115]. Knockout ACSL3 is lethal to mice because FAs are ligands for the Peroxisome proliferator-activated receptors (PPARs) which are transcription factors of nuclear hormone receptor superfamily[116]. The absence of acyl-CoA enhances the activity of PPAR and increases transcription of PPAR target genes [81] which suggests that ACSL3 could mediate hepatic lipogenesis by controlling transcription.

ACSL4 is prominently expressed in brain, also highly expressed in the adrenal gland, ovary, and testis. The preference of ACSL4 is polyunsaturated FAs, such as 20:4ω6 and 20:5ω3 [117]. Lack of ACSL4 in neural development by mutations could impair the transport and function of synaptic vesicle and accumulate axonal aggregates, thereby cause X-linked mental retardation [118]. ACSL4 especially regulates eicosanoid metabolism, overexpression ACSL4 significantly increases the synthesis of 20:4ω6-CoA and its incorporation into phospholipid and TAG, and decreases Prostaglandin E2 (PGE2) secretion. Conversely, inhibiting ACSL4 activity increases PGE2 release [119]. Upregulation of ACSL4 in ACSL4- negative LNCaP cells promotes cell proliferation, migration, and invasion, while ablation of endogenous ACSL4 decreases cell proliferation, migration, and invasion[17]. The ACSL4 expression is increased in castration resistant as compared with hormone naive prostate cancer[17]. There is an inverse relationship between AR and ACSL4 expression. The ACSL4 expression is associated with hormone-independent cell growth and is able to induce castration resistance prostate cancer, so ACSL4 may be useful as a biomarker for CRPC [120].

ACSL5 is prominently expressed in the intestinal mucosa, and lower expressed in lung, liver, adrenal gland, adipose tissue, kidney[121]. The expression of ACSL5 is higher during fasting and lower with refeeding[111]. The KO of ACSL5 decreases total ACSL activity 60% in jejunum without changing the mRNA expression of the other isoforms. SiRNA against ACSL5 decreases incorporation of 18:1 into TAG, phospholipids, and cholesterol esters in primary rat hepatocytes[122].

ACSL6 is highly present in the plasma membrane of brain cells [123]. The KD of ACSL6 in mouse neuroblastoma cells inhibits proliferation and neurite outgrowth [124]. Overexpressed ACSL6 in PC12 cells increases the accumulation of 22:6ω3 and the incorporation of 18:1, 20:4ω6, and 22:6ω3 into phospholipids and TAG. These studies suggest that ACSL6 play a primary role in docosahexaenoic acid metabolism and phospholipid synthesis during neurite outgrowth [125].

2.6 The proportional acyl-CoA profile

Yang et al. [97] recently reported a study measured seven saturated fatty acyl-CoA, one monounsaturated fatty acyl-CoA and three polyunsaturated fatty acyl-CoA in healthy liver cells (HepG2) and healthy prostate cells (PNT2). A couple of common monounsaturated and polyunsaturated fatty Acyl-CoAs were not examined, so the relative myristoyl-CoA was higher than reality. There were no fatty acid metabolic analyses performed on healthy human livers because wedge biopsies of the liver require surgery. Instead, obese human liver and healthy rat liver biopsies are available.

The levels of myristoyl CoAs and palmitoyl CoAs were higher in HepG2 cells than PNT2 cells indicating a higher activity of myristic and palmitic acid metabolism in hepatic cells. These data were collected from four different publications. The relative proportion of 14:0 and 16:0 (or C14:0 CoA and C16:0 CoA) was compared. Brain and plasma FA profiles reflect dietary fat. There are only very limited amounts of free FAs and Fatty Acyl-CoAs in our body since they are intermediate and quickly used as fuel molecules or added onto proteins to create lipoproteins or

as building blocks of phospholipids and glycolipids. In obese livers, the saturated FAs are much higher than healthy rat livers.

2.7 Myristoyl/palmitoyl-CoA facilitates the biosynthesis of the cytoplasmic membrane

Fatty acids, as the building block of cellular membranes, are integral components of phospholipids that function to maintain cellular integrity and modulate the activities of many membrane associated proteins[45]. Dietary saturated FAs regulate the composition of ceramides, sphingolipids, and other phospholipids in cancer cells [25]. Ceramides are found in high concentrations within the cell membrane since they are composed of sphingosine and fatty acids that make up sphingomyelin, one of the major lipids in the lipid bilayer (Figure 8). Ceramides, generated by hydrolysis of plasma membrane sphingomyelin, are crucial for programmed cell death, autophagy and inflammation[126].

Ceramide associated with myristoylated Src determine the formation of detergent resistant lipid raft microdomains in the cytoplasmic membrane [126]. Exogenous FAs altered ceramide compositions and increased myristoylated protein, particularly an increase of C16:0 ceramide. Lipid rafts are the major platform where proteins can associate and initiate oncogenic signaling [127]. Ceramides are one of the important elements in assembling lipid rafts in the cellular membrane [126]. The ceramide enriched membrane domain promotes reorganization of receptor molecules, potassium channels or recruits intracellular signal mediators for effective cell signal initiation or transduction [128].

CHAPTER 3

Protein myristoylation facilitates the activity of oncogenic proteins and oncogenic signaling

3.1 Protein N-myristoylation

The biosynthesis of acyl-CoAs derived from the activation of exogenous fatty acids from high-fat diets or de novo synthesized fatty acids provide substrates for ATP synthesis in the fatty acid oxidation, protein lipidation including acylation, and lipid biosynthesis, and other biological process [81]. Two most common forms of protein acylation are myristoylation and palmitoylation [129]. The attachment of myristate (14-carbon saturated fatty acid) and palmitate (16-carbon saturated fatty acid) is an essential lipid modification that targets proteins to the cytoplasmic membrane to facilitate molecular functions. Of those, protein myristoylation is one of the important processes in the maintenance of oncogenic protein activity and oncogenic signaling in cancer progression.

N-myristoylation reaches 1-4% of all proteins to be predicted in eukaryotic proteome [130] or 0.5-0.8% of all proteins in the genome [131]. In a combination of Click chemistry and proteomics analysis, more than 100 proteins were identified to be myristoylated in the mammalian cells [132], including N-myristoylated proteins, apoptotic cleaved proteins, and modification of proteins at lysine due to the amine group. Protein myristoylation provides an anchorage for the association with cytoplasmic membrane, which is essential for its function [129]. Among those, numerous proteins have been characterized in the oncogenic process.

N-myristoylation regulates the localization of its substrates to the specific membrane where the protein can regulate signal transduction pathways including small G proteins and many kinases [133]. Thus, N-myristoylation plays a major role in cell communication and metastatic prostate cancer. Additionally, N-myristoylation is necessary but not sufficient to target a protein to the membrane [134]. A palmitate or a polybasic motif will provide the second signal for myristoylated proteins [135].

3.2 Myristoyl-protein promotes tumor progression

Recently, at least 30 proteins have been identified that are myristoylated via post-translational modification during apoptosis [136]. N-myristoylation has been demonstrated to be essential for the viability and survival of humans, plants, fungal, and many other organisms[137, 138] since it's important for membrane association and function of certain proteins. The activity of many oncogenes can be extremely enhanced by myristoylation, such as Src and AKT.

3.2.1 Myristoylation of proto-oncogene tyrosine-protein kinase Src

Src family kinases (SFKs) are well-known regulators of diverse signal transduction pathways. Proto-oncogene tyrosine-protein kinase Src is essential for normal mitotic cycling and overactive in many cancers including prostate[139]. Myristoylation is conserved in the SH4 domain and regulates SFKs intracellular trafficking[140]. N-myristoylated Src, change the membrane binding affinity by regulating the number of positive charges on the surface, during which the electrostatic and hydrophobic interactions function together to anchor the protein to a membrane[129]. Tyrosine phosphorylation controls Src activity and regulates Src intramolecular interactions. SH2 and SH3 Domains of Src-mediated protein–protein interactions with

sequences containing phosphotyrosine and proline-rich motifs[141]. It switches signaling on and off by phosphorylation and dephosphorylating.

The upregulation of the c-Src has been observed in about 50% of tumors from colon, liver, lung, breast, prostate and the pancreas[142]. The overexpression of c-Src leads to transforming normal cells into cancer cells and the enhanced angiogenesis and sever epithelial cells proliferation. One mechanism is genetic mutations that result in the increased activity or the constitutively activated c-Src. Compared with normal prostate cells, members of the SFK, Src, Fyn, and Lyn are overexpressed in malignant prostate cells DU145 and LNCaP [143]. When the primary prostate cells were treated with Saracatinib (AZD0530), which is an inhibitor of Src, reduced cell proliferation and migration can be observed in vitro [144]. The localization of SFKs at the cytoplasmic micro domain is critical for cell signaling mediation, exhibiting activity, and illustrating their tumorigenic potential in cancer cells. Src is mainly localized in the plasma membrane in normal cells to phosphorylate its downstream targets, myristoylation is responsible for Src targeting membrane. However, mutation of the N-myristoylation site of Src, Glycine2 switching to alanine (G2A), causes the reduction or loss of membrane binding. Non-myristoylated Src mutants diffuse into the cytoplasm and form aggregates; therefore Src(G2A) do not mediate cellular transformation[145]. Targeting myristoylation of SFKs has an advantageous inhibitory effect on proliferation of tumorigenic cells in advanced prostate cancer patients. Also, the use of a Src kinase inhibitor is a potential way of reducing the progression of prostate cancers.

3.2.2 Myristoylated AKT(mAKT)

AKT, a serine/threonine kinase, is a well-known oncogene, also known as Protein kinase B (PKB). Stimulation of AKT requires a functional phosphatidylinositol 3-kinase by growth factors or insulin[146]. Myristoylated AKT is that a Src myristoylation sequence (first nine amino acids sequence) was added in front of AKT so than AKT can be directed to membranes and become constitutively active AKT. In LNCaP cells, a myristoylated AKT fusion protein was found to be highly enriched in lipid rafts compared with wild-type AKT [147].

Since high-fat diet provides elevated amount of exogenous myristic acid, we examine if exogenous FAs accelerate mAKT-mediated prostate tumor progression by using a previously established prostate regeneration cancer model (Figure 9). Host mice carrying mAKT induced prostate tumors were fed a low-fat diet (LFD, 10% fat), 45% fat or a high-fat diet (HFD, 60% fat) (Table 7). All diets were designed to contain the same amount of total calories, and both groups of mice showed no significant difference in total amount of calories consumed during the experimental period as reported in other studies. The composition of individual FAs varied between the diets. For example, compared 45% fat diets with 10% fat diets, the amount of MA increased 10-fold and PA increased ~6-fold (Table 8).

Hematoxylin and Eosin(H&E) staining to differentiate nucleus and cytoplasm, and RFP positive corresponded to the location of mAKT transformation. The regenerated prostate tissue from mAKT in the 10% fat diet group contained low-grade PIN lesion. In contrast, mAKT regenerated grafts in the 45%, and 60% fat diet groups showed higher grade tumor pathologically. We observe severe cell proliferation within a prostate tubule at the HFD group. Although the expression levels of AKT or AR showed similarly between LFD with HFD, the tubules in the

regenerated tumors were comprised of more CK8+ luminal tumorigenic cells in HFD than that in LFD. These data suggest that HFD accelerates mAKT-mediated prostate tumorigenesis. E-Cadherin/Vimentin is staining for Epithelial-mesenchymal transition (EMT) which usually occurs during embryonic development and tumor progression. We did not observe a visible difference in tumors between LHD and HFD (Figure 10).

CHAPTER 4

NMT as a potential drug target to treat prostate cancer

4.1 NMTs catalyze protein myristoylation

N-myristoyltransferases (NMT) is a monomeric enzyme belonging to the N-acetyltransferase superfamily [148]. To date, nineteen NMTs from mammalian and fungal cells have been identified. Most higher eukaryotes have two NMTs (NMT1 and NMT2) and share very similar sequence identity and enzyme function[133].

N-myristoyltransferase (NMT) catalyzes the transfer of a myristate to an N-terminal glycine of various cellular proteins by covalent bond (Figure 11) [149]. N-myristoylation site of proteins is destined to begin with the sequence: Methionine-Glycine. The initiating methionine in the N-terminal is removed by the co-translational process, and myristoyl group is attached to Gly-2 via an amide bond [129]. The preference of the peptide substrate is glycine at position 2, serine or threonine at position 6, and basic amino acids like lysine or arginine at positions 7 and 8 [129]. Myristoyl group is specially attached to the N-terminus of the protein substrate. The binding affinity of longer or shorter acyl-CoAs is low[150].

Under most circumstances, N- myristoylation is a co-translational modification in cells that happens before the polypeptide chain detached to the ribosome [151]. Co-translational addition of the myristate occurs in almost 80% of myristoylated proteins. An N-terminal Gly is exposed because of N-terminal methionine excision process during protein biosynthesis. However, this

modification can also occur later on a newly exposed N-terminal Gly after a proteolytic cleavage during apoptosis or by removal of a leader peptide from bacterial proteins in the eukaryotic host cells [152]. Thus, N-Myristoylation is a co- or post-translational modification.

4.2 NMT as an antifungal target

NMT has been reported as a novel and potent antifungal, antiparasitic, and anticancer target. Plenty of NMT inhibitors have been reported recently, as promising antifungal, antiparasitic, or anticancer agents[153]. Fungal infections have been received significant attention due to the limitation of current treatment, for instance, resistance development, low selectivity (severe host toxicity), and limited activity spectrum [154].Then research found that loss of NMT is lethal for fungi since N-myristoylation is necessary to protein structural conformation and thermos-stability [155]. Although mammalian and fungal NMTs have overlapping, due to their distinct substrate specificity, NMT has been characterized as a potential antifungal and antiparasitic drug target. Proteins from other microorganisms such as parasites and bacteria can undergo N-myristoylation in the host [131].

4.3 NMT as an anti-cancer target

In humans, two homologs of NMT, HsNMT1, and HsNMT2, are potential chemotherapeutic targets in cancer and autoimmune disorders[156]. Compared with two distinct family members of NMT, 58kDa of human NMT2 protein has 96% and 77% sequence overlapping with mouse NMT2 and human NMT1 [23, 157]. NMT1 is the most abundant enzyme in mammalian cells [158]. The requirements for both protein substrate and myristoyl group specificity of NMT are restricted. The expression of NMT is considerably elevated in several cancers, originating in the

prostate colon[158], stomach, gallbladder, brain, and breast, and loss of NMT has been shown to induce apoptosis in cancerous cell lines and reduce tumor volume in murine xenograft models for cancer[159]. This review highlights recent advances for new natural and synthetic NMT inhibitors, focusing on their biological activity, selectivity, and their anticancer activities.

NMT catalyzes the reaction of N-Gly-peptide with myristoyl-CoA in Bi-Bi reaction mechanism [160]. The protein interacting with two substrates are confirmed by a co-crystal structure of NMT, myristoyl-CoA analog and peptide inhibitors based on NMT protein of *Saccharomyces cerevisiae* species [161]. Several NMTs inhibitors have been well-characterized so far. These inhibitors are designed to target two binding sites to interfere the myristoylation process (Figure 12) [139]. As a result, myristoylation proteins are inhibited.

4.3.1 Inhibitors of targeting peptide binding site.

An NMT inhibitor named as "Compound 1" was identified by Edward Tate's group [132]. This compound is a dual NMT1/2 inhibitor. Based on X-ray crystallography (2.1A resolution), "Compound 1" competes with protein substrate and binds at the protein substrate-binding site in the presence of myristoyl-CoA [132]. The compound inhibits over 100 protein N-myristoylation. It induces cell cycle arrest, ER-stress, and apoptosis in breast and colon cancer cells [136]. The IC50 value of compound 4 is 0.005 μM against HsNMT.

A drug screening based assay lead to an identification of 32 compounds with chemotype of cyclohexyl-octahydropyrrolo[1,2-*a*]pyrazine. Among those, COPP-24 showed inhibitory effect toward human NMT-1 with competitive inhibition on the binding site of the peptide substrate

and with non-competitive inhibition of targeting the myristoyl-CoA site [162]. In particularly, cyclohexyl moiety of COPP24 is positioned at the plane of the peptide substrate based on the docking analysis of the X-ray structure of the yeast NMT. COPP24 possesses IC_{50} of 6 μM in inhibition of human NMT in vitro [162]. The inhibitory effect of COPP24 is further confirmed by a cell-based assay with blockade of Myr-GFP location at the cytoplasmic membrane. COPP24 exhibits suppression of proliferation of numerous cancer cells.

4.3.2 Inhibitors of targeting myristoyl-CoA binding site

Analogs of myristoyl-CoA are also designed as competitive inhibitors to inhibit NMT activity. One sub-type is the inhibitors containing the coenzyme A group, but resistant to be released due to the chemical modification. Sharma's group report that myristoyl coenzyme A derivatives, such as S-(2-ketopentadecyl)-CoA and 2-dodecylglycidoyl-S-CoA. The similarity of the myristoyl group shows high affinity to access the myristoyl-CoA binding site. The compounds show IC_{50} toward human NMT of 60 nM and 450 nM [163].

The other subtype of myristoyl-CoA mimics is the compounds with myristoyl group without coenzyme A moiety. The myristoyl group provides affinity for competition with the myristoyl-CoA binding site, while the moiety of coenzyme A is replaced by aromatic ring group with a similarity in structure. We screen a panel of LCL compounds synthesized for targeting ceramidase [164]. D-NMAPPD, N-[(1R,2R)-2-hydroxy-1-(hydroxymethyl)-2-(4-nitrophenyl)ethyl]-tetradecanamide, also named B13 (or LCL4) and LCL204 are identified with IC_{50} of 77.6 and 8.7 μM of potential inhibition of NMT1 activity, respectively. B13 shows an inhibitory effect in suppression of Src kinase myristoylation and exhibited limited toxicity to

normal cells and no observed pathological in liver and kidney, or lung tissue, but significantly inhibited xenograft tumors (Unpublished data). The SAR analysis shows that the number of acyl group with 14 carbon is essential for the inhibitory effect. Longer or shorter acyl chains exhibits no inhibitory effect (unpublished data). Due to the hydrophobicity of the myristoyl group, these analogs of myristoyl-CoA might require to be modified for solubility in the pre-clinic test. Additionally, a special delivery system such as nanoparticle should be considered as a delivery vehicle.

4.3.2 Other compounds of unknown targeting sites

Other compounds of unknown targeting sites are also reported to have the inhibitory effect in NMT activity. These compounds have been summarized in the review article [153], including monocyclic and bicyclic compounds (Table 9). Some of these compounds have a higher value of IC50 on NMT enzyme than IC50 on cells, suggesting non-specific cytotoxic issues or off-target.

4.3.4 Future consideration of using NMT inhibitors for the treatment of cancers.

An array of proteins has been reported to be myristoylated. Targeting NMT activity will un-avoidably impact the activity of a complexity of proteins. Therefore, the toxicity of inhibitors designed to target NMT activity needs to be carefully assessed before being used in a clinic trial. Future study should focus on if cancer cells have particular myristoylated protein pattern in comparison with normal cells. Cancer cells have been well-documented in the elevation of fatty acid metabolism [165]. Additionally, NMTs expression is also aberrantly elevated in some

cancers types. It is possible that with an elevation of either NMTs activity or the amount of myristoyl-CoA supply, cancer cells develop the addiction to myristoyl-proteins to promote proliferation of cancer cells. The differential activity of NMT activity will open a window to use the NMT inhibitors for the treatment of cancer progression.

CHAPTER 5

Conclusion

Dietary fat is a major source of fatty acids. High-fat diets provide a tremendous amount of fatty acids for the acceleration of oncogenic signaling. Increase myristic acid from the high-fat diet, leading to myristoylated mAKT/Src. Protein modification and abnormal expressions are associated with the cause and progression of cancer (Figure 13). The expression and activity of Src family kinases (SFKs) are highly elevated in numerous human cancers, including prostate cancer. SFKs are pleiotropic activators in several signal transduction pathways. Targeting SFKs has a favorable inhibitory effect on proliferation of tumorigenic cells and bone metastasis in advanced castration-resistant prostate cancer patients. Epidemiologic studies suggested that systemic metabolic disorders like obesity might increase the risk of prostate cancer. Exogenous MA and PA led to an alteration of ceramide compositions. Fatty acid de novo synthesis and lipid salvage pathway could contribute to the growth of tumors. Alterations of Fatty acid metabolism have been demonstrated in a variety of cancers, including prostate cancer to promote the growth and survival of cancer cell. Exploitation of the dysregulation in lipid metabolic pathways in PCa could be a promising method to develop new therapeutics. Inhibition of ACSL inhibited FAs entry bioactivity pool, ACSL is too complex to be an anticancer drug target. Inhibition of acyl-CoA biogenesis inhibited myristoylation of Src kinase, and its oncogenic signaling and cancer cell proliferation. The inhibition of myristoylation as a therapeutic strategy in targeting mAKT/Src-mediated tumorigenesis.

Reference

1. Haas, G.P., et al., *The worldwide epidemiology of prostate cancer: perspectives from autopsy studies.* The Canadian journal of urology, 2008. **15**(1): p. 3866.
2. Zhou, C.K., et al., *Prostate cancer incidence in 43 populations worldwide: an analysis of time trends overall and by age group.* International Journal of Cancer, 2016. **138**(6): p. 1388-1400.
3. Speight, J.L., K. Shinohara, and M. Mack Roach III, *Radiation Therapy for Prostate Cancer-A Patient Guide.*
4. Sfanos, K.S., et al., *A molecular analysis of prokaryotic and viral DNA sequences in prostate tissue from patients with prostate cancer indicates the presence of multiple and diverse microorganisms.* The Prostate, 2008. **68**(3): p. 306-320.
5. Boudadi, K. and E.S. Antonarakis, *Resistance to novel antiandrogen therapies in metastatic castration-resistant prostate cancer.* Clinical Medicine Insights. Oncology, 2016. **10**(Suppl 1): p. 1.
6. Hotte, S.J. and F. Saad, *Current management of castrate-resistant prostate cancer.* Current Oncology, 2010. **17**(Suppl 2): p. S72-S79.
7. Crawford, E.D., *Epidemiology of prostate cancer.* Urology, 2003. **62**(6): p. 3-12.
8. Pollard, M. and P.H. Luckert, *Promotional effects of testosterone and high fat diet on the development of autochthonous prostate cancer in rats.* Cancer letters, 1986. **32**(2): p. 223-227.
9. Rocha, G., et al., *A role for the Akt/mTOR pathway in the increased tumor growth of high-fat diet-induced obesity mice.* European Journal of Cancer Supplements, 2008. **6**(9): p. 32.
10. Huang, M., et al., *A high-fat diet enhances proliferation of prostate cancer cells and activates MCP-1/CCR2 signaling.* The Prostate, 2012. **72**(16): p. 1779-1788.
11. Vykhovanets, E.V., et al., *High-fat diet increases NF-κB signaling in the prostate of reporter mice.* The Prostate, 2011. **71**(2): p. 147-156.
12. Howlader, N., et al., *SEER Cancer Statistics Review, 1975–2012, National Cancer Institute. Bethesda, MD. 2015*. 2015.
13. Jemal, A., et al., *Annual report to the nation on the status of cancer, 1975–2014, featuring survival.* JNCI: Journal of the National Cancer Institute, 2017. **109**(9).
14. Mouraviev, V., B. Evans, and T.J. Polascik, *Salvage prostate cryoablation after primary interstitial brachytherapy failure: a feasible approach.* Prostate Cancer Prostatic Dis, 2005. **9**(1): p. 99-101.
15. Lu-Yao, G.L., et al., *Outcomes of localized prostate cancer following conservative management.* JAMA, 2009. **302**(11): p. 1202-1209.
16. Semenas, J., N. Dizeyi, and J.L. Persson, *Enzalutamide as a second generation antiandrogen for treatment of advanced prostate cancer.* Drug Des Devel Ther, 2013. **7**: p. 875-881.
17. Wu, X., et al., *ACSL4 promotes prostate cancer growth, invasion and hormonal resistance.* Oncotarget, 2015. **6**(42): p. 44849.

18. Hassanipour-Azgomi, S., et al., *Incidence and mortality of prostate cancer and their relationship with the Human Development Index worldwide.* Prostate International, 2016. **4**(3): p. 118-124.
19. Shimizu, H., et al., *Cancers of the prostate and breast among Japanese and white immigrants in Los Angeles County.* British journal of cancer, 1991. **63**(6): p. 963.
20. Last, A.R. and S.A. Wilson, *Low-carbohydrate diets.* Am Fam Physician, 2006. **73**(11): p. 1951-1958.
21. TOSHIMA, H., *Coronary artery disease trends in Japan.* Japanese circulation journal, 1994. **58**(3): p. 166-172.
22. Lassed, S., et al., *Diet, Lifestyles, Family History, and Prostate Cancer Incidence in an East Algerian Patient Group.* BioMed Research International, 2016. **2016**.
23. Ng, M., et al., *Global, regional, and national prevalence of overweight and obesity in children and adults during 1980–2013: a systematic analysis for the Global Burden of Disease Study 2013.* The Lancet, 2014. **384**(9945): p. 766-781.
24. Grundy, S.M., *Obesity, metabolic syndrome, and cardiovascular disease.* The Journal of Clinical Endocrinology & Metabolism, 2004. **89**(6): p. 2595-2600.
25. Louie, S.M., et al., *Cancer cells incorporate and remodel exogenous palmitate into structural and oncogenic signaling lipids.* Biochimica et Biophysica Acta (BBA)-Molecular and Cell Biology of Lipids, 2013. **1831**(10): p. 1566-1572.
26. Balaban, S., et al., *Obesity and cancer progression: is there a role of fatty acid metabolism?* BioMed research international, 2015. **2015**.
27. Gong, Z., et al., *Obesity, diabetes, and risk of prostate cancer: results from the prostate cancer prevention trial.* Cancer Epidemiology and Prevention Biomarkers, 2006. **15**(10): p. 1977-1983.
28. Vidal, A.C., et al., *Obesity increases the risk for high-grade prostate cancer: results from the REDUCE study.* Cancer Epidemiology and Prevention Biomarkers, 2014. **23**(12): p. 2936-2942.
29. Andersson, S.-O., et al., *Body size and prostate cancer: a 20-year follow-up study among 135006 Swedish construction workers.* Journal of the National Cancer Institute, 1997. **89**(5): p. 385-389.
30. McGee, D.L. and C.J. Crespo, *Body mass index and the risk of prostate cancer.* Open Access Medical Statistics, 2012.
31. Giovannucci, E., et al., *Body mass index and risk of prostate cancer in US health professionals.* Journal of the National Cancer Institute, 2003. **95**(16): p. 1240-1244.
32. Baillargeon, J., et al., *Obesity, adipokines, and prostate cancer in a prospective population-based study.* Cancer Epidemiology and Prevention Biomarkers, 2006. **15**(7): p. 1331-1335.
33. Engeland, A., S. Tretli, and T. Bjørge, *Height, body mass index, and prostate cancer: a follow-up of 950 000 Norwegian men.* British journal of cancer, 2003. **89**(7): p. 1237-1242.
34. Bonn, S.E., et al., *Body mass index and weight change in men with prostate cancer: progression and mortality.* Cancer Causes & Control, 2014. **25**(8): p. 933-943.
35. Dickerman, B.A., et al., *Weight change, obesity, and risk of prostate cancer progression among men with clinically localized prostate cancer.* International Journal of Cancer, 2017.

36. Nilsen, T.I.L. and L.J. Vatten, *Anthropometry and prostate cancer risk: a prospective study of 22,248 Norwegian men.* Cancer Causes and Control, 1999. **10**(4): p. 269-275.
37. Barqawi, A.B., et al., *Observed effect of age and body mass index on total and complexed PSA: analysis from a national screening program.* Urology, 2005. **65**(4): p. 708-712.
38. Zumoff, B., *Hormonal abnormalities in obesity.* Acta Medica Scandinavica, 1987. **222**(S723): p. 153-160.
39. Neugut, A.I., A.C. Chen, and D.P. Petrylak, *The "skinny" on obesity and prostate cancer prognosis*. 2004, American Society of Clinical Oncology.
40. Lo, J.C., et al., *Obesity does not promote tumorigenesis of localized patient-derived prostate cancer xenografts.* Oncotarget, 2016. **7**(30): p. 47650-47662.
41. Allott, E.H. and S.D. Hursting, *Obesity and cancer: mechanistic insights from transdisciplinary studies.* Endocrine-related cancer, 2015. **22**(6): p. R365-R386.
42. Giovannucci, E., et al., *A prospective study of dietary fat and risk of prostate cancer.* Journal of the National Cancer Institute, 1993. **85**(19): p. 1571-1579.
43. Le Marchand, L., et al., *Animal fat consumption and prostate cancer: a prospective study in Hawaii.* Epidemiology, 1994. **5**(3): p. 276-282.
44. Michaud, D.S., et al., *A prospective study on intake of animal products and risk of prostate cancer.* Cancer Causes & Control, 2001. **12**(6): p. 557-567.
45. Kolonel, L.N., A.M. Nomura, and R.V. Cooney, *Dietary fat and prostate cancer: current status.* Journal of the National Cancer Institute, 1999. **91**(5): p. 414-428.
46. Fizazi, K., *The role of Src in prostate cancer.* Annals of Oncology, 2007. **18**(11): p. 1765-1773.
47. Brasky, T.M., et al., *Serum phospholipid fatty acids and prostate cancer risk: results from the prostate cancer prevention trial.* American journal of epidemiology, 2011. **173**(12): p. 1429-1439.
48. Männistö, S., et al., *Fatty acids and risk of prostate cancer in a nested case-control study in male smokers.* Cancer Epidemiology and Prevention Biomarkers, 2003. **12**(12): p. 1422-1428.
49. Kurahashi, N., et al., *Dairy product, saturated fatty acid, and calcium intake and prostate cancer in a prospective cohort of Japanese men.* Cancer Epidemiology and Prevention Biomarkers, 2008. **17**(4): p. 930-937.
50. Park, S.-Y., et al., *Circulating fatty acids and prostate cancer risk in a nested case–control study: the Multiethnic Cohort.* Cancer Causes & Control, 2009. **20**(2): p. 211-223.
51. Shannon, J., et al., *Erythrocyte fatty acids and prostate cancer risk: a comparison of methods.* Prostaglandins, Leukotrienes and Essential Fatty Acids, 2010. **83**(3): p. 161-169.
52. Ukoli, F.A., et al., *The association of plasma fatty acids with prostate cancer risk in African Americans and Africans.* Journal of Health Care for the Poor and Underserved, 2010. **21**(1A): p. 127-147.
53. Chavarro, J.E., et al., *Blood levels of saturated and monounsaturated fatty acids as markers of de novo lipogenesis and risk of prostate cancer.* American journal of epidemiology, 2013: p. kwt136.
54. Crowe, F.L., et al., *Fatty acid composition of plasma phospholipids and risk of prostate cancer in a case-control analysis nested within the European Prospective Investigation*

into Cancer and Nutrition. The American journal of clinical nutrition, 2008. **88**(5): p. 1353-1363.
55. Jackson, M.D., et al., *Associations of whole-blood fatty acids and dietary intakes with prostate cancer in Jamaica.* Cancer Causes & Control, 2012. **23**(1): p. 23-33.
56. Pelser, C., et al., *Dietary fat, fatty acids, and risk of prostate cancer in the NIH-AARP diet and health study.* Cancer Epidemiology and Prevention Biomarkers, 2013. **22**(4): p. 697-707.
57. Crowe, F.L., et al., *Circulating fatty acids and prostate cancer risk: individual participant meta-analysis of prospective studies.* Journal of the National Cancer Institute, 2014. **106**(9): p. dju240.
58. Farazi, T.A., G. Waksman, and J.I. Gordon, *The biology and enzymology of proteinn-myristoylation.* Journal of biological chemistry, 2001. **276**(43): p. 39501-39504.
59. Rioux, V., P. Lemarchal, and P. Legrand, *Myristic acid, unlike palmitic acid, is rapidly metabolized in cultured rat hepatocytes.* The Journal of nutritional biochemistry, 2000. **11**(4): p. 198-207.
60. Kromhout, D., W. Saris, and C.H. Horst, *Energy intake, energy expenditure, and smoking in relation to body fatness: the Zutphen Study.* The American journal of clinical nutrition, 1988. **47**(4): p. 668-674.
61. Zock, P.L., J.H. de Vries, and M.B. Katan, *Impact of myristic acid versus palmitic acid on serum lipid and lipoprotein levels in healthy women and men.* Arteriosclerosis, Thrombosis, and Vascular Biology, 1994. **14**(4): p. 567-575.
62. Huber, A.H. and A.M. Kleinfeld, *Unbound Free Fatty Acid Profiles in Human Plasma and the Unexpected Absence of Unbound Palmitoleate.* Journal of Lipid Research, 2017: p. jlr. M074260.
63. Currie, E., et al., *Cellular fatty acid metabolism and cancer.* Cell metabolism, 2013. **18**(2): p. 153-161.
64. Glatz, J.F., J.J. Luiken, and A. Bonen, *Membrane fatty acid transporters as regulators of lipid metabolism: implications for metabolic disease.* Physiological reviews, 2010. **90**(1): p. 367-417.
65. Abumrad, N., C. Coburn, and A. Ibrahimi, *Membrane proteins implicated in long-chain fatty acid uptake by mammalian cells: CD36, FATP and FABPm.* Biochimica et Biophysica Acta (BBA)-Molecular and Cell Biology of Lipids, 1999. **1441**(1): p. 4-13.
66. Bonen, A., et al., *A null mutation in skeletal muscle FAT/CD36 reveals its essential role in insulin-and AICAR-stimulated fatty acid metabolism.* American Journal of Physiology-Endocrinology and Metabolism, 2007. **292**(6): p. E1740-E1749.
67. Pascual, G., et al., *Targeting metastasis-initiating cells through the fatty acid receptor CD36.* Nature, 2017. **541**(7635): p. 41-45.
68. Simantov, R., M. Febbraio, and R. Silverstein, *The antiangiogenic effect of thrombospondin-2 is mediated by CD36 and modulated by histidine-rich glycoprotein.* Matrix Biology, 2005. **24**(1): p. 27-34.
69. Sargiannidou, I., C. Qiu, and G.P. Tuszynski. *Mechanisms of thrombospondin-1-mediated metastasis and angiogenesis*. in *Seminars in thrombosis and hemostasis*. 2004. Copyright© 2004 by Thieme Medical Publishers, Inc., 333 Seventh Avenue, New York, NY 10001, USA.
70. Firlej, V., et al., *Thrombospondin-1 triggers cell migration and development of advanced prostate tumors.* Cancer research, 2011. **71**(24): p. 7649-7658.

71. Chen, P.-C., et al., *Thrombospondin-2 promotes prostate cancer bone metastasis by the up-regulation of matrix metalloproteinase-2 through down-regulating miR-376c expression.* Journal of hematology & oncology, 2017. **10**(1): p. 33.
72. Roepstorff, C., et al. *Studies of plasma membrane fatty acid-binding protein and other lipid-binding proteins in human skeletal muscle*. in *PROCEEDINGS-NUTRITION SOCIETY OF LONDON*. 2004. Cambridge Univ Press.
73. Clarke, D.C., et al., *Overexpression of membrane-associated fatty acid binding protein (FABPpm) in vivo increases fatty acid sarcolemmal transport and metabolism.* Physiological genomics, 2004. **17**(1): p. 31-37.
74. Haunerland, N.H. and F. Spener, *Fatty acid-binding proteins–insights from genetic manipulations.* Progress in lipid research, 2004. **43**(4): p. 328-349.
75. Newberry, E.P., et al., *Decreased hepatic triglyceride accumulation and altered fatty acid uptake in mice with deletion of the liver fatty acid-binding protein gene.* Journal of Biological Chemistry, 2003. **278**(51): p. 51664-51672.
76. Das, R., et al., *Expression pattern of fatty acid-binding proteins in human normal and cancer prostate cells and tissues.* Clinical cancer research, 2001. **7**(6): p. 1706-1715.
77. De Santis, M.L., et al., *Adipocyte-fatty acid binding protein induces apoptosis in DU145 prostate cancer cells.* J Exp Ther Oncol, 2004. **4**(2): p. 91-100.
78. Hammamieh, R., et al., *Molecular impacts of antisense complementary to the liver fatty acid binding protein (FABP) mRNA in DU 145 prostate cancer cells in vitro.* Journal of experimental therapeutics & oncology, 2004. **4**(3).
79. Vergnes, L., et al., *Heart-type fatty acid-binding protein is essential for efficient brown adipose tissue fatty acid oxidation and cold tolerance.* Journal of Biological Chemistry, 2011. **286**(1): p. 380-390.
80. Coe, N.R., M.A. Simpson, and D.A. Bernlohr, *Targeted disruption of the adipocyte lipid-binding protein (aP2 protein) gene impairs fat cell lipolysis and increases cellular fatty acid levels.* Journal of lipid research, 1999. **40**(5): p. 967-972.
81. Grevengoed, T.J., E.L. Klett, and R.A. Coleman, *Acyl-CoA metabolism and partitioning.* Annual review of nutrition, 2014. **34**: p. 1-30.
82. Schaffer, J.E. and H.F. Lodish, *Expression cloning and characterization of a novel adipocyte long chain fatty acid transport protein.* Cell, 1994. **79**(3): p. 427-436.
83. Chiu, H.-C., et al., *Transgenic expression of fatty acid transport protein 1 in the heart causes lipotoxic cardiomyopathy.* Circulation research, 2005. **96**(2): p. 225-233.
84. Wu, Q., et al., *FATP1 is an insulin-sensitive fatty acid transporter involved in diet-induced obesity.* Molecular and cellular biology, 2006. **26**(9): p. 3455-3467.
85. Doege, H., et al., *Targeted deletion of FATP5 reveals multiple functions in liver metabolism: alterations in hepatic lipid homeostasis.* Gastroenterology, 2006. **130**(4): p. 1245-1258.
86. Hubbard, B., et al., *Mice deleted for fatty acid transport protein 5 have defective bile acid conjugation and are protected from obesity.* Gastroenterology, 2006. **130**(4): p. 1259-1269.
87. Menendez, J.A. and R. Lupu, *Fatty acid synthase and the lipogenic phenotype in cancer pathogenesis.* Nature Reviews Cancer, 2007. **7**(10): p. 763-777.
88. Kusakabe, T., et al., *Fatty Acid Synthase Is Expressed Mainly in Adult Hormone-sensitive Cells or Cells with High Lipid Metabolism and in Proliferating Fetal Cells1.* Journal of Histochemistry & Cytochemistry, 2000. **48**(5): p. 613-622.

89. Wu, X., et al., *Lipid metabolism in prostate cancer.* American journal of clinical and experimental urology, 2014. **2**(2): p. 111.
90. Medes, G., A. Thomas, and S. Weinhouse, *Metabolism of neoplastic tissue. IV. A study of lipid synthesis in neoplastic tissue slices in vitro.* Cancer research, 1953. **13**(1): p. 27-29.
91. Beloribi-Djefaflia, S., S. Vasseur, and F. Guillaumond, *Lipid metabolic reprogramming in cancer cells.* Oncogenesis, 2016. **5**(1): p. e189.
92. Kuhajda, F.P., *Fatty acid synthase and cancer: new application of an old pathway.* Cancer research, 2006. **66**(12): p. 5977-5980.
93. Mashima, T., H. Seimiya, and T. Tsuruo, *De novo fatty-acid synthesis and related pathways as molecular targets for cancer therapy.* British journal of cancer, 2009. **100**(9): p. 1369-1372.
94. De Schrijver, E., et al., *RNA interference-mediated silencing of the fatty acid synthase gene attenuates growth and induces morphological changes and apoptosis of LNCaP prostate cancer cells.* Cancer research, 2003. **63**(13): p. 3799-3804.
95. Duke, G., et al., *LBP-515-Establishing the foundation for a novel, first-in-class, fatty acid synthase inhibitor, TVB-2640, for the treatment of NASH.* Journal of Hepatology, 2017. **66**(1): p. S99-S100.
96. Dean, E.J., et al., *Preliminary activity in the first in human study of the first-in-class fatty acid synthase (FASN) inhibitor, TVB-2640*. 2016, American Society of Clinical Oncology.
97. Yang, X., et al., *Development of a Novel Method for the Determination of Acyl-CoA Compounds by Liquid Chromatography Mass Spectrometry to Probe the Metabolism of Fatty Acids.* Analytical Chemistry, 2016.
98. Morita, Y.S., K.S. Paul, and P.T. Englund, *Specialized fatty acid synthesis in African trypanosomes: myristate for GPI anchors.* Science, 2000. **288**(5463): p. 140-143.
99. Horton, J., J. Goldstein, and M. Brown. *SREBPs: transcriptional mediators of lipid homeostasis*. in *Cold Spring Harbor Symposia on Quantitative Biology*. 2002. Cold Spring Harbor Laboratory Press.
100. Ettinger, S.L., et al., *Dysregulation of sterol response element-binding proteins and downstream effectors in prostate cancer during progression to androgen independence.* Cancer research, 2004. **64**(6): p. 2212-2221.
101. Williams, K.J., et al., *An essential requirement for the SCAP/SREBP signaling axis to protect cancer cells from lipotoxicity.* Cancer research, 2013. **73**(9): p. 2850-2862.
102. Shpilka, T., et al., *Lipid droplets and their component triglycerides and steryl esters regulate autophagosome biogenesis.* The EMBO journal, 2015: p. e201490315.
103. Bamba, V. and D.J. Rader, *Obesity and atherogenic dyslipidemia.* Gastroenterology, 2007. **132**(6): p. 2181-2190.
104. Park, J.B., et al., *Phospholipase signalling networks in cancer.* Nature Reviews Cancer, 2012. **12**(11): p. 782-792.
105. Richmond, G.S. and T.K. Smith, *Phospholipases A1.* International journal of molecular sciences, 2011. **12**(1): p. 588-612.
106. Sonoda, H., et al., *A Novel Phosphatidic Acid-selective Phospholipase A1That Produces Lysophosphatidic Acid.* Journal of Biological Chemistry, 2002. **277**(37): p. 34254-34263.
107. Coleman, R.A. and E. Haynes, *Selective changes in microsomal enzymes of triacylglycerol and phosphatidylcholine synthesis in fetal and postnatal rat liver.*

Induction of microsomal sn-glycerol 3-phosphate and dihydroxyacetonephosphate acyltransferase activities. Journal of Biological Chemistry, 1983. **258**(1): p. 450-456.
108. Ellis, J.M., et al., *Mouse cardiac acyl coenzyme a synthetase 1 deficiency impairs fatty acid oxidation and induces cardiac hypertrophy.* Molecular and cellular biology, 2011. **31**(6): p. 1252-1262.
109. Durgan, D.J., et al., *Distinct transcriptional regulation of long-chain acyl-CoA synthetase isoforms and cytosolic thioesterase 1 in the rodent heart by fatty acids and insulin.* American Journal of Physiology-Heart and Circulatory Physiology, 2006. **290**(6): p. H2480-H2497.
110. Li, L.O., et al., *Liver-specific loss of long chain acyl-CoA synthetase-1 decreases triacylglycerol synthesis and β-oxidation and alters phospholipid fatty acid composition.* Journal of Biological Chemistry, 2009. **284**(41): p. 27816-27826.
111. Mashek, D.G., L.O. Li, and R.A. Coleman, *Rat long-chain acyl-CoA synthetase mRNA, protein, and activity vary in tissue distribution and in response to diet.* Journal of lipid research, 2006. **47**(9): p. 2004-2010.
112. Ellis, J.M., et al., *Adipose acyl-CoA synthetase-1 directs fatty acids toward β-oxidation and is required for cold thermogenesis.* Cell metabolism, 2010. **12**(1): p. 53-64.
113. Kanter, J.E., et al., *Diabetes promotes an inflammatory macrophage phenotype and atherosclerosis through acyl-CoA synthetase 1.* Proceedings of the National Academy of Sciences, 2012. **109**(12): p. E715-E724.
114. Poppelreuther, M., et al., *The N-terminal region of acyl-CoA synthetase 3 is essential for both the localization on lipid droplets and the function in fatty acid uptake.* Journal of lipid research, 2012. **53**(5): p. 888-900.
115. Bu, S.Y., M.T. Mashek, and D.G. Mashek, *Suppression of long chain acyl-CoA synthetase 3 decreases hepatic de novo fatty acid synthesis through decreased transcriptional activity.* Journal of Biological Chemistry, 2009. **284**(44): p. 30474-30483.
116. Tyagi, S., et al., *The peroxisome proliferator-activated receptor: A family of nuclear receptors role in various diseases.* Journal of advanced pharmaceutical technology & research, 2011. **2**(4): p. 236.
117. Kang, M.-J., et al., *A novel arachidonate-preferring acyl-CoA synthetase is present in steroidogenic cells of the rat adrenal, ovary, and testis.* Proceedings of the National Academy of Sciences, 1997. **94**(7): p. 2880-2884.
118. Meloni, I., et al., *FACL4, encoding fatty acid-CoA ligase 4, is mutated in nonspecific X-linked mental retardation.* Nature genetics, 2002. **30**(4): p. 436-440.
119. Golej, D.L., et al., *Long-chain acyl-CoA synthetase 4 modulates prostaglandin E2 release from human arterial smooth muscle cells.* Journal of lipid research, 2011. **52**(4): p. 782-793.
120. Monaco, M.E., et al., *Expression of Long-chain Fatty Acyl-CoA Synthetase 4 in Breast and Prostate Cancers Is Associated with Sex Steroid Hormone Receptor Negativity.* Translational Oncology, 2010. **3**(2): p. 91-98.
121. Meller, N., et al., *Targeting of Acyl-CoA synthetase 5 decreases jejunal fatty acid activation with no effect on dietary long-chain fatty acid absorption.* Lipids in health and disease, 2013. **12**(1): p. 88.
122. Bu, S.Y. and D.G. Mashek, *Hepatic long-chain acyl-CoA synthetase 5 mediates fatty acid channeling between anabolic and catabolic pathways.* Journal of lipid research, 2010. **51**(11): p. 3270-3280.

123. Fujino, T. and T. Yamamoto, *Cloning and functional expression of a novel long-chain acyl-CoA synthetase expressed in brain.* Journal of Biochemistry, 1992. **111**(2): p. 197-203.
124. Kim, H.C., et al., *RNA interference of long-chain acyl-CoA synthetase 6 suppresses the neurite outgrowth of mouse neuroblastoma NB41A3 cells.* Molecular medicine reports, 2009. **2**(4): p. 669-674.
125. Marszalek, J.R., et al., *Long-chain acyl-CoA synthetase 6 preferentially promotes DHA metabolism.* Journal of Biological Chemistry, 2005. **280**(11): p. 10817-10826.
126. Gulbins, E. and R. Kolesnick, *Raft ceramide in molecular medicine.* Oncogene, 2003. **22**(45): p. 7070-7077.
127. Mollinedo, F. and C. Gajate, *Lipid rafts as major platforms for signaling regulation in cancer.* Advances in biological regulation, 2015. **57**: p. 130-146.
128. Bollinger, C.R., V. Teichgräber, and E. Gulbins, *Ceramide-enriched membrane domains.* Biochimica et Biophysica Acta (BBA)-Molecular Cell Research, 2005. **1746**(3): p. 284-294.
129. Resh, M.D., *Fatty acylation of proteins: new insights into membrane targeting of myristoylated and palmitoylated proteins.* Biochimica et Biophysica Acta (BBA)-Molecular Cell Research, 1999. **1451**(1): p. 1-16.
130. Martinez, A., et al., *Extent of N-terminal modifications in cytosolic proteins from eukaryotes.* Proteomics, 2008. **8**(14): p. 2809-2831.
131. Maurer-Stroh, S. and F. Eisenhaber, *Myristoylation of viral and bacterial proteins.* Trends in microbiology, 2004. **12**(4): p. 178-185.
132. Thinon, E., et al., *Global profiling of co- and post-translationally N-myristoylated proteomes in human cells.* Nat Commun, 2014. **5**: p. 4919.
133. Giglione, C., S. Fieulaine, and T. Meinnel, *N-terminal protein modifications: Bringing back into play the ribosome.* Biochimie, 2015. **114**: p. 134-146.
134. Pool, C.T. and T.E. Thompson, *Chain length and temperature dependence of the reversible association of model acylated proteins with lipid bilayers.* Biochemistry, 1998. **37**(28): p. 10246-10255.
135. Burnaevskiy, N., et al., *Proteolytic elimination of N-myristoyl modifications by the Shigella virulence factor IpaJ.* Nature, 2013. **496**(7443): p. 106-109.
136. Thinon, E., et al., *N-Myristoyltransferase Inhibition Induces ER-Stress, Cell Cycle Arrest, and Apoptosis in Cancer Cells.* ACS chemical biology, 2016. **11**(8): p. 2165-2176.
137. Frearson, J.A., et al., *N-myristoyltransferase inhibitors as new leads to treat sleeping sickness.* Nature, 2010. **464**(7289): p. 728-732.
138. Wright, M.H., et al., *Global analysis of protein N-myristoylation and exploration of N-myristoyltransferase as a drug target in the neglected human pathogen Leishmania donovani.* Chemistry & biology, 2015. **22**(3): p. 342-354.
139. Wright, M.H., et al., *Protein myristoylation in health and disease.* Journal of chemical biology, 2010. **3**(1): p. 19-35.
140. Shoji, S. and Y. Kubota, *Function of protein myristoylation in cellular regulation and viral proliferation.* Yakugaku zasshi: Journal of the Pharmaceutical Society of Japan, 1989. **109**(2): p. 71-85.
141. Parsons, S.J. and J.T. Parsons, *Src family kinases, key regulators of signal transduction.* Oncogene, 2004. **23**(48): p. 7906-7909.

142. Dehm, S.M. and K. Bonham, *SRC gene expression in human cancer: the role of transcriptional activation.* Biochemistry and cell biology, 2004. **82**(2): p. 263-274.
143. Nam, S., et al., *Action of the Src family kinase inhibitor, dasatinib (BMS-354825), on human prostate cancer cells.* Cancer research, 2005. **65**(20): p. 9185-9189.
144. Chang, Y.-M., et al., *Survey of Src activity and Src-related growth and migration in prostate cancer lines*. 2006, AACR.
145. Kamps, M.P., J.E. Buss, and B.M. Sefton, *Mutation of NH2-terminal glycine of p60src prevents both myristoylation and morphological transformation.* Proceedings of the National Academy of Sciences, 1985. **82**(14): p. 4625-4628.
146. Kohn, A.D., et al., *Expression of a constitutively active Akt Ser/Thr kinase in 3T3-L1 adipocytes stimulates glucose uptake and glucose transporter 4 translocation.* Journal of Biological Chemistry, 1996. **271**(49): p. 31372-31378.
147. Adam, R.M., et al., *Cholesterol sensitivity of endogenous and myristoylated Akt.* Cancer Research, 2007. **67**(13): p. 6238-6246.
148. Vetting, M.W., et al., *Structure and functions of the GNAT superfamily of acetyltransferases.* Archives of biochemistry and biophysics, 2005. **433**(1): p. 212-226.
149. Xu, M., et al., *Roles of protein N-myristoylation and translational medicine applications.* Critical Reviews™ in Eukaryotic Gene Expression, 2015. **25**(3).
150. Johnson, D.R., et al., *Genetic and biochemical studies of protein N-myristoylation.* Annual review of biochemistry, 1994. **63**(1): p. 869-914.
151. Deichaite, I., et al., *In vitro synthesis of pp60v-src: myristylation in a cell-free system.* Molecular and cellular biology, 1988. **8**(10): p. 4295-4301.
152. Martin, D.D., E. Beauchamp, and L.G. Berthiaume, *Post-translational myristoylation: Fat matters in cellular life and death.* Biochimie, 2011. **93**(1): p. 18-31.
153. Zhao, C. and S. Ma, *Recent Advances in The Discovery of N-Myristoyltransferase Inhibitors.* ChemMedChem, 2014. **9**(11): p. 2425-2437.
154. Georgopapadakou, N.H., *Antifungals targeted to protein modification: focus on protein N-myristoyltransferase.* Expert opinion on investigational drugs, 2002. **11**(8): p. 1117-1125.
155. Duronio, R.J., et al., *Disruption of the Yeast N-Myristoyl Transferase Gene Recessive Lethality.* Science, 1989. **243**(4892): p. 796.
156. Rampoldi, F., et al., *Immunosuppression and aberrant T cell development in the absence of N-myristoylation.* The Journal of Immunology, 2015. **195**(9): p. 4228-4243.
157. Raju, R.V., et al., *Molecular Cloning and Biochemical Characterization of Bovine Spleen Myristoyl CoA: ProteinN-Myristoyltransferase.* Archives of biochemistry and biophysics, 1997. **348**(1): p. 134-142.
158. Selvakumar, P., et al., *Potential role of N-myristoyltransferase in cancer.* Progress in lipid research, 2007. **46**(1): p. 1-36.
159. Das, U., et al., *Inhibition of protein N-myristoylation: a therapeutic protocol in developing anticancer agents.* Current cancer drug targets, 2012. **12**(6): p. 667-692.
160. Rudnick, D.A., et al., *Kinetic and structural evidence for a sequential ordered Bi Bi mechanism of catalysis by Saccharomyces cerevisiae myristoyl-CoA:protein N-myristoyltransferase.* J Biol Chem, 1991. **266**(15): p. 9732-9.
161. Bhatnagar, R.S., et al., *Structure of N-myristoyltransferase with bound myristoylCoA and peptide substrate analogs.* Nat Struct Biol, 1998. **5**(12): p. 1091-7.

162. French, K.J., et al., *Cyclohexyl-octahydro-pyrrolo [1, 2-a] pyrazine-based inhibitors of human N-myristoyltransferase-1.* Journal of Pharmacology and Experimental Therapeutics, 2004. **309**(1): p. 340-347.
163. Bajaj, G., et al., *Inhibitors of NMT: A New Class of Chemotherapeutic Drugs.* Drug Design Reviews-Online, 2004. **1**(4): p. 347-354.
164. Szulc, Z.M., et al., *Novel analogs of D-e-MAPP and B13. Part 1: synthesis and evaluation as potential anticancer agents.* Bioorg Med Chem, 2008. **16**(2): p. 1015-31.
165. Menendez, J.A. and R. Lupu, *Fatty acid synthase and the lipogenic phenotype in cancer pathogenesis.* Nat Rev Cancer, 2007. **7**(10): p. 763-77.
166. Shen, M.M. and C. Abate-Shen, *Molecular genetics of prostate cancer: new prospects for old challenges.* Genes & development, 2010. **24**(18): p. 1967-2000.
167. Untawale, S., et al., *Body mass index and risk of pancreatic cancer in a Chinese population.* PloS one, 2014. **9**(1): p. e85149.
168. Hanyuda, A., et al., *Body mass index and risk of colorectal cancer according to tumor lymphocytic infiltrate.* International journal of cancer, 2016. **139**(4): p. 854-868.
169. Rauscher, G.H., S.T. Mayne, and D.T. Janerich, *Relation between body mass index and lung cancer risk in men and women never and former smokers.* American journal of epidemiology, 2000. **152**(6): p. 506-513.
170. Gao, Y., et al., *Body mass index is positively associated with endometrial Cancer in Chinese women, especially prior to menopause.* Journal of Cancer, 2016. **7**(9): p. 1169.
171. Campbell, P.T., et al., *Body Mass Index, Waist Circumference, Diabetes, and Risk of Liver Cancer for US Adults.* Cancer Research, 2016. **76**(20): p. 6076-6083.
172. Engeland, A., S. Tretli, and T. Bjørge, *Height and body mass index in relation to esophageal cancer; 23-year follow-up of two million Norwegian men and women.* Cancer Causes & Control, 2004. **15**(8): p. 837-843.

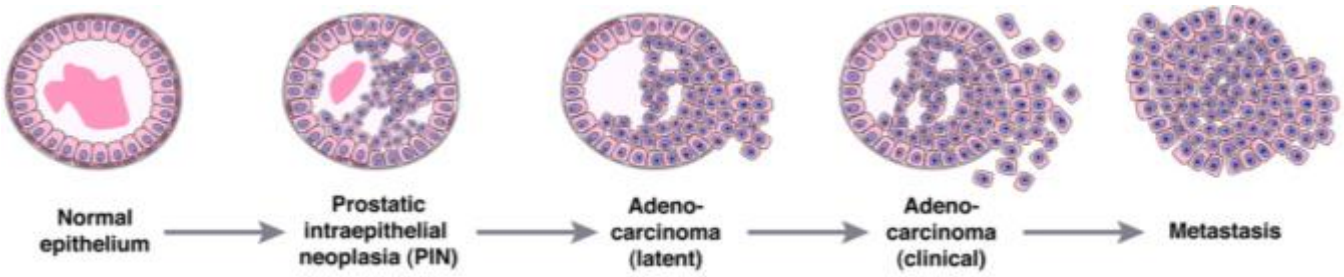

Figure 1. **Prostate cancer progression in a tubule level**[166].

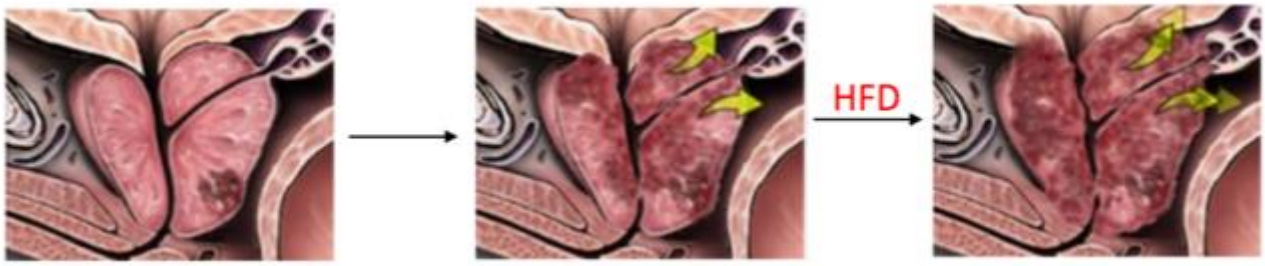

Figure 2. **Prostate cancer progression in a tissue level.** Prostate cancer begins with abnormal epithelial proliferation and prostatic intraepithelial neoplasia. Most tumors begin to form in proximity to the outer surfaces of a prostate gland. Some tumors can grow to a volume where they start to obstruct urination, progression to invasive carcinoma and metastases mainly in the skeleton. Aggressive tumors can eventually break the capsule and infiltrate nearby organs. If tumor cells separate and enter the blood stream, prostate cancer may spread to other parts of the body. Eventually, the stage called castration-resistant prostate cancer (CRPC) is the incurable state of prostate cancer [5].

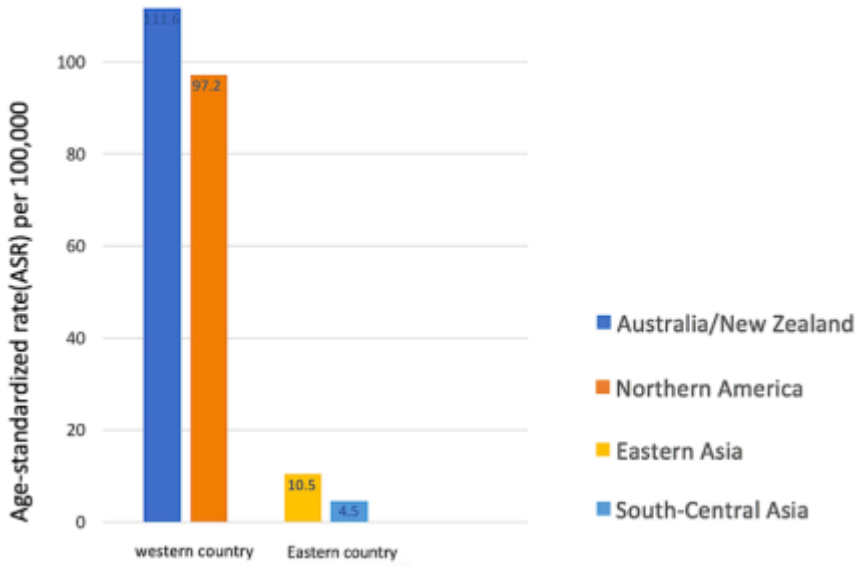

Figure 3. **Prostate cancer incidence in 2012.** The age-standardized rate of prostate cancer is a weighted average of the age-specific rates per 100,000 persons, where the weights are the proportions of persons in the corresponding age groups of the WHO standard population. Western countries like Australia, have a much higher incidence and mortality rate of prostate cancer than Eastern countries.
(http://globocan.iarc.fr/old/FactSheets/cancers/prostate-new.asp)

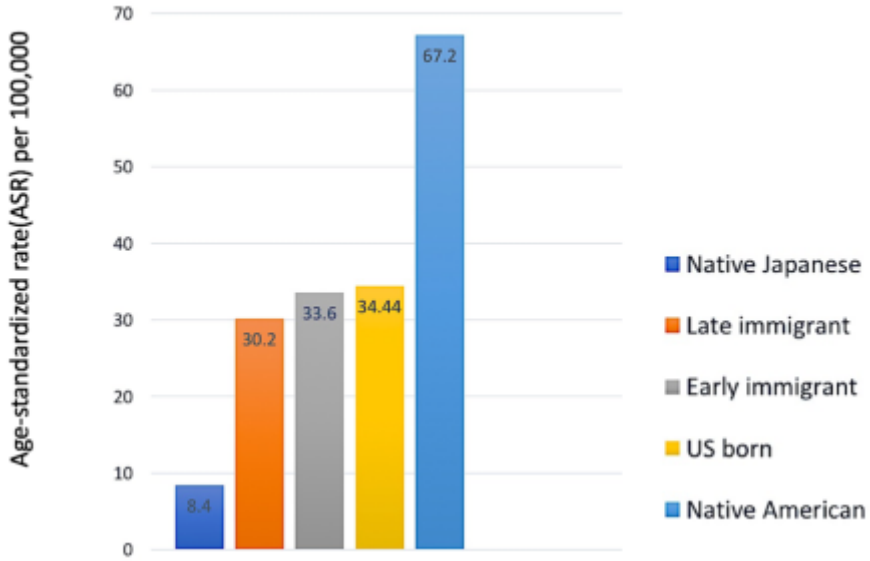

Figure 4. **A case study of prostate cancer on Japanese descent in US**[19]. The prostate cancer incidence of Japanese immigrants to the Unite State represents a less-differentiated to that of the general American population. This rate is higher than the native Japanese population, strongly suggesting that the environment, lifestyle, and diet play an important role in contributing to the initiation and progression of prostate cancer.

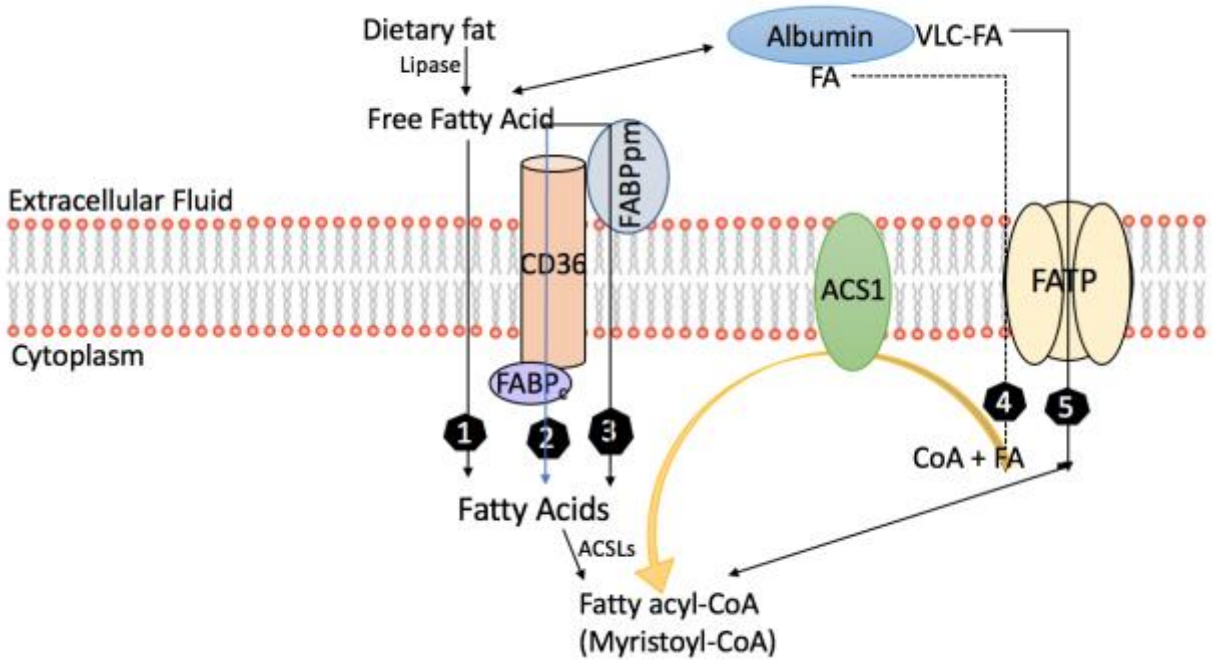

Figure 5. **Myristic acid/myristoyl-CoA Transporters.** Fatty acids can across the plasma membrane by either passive diffusion or facilitate diffusion (route 1-5). 1) Fatty acids could dissociate with albumin and cross the plasma membrane by passive diffusion. 2) $FABP_{pm}$ is a peripheral membrane protein which could act alone or together with CD36, facilitate FAs translocate into cells. 3) CD36 itself could also mediate FAs transport cross the lipid bilayer. Once FAs get into the cells, FAs are bound with $FABP_c$. 4) Fatty acid transporter protein (FATP) belongs to Acyl-CoA synthetase (ACS) superfamily which can transport VLC-FAs and converts then directly into fatty acyl-CoA. 5) A minority of FAs are thought to be transported by FATP1 and rapidly activated by plasma membrane ACS1 to form acyl-Co esters. [65].

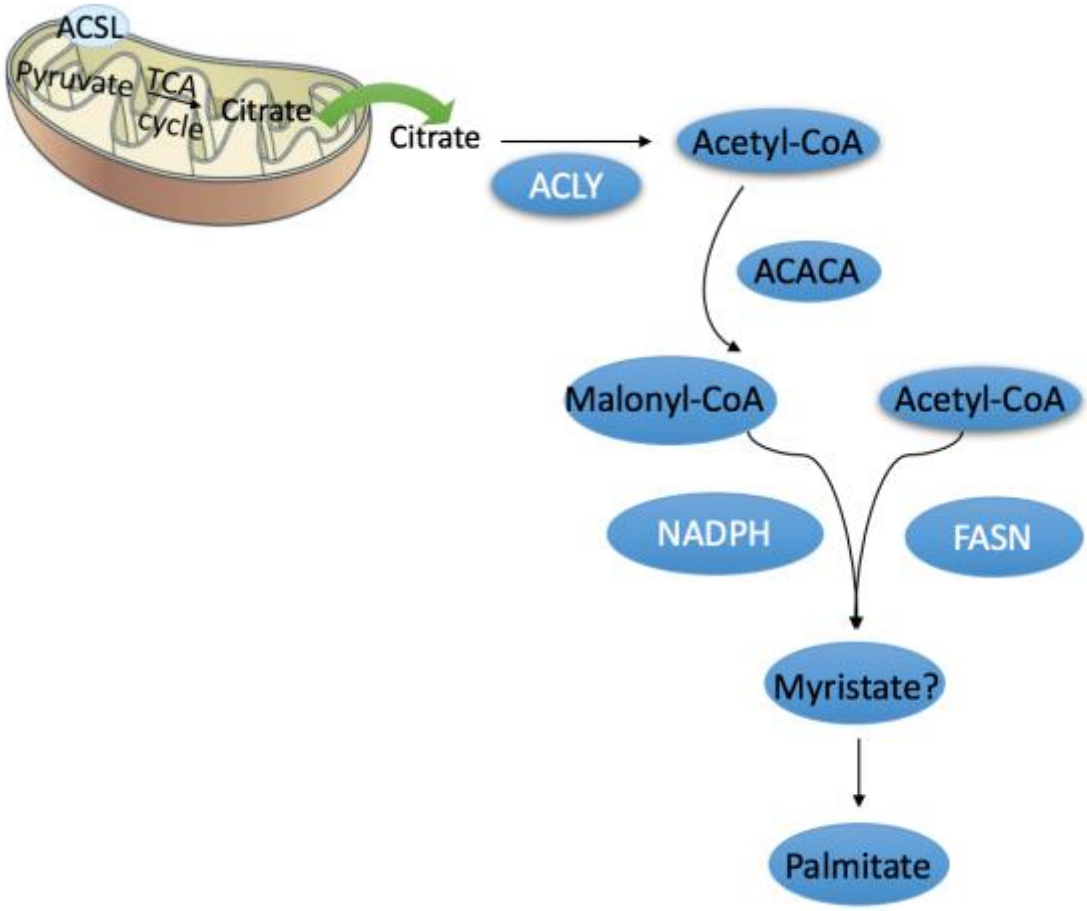

Figure 6. **Fatty acid de novo synthesis** [87]. When the acetyl-CoA in the mitochondria starts to accumulate, FA de nove synthesis starts with high levels of citrate and ATP. Fatty acid synthetase (FASN) is a single polypeptide chain and contains seven different catalytic domains that catalyze the assembly of fatty acid chains via fatty acid synthase. In the loading step, acetyl-CoA and malonyl-CoA are attached to acyl carrier protein (ACP). Then, both acetyl-CoA and malonyl-CoA are condensed by fatty acid synthase to from acetoacetyl-ACP. The third step is reduction through which NADPH is oxidized to form hydroxybutyryl ACP. Next is dehydration which removes a hydroxyl group to form a double bond, thereby forming a crotonyl attached to ACP and releasing an H_2O. Then the second reduction step is to form butyryl-ACP, NADPH as a source of e- and H+. The last four steps are repeated six more times with malonyl-ACP to elongate the chain until palmitate is produced.

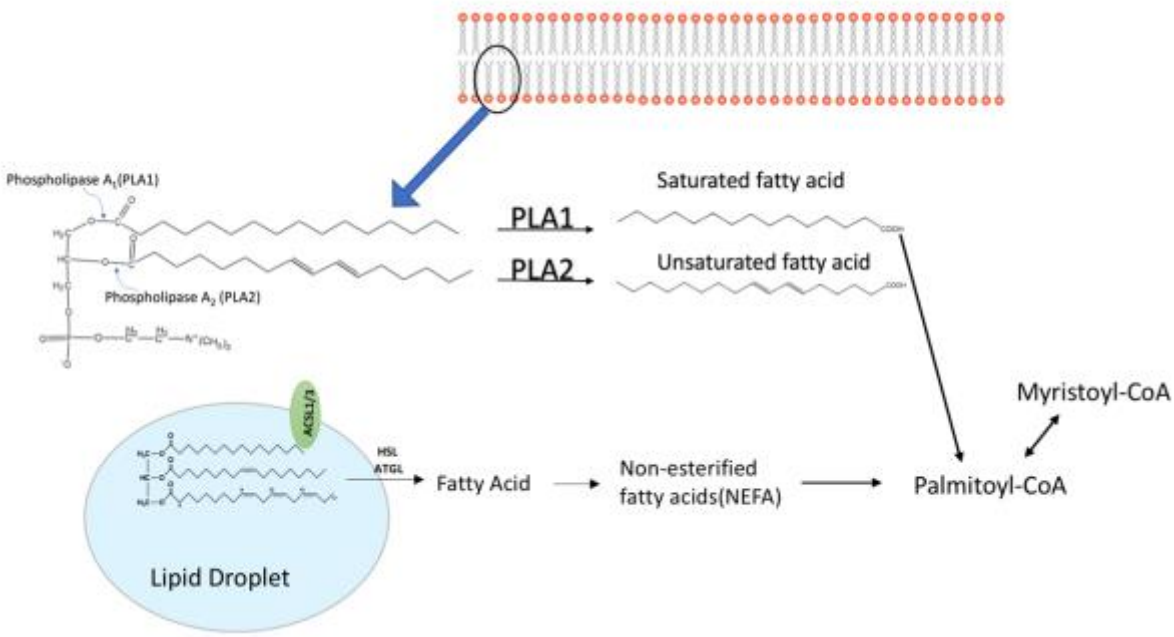

Figure 7. **Lipid Salvage Pathway**

Phospholipases hydrolyze phospholipids by cleaving fatty acyl ester bonds, release saturated FAs, unsaturated FAs and lysophospholipids [105]. Lipoprotein lipase breaks down triglycerides and allows them to be taken up by the receptors and fatty acid transporters. Some fatty acids are then restored as triglycerides inside the adipocyte lipid droplets. When FAs are needed, hormone-sensitive lipases break down the triglyceride producing FAs which are then released into circulation and are able to be oxidized or associated with certain proteins[103]. Although lipid salvage pathway is not the primary source of myristate, it may slightly contribute to the biogenesis of myristic acid.

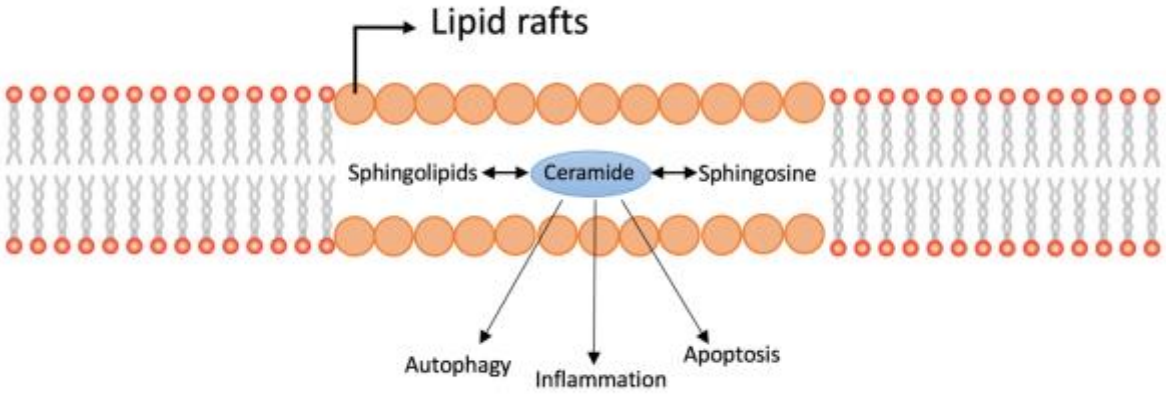

Figure 8. **Ceramide metabolism**

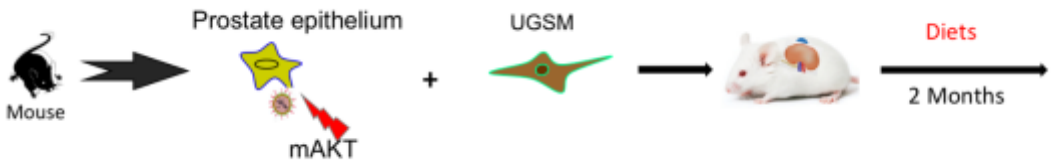

Figure 9. **Schematic of the in vivo prostate regeneration assay.** *Prostate epithelial cells derived from C57BL/6J mice were transduced with mAKT and mixed with UGSM and implanted under the renal capsule. Host SCID mice were feed with a 10%, 45% and 60% fat diet for 8 weeks. Total calorie intake was not significantly different between the diet groups*

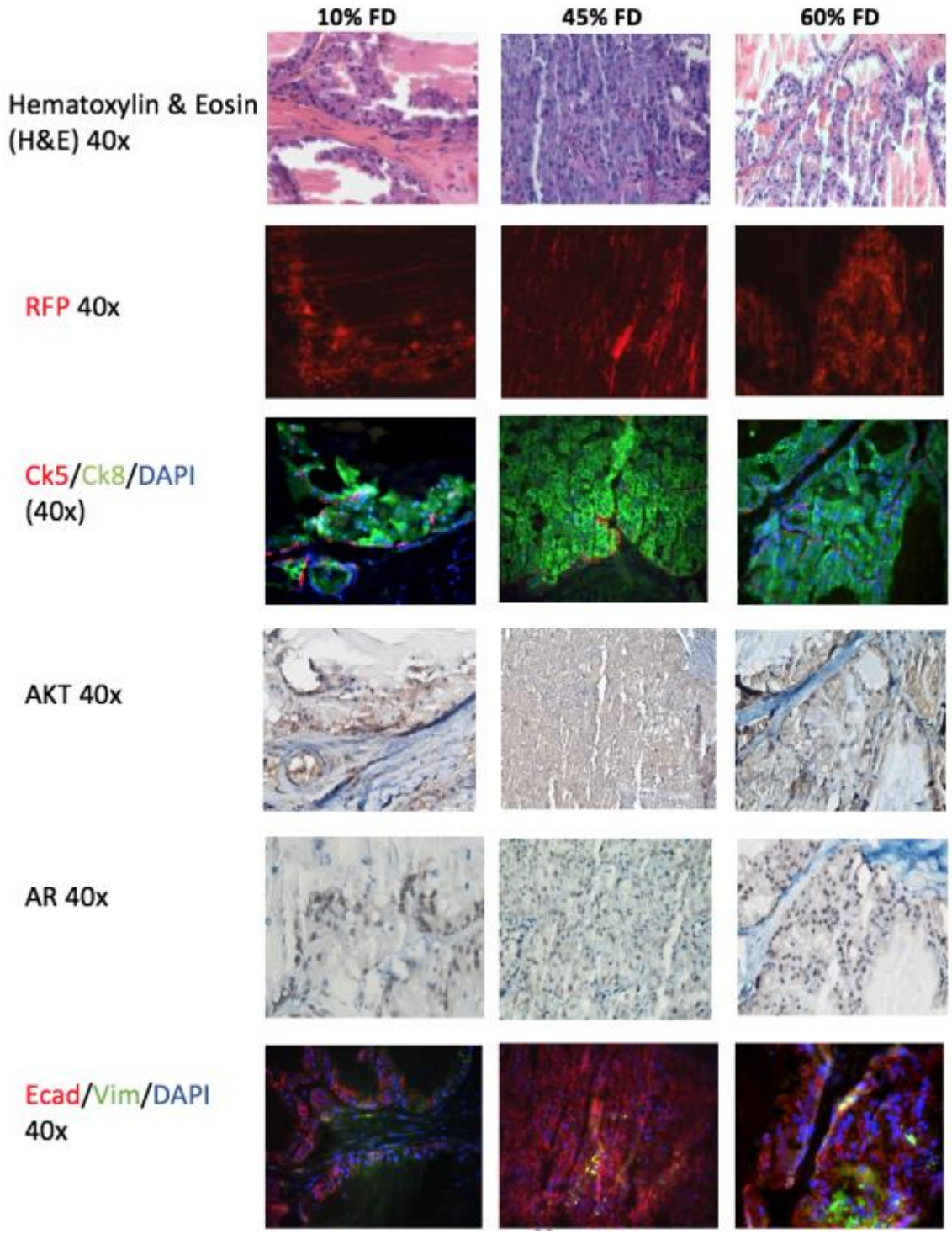

Figure 10. Representative H&E (panoramic view, scale bar, 400 μm), RFP fluorescence (a marker for lentiviral infection) and co-staining of CK5(red), a basal epithelial cell marker, CK8(green), a luminal epithelial cell marker and DAPI of tumors from and IHC staining (selected tumorigenic region, scale bar, 100 μm) of AR and AKT, co-staining of E-Cadherin(red), an Epithelial cell marker, vimentin(green), a mesenchymal cell marker and DAPI.

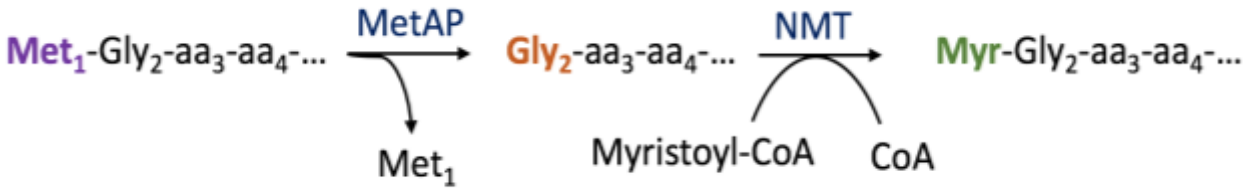

Figure 11. **N-Myristoylation**, as co-translational N-terminal protein modifications, which are catalyzed by two enzymes. Methionine aminopeptidase (MetAP) which ensure the N-terminal methionine excision process, followed by N-myristoyltransferase that acylates proteins.

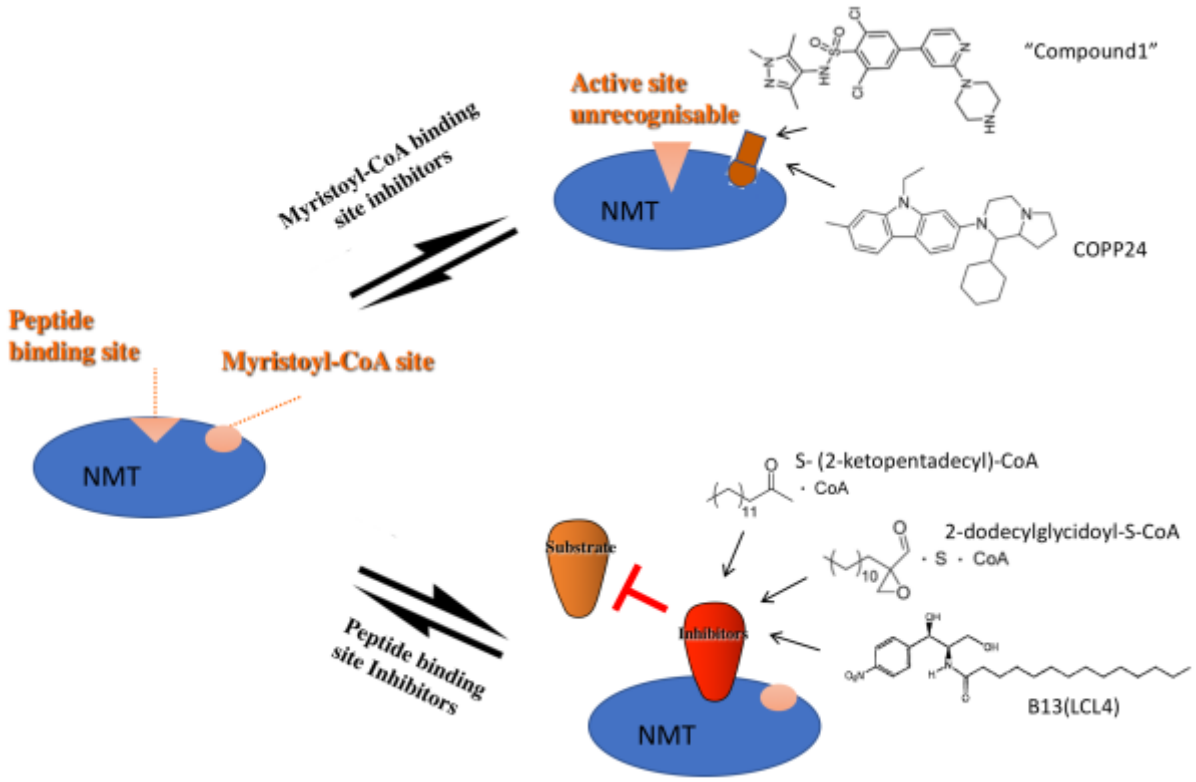

Figure 12. **Process of Inhibition NMT Enzyme Catalysis**

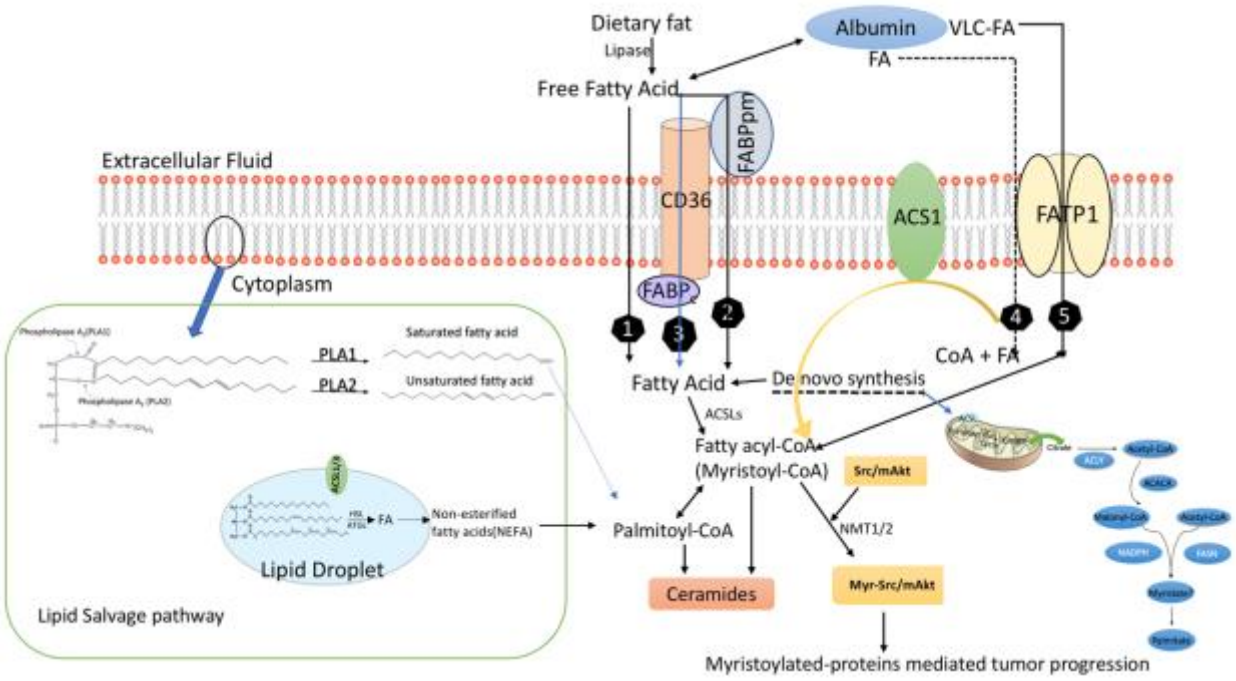

***Figure 13*. Schematic representation of metabolism of dietary myristic acid facilitates prostate cancer progression.** Myristic acid could be derived from Lipid salvage pathway and de novo synthesis, and diet. Myristic acid undergo to myristoylated protein or elongation to palmitoyl-CoA, and further incorporated in ceramide synthesis.

	Europe	US	Japan	South Korean
Total Fat Intake (kcal%)	36%	35%	22%	21%
Saturated Fatty Acids	14%	12%	7%	7%
Monounsaturated Fatty Acids	16%	19%	9%	9%
Polyunsaturated Fatty Acids	6%	5%	6%	5%

Table 1. **Dietary fat comparison.** Western diets contain a high intake of fat and several FAs. The standard American diet is about 50% carbohydrate, 15% protein, and 35% fat [20]. The average Japanese diet is about 60% carbohydrate, 15% protein, and 25% fat [21] which indicates the lower total fat and lower saturated FAs.

Prostate Cancer	BMI kg/m^2	Cancer Risk Ratio	Reference
White males over 40 years	BMI ≥ 25.5 compared with<25.5	1.041 (95% CI:1.007, 1.077)	[30]
Younger (<60 years old)	BMI ≥ 30 compared with 23-24.9	0.52 (95% CI:0.33, 0.83)	[31]
Had a family history of prostate cancer		0.74 (95% CI:0.45, 1.19)	[31]
Low-grade PCa (Gleason <7)	BMI ≥ 30 compared with<25	0.79 (P = 0.01)	[28]
High-grade PCa (Gleason≥ 7)		1.28 (P =0.042)	[28]
Low-grade PCa (Gleason <7)	BMI ≥ 30 compared with<25	0.82 (95% CI= 0.69, 0.98)	[27]
High-grade PCa (Gleason≥ 7)		1.29 (95% CI =1.01, 1.67)	[27]
Obese men compared to normal weight men	BMI ≥ 30 compared with<25	1.2 (95% CI = 0.9, 1.6)	[36]
		1.47 (95 % CI= 1.03, 2.10)	[34]

Table 2. **The correlation between body mass index and prostate cancer risk.**

Cancer type		BMI kg/m^2	Cancer Risk Ratio	Reference
Pancreatic Cancer	Smokers	BMI < 18.5 compared with 21.5-22.4	1.99 (95%CI: 1.03, 3.84)	[167]
	Non-smokers	BMI ≥27.5 compared with 21.5-22.4	1.75 (95 %CI: 0.93–3.3)	
Colorectal Cancer		18.5–22.5	1]Ref[	[168]
		22.5–24.9	1.22 (95% CI: 1.03, 1.44)	
		25–27.5	1.36 (95% CI: 1.15, 1.61)	
		27.5–29.9	1.39 (95% CI: 1.15, 1.69)	
		≥30	1.61)95% CI: 1.33, 1.94(	
Lung Cancer	Non-smokers	BMI ≤ 21.26 compare with > 30.84.	2.4 (95%CI: 1.1, 6.0)	[169]
	Smokers	BMI ≤ 21.26 compare with > 30.84.	3.2 (95%CI: 1.3, 8.1)	
Endometrial Cancer	Overweight (10-15%)	BMI=23-27.49 compare with normal weight (BMI=18.5-22.9)	2.6 (95%CI: 1.9, 3.5)	[170]
	Obese (2-3%)	BMI>27.50compare with normal weight (BMI=18.5-22.9)	3.5 (95%CI: 2.2, 5.4)	
Liver Cancer		For every 5 kg/m^2 increase in BMI	1.32 (95% CI: 1.25, 1.38)	[171]
Esophagus Cancer	Overweight (men)	BMI=25-29 compare with normal weight (BMI=18.5-24.9)	1.80) 95% CI: 1.48-2.19(	[172]
	Obese (men)	BMI ≥30 compare with normal weight	2.58) 95% CI: 1.81-3.68(	
	Overweight (women)	BMI=23-27.49 compare with normal weight (BMI=18.5-22.9)	1.64) 95%CI: 1.08-2.49(	
	Obese (women)	BMI>27.50compare with normal weight	2.06) 95% CI: 1.25-3.39(	

Table 3. **The correlation between body mass index and the risk of cancers. Supplementary data.**

	Fatty Acid	Risk Ratio	Reference
Saturated FA	C14:0 Myristic	Odds Ratio(OR)= 1.93; 95% confidence interval(CI)=1.02 - 3.64	[48]
		OR=1.62, 95% CI = 1.15 - 2.29	[49]
		No association	[55]
	C16:0 Palmitic	OR=0.53; 95% CI= 0.19-1.54	[55]
		OR=1.53, 95% CI = 1.07 - 2.20	[49]
	C18:0 Stearic	OR=0.88, 95% CI = 0.78 - 1.00	[57]
		OR=1.35, 95% CI = 0.94 - 1.94	[49]
Monounsaturated FA	C14:1	OR=1.74, 95% CI = 1.15 - 2.65	[53]
	C16:1 Palmitoleic	OR=1.97, 95% CI = 1.27 - 3.06	[53]
	C18:1 Oleic	OR=1.47, 95% CI = 0.95 - 2.26	[53]
Polyunsaturated FA	C18:2 Linoleic	OR= 0.17, 95% CI = 0.04 - 0.68	[48]
	C18:3 Linolenic	No association	[48]
	C20:5 Eicosapentanoic	OR = 1.14, 95% CI = 1.01 - 1.29	[57]
	C22:6 Docosapentaenoic	OR = 1.16, 95% CI = 1.02 - 1.33	[57]

Table 4. Different fatty acids vs Prostate cancer risk. Specifically, some studies reported blood levels of myristic acid were associated with higher prostate cancer incidence, but several studies showed there is no association between MA and PCa risk. Also, the correlation between prostate cancer risk and serum palmitic acid level is controversial. Lower blood levels of MUFAs in the present study are associated with lower risk. Moreover, most studies are in agreement with that EPA and DHA inhibited prostate cancer cell proliferation, whereas the n-6 fatty acids linoleic acid and arachidonic acid stimulated proliferation. Whereas an exception has been report that the modest associations of EPA (OR = 1.14, 95% CI = 1.01 - 1.29), and DHA (OR = 1.16, 95% CI = 1.02 - 1.33) with prostate cancer.

Stimulation of FA uptake		Wild Type CD36	Knock out CD36	Overexpress CD36
Oxidation-enhancing agent	cardiac myocytes	+150%	+20%	
	skeletal muscle	+77%	+13%	
Insulin		+60%	+21%	
Muscle contraction		+100%		+400%

***Table 5*. Fatty acid uptake during metabolic challenges.**

FABP isoform	Tissue distribution	Knockout model
Fabp1	liver and intestine	reduces hepatic FA binding capacity, total liver lipid content remains unchanged
Fabp3	heart, skeletal muscle, and brain	defective FA oxidation more reliant on glucose as a substrate for energy production. extremely cold intolerant.
Fabp4	adipocyte	increases the cytosolic content of free FA

Table 6. **Tissue distribution and loss-of-function studies of fatty acid binding protein.**

Diets	10% fat diet	45% fat diet	60% fat diet
Protein (kcal%)	20	20	20
Carbohydrate (kcal%)	70	35	20

Table 7. **Nutrition facts of mice diet.** Each diet shares the same amount of total calories and protein.

	10% fat diet	45% fat diet	60% fat diet
C2, Acetic	0	0	0
C4, Butyric	0	0	0
C6, Caproic	0	0	0
C8, Caprylic	0	0	0
C10, Capric	0.0	0.1	0.1
C12, Lauric	0.0	0.2	0.2
C14, Myristic	0.2	2.0	2.8
C14:1, Myristoleic	0	0	0
C15	0.0	0.1	0.2
C16, Palmitic	6.5	36.9	49.9
C16:1, Palmitoleic	0.3	2.4	3.4
C16:2	0	0	0
C16:3	0	0	0
C16:4	0	0	0
C17	0.1	0.7	0.9
C17:1	0	0	0
C18, Stearic	3.1	19.8	26.9
C18:1, Oleic	12.6	64.4	86.6
C18:2, Linoleic	18.3	56.7	73.1
C18:3, Linolenic	2.2	4.3	5.2
C18:4, Stearidonic	0	0	0
C20, Arachidic	0.0	0.3	0.4
C20:1,	0.1	1.1	1.5
C20:2	0.2	1.4	2.0
C20:3	0.0	0.2	0.3
C20:4, Arachidonic	0.1	0.5	0.7
C20:5, Eicosapentaenoic	0	0	0
C21:5	0	0	0
C22, Behenic	0	0	0
C22:1, Erucic	0	0	0
C22:4, Clupanodonic	0	0	0
C22:5, Docosapentaenoic	0.0	0.2	0.2
C22:6, Docosahexaenoic	0	0	0
C24, Lignoceric	0	0	0
C24:1	0	0	0

Table 8. **Fatty acid components in 10, 45, 60% fat diets.** Myristic acid is ten and fourteen times higher in 45% and 60% fat diet than 10% fat diet. Palmitic, stearic, oleic and linoleic are all around six or eight folds higher in 45% and 60% fat diet compared with 10% fat diet, respectively.

Group of Inhibitors		**Name**	**IC50 (against hNMT and/or tested cells)**
Monocyclic compounds	Metal complexes	Cu(II) complexes	12.2 μM to NMT
		Mn(III) complexes	16.1 μM to NMT
	Benzenesulfonamides	N-heterocyclic sulphonamide derivative	4 μM to NMT
	a,b-unsaturated ketones	Thiol alkylators	4 μM to NMT
		Thiol alkylators	1.5μM to NMT
		6-arylidene-2-dimethylaminomethylcyclo-hexanone hydrochlorides	500 μM to NMT 2.18-2.4 uM to cells
Bicyclic compounds		Core structure: 3-arylidene-1-(4-nitrophenylmethylene)-2-oxo-3,4-dihydro-1H-naphthalenes	54± 2 μM to NMT 0.95-3.2 uM to cells
		Core structure: 3-arylidene-1-(4-nitrophenylmethylene)-2-oxo-3,4-dihydro-1H-naphthalenes	54± 2 μM to NMT 1.1-4.8 uM to cells

***Table 9*. NMT inhibitors with un-characterized targeting location of NMT enzymes** [153]**.**