BER and Folate Deficiency: A comprehensive overview of DNA base excision repair and the effect of dietary folate

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Abstract

Folate is the naturally occurring form of water-soluble B vitamin that is found in foods such as leafy vegetables, fruits, legumes, etc. Dietary supplementation of folate has shown to be protective against neural tube defects and other congenital disorders, and of recent, its role in carcinogenesis has been of special interest. Though mechanistically unclear, a positive correlation has been observed between folate deficiency in the diet and decrease function of DNA base excision repair pathways. DNA base excision repair, commonly referred to as BER, is an important cellular process that is responsible for the removal and repair of individual damaged nitrogenous bases, effectively restoring proper DNA sequence and stability. Folate is believed to be somehow involved in the mechanisms by which BER enzymes function. It has been shown in animal model studies that by decreasing folate in the diet, the cell’s capability for BER is also decreased. Interestingly, more recent studies have indicated that under specific conditions folate deficiency may be associated with an up-regulation of apoptotic activity, suggesting a potential for therapeutic application of folate deficient diets. Further research is still needed, especially in the determining the specific mechanism by which folate affects BER and the enzymes associated with this pathway.
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In all cells, spontaneous and induced DNA mutations occur. The mechanisms by which cells repair these damaged DNA sequences is an area of much interest for research scientists, especially analyzing the role environment plays in the rate at which these mechanisms function. DNA repair is a key component in reducing harmful DNA mutations that can alter the cell’s overall function, increasing the risk of diseases such as cancer. Normal eukaryotic cells have several mechanisms by which they carry out DNA repair. One such mechanism is excision of the damaged section of DNA. There are three types of excision that have been identified as part of the cell’s DNA repair functionality; nucleotide excision repair, base excision repair, and mismatch repair. Base excision repair (BER) and the enzymes that carry out this form of DNA repair are of particular interest to the field of Nutrition. By controlling certain environmental factors, such as diet, the rate and efficiency of repair can be altered. Folate is one nutrient in the diet that has been observed to have an effect on BER, and is therefore a possible area of research in disease prevention. Alteration of DNA repair pathways is especially significant in conditions that are characterized by DNA damage, namely cancer. This paper will give an overview of DNA replication and repair focusing on the functionality of BER pathways, as well as discuss the current research of disease prevention through nutritional management of BER, specifically by dietary folate manipulation.

**DNA Replication and Repair**

Deoxyribonucleic acid (DNA) contains the genetic information necessary for the development and growth of all live cells, with the exception of RNA viruses. DNA is heritable, and therefore its replication must be carefully monitored and regulated to avoid over expression
and to repair any mutations that may arise throughout the process. This is necessary for the survival of the individual, as genetic stability is essential for life. As proven through experimentation by Meselson-Stahl, double helical DNA replication is semi-conservative, meaning that during replication one parental strand serves as a template for the synthesis of a new strand. The cell division that occurs during mitosis results in the formation of two daughter cells, each with their own DNA consisting of one conserved strand and one newly formed strand of complementary DNA. Maintaining one conserved strand improves the fidelity of DNA replication and therefore helps maintain genetic stability (Alberts, 2002).

DNA replication is designed in such a way as to avoid extreme genetic variation, and yet alterations in the DNA sequence still arise. To help insure that the random variances in DNA sequence do not accumulate as mutations, the cell has developed several repair mechanisms. As mentioned above, each new daughter cell will have DNA composed of two strands, one strand that had been used as a template and one that is synthesized as its complement. All genetic information of a cell has stored within double stranded helix of DNA. If one strand becomes damaged by chance, the complementary strand can be used to correct it. Due to the fact each strand is an intact copy of the same information present on that segment of DNA, fidelity of DNA replication is better insured. The complementary nature of DNA is an important characteristic that is used by DNA repair systems, though different DNA repair systems will use different repair enzymes depending on the type of DNA damage present. Two of the most common pathways of DNA repair that exist within cells are base excision repair, BER, and nucleotide excision repair, NER (Alberts, 2002).

In both BER and NER pathways, the damaged section of the DNA is recognized and then removed, and with the use of DNA polymerase, the undamaged strand is used as a template
to restore the original sequence on the strand in which the damage occurred. BER and NER
differ in the way in which the damage is removed from the DNA. Nucleotide excision repair is
the mechanism that is employed to repair damage incurred by large changes in the structure of
DNA. Rather than scanning the DNA for a specific base change, the multienzyme complex
involved in NER will recognize “bulky lesions”, distortions in the double helix structure of the
DNA, and cleave the DNA on both sides of the distortion. This leaves a large gap that is then
filled by DNA polymerase and resealed with DNA ligase. Base excision repair is much more
specific. Unlike NER, the BER pathway involves the removal and repair of a single damaged
base. The BER pathway involves several different enzymes known as DNA glycosylases, each
of which can recognize a specific type of altered base in DNA and catalyze its hydrolytic
removal (Alberts, 2002).

BER

DNA damage can be a result of many factors; spontaneous hydrolysis, oxidation, and
alkylation occur during normal physiological metabolism and are all pathways in which a single
DNA base may be damaged or lost. Accumulation of such base damages has been correlated
with many disorders including cancer, atherosclerosis, neurodegenerative disease, and aging
(Liu, Prasad, Beard, Kedar, Hou, Shock, & Wilson, 2007). BER removes DNA bases that have
been damaged, therefore preventing this accumulation. There have been two major subpathways
of BER that have been identified; one involving the replacement of one nucleotide in the repair
process, known as single-nucleotide BER (SN-BER), and another that replaces two or more
replacement nucleotides, known as long-patch BER (LP-BER) (Liu et.al., 2007).

SN-BER is typically initiated by a spontaneous base loss or by DNA glycosylase
cleaving the N-Glycosidic bond of the damaged base, resulting in an abasic site on the DNA (Liu
et.al., 2007). Following this initial event the 5’-side of the abasic site on the damaged DNA strand will be incised by apurinic/apyrimidinic endonuclease (APE), which leads to the formation of a one-nucleotide gap (1-nt gap) with 3’-hydroxyl and 5’-deoxyribose phosphate group (dRP) at the edges. This dRP group will be removed by the lyase activity of DNA polymerase β (Pol β) while it fills the newly formed gap. DNA ligase re-seals the nicked DNA, restoring its integrity. In cases where the dRP group is unable to be removed by lyase due to alteration by oxidation or reduction, the LP-BER sub pathway way will be initiated. This sub pathway involves the replacement of 2-12 nucleotides on the damaged strand. A multinucleotide flap that includes the 5’-dRP group is created by Pol β, and this flap is then cleaved by flap endonuclease 1. *In vivo* and *in vitro* studies have shown that SN-BER accounts for the majority of BER activity in mammalian cells (Liu et.al., 2007).

The DNA glycosylase enzymes used in BER have two different mechanistic classes: monofunctional glycosylases and bifunctional glycosylases. These two classes of glycosylases differ in function in respect to the catalytic mechanism they use to remove damaged bases. The catalysis performed by monofunctional glycosylases involves a single-step displacement of the damaged base, which is done by an attack of an activated water molecule on the C1’ of the substrate. Monofunctional glycosylases function in removing the nitrogenous base, but cannot cut the DNA backbone, and therefore they need another enzyme to assist them in completing BER. Bifunctional DNA glycosylases are similar to the monofunctional glycosylases in the sense that both classes excise the lesion base, but bifunctional glycosylases use an amine nucleophile of the protein to attack C1’ instead of water. This causes the formation of a covalently linked enzyme-substrate complex that will go through a multistep reaction cascade, eventually leading to severance of the DNA backbone on the 3’ side. Bifunctional glycosylases are enzymes that
remove damaged DNA bases and are able to cut the sugar-phosphate backbone of the DNA at the same time (Fromme, Banerjee, & Verdine, 2004).

DNA glycosylases are further classified by the structural superfamily to which they belong. The UDG, AAG, and MutM/Gpg superfamilies are named based on their similarities to uracil DNA glycosylase (UDG), alkyladenine DNA glycosylase (AAG), and bacterial 8-oxoguanine DNA glycosylase (MutM/Fpg) respectively. The fourth structural superfamily of glycosylases is HhH-GPD, which is named for its characteristic active site motif. The UDG and AAG superfamilies are characterized as being compact single-domain enzymes that have relatively small DNA-interaction surfaces, while the proteins of the MutM/Fpg and HhH-GPD superfamilies have multiple domains. Some of the members of the MutM/Fpg and the HhH-GPD superfamilies have structural metal ions or clusters, which assist in specialized biological functions. Though structurally different, all four of the superfamilies bind primarily to the lesion-containing strand of DNA and have an extrahelical recognition pocket in which the damage base is inserted (Fromme et. al., 2004).

The mechanism by which DNA glycosylases recognize and cleave damaged nitrogenous bases is thought to be mediated by base flipping. Base flipping is the rotation of the damaged bases 180° so that it is no longer facing the hydrophobic, inner region of the DNA double helix. This allows for recognition and for the correct base to be inserted into the DNA sequence. In a specific example, human uracil DNA glycosylase (UDG) has been shown to incorporate base flipping in its removal of uracil residues from DNA. UDG recognizes the uracil that has been inserted in the DNA and then through a suggested push-pull mechanism, the uracil and the 5′ phosphate are rotated 180° from their starting position. By the uracil being flipped, an Arg272 residue was inserted into the newly unoccupied space within the DNA. This insertion secures
the correct positioning for the gap to be filled by DNA polymerase, helping with the stability of the mechanism. An AP endonuclease enzyme will recognize the site where base flipping has occurred, at which point it will cut the DNA backbone removing the damaged base and repairing the strand sequence (Roberts & Cheng, 1998).

**BER in the mitochondria**

In each of the mitochondria found within a mammalian cell, there are at least two to five copies of circular supercoiled mitochondrial DNA (mtDNA). This DNA is important, as it is responsible for encoding the components of the electron transport chain. Though it was initially thought that there was no level of repair involved in mtDNA, BER repair proteins identical to those involved in nuclear DNA repair or to the isoforms of these nuclear proteins have been identified in mitochondria. BER has been observed in mtDNA and has shown to be an efficient way to remove DNA damage (Maynard, Schurman, Harboe, de Souza-Pinto & Bohr, 2010).

The organization of mtDNA differs than that of nuclear DNA (nDNA) in that it is not associated or protected with histones. It is instead associated with the inner mitochondrial membrane, which is where the activity of the electron transport chain takes place. The electron transport chain generates reactive oxygen species (ROS), and the mtDNA’s nearness to this makes it more prone to oxidative damage than nDNA. MtDNA was observed to have a higher steady-state level of oxidative damage in comparison to nDNA. The oxidative stress produced by mitochondria is believed to have a significant role in human aging, cancer, and neurodegeneration. As proposed by the mitochondrial theory of aging, the mutations that are caused by ROS will accumulate in the cell, which will lead to damage of respiratory chain proteins, which will generate more ROS, leading to higher mutation rates (Maynard et. al.,
2010). The evidence that BER occurs not only within nuclear DNA, but also in mitochondrial DNA suggests another degree of control that can be manipulated by environmental factors.

**Clinical Manifestation of Dysfunctional BER**

BER deficiencies are believed to be associated with the observed increase of double strand breaks that accumulate in senescent cells. The increased concentration of irreparable double-strand breaks may be as a result of oxidized nucleotides being incorporated into DNA sequences, which undergo an incomplete repair process due to inefficient BER that is observed in aging cells. The mechanism by which BER functions may also be a cause for the increased observance of DNA double strand breaks; the repair pathway creates a nick in the DNA strand in order to remove a damaged base, and a lesion that is not resealed could possibly become a strand break due to its structural instability. As cells age, there is an observed decrease in double strand break repair as well, so these breaks accumulate and eventually lead to the cell’s death (Rai, Onder, Young, McFaline, Pang, Dedon & Weinberg, 2009).

Aberration in the functionality of BER within the nuclear and the mitochondrial DNA can cause certain disease phenotypes, many of which have been studied in animal models. Such animal studies included knockout mice, in which specific genes coding for the core proteins of BER were essentially removed. It was observed that knockout strains of Xrcc1, Pol β, Ape/Hap1, Fen1, and DNA ligase were all embryonic lethal (Maynard et. al., 2010). In a human study that examined tumors, it was observed that 30% of all tumors had a variation within their pol β proteins. Of the tumors that arose by dysfunctional pol β proteins, approximately half were due to a single amino acid change, further suggesting a decrease in BER function occurred (Maynard et. al., 2010). BER has also been proposed to have some involvement in Alzheimer’s
disease (AD); deficient BER coupled with mitochondrial deletions, and increased 8-oxoG levels have all been observed in AD patients (Maynard et. al., 2010).

Neurodegenerative diseases such as Parkinson’s disorder and dementia have been associated with oxidative stress and the rate of neuronal death observed in such disorders is thought to be affected by BER due to the evidence that BER plays a role in nDNA and mtDNA repair in neurons (Maynard et. al., 2010). Miscoding in DNA sequences can also arise from oxidative damage. The oxidative stress placed on a cell leads to oxidative base damage, and it is through this mechanism that mutations arise. These mutations that occur when damaged bases fail to be removed are thought to lead to disease such as cancer (Krokan, Standal & Slupphaug, 1997). Though BER deficiencies have not been established as a direct cause of cancer, damage that would generally be repaired by the BER pathway has been seen as a dominant cause of mutation in certain cases of this disease. Decreased BER function can reduce the stability of DNA, which makes a cell more prone to aberrant genotypic and phenotypic manifestations.

**Nutrient and Gene Interactions**

Nutrigenomics is the field of study concerned with the role of nutrients in gene expression, as well as the evolutionary aspect of diets (Simopoulos, 2010). This is a growing field of research as the role of diet in disease prevention and care are beginning to be better understood. The research that focuses on the role genetic variation plays in dietary response is known as nutrigenetics. Together, nutrigenomics and nutrigenetics can be used for the prevention and management of chronic diseases based on nutrition's role as an environmental factor. Mechanisms by which genes affect nutrient absorption, metabolism and excretion, and to what degree nutrients influence gene expression need to be defined to better understand nutrient gene-interactions and their phenotypic expression. Coronary heart disease (CHD), hypertension,
diabetes, cancer, and obesity are all multigenetic and multifactorial, meaning these phenotypes could be caused by different combinations of genes and their interaction with various environmental factors, such as nutritional composition of the diet (Simopoulos, 2010).

Gene-nutrient interactions play a role in determining the nutritional requirements required for an individual. The nutritional status of an organism depends on multiple environmental aspects such as supply, bioavailability, and consumption, as well as the genetically controlled aspects of digestion, absorption, distribution, transformation, and storage. Excretions by proteins, as in receptors, carriers, enzymes, and hormones, are also affected by genetic variation. By taking these nutrient-gene interactions into consideration, the recommendations for specific dietary elements can be more accurately estimated. One such nutrient that has been studied in regards to its nutrigenetic expression is folate, the water-soluble B vitamin occurring naturally in food (Simopoulos, 2010).

Folate and its role in the diet have been investigated due to its believed role in a variety of disorders such as cancer, neural tube defects, and coronary heart disease (Simopoulos, 2010). Observations of an increased risk in the initiation of several types of cancer that was associated with a reduced intake of folate rich foods like vegetables and fruits has made it an area of special interest for research in regards to its role in carcinogenesis (Ulrich, 2005). Folate has been shown to have many important functions within a cell. It assists in the mechanisms by which DNA synthesis occurs; folate acts as the one-carbon unit donor for nucleotide synthesis. It also assists in cell cycle regulation; folate acts as a one-carbon donor that is essential for methylation reactions (Ulrich, 2005). The physical manifestation of dietary folate manipulation, therefore, should be observed in these pathways.
Through its role in DNA methylation, folate has been linked to carcinogenesis. During the process of gene transcription, methylation plays an important role in regulation. Cytosines within in CpG sites are often methylated as part of transcriptional regulation, which in turn effectually shuts the gene off. Dysregulation of the methylation can lead to hypomethylation of DNA and promoter-specific hypermethylation. With the occurrence of these events, there is a positive correlation in the initiation of the carcinogenic process observed. In both animal models and human experimental studies, DNA hypomethylation has been associated with low intakes of folate (Ulrich, 2005).

Deficiency of folate has also been shown to cause damage to the DNA due to its effects on nucleotide synthesis. It has been observed that when folate concentration is low, uracil is incorporated into the DNA of the cell, which can have deleterious effects. The hypothesized method to which folate deficiency is linked to carcinogenesis is through the mechanism of BER pathways. To restore the correct DNA sequence and structure, BER would occur with the assistance of uracil DNA glycosylase (UDG), but down regulation of the BER pathway has been observed in a folate deficient environment (Ulrich, 2005). Due to the nature of BER, there is also an inborn risk of chromosomal damage and translocations that can occur. BER induces DNA strand breaks, which can cause genomic instability, therefore enhancing carcinogenic progression (Ulrich, 2005).

**Studies: BER and Folate**

Methyl metabolism in the cell depends on the folate status of the environment. Folate has been shown to be important in the production of S-adenosylmethionine (SAM), which is the primary methyl donor for DNA methylation. Folate, in the form of 5-methyltetrahydrofolate (5-methyl THF) is a methyl donor used in the remethylation of homocysteine back to methionine,
which is then converted SAM. In a cell that is folate deficient, SAM levels may be depleted causing hypomethylation and may result in an increase of tumorigenesis. Another important mechanism in which folate is relied on is DNA synthesis and repair. Folate in the form of 5,10, methylenetetrahydrofolate is the methyl donor that is utilized in the conversion of deoxyuridine monophosphate (dUMP) to thymidine monophosphate (TMP). In the case of folate deficiency, the methylation of dUMP to TMP is blocked, which leads to an increase in cellular concentration of deoxyuridine triphosphate and an increase in uracil misincorporation into DNA molecules in the place of thymine (Duthie, Narayanan, Brand, Pirie & Grant, 2002). The BER pathway, which will remove the misincorporated uracil, generally overcomes this but in the case where folate deficiency persists, there is no methyl donor to convert dUMP to TMP, and therefore the uracil misincorporations will continue occurring. Misincorporation of uracil into DNA is in itself mutagenic, but also can result in further DNA instability due to the nature of the repair cycle that is employed to remove such aberrations (Duthie et.al., 2002). Folate deficiency is associated with an accumulation of DNA strand breaks, mutations, and chromosomal instability. These associations suggest that there is some mechanism with which folate deficiency leads to an inability to repair DNA damage and therefore a possible mechanistic connection between folate deficiency and cancer (Cabelof, Raffoul, Nakamura, Kapoor, Abdalla & Heydari, 2004). To identify this underlying mechanism, studies have been completed to directly determine the effect of folate deficiency on base excision repair capability.

In a study completed by Cabelof et. al, (2004), an animal model using mice was designed to observe the affect of folate deficiency on the BER pathway. The mice were randomly divided into two different dietary groups: one being a control with adequate folate supplementation and the other being the experimental group with a folate deficient diet. With
careful monitoring for toxicity, these two groups of mice were fed their experimental diets for eight weeks, at which point they were anesthetized by CO₂ and sacrificed by cervical dislocation. The blood and tissue were collected and analyzed for serum folate levels, aldehydic base lesions, UDG activity, AP endonuclease activity, BER capacity, and the relative number of 3’-OH group-containing DNA strand breaks.

In mice fed the folate deficient diet, it was expected that an increase BER activity would be observed due to it being the primary pathway in which folate deficiency-induced damage is removed from DNA. This, however, was not shown in the results. It was also expected that an induction of β-pol would be occur in response to the folate deficiency because β-pol levels are rate determining in BER, but instead a lack of β-pol inducibility was observed. This lack of induction of the BER pathway supports the hypotheses that folate deficiency results in the inability to perform DNA damage repair. In monitoring UDG activity, there was an increased UDG response to folate deficiency observed, representing a dysregulation in BER. UDG was upregulated, but there was no increase in actual BER activity or β-pol. This could be genotoxic as a result of the possible accumulation of DNA repair intermediates that may occur if prolonged folate deficiency persists. There was no significant effect on AP endonuclease protein levels observed with deficient folate, but this is not a rate limiting step in BER such as β-pol is, so its lack of response may not be physiologically relevant. There was no accumulation of abasic sites observed, which indicates that even though there was no significant change in AP endonuclease levels, it was still able to process the abasic sites and generate single strand breaks.

Using the randomized oligonucleotide-primed synthesis (ROPS) assay, an increase in the accumulation of single strand breaks in response to folate deficiency was observed. This increase in detected single strand breaks is thought to be caused by inadequate β-pol activity
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following an efficient removal of uracil. Without completion of the rate limiting step by β-pol, the strand break that occurs during repair is unable to be ligated, and therefore will persist. There was even a greater increase of strand breaks in β-pol-haploinsufficient mice that were fed folate deficient diets. There was also an increase in aldehydic base lesions also observed in β-pol-haploinsufficient mice that was not observed in the wild type mice, suggesting that haploinsufficiency in β-pol reduces the processing efficiency of these lesions. This data shows that folate deficiency induces a level DNA damage that BER is unable to be overcome due to the inability to induce β pol, resulting in a BER deficiency.

In mouse study completed by Ventralla-Lucente et.al. (2010), the function of folate deficiency in specific regard to tumorigenesis in colon and liver tissue was monitored. There was special emphasis placed on understanding the effects of exposing β-pol-haploinsufficient mice to a carcinogen, 1,2-dimethylhydrazine (DMH). Results from these experiments were evaluated to see if there was any acceleration in the development or increased aggressiveness of pre-neoclastic lesions in the liver or colon of the mice in the study. These observations were made using wild type (WT) mice and mice that were heterozygous for the DNA polymerase β gene (β-pol+/-), the genotype for β-pol-haploinsufficiency. These genotypically different mice were then randomly assigned to one of two dietary groups: one that had a diet with adequate folate supplementation and one that was folate-deficient. After one week of the assigned diet, randomly selected mice from both the folate adequate diet and the folate deficient diet were injected interperitoneally with DMH. After being anesthetized under CO₂ asphyxiation, the mice organs and tissues were examined for grossly visible lesions, including colon and liver tissues. As shown in previous studies, folate deficiency induced a deficiency of DNA polymerase β gene expression, and therefore a concurrent decline in BER capacity.
In order to evaluate the effect of β-pol-haploinsufficiency on carcinogenesis, aberrant crypt foci (ACF) were used as the basis for measuring the early indications of the initiation of colon cancer in the mice. All DMH-treated animals had DMH-induced ACF observed. In the population of wild type mice, the mice that were folate deficient showed a significant increase in ACF formation when in comparison the their folate adequate counterparts. This suggests that in the presence of DMH, folate deficiency predisposed mice to increased colon carcinogenesis.

Data collected in regards to the β-pol-haploinsufficient mice population showed that that DMH-induced ACF was found to be significantly higher in mice with folate adequate diets than their wild type counterparts. For the β-pol-haploinsufficient mice that folate deficient, however, there was a significant reduction in ACF formation in β-pol+/- mice when compared with their wild type counterparts who were folate deficient. These results seemed counterintuitive, so further testing the sample population was completed.

In order to determine to effect of prolonged folate deficiency on ACF progression, wild type mice were kept on their specific diets for forty weeks past their DMH treatment. It was observed that there was no significant change in crypt multiplicity for either of the groups studied. There was, however, a significant increase of crypts per focus observed in folate deficient wild type mice in comparison to their folate adequate wild type counterparts. The ACF in the folate deficient mice were also observed to have a well-defined elevation above the surrounding colon mucosa, which is unlike the folate adequate mice that were observed to have a flat surrounding surface. This data suggests that folate deficiency increases the number of ACF in response to DMH at an early stage and that it adversely impacts further development of aberrant foci and microscopic adenoma. This process could be slowed down where β-pol is deficient, however. Evidence of the β-pol+/- conferring protective qualities to mice exposed to
DMH was observed upon dissection of their liver forty weeks after treatment with DMH. In folate deficient wild type-DMH treated mice and folate adequate β-pol+/- DMH treated mice, there were gross changes in the liver in the formation of tumors. This is unlike the results of the folate adequate wild type mice and the folate deficient β-pol+/- mice, which showed no visible changes in their liver pathology.

By using microarray analysis, the differential gene expression among the mice could be determined. β-pol-haploinsufficiency combined with DMH treatment in mice resulted in more extensive differential gene expression than compared to the wild type untreated mice. Further gene expression analysis showed that there is a reduction in DNA repair gene activity and an up-regulation in apoptotic gene activity in the folate deficient β-pol+/- DMH treated mucosal tissue. This is much different than WT-untreated mucosal tissue that showed either no difference or a slight up-regulation in DNA repair and a down-regulation in proapoptotic gene expression and the folate deficient-wild type DMH treated samples that showed either no difference or an up regulation in DNA repair and an up-regulation in apoptosis. This is of significance because it gives insight to the ACF and tumor formation reduction that was observed in the folate deficient β-pol+/- DMH treated mice. The folate deficiency and β-pol-haploinsufficiency compromise the BER pathway, hence the reductions in DNA repair gene expression. This suggests that the reduced tumorigenesis that was observed is due to an increase in apoptosis, which is supported by the up-regulation of apoptotic gene activity that was observed in gene expression analysis of the folate deficient β-pol+/- DMH treated colon mucosal cells. This hypothesis was further confirmed by completing apoptotic and proliferative assays. This study indicates new possible direction for designing potential therapeutic pathways, showing that although dysfunction BER
is usually associated with an increased cancer risk, if specific nutrient variations are made in the correct setting, these alterations could cause a protective effect.

**Cloning BER Genes**

Folate deficiency and its relationship in the BER pathway are of interest to Dr. Diane Cabelof and her laboratory in the Nutrition and Food Science Department and Wayne State University, and this is where I chose to complete my directed study. It is clear that there is a distinct role folate plays in the regulation of BER, and if the exact mechanism in which it occurs was better understood, it could possibly be developed and marketed for therapeutic purposes. This process of elucidating the exact mechanism is complicated, however, due to the fact that there are many proteins involved in BER and many genes that encode for them. The focus can be first narrowed down to a group of core BER proteins that contribute to the main functionality of this repair pathway. These would include: Ung, Smug, Mbd4, Tdg, Apex 1, β-pol, Xrcc1, Ligase I, and Parp I. As part of working in the Cabelof laboratory, cloning of these genes was an assigned task and a major focus of my work.

In order for these genes to be cloned, they first needed to be isolated from the genomes of the mouse tissue cells that we worked with. This began with the process of isolating genomic DNA from tissue cells. When working in the lab, a DNA extraction and isolation kit that includes the necessary reagents is utilized. The basic process in which DNA isolation occurs begins with the lysing of cells. Since we work with mouse tissue samples, the tissue of interest has to first be prepared. Preparation of tissue samples can be done using several techniques. Categories of tissue preparation techniques include mechanical, digestion, or instrumental extraction. Though each method has its own set of drawbacks and benefits, the mechanical technique is most often utilized for the mouse tissues. This involves the homogenization of
tissues using a small probe-style blender that has a set of small blades, which causes vigorous mixing, leading to the break down of the cells (Yu & Cohen, 2004). A buffer solution is added to the homogenate, resulting in a semisolid that will be centrifuged. Once centrifuged, the supernatant can be decanted as the desired DNA is in the pellet that is formed during centrifugation. This pellet is resuspended in lysis buffer. The next step includes addition of enzymes that remove proteins and other associated components of the DNA. A detergent or surfactant is used to break up membrane lipids, a protease cuts the proteins, and an RNAase works to cut RNA. After this, the “naked” DNA is precipitated out of solution. This is done with the use of an alcohol; DNA is insoluble in alcohol, so it will aggregate together and forms a pellet upon centrifugation.

After the DNA has been purified, gene isolation can be carried out. To isolate the gene of interest, restriction enzymes specific to the gene’s segment on DNA must be identified. Restriction enzymes recognize specific sequences on DNA and cleave DNA only at these recognition sites, allowing for selection of certain genes and their separation from the DNA strand. Many of these recognition sites are palindrome sequences, meaning the forward sequence is equal to the reverse compliment. These same restriction enzymes used to cut mouse DNA will be used when cutting the plasmid in which we wish to insert our gene to be cloned. By doing so, we know that the same recognition sites along the tissue and plasmid DNA are being cut, therefore increasing the probability of recombinant DNA formation (“Restriction Enzymes”, 2012).

Annealing together two separate fragments of DNA from different origins forms recombinant DNA. Recombinant DNA is important to observe nutrient folate concentration effects on BER genes because it allows for individual genes to be recombined into a non-coding
strand of DNA, a plasmid. The effects on the single gene can therefore be observed without being confounded with other elements the genome. By forming recombinant DNA with a bacterial plasmid, the gene containing plasmid can be taken up into the cell, resulting in DNA transformation, at which point our gene of interest can be replicated by the normal mechanism found within the cell. After several rounds of replication, after the bacterial cells will be plated on an agar medium.

Once the cells are plated, we screen for our desired recombinant plasmids. These are the plasmids that had successful insertion of a BER gene. This can be done using different methods, one of which being X-gal screening. The basis for selection is the inactivation of the β-galactosidase gene. There are unique restriction sites found within these gene in a plasmid, so restriction enzymes will cleave the DNA at these locations, and with the introduction and insertion of the restriction fragment containing our BER gene, the β-galactosidase gene will be disrupted and no longer be functional. We place our bacterial cells on a plate containing X-gal, which in the presence of functional β-galactosidase gene will be cleave and cause a blue precipitate to be formed and the colonies will be blue. Our desired recombination plasmids, however, will have a disrupted β-galactosidase gene, and therefore the colonies that grow from them will be white due to their inability to cleave X-gal. Using a small pipette tip or a sterilized inoculating loop, the recombinant colonies can be selected from the plate and place in new media to grow (“Metabolism”, 2012).

To ensure that the correct gene has been inserted and replicated within the plasmid, samples may be analyzed using agarose gel electrophoresis. Gel electrophoresis is an analytical technique that is used to separate DNA fragments based on their size. A gel is set and then the base pair ladder and DNA samples are placed in the wells of the gel, at which point the
apparatus is closed and electrical current is made to flow through the gel. Because of the negative charge carried on DNA, the fragment of DNA will move down the gel towards to positive end of the electric field, known as the anode. Smaller particles migrate farther down the gel because they have less resistance due to their size. The base ladder is a known sample with specifically sized fragments that allow for size comparison of the unknown samples (“DNA Sequencing”, 2012). By knowing the expected size of our desired gene fragment, we can run a gel and see if the sample does include a DNA fragment containing the desired gene.

Agarose gel visualization is based on the fluorescent properties of ethidium bromide in the presence of ultraviolet light. A small amount of ethidium bromide is added to the dissolved agarose immediately before it is poured into the gel mold. A loading dye is added to each DNA sample before it is loaded into the gel, after which the gel is run for a specific amount of time dependent on sample size and variety. Once run to completion, the gel can be placed on a UV transluminator, which exposes the ethidium bromide to UV light causing the gel to glow. The loading dye used for the samples creates a dark banding pattern, allowing for the individual bands of DNA to be visualized and compared to the base ladder for size determination (“Genetics”, 2012).

After running a gel and successfully identifying samples that our desired gene has been introduced, the segment can be amplified using polymerase chain reaction, PCR. This allows for the amplification of only the specific segment of DNA that contains our gene of interest. It is necessary to first find forward and reverse primers that match the nucleotide sequence at the ends of the gene segment we wish to clone. To find the primers for PCR, we complete a search in the PrimerBank, which allows us to specify the gene we wished to amplify and the species in which it was derived. By completing this search, we select the best primer set for each gene based on
the melting temperature and its potential homology with other primers. The selection of the correct primers is integral for the success of PCR ("Polymerase Chain Reaction", 2012).

PCR in the lab was completed with the use of a thermocycler, which carried out an automatic program of specific temperatures that promote the amplification of our selected gene. Each sample we wish to amplify must contain a template, RNA primers, DNA polymerase, dNTPs, and a buffer solution ("Polymerase Chain Reaction", 2012). For our amplifications, Taq polymerase was used as our DNA polymerase, which was provided in an amplification kit that also included dNTP and buffer solution. After running the specified PCR program in the thermocycler, we took our samples and completed another analytical separation using agarose gel electrophoresis, allowing us to determine if our correct fragment of DNA had been amplified. If it was determined that the correct fragment had been cloned, the sample containing this DNA could be frozen and saved for later use and testing.

Discussion

DNA repair mechanisms, especially BER, are a target of much research for disease prevention and treatment. It is through these mechanisms that continued life is possible. Accumulation of DNA base damage or chromosomal mutations threatens the stability of the DNA molecule, and without genomic stability, a cell will no longer have the ability to carry out necessary metabolic processes. BER is an important mechanism in which individually damaged bases are removed and replaced, in effect restoring proper DNA sequence. Though primarily a beneficial and functional pathway, BER can also pose a threat to genomic stability due to the nature of its repair mechanism, which requires a strand break to remove the damaged base. If there is a failure to ligate this singe nucleotide gap, there is potential for double strand breaks to occur, which is even more deleterious to DNA stability.
Folate is an important water-soluble B vitamin that has function and use throughout the cell. Though commonly associated with its role in prevention of congenital disorders, such as neural tube defects, folate and its role in carcinogenesis is becoming a growing area of study. Its role in the BER pathway of cells is of particular interest due to the folate deficiency studies that have been conducted in animal models and its effects that have been observed. It has been shown that mice, fed diets with adequate levels of folate result in better functioning BER pathways when no other confounding elements are present, which decreases the risk for DNA mutation related diseases. It has also been shown that under specific conditions (i.e. β-pol-insufficiency), a diet deficient in folate could have protective effects against the rate of mutations and therefore reduce the risk of cancer formation. More study is needed to better understand the specific mechanisms that are at work in the cell that have produced these results.

Through working with genes encoding for BER proteins, a clearer picture may be drawn in relation to the mechanism in which folate effects DNA repair and the phenotypic manifestation of diseases such as cancer. As seen with the studies discussed previously, folate deficiency in specific cellular environments could have beneficial and possibly therapeutic effects to combat carcinogenesis. This is a promising area of study in the field of Nutrigenomics, especially in regards to individuals that are already genetically predispositioned to certain disease. The genetic makeup of all natural organisms is predetermined, meaning that after initial cell division and differentiation, little can be done to the genome that will directly reduce the risk of major disease in those individuals that are predisposed. However, the results of studies involving folate deficiency show its ability to affect major cellular repair pathways, suggesting that by changing the environmental factors, gene expression can be controlled. Alteration of gene environment through diet could be a simple and more holistic way of disease prevention. It
could also lead to the development of treatment methods. As observed in the animal model of β pol +/- mice that were fed a folate deficient diet and then exposed the DMH, tumorigenesis was declined in these animals. A potential combination of nutrition from the diet and other environmental factors may actually decrease the rate of disease that occurs and lessen its effects. It is important to continue research that combines the knowledge of gene functionality and nutrition’s role as an environmental factor in order to better understand the synergistic mechanisms that are occurring within the body.
References


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