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Targeting Metastatic Breast Cancer with Statin Drugs and CoQ10

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Targeting Metastatic Breast Cancer with Statin Drugs and CoQ10

By
Jacob Garrett Thrasher

A thesis submitted to the faculty of The University of Mississippi in partial fulfillment of the requirements of the Sally McDonnell Barksdale Honors College.

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ABSTRACT

Jacob Garrett Thrasher: Targeting Metastatic Breast Cancer with Statin Drugs
(Under the direction of Dr. Yu-Dong Zhou and Dr. Dale George Nagle)

Statin drugs are commonly prescribed to lower cholesterol levels in patients, but recently statins have been under investigation as a potential anti-cancer drug. Statins are HMG-CoA reductase inhibitors and subsequently inhibit the mevalonate pathway and cholesterol synthesis. Since cholesterol trafficking is vital in the growth and metastasis of cancer, statin drugs are being studied to explore their effects on cancer cells. CoQ10, one of the products from the mevalonate pathway, also has shown some potential anti-cancer properties, but its use to combat cancer is controversial. While some studies have shown that CoQ10 supplements can be synergistic with other cancer treatment methods, the biochemical mechanisms of CoQ10’s potential anti-cancer properties are poorly understood. In this experiment, the anti-cancer potentials of lovastatin and CoQ10 were studied using the breast cancer cell lines BoM-1833 (BoM) derived from MDA-231, MCF7/BoM, and T47D. The IC$_{50}$ values for lovastatin were found to be $4.89 \times 10^{-06}$ M (BoM) and $4.06 \times 10^{-06}$ M (MCF7/BoM). The IC$_{50}$ values for CoQ10 were found to be $5.46 \times 10^{-07}$ M (BoM) and $8.86 \times 10^{-07}$ M (MCF7/BoM). IC$_{50}$ values for lovastatin and CoQ10 with the cell line T47D were not found because the drugs did not sufficiently inhibit the growth of the T47D cells.
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LIST OF ABBREVIATIONS

ADAMTS1 A disintegrin-like and metallopeptidase with thrombospondin type 1 motif 1
ATP Adenosine triphosphate
CTGF Connective tissue growth factor
CXCR4 Chemokine CXC motif receptor-4
DMSO Dimethyl sulfoxide
ER Estrogen receptor
EtOH Ethanol
FBS Fetal bovine serum
HER-2 Human estrogen receptor-2
HMG-CoA β-hydroxy β-methylglutaryl coenzyme A
IC$_{50}$ Half maximal inhibitory concentration
IL-11 Interlukin-11
MEK1 Mitogen-activated protein kinase-1
MMP1 Matrix metalloproteinase-1
OATP2 Organic anion transporter protein-2
OPG Osteoprotegerin
OPN Osteopontin
PR Progesterone receptors
RANKL Receptor activator of nuclear factor kappa-B ligand
Rheb Ras homologs enriched in brain
SRB Sulforhodamine B
TCA Trichloroacetic acid
1 Introduction

1.1 The Value of a Biochemical Approach to Cancer Research

Studying cancer and looking for new therapies can be approached from many different perspectives such as genetic approaches, biological approaches, and biochemical approaches. Cancers utilize altered metabolic pathways, so studying the biochemistry of cancers is a great way to understand how and why these altered pathways work and potentially target these pathways for treatment. One of the most recognized altered biochemical tumor pathways is known as the Warburg effect.1 This altered pathway affects the metabolic state of cancer by changing the means of energy production in cancer cells.1 Normal cells produce ATP through oxidative phosphorylation, but the Warburg effect is where cancer cells predominantly produce their ATP through an increased rate of aerobic glycolysis.1 Cholesterol metabolism is another pathway that is disrupted in cancer cells and this altered metabolism supports the uncontrolled cell growth which is a key feature of cancer. This cholesterol metabolic change causes high concentrations of cholesterol in mitochondria which in turn may contribute to the Warburg effect while changing the chemistry of the mitochondrial membrane to prevent mitochondrial apoptosis.2 Better understanding these compromised pathways open up doors for new drug targets and a better overall understanding of cancers.

1.2 Metastasis

The most life-threatening attribute of cancer is metastasis, the spread and vigorous growth of cells from the primary neoplasm to distant organs. The spread of cancer to distal organs is not a random process, but one that is controlled by the properties of both the
tumor and the micro-environment of the target organ. The first explanation for this non-random pattern of metastasis was published in 1889 by Stephen Paget and is known as the “seed and soil” hypothesis. He stated that the outcomes of metastasis depended on the properties of the specific tumor cell, the ‘seed,’ and the environment of specific organs, the ‘soil.’ The ‘seed and soil’ hypothesis has stood strong for over 100 years, and is the foundation for modern metastatic studies.

Metastasis occurs through a complex multistep process: invasion of cancer cell through the basement membrane, invasion of the vascular system, survival in cardiovascular circulation, attachment to blood vessel walls, extravasation to an organ, and the colonization and growth of a neoplasm. Each one of these steps have to be successfully executed for the cancer to spread to a new organ, and abrogation of any of the steps in this process can halt the metastasis. Advances in understanding the mechanisms behind metastasis are not advanced as other developments in the cancer field; therefore, there are many opportunities for new therapies to arise from metastatic studies.

The success of metastasis is dependent on both the properties of the tumor cells and the properties of the target site. The ability for tumors to be heterogeneous promotes a cancers ability to metastasize. At the beginning of initial tumor formation, most neoplasms are already heterogeneous and contain multiple subpopulations of cancerous cells. Only a few cells from the primary tumor need to possess metastatic qualities for a distal neoplasm to form. Some cells in the primary tumor may contain some metastatic properties, but only the cells that possess all the necessarily qualities for metastasis will actually metastasize. Metastases can have unicellular or multicellular origins just like primary neoplasms, and therefore neoplasms can also be heterogeneous.
Table 1.1 Each sequential step of metastasis is likely regulated by mutations and modifications in the cancer cell. Only cells that have the mutations (symbolized by ✔) for all the steps will successfully undergo metastasis and form a secondary neoplasm. Metastasis can be blocked at any step which would prevent the secondary neoplasm from forming. Not only must the cell have the mutations for the traits above, the cell also must find the proper metastatic site.⁵

Figure 1.1. A primary neoplasm going through the sequential metastatic steps to form a secondary neoplasm.
1.3 Organotropism

Metastasis is a non-random process contrary to previous belief. Recent studies have confirmed the process is specific and relies on intricate tumor-stroma interactions.\(^5\) The ability for certain cancers to spread to specific organs is known as organotropism, and each type of cancer has a specific organotropic pattern. In the 1970s, Fider and co-workers observed in a metastatic assay that the cancer cells derived from a specific secondary site have heightened metastatic affinity to the specific organ.\(^6\) Tumor gene expression profiling studies support the organotropism hypothesis by revealing distance gene expression patterns for organ-specific secondary neoplasms.\(^7,8\) Breast cancer is responsible for over 40,000 deaths annually in the United States, most of which are due to metastasis to other organs.\(^9\) Breast cancer usually metastasizes to lung, bone, brain, or liver tissue. Breast cancer metastasis to the kidney, spleen or uterus tissue is very rare.\(^9\)

Figure 1.2. *The most common metastatic sites for breast cancer.*
1.4 Bone Metastatic Breast Cancer

Bone metastasis is one of the most studied organotropisms because bone metastasis is extremely common in late stage breast, lung, and prostate cancers and because of the way normal bones maintain homeostasis.\(^9\) Two cell types, bone-building osteoblasts and bone-degrading osteoclasts, maintain bone homeostasis and their balance is destabilized when cancers metastasize to the bone.\(^9\) Under non-cancerous conditions, osteoblasts and osteoclasts actively remodel bone to preserve the strength of the skeletal system, but loss of their homeostasis leads to diseases such as osteoporosis and osteopetrosis. Breast cancer can tip the balance to generate either in favor of osteoblasts or osteoclasts, but most often the bone metastases are osteolytic.\(^9,10\)

Breast cancer cells are often not able to carry out bone alteration because these metastatic cells are not specialized to do so. Yet, bone metastatic cells secrete factors to promote the production of receptor activator of nuclear factor kappa-B ligand (RANKL), an essential signaling molecule for osteoclast differentiation, and reduce the expression of osteoprotegerin (OPG), a decoy receptor for RANKL.\(^10,11\) By altering the production of RANKL and OPG, the bone metastatic breast cancer cells promote the activation of osteoclasts which leads to bone degradation.

1.5 MDA-MB-231

The MDA-MB-231 is a bone metastatic breast cancer cell line that is triple negative meaning that estrogen receptors (ER) and progesterone receptors (PR) are not present and they do not overexpress human epidermal growth factor receptor-2 (HER-2).\(^12,13\) Bone metastatic sub-populations of MDA-MB-231 were genetically profiled and have been
found to contain bone metastasis gene signatures like osteopontin (OPN), connective tissue growth factor (CTGF), interleukin 11 (IL-11), CXC motif chemokine receptor 4 (CXCR4), matrix metalloproteinase-1 (MMP1), and ADAM metallopeptidase with thrombospondin type 1 (ADAMTS1).\textsuperscript{5,14} This cell line also has high expressions of Ras and Rho family proteins.\textsuperscript{13} The Ras and Rho family proteins are small signaling G proteins which are known as GTPases.\textsuperscript{13}

This cell line is an epithelial breast cancer line from a 51-year-old Caucasian female. The line was established from a pleural effusion of the woman who had a metastatic mammary adenocarcinoma.\textsuperscript{13} MDA-MB-231 is a highly invasive and aggressive line of triple-negative breast cancer that belongs to the claudin-low molecular subtype classification.\textsuperscript{12} Morphologically, these cells are endothelial-like and have stellate projections that can bridge cell colonies.\textsuperscript{15} The MDA-MB-231 cell line is commonly used for drug discovery studies and is an established tool for bone metastasis research.\textsuperscript{16}

\subsection*{1.6 Cholesterol and the Mevalonate Pathway}

Cholesterol is a vital part of membrane bilayers because it plays key roles in their structure, stability, and function. Some specific functions of cholesterol are regulating the activity of membrane-bound transporters, ion channels, and signaling molecules along with being the precursor in steroid hormone and bile acid synthesis.\textsuperscript{17} Although cholesterol comes from diet and ends up as many different products in different cells, the main pathway used to make cholesterol for cellular functions is its \textit{de novo} synthesis from acetyl-CoA.\textsuperscript{17} This pathway is known as the mevalonate pathway, illustrated in Figure 1, and produces not only cholesterol but also dolichol, ubiquinol, and isoprenoids. The rate limiting step
of the mevalonate pathway is the reduction of HMG-CoA to mevalonate which is catalyzed by HMG-CoA reductase. To get HMG-CoA, two acetyl CoAs react with thiolase as the catalase to produce acetoacetyl-CoA which is then catalyzed by HMG-CoA synthase to make HMG-CoA. Mevalonate kinase then phosphorylates the mevalonate to pyrophosphomevalonate which is then converted to isopentenyl pyrophosphate by the enzyme mevalonate-5-pyrophosphate decarboxylase. Isopentenyl pyrophosphate can reversibly convert to dimethylallylpyrophosphate which can in turn react with isopentenyl pyrophosphate with the aid of the enzyme farnesyl diphosphate synthase to make the 10-carbon isoprenoid geranyl pyrophosphate. Another unit of isopentenyl pyrophosphate is added to generate farnesyl pyrophosphate which can then accept another isopentenyl pyrophosphate to synthesize geranylgeranyl pyrophosphate, or the isoprenoid geranyl pyrophosphate can be used to synthesize cholesterol, squalene, dolichol ubiquinone and other products.

Farnesyl pyrophosphate and geranylgeranyl pyrophosphates are used to prenylate proteins in the Ras, Rho, and Ras homolog enriched in brain (Rheb), family which are overexpressed in cancers and cause unregulated growth. These isoprenoids act as lipid anchors for signaling proteins including the GTPases Ras and Rho families which have been identified as oncogenes. In cancers, the mevalonate pathway is upregulated so more cholesterol is also produced. Geranylgeranyl pyrophosphates are also used in the synthesis of CoQ10, an important factor in mitochondrial respiration. Enhanced cholesterol requirements are very closely associated with cell proliferation and tumor growth.
Figure 1.3 Mevalonate pathway with statin drug target and isoprenylated proteins labeled.
1.7 Statins

Statins are a class of drug commonly prescribed for cholesterol reduction. There are currently seven FDA approved statin drugs which are given to patients to control cholesterol levels and reduce the risk of stroke, heart attack, and death by heart disease.\textsuperscript{23} The serious adverse effects of statin treatment are rhabdomyolysis (0.44 cases per 10,000 patients) and liver failure (1 case per million patients) are very rare.\textsuperscript{14,23} Statins are a 3-hydroxy-3-methyl-glutaryl-coenzyme A (HMG-CoA) reductase inhibitors which reversibly inhibit the conversion of HMG-CoA to mevalonate. Inhibiting this conversion inhibits cholesterol biosynthesis because mevalonate is the rate-limiting step of the reaction.\textsuperscript{14} Although statins have a similar basic structure, they have variable moieties and therefore different properties. The lipophilic statins are lovastatin, simvastatin, fluvastatin, pitavastatin, and atorvastatin.\textsuperscript{7,14} The hydrophilic statins are pravastatin and rosuvastatin.\textsuperscript{7,14} The solubility of the drug affects how the drugs enter liver cells; the lipophilic statins enter the cells by diffusion and the hydrophilic statins enter the cells via the organic anion transporter protein 2 (OATP2).\textsuperscript{7} Because the statins have different chemical structures and different pharmacokinetics, it is plausible that specific statins will differentially interact with the biochemical processes in different cancers.

\textit{In vivo} studies done mostly on rodent models, show support for the potential role of statins in tumor suppression. In one rodent model-based breast cancer study, rats on simvastatin had a lower incidence rate of radiation-induced mammary tumors\textsuperscript{24} and another study showed lovastatin reduced sarcomatoid mammary carcinoma formation and metastasis in a mouse model.\textsuperscript{10} Lipophilic statins were also found to possess significant
antitumor properties in mice by decreasing phosphorylated mitogen-activated protein kinase-1 (p-MEK1) and mitogen activated protein kinase 2 (MEK2) levels that act in Ras and Raf cascades that drive cell proliferation.\textsuperscript{11} Anti-cancer properties of statins in animal models were also shown for ovarian tumors,\textsuperscript{18} mammary tumors,\textsuperscript{25} lung tumors,\textsuperscript{26} and prostate tumors.\textsuperscript{27}

\textbf{Figure 1.4} \textit{Hydrophilic and lipophilic statin drug structure.}
1.8 Lovastatin

Lovastatin is a lipophilic 3-hydroxy-3-methyl-glutaryl-coenzyme A (HMG-CoA) reductase inhibitor. This drug reduces the synthesis of intracellular cholesterol and the isoprenoids farnesyl pyrophosphate and geranylgeranyl pyrophosphate by inhibiting the conversion of HMG-CoA to mevalonate.\textsuperscript{18} The empirical formula of lovastatin is $C_{24}H_{36}O_5$ and has a molecular weight of 404.547 g/mol.

Lovastatin is commonly prescribed to reduce cardiovascular disease incidences and to treat hyper-cholesterolemia.\textsuperscript{18} Lovastatin can be found naturally in certain mushrooms (e.g., oyster mushrooms) such as \textit{Pleurotus ostreatus},\textsuperscript{28} but the natural product was originally isolated from \textit{Aspergillus terreus}.\textsuperscript{29} In its native form, lovastatin is inactive and is in what is known as its lactone form. The lactone ring in the inactive form is hydrolyzed \textit{in vivo} to the $\beta$-hydroxy acid form of lovastatin which is the active form.\textsuperscript{30} The $\beta$-hydroxy acid site of the active form of lovastatin resembles the structure of HMG-CoA, therefore can bind to HMG-CoA reductase and act as a reversible inhibitor to block the function of the enzyme and reduce the amount of mevalonate.\textsuperscript{31}

![Figure 1.5](image_url)

\textbf{Figure 1.5.} The inactive lactone form of lovastatin and the active the $\beta$-hydroxy acid form of lovastatin.\textsuperscript{31}
Figure 1.6. Structural similarity between HMG-CoA and the active form of lovastatin, and the mechanism of inhibition.\textsuperscript{31}

Lovastatin also reduces the amount of CoQ10 produced because farnesyl pyrophosphate from the mevalonate pathway is used for its synthesis.\textsuperscript{19} Lovastatin also decreases the levels of CoQ10’s lipoprotein transport carriers.\textsuperscript{32} In animal studies, CoQ10 levels in both blood and tissues were depleted after lovastatin treatment.\textsuperscript{32} In some patients on high-dose lovastatin therapy, mitochondrial function was also decreased in patients who had low levels of CoQ10 in the muscle.\textsuperscript{33} This mitochondrial depletion of CoQ10 can cause side effects in some patients such as muscle aches and weakness which may prevent some patients from tolerating lovastatin and other statin therapy.\textsuperscript{33}
2 Methods

2.1 Introduction to cell viability testing

Cell viability testing is the most commonly used assay to determine the number of viable cells in multi-well plates. This method is often used to screen compounds to determine if those molecules can inhibit cell proliferation and show cytotoxic effects that cause cell death. Some more elaborate cell viability methods can be used to monitor metabolic activity, enzymatic activity, and protease activity. The method used in this experiment, the sulforhodamine B method, is discussed in the methods section. This experiment tests the effects of lovastatin (Mevacor®) and CoQ10 at different concentrations on different breast cancer cell lines, MDA-MB-231, BoM-1833 (BoM) derived from MDA-MB--231, MCF7/BoM, and T47D.

2.2 Cell lines and cell culture

Human breast tumor T47D, and MDA-MB-231 cells were purchased from ATCC. The MDA-MB-231-derived subclones BoM-1833 (BoM, bone metastatic) and MCF7/BoM were generated by Dr. J. Massagué’s group at the Memorial Sloan Kettering Cancer Center, New York City, New York. The cells were maintained in RPMI 1640 media with L-glutamine (2 mM) (Corning), supplemented with fetal bovine serum [FBS, 10% (v/v), Hyclone], penicillin (50 units/mL) and streptomycin (50 µg/mL) (Lonza) (referred to as ‘complete media’).
2.3 Preparation of Lovastatin and CoQ10 solutions

Both CoQ10 and lovastatin were purchased from Sigma-Aldrich, dissolved in specified solvents to obtain stock solutions (CoQ10: 1 mM in EtOH; and lovastatin: 4 mM in DMSO), and stored at –20°C. In serum-free media, 100 µL of lovastatin solution was prepared as a 2x working solution (final concentrations in a volume of 200 µL at 0.03 µM, 0.1 µM, 0.3 µM, 1 µM, 3 µM, 10 µM, and 30 µM, respectively). For CoQ10, the final concentrations are 0.1 µM, 0.3 µM, 1 µM, 3 µM, and 10 µM.

2.4 Sulforhodamine B (SRB)-based cell viability assay

Cells were seeded at the density of 30,000 cells/well into 96-well plates in a volume of 100 µL/well complete media and incubated at 37°C in a humidified environment that contains 5% CO₂:95% Air for 24 hours. After incubation, test compounds, CoQ10 and lovastatin, diluted separately in serum-free RPMI 1640 media with L-glutamine (2 mM) and penicillin/streptomycin were added in a volume of 100 µL/well. The compounds were prepared as stock solutions in EtOH (for CoQ10) or DMSO (for lovastatin), and the final solvent concentrations were ≤ 0.25% (v/v). The incubation continued for another 48 h and the cells were fixed with trichloroacetic acid (TCA) by replacing 100 µL conditioned media with an equal volume of 20% TCA in 1x PBS. After 1 h at 4°C, the plates were washed four times with tap water and air dry. The fixed cells were stained with 0.4% SRB (w/v, 1% acetic acid) in a volume of 100 µL/well at room temperature for 10 min. The plates were washed four times with 1% acetic acid and air dry. A Trizma® base solution (10 µM) was added in a volume of 200 µL/well to solubilize SRB. The plates were read at 490 nm
with background at 630 nm subtracted. Cell viability data are presented as ‘% Control’ calculated using the formula where OD is the optical density:

\[
\text{% Control} = \left( \frac{\text{OD}_{\text{compound}}}{\text{OD}_{\text{control}}} \right) \times 100
\]

The % Control data can be used to calculate the ‘% Inhibition’ using the formula:

\[
\text{% Inhibition} = \left[ 1 - \left( \frac{\text{OD}_{\text{compound}}}{\text{OD}_{\text{control}}} \right) \right] \times 100
\]
3 Results and Discussion

3.1 Percent Inhibition curves

The percentage inhibition of lovastatin and CoQ10 at different concentrations in different cell lines was graphed to show the inhibition as it related to concentration. These graphs were generated using the software Prism 7 (GraphPad).

Figure 3.1. Percentage inhibition of BoM cell line by different concentrations of lovastatin. C is the control which is the cell line in media with no added lovastatin. Data shown are average ± standard deviation (n=4). The IC$_{50}$ value for lovastatin on this cell line is 4.89×10$^{-6}$ M.
Figure 3.2. Percentage inhibition of MCF7/BoM cell line by different concentrations of lovastatin. C is the control which is the cell line in media with no added lovastatin. Data shown are average ± standard deviation (n=4). The IC$_{50}$ value for lovastatin on this cell line is 4.06×10$^{-6}$ M.
Figure 3.3. Percentage inhibition of T47D cell line by different concentrations of lovastatin. C is the control which is the cell line in media with no added lovastatin. Data shown are average ± standard deviation (n=4). There is no IC$_{50}$ value for lovastatin treatment of this cell line.
Figure 3.4. Percentage inhibition of BoM cell line by different concentrations of CoQ10. 

C is the control which is the cell line in media with no added CoQ10. Data shown are average ± standard deviation (n=4). The IC$_{50}$ value for CoQ10 on this cell line is $5.46 \times 10^{-7}$ M.
Figure 3.5. Percentage inhibition of MCF7/BoM cell line by different concentrations of CoQ10. C is the control which is the cell line in media with no added CoQ10. Data shown are average ± standard deviation (n=4). The IC$_{50}$ value for CoQ10 on this cell line is 8.86×10$^{-7}$ M.
Figure 3.6. Percent inhibition of T47D cell line by different concentrations of CoQ10. C is the control which is the cell line in media with no added CoQ10. Data shown are average ± standard deviation (n=4). The IC$_{50}$ value for CoQ10 on this cell line is $2.15 \times 10^{-7}$ M.

The above graphs illustrate the inhibitory effects lovastatin and CoQ10 have on the breast cancer cell lines BoM, MCF7/BoM, and T47D. The inhibition data for MDA-MB-231 was not included because the high cell concentration produced too much stress for this cell line. The cells were unhealthy after the first incubation and the media turned yellow, so the data was excluded from this experiment.
The lovastatin inhibition graphs for BoM and MCF7/BoM cell lines show sigmoidal curves which both reach 98% inhibition at high concentrations (30 \( \mu \text{M} \)) of lovastatin. For the T47D cell line, the lovastatin treatment barely inhibited the cell with the highest concentration of lovastatin (30 \( \mu \text{M} \)) only inhibiting growth by 22%.

The CoQ10 inhibition graphs for BoM and MCF7/BoM also show sigmoidal curves reaching about 98% inhibition at the maximum concentration of CoQ10 (10 \( \mu \text{M} \)). The T47D cell line showed a sigmoidal curve when treated with CoQ10, but was only inhibited by 91% at the highest concentration of CoQ10 (10 \( \mu \text{M} \)).

The half-maximal inhibitory concentration, IC\(_{50}\), was determined from the percent inhibition graphs. The IC\(_{50}\) is the concentration of a particular drug that is needed to inhibit a biological process, in this case cell growth, by half. The lower the value of the IC\(_{50}\) of a drug means the drug is more potent. The IC\(_{50}\) values are listed in the chart below.

<table>
<thead>
<tr>
<th>Cell line</th>
<th>IC(_{50}) Lovastatin</th>
<th>95%CI Lovastatin</th>
<th>IC(_{50}) CoQ10</th>
<th>95%CI CoQ10</th>
</tr>
</thead>
<tbody>
<tr>
<td>BoM</td>
<td>4.89×10(^{-6}) M</td>
<td>n/a*</td>
<td>5.46×10(^{-7}) M</td>
<td>4.56×10(^{-7}) -6.35×10(^{-7})</td>
</tr>
<tr>
<td>MCF7/BoM</td>
<td>4.06×10(^{-6}) M</td>
<td>n/a*</td>
<td>8.86×10(^{-7}) M</td>
<td>8.517×10(^{-7}) -9.56×10(^{-7})</td>
</tr>
<tr>
<td>T47D</td>
<td>n/a</td>
<td>n/a</td>
<td>2.15×10(^{-7}) M</td>
<td>n/a*</td>
</tr>
</tbody>
</table>

Table 3.1. IC\(_{50}\) values and 95% CI for lovastatin and CoQ10 on different breast cancer cell lines. There is no IC\(_{50}\) value for lovastatin with the T47D cell line because the compound did not completely inhibit the cell replication at high concentrations. *There is no 95% CI for these IC\(_{50}\) values because the IC\(_{50}\) was determined from the best-fit curve.
The IC$_{50}$ values for lovastatin were similar for both the BoM and MCF7/BoM cell lines, 4.89×10$^{-6}$ M and 4.06×10$^{-6}$ M respectively. There was no IC$_{50}$ value for lovastatin for the T47D cell line and at the highest concentrations of lovastatin, there was no more than 22% inhibition. The IC$_{50}$ values for CoQ10 were 5.46×10$^{-7}$ M for BoM, 8.86×10$^{-7}$ M for MCF7/BoM, and 2.15×10$^{-7}$ M for T47D. Treatment with CoQ10 inhibited T47D up to 91% at the concentration of 10 µM, so the IC$_{50}$ value was estimated using the best fit sigmoidal curve from Figure 3.6.

One difference between T47D and the other cell lines is that BoM and MCF7/BoM are estrogen independent, bone metastatic cell lines, which T47D is estrogen dependent and not bone metastatic. The mevalonate pathway could possibly play a bigger role in cell replication and growth for the BoM and MCF7/BoM cell lines compared to the role it plays in T47D. Because both lovastatin and CoQ10 inhibit BoM and MCF7/BoM, cholesterol regulation seems important for these bone metastatic cell lines.

The inhibition caused by lovastatin could be due to a couple of different reasons. First, inhibiting HMG-CoA reductase inhibits the farnesyl pyrophosphate synthesis which is a precursor for the synthesis of many molecules including CoQ10. Decreasing the amount of CoQ10 available will inhibit ATP synthesis through oxidative phosphorylation, although this would probably not affect cancer cells as much considering their glycolytic nature. To see if the inhibition caused by lovastatin is related to CoQ10 production from the mevalonate pathway, a drug combination assay could be done. If adding CoQ10 to cells treated with lovastatin reverses the inhibition effects, then the cell growth inhibition could likely be caused by the lovastatin preventing production of CoQ10.
Inhibiting HMG-CoA reductase inhibits the production of farnesyl pyrophosphate and gernylgernyl pyrophosphate which are vital in the post-translational modification of many GTPases specifically those in the Ras and Rho family which are upregulated in cancers.\textsuperscript{34,35} These isoprenoids act as lipid attachments for intracellular signaling molecules like Rho and Ras GTPases. Because Ras and Rho play significant roles in cancers by promoting cell cycle progression, resisting apoptosis, neovascularization, and promoting metastasis, preventing necessary post-translational modification for these proteins will inhibit cancer cell growth.\textsuperscript{35} Perhaps the isoprenylation of GTPases is more vital for cell survival in the BoM and MCF7/BoM cell lines than it is in the T47D cell line, but an assay that specifically quantifies the isoprenylation of GTPases in the presence and absence of lovastatin for the different cell lines would be needed to draw that conclusion.

The inhibition caused by CoQ10 is more elusive in explanation and its beneficial effects on cancer is highly controversial. Several human studies have tested adding CoQ10 supplements with traditional chemotherapy which showed CoQ10 aiding in the prevention of metastasis, improved quality of life, and in some patients, apparent partial remission.\textsuperscript{36} The mechanisms explaining these effects of CoQ10 are poorly understood. One study found that CoQ10 inhibits the activity of matrix metalloproteinase 2 (MMP-2), an important molecule in metastasis. The MMP-2’s activity is mediated by mitochondrial ROS and CoQ10 is a mitochondrial ROS regulator, so CoQ10 inhibits MMP-2’s activity most likely through ROS regulation.\textsuperscript{37} Addition of CoQ10 could possibly regulate other reactions through altering ROS production, but many more specific assays would need to be performed before drawing this conclusion. Another possible explanation could lie in membrane dynamics. The compound CoQ10 is found contained between membrane
bilayers, so very high concentration of extracellular CoQ10 could have caused the inhibition in this specific experiment by altering membranes. Membrane surface analysis using methods such as cryo-electron microscopy, laser scanning microscopy, or total internal reflection fluorescence may help to address this issue.
4 Conclusion

These experiments tested the effects of lovastatin and CoQ10 on the breast cancer cell lines, MDA-MB-231, BoM-1833 (BoM) derived from MDA-231, MCF7/BoM, and T47D. Percent inhibition curves based on concentration were generated, and IC$_{50}$ values were obtained. The MDA-MB-231 cell line was too stressed during the experiment, so the percent inhibition data for it was discarded because it was not reliable. The IC$_{50}$ values for lovastatin were found to be 5.46×10$^{-07}$ M for BoM and 4.06×10$^{-06}$ M for MCF7/BoM, but there was no IC$_{50}$ value for the T47D trial because lovastatin did not sufficiently inhibit the cell lines growth. The IC$_{50}$ values for CoQ10 were 5.46×10$^{-07}$ M for BoM, 8.86×10$^{-07}$ M for MCF7/BoM, and 2.15×10$^{-07}$ M for T47D. These results suggest that lovastatin and CoQ10 are both viable drugs to further investigate as potential anti-cancer drugs.
5 References


