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Effect Of Folate Deficiency On Mtor Signaling Network On The Liver Of Wild Type Mice

Essra Moussawi
Wayne State University,

EFFECT OF FOLATE DEFICIENCY ON mTOR SIGNALING NETWORK ON THE LIVER OF WILD TYPE MICE

by

ESSRA MOUSSAWI

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_____

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DEDICATION

I would like to dedicate my thesis to my family who has continuously supported me in my endeavors. A special thanks to my Mother, I would not be where I am today had I not had my mother's continuous support and mentorship.

ACKNOWLEDGMENTS

It is with immense gratitude that I acknowledge the support and help of my mentor Dr. Ahmad R. Heydari who has supported me since my undergraduate studies. He has provided me with support and resources in order to complete the work that was needed for my thesis. In addition I would like to sincerely thank Dr. Archana Unnikrishnan as she has also been a mentor, supporter and has given me much of her valuable time and efforts in order to guide me throughout the process; it has been an honor to get to know and be able to work with both Dr. Heydari and Dr. Unnikrishnan. Lastly, I would like to extend appreciation to my lab members: Ali, Amanda, Rawya, Safa, Sukayina and Tom.

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CHAPTER 1

INTRODUCTION

Nutritional health has always been of important concern to humanity. We are a society built around the preservation of life. For centuries we have been a part of the “Battle of the Fittest”. With modern technology, and research we are apart of an era in which we are able to understand and/or observe the effects of bioactive food components, such as folate, on the human genome. Such insight is accomplished through the study of nutrigenomics. It is of great importance that we understand the components of the foods consumed effect the human body on the surface, deep within deep within our physiological begins, and on a molecular biology level. Recent studies have indicated an inverse relationship between dietary intake of folate, and the development of cancer [1]. As one increases their intake level of folate, one is inadvertently decreasing the risk of cancers such as liver, pancreatic, stomach, colon, and breast cancer [1]. Studies have suggested that the activation and inactivation of autophagy may benefit against the progression of cancer cells [41]. Thus, it is important for researchers to understand the mechanism that surrounds folate restriction and the maturation of cancer, in essence to the effects of autophagy by observing the mTOR signaling pathway.

A. Folate

Folate is a naturally occurring water-soluble vitamin, belonging to the vitamin B-complex group. Folate is found in plant-based foods such as, fruits, legumes, lentils, beans, green leafy vegetables, and other foods [3,14]. Folic acid is the

synthetically produced form of folate used in fortified foods. Individuals dependent on receiving nutrients from fast foods, or diets low in fruits and vegetables are under consuming folate, and thus they are putting themselves at risk for developing diseases and/or dangerous health conditions [3]. Additionally folate is thought to be the most common vitamin deficiency not only in the United States but worldwide [4]. Folate is a cofactor involved in several mechanistic pathways, such as synthesis of DNA, repairs of DNA and methylation of DNA. However the details of the folate pathway and its effects are still unknown. Folate has been associated with the etiology of birth defects, and many chronic diseases such as cardiovascular, neurological degeneration and various cancers.

B. Role of Folate in One-Carbon Metabolism

Discovered in the 1940s, folate is an essential cofactor for cell growth in living organisms. Folic acid is a cofactor in one-carbon metabolism [5]. Folate behaves as a carrier in one-carbon metabolism, and it is essential for cellular functions such as cell division, DNA repair, and the regulation of amino acid homocysteine [11]. It is of utter importance to understand the mechanism behind folic acid's ability to alter the one-carbon metabolism. Folic acid is found at different oxidative states in a cell. The different oxidative states are known as folate, dihydrofolate (DHF) and tetrahydrofolate (THF) [6]. The cofactor known as 5-methylenetetrahydrofolate (5-methyl THF) is the main factor that is involved in one-carbon metabolism. The enzyme known as methylenetetrahydrofolate reducatase (MTHER) is the enzyme responsible for causing an irreversible conversion of 5,10-methylene THF to 5-methyl THF. The main functions that are

a result of folate metabolism are: one-carbon transfer in the methionine cycle, synthesis of thymidylate and purines (adenine and guanine) and DNA methylation [7,9,10].

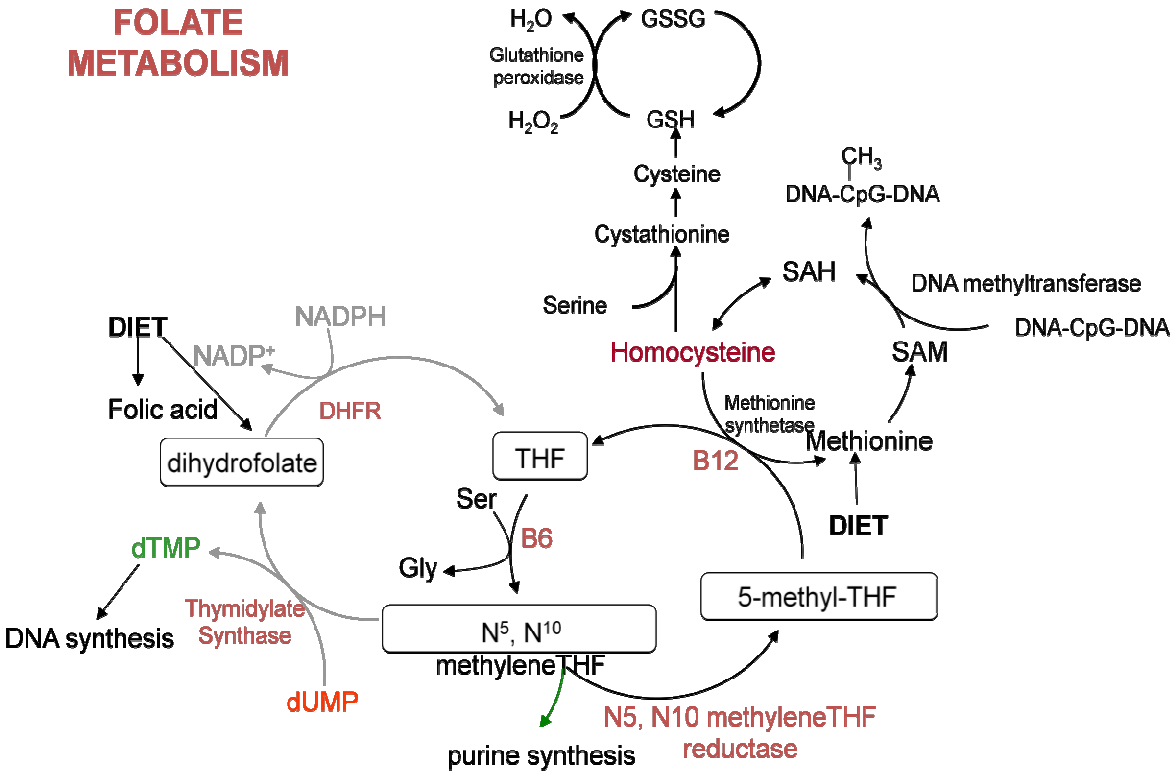


Figure 1-1: Folate and its Metabolism (2)

In the methionine pathway the function of 5-methyl THF is to remethylate the amino acid homocysteine to its essential amino acid for “methionine”. Methionine is then broken down to the methyl group donor s-adenosylmethionine (SAM).

Levels of purines adenosine and guanosine are relatively adequate, as 5-methyl THF is the dominant source used as a methyl donor. Through the pyrimidine biosynthesis pathway, the production of thymidine is established involving an exchange of deoxyuridine monophosphate (dUMP) to thymidine monophosphate (TMP). The enzyme thymidylate synthase (TS) catalyzes the reaction. All of these reactions are essential for a continued production of nucleotides, and further more to ensure that DNA proliferation, as well as repair activities are appropriately maintained. A folate restricted diet will have a negative impact on these reactions causing altering the reasons that need to take place and ultimately affecting the well being of individuals [9,11].

C. Folate Deficiency Leading to Health Concerns

There are many factors to take into consideration when in the process of understanding the underlying cause of a disease of health concern. It is the battle between cause and effect, which may be due to genetic, environmental or dietary susceptibility. Nutrition has been considered to play a vital role on an individual's health [4]. The correlation between an individual's degenerating health may not always be a straight path of cause and effect. Many times there are a series of indirect reactions and occurrences that lead up to a person's development of their disease or condition. Through careful epidemiological studies, researchers have suggested that folate restriction is the indirect cause of poor health. Folate restriction has shown to increase homocystine (tHcy) levels, which in turn has increased the risk for abortion, birth defects, low birth weights and cardiovascular disease. Folate is responsible for DNA and RNA synthesis, growth, cognitive

functions and proliferation [14]. Understanding the health concerns associated with folate restriction is important because it plays a serious role is diseases such as Alzheimers, various cancers, cardiovascular diseases and neural tube defects. These are amongst just some of the possible diseases and conditions that come from folate restriction [15]. Folate restriction is considered to be the most common vitamin deficiency worldwide that can severely alter an individual's health condition because of the molecular role it plays within the body [4,13].

1. Vitamin B_{12} Deficiency and Anemia

Anemia is the inadequacy of red blood cell production due to the inability of hemoglobin generation, which in return leaves the organism with insufficient levels. The primary function for red blood cells is to provide nourishment of oxygen to the cells, through the transportation of hemoglobin [18]. When hemoglobin levels are altered, the oxygen delivery to cells is altered as well. An inadequacy of folate levels may severely impact the de novo pathway of DNA and RNA synthesis, thus causing a disruption in cell division and/or may cause deformed red blood cells [18].

An alarming concern is the ability of folic acid to mask anemia caused by vitamin B_{12} deficiency as past studies have suggested. Case reports have suggested that a consumption of ≥5000 µg of folic acid daily will mask the anemia caused by vitamin B_{12} [22]. This is important finding, because anemia caused by vitamin B_{12} deficiency if not detected early on-set may lead to peripheral neuropathy, which is the disease or damage of nerves [22]. Studies in India have reported anemia to be the seventh most common death amongst

Indian women working in factories, according to the factory medical officer's reports [20].

2. Neural Tube Defects

Neural tube defects (NTDs) occur when the neural tube of the brain and/or the spinal cord does not successfully close during the early development stages of an embryo resulting in damage of the embryo neural tissues. This may result in death depending on the severity and location of the lesion [22,24].

Insufficient amounts of dietary folate are known to be the cause of neural tube defects, miscarriages, and premature births. Thus after many studies conducted by various researchers the CDC has recommended that women with prior history of NTD consume a daily amount of 4000 µg prior to conceiving. Consuming folate is essential prior to conception, since the neural tubes close early during the growth of the embryo (28 days after conception). Additionally the U.S Public Health has recommended that all women consume a daily dose of 400 µg of folate [22,25].

3. Cancer and Epigenetics

Through the studies of epidemiology, researchers have found a strong correlation between folate deficiency and the risk of developing cancerous diseases [22,25]. As previously mentioned, studies have shown that folate restriction may increase the risk of cancer development in the liver, pancreas, stomach, colon and breast regions [1]. Whilst suggesting that folate consumption through regular diet of fruits and vegetables will have auspicious effects. Most studies have shown that there is a strong link between folate consumption and

colorectal cancer [22]. Studies relating to folate consumption and epigenetic patterns are being conducted, in order to better understand the link between the two.

Understanding folate's relation to epigenetics is important because of the metabolic roles folate plays in both the DNA synthesis and DNA methylation. Researches believe that an adequate consumption of folic acid may prevent development of tumor cells by providing an adequate amount of methyl groups to enhance DNA repair during the occurrence of DNA damage. On the other hand other researches have suggested, that high consumption of folic acid may accelerate the development of pre-existing tumors thus suggestion that early exposure to folic acid should be observed in order to maintain normal physiological activities of DNA synthesis and DNA methylation. If we gain a better understanding of epigenetics we will have a better understanding of the health concerns that come along with folate deficiency [25].

D. Roles of Autophagy and mTOR Signaling Pathway

The mTOR (mammalian target of rapamycin) is a serine/threonine protein kinase that plays a very important role in signaling pathways which in return controls cells growth, cell proliferation, cell motility, cell survival, protein synthesis and transcription [31]. mTOR activity is supervised by a series of amino acids, mainly leucine, as well as growth factors and AMP-activated protein kinase as an overall energy supplier [30]. mTOR senses cellular nutrient, oxygen and energy levels.

The mTOR signaling pathway can easily be affected by various factors, including factors that may cause an alteration in the nutrient transport, energy metabolism, protein availability and purine biosynthesis. In the state of a disease, the mTOR signallng pathway is deregulated, causing the homeostatsis of an organism to be compromised [31]. Such diseases include the following: cancer, metabolic disorder such as diabetes, and ageing. When the mTOR pathway is deregulated proliferation of cancer cells are stimulated and further damage is inflected upon the organism [31]. During meticulous stages of tumor and cancer development, the signaling mechanism in upstream and downstream of the substrates have a great affect on the mTOR-signaling pathway [30].

Autophagy is the cell degradation of unnecessary and dysfunctional intracellular components through the process of lysosome in the eukaryotic system. During autophagy, the cytoplasmic cellular components are engulfed by lysosomes. Reports have indicated that autophagy plays a very important role in pathology, leading to the belief that both activation and inactivation of autophagy may benefit cancer cells [40]. If a cell is unable to activate autophagy, protein synthesis rises over degradation of protein and tumor growth will continue. However, if autophagy is being activated this could lead to an effective method of eliminating infectious components that gain access into the cytosol of the cell [39, 40].

The protein Beclin, a tumor suppressor, is required during the initial regulation of autophagy [36]. Autophagy related protein 5 (ATG5) is an E3 ubiquitin ligase protein that is necessary for autophagy and is activated by ATG7.

ATG5 is additionally involved in preservation of the quality of the mitochondria after oxidative damage has been inflected, as well as cellular longevity [37, 38]. The light-chain 3 (LC3) can be used to monitor autophagy activity and two distinct bands are shown: LC3-I and LC3-II. The amount of autophagy is correlated with LC3-II [40]. During autophagy, the cytoplasmic form LC3-I is drafted to the autophagososmes, where LC3-II is produced by site-specific proteins proteolysis and lipidation, which remains associated with autophagososmes until it is fused with lysosome then LC3 is degraded [39, 40]. Autophagy related protein 12 (ATG12) is a protein involved in promotion of apoptosis. It is involved in vesicle formation of autophagy, and ATG12-ATG5 act as E3-like enzymes, which are required for lipidation [38]. Autophagy related protein 3 (ATG3) is a protein involved in autophagy, degradation and turnover and recycling of the cytoplasmic materials in eukaryotic cells. Additionally ATG3 regulates autophagy during the death of a cell [43].

There have been many suggestions which link autophagy and cancer [39]. Figure 1-2 is an illustration of the autophagy pathway in the mTOR signaling pathway. Through the mTOR pathway autophagy can be induced, helping to fight against cancer [42]. In specific tumor and cancer stages, certain autophagy-related proteins have a major effect on the mTOR signaling pathway. It is important to identify the substrates and proteins along with how they significantly contribute to the regulation of the mTOR signaling pathway, during a folate deficient status.

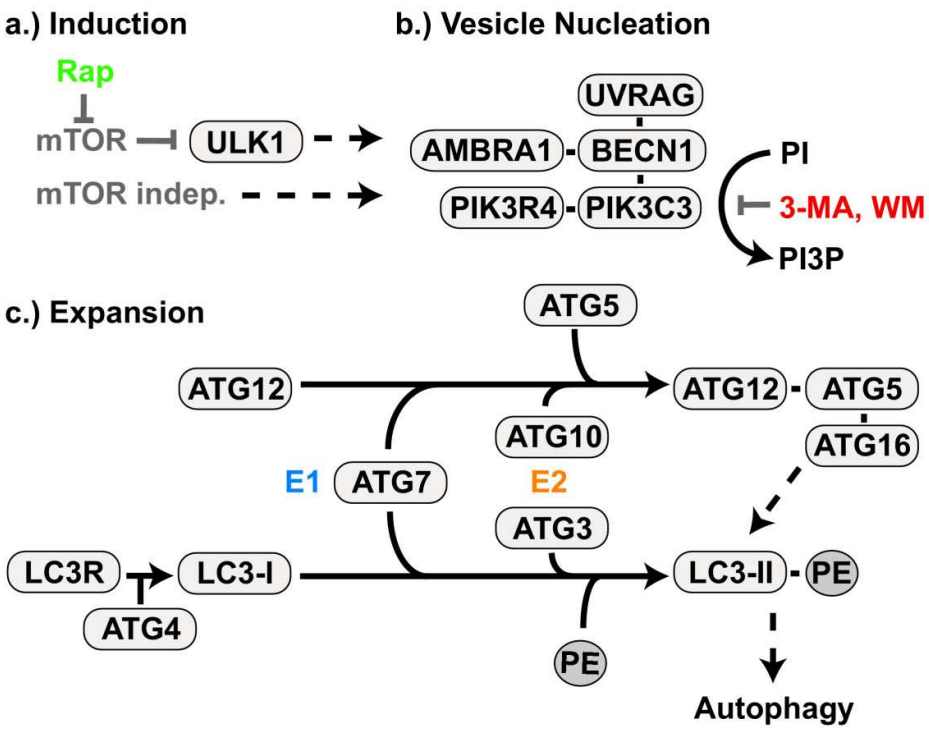


Figure 1-2: Illustration of mTOR Signaling Pathway [42].

CHAPTER 2

SPECIFIC AIMS

In order to gain an understanding of the mechanism behind the effects of folate restriction on tumorigenesis, it is crucial we examine the mechanism behind folate, and the autophagy related proteins in the mTOR signaling pathway. Upon deregulation of the mTOR signaling pathway, proliferation of cancer cells are stimulated causing extensive damage. Specific proteins involved in the autophagy of the mTOR pathway affect the growth and development of cancer and tumors. We hypothesize that dietary folate alters the mTOR signaling pathway in the liver of wild type mice protecting them against tumorigenesis, which have been treated with a carcinogenic substance.

The following are the specific aims of the research:

Specific Aim 1: To examine the effects of folate deficiency against tumor growth in the liver of wild type mice through measurement of ACF counts per mouse.

Specific Aim 2: To examine the effects of folate deficiency on the signaling pathway of the substrates involved in the regulation of autophagy in mTOR signaling pathway in wild type mice that have been treated with DMH, a cariogenic substance.

CHAPTER 3

METHODS

A. Animals

The experiments were performed on young male wild type mice 6 weeks of age. Mice came from a C57BL/6-specfic pathogen-free strain. All procedures were performed in accordance with the National Institutes of Health guidelines for the use and care of laboratory mice. All mice survived, and no retardation was developed as a result of dietary intake. Mice used during this experimental trial were backcrossed into the C57BL/6 strain for a minimum of 20 generations. In order to determine the genotype of all mice Southern blot analysis was performed as described by Cabelof *et. al.* Animal protocol was given approval through the Wayne State University Animal Investigation Committee [15]. All mice during this experiment were maintained on a 12-hr light/dark cycle and given water *ad libitum* [15].

B. Diet and Carcinogenic Treatment

After a weeklong acclimation process, wild type (WT) mice were randomly delegated to the following dietary groups: folate adequate (FA), and folate deficient (FD) AIN93G-purified isoenergetic diet (Dyets, Inc., Lehigh Valley, PA). The FA dietary group received a folate adequate diet composed of 2 mg/kg of folic acid, while the folate deficient group received a 0 mg/kg of folic acid. 1% succinyl sulfathiazole was used in all diets for experimental purposes, and all diets were stored at -20 °C [15]. One week proceeding to dietary ingestion, mice were randomly selected from both FA and FD groups and injected

intraperitoneally with the carcinogenic 1,2-dimethylhydrazine HCL (DMH, 30 mg/kg body weight) in 10 mmol/liter of $NaHCO_3$ (Fisher Scientific) once a week for the duration of 6 weeks. Both dietary intake and body weight were recorded twice a week to monitor for signs of toxicity and/or weight loss, and diets continued for the duration of 12 weeks [15].

C. Aberrant Colonic Crypt (ACF) Analysis

Animals were anesthetized under CO_2 asphyxiation the abdominal cavity was exposed and the colon was extracted, cleansed with cold phosphate-buffered saline, cut longitudinally, and fixed flat overnight in 10% neutral buffered formalin. The colonic crypts were stained with 2 g/liter of methylene blue in phosphate-buffered saline for 5 min. The number of ACF and aberrant crypts per foci were observed by light microscopy at a magnification of $^{X}10$ in a blinded manner [15].

D. Western Blot Analysis

Western blot analysis was performed during this experiment, using 200 µg of nuclear protein. Upon completion of SDS-PAGE, the region containing the protein(s) of interest was extracted and primed for Western blot analysis. Remaining portion of the gel was dyed with a GelCode blue stain reagent (Pierce Biotechnology) to establish equivalent protein loading in each of wells. As an internal control for protein loading, membranes were re-probed with anti-Lamin B antibody (Santa Cruz Biotechnology, Santa Cruz, CA). The bands were visualized and quantified using a Chemilmager^{TM} System (Alphalnnotech, San Leandro, CA) proceeding incubation in SuperSignal® West Pico

Chemiluminescent Substrate (Pierce Biotechnology). Data are shown as the integrated density value of band per µg of protein loaded [15].

E. Statistical Analysis

Statistical significance between means was determined using a “unpaired t-test”, after quantification of protein data. *P* values representing a value less than 0.05 were considered statistically significant. Significant values will be marked by “*” on graphs, where significant p value is present.

CHAPTER 4

FIGURES

Figure 4-1: Experimental Design. Wild type (WT) mice were fed either a folate adequate (2 mg/kg, FA) diet or a folate deficient (0 mg/kg, FD) diet for the duration of 12 weeks. All WT mice in this experiment were treated with a carcinogenic at 6 weeks old (young mice). Proceeding the first week of dietary intake, all groups then followed the same treatment and were injected with 30 mg/kg body weight of 1,2-dimethylhydrazine (DMH), a colon and liver carcinogen, for a period of 6 weeks. After 6 weeks of consecutive treatment, the animals were sacrificed using a CO_2 asphyxiation chamber on week 12.

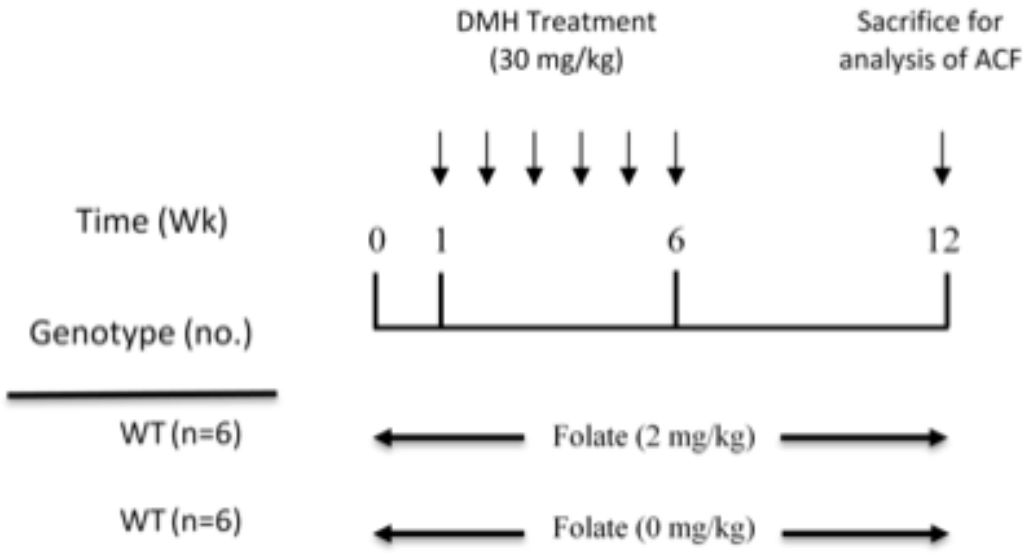


Figure 4-2: ACF Formation. Wild Type (WT) mice received either folate adequate (FA) or folate deficient (FD) diet. Additionally both diet groups FA and FD were subjected to intraperitoneal treatment with 1,2-dimethylhydrazine (DMH) for 6 weeks at 30 mg/kg body weight. After sacrifice, colons were processed as described under “Methods”. Colons were analyzed under light microscopy to visualize the number of ACF per mouse colon (ACF/mouse).

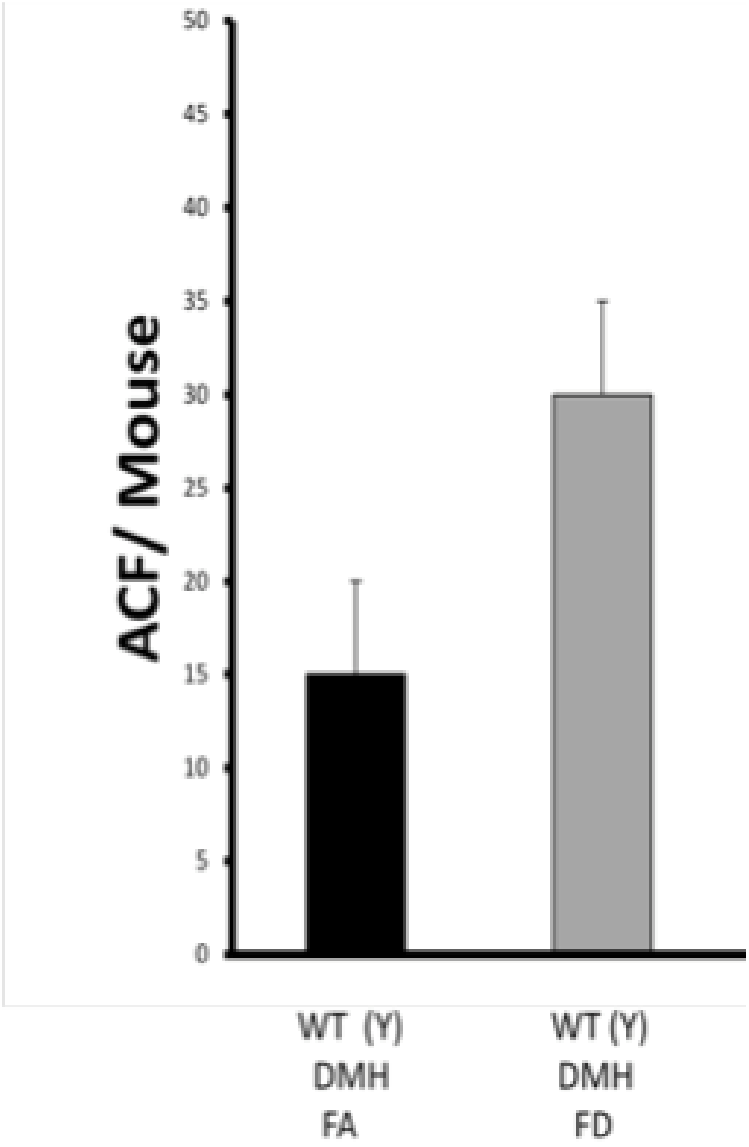


Figure 4-3: Effect of folate deficiency and DMH treatment on protein levels of Beclin in young WT mice. This figure is a representation of the analysis of the Beclin protein levels in mucosa of young (Y) WT mice that received either folate adequate (FA) or folate deficient (FD) diet, and were subjected to intraperitoneal treatment with DMH for 6 weeks at 30 mg/kg body weight (DMH). The protein levels were quantified using Western blot analysis. Values represent an average (±SEM) of data obtained from 3 mice of each group and are representative of separate identical experiments. Values with different letter superscripts indicate significant differences at $p>0.05$.

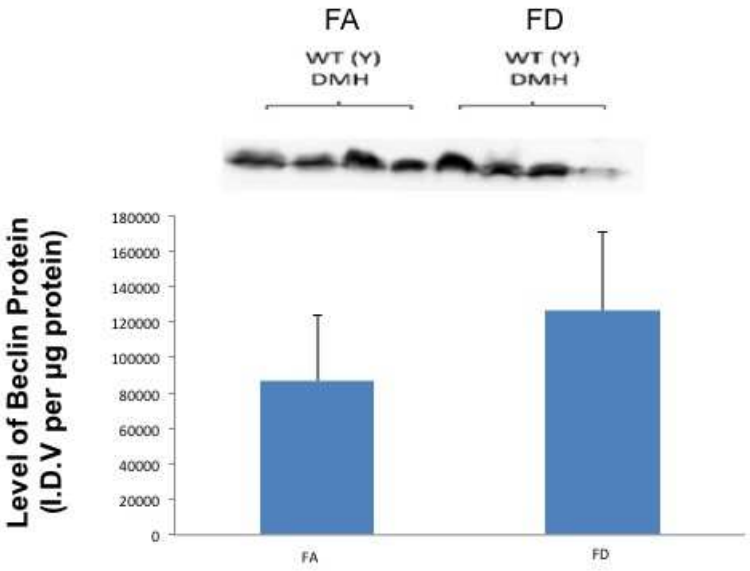

FA
FD
WT (Y)
DMH
WT (Y)
DMH
Level of Beclin Protein
(I.D.V per µg protein)
180000
160000
140000
120000
100000
80000
60000
40000
20000
0
FA
FD

Figure 4-4: Effect of folate deficiency and DMH treatment on protein levels of ATG5 in young WT mice. This figure is a representation of the analysis of the ATG5 protein levels in mucosa of young (Y) WT mice that received either folate adequate (FA) or folate deficient (FD) diet, and were subjected to intraperitoneal treatment with DMH for 6 weeks at 30 mg/kg body weight (DMH). The protein levels were quantified using Western blot analysis. Values represent an average (±SEM) of data obtained from 3 mice of each group and are representative of separate identical experiments. Values with different letter superscripts indicate significant differences at p>0.05.

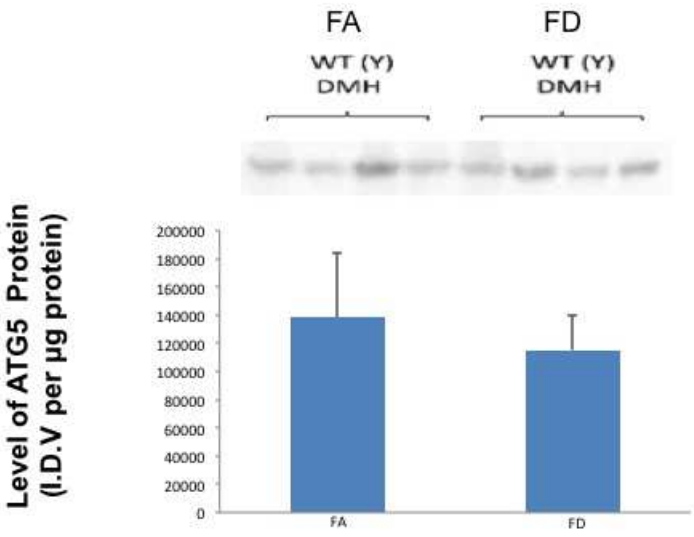


Figure 4-5: Effect of folate deficiency and DMH treatment on protein levels of LC3 in young WT mice. This figure is a representation of the analysis of the LC3 protein levels in mucosa of young (Y) WT mice that received either folate adequate (FA) or folate deficient (FD) diet, and were subjected to intraperitoneal treatment with DMH for 6 weeks at 30 mg/kg body weight (DMH). The protein levels were quantified using Western blot analysis. Values represent an average (±SEM) of data obtained from 3 mice of each group and are representative of separate identical experiments. Values with different letter superscripts indicate significant differences at p>0.05.

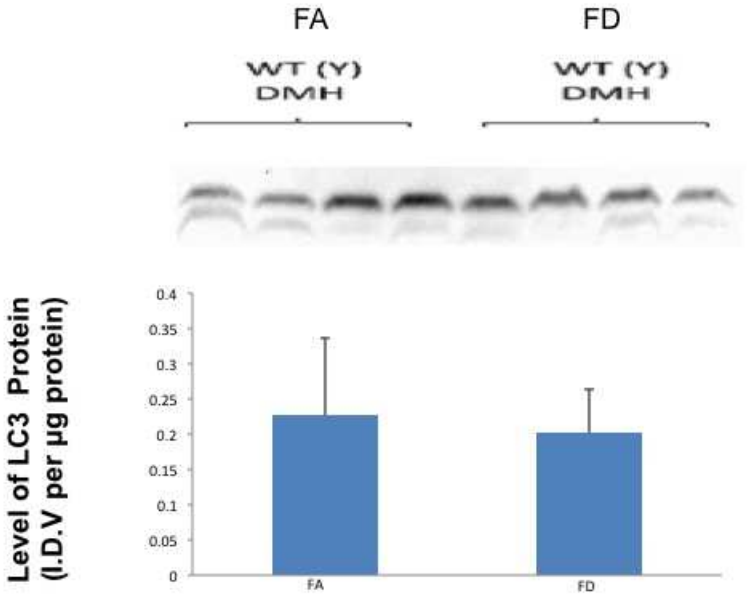


Figure 4-6: Effect of folate deficiency and DMH treatment on protein levels of ATG12 in young WT mice. This figure is a representation of the analysis of the ATG12 protein levels in mucosa of young (Y) WT mice that received either folate adequate (FA) or folate deficient (FD) diet, and were subjected to intraperitoneal treatment with DMH for 6 weeks at 30 mg/kg body weight (DMH). The protein levels were quantified using Western blot analysis. Values represent an average (±SEM) of data obtained from 3 mice of each group and are representative of separate identical experiments. Values with different letter superscripts indicate significant differences at p>0.05.

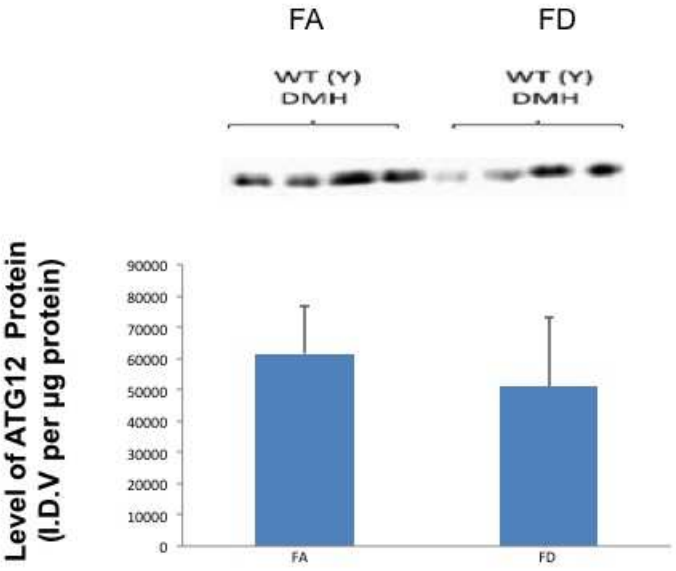
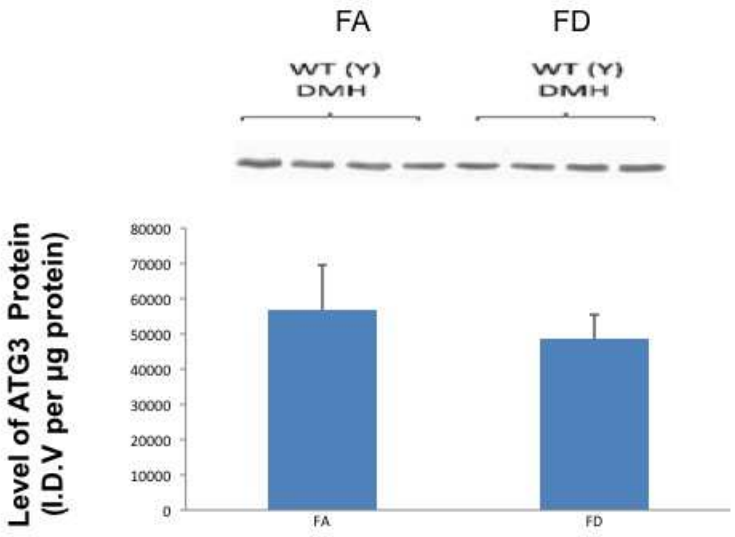


Figure 4-7: Effect of folate deficiency and DMH treatment on protein levels of ATG3 in young WT mice. This figure is a representation of the analysis of the ATG3 protein levels in mucosa of young (Y) WT mice that received either folate adequate (FA) or folate deficient (FD) diet, and were subjected to intraperitoneal treatment with DMH for 6 weeks at 30 mg/kg body weight (DMH). The protein levels were quantified using Western blot analysis. Values represent an average (±SEM) of data obtained from 3 mice of each group and are representative of separate identical experiments. Values with different letter superscripts indicate significant differences at p>0.05.



CHAPTER 5

RESULTS AND DISCUSSION

The purpose of our study was to determine the effects of folate deficiency against tumor growth in wild type mice, which were treated with colon and liver carcinogen, through the measurement of ACF counts per mouse, in addition too the effects of folate deficiency on autophagy regulation in the mTOR signaling pathway. This was done through the observation autopohagy related proteins Becline, ATG5, LC3, ATG12 and ATG3, which all play a crucial role in the autophagy pathway. Through the knowledge of previous studies, a correlation has linked folate deficiency to carcinogenesis. Previous studies have also linked autophagy to cancer, using the mTOR pathway to analyze autophagy's effects on cancer we have seen that in specific tumor and cancer stages, certain autophagy related proteins help prevent cancer. It is important to identify the key components and observe their specific roles contributing to the regulation of the mTOR signaling pathway during a state of folate restriction and turmogenesis.

Data collected from our laboratory, and microarray analysis have observed the effects folate deficiency displaces upon the expression of the mammalian target of rapamycin (mTOR) signaling pathway in the colon of young wild type mice, which were treated with carcinogen 1,2-dimethyhydrazine (DMH). The mTOR signaling pathway, as seen in figure 1-4, plays a crucial role cell growth, proliferation and survival incorporating both extracellular and intracellular signals. Studies have shown that the mTOR pathway has been trigged by various cellular activities such as: tumor growth, insulin resistance (IR) and/or

adipogenesis. Additionally the mTOR pathway can be deregulated during the development of cancer, causing stimulation to cancer cells, which further causes damage. An experimental design, as illustrated by figure 4-1, was developed by our laboratory to determine whether folate deficiency would protect against the progression of tumorigenesis, with young wild type (WT) mice that were treated with a carcinogen beginning at 6 weeks of age. Mice were divided into two dietary groups folate adequate (FA) and folate deficient (FD), for the duration of 12 weeks. The FA group were fed 2 mg/kg of dietary folate, while the FD group was fed 0 mg/kg dietary folate. All WT mice were injected with 30 mg/kg body weight of 1,2-dimethylhydrazine (DMH), a colon and liver carcinogen, for a period of 6 weeks. After 6 weeks of consecutive treatment, the animals were sacrificed using a CO_2 asphyxiation chamber on week 12.

After scarification of the animals the abdominal cavity was exposed and the colon was carefully and properly extracted from the body in order to analyze the number of Aberrant Colonic Crypt (ACF). Mice colon was analyzed under light microscopy, as illustrated by figure 4-2, an image explaining the ACF count per mouse in colon (ACF/mouse). Previous studies showed that untreated wild type mice showed no sign of ACF formation in their colon. In our studies, our ACF findings, as illustrated by figure 4-2, the young WT mice in the folate deficiency (FD) group, whom were treated with DMH, showed a significant increase in ACF (~50%) as compared to animals that were on folate adequate (FA) diets. This was an indication that folate deficiency leads to a significant increase in colon carcinogen in response to DMH. As both the FA and FD groups

were young wild type mice, this finding indicated that age was not a factor in the development of colon carcinogenesis.

Using the data collected from our experimental study, we will provide a comparison amongst the two dietary groups in order to show a better perception of the upstream and downstream signaling pathway of these different substrates, which are involved in the autophagy of the mTOR pathway. Figures 4-3 to 4-7 will draw a comparison between the different dietary groups. No other comparisons will be shown as all mice used in this experiment were of wild type (WT) strain and young (“Y”) mice in age, and all mice were administered with the DMH treatment for the duration of 6 weeks.

Macroautophagy is the primary pathway that occurs in damaged cell organelles and unused proteins. Macroautophagy uses the involvement of a double membrane formation around the cytoplasm of the substrate resulting in autophagosome, which is induced by class III PIK3 (phosphoinsitide-3-kinase). PIK3 is the autophagy related gene that includes Becline1 (ATG6), ATG4, ATG12, ATG5, ATG12 and ATG16 are additionally involved in the autophagy pathway in mTOR. Autophagosome voyages through the cytoplasm of the cell to a lysosome and both the organelles and amalgamate. Due to the acidic nature of lysosome the organelles go through hydrolases breaking down the waste material.

The autophagy protein Beclin is a tumor suppressor, which is required during the initial regulation of autophagy [36]. Folate deficiency and DMH treatment on the autophagy related protein Beclin is illustrated by figure 4-3 using

young WT mice. The figure for Beclin shows an insignificant difference of protein levels with treated young WT mice between the FA and FD diet. Figure 4-3 shows a $p > 0.05$, Beclin is reported at 0.46.

Autophagy related protein 5 (ATG5) is a E3 ubiquitin ligase protein that is necessary for autophagosome elongation. ATG5 is additionally involved in the maintenance of the quality of the mitochondria after oxidative damage has been inflected upon it, as well as cellular longevity. Folate deficiency and DMH treatment on the autophagy related protein ATG5 is illustrated by figure 4-3 using young WT mice did not show a significant difference. The p-value for ATG5 is reported at 0.62.

The light-chain 3 (LC3), autophagy protein is used to monitor the activities of autophagy, using two bands LC3-I and LC3-II for distinction. LC3-I band is used for autophagososmes, while the second band LC3-II to indicated the amount of autophagy within the protein that is drafted for proteolysis and lipidation, which is fused with lysosome before it is degraded. Folate deficiency and DMH treatment on the autophagy related protein LC3 is illustrated by figure 4-3 using young WT mice. The autophagy protein LC3 is p value was 0.83 which, indicated there is no significant difference between the dietary groups in treated young WT mice.

Autophagy related protein 12 (ATG12) is involved in the promotion of apoptosis. It is also involved the vesicle formation of autophagy. Folate deficiency and DMH treatment on the autophagy related protein ATG12 is illustrated by figure 4-3 using young WT mice. There was an insignificant

difference between the dietary groups that were illustrated by figure 4-3, as the p-value was at a 0.67.

Autophagy-related protein 3 (ATG3) is involved in the autophagy, degradation and turnover and recycling of the cytoplasmic materials in eukaryotic cells. In addition ATG3 protein regulates autophagy during the death of a cell. Folate deficiency and DMH treatment on the autophagy related protein ATG3 is illustrated by figure 4-3 using young WT mice, after running an unpaired t-test the p-value was at a 0.53 indicating an insignificant difference between the treated FA and FD dietary groups.

CHAPTER 6

CONCLUSION

A respective some of studies linked a correlation between folate restriction, aging and carcinogenesis, suggesting that folate restriction can reduce the progression of cancer with the aid of apoptosis through autophagy in the mTOR signaling pathway. It is important that we understand the folate pathway, the mTOR signaling pathway and the role autophagy plays in cancer. Previous studies have found folate deficiency in young WT mice resulted in a significant increase in ACF formation in comparison to the FA counter-group. This finding suggested that folate deficiency resulted in a significant increase in colon tumorogenesis in response to DMH treatment. Our data agrees with these findings, as they have suggested that folate deficiency in DMH treated young WT mice resulted in a significant increase (~50%) in ACF formation as compared to the FA counter-group, illustrated by figure 4-2. These findings suggested that apoptosis my play a significant role in anti-cancer progression. However, when we conducted our western blot analysis on autophagy related proteins comparing the two dietary groups (FA ad FD) no significant differences were observed, according to the *p-values* obtained. This finding may suggest these specific autophagy proteins are not affected by dietary folate. Our studies are insufficient and need to conduct further studies to further understand the mechanism behind folate deficiency's affect on autophagy in the mTOR pathway and how it plays a role in cancer prevention.

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41. Clelia Miracco, MDa, Corresponding Author Contact Information, E-mail the Corresponding Author, Gabriele Cevenini, MEngb, Alessandro Franchi, MDc, Pietro Luzi, MDa, Elena Cosci, BSca, Vasileios Mourmouras, MDa, Irene Monciatti, BSca, Susanna Mannucci, BSca, Maurizio Biagioli, MDd, Marzia Toscano, MSce, Daniele Moretti, PhDe, Roberto Lio, MDa, Daniela Massi, MDc. "Beclin 1 and LC3 Autophagic Gene Expression in Cutaneous Melanocytic Lesions ☆." *Human Pathology*. Elsevier, 2014.

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ABSTRACT

EFFECT OF FOLATE DEFICIENCY ON mTOR SIGNALING NETWORK ON THE LIVER OF WILD TYPE MICE

by

ESSRA MOUSSAWI

August 2014

Advisor: Dr. Ahmad R. Heydari

Major: Nutrition and Food Science

Degree: Masters of Science

Through various studies of dietary nutrients on the affects of the physiological and molecular pathways are a key study in understanding the interaction between dietary nutrients and the human genome that may severely impact the development of various cancers. A primary approach is observing the dietary nutrients, that we consume and how it plays a role in the cellular pathway of our bodies through experimental methods. Folate deficiency (FD) has shown, through studies, to play a role anti-cancer progression. Our goal was to observe the autophagy related proteins in the mTOR signaling pathway as it was suggested that mTOR may induce autophagy, which in return could help reduce the progression of cancer by apoptosis of cancer cells. We anticipated to see an increase in the protein levels of autophagy related proteins, with our DMH treated FD dietary experimental group. We hypothesize that dietary restriction will increase the protein levels of autophagy proteins in the mTOR signaling pathway, acting as an anti-cancer aid.

AUTOBIOGRAPHICAL STATEMENT

ESSRA MOUSSAWI

Education

Graduating-August 2014	**Master of Science in Nutrition and Food Science**
December 2011	**Bachelor of Science in Nutrition and Food Science**

Awards, Memberships & Volunteer Work

Member of Golden Future for South Africa Graduate Students, Wayne State University 2014 to Present
Volunteer HUDA Clinic Detroit, 2014 to Present
Volunteer American Red Cross, 2010 to Present
Member of American Student Dental Association, 2009 and 2010
Volunteer Detroit Partnership, 2009 and 2010