Introduction

Cancer remains one of the most damaging diseases in the modern world. Longer life expectancy has lead to increased numbers of cancer patients, despite improvements in living conditions and healthcare. In Canada, an estimated 177,800 cases of cancer were diagnosed, and 75,000 cancer patients will die in 2011. Of the 84,400 new cases estimated for Canadian women in 2011, 23,400 (27.6 %) will be cases of breast cancer (CCS 2011). Clearly, cancer treatments (particularly that of breast cancer) are of high value to society. Although significant improvements in treating cancer have been made, the amount of research to be done is still significant. Greater knowledge about the underlying mechanisms of cancer have led to even greater uncertainty about its nature. It is for this reason that cancer is the dominant area of research in medical and scientific communities today. The term “cure for cancer” remains the epitome of scientific discoveries.

Breast Cancer and Metastasis

As mentioned above, breast cancer is the most commonly diagnosed type of cancer in women. Tumors are formed when cells begin to multiply at an unchecked rate. These unchecked cells form tumors. The cause of initial tumor growth has been heavily researched, and contributing factors are too many to name here. Although tumors are the initial form of cancer, in organs like the breast, which are not critical to bodily function, the primary tumor is rarely the cause of death (Chambers et al 2002). Tumors in the breast most commonly develop from the epithelial tissue lining the breast duct. These are known as *adenocarcinomas*, and are particularly dangerous because of their predilection to metastasis (Pantel and Brakenhoff 2004). The ability of a primary tumor to metastasize has been linked to a number of factors, primarily gene expression (van’t Veer 2002), associated cells (Karnoub et al 2007), and the state of the tissues around the tumor (McDaniel et al 2006). In breast ductal carcinomas, tumors become especially prone to metastasis when they breach the basal membrane of the duct, and enter the

lymphatic system or the bloodstream, where even a single cell can be carried to other parts of the body and establish a secondary tumor (Place et al 2011). These disseminated cells pose an additional risk to patients, since they remain in the body even after the primary tumor is removed, and is one of the contributing factors towards relapse after seemingly successful treatment (Klein et al 2002). The location of secondary tumors, is also a study of much research, and there have been many factors suggested which may contribute to the preference of some metastasized cells to certain tissues, but they are too many to name here. The common sites of metastasis for human breast cancer are the lungs, bones, and liver (Place et al 2011).

Resveratrol

Resveratrol is a nonsteroidal anti-inflammatory drug (NSAID), which is found in a number of plants. It was isolated in 1974 from *Senna quinquangulata* in Peru (Jang et al 1997), but has been found in a number of other plants, including in many considered food. The most notable source of natural resveratrol is *Vitis vinifera* (the common grape). Research suggests that resveratrol is produced by grapes to protect the plant against fungal infections (making resveratrol a “phytoalexin”)(Dercks and Creasy 1989). Resveratrol was first applied to cancer research in 1997 by Jang *et al*, where they studied the effectiveness of resveratrol on cyclooxygenase (COX) activity. COX primarily regulates the production of prostaglandins and other inflammatory substances, which are often upregulated in cancer cells to increase tumour proliferation. The connection between COX suppression and resveratrol pointed towards a possible chemopreventive role for resveratrol (Jang et al 1997). Initially only COX-1 was believed to be suppressed, but later research indicated that COX-2 was also suppressed by resveratrol treatment. Another promising sign for the usefulness of resveratrol was that suppressed both the transcription and activity of the COX-2 enzyme (Subbaramaiah et al 1998).

Later research focused on the ability of resveratrol to induce apoptosis. The first experiments treated both p53 and p53-deficient cell lines with resveratrol, and discovered that

treatment with resveratrol induced apoptosis in mouse embryo cell lines with functioning p53, but not in p53-deficient lines. Treatment with 30 μM of resveratrol showed the highest levels of p53 activity, with activity peaking at 24 hours after treatment (Huang et al 1999). Later studies showed p53-independent apoptosis was increased in human colon cancer cell lines, indicating that resveratrol has multiple mechanisms to induce apoptosis (Mahyar-Roemer et al 2001). This experiment used resveratrol concentrations of up to 100 μM, and found that mitochondrial proliferation occurred in response to treatment with resveratrol. While some research indicated that p53 is not always required, further research was being performed to understand how resveratrol is able to induce apoptosis via p53. Phosphorylation of p53 at Ser 15 was shown to be induced by resveratrol, which is believed to stabilize p53. This phosphorylation occurs via mitogen-activated proteins (MAPs), including kinases such as c-Jun NH_2-terminal kinases (JNKs), Extracellular-signal-regulated protein kinases (ERKs) and p38 kinase. JNKs, ERKs, and p38 all were shown to have an effect on p53 phosphorylation levels induced by resveratrol, providing a possible segment of the signal pathway between resveratrol and p53 (She et al 2001). Studies that followed shed more light on the complexities of the resveratrol-induced p53 apoptotic pathway, including acetylation following phosphorylation of p53, and the interfering effect of estrogen on apoptosis (Zhang et al 2004).

A breakthrough in resveratrol research occurred in 2006 when the receptor for resveratrol was isolated. It was found that Integrin αVβ3 contains a receptor site for resveratrol. This was shown to be true in both MCF-7 (estrogen-receptor α positive) and MDA-MB-231 (estrogen receptor α negative) cell lines. Due to the similarity between resveratrol and estrogen (and the interference shown above), it was suspected that resveratrol might have been dependant on estrogen-receptor α. However, this study found that induction of p53 phosphorylation via JNK/ENK activity was induced in both cell lines. The specific receptor site

was found using anti-αVβ3 antibodies, which prevented resveratrol binding, and therefore resveratrol-mediated apoptosis (Lin et al 2006).

Gradually, studies began to emerge which showed that resveratrol had potential as a chemotherapeutic compound. For obvious reasons, the ability of resveratrol to induce apoptosis makes it a potentially useful compound for treating cancer patients. Along with this new research focus, resveratrol was linked to increased COX-2 expression in breast cancer cells. It was found that ERK activity also contributed to an accumulation of COX-2 in the nucleus, where it could be seen to be accumulating in the same location as phosphorylated p53. Data has been presented which show both pro- and anti-apoptotic activity by COX-2, as well as several proposed relationships between COX-2 and p53. However, it was clear that resveratrol treatment led to increased COX-2 activity, and that it was overlapping with p53 activity (Tang et al 2006).

Recently, research into the effects of resveratrol has exploded. The scope has both widened (in the potential application of the drug) and narrowed (seeking answers about more and more specific sections of the activity of the drug). New research is looking into resveratrol's ability to block carcinogens, boost antioxidants, arrest the cell cycle, stop angiogenesis, suppress metastasis, and sensitize tumours before other chemotherapies (Kundu and Surh 2008). Another area of research which is rapidly gaining information is that of *in vivo* trials. A number of organisms have been tested and seen lifespan increases when treated with resveratrol (including yeast, nematodes, insects, and fish). A field of study which is connected to this and has implications for humans is the levels of resveratrol found in various dietary sources (Baur and Sinclair 2006).

Measuring Cell Proliferation

To measure the proliferation of cells, it is necessary to have a way of measuring which cells are alive and which are dead. While it is very difficult to make such a judgement using traditional visual cell-counts, measuring the metabolic activity of the cells is a reliable way of

determining their activity. XTT Assays rely on the cleavage of XTT (a tetrazolium salt) to water soluble formazan by dehydrogenase enzymes in metabolically active cells. The amount of formazan dye in the well is directly equivalent to the number of metabolically active cells (Scudiero et al 1988). The amount of formazan in solution can easily and quickly be measured using absorbance reading, which is particularly quick when a spectrophotometer is used which can process a 96-well plate.

Visualizing Mitotic Arrest

To observe the state of cells in mitosis simply visualizing the DNA using DAPI (MB 2006). 4',6-diamidino-2-phenylindole (DAPI) binds to DNA in the A-T minor groove (Kapuscinski 1995). When this binding occurs, DAPI fluoresces strongly, leading to its common use in DNA staining in many capacities. Its level of fluorescence is suitable for direct observation of nuclear morphology of a cell (Collins et al 1997).

Visualizing Autophagy

Autophagy is an alternative form of programmed cell death which also plays an important role in maintaining homeostasis in the body. Autophagy is tied with apoptosis in several ways, although generally, autophagy is more common in cells not destined for total destruction, while apoptosis is an irreversible process (Eisenber-Lerner and Kimchi 2009). Autophagy is a process which is used primarily for maintenance of cells, especially during times of metabolic stress, when components of cells can be scavenged for energy. This is completed by engulfing subcellular components by vacuoles or lysosomes, where they are digested to produce energy (Klionsky and Emr 2000). This connection with cell survival makes autophagy a double-edged sword for cancer cells. Despite several exceptions, such as the role of autophagic factors in stopping virus-induced apoptosis (Liang et al 1998), cancer cells generally downregulate autophagic genes, and some autophagic factors have been linked strongly to antitumor activity (Liang et al 1999). Autophagy is closely tied with the ability to sort vacuoles directly from the Golgi, diverting vesicles to vacuoles or lysosomes for digestion. This sorting is

reliant on the application of unique protein “tags”. A number of protein complexes are involved in the Golgi network, and most research to this point has been based on *E. coli* autophagic genes, which tend to be highly conserved across many mammalian species (Tanida et al 2004). One of the proteins which is involved in the application of unique autophagic tags is Beclin 1 (Furuya et al 2005). *Beclin-1* down regulation was also found to be present in a number of cancers, including breast carcinomas (Aita et al 1999). Beclin 1 is believed to be integral to the initiation of autophagy, via complex with Vsp-34, and is therefore the natural marker to measure the progress of autophagy in cells. This can be done through a western blot, to separate and label Beclin 1 with a labelled antibody (Liang et al 1999).

This presence of Beclin-1 can be verified via western blot, a well-established process for sorting proteins by SDS-PAGE, and then labelling using specific antibodies (in this case anti-Beclin antibodies derived from rabbits).

Protocol

To get an accurate understanding of the effect of resveratrol on breast cancer cells, two types of cells were chosen: one aggressively metastatic, and one that is not. The presence of Apoptosis was confirmed via western blot for Caspase-3. This method was chosen because Caspase-3 is a well known marker of apoptosis, and immunofluorescence is easily performed with they laboratory equipment available at Concordia.

As with all research, cost was also a prohibitive factor, and the experiment was also designed to keep costs at a manageable level. This played a factor in the choice of the cell lines, as they are provided in-house by Dr. Deborah Hemmerling. Cost was also a factor in choosing the tests to run. The balance between cost and accuracy, as well as limitations on what equipment is available is an important one to make in any laboratory situation, where being cost effective is very important. This helped guide the decision to use an XTT assay - which does not involve dangerous isotopes, or complicated flow cytometry equipment- a logical one.

Timeframe, Location, and Partners

This experiment was carried out in completion of Biology 488/489: Independant Research at Concordia University College of Alberta (CUCA). The lab portion of this experiment ran from November 2012 until its completion by March 15, 2013. The experiment was performed along with Alexandra Bennett, who will be performing the same tests using another drug linked to cancer cell death: Vinorelbine. This partnership with CUCA allowed for supervision under Dr. Deborah Hemmerling, who served as Faculty Advisor, and who has experience researching cancer cells and the effect of various drugs on their cell death. As part of the Bio 488/489 program, lab space and equipment were provided for use at no cost.

Cell Culture

Two cell lines were provided by Dr. Hemmerling, and both are commonly used in research in the field. MDA-MB-231 is a highly metastatic adenocarcinoma cell line, derived from a 51 year old caucasian woman (ATCC 2007). MCF7 is a non-metastatic adenocarcinoma derived from a 69 year old caucasian woman (ATCC 2009). Both cell lines were derived from pleural effusions, are epithelial in morphology, and grow adherently in cell culture. Both have become standard cell lines for research on breast cancer. Both cell lines are also easily cultured, and replicate quickly.

Both cells will be cultured in RPMI 1640 media, with L-glutamine and Phenol Red, produced by Gibco, with 10% Fetal Bovine Serum (FBS). Cells will be cultured in Corning T75 cm^2 cell culture flasks at 37 ^oC and 5% CO_2. Two main cell cultures will be maintained for the course of the experiment, and these cultures will be replenished with RPMI + 10% FSB as needed. As these cultures become crowded (80-85 % confluence), the cultures will be trypsinized and samples taken for subculturing and incubation, and the remainder recultured in a Corning T75 flask at a 1:10 a cell-to-medium ratio.

Cell Proliferation (XTT) Assay

For the XTT assay, 96-well plates will be seeded with a starting concentration of 1×10^{-5} cells/mL for MDA-MB-231 cells and 2×10^{-5} cells/mL for MCF-7 cells. Two plates with 10 replicates per cell line will be set up, 5 replicates of treatment and 5 control. The cells were allowed to adhere for 24 hours in 100 µl of media at which point the treatment was applied (either DRUG or CNTL). The wells designated for DRUG were treated with 100 µl of 30 µM resveratrol solution, replacing the old media, and the wells designated CNTL were treated with 100 µl of fresh RPMI to replace the old media. One plate was treated for 24 hours, and then analyzed. The second plate was treated for 48 hours. To determine the amount of viable cells per well, Biological Industries' XTT based Cell Proliferation kit will be used following the manufacturer's protocols (BI 2012). The proliferation was measured using a spectrophotometer, which provided a printout that contained the absorbance levels of each individual well. A well of

blank media was included as a zero reference. The values were then compiled in a spreadsheet for analysis. One run (5 wells DRUG, 5 wells CNTL per cell line) was performed in November, and 2 more were performed in March. The data was compiled in Microsoft Excel, and One-Way ANOVA was performed to determine statistical significance. Some values were noticeably higher (two- or threefold the absorbance), likely due to bacterial contamination. These values were excluded from the analysis (see "Results" below).

DAPI Staining

MDA-MB-231 and MCF-7 cells were seeded for 24 hours in 6-well plates containing glass coverslips (starting concentration: 1 x 10^{-5} cells/mL for MCF-7 and 2 x 10^{-5} cells/mL for MDA-MB-231; 2 mL per well). After 24 hours to establish growth, a treatment of 30 μM resveratrol was applied to 3 of the wells on each plate, while the other 3 wells had their media replaced with fresh media for control. One plate was incubated with drug/control treatment for 24 hours, and the second plate was incubated for 48 hours, after which the coverslips were removed and the cells were fixed, washed with PBS and stained with DAPI (Invitrogen), and mounted to microscope slides. Mitotic cells were visualized and a rough count was taken by counting 100 cells in a horizontal line, recording the number of cells which were deemed to be mitotic.

Western Blot

Two 12-well plates for each cell line were seeded with a starting concentration of 1 x 10^{-5} cells/mL MDA-MB-231 and 2 x 10^{-5} cells/mL MCF-7 cells. The cells were left to adhere for 24 hours, and then, after the old media was removed, 6 wells on each plate were treated with 30 μMresveratrol solution, and 6 wells were treated with fresh media as control. One plate was treated for 24, and another for 48 hours. After the treatment time had elapsed, the media will be removed and the cells were washed with 100 μl 2X SSB (100 mM Tris-Cl pH 6.8, 4% (w/v) SDS, 0.2% (w/v) bromophenol blue, 20% (v/v) glycerol, 200 mM dithiothreitol) and collected. This collected SSB solution was then boiled to lyse cells, labelled, and frozen to be run later. The

SDS-PAGE gels were poured using a 5% acrylamide stacking gel and a 12% acrylamide separating gel. The presence of Beclin-1 was detected using indirect antibody detection. Rabbit anti-Beclin-1 (Cell Signaling Technology) diluted 33 : 100,000 with a milk-TBST solution. Anti-Rabbit IgG-peroxidase (Sigma-Aldrich) diluted 33 : 100,000 with a milk-TBST solution was used as a secondary antibody. Due to logistical error, the Anti-Rabbit peroxidase available was of inferior quality, and did not produce easily identifiable bands. As a result, it is difficult to verify the accuracy of data that was collected in this experiment.

Thanks to Alexandra Bennett (private correspondence) for information on protocols, and advice on cell culturing, and to Dr. Hemmerling for assistance during Western Blots.

Results, Observations, and Data

Cell Culture

Due to a protocol error, the media used in the first portion of the experiment (November-December 2012) was RPMI 1640 with 5% FBS, when the correct media was RPMI 1640 with 10% FBS. This error was corrected before the cell cultures were restarted in January using the RPMI + 10% FBS. This error led to suboptimal growth, which was especially noticeable in the MCF-7 culture, where it was sometimes difficult to collect enough cells to perform tests. Once the correct media was used, there was noticeable improvement in the growth of the MCF-7 cultures.

Cell Proliferation Assay

Table 1: Proliferation Assay Data for MDA-MB-231 Cells Treated with 30 μM Resveratrol for 24 Hours

Replicate	Treatment	Control
#1 (Nov 28)	0.352	0.311
#2 (Mar 7)	0.135	0.160
#3 (Mar 7)	0.188	0.232
Total Means:	0.225	0.234

Raw Data can be found in Appendix 1

Table 2: Proliferation Assay Data for MDA-MB-231 Cells Treated with 30 μMResveratrol for 48 Hours

Replicate	Treatment	Control
#1 (Nov 28)	0.376	0.317
#2 (Mar 7)	0.213	0.245
#3 (Mar 7)	0.243	0.334
Total Means:	0.277	0.298

Raw Data can be found in Appendix 1

Table 3: Proliferation Assay Data for MCF-7 Cells Treated with 30 μMResveratrol for 24 Hours

Replicate	Treatment	Control
#1 (Nov 28)	0.052*	0.046
#2 (Mar 7)	0.044	0.092
#3 (Mar 7)	0.132	0.142
Total Means:	0.076	0.093

Raw Data can be found in Appendix 1
*A value was omitted due to contamination

Table 4: Proliferation Assay Data for MCF-7 Cells Treated with 30 μMResveratrol for 48 Hours

Replicate	Treatment	Control
#1 (Nov 28)	0.067*	0.053**
#2 (Mar 7)	0.031	0.088
#3 (Mar 7)	0.123	0.179
Total Means:	0.074	0.107

Raw Data can be found in Appendix 1
*2 values were omitted due to contamination
**A value was omitted due to contamination

This data was analyzed for statistical significance using One-way ANOVA.

DAPI Staining

The entire set of 24 hour samples was destroyed due to a misinterpretation of the staining protocol. The majority of the 48 hour slides were successfully stained, except for the MDA-MB-231 Treated Samples. The 231 Control slides were some of the most clearly visualized, but without a corresponding Treatment count to compare to, they have no statistical value. This resulted in only 2 useful counts: MCF-7 48 Hour Treatment (30 μMresveratrol) and Control. Although these results were disappointing, the nature of quantifying mitotic cells visually is one that is far from trustworthy, and is particularly weak to the bias of the researcher. Due to this inherent difficulty, this procedure was never intended to be a major statistical contributor, but mostly act as a means of visualizing mitosis, and to obtain some visual representation of what was occurring. As a result, the data is available from this procedure is limited, but the pictures that were obtained remain useful.

Table 5: Mitotic Cell Count of DAPI Stained MCF-7 Cells After 48 Hours of 30 μMresveratrol Treatment

Replicate	**Treatment (mitotic cells per 100 cells)**	**Control (mitotic cells per 100 cells)**
#1	15	10
#2	13	3
#3	n/a*	6
Mean	14	6.3

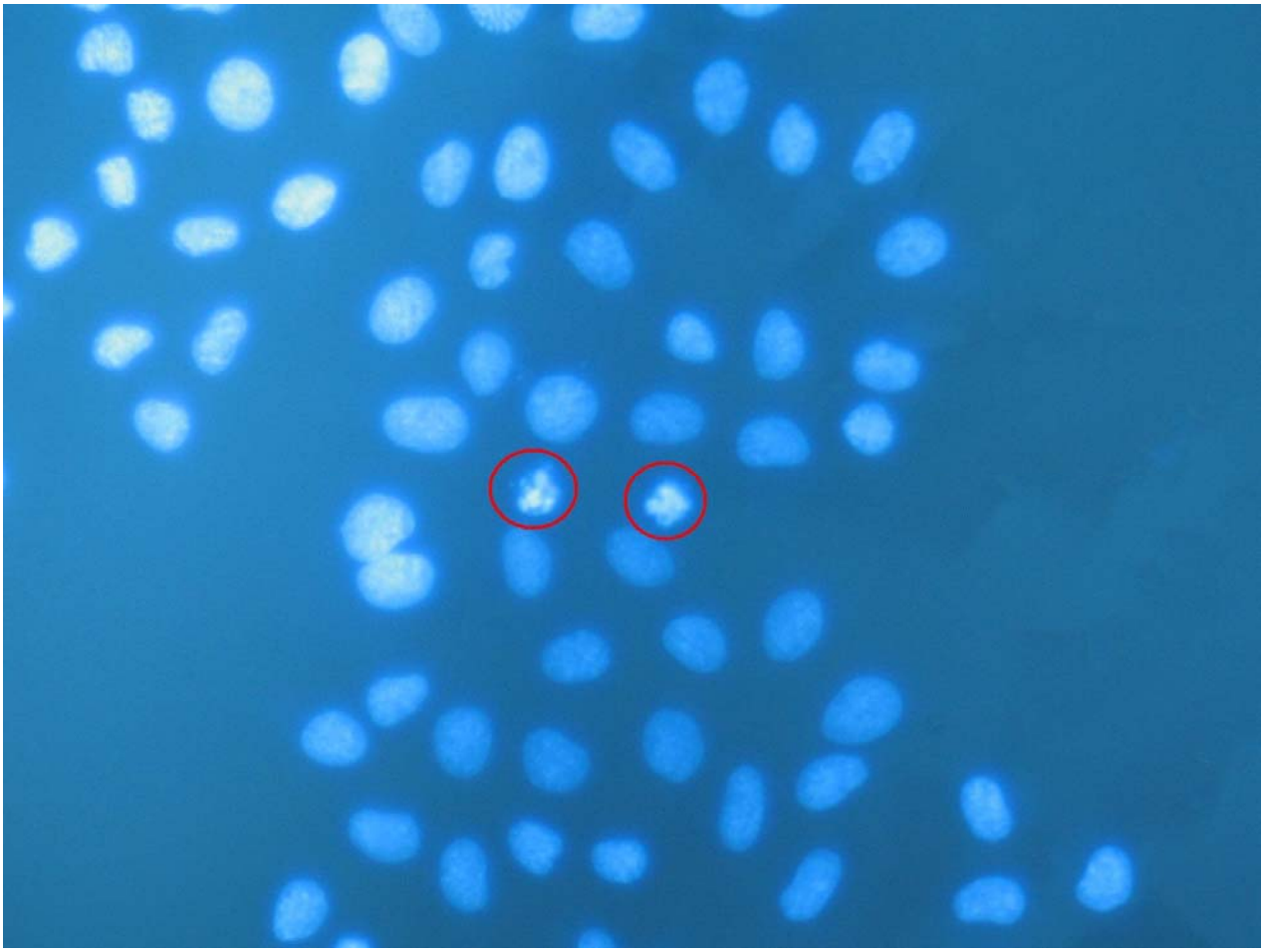

Figure 1: Mitotic MCF-7 Cells (circled) note the tightened chromosomes and fragmented nucleus

Western Blot

The results for the Western Blot are highly subjective, as the secondary antibody used did not produce easily discernable results. What is recorded here is only the best interpretation of the results.

Table 6. Results of MDA-MB-231 Western Blot.

Marker (Cut)	48 Hour Drug	48 Hour Drug	48 Hour Control	48 Hour Control	24 Hour Drug	24 Hour Control	24 Hour Control	24 Hour Drug	Marker
Marker	Faint Band	Faint Band	Band	Band	No Band	Faint Band	Band	Band	Marker

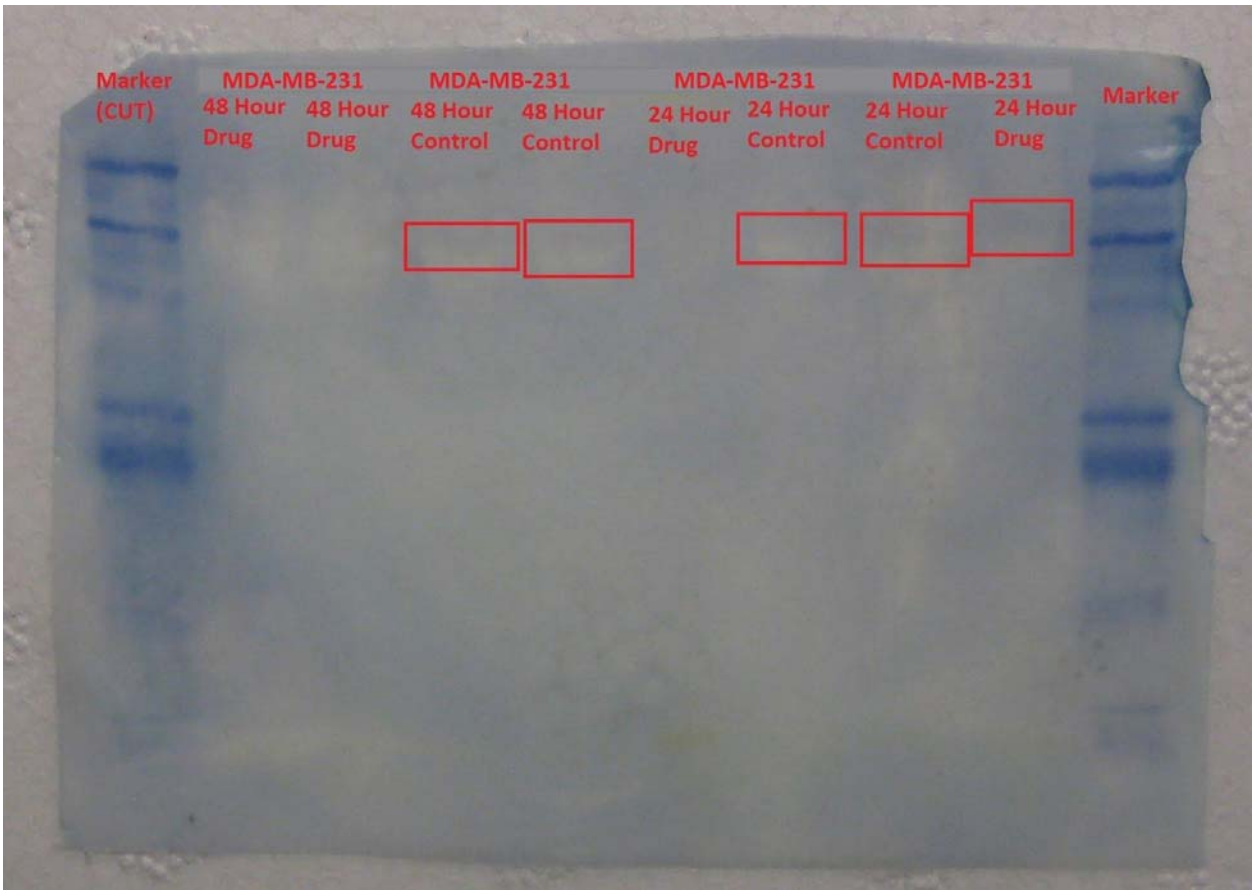

Figure 2. The Western Blot results for MDA-MB-231 cells treated with resveratrol (“drug”) and controls. The bands (representing the presence of Beclin-1) which were visible are highlighted with red boxes.

Table 6. Results of MCF-7 Western Blot after 24 Hours.

Marker	24 Hour Drug	24 Hour Drug	24 Hour Drug	24 Hour Control	24 Hour Control	24 Hour Control
Marker	Band	Faint Band	Band	Faint Band	Faint Band	Faint Band

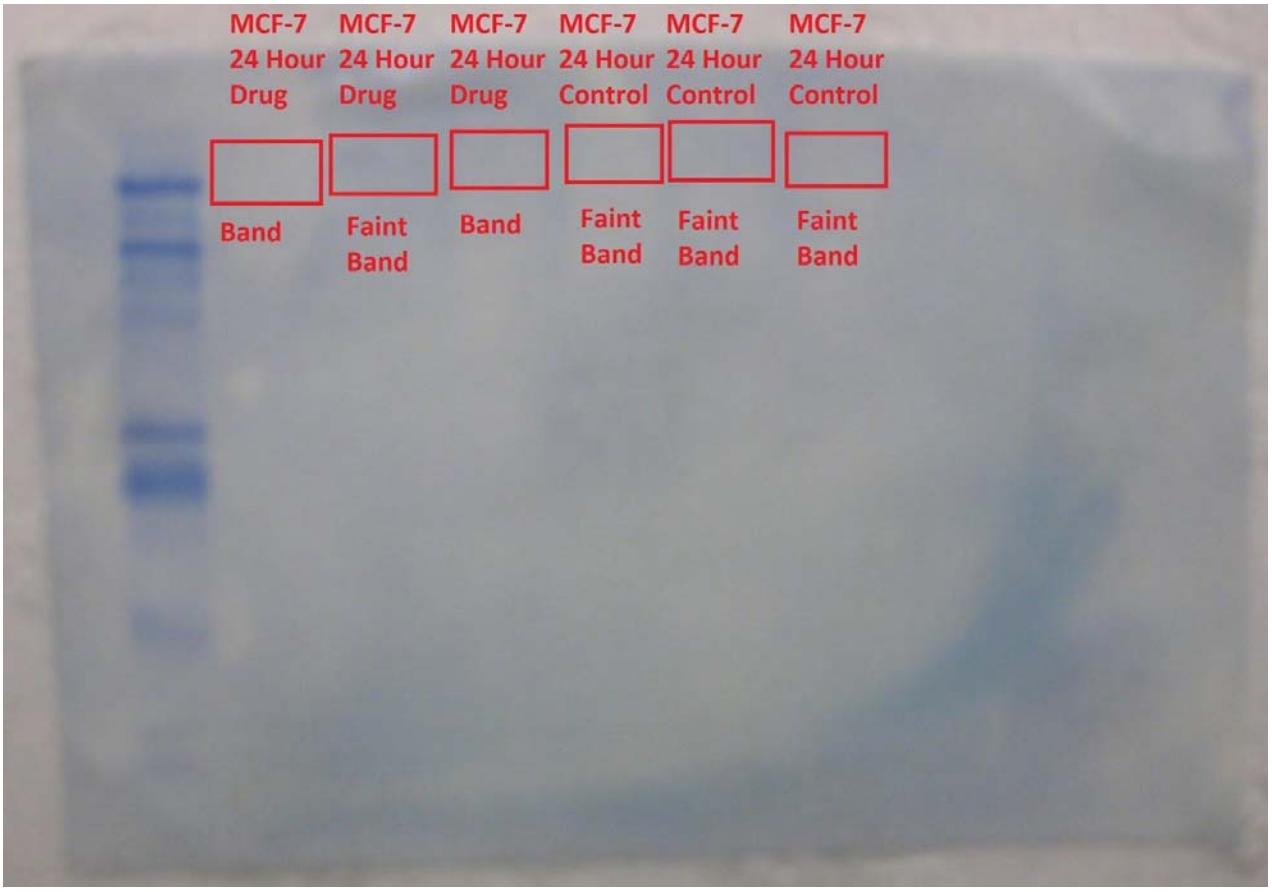

Figure 3. MCF-7 Western Blot results after 24 Hours. Bands indicate the presence of Beclin-1. Although the bands were faint, they were visible at the time of recording, however, they did not capture well in the photograph.

Table 7. Results of MCF-7 Western Blot after 48 hours.

Marker	Marker	48 Hour Drug	48 Hour Drug	48 Hour Drug	48 Hour Control	48 Hour Control	48 Hour Control
Marker	Marker	Faint Band	Band	Band	Faint Band	No Band	No Band

Figure 3. MCF-7 Western Blot results after 48 Hours. Bands indicate the presence of Beclin-1. Although the bands were faint, they were visible at the time of recording, however, they did not capture well in the photograph.

Analysis

Proliferation Assay

One-way ANOVA was used to determine the statistical significance of the data collected from the XTT Assays performed. An α of 0.05 was chosen, in line with standard research practises of the day. The results of the ANOVA are shown in Table 8 below.

Table 8. Analysis of Variance Calculation Results for the XTT Assay data for treatment with 30 μM of resveratrol.

Treatment	**Treatment Average**	**Control Average**	**F**	**P-value**	**α**	**null hypothesis?**
MCF-7 24 Hours	0.0759	0.0934	0.1944	0.682	0.05	fail-to-reject
MCF-7	0.0738	0.1076	0.5066	0.516	0.05	fail-to

48Hours						-reject
MDA-MB-231 24Hours	0.675	0.703	0.014	0.911	0.05	fail-to-reject
MDA-MB-231 48Hours	0.278	0.298	0.134	0.732	0.05	fail-to-reject

Raw Data in Appendix 2.

As this was the test that produced the results that are the least questionable, this result is very unfortunate from a statistical significance standpoint. There are trends that suggest that resveratrol had an effect on the growth of the cells that were treated (the treatments all showed

lower levels of growth than their respective control values). However, casual comparisons of values are not enough to claim that there is a legitimate cause and effect relationship present. For any result to be accepted as conclusive, it must be deemed statistically significant according to the protocols accepted by the general scientific community. Although it is very frustrating to see that any amount of work results in no verifiable results, the rules of statistical significance must be followed to maintain the credibility of the entire scientific process, and cannot be ignored.

DAPI Staining

The DAPI staining results are considerably more subjective than the XTT results. Although the results do suggest that there are higher levels of apoptosis in the treated (mean = 14 apoptotic cells/100 cells) than the control (mean = 6.3 apoptotic cells/100 cells) cell cultures. However, these results are inherently vulnerable to researcher bias, and the interpretation of what would be considered apoptotic cell was purely subjective. The goal of the test was to visualize apoptosis, and the image in Figure 1 shows that with a fair degree of certainty, there was apoptosis taking place.

Western Blot

Western Blots are typically a binary result. Either the protein of interest is present, or it isn't. However, the poor secondary antibody made this test considerably more difficult to interpret. Unfortunately, even the readings that are considered to be "bands" could have quite easily been mistaken, as they were quite faint. It is for that reason that neither the data supporting nor discrediting the effectiveness of resveratrol can be taken to be anything more than a suggestion. In this case, there appears to be Beclin-1 present in the MCF-7 cells that were treated with resveratrol (4 "Band", 2 "Faint Band), while the MCF-7 Controls appear to show less evidence of apoptosis (4 "Faint Band", 2 "No Band"). The MDA-MB-231 cell cultures appear to show greater signs of apoptotic activity in the control samples (4 "Band") than the samples that were treated with resveratrol (3 "Faint Band", 1 "No Band"). However, as stated

before, these results are highly subjective, and cannot be taken to be proof of any specific response.

Conclusions

Although a number of the tests performed produced results that *suggest* there is an increase in apoptosis when MCF-7 and MDA-MB-231 cell cultures are treated with 30 μMresveratrol (particularly the XTT Assay data), nothing can be accepted as definitive. The XTT Assay failed to meet the required level of variance for statistical significance, which is one of the keys to the entire scientific process. The Western Blots did not produce clear enough results to make any definitive statements about the protein of interest (Beclin-1).

Although the lack of results was disappointing from a research perspective, from a personal perspective, the experience was still very valuable. It shed a very revealing light on the life of a researcher, particularly on the attention to detail that must be maintained to produce reliable results. With this knowledge, my appreciation for scientific researchers has grown immensely, which I believe is a valuable lesson that fulfills the ultimate goal of an undergraduate independent study like this one.

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Appendix 1. XTT Assay Raw Data

Resveratrol- 24 Hour #1 (Nov 28)	Blank:	0.099	
MCF-7		MDA-MB-231	
Treatment	Control	Treatment	Control
	0.144	0.451	0.397
0.174	0.149	0.567	0.405
0.160	0.147	0.413	0.465
0.109	0.145	0.395	0.393
0.161	0.139	0.429	0.392
Blank-Adjusted	Blank Adjusted	Blank-Adjusted	Blank Adjusted
	0.045	0.352	0.298
0.075	0.050	0.468	0.306
0.061	0.048	0.314	0.366
0.010	0.046	0.296	0.294
0.062	0.040	0.330	0.293
Resveratrol- 24 Hour #2 (Mar 7)	Blank:	0.132	
MCF-7		MDA-MB-231	
Treatment	Control	Treatment	Control
0.158	0.185	0.235	0.246
0.186	0.244	0.275	0.271
0.173	0.225	0.278	0.295
0.177	0.224	0.277	0.291
0.185	0.242	0.272	0.355
Blank-Adjusted	Blank Adjusted	Blank-Adjusted	Blank Adjusted
0.026	0.053	0.103	0.114
0.054	0.112	0.143	0.139
0.041	0.093	0.146	0.163
0.045	0.092	0.145	0.159
0.053	0.110	0.140	0.223
Resveratrol- 24 Hour #3 (Mar 7)	Blank:	0.132	
MCF-7		MDA-MB-231	
Treatment	Control	Treatment	Control
0.206	0.180	0.268	0.289
0.259	0.305	0.314	0.353
0.278	0.303	0.321	0.375
0.269	0.279	0.328	0.361
0.308	0.305	0.367	0.443
Blank-Adjusted	Blank Adjusted	Blank-Adjusted	Blank Adjusted
0.074	0.048	0.136	0.157
0.127	0.173	0.182	0.221
0.146	0.171	0.189	0.243
0.137	0.147	0.196	0.229
0.176	0.173	0.235	0.311

Figure 1. Raw XTT Assay Data for MDA-MB-231 Cell Cultures.

Resveratrol- 48 Hour #1 (Nov 29)	Blank:	0.100	
MCF-7		MDA-MB-231	
Treatment	Control	Treatment	Control
0.165	0.174	0.682	0.430
0.155	0.151	0.380	0.436
0.182	0.147	0.452	0.396
	0.147	0.390	0.404
	0.146		
Blank-Adjusted	Blank Adjusted	Blank-Adjusted	Blank Adjusted
0.065	0.074	0.582	0.330
0.055	0.051	0.280	0.336
0.082	0.047	0.352	0.296
	0.047	0.290	0.304
	0.046		

Resveratrol- 48 Hour #1 (Nov 29)	Blank:	0.143	
MCF-7		MDA-MB-231	
Treatment	Control	Treatment	Control
0.176	0.227	0.344	0.344
0.158	0.244	0.377	0.379
0.180	0.223	0.348	0.377
0.161	0.204	0.354	0.406
0.195	0.255	0.359	0.432
Blank-Adjusted	Blank Adjusted	Blank-Adjusted	Blank Adjusted
0.033	0.084	0.201	0.201
0.015	0.101	0.234	0.236
0.037	0.080	0.205	0.234
0.018	0.061	0.211	0.263
0.052	0.112	0.216	0.289

Resveratrol- 48 Hour #1 (Nov 29)	Blank:	0.143	
MCF-7		MDA-MB-231	
Treatment	Control	Treatment	Control
0.211	0.257	0.361	0.405
0.285	0.305	0.383	0.467
0.278	0.325	0.353	0.497
0.257	0.303	0.391	0.500
0.299	0.422	0.443	0.517
Blank-Adjusted	Blank Adjusted	Blank-Adjusted	Blank Adjusted
0.068	0.114	0.218	0.262
0.142	0.162	0.240	0.324
0.135	0.182	0.210	0.354
0.114	0.160	0.248	0.357

Figure 2. Raw XTT Assay Data for MCF-7 Cell Cultures.

	MCF-7 24 Hours	
Replicate	Treatment	Control
#1 (Nov 28)	0.052	0.046
#2 (Mar 7)	0.044	0.092
#3 (Mar 7)	0.132	0.142
	MCF-7 48 Hours	
Replicate	Treatment	Control
#1 (Nov 29)	0.067	0.053
#2 (Mar 8)	0.031	0.088
#3 (Mar 8)	0.123	0.179
	MDA-MB-231 24 Hours	
Replicate	Treatment	Control
#1 (Nov 28)	0.352	0.311
#2 (Mar 7)	0.135	0.16
#3 (Mar 7)	0.188	0.232
	MDA-MB-231 48 Hours	
Replicate	Treatment	Control
#1 (Nov 29)	0.376	0.317
#2 (Mar 8)	0.213	0.245
#3 (Mar 8)	0.243	0.334

Figure 3. Mean XTT Values used for Analysis of Variance.

Appendix 2. Full Analysis of Variance Results

Anova: Single Factor	MCF-7 24 Hours					
SUMMARY						
Groups	*Count*	*Sum*	*Average*	*Variance*		
Treatment	3	0.2278	0.075933333333333	0.002374413333333		
Control	3	0.2802	0.0934	0.00233436		
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.000457626666667	1	0.000457626666667	0.194371924181245	0.682083082967309	7.70864742132169
Within Groups	0.009417546666667	4	0.002354386666667			
Total	0.009875173333333	5				

Figure 1. ANOVA Results of 24 Hour MCF-7 Treatments.

Anova: Single Factor	MCF-7 48 Hours					
SUMMARY						
Groups	*Count*	*Sum*	*Average*	*Variance*		
Treatment	3	0.221333333333333	0.073777777777778	0.002147148148148		
Control	3	0.32	0.106666666666667	0.004266893333333		
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.001622518518519	1	0.001622518518519	0.505927042474866	0.516187703418056	7.70864742132169
Within Groups	0.012828082962963	4	0.003207020740741			
Total	0.014450601481482	5				

Figure 2. ANOVA Results of 48 Hour MCF-7 Treatments.

Anova: Single Factor	MDA-MB-231 24 Hours					
SUMMARY						
Groups	*Count*	*Sum*	*Average*	*Variance*		
Treatment	3	0.675	0.225	0.01277796		
Control	3	0.7032	0.2344	0.00576444		
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.00013254	1	0.00013254	0.014295884027957	0.910592031279959	7.70864742132169
Within Groups	0.0370848	4	0.0092712			
Total	0.03721734	5				

Figure 3. ANOVA Results of 24 Hour MDA-MB-231 Treatments.

Anova: Single Factor	MDA-MB-231 48 Hours					
SUMMARY						
Groups	*Count*	*Sum*	*Average*	*Variance*		
Treatment	3	0.8326	0.277533333333333	0.007493773333333		
Control	3	0.8953	0.298433333333333	0.002251843333333		
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.000655215	1	0.000655215	0.134463528047652	0.73242219181318	7.70864742132169
Within Groups	0.019491233333333	4	0.004872808333333			
Total	0.020146448333333	5				

Figure 4. ANOVA Results of 48 Hour MDA-MB-231 Treatments.