Epigenetic Effect of Food for Cancer Management

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Abstract

Epigenetics is the study of inherited changes in phenotype or gene expression, caused by mechanisms other than changes in the underlying DNA sequence. The role of food as an effective epigenetic factor is discussed in this review. Understanding the mechanism of epigenetic resetting could be exploited to deal with adult diseases such as cancers, or in 'rejuvenating' aged cells, linked with an accumulation of aberrant epigenetic marks. The significance of the information would also depart from well-known and generally accepted environmental effects on the unborn fetus in mother's womb or other maternal effects, mediated by the many provisions in the zygote during embryogenesis, and after birth, through mother's milk. In addition, in-depth understanding also provide vital information to find how to erase aberrant epigenetic marks that may underlie some diseases in adults and also provide opportunities to address whether germ cells can acquire new epigenetic marks through environmental or dietary influences on parents that may escape erasure and be transmitted to subsequent generations, with potentially undesirable consequences.

KEYWORDS: Epigenetic effect of food, mechanism, inheritance, cancer

Introduction:

Epigenetics is the study of inherited changes in phenotype or gene expression, caused by mechanisms other than changes in the underlying DNA sequence, hence the name epi- (Greek: επί- over, above) -genetics. Considering chromatin-based information, epigenetic marks include DNA and histone modifications, histone variants, non-histone chromatin proteins, nuclear RNA as well as higher-order chromatin organization. Epigenetics is a switch to turn genes on and off by a mechanism using chemical tags the epigenetic marks, attaching to DNA directing the cell either to use or ignore a particular gene. One such epigenetic mark is a methyl group having the capability to block (by methylation) the attachment of proteins which normally turn the genes on by fastening to DNA. Scientific observations give enough evidence for the inheritance of the epigenetic effect such as:

1. Offspring inheriting the altered traits due to their parents' past experiences; *e.g*., historical incidents of famine have resulted in health effects on the children and grandchildren of individuals who had restricted diets (van Berge-Henegouwen, and Mulder, 1993; van Berge-Henegouwen *et al*., 2011).
2. The epigenetic effect of DNA changes (mutations) during embryogenesis, which in turn sometimes even before the birth of the child resulting in the manifestation of childhood cancers (Esteller, 2007; Hackett *et al*., 2013).

Classic genetics can explain neither the diversity of individual identity within a population nor does classic genetics explain how, despite their identical DNA sequences, monozygotic twins or cloned animals can have different characters and different susceptibilities to diseases. The concept of Epigenetics offers atleast a partial explanation of these phenomena. The concept of Epigenetics first introduced by C.H. Waddington (1939) is now defined as "Heritable changes in gene expression that are not due to any alteration in the DNA sequence". Epigenetics has many and varied potential in medical applications since, ultimately it turns out to have a greater role in prevention of diseases than classical genetics. Researchers so far have found epigenetic clues for management of not only to cancer, but also cardiovascular disease, diabetes, lupus and even autism (Bird, 2007).

It is believed that unlike many cancers in adults, childhood cancers are not strongly linked to lifestyle or environmental risk factors. However, it is not the individual whose lifestyle matters in this case. Mother's conditions matters, either the food that the mother eats or the environment in which she lives or may be both. Nevertheless, it is hypothesized by Ballal and Gupta (2012) that many epigenetic factors such as food, light, toxins, narcotics, etc., to which a lady is directly or indirectly exposed during pregnancy, will have impact on the child and may suffer with cancer.

Epigenetic Changes can be Inherited

In general epigenetic changes are not inherited in the next generation; it is thought that, between each generation the epigenetic marks are erased in cells called primordial germ cells (PGC), the precursors to sperms and eggs. This 'reprogramming' of genes before fertilization helps the genes to read afresh for every new person. However, in the rare circumstances it is found that methylation can still 'escape' such reprogramming process and passed onto the offspring leading to aberrant genome. Such aberrant methylation could accumulate at genes during lifetime, in response to environmental factors such as chemical exposure or nutrition and can cause abnormal use of genes, leading to diseases (Hackett *et al*., 2013).

DNA methylation

One of the most fundamental questions in the control of gene expression in mammals is, how epigenetic methylation patterns of DNA and histones are established, erased, and recognized (Esteller, 2007; 2008**)**. The best-known epigenetic marker is DNA methylation. The initial finding of global hypomethylation of DNA in human tumors was soon followed by the identification of hypermethylated tumor-suppressor genes, and then, more recently the discovery of inactivation of microRNA (miRNA) genes by DNA methylation.

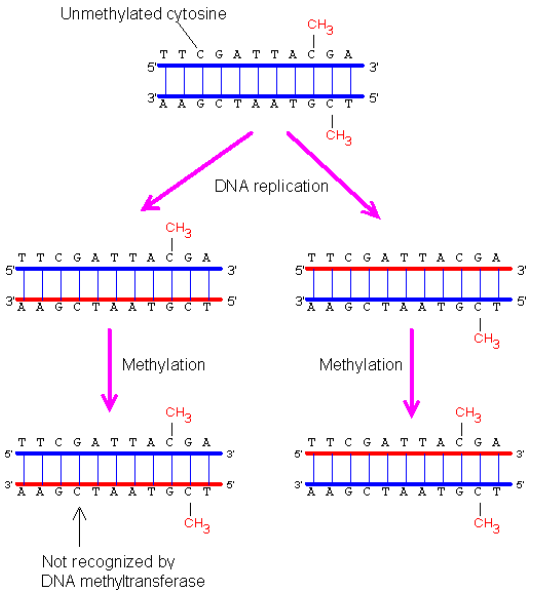

Fig-1. Inheritance of the DNA methylation pattern. The DNA methyltransferases can methylate only the CG sequence paired with methylated CG. The CG sequence not paired with methylated CG will not be methylated. Hence, the original pattern can be maintained after DNA replication.

This (Fig-1) demonstrates how epigenetic changes can modify gene expression that led to human epigenome projects and epigenetic therapies. Moreover, we have understood now, that DNA methylation occurs in a complex chromatin network and influenced by the modifications in histone structure, commonly disrupted in cancer cells **(**Pushkala and Gupta, 2011).

The development of cancer has been associated with epigenetic alterations such as aberrant histone deacetylase (HDAC) activity. It was recently reported that valproic acid is an effective inhibitor of histone deacetylases and as such induces tumor cell differentiation, apoptosis, or growth arrest **(**Pushkala and Gupta, 2011). Due to post-translational modifications of the N-terminal tails of histone and methylation of cytosine residues in the DNA, chromatin undergoes a variety of chemical modifications. Core histones are characterized by the presence of a histone fold domain and N-terminal tails of variable length that are subject to extensive post-translational modifications.

Histone modifications include: acetylation, methylation, phosphorylation, ubiquitylation, glycosylation, ADP-ribosylation and carbonylation. Many amino acids of histones are modified, including lysine residues that may be acetylated, methylated or coupled to ubiquitin; arginine residues may get methylated; and serine or threonine residues could be phosphorylated. Many of the modifications such as methylation of DNA and acetylation of histones can affect the other translation processes. They are positively or negatively correlated with specific transcriptional states or specific organization of repressive or open chromatin.

Histone methylation is a post-translational modification of histones which takes place on the side chains of both lysine (K) and arginine (R) residues. Histone methylation is a reversible process which is catalysed by histone methyltransferases (HMT), such as PRMT1 or Suv39H whereas histone demethylation is catalysed by histone demethylases, such as LSD1 or Jumanji domain-containing proteins. The regulational consequence of histone methylation on transcriptional state of a gene depends on the methylated residue and degree of methylation. Lysine can indeed be mono-, di- or tri-methylated.

The modulation of chromatin condensation can be achieved via reversible acetylation on the lysine residues of histone tails. The acetylation

reaction consists in the transfer of an acetyl group from acetyl coenzyme A (acetyl-coA) on the ε-amino group of the lysine residue, neutralizing the positive charge. This process results from a balance between the activities of two families of antagonistic enzymes: histone deacetylases (HDACs) and histone acetyltransferases (HATs), respectively removing or adding acetyl groups into core histone (Fig-2).

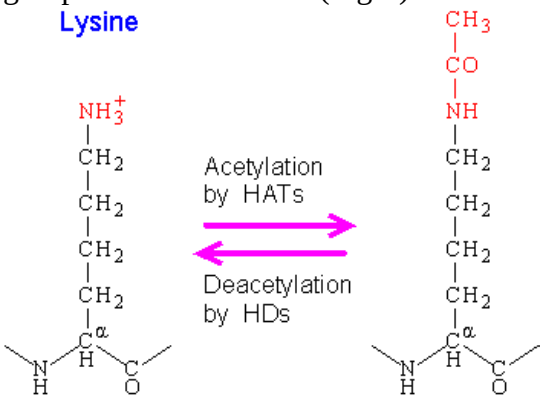

Fig-2. Showing acetylation and deacetylation of lysine

Histone phosphorylation occurs on serine and threonine residues and influences transcription, chromosome condensation, DNA repair and apoptosis. For example, phosphorylation of serine 10 and serine 28 on the tail of histone H3 (H3 phospho Ser10 or H3 phospho Ser28) occurs early in mitosis when chromosome condensation is induced during S-phase. Mammalian DNA methylation is intricately connected to the presence of unmodified lysine 4 and methylated lysine 9 residues in histone H3. An interconnected network of methyltransferases, demethylases, and accessory proteins is responsible for changing or maintaining the modification status of specific regions of chromatin. The structural and functional interactions among members of this network are critical to processes that include imprinting and differentiation, deregulation of which is associated with disorders ranging from inflammation to cancer (Cheng and Blumenthal, 2010).

Balance between HAT(histone acetyltransferases) **and HDAC (**histone deacetylases)

Transcription in a micro-environment is a multi-step process in which, information in the genes is used to manufacture proteins. Proteins in turn, direct cell activity and so impaired expression of the genes at the appropriate time could be a causal factor to promote body disorders. Lamins are major components of the nucleoskeleton in the cell nucleus. They polymerize and depolymerize during the cell cycle, resulting in deformation and reformation of the nuclear envelope. This dynamic behavior of lamins is regulated by the enzyme protein phophatase (PP1), which dephosphorylates lamins at the end of mitosis, enabling their incorporation into reassembling the nuclear envelope. Any irregulation in this process of addition or removal of phosphate group on/from the protein can lead to excessive and insufficient programmed cell death, which may further create certain pathological conditions (Gupta and Saumyaa, 2008). Since the emergence of epigenetic concept in biological sciences, statements such as, "You are what you eat" and "You are what your mother ate" (Ballal and Gupta, 2012) is getting scientific foundation with experimental as well as epidemiological support (Gupta *et al*., 2010; Hackett *et al*., 2013). The food we eat has the ability to turn on or off certain genes *e.g*., if you eat certain anticancerous food (Broccoli), may turn on the "good genes" that activate detoxification

pathways in the lungs, thereby reducing the risk of lung cancer. The acetylation of histones by histone acetyltransferases (HATs) makes DNA more accessible to transcription factors during transcription. On the contrary, deacetylation of histones by histone deacetylases (HDACs) restricts the access of transcription factors to DNA. However, the balance between HAT and HDAC activities that exists in normal cells may be disrupted in cancer cells. Sulforaphane (SFN), SFN metabolites inhibit HDAC activity in cultured cancer cells, in animal models and SFN-rich Broccoli sprouts inhibited HDAC activity in peripheral blood mononuclear cells of human volunteers. Cruciferous vegetables are unique in that, as they have rich source of sulfur-containing compounds known as glucosinolates. Chopping or chewing cruciferous vegetables results in the formation of bioactive glucosinolate hydrolysis products, such as isothiocyanates and indole-3-carbinol. A case-control study found that urinary isothiocyanate excretion was significantly lower in Chinese women diagnosed with breast cancer than in a cancer-free control group. There also a growing evidence for transplacental cancer chemopreventive effects of indole 3 carbinol (I3C) and other dietary modulators involving changes in DNA promoter methylation. Isothiocyanates, particularly SFN, are potent inducers of phase II enzymes in cultured human cells, including UDP-glucuronosyl transferases (UGTs), NADPH quinone oxidoreductase (NQO) and glutamate cysteine ligase playing an important role in protecting cells from DNA damage by carcinogens and reactive oxygen species. A number of isothiocyanates also are found to be inducing cell cycle arrest in cultured cells, inhibit proliferation and induce apoptosis in a number of cancer cell lines (Higdon *et al*., 2007). On the other hand, you might turn on some "bad genes" that could eventually lead to cancer. The type of food makes difference in our genetic and non-genetic inheritance. Chronobiologists have even gone one step ahead and made a statement, "We are not what we eat but when we eat" (Udupa and Gupta, 2009; Gupta and Pushkala, 2012).

Fasting can change Gene expression

Eating what and when make all the difference in our health, aging, behavior and prevention and treatment of diseases. Gupta and his team at CCMB, Hyderabad, made a pilot study on the beneficial effect of fasting and surprised to find that during fasting, lot of metabolic energy is saved. He gave scientific proof for the benefits of fasting, ranging from energy conservation to cancer prevention. During the experiments, which subjected rats to fasting, it was found that the turnover or replacement of internal lining cells, which required a lot of energy, was completely stopped. Due to starvation for short period, fluidity and surface area of plasma membrane projecting in the lumen of rat intestine increased. Biochemical estimations show a decrease in the levels of cholesterol and proteins with respect to phospholipids (Waheed *et al*., 1998b). d-glucose transport through the membranes also showed an increase (Gupta and Waheed, 1992). In addition to glucose, L-proline, glycine and L-glutamic acid which represent imino, glycine and acidic systems respectively also increase significantly in Na+-dependent pathway whereas transport of

L-lysine representing basic system increased significantly in Na+-independent pathway during starvation (Waheed and Gupta, 1997). The ratios of cholesterol/phospholipid (mol/mol), sphingomyelin /phosphatidylcholine (mol/mol), protein/lipid (w/w), and free fatty acids (w/w) decreased, whereas the total phospholipid (w/w) ratio and the double-bond index increased the intestinal membranes of the starved rat, compared to that of the well-fed rat. Analyses of fatty acids showed higher percentage of stearic and arachidonic acids whereas oleic and linoleic acids decreased under starvation (Waheed *et al*., 1998a).

Mithieux and his group (2009; 2013) suggest that fasting intestine contributes in two ways to maintain blood glucose level, directly by absorbing more glucose and indirectly by providing lactate and alanine. Habold *et al*., (2005) noted an increase in the content of gluconeogenic enzymes and glucose transporter level in the rat intestine after prolonged fasting. Intestine may help in gluconeogenesis (Mithieux, 2010; Pai *et al*., 2010) that occurs during fasting, by providing higher amounts of lactate (Pai *et al*., 2010). Further, transport of glucose indicating that transporter proteins are spared during this protein breakdown short term fasting. There was also no physiological cell death, and intestinal cells became more efficient in absorbing nutrients, however, it was found that fasting up to three days stopped 'physiological cell death' completely. Biochemical markers for apoptosis such as increased transglutaminase activity and DNA fragmentation are clearly discernible in normally fed animals (Habold *et al*., 2006). The percentage of cells labeled immune-histochemically by antibody against transglutaminase decreased during starvation while DNA fragmentation was absent (Luciano *et al*., 1995). Fasting for one or two days intermittently over a period of one month was always beneficial. American scientists had recently studied the effect of fasting on cancer prevention and showed that cancer cells required more food, and so, they were the ones to die first during fasting. Whereas the normal cells could sustain the shock (of fasting), and when food was available to them, they again worked efficiently (Waheed and Gupta,1997).

Short-term starvation (or fasting) changes gene expression, and multiple cycles of fasting promote differential stress sensitization in a wide range of tumors and could potentially replace or augment the efficacy of certain chemotherapy drugs in the treatment of various cancers (Lee *et al*., 2012).

Food and cancer

Breast cancer is one of the most dreadful diseases; nevertheless, by change of life style it can be prevented easily (Pushkala and Gupta, 2011). Out of many risk factors, which aids in development of breast cancer food have been considered (Gupta *et al*., 2010; Pushkala and Gupta, 2012). Some diets are better than others in prevention of breast cancer. For prevention, not only individuals but governments should adopt certain measures like, restriction on food additives; encourage people to eat less processed and salty food and whole grains including fresh vegetables and fruits. Headlines continue to raise concerns over the health effects meat consumption. In recent years, high profile studies have linked meat consumption, red or processed meats

(Ballal and Gupta, 2012), to increased risks of various diseases, including cancer, diabetes, and heart disease. The World Cancer Research Fund published a report in 2007 that directly linked diet to cancer, specially mentioned of alcohol and red and processed meats, those pose particular risks.

The post-translational epigenetic modification of histone proteins plays an important role in controlling cell fate by directing essentially all DNA-associated nuclear processes. Misregulation and mutation of histone modifying enzymes is one of the hallmarks of tumorigenesis. However, how these different epigenetic modifications lead to tumor initiation and/or progression remains poorly understood (Johnsen, 2012). The role of nutrients affecting gene expression through interaction with genetic polymorphism and modulate the methylation of DNA has received considerable attention recently because, the disruption of homeostasis leads to major health problems such as risk of heart disease, neural tube defects, and cancer. Such disruption can occur as a result of deficiencies of the two essential micronutrients involved in this metabolism: folate and cobalamin (vitamin B12). Unlike mutations, DNA methylation and histone modifications are reversible (Das and Singal, 2004).

Epigenetic alterations allow the cancer cell to adapt to changes in its microenvironment, but dormant, hypermethylated tumor-suppressor genes can be awakened with drugs (Grant *et al*., 2007). Zolinza (vorinostat) suberoylanilidine hydroxamic acid is the first HDAC inhibitor approved by the US. FDA., an epigenetic drug approved in 2006, works by helping the cell to package the DNA correctly around the histones, unwinding genes that control cell growth or constricting those that invite cell division with abandon. More than a dozen other drugs that modify the way DNA spools around histones are in development. Most of them, like Zolinza, have shown an effect in T-cell lymphomas, but studies are also under way to test the agents for Hodgkin disease, leukemia, and other forms of cancer. However, it is not entirely understood how these histone-targeting drugs operate. Generally, they help DNA to unwind from the histones. Opening of DNA may be good or bad. The drugs may be affecting more than the cancer-feeding genes, and they may be affecting other cells.

The DNA-methylation and histone-modification patterns associated with the development and progression of cancer have potential clinical advantage. DNA hypermethylation markers are under study as complementary diagnostic tools, prognostic factors, and predictors of responses to treatment. For instance, the glutathione S-transferase gene (GSTP1) is hypermethylated in 80 to 90% of patients with prostate cancer, but it is not hypermethylated in benign hyperplastic prostate tissue. Analysis of hypermethylation of the CpG island has potential diagnostic applicability for carriers of high-penetrance mutations in tumor-suppressor genes. For example, identification of DNA hypermethylation in a breast-biopsy specimen from a carrier of a BRCA1 mutation could be useful when the pathological diagnosis is uncertain, because hypermethylation of the CpG island is an early event in the development of cancer. Analysis of several hypermethylated genes detects twice as many tumor cells in breast ductal fluids as conventional cytologic analysis, and hypermethylated genes can be found in exfoliated cells at different

stages in the development of cervical cancer. The application of DNA-hypermethylation markers as tumor markers in routine clinical practice will require rapid, quantitative, accurate, and cost-effective techniques and objective criteria for selection of the genes that are applicable to different tumor types.

Conclusion

Nutritional epigenetics is not yet developed and so far the possibility of developing a treatment or discovering preventative measures of cancer is not yet possible. However, it is very exciting and promising field. Current knowledge in nutritional factors involved in changing the gene expression is not enough, and further studies are needed to expand the available resources and better understanding of the use of nutrients or bioactive food components for maintaining health and preventing diseases through modifiable epigenetic mechanisms or using effective factors for specific gene expression . Recently, two thought provoking reviews were published by Jacob Peedicayil (2008; 2012) which explained the potential role of epigenetic biomarkers, that is, epigenetically altered genes and/or expression patterns of proteins or metabolites, in psychiatric disorders. He also emphasized that, establishing a connection between biomarkers and the disease process; determining the predictive quality of the biomarkers; determining the effects of disease; heterogeneity on the biomarkers; and identifying sample sources for the biomarkers that are easily accessible for testing are prerequisite.

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