MQP-BIO-DSA-5930

MTOB AS A CANCER TREATMENT: THE EFFECT OF 4-METHYLTHIO-2-OXOBUTYRIC ACID ON CANCER CELL MIGRATION

A Major Qualifying Project Report

Submitted to the Faculty of the

WORCESTER POLYTECHNIC INSTITUTE

in partial fulfillment of the requirements for the

Degree of Bachelor of Science

in

Biology and Biotechnology

by

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Daniel Parker

January 25, 2010

APPROVED:

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Steven Grossman, MD, PhD
Program in Cancer Biology
Umass Medical Center
Major Advisor

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David Adams, PhD
Biology and Biotechnology
WPI Project Advisor

ABSTRACT

The main source of morbidity and mortality in cancer is the metastasis of cancer once it has developed. 4-methylthio-2-oxobutyric acid (MTOB) is a drug shown to be effective in inducing apoptosis in cancer cells regardless of the presence of active p53 tumor suppressor, due to its interaction with CtBP transcription repressor. The purpose of this MQP was to determine whether MTOB is an effective treatment for reducing cancer cell migration. This was done using wound healing assays to characterize cancer cell migration in the presence or absence of MTOB. The results show that the migration of U2OS, HCT116 -/-, and MCF7 cancer cells was reduced by 10 mM MTOB treatment.

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ACKNOWLEDGEMENTS

First, I would like to thank Steven Grossman MD, PhD for the generous use of his lab at the UMass Medical School during my time working on this project. Second, I would like to thank Mike Straza for his guidance on this project and for providing me with direction. This project would not have been possible without Seema Paliwal PhD with respect to her help and training on many of the techniques used. In addition I would like to thank Ding Ding Shi PhD and Roman Kulikov PhD, for all of their insight that helped me along the way during my time in the lab, as well as Ramesh Kovi PhD for allowing me the use of his thesis as background for my project. Finally I would like to thank David Adams PhD for his guidance through this project, with help editing the report, and with providing me with the opportunity to find a lab to work in.

BACKGROUND

Cancer Metastasis

It is widely known that cancer cells migrate and can metastasize to areas of the human body that differ from where they originated. This metastasis is caused by two main factors: 1) the migration of cells that spread locally, and 2) the detachment of cells that are then released into the bloodstream or lymphatic system that spread systemically. Cells that spread via the circulatory system can localize in other major body systems and cause serious complications for cancer patients (Sadanandam, et al., 2010). Thus, research into cancer cell migration, and treatments for it, are crucial.

Cell Migration

The spread of cancer cells through metastasis is highly dependent on cell motility. Without the inherent ability of the cells to migrate, the cells remain in one place only, and grow without spreading (Gupta et al., 2005). Of all the mechanisms that induce cells to migrate, the activity of the corepressor C-terminal Binding Protein-2 (CtBP2) (discussed below) is critical, in both p53-dependent and p53-independent cancer lines, due in part to its transcriptional regulation of genes related to proliferation, invasion, and metastasis, and due to its interaction with adhesion proteins such as PTEN and E-Cadherin (Zhang et al., 2006).

C-Terminal Binding Proteins

CtBP is a protein that stimulates tumor cell migration. It localizes mainly to the nucleus, and is well known to act as an active repressor and corepressor of the transcription of genes, many of which inhibit the proliferation, invasion, and metastasis of cancer (Chinnadurai, 2009). The genes repressed by CtBP can be grouped largely by their main effects on cell growth and motility.

The first group of genes targeted by CtBP control cellular proliferation. The genes mainly included in this are the cell cycle inhibitors $p16^{INK4a}$ and $p15^{INK4b}$, two genes that directly affect the proliferation of cells by causing G-1 phase cell cycle arrest (Scaini et al., 2009). CtBP represses the expression of p16 and p15, thus increasing mitosis.

Another cellular proliferation gene targeted by CtBP is the phosphatase and tensin homolog (PTEN) (Chinnadurai, 2009). PTEN acts as a tumor suppressor through its interaction and inhibition of the AKT pathway. The AKT pathway is a powerful source of cellular proliferation through activation of the cell cycle and growth (Tamura et al., 1999). CtBP inhibits PTEN transcription, thereby increasing the AKT pathway and tumor proliferation.

In addition to inhibiting genes which cause cell cycle arrest and thus increasing cellular proliferation, CtBP also represses many genes that inhibit cellular migration. One gene affected is the aforementioned PTEN which has been shown to cause increased cellular migration, in this case CtBP represses PTEN to increase cellular migration (Bowen et al., 2009). Along with the repression of PTEN, another target of CtBP repression is the E-Cadherin, a powerful cellular adhesion protein, that when repressed increases cell mobility (Zhang et al., 2006).

A final way in which CtBP acts to increase oncogenesis is through its interactions with many genes that would normally trigger apoptosis, thus increasing cell survival. This is shown

through its repression of such powerful apoptosis-inducing genes as PERP, Bax, and NOXA. By inhibiting these genes directly, as well as through its interaction with mdm2 causing repression of p53, CtBP is a strong cause of the survival of cells that would under normal circumstances undergo apoptosis (Chinnadurai, 2009). A summary of the role of CtBP in oncogenesis is shown in **Figure-1**, and the protein remains under continuous research.

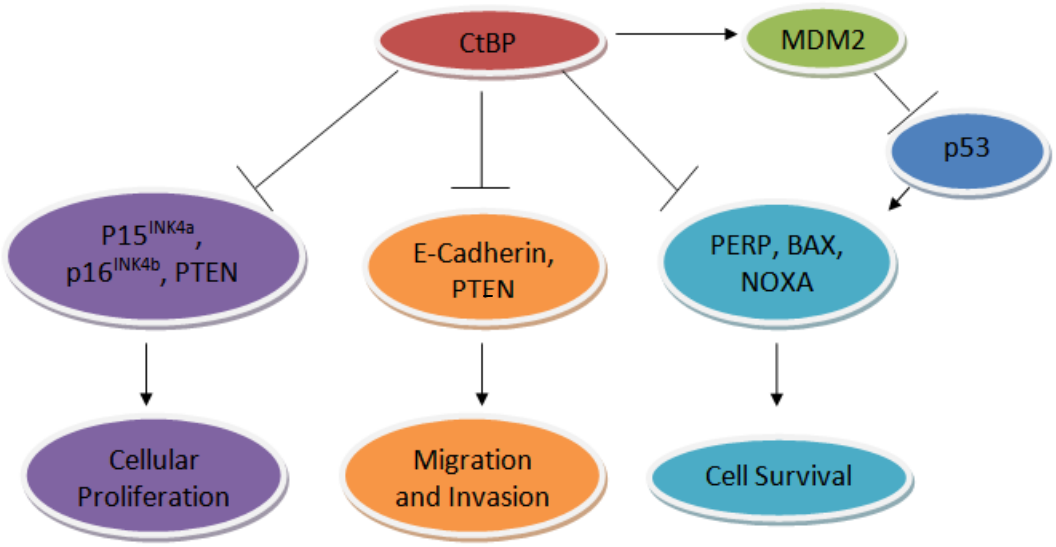

Figure-1: Interaction of CtBP with Common Tumor Suppressors. Shown above is the repressor activity of CtBP upon tumor suppressors such as $p15^{INK41}$, $p16^{INK4b}$ and PTEN, causing increased cellular proliferation. In addition to this it is known that CtBP represses E-Cadherin and PTEN which causes increased cell mobility. And finally it is shown that CtBP upregulates MDM2, a suppressor of p53; CtBP also represses PERP, Bax, and NOXA, targets for upregulation by p53.

MTOB

In the Grossman Lab at Umass Medical School (Worcester), in the search for active repressors of CtBP for use as cancer therapeutics it was found that ARF, a product of the PTEN gene, targets CtBP for nuclear localization and proteasome degradation (Paliwal et al., 2006). During the course of these investigations it was also found that the compound MTOB, the penultimate compound in the methionine salvage pathway, is able to bind to CtBP and repress its

activity (Straza et al., submitted). Alone, MTOB in large doses is able to cause significant apoptosis *in vitro*, and has been shown to be an effective tool for reducing the survivability of cancer cells (Tang et al., 2006). Ongoing research in the Grossman Lab shows that MTOB is both a safe and effective treatment *in vivo* for reducing the size and spread of tumors. With this in mind, this MQP was performed to determine the effectiveness of MTOB against cancer cell migration.

Cancer Cell Lines

To show MTOB's effectiveness in a large range of cancer types, multiple cancer lines were studied in this project. The first of these was the osteosarcoma cell line U2OS. U2OS cells are known to be an invasive line of osteosarcoma cells showing a large amount of cellular migration when plated (Cho et al., 2007). This line of cells is used primarily as a model for human bone cancers. These cells are positive for the tumor suppressor p53, a powerful regulator of cell cycle arrest and apoptosis. U2OS cells were originally isolated from a differentiated sarcoma from the tibia of a 15 year old girl in 1964 (U2OS Product Description, 2009).

The next line of cells used in these experiments was the MCF7 cell line. The MCF7 line of breast adenocarcinoma was used to support the p53 positive data of the U2OS cell line. MCF7 cells are a metastatic cancer line that is both p53 positive and shows a significant amount of cell migration (Khanfar et al., 2009). This cell line was isolated from an adenocarcinoma in a pleural effusion of a 69 year old female (MCF7 Product Description, 2009).

The third cell line used in this MQP was the HCT116 -/- cell line. p53 is one of the most important suppressors, and inactivated by mutation or by alteration in its pathway in almost 50%

of all human cancers (Zilfou & Lowe, 2009). The HCT116 -/- cell line has a point mutation causing the cancer line to be p53 negative. Without p53, many of the common chemotherapy drugs that stimulate p53 activity are ineffective. These HCT116 cells were isolated from an adult male colorectal carcinoma (HCT116 Product Description, 2009). Each of these cell lines is known to actively metastasize through the migration of the cells.

PROJECT PURPOSE

The Background section shows that cancer cell metastasis is an important issue in designing drugs to block tumors. C-terminal Binding Protein (CtBP) is a protein previously shown to increase tumor cell division, migration, and survival. MTOB is a known inhibitor of CtBP. This MQP project is directed to show that MTOB is an effective treatment for both killing cancer cells and stopping cancer cell metastasis. The hypothesis tested in this MQP is that cancer cell migration is drastically reduced after treatment with MTOB. Three cell lines (U2OS, HCT116 -/-, and MCF7) will be plated on 6-well plates. In each well, three scratches will be made to create space into which the cells can migrate, and the cells will then be photographed over time through a microscope. Three wells will be treated with 10 mM NaCl as a control and another three wells will be treated with 10 mM MTOB. After 8 or 16 hours, the wells will be photographed again to show the difference in scratch size. This will be used to confirm the hypothesis that the cells treated with MTOB will migrate less than the cells treated with the NaCl control.

METHODS

Cell Culture

Cell Lines

The cell lines used during these experiments were U2OS, HCT116-/-, and MCF7. The U2OS cells were provided by Seema Paliwal, and the HCT116 -/- and MCF7 cells were provided by Mike Straza. The U2OS cells are an osteosarcoma line that is p53 positive. The HCT116 -/- cells are a line of human colorectal carcinoma cells that are mutated with a point mutation in p53. The MCF7 cells are a line of breast cancer cells that are also p53 positive.

Cell Media

The U2OS cells were cultured on T75 flasks using DMEM High Glucose media supplemented with 10% FBS and 1% Pen-Strep. The HCT116 -/- cells were cultured in T25 flasks using McCoy's Media supplemented with 10% FBS and 1% Pen-Strep. The MCF7 Cells were cultured in T25 flasks using DMEM Low Glucose media supplemented with 10% FBS and 1% Pen-Strep.

Cell Splitting

Each cell line was split by first washing with PBS, and then trypsin with EDTA was used to remove the adherent cells from each plate. Media was then added and the cells were then centrifuged at 2000 rpm for 5 minutes. Cells were then plated at the desired dilution into the same or additional flasks.

Wound Healing Assays

Cell Plating

Cells were plated on 6-well plates and run in triplicate. Three wells represented negative controls using NaCl-supplemented media at 10 mM, while three wells of 10 mM MTOB-treated cells represented the experimental group. Each well had a series of three scratches made using a sterile P1-200 micropipette tip. These scratches were then photographed using a microscope to provide a baseline size for each "wound". After 8 or 16 hours, the media was aspirated off the plates, and each wound was photographed in 15 unique places to display the "wound" size after the specified time had passed. The arrangement of wells for each of the wound healing assays is shown in **Figure-2**.

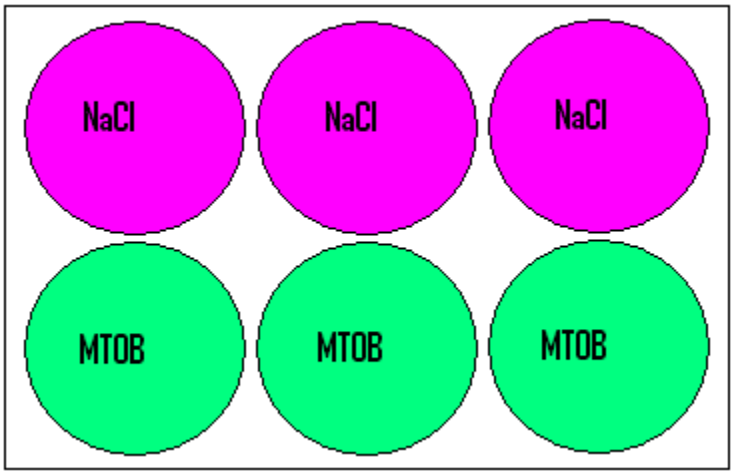

Figure-2: Diagram of the 6-Well Plate Organization for a Wound Healing Assay.

Treatment with MTOB

A 40 mM stock of MTOB was created in each of the media used for each cell line. This was done by adding 62 mg of MTOB to 10 mL of each medium. In addition to this, the control stock was created for each media by adding 23 mg of NaCl to 10 mL of each medium. The

resulting concentrated solution was stored in 15mL falcon tubes, and kept at 4^{o}C until use. The media was then diluted 1:4 for a final concentration of 10 mM in each well.

Data Analysis

Each photograph was imported to Adobe Photoshop, and measured for wound size. The average size of the original wound was then compared with the size of the wound after migration, and the distance moved and percent wound closure for each well was calculated and averaged.

RESULTS

As stated before, the purpose of this MQP was to show the reduction in cellular migration of three different tumor cell lines after being treated with the prospective cancer drug MTOB. A series of repetitive wound healing assays were conducted at the 8 hour and 16 hour time points for each cell line.

U2OS Migration

The first cell line to be tested for MTOB effect was the U2OS osteosarcoma cell line. The raw data was collected by taking photographs of the scratches ("wounds") in each well both before and after treatment. An image of a scratch in each well was taken at time zero and used to form a baseline size of each scratch in each of the wells. The wells were then treated with the NaCl control or the MTOB treatment at 10 mM, and allowed to run for 8 or 16 hours depending on the frame of the experiment being run. After the allotted time had passed, the medium in each well was removed, and the scratches photographed again, this time in 15 unique locations to allow correction for any size discrepancy between the final distances that each "wound" closed to. These photographs were then analyzed in Adobe Photoshop to calculate the distance moved by the cells in each well.

Figure-3 shows the difference in cell migration after 16 hours in a U2OS migration assay. Using Adobe Photoshop, these pictures were measured for the average distance between the edges, and compared between MTOB-treated (figure right side) and control images (figure left side). It is clearly evident in these pictures that the MTOB treatment (D) inhibits cell migration into the clear zone compared to untreated cells (C). These cells showed a 43% reduction in migration after 8 hours, and a 55% reduction in migration after 16 hours, normalized

to the NaCl treated control cells (**Figure-4**). The raw unprocessed data can be found in the Appendix at the end of the report.

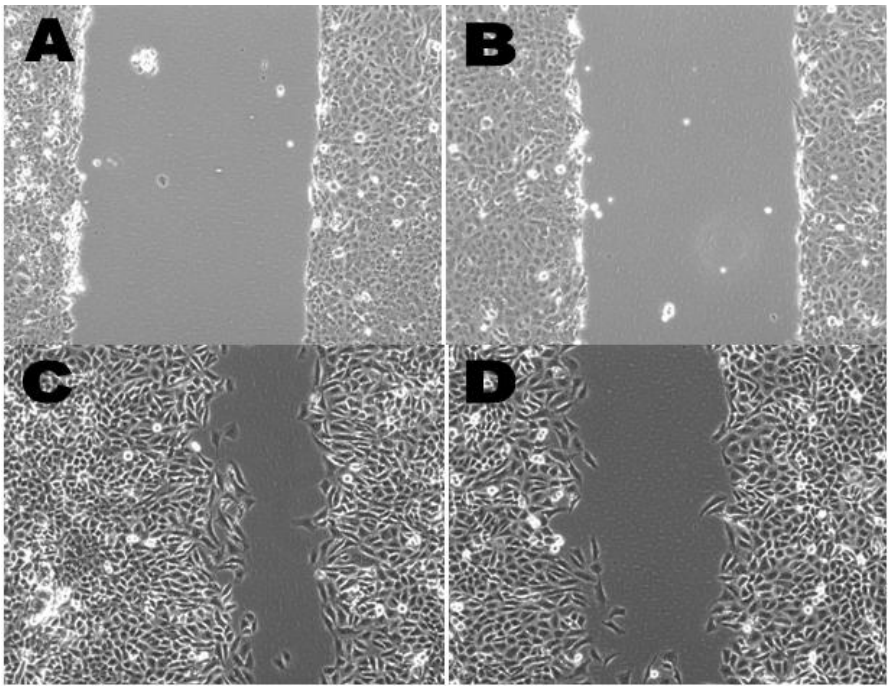

Figure-3. Measurement of U2OS Cellular Migration at 16 Hrs. Cells treated with NaCl are shown at time zero (A) and after 16 hours (C). Also shown are cells treated with MTOB at time zero (B) and after 16 hours (D).

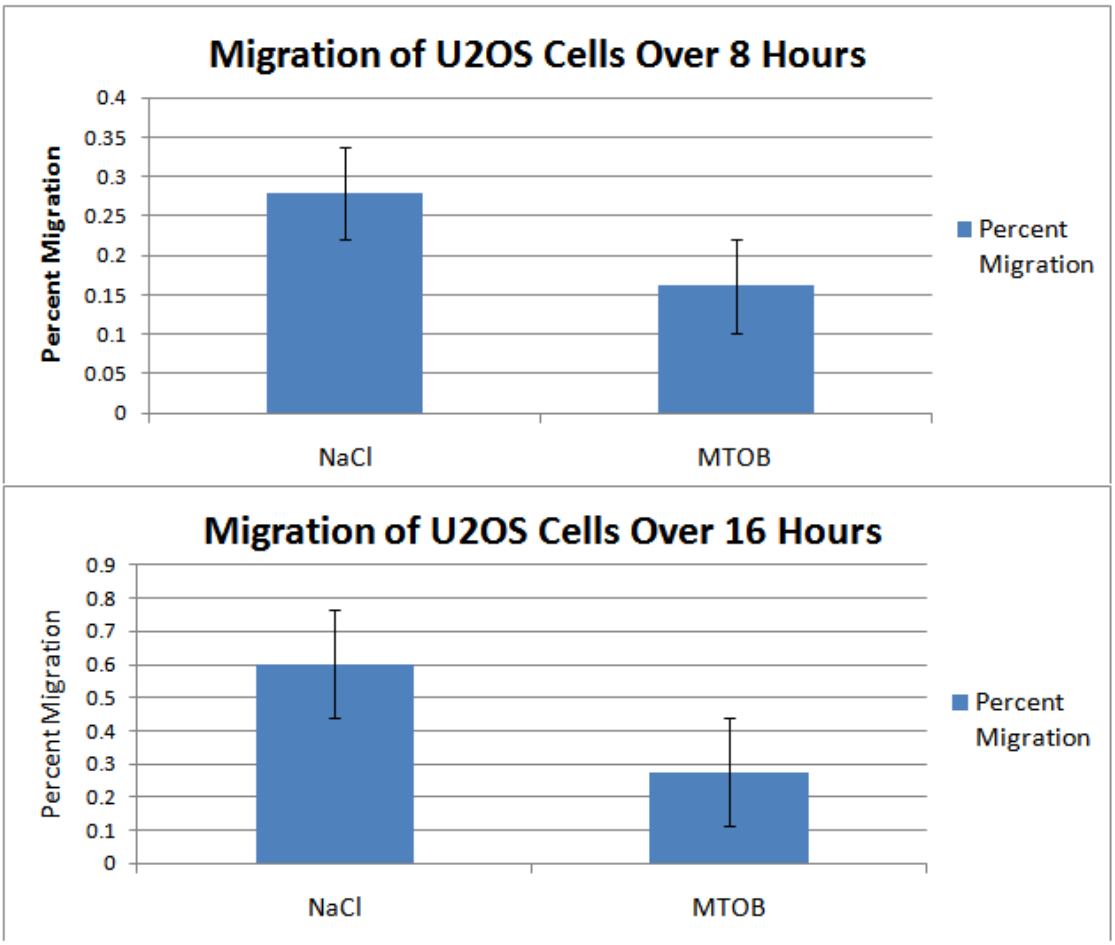

Figure 4. MTOB Inhibition of U2OS Tumor Cell Migration. U2OS cells were treated with 10 mM MTOB or 10 mM NaCl as a control, and then allowed to migrate for 8 hours (above) or 16 hours (below) into a scratched area on the microtiter well. Each histobar denotes the mean of 12 determinations for 8 hours, and N=6 for 12 hours. Error bars denote standard error.

HCT116 -/- Migration

In addition to the U2OS tumor cell line, a p53-negative cell line was used in this MQP, the HCT116 -/- cell line from a human colorectal carcinoma. These cells showed a 49% reduction in migration after 8 hours, and a 31% reduction in migration after 16 hours, when

normalized to the saline-treated control (**Figures 5 and 6**). Thus MTOB can reduce the migration of both a p53 positive and a p53 negative cancer cell line.

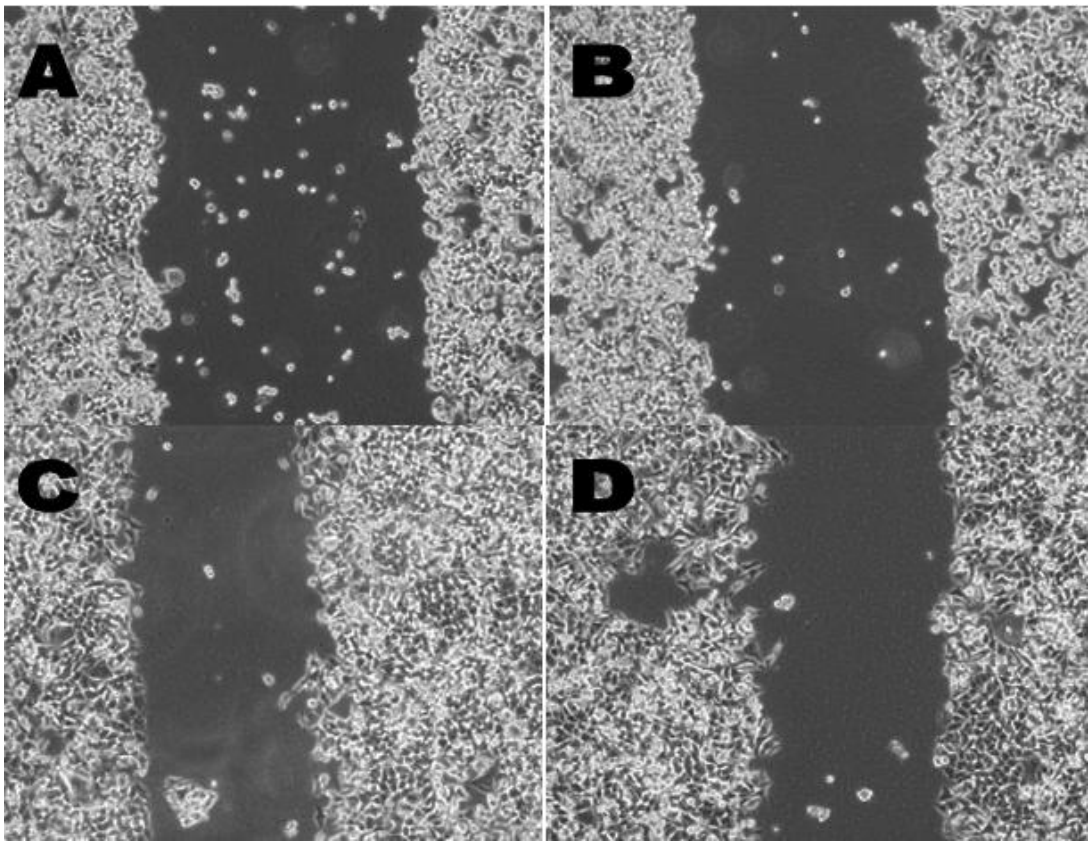

Figure-5. HCT116 -/- Cell Migration at 16 Hours. HCT116 -/- cells were treated with NaCl at time zero (A) and after 16 hours (C). Also shown are cells treated with MTOB at time zero (B) and after 16 hours (D).

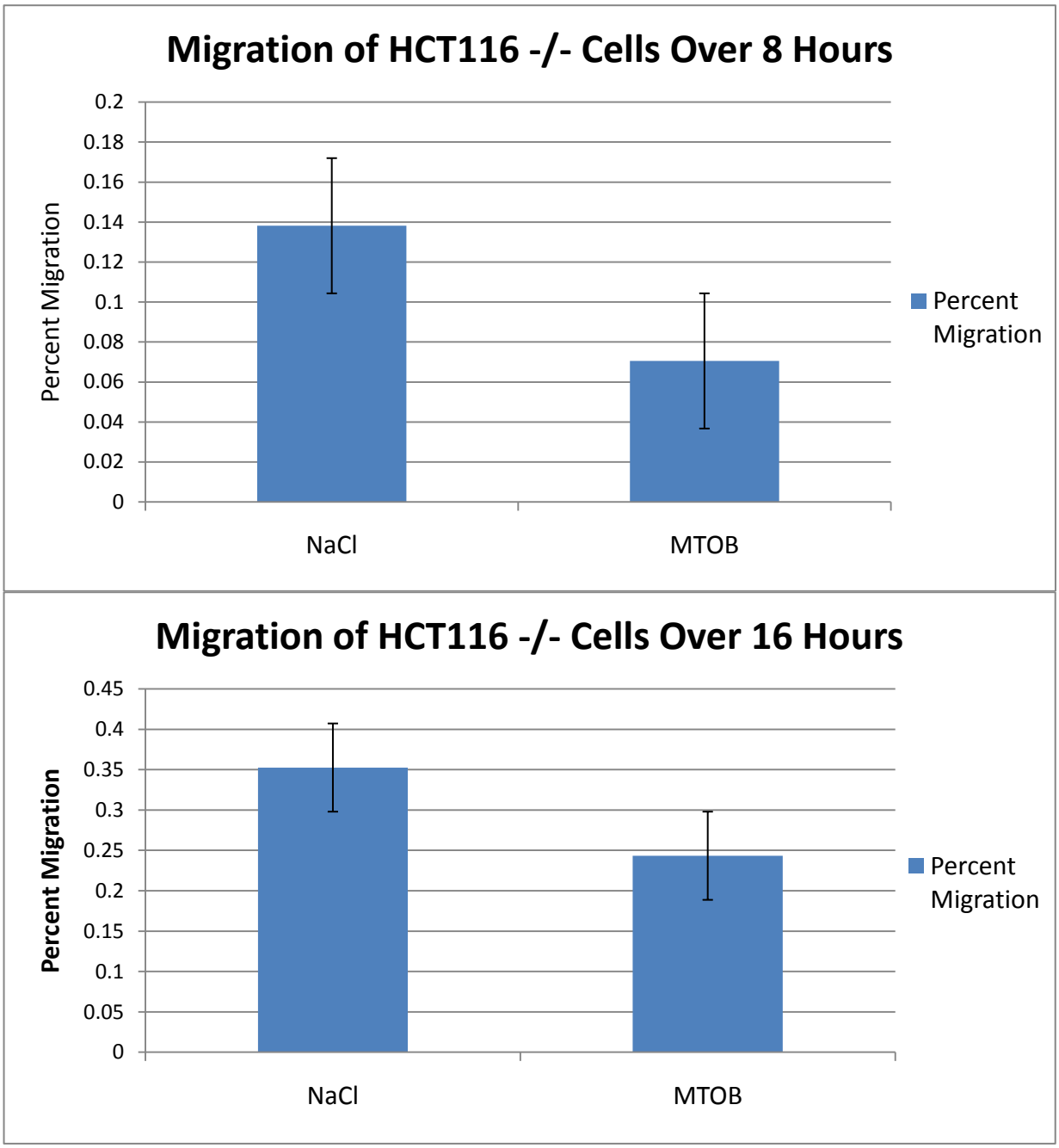

Figure 6. Summary of HCT116 -/- Cell Migration Results. HCT116 -/- cells were treated with 10 mM MTOB or 10 mM NaCl as a negative control, then allowed to migrate for 8 hours (above) or 16 hours (below) into a scratched surface. Each histobar denotes the mean of 6 determinations for 8 hours, and N=9 for 16 hours. Error bars denote standard error.

MCF7 Migration

The final cell line used during this MQP was the MCF7 breast adenocarcinoma. This line was used as an additional p53 positive cell line to show the reduction in cellular migration caused by MTOB. These cells are grown in a low glucose DMEM media as opposed to the high glucose media used for the U2OS cells. **Figure-7** shows images of the MCF7 migration at 16 hours. The graphs in **Figure-8** show that there was a 49% reduction in migration after 8 hours, and a 29% reduction in cellular migration after 16 hours when normalized to the saline controls.

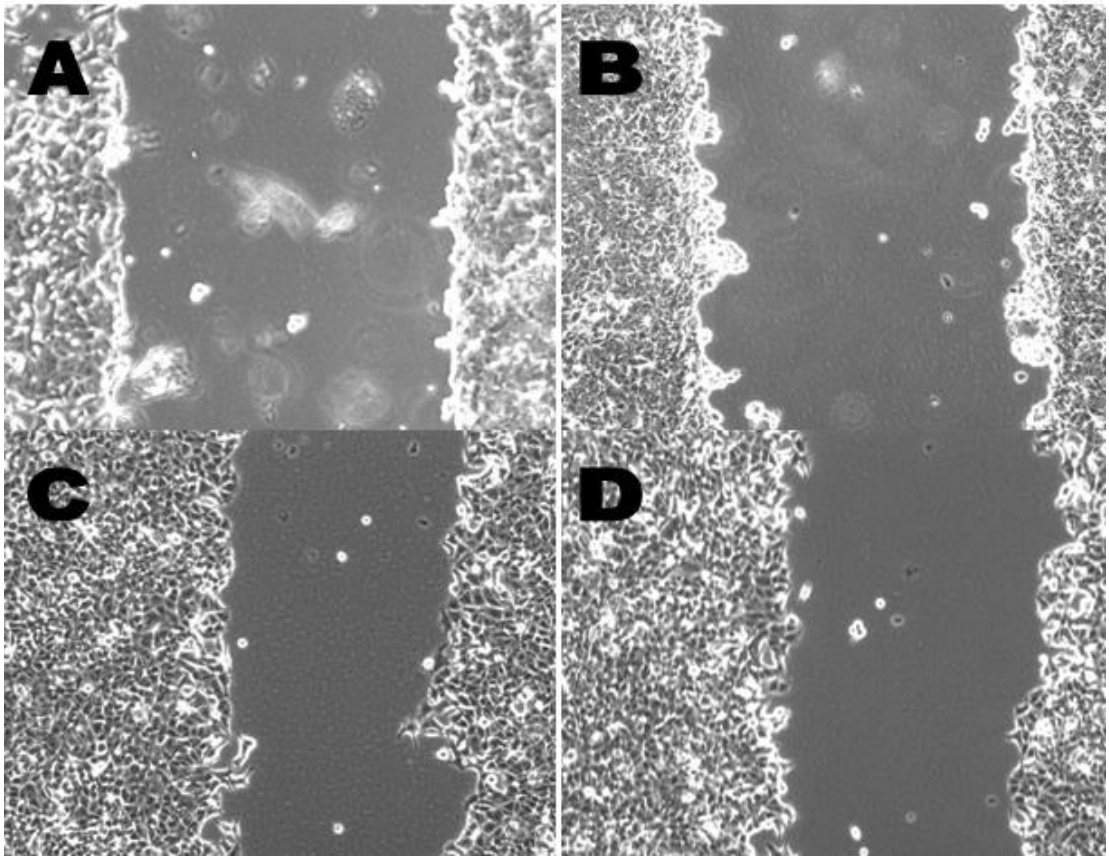

Figure 7. MCF7 Cell Migration After 16 Hours. MCF7 cells treated with NaCl at time zero (A) and after 16 hours (C). Also shown are cells treated with MTOB at time zero (B) and after 16 hours (D).

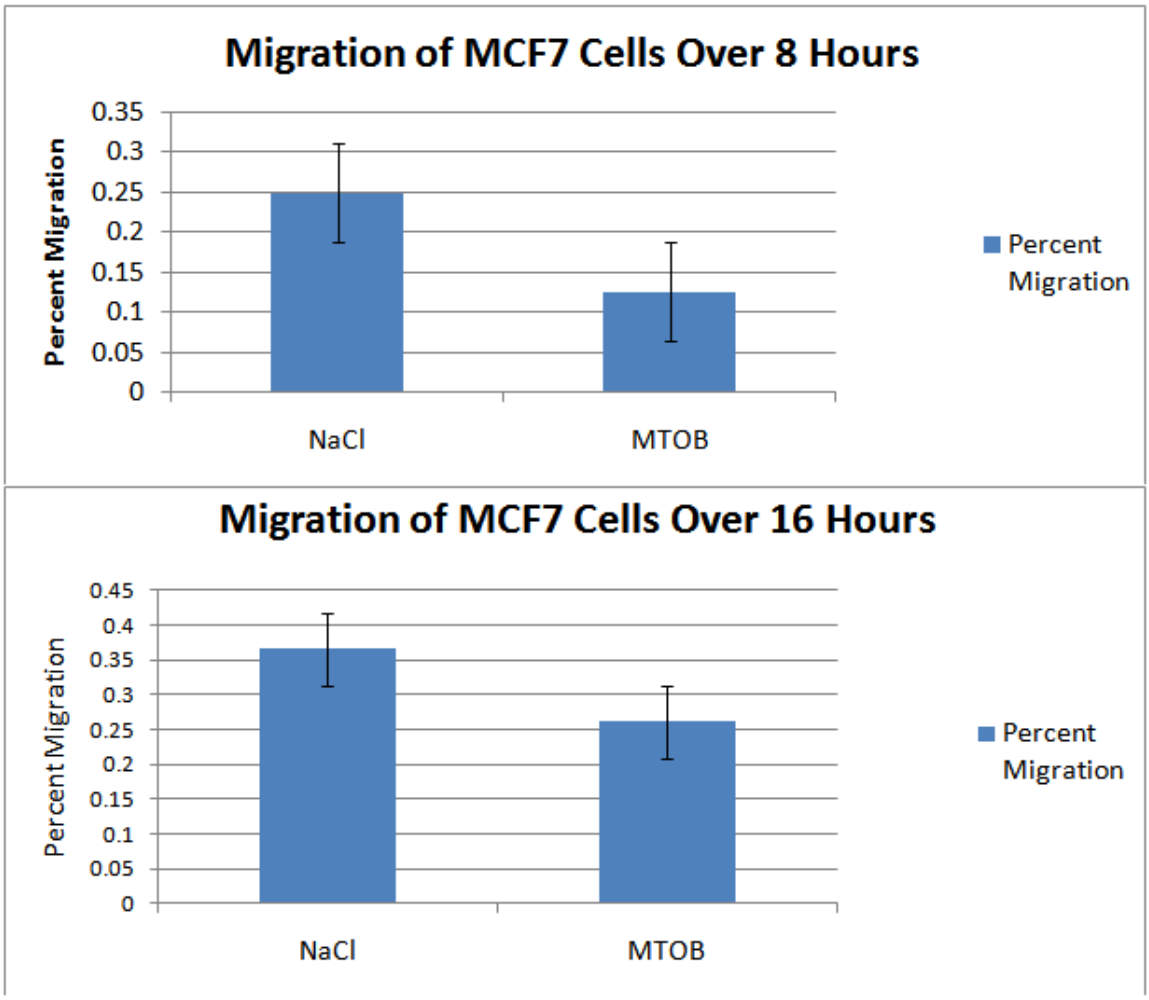

Figure 8. Summary of MCF7 Migration Results. MCF7 cells were treated with 10 mM MTOB or 10 mM NaCl as a negative control, and then allowed to migrate for 8 hours (above) or 16 hours (below) into a scratched surface. Each histobar denotes the mean of 6 determinations for 8 hours, and N=9 for 16 hours. Error bars denote standard error.

Final Analysis

Once all the migrations for each cell line were completed, they were compiled into **Figure-9**. These numbers were then normalized to the NaCl control values for each repetition of each experiment.

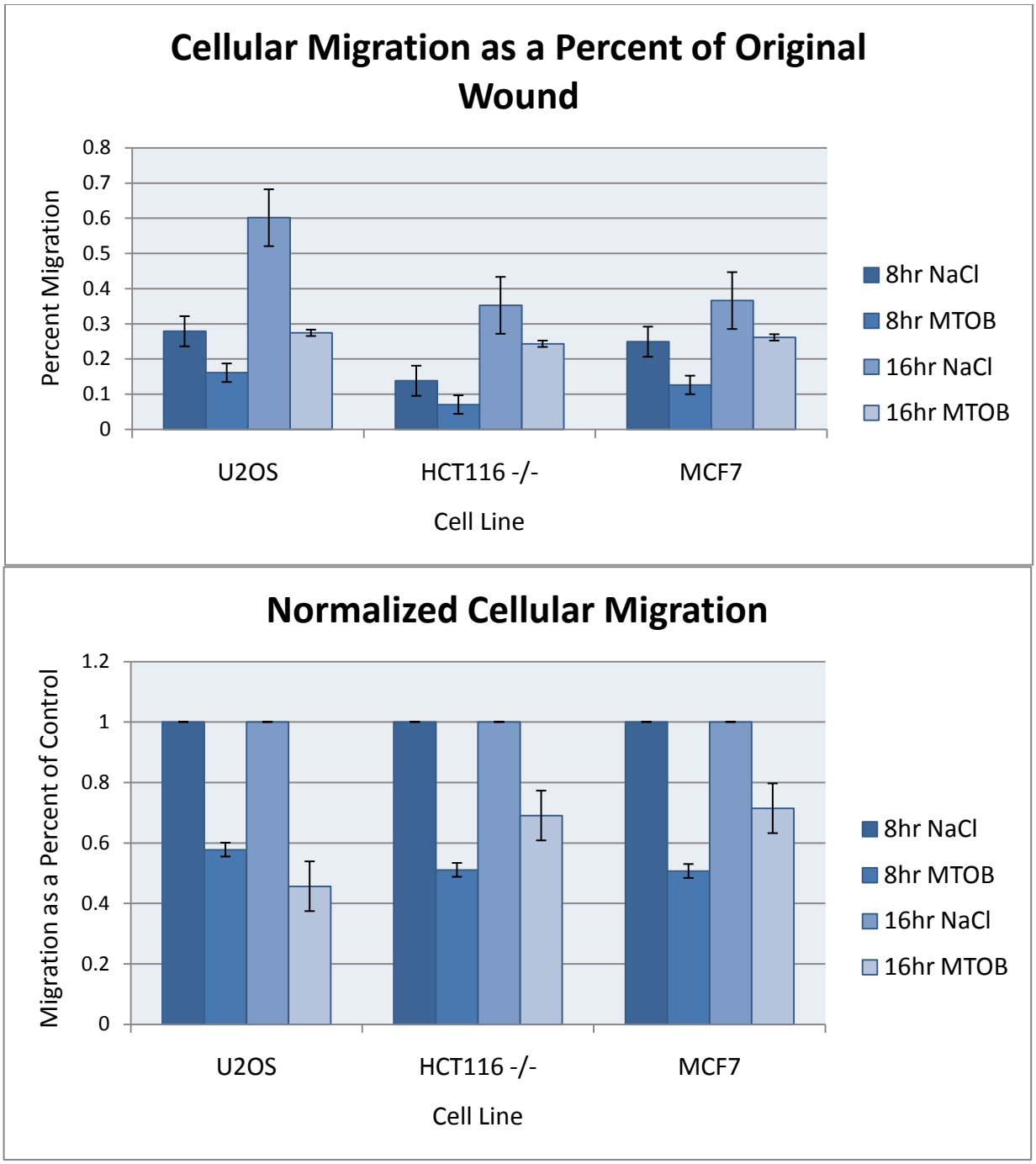

Figure 9. Compilation of Normalized Cell Migration Results. The results from each cell line were combined into a single graph (top), and then normalized to the saline control values (bottom).

DISCUSSION

Major Conclusions

The hypothesis that MTOB reduces cellular migration in multiple cell lines is supported by the summary results in Figure 9. This figure shows a significant reduction in migration at both 8 hours and 16 hours in the U2OS, HCT116 -/-, and MCF7 cell lines. These results support the initial hypothesis that MTOB reduces the migration, and thus possibly the metastasis, of cancer cells regardless of the presence of p53. Thus, MTOB may be a useful therapeutic in reducing neoplastic invasion and migration of cancer cells. In conjunction with this evidence supporting the effectiveness of MTOB in killing cancer cells independent of the presence of p53, the viability of MTOB as an efficient cancer treatment is very much a possibility.

Complications

Despite the effectiveness of the wound healing assay as a way to estimate cell migration accurately, mistakes are inevitable. During the course of these experiments some of the runs had to be discarded and were impossible to analyze due to errors in plating or procedure. These errors included mistakes in the confluence at plating, causing the wells to reach confluence at different times; or the usage of incorrect media for each plate.

Some complications were unavoidable. Most cell lines at some point of continuous culture reach their passage limit and die. In this project, for the MCF7 cell line, there was a time at which both the working stock and frozen stock cells died. Another complication that occurred was the receipt of unusable media, causing massive loss of cell lines across the board. Except for these complications, the experiments used in this MQP were efficient and effective in producing the expected results in support of the hypothesis.

Future Experiments

The future of MTOB research is focused on finding analogs that are more effective at lower dosages. Due to the high 10 mM dose of MTOB required, while reachable in a mouse model, treatment in the human body would be extremely expensive and inefficient. The prospect of an analog of MTOB with only minor changes that could exponentially increase its effectiveness is a desired goal. In addition to the analog research, studies testing the effectiveness of MTOB in conjunction with other current known cancer treatments should be performed. Cancer is usually treated with a combination of drugs rather than a single drug. This research might also lead to interest from drug companies providing their drugs that might work well in conjunction with MTOB. The final future experiment to be mentioned is an expansion of the ongoing research of the Grossman Lab with MTOB as an *in vivo* treatment to show its effectiveness and safety in an animal model. Following this research, MTOB may yet be developed as a safe and effective treatment for cancer, independent of p53 status of the tumor.

BIBLIOGRAPHY

Bowen, K., Doan, H., Zhou, B., Wang, Q., Zhou, Y., Rychahou, P., et al. (2009) PTEN loss induces epithelial--mesenchymal transition in human colon cancer cells. *Anticancer Research*, 29(11): 4439-4449.

Chinnadurai, G. (2009) The transcriptional corepressor CtBP: a foe of multiple tumor suppressors. *Cancer Research*, 69: 731-734.

Cho, H., Lee, T., Park, J., Park, K., Choe, J., Sin, D., et al. (2007) Disulfiram suppresses invasive ability of osteosarcoma cells via the inhibition of MMP-2 and MMP-9 expression. *Journal of Biochem Molecular Biology*, 40(6): 1069-1076.

Gupta, P., Mani, S., Yang, J., Hartwell, K., & Weinberg, R. (2005) The Evolving Portrait of Cancer Metastasis. *Cold Spring Harbor Symp Quart Biology*, 70: 291-297.

HCT116 Product Description (2009) Retrieved 1 1, 2010, from American Type Culture Collective: http://www.atcc.org/ATCCAdvancedCatalogSearch/ProductDetails/tabid/452/Default.aspx?ATCCNum=CCL-247&Template=cellBiology

Khanfar, M. A., Youssef, D. T., & El Sayed, K. A. (2009) Semisynthetic Latrunculin Derivatives as Inhibitors of Metastatic Breast Cancer: Biological Evaluations, Preliminary Structure-Activity Relationship and Molecular Modeling Studies. ChemMedChem.

MCF7 Product Description. (2009) Retrieved Jan 1, 2010, from American Type Culture Collection: http://www.atcc.org/ATCCAdvancedCatalogSearch/ProductDetails/tabid/452/Default.aspx?ATCCNum=HTB-22&Template=cellBiology

Paliwal, S., Pande, S., Kovi, R., Shapless, N., Bardeesy, N., & Grossman, S. (2006) Targeting of C-Terminal Binding Protein by ARF results in p53-independent Apoptosis. *Molecular Cell Biology*, 26(6): 2360-2372.

Paliwal, S., Kovi, R., Nath, B., Chen, Y., Lewis, B., & Grossman, S. (2007) ARF antagonizes cancer cell migration via interaction with CtBP corepressor. *Cancer Research*, 67(19): 9322-9329.

Sadanandam, A., Varney, M., Singh, S., Ashour, A., Moniaux, N., Deb, S., et al. (2010) High gene expression of semaphorin 5A in pancreatic cancer is associated with tumor growth, invasion and metastasis. *Int J Cancer* [e-pub ahead of print].

Scaini, M., Rossi, E., de Siqueira Torres, P., Zullato, D., Callegaro, M., Casella, C., et al. (2009) Functional impairment of p16(INK4A) due to CDKN2A p.Gly23Asp missense mutation. *Mutation Research*, 671(1-2): 26-32.

Straza, M., Kovi, R., Paliwal, S., Messina, M., Trench, P., & Grossman, S. (Submitted) C-Terminal Binding Protein 2 is a cellular target of the cytotoxic compound methylthio-2-oxobutyruc acid (MTOB). *Cancer Research*.

Tamura, M., Gu, J., Danen, E., Takino, T., Miyamoto, S., & Yamada, K. (1999). PTEN interactions with focal adhesion kinase and suppression of the extracellular matrix-dependent phosphatidylinositol 3-kinase/Akt cell survival pathway. *J Biol Chem*, 274: 20693-20703.

Tang, B., Kadariya, Y., Murphy, M. E., & Kruger, W. D. (2006) Themethionine salvage pathway compound 4-methylthio-2-oxobutanate causes apoptosis independent of down-regulation of ornithine decarboxylase. *Biochemical Pharmacology*, 72(2): 806-815.

U2OS Product Description (2009) Retrieved January 1, 2010, from American Type Culture Collection: http://www.atcc.org/ATCCAdvancedCatalogSearch/ProductDetails/tabid/452/Default.aspx?ATCCNum=HTB-96&Template=cellBiology#aProp506f5

Zhang, Q., Wang, S., Nottke, A., Rocheleau, J., Piston, D., & Goodman, R. (2006) Redox sensor CtBP mediates hypoxia-induced tumor cell migration. *Proc Natl Acad Sci USA* , 103(24): 9029-9033.

Zilfou, J. T., & Lowe, S. W. (2009) Tumor Suppressive Functions of p53. *Cold Spring Harbor Perspect Biology*.

APPENDIX

U2OS 8-21-09

U2OS			Control			MTOB	
82109		C1	C2	C3	M1	M2	M3
	1	6	6	7.75	7	6.25	5.75
	2	6.5	6.25	7.75	7.25	6.25	5.75
	3	6.25	6	7.25	7.25	5.75	6
	4	6.25	6	7.5	7.5	6	5.75
	5	6	6.25	7.25	7	6.25	5.75
	Avg 0	6.2	6.1	7.5	7.2	6.1	5.8
	1	3.5	3.25	3.75	5.25	4.5	4.25
	2	4	3.75	4.5	5.75	4.25	4.75
	3	4	3.5	5.5	5.25	5.25	4.25
	4	4	3.25	5.25	6	4.25	4.75
	5	3.75	3.5	4.75	6.25	4.75	4
	6	4.5	3.25	5	5.75	4.5	4.5
	7	4	4.25	4.75	5.75	4.5	4.25
	8	3	4.25	4.25	6	4.75	4.5
	9	3	4.5	4.75	5.75	4.75	4.75
	10	3.75	4.75	4.5	6.25	5.5	5
	11	4	5	4.75	6.5	4.75	4.25
	12	4.25	4.5	3.5	6.75	5.25	4.5
	13	4.25	4.5	4.5	6.25	5	4.5
	14	4.5	4.75	5.25	5.75	5.25	4.75
	15	4.25	4.5	4	6	5	4.75
	Avg 8	3.916667	4.1	4.6	5.95	4.816667	4.516667
	d moved	2.283333	2	2.9	1.25	1.283333	1.283333
	%space	0.63172	0.672131	0.613333	0.826389	0.789617	0.778736
	%moved	0.36828	0.327869	0.386667	0.173611	0.210383	0.221264
	Avg %		0.360938			0.201753	
	Std dev		0.030078			0.024971	

U2OS			Control			MTOB	
82109		C1	C2	C3	M1	M2	M3
	1	5.5	8.5	7.25	8.5	9	6.25
	2	6.25	9	8.5	8.75	9	7.25
	3	6	8.75	7.25	8.5	8.5	6.5
	4	6.25	8.5	7.5	8.5	8.5	7
	5	6	8.25	8	8.25	8.25	6.5
	Avg 0	6	8.6	7.7	8.5	8.65	6.7
	1	5	5.25	4.5	7	7.5	5.75
	2	4.5	6.25	4.75	7	7.5	5.25
	3	4.25	6.25	4.5	6.75	7.75	5.5
	4	4.5	6.75	5	7	7.25	6
	5	4.25	7	6.25	7	7.75	5.5
	6	4.25	7.25	6.25	7.25	8.25	5.75
	7	4	7.5	6	7.5	8	5.75
	8	5.25	7	6.25	6.75	7.75	6.25
	9	5	7	6	7.25	8	6.5
	10	4.75	7.25	6	7.25	7.25	5.75
	11	4.75	7	5.75	7.75	6.75	6.5
	12	4.5	5.5	6.5	7.25	6.75	6.5
	13	4.5	5.75	6	7.75	7	6.25
	14	5	6.5	6	7	6.5	5.5
	15	4.5	6	5.75	7.5	6.75	5.5
	Avg 8	4.6	6.55	5.7	7.2	7.383333	5.883333
	d moved	1.4	2.05	2	1.3	1.266667	0.816667
	%space	0.766667	0.761628	0.74026	0.847059	0.853565	0.878109
	%moved	0.233333	0.238372	0.25974	0.152941	0.146435	0.121891
	Avg %		0.243815			0.140422	
	Std dev		0.01402			0.016375	

U2OS 8-26-09

U2OS			Control			MTOB	
82609		C1	C2	C3	M1	M2	M3
8hr	1	9.25	8.25	8	9	8.25	8.75
	2	9.5	8.5	8	9.25	8.5	8.75
	3	9	8	8	9.25	8.5	9.25
	4	9	8.25	7.75	9	8.75	8.75
	5	9.25	7.75	8	8.75	8.5	8.5
	Avg 0	9.2	8.15	7.95	9.05	8.5	8.8
	1	6.75	6.5	6	8	7.5	7.75
	2	7	6.5	5.5	8	7.75	8.25
	3	6.5	6	5	8.25	7	7.25
	4	6.5	6.5	5.5	8	7.25	7.25
	5	6.75	6.25	5.75	8.5	7.25	7
	6	6.5	6.25	6	8.5	7.25	7
	7	6.25	5.75	5.5	8.25	7.5	7.75
	8	6.75	5.5	6	8.25	7	7.5
	9	7	5.5	5.75	8	7.25	7.75
	10	7	6	6	7.75	7.25	7.5
	11	6.75	6	5.75	7.75	7	6.75
	12	7.25	6	5.5	8	7	8
	13	7	6.25	5.75	7.75	7	7.25
	14	6.75	6	5.5	7.75	7.25	7.75
	15	7.25	5.5	5.25	8	7	7
	Avg 8	6.8	6.033333	5.65	8.05	7.216667	7.45
	d moved	2.4	2.116667	2.3	1	1.283333	1.35
	%space	0.73913	0.740286	0.710692	0.889503	0.84902	0.846591
	%moved	0.26087	0.259714	0.289308	0.110497	0.15098	0.153409
	Avg %		0.269964			0.138296	
	Tot Std dev		0.016763			0.024105	

U2OS			Control			MTOB	
82609		C1	C2	C3	M1	M2	M3
8hr B	1	7.25	7.5	8	6.25	6.5	11
	2	7.25	7.75	7.75	6.75	6.75	11
	3	7.75	7.25	8	6.5	6.5	11.25
	4	7.75	7.5	8	6.5	6.25	10.75
	5	8	7.5	8	6	6.75	10.5
	Avg 0	7.6	7.5	7.95	6.4	6.55	10.9
	1	6	5.5	6.5	5.75	5.5	9.25
	2	6	5.5	6.25	5.5	5.75	9.5
	3	6.25	5	6.25	5.25	5.5	9.25
	4	6	5.75	6.5	5.5	6	9.25
	5	6.25	5.75	5.75	5.5	5.75	9
	6	6.25	5.5	6.25	6	5.75	8.75
	7	5.75	5.5	5.75	5.25	5.5	8.5
	8	6.25	5.75	6	5.75	5.5	8.25
	9	5.5	5.25	6.25	5.5	5.25	8
	10	6	5.5	5.75	5.5	5.75	8
	11	6.25	5.75	5.5	5.5	5.75	7.75
	12	6.25	5.75	5.5	6	5.75	7.5
	13	5.25	5.5	6	5.5	5.75	8
	14	5.75	5.75	5.25	5.75	5.5	8.25
	15	6	6	5.5	5.5	5.5	7.75
	Avg 8	5.983333	5.583333	5.933333	5.583333	5.633333	8.466667
	d moved	1.616667	1.916667	2.016667	0.816667	0.916667	2.433333
	%space	0.787281	0.744444	0.746331	0.872396	0.860051	0.776758
	%moved	0.212719	0.255556	0.253669	0.127604	0.139949	0.223242
	Avg %		0.240648			0.163598	
	Std dev		0.024205			0.05202	

U2OS			Control			MTOB	
82609		C1	C2	C3	M1	M2	M3
16 hr	1	9.25	9.25	9	9.25	8.25	9.25
	2	9.25	9.25	9.25	9	8.75	9.25
	3	9.25	9	9	9	8.25	9
	4	9.75	9	9	8.75	8.5	9
	5	9.25	9.25	8.75	8.75	8.25	9.25
	Avg 0	9.35	9.15	9	8.95	8.4	9.15
	1	6	4.5	4	6	5.75	5.75
	2	6.5	4.75	2.25	5.75	5	6.5
	3	6.5	5.5	2.25	6.25	5	6.5
	4	6.5	5.25	3	6	5.5	6.25
	5	6.25	5.25	3.25	6	5.5	6.5
	6	5	6	3.25	6.25	5.25	6.5
	7	4.5	5	3.75	6.25	5	6.75
	8	5.25	6	2.5	7	5	6.75
	9	5.25	6.5	2.5	5.5	5	5.5
	10	4.75	5.75	3.75	7	5.25	6.75
	11	5.5	6.5	2.25	7.25	5.5	6
	12	5.5	5.75	3.25	7.25	4.5	6.75
	13	6	5	4	6.25	5	5.75
	14	5.25	4.5	4	6.5	4.5	6.5
	15	5	5.25	3	7	4.25	6.75
	Avg 16	5.583333	5.433333	3.133333	6.416667	5.066667	6.366667
	d moved	3.766667	3.716667	5.866667	2.533333	3.333333	2.783333
	%space	0.597148	0.593807	0.348148	0.716946	0.603175	0.695811
	%moved	0.402852	0.406193	0.651852	0.283054	0.396825	0.304189
	Avg %		0.486966			0.328023	
	Std dev		0.142805			0.060515	

U2OS			Control			MTOB	
82609		C1	C2	C3	M1	M2	M3
16hr b	1	10	9.25	5.5	7	8.5	7.25
	2	9.25	9	5.75	7.25	8.75	7.25
	3	9.75	9	6	7	8.75	7.25
	4	9.75	9	5.5	7.25	8.75	7.5
	5	9.25	8.75	6	7.25	8	7.75
	Avg 0	9.6	9	5.75	7.15	8.55	7.4
	1	3.75	4	2	6	6	6.5
	2	3	3.5	2.25	6	6.75	6.75
	3	3	2.75	1.25	5.75	6.25	6.5
	4	3	3.25	2	5.75	6	5.75
	5	2.5	3.25	1.5	5.75	7	6.5
	6	2.25	3.75	2.25	6	6.25	6.25
	7	1.25	3	1	5.75	6	6.25
	8	1.5	2.75	1.5	6	5.5	6
	9	1	3.25	0.75	5.5	6.25	6
	10	1.25	3	1.5	5.25	5.5	6.5
	11	1.5	3	0.75	5.5	5.75	6
	12	2.5	3.75	1.5	5.75	5.5	5.75
	13	3.75	4	1	5.75	6	5.5
	14	4.25	2.25	0	6.5	5.25	5
	15	3.5	3	0.5	6.25	6	5.75
	Avg 16	2.533333	3.233333	1.316667	5.833333	6	6.066667
	d moved	7.066667	5.766667	4.433333	1.316667	2.55	1.333333
	%space	0.263889	0.359259	0.228986	0.815851	0.701754	0.81982
	%moved	0.736111	0.640741	0.771014	0.184149	0.298246	0.18018
	Avg %		0.715955			0.220858	
	Std dev		0.067435			0.067049	

HCT116 -/- 8-13-09

HCT -/-		Control Wells			MTOB Treated Wells		
81309		C1	C2	C3	M1	M2	M3
	0	1.875	2	1.625	2	1.75	1.75
	0	2.25	1.75	1.5	1.5	2	1.875
	0	1.625	1.875	1.625	1.625	2	1.625
	0	1.875	1.75	1.625	1.75	1.75	1.875
	0	1.625	2	1.875	1.75	1.875	1.75
	Avg 0	1.85	1.875	1.65	1.725	1.875	1.775
	1	1.375	1.375	1	1.25	1.75	1.5
	2	1.25	1.25	1.125	1.25	1.375	1.75
	3	1.25	1	1.125	1.25	1.5	1.5
	4	1.375	1.25	1.125	1.375	1.375	1.625
	5	1.125	1.125	1.25	1.375	1.25	1.25
	6	1.25	1.375	1.125	1.5	1.5	1.375
	7	1.375	1.375	1.25	1.125	1.375	1.5
	8	1.375	1.25	1.125	1.375	1.5	1.75
	9	1	1.375	0.875	1	1.5	1.375
	10	1.125	1	1	1.375	1.25	1.5
	11	1.375	1.25	1	1.25	1.5	1.5
	12	1	1.25	1	1.5	1.625	1.125
	13	1.25	1.125	1.125	1.25	1.25	1.375
	14	1.25	1.25	1.25	1.375	1.25	1.375
	15	1.25	0.75	1.25	1.25	1.375	1
	Avg 16	1.241667	1.2	1.108333	1.3	1.425	1.433333
	D moved	0.608333	0.675	0.541667	0.425	0.45	0.341667
	% space	0.671171	0.64	0.671717	0.753623	0.76	0.807512
	% moved	0.328829	0.36	0.328283	0.246377	0.24	0.192488
	Avg %		0.339037			0.226288	
	Std Dev		0.018156			0.029445	

HCT116 -/- 8-21-09

HCT -/-			Control			MTOB	
82109		C1	C2	C3	M1	M2	M3
	1	7.5	7.75	7.5	8.5	10.5	8.75
	2	10	8.25	6.75	9	10.75	9
	3	9.5	9.25	7	9.25	10.25	8.5
	4	9.5	8.5	7.5	8.75	10	9
	5	9.25	9.5	7.75	9.25	10.5	8.5
	Avg 0	9.15	8.65	7.3	8.95	10.4	8.75
	1	7.75	8	6.25	8.75	10	9.5
	2	8	7	6.75	8.75	9.5	8.75
	3	7.5	7.25	6.25	9	9.5	8.75
	4	7.5	7.5	7	8.75	9	8.25
	5	7.25	8	6.75	8.25	9.25	8
	6	7.25	7	6.5	8	9	8.75
	7	8	8.25	6.5	8.5	9.25	8.75
	8	7	8.5	6	8	9.25	8.25
	9	8	8	6.25	8	9.75	7.5
	10	7	7.25	6.5	7.75	9.25	7
	11	8.5	6.75	6.5	8.5	10	8
	12	7.5	7.5	6.25	8.25	10	7.5
	13	8.25	7.75	5.75	8.25	9.5	7.75
	14	8	7.25	6	8.5	10	8.5
	15	7.25	7.25	6.75	7.5	10	8.25
	Avg 8	7.65	7.55	6.4	8.316667	9.55	8.233333
	d moved	1.5	1.1	0.9	0.633333	0.85	0.516667
	%space	0.836066	0.872832	0.876712	0.929236	0.918269	0.940952
	%moved	0.163934	0.127168	0.123288	0.070764	0.081731	0.059048
	Avg %		0.13813			0.070514	
	Std dev		0.022431			0.011344	

HCT116 -/- 8-31-09

HCT -/-			Control			MTOB	
83109		C1	C2	C3	M1	M2	M3
	1	2	1.75	2	2	1.75	2
	2	2.25	1.875	1.625	2.125	1.875	2
	3	1.625	2	1.875	1.875	1.625	1.875
	4	1.875	1.875	1.625	1.875	1.75	2
	5	1.875	2	2	1.625	1.875	1.75
	Avg 0	1.925	1.9	1.825	1.9	1.775	1.925
	1	1.5	1.5	1.125	1.375	1.25	1.625
	2	1.375	1.375	1.25	1.375	1.5	1.875
	3	1.375	1.125	1.25	1.375	1.625	1.625
	4	1.5	1.375	1.25	1.5	1.5	1.75
	5	1.25	1.25	1.375	1.5	1.375	1.375
	6	1.375	1.5	1.25	1.625	1.625	1.5
	7	1.5	1.5	1.375	1.25	1.5	1.625
	8	1.25	1.125	1	1.25	1.375	1.625
	9	0.875	1.25	1	1.625	1.375	1.25
	10	1	0.875	0.875	1.25	1.125	1.375
	11	1.25	1.125	0.875	1.125	1.375	1.375
	12	0.875	1.125	0.875	1.375	1	1
	13	1.125	1	1	1.125	1.125	1.25
	14	1.125	1.125	1.125	1.25	1.125	1.25
	15	1.125	0.625	1.125	1.125	1.25	1.25
	Avg 16	1.233333	1.191667	1.116667	1.341667	1.341667	1.45
	d moved	0.691667	0.708333	0.708333	0.558333	0.433333	0.475
	%space	0.640693	0.627193	0.611872	0.70614	0.755869	0.753247
	%moved	0.359307	0.372807	0.388128	0.29386	0.244131	0.246753
	Avg %		0.373414			0.261581	
	Std dev		0.01442			0.027984	

HCT -/-			Control			MTOB	
83109		C1	C2	C3	M1	M2	M3
	1	1.875	1.625	1.875	1.875	1.625	1.875
	2	2.125	1.75	1.5	2	1.75	1.875
	3	1.75	1.875	1.75	1.75	1.75	1.75
	4	2	2	1.75	2	1.875	2.125
	5	2	1.875	2	1.75	2	1.875
	Avg 0	1.95	1.825	1.775	1.875	1.8	1.9
	1	1.625	1.625	1.25	1.5	1.375	1.75
	2	1.25	1.25	1.125	1.625	1.375	1.75
	3	1.5	1.25	1.375	1.5	1.75	1.75
	4	1.375	1.25	1.125	1.375	1.375	1.625
	5	1.375	1.375	1.5	1.625	1.5	1.5
	6	1.25	1.375	1.125	1.5	1.5	1.375
	7	1.625	1.375	1.5	1.375	1.625	1.75
	8	1.125	1	1	1.5	1.25	1.5
	9	1	1.375	1.125	1.75	1.5	1.375
	10	1	1	1	1.125	1	1.25
	11	1.375	1.25	1	1.25	1.5	1.5
	12	1	1	0.75	1.25	1.125	0.875
	13	1.25	1.125	1.125	1.25	1.25	1.375
	14	1	1	1	1.125	1	1.125
	15	1.25	1	1.25	1.25	1.375	1.375
	Avg 16	1.266667	1.216667	1.15	1.4	1.366667	1.458333
	d moved	0.683333	0.608333	0.625	0.475	0.433333	0.441667
	%space	0.649573	0.666667	0.647887	0.746667	0.759259	0.767544
	%moved	0.350427	0.333333	0.352113	0.253333	0.240741	0.232456
	Avg %		0.345291			0.242177	
	Std dev		0.01039			0.010512	

MCF7 8-14-09

MCF7			Control			MTOB	
81409		C1	C2	C3	M1	M2	M3
	0	1.75	2	1.625	2.25	1.875	1.625
	0	1.625	1.875	1.5	2.125	1.75	1.625
	0	2	1.625	1.875	2	1.875	1.75
	0	1.875	1.875	1.5	2.125	1.875	1.5
	0	1.875	2	1.75	2	1.75	1.625
	Avg 0	1.825	1.875	1.65	2.1	1.825	1.625
	1	1.25	1.75	1.25	1.75	1.75	1.5
	2	1.5	1.625	1.375	1.625	2	2
	3	1.625	1.25	1.375	1.875	1.75	1.625
	4	1.75	1.5	1.125	1.75	1.875	1.5
	5	1.5	1.375	1.5	1.625	1.625	1.5
	6	1.375	1.25	1	1.875	1.875	1.625
	7	1.25	1.5	1.25	1.875	1.625	1.5
	8	1.5	1.5	1	1.75	1.625	1.375
	9	1.625	1.625	1.5	1.75	1.5	1.25
	10	1.375	1.375	1.25	1.75	1.5	1.5
	11	1.375	1.75	1.375	1.625	1.5	1.25
	12	1.5	1.5	1	1.625	1.5	1.25
	13	1.25	1.75	1.375	1.5	1.375	1.375
	14	1.5	1.375	1.25	1.625	1.375	1.25
	15	1.375	1.5	1.25	1.625	1.25	1.25
	Avg 16	1.45	1.508333	1.258333	1.708333	1.608333	1.45
	Distance	0.375	0.366667	0.391667	0.391667	0.216667	0.175
	%space	0.794521	0.804444	0.762626	0.813492	0.881279	0.892308
	%moved	0.205479	0.195556	0.237374	0.186508	0.118721	0.107692
	Avg %		0.212803			0.137641	
	Std Dev		0.02185			0.042678	

MCF7 8-26-09

MCF7			**Control**			**MTOB**	
82609		C1	C2	C3	M1	M2	M3
8hr	1	8.5	9.25	9.5	9.5	8.5	12.25
	2	8.75	9	9.75	9.75	8.5	12.25
	3	8.75	8.75	8.5	10	8	12.5
	4	8.5	9	9.25	9.25	8.25	12.25
	5	9	8.75	9	9.75	7.75	12.25
	Avg 0	8.7	8.95	9.2	9.65	8.2	12.3
	1	4.5	6.75	6.5	9.75	8.25	10.5
	2	4.75	6.75	6	9.5	7.75	10.25
	3	4.5	6.5	5.75	9.5	7.75	10.75
	4	4.5	6.25	6	9.25	7.5	11.25
	5	4.75	6.75	6.25	9	8.25	11
	6	5	6	6.25	9	7.75	11
	7	5.25	6	6.25	9.5	8	10.75
	8	5.25	6.75	5.75	9.25	7.5	10.75
	9	5.25	6.75	6.25	9.25	7.25	10.25
	10	4.5	6.5	6.5	9	7.25	11
	11	4.75	6.5	6.5	9.25	7	11.5
	12	5.5	5.75	6.5	8.75	7.25	11.5
	13	5.25	5.75	6.5	9.25	7	10.75
	14	5	5.5	6.25	9	7.5	10.5
	15	5.5	5.5	6	9.5	7.25	10
	Avg 8	4.95	6.266667	6.216667	9.25	7.55	10.78333
	d moved	3.75	2.683333	2.983333	0.4	0.65	1.516667
	%space	0.568966	0.700186	0.675725	0.958549	0.920732	0.876694
	%moved	0.431034	0.299814	0.324275	0.041451	0.079268	0.123306
	Avg %		0.351708			0.081342	
	Std dev		0.069779			0.040967	

MCF7			Control			MTOB	
82609		C1	C2	C3	M1	M2	M3
8hr b	1	8	8.5	7.75	11.25	10.25	7
	2	8.25	8.25	8	12	10.5	7.25
	3	8.5	8	7.75	11.75	10	6.75
	4	8	8	7.5	12	11	6.75
	5	8.25	8.5	8	12	10.25	7.5
	Avg 0	8.2	8.25	7.8	11.8	10.4	7.05
	1	7	8	7	11.5	8	6
	2	7.25	7.5	6.5	11	8.25	5.75
	3	6.75	7	7	10	8	6.5
	4	6	7	6.5	10.25	8.5	6.25
	5	6.75	6.75	6.75	10.5	8.5	6.5
	6	5.75	6.5	6.25	11	8	5.75
	7	6.25	6.75	6.5	10	7.75	6.5
	8	7	6.25	6.25	9.5	8.5	6.25
	9	7	6.5	6.25	9.5	8.25	5.5
	10	6.5	7.25	5.75	10	8.75	5.75
	11	6.25	7.25	5.5	9.75	9.25	5.75
	12	7	6.75	6	10.25	8.75	6
	13	6.75	6.5	6	10	8.5	6
	14	7	6.25	5.75	9.5	8	6.5
	15	7.5	6.25	6	9.5	7.75	6
	Avg 8	6.716667	6.833333	6.266667	10.15	8.316667	6.066667
	d moved	1.483333	1.416667	1.533333	1.65	2.083333	0.983333
	%space	0.819106	0.828283	0.803419	0.860169	0.799679	0.86052
	%moved	0.180894	0.171717	0.196581	0.139831	0.200321	0.13948
	Avg %		0.183064			0.159877	
	Std dev		0.012573			0.035026	

MCF7			Control			MTOB	
82609		C1	C2	C3	M1	M2	M3
16 hr	1	6.75	9	9.25	9.25	9.5	8.5
	2	8.25	8.75	9.5	10	9.5	8
	3	7.75	9.25	9	10	9.25	8.25
	4	8	8.5	9.25	10.25	9.75	8.75
	5	7.25	9	9	9.25	9	8.5
	Avg 0	7.6	8.9	9.2	9.75	9.4	8.4
	1	5.25	5.75	5.5	7.5	7.5	7
	2	5.25	6	5.75	7.5	7	6.75
	3	5.75	6	4.75	8	7.25	6.75
	4	5.75	5.75	5.5	7.75	8	6.25
	5	5.25	6	5.5	6.75	7.5	6.25
	6	5.25	5.5	5.5	7.5	8	6.5
	7	4.75	6.25	4.75	6.75	8.25	6
	8	5	6.5	4.75	6.25	7	6
	9	4.75	6.25	5	6.5	7.5	6.25
	10	5.75	6	5.25	6.5	7.5	5.75
	11	5.5	5.5	5	7.25	7	6
	12	5.25	5.25	5	6.5	7.75	6.25
	13	5	5.75	4.5	7.25	7	6
	14	5	5.5	4.75	7.25	7.25	6.5
	15	5	5.75	4.75	7.5	7.5	6.25
	Avg 16	5.233333	5.85	5.083333	7.116667	7.466667	6.3
	d moved	2.366667	3.05	4.116667	2.633333	1.933333	2.1
	%space	0.688596	0.657303	0.552536	0.729915	0.794326	0.75
	%moved	0.311404	0.342697	0.447464	0.270085	0.205674	0.25
	Avg %		0.367188			0.24192	
	Std dev		0.07126			0.032957	

MCF7			Control			MTOB	
82609		C1	C2	C3	M1	M2	M3
16hr b	1	10.5	10	8.75	9.75	9.5	10.5
	2	10.25	9.5	8.5	9.5	10.25	11
	3	10	9.25	9	9.25	9.5	11
	4	10	9.5	8.75	9.75	9.75	10.5
	5	9.75	9.75	8.5	10	9.5	10.25
	Avg 0	10.1	9.6	8.7	9.65	9.7	10.65
	1	7	5.5	5	7.75	7	8
	2	6.5	5.75	5.5	8	7.5	7.75
	3	6.75	6	4.75	8	7.75	7.5
	4	6.5	6.25	5.25	7.5	8.25	8
	5	6.25	6.25	6	7	8	7.5
	6	6.75	6.5	6	7.25	7.5	7.75
	7	6.25	6.5	5	6.75	7.25	7
	8	6	7	4.75	6	7	7.5
	9	6.5	6	5.25	6.5	7	6.5
	10	6	6	4.75	6.5	7.5	6.75
	11	6.5	6.75	5.25	7.25	6	6
	12	6.25	6.25	5.75	6.75	7.5	6.75
	13	7	7	5.5	6.5	7	6.5
	14	6.75	6.5	5	6.5	7	7
	15	6.25	6.5	5.25	6.25	8	7.75
	Avg 16	6.483333	6.316667	5.266667	6.966667	7.35	7.216667
	d moved	3.616667	3.283333	3.433333	2.683333	2.35	3.433333
	%space	0.641914	0.657986	0.605364	0.721934	0.757732	0.677621
	%moved	0.358086	0.342014	0.394636	0.278066	0.242268	0.322379
	Avg %		0.364912			0.280904	
	Std dev		0.026967			0.040131	