TOURO COLLEGE & UNIVERSITY SYSTEM

The Science Journal of the Lander College of Arts and Sciences

Volume 9
Number 1 *Fall 2015*

1-1-2015

Epigenetics as a Cure for Cancer

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Abstract

Epigenetics is an emerging research topic that is being tested as a potential cure for cancer. Epigenetics is a non-genetic influence that shapes the phenotype. Epigenetics effects gene expression, but does not cause any changes in the DNA. DNA methylation patterns is one such epigenetic change in the cell that has huge potential for cancer treatment. Scientists have observed that many cancerous genes express signs of either hypermethylation or hypomethylation. The key for the treatment is that epigenetic changes are reversible, which opens the door to potential drugs to cure cancer and other diseases.

Introduction

Epigenetics literally means "above" the gene and is a new study of research in biology that explains how environmental factors impact one's biology. Epigenetic mechanism explains how there are over 220 different cell types in an adult organism even though they all have the same DNA. All cells in each organism contains the same DNA, but their cell function differs in different cells, based on "qualitative and quantitative differences in gene expression." (Gibney, Nolan, 2010). Bone cells, skin cells, heart cells, all have the same DNA, but their functions are completely different. This cell differentiation is a specific pattern of gene expression where certain parts of the DNA are turned on or off in each type of cell. This specific pattern of gene expression is passed down from cell to cell. Epigenetics is defined as "the study of mitotically (and potentially meiotically) heritable alterations in gene expression that are not caused by changes in DNA sequence (Gibney, Nolan, 2010). One researcher compares genes and epigenetics to a computer, where genes are the hardware and epigenetics is the software telling the hardware how to function. There is a certain balance of epigenetics that is normal and healthy for the cell, but when these balances are changed and the epigenetic mechanisms become unregulated there may be alterations in gene expression, and can ultimately lead to cell transformation and malignant outgrowths. There are three major types of epigenetic changes; DNA methylation, Covalent Posttranslational Histone Modification, and Small Inhibitory RNA-mediated signaling Pathways. Mainly, Epigenetic processes, including DNA methylation and histone modification, influence gene expression at the transcription level.

DNA methylation is the most studied epigenetic factor and that is what will be discussed in this paper. DNA methylation occurs through the covalent addition of a methyl group (CH3) to the 5 position of a cytosine generating 5-methylcytosine (Stein, Davis, 2012). Methyl groups protrude into the major groove of DNA and change the biophysical characteristics of the DNA. Cells can methylate and demethylate DNA, which effects specific gene expression. These epigenetic changes can experience multigenerational inheritance; not only does it change the phenotype of the first individual, but the methylation can pass onto further generations.

Materials and Methods

In order to understand how epigenetics can be a potential cure for cancer, many journals and articles were retrieved from Touro College's library database. Those articles and the references they listed formed the basis for exploring this topic.

Correlation between DNA methylation and gene silencing

An experiment with mice and coat color phenotype and body weight measurement exposed the correlation between methyl groups and silencing of gene expression. A group of pregnant mice that were genetically identical were assembled, half the group were fed a diet rich in methyl groups (here soy), and the control half their regular diet. All these mice had the agouti gene, which when expressed gives the mouse a yellow color and causes obesity. The offspring of the mice that had the methyl rich diet were thin and brown, whereas the offspring of the mice that did not eat the methyl groups remained yellow and fat. They both have the agouti gene, but the brown mice had a methyl group attached to the agouti gene which shut it down. Bisulfite sequencing methylation analysis of CpG sites in the promoter region of the agouti gene (Avy IAP) showed a statistical increased average percentage of cells methylated of the mice given the methyl rich diet. This was a simple experiment to see how DNA methylation affects gene expression because it was easy to see when the gene was on and off based on the phenotype color switch and body measurement. Also, this epigenetic tag was passed down to the offspring's of these mice, and continued until they ate a diet without enough methyl groups. Soy has an active ingredient that methylates DNA. This research may explain why there is a lower incidence of cancer among Asians compared to Westerners, because Asians eat diets rich in soy and may explain why Asians who emigrate to America are more likely to develop cancer than their family back home. (Dolinoy, et. al. 2006)

DNA methylation causes gene silencing

Although a correlation was noted between gene activation and methylation, the authors wanted to prove conclusively that DNA methylation is the cause of the gene silencing. They were not positive yet which was the cause and which was the effect, they though that it might be possible for the gene inactivation to cause the DNA methylation. An experiment was carried

out that proved that DNA methylation caused gene inactivation. The experiment was conducted on the E2a region of Ad2 DNA. The region was cloned, and then half of the gene was methylated in vitro with HpaII DNA methyltransferase and then microinjected into the nuclei of X. laevis oocytes. The key is that the scientists ensured that the methylated DNA only remained methylated for 24 hours after microinjection. There was no synthesis of Ad2 specific RNA in the methylated DNA cells until 24 hours after injection. The control did however readily express the Ad2 specific RNA. The experiment results demonstrate that methylation plays an actual role in causing genome inactivation because at the end of the 24 hour period, there is no genome expression, yet that does not cause the methylation to continue, which proves that methylation is not a consequence of a lack of gene expression. The gene expression inactivation only occurred in the narrow window that the Gene was methylated (Doerfler, 1981).

How it causes gene silencing

Cytosine methylation inhibits the transcription of genes. When there is an extra methyl group on the DNA, it becomes inaccessible for protein binding for gene expression. The methyl group interferes with the binding of the protein to its specific DNA to transcribe it.

Cancer

Typically, it was believed that cancer was caused by genetic defects such as, mutations, amplifications, deletions and translocations, which affected the cancer cell and provided it with the advantage to survive and metastasize. However, today, it is clear that there is another system of equal importance that is liable in causing cancer, and that is epigenetic marks. Today cancer is considered a genetic and epigenetic disease. This epigenetic alterations which can cause cancer have been termed epimutations. This is extremely important because damage to a cell's DNA is permanent, whereas epigenetic change is reversible. (Stein, Davis, 2012).

Proof that epigenetic changes can cause cancer

Take arsenic which is known to cause cancer of the skin, liver, lung, and bladder, but actually does not cause DNA mutations, as demonstrated in standard mutagenic assays. However in 2010, researchers found that arsenic caused cancer by epigenetic mechanisms. This was a groundbreaking epigenetic mechanism that cause cancer, and that is a major breakthrough in terms of finding a cure. One group of researchers studied CpG islands of over 14000 genes in people who were exposed to high levels of arsenic, verified by their urinary arsenic levels. It was found that these genes were epigenetically modified from the arsenic exposure, and researchers found that a tumor suppressor gene was silenced. This is called a "tumor suppresorome," which is a group of 17 confirmed or recognized tumor suppressor genes that are silenced in human cancers, most of these through abnormal methylation patterns. (Stein, Davis, 2012)

Two ways that DNA Methylation can have an impact on carcinogenesis

One is by hypomethylation and one is by hypermethylation. Hypomethylation is a decrease in the normal amount of methylation on DNA. Hypomethylation usually occurs at repetitive chromosomal sequences where DNA is normally methylated. Researchers found that four out of five cancer patients had significant hypomethylation in their cancer cells as compared to their neighboring cancer free cells. Hypomethylation, in decreasing the normal amount of methylation of the gene, decreases the normal amount of gene silencing, i.e. increases gene expression. This can result in an activation of genes with growth and tumor promoting functions (Lund, Lohuizen, 2004). Additionally, hypermethylation, or the increase in the amount of DNA methylation can cause a negative impact on the cells, and can lead to carcinogenesis. This hypermethylation is found at CpG islands, which are most likely found in the 5' regulatory region of the gene, which are not methylated. Here the extra methylation causes gene silencing, and tumor suppressor genes are being silenced (Feinberg, 2001). In one experiment, it was revealed that the mRNA for RL7NX3, a tumor suppressor gene involved in several cancers, is suppressed in primary cutaneous melanoma and in metastatic tumors. CpG Island, which are usually not methylated and transcriptionally active, have been found to be abnormally hypermethylated during the development of cancer, particularly in tumor suppressor genes. Genes involved in cell cycle regulation, DNA repair, (to name two) are very epigenetically susceptible (Stein, Davis, 2012).

Hypermethylation of GSTP1 and prostate cancer

Methylation of GSTP1 is the most common epigenetic alteration described in several tumors, such as prostate cancer, endometrial cancers, and breast cancers. GSTP1 catalyzes the s conjugation of GSH, which protects the cell from cytoxic and carcinogenic agents. Methylation of GTSP1 gene occurred in 90% of prostate cancer tissue from surgical specimens. In this experiment, the tissue samples from 144 patients who were having a prostate biopsy were examined. Forty two out of the 144 patients were diagnosed with prostate cancer. Additionally, there was hypermethylation of the GTSP1 gene in 31 out of those 42 patients, and only in 2 out of the 102 patients who did not have cancer. Since epigenetics is a cause of carcinogenesis, and, therefore, cells will exhibit hypermethylation at the onset of the cancer, there is potential for using hypermethylation as a biomarker to detect the cancer. These genes, and other genes that exhibit hypermethylation in the early onset of cancer, may eventually be useful as a biomarker to detect prostate cancer. However, methylation of

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Table 1. Selected hypermethylated genes in human cancers.

Gene or gene product	Function	Tumor type
Rb	Cell cycle regulation	Retinoblastoma
APC	Wnt signal trasduction	Colorectal cancer and others
p14/ARF	Cell cycle regulation	Colorectal cancer
p15/*CDKN2B*	Cell cycle regulation	Leukemias
p16/*CDKN2A*	Cell cycle regulation	Melanoma cancer and others
BRCA1	DNA repair	Breast, ovarian cancer
VHL	Tumor suppressor	Renal cell cancers
hMLH1	DNA mismatch repair	Colorectal, endometrial and melanoma cancer
ER-	Estrogen receptor-	Breast cancer
GSTP1	Phase II metabolic enzyme (detoxifier)	Prostate, endometrial, breast cancer

Table 1 shows a list of that when hypermethylated become cancerous. This means that it may be possible to use hypermethylation as a biomarker to detect cancer early on.

the GTSP1 gene may indicate another cancer, and does not indicate prostate cancer, specifically. However there is potential for mapping out hypermethylation of other genes and using a multi gene profile to improve specificity for cancers. Azacytidine has been found to reverse the hypermethylation of the GTSP1 gene, and reactivate transcription (Miano et. al. 2007).

Epigenetic tests on identical twins

The best way to study epigenetics is on monozygotic twins because they have the same genetic material, but still have difference in cell expression and their phenotype. There is a high discordance rate between identical twins for many diseases, even those thought to be genetic. Epigenetic changes through environmental variances can be the answer to the phenotypic differences of identical twins. Researchers in Madrid studied 80 identical twins in a range of 3 to 74 years old. Thirty five percent of the twin pairs had significantly different methylcytosine genomic content. Researchers observed that the difference in the methylcytosine pattern for the twins increased with the age of the twins. The youngest twin pair had their epigenetic pattern the most similar, whereas the oldest pair's epigenetic pattern was very different. Also, they found that twins who had not grown up together had the greatest difference of 5' methyl cytosine levels. These differences in methylation of their cells is what causes the gene expression and phenotype of monozygotic twins to differ. This study indicates that methylation patterns are one reason why twins with the same DNA may have different phenotypes. Additionally, the fact that the epigenetic differences were more distinct in twins who spent less of their lives together indicates that major role the environment plays into translating a general genotype into different phenotypes detectable. External factors play a great role in gene expression by changing the pattern of epigenetic modifications (Fraga, et. al. 2005).

Epigenetics and age correlation

For every cell division there is a potential for epigenetic damage. As a person ages, their cells are forced to divide again and again to replenish any tissue damage that may have occurred. Each time this happens there is a chance for epigenetic damage through an altering of the normal methylation pattern of the cell. Scientists can take tissues from an older person and can estimate by looking at the epigenetic patterns of the DNA in a particular tissue how old the person is, because these epigenetic changes accumulate as a person ages. For a cell, aging is counted by how many times the cell has divided. The more damage a cell has, the more times it is forced to divide and replenish, and the more chances that there is going to be an abnormal pattern of methylation in this cell. For example, smoking and sun exposure can cause skin tissue injury. When a person's skin peels from a sunburn its cells repair that damage by dividing and replenishing those cells. In these cases, the sun and cigarette exposure can cause actual DNA damage, but they also just damage the tissue which leads to cellular repair of that injury through cell division, and leads to a gradual accumulation of epigenetic damage. Lungs of heavy smokers can look 20 times older than nonsmokers. This can explain why there is a higher incidence of cancer among older individuals. With age, the amount of exposure he or she has had to environmental toxins increases, which can ultimately lead to an abnormal methylation pattern (Nova, 2007).

Epigenetic therapy

Epigenetic therapy works to repair the normal methylation pattern of the cell. This is done through DNA methylation inhibition. 5'Azacytidine and 5-aza-2'-deozycytidine (Decitabine/DAC), the two most advanced drugs for epigenetic therapies for cancer. Both are in clinical use (Silverman, et. al. 2006). They are DNA methyltransferase inhibitors and work by trapping DNA methyl transferases thereby inhibiting methylation, and restoring the gene's normal expression. DNA methyl transferases, known as DMTAs, are the enzymes that are responsible for DNA methylation. DAC incorporates into DNA and forces the methyl transferases to form irreversible covalent bonds to DNA. These methyl transferases are then targeted for deletion in the proteasome. Then, cells divide, without DMTA and there is now DNA hypomethylation and reactivation of genes that were silenced from the extra methyl groups. However, DAC can also cause cytotoxicity when given in high doses, and therefore dosage must be monitored. A positive side to epigenetic therapy of cancer is the minimal side effects which is due to the fact that scientist can give low doses of the medication with successful results. Whereas for cancer drugs scientist have to give the highest dose possible of the medication to kill all the harmful cells, epigenetic therapy, does not necessitate high doses of the drug because it is not aiming to kill all the cells, but rather just to change the abnormal methylation patterns of the cancer cells. DAC was not tested

on young children or on pregnant women. The drug does not target normal methyl tags for two reasons. Firstly, drugs target the area of the most cellular divisions, and in this case, they are going to target the cancer cells and leave the normal cells alone. Additionally, modification of epigenetic patterns has a large effect on cancer cells but will hardly have any long term effect on the behavior of normal cells. When the drug was stopped the normal cells returned to their normal epigenetic pattern, while the cancer cells, now healthy cells with normal methylation patterns, did not revert to tumorigenesis. There is potential for all other cancers that have an epigenetic origin. Myelodysplastic syndrome, also known as MDS, and Acute Myelogenous Leukemia, also known as ALS are easier targets for these drugs because the cancer cells are in the blood and have easy access to drugs. (Issa, 2013) These drugs do not work with solid tumors yet. A reason that is likely is because it is much harder to incorporate the drugs into these cells, which are multiplying much more slowly than hematological cancers. Right now, these drugs are being tested with solid state tumors and the results have been promising. In the laboratory the scientists have been able to adjust the abnormal epigenetic pattern of the solid state tumor with these drugs, and are working to mimic these results in the body. The catch is that these drugs that prevent methylation can reactivate expression of multiple silenced genes, even genes that we want silenced, like oncogenes, which are genes that have the potential to cause cancer. Therefore, the demethylation can be harmful as well as helpful. For example, oncogene NT5E is transcriptionally silenced by methylation in breast cancer. Therefore hypomethylating that area would activate that oncogene and not be helpful. This is why in the future it is important to have some sort of epigonomic profiling- to identify potentially deleterious silenced genes before using epigenetic therapy to pharmacologically reverse resistance (Hatzmichael, Crook, 2013).

MDS, AML and other lymphomas

Myelodysplastic syndrome, or MDS, and Acute Myelogenous Leukemia, are cancers of the bone marrow. MDSs are hematological disorders that usually progresses into AML. Years ago, patients diagnosed with MDS were basically given a death sentence, there was no cure, and no hope for any remission. It was soon realized that MDS was epigenetic in origin. Researchers thought to study the epigenetic pattern in patients with MDS because of the fact that the patients with the disease have an average age of 70. Diseases that target older individuals likely have an epigenetic component in it. MDS patients have aberrant methylation of their CpG loci and silencing of multiple genes. MDS was therefore an ideal candidate for epigenetic therapy, and indeed results were positive. Decitabine was given to over 100 patients for Acute Myelogenous Leukemia. In fifty percent of the patients the disease disappeared fully. Twenty five percent achieved some improvements. However, another 25% did not respond to the drug, or the drug worked for a short amount of time but then stopped working (Nova, 2007).

Conclusion

All in all, the discovery that many cancers have an epigenetic origin, has opened a new path for potential cures for cancer. This was possible because epigenetic abnormalities are reversible. It is integral to realize that everything one does affects his or her health, and the health of future generations. One's diet and what one is exposed to can affect his epigenome and subsequently his future generation's phenotype. We are not just the product of our biology, we are also its cause.

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