

Malignancy and methylation: the link between epigenetics and cancer

Cancer is one of the most significant health concerns that we are faced with today, both in this country and throughout the world. One in 4 deaths in the United States is due to cancer and the American Cancer Society predicts that this year there will be a total of 1,638,910 new cancer cases and 577,190 deaths from cancer.¹ Because of the vast prevalence and morbidity of this disease, huge amounts of money and resources are always put towards cancer research. Along with the advent of new sequencing technology many recent efforts have revealed new insights about the risk and epidemiology of certain cancers related to genetics. Many different SNPs and gene mutations have been studied in relation to cancer risk, some of the most famous examples being the *BRCA1* and *BRCA2* tumor suppressor genes. Germline mutations that inactivate these genes increase a woman's cancer risk and according to website of the personal genomics company 23andme, being a carrier of the 185delAG BRCA mutation increases a woman's risk of breast cancer from 12% to 60% and her risk of ovarian cancer from less than 2% to about 40%.² Genetic testing is now available to check your individual risk. While many genetic predispositions to cancer are purported, recent research has indicated that the complexity of carcinogenesis cannot be down to genetic changes alone, but that *epigenetic* changes may play a significant, and possibly larger role.

The term “epigenetics” refers to changes in gene expression or cellular phenotype that are caused by mechanisms other than changes in the underlying DNA sequence. More detail will be covered later one, but one of the main types of epigenetic change is DNA methylation, whereby a methyl group is added to particular area of the genetic code, essentially silencing the expression of a gene. In a paper by Wong et al. published in *Cancer Prevention Research*, it was found that constitutional methylation of the promoter region for *BRCA1* is associated with similar cancer pathology that is seen in women with the actual *BRCA1* mutation.³ Overall, the past decade has highlighted a central role of epigenetic processes in cancer causation since the genes that are commonly mutated in human cancers can undergo epigenetic changes that silence them to the same effect.⁴ However, unlike other mutations, epigenetic changes do not alter the underlying sequence of the genetic code thus could potentially be more reversible and transient and responsive to the right types of therapy. This paper will discuss the overarching concept of epigenetics, then look at various ways that epigenetic changes have been linked to human cancers and finally discuss possible clinical implementations of this knowledge for prevention, prognosis and treatment.

Epigenetics refers to the series of mechanisms by which gene expression can be altered without any modification in the actual underlying sequence of bases in DNA. Epigenetics was first considered within the realm of developmental biology and used to explain how cells with identical DNA sequences could differentiate into totally different cell types, or why there is often some degree of phenotypic variation among identical twins or how the genes on only one X chromosome are expressed in females. Two of

the main forms of epigenetic change are DNA methylation and histone modification. In the nuclei of eukaryotic cells, DNA is folded and packed in with histone and nonhistone proteins to form a dynamic polymer called chromatin.⁵ Both DNA methylation and histone modifications help to organize the genome into regions with different transcriptional potential. DNA methylation is the addition of a methyl group to the cytosine ring at the 5' position of a CpG dinucleotide. S-adenosyl methionine acts as the methyl donor and the overall reaction is catalyzed by enzymes called DNA methyltransferases (DNMTs). These CG dinucleotide pairs occur in the DNA at a frequency five times lower than what we would expect statistically, however the cytosine in these CpG sequences is almost always methylated.⁶ However, in certain parts of the genome known as "CpG islands" CpG dinucleotides are found in a higher concentration but almost always unmethylated. CGIs are found at the 5' promoter region of about half of the genes in the human genome and thus strongly control the expression of these genes.⁶ We will see later on that aberrant methylation of CGIs is one of the more common mechanisms by which epigenetics affects cancer development through gene silencing. The methylation of CpG sequences does not interfere with base pairing but does interfere with the histone acetylation process that usually 'loosens' nucleosomes allowing the DNA to be accessed by RNA polymerase for transcription, which means that highly methylated areas will be transcriptionally silent. Another form of epigenetic change is "histone modification" in which different molecules can attach to the "tails" of histone proteins, thereby altering the activity of the DNA that is wrapped around them.

Methylation of H3 and H4 is strongly correlated with concurrent methylation of the underlying DNA.

Figure 1 shows how epigenetic changes are related to the structure of chromatin. Euchromatin (genes “turned on”) has high histone acetylation, low DNA methylation and H3-K4 (4th lysine on histone 4) methylation. On the contrary, heterochromatin (genes “turned off”) has low histone acetylation, dense DNA methylation (not shown in figure 1) and H3-K9 methylation.

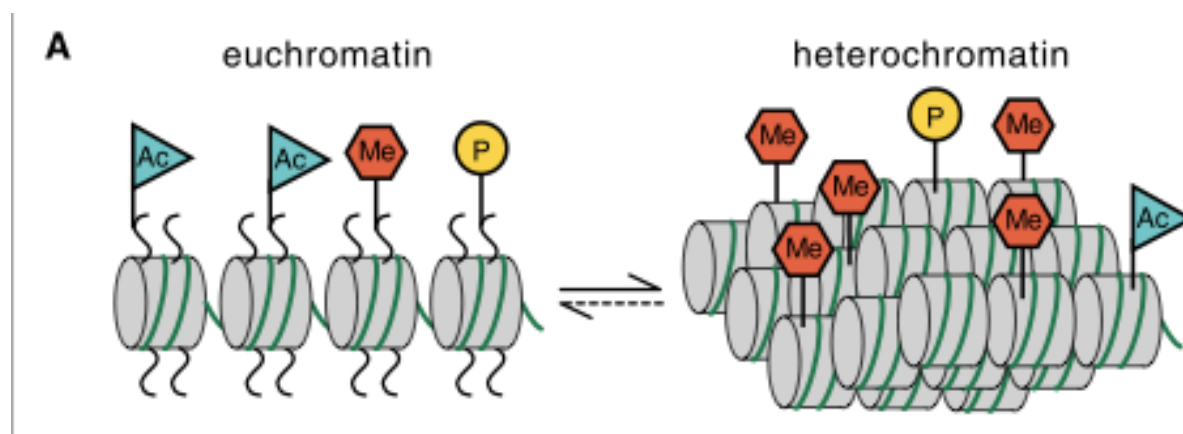


Figure 1. Models for euchromatic or heterochromatic histone tail modifications

Epigenetics has been linked with cancers through a few different mechanisms including global hypomethylation, promoter-specific hypermethylation, as well as possible interplay with pathogen induced inflammation and infectious disease.⁶ These aberrations from the norm favor the growth of neoplastic (cancerous) cells, leading to uncontrolled cell proliferation and tumor growth. DNA methylation serves as an alternate way of turning off tumor suppressor genes that are implicated in cancers and thus

exhibits that same overall effect that a mutation in the actual oncogene would. Several convincing examples of this have been reported in the literature, including methylation of the DNA repair gene *MGMT* in gliomas and colorectal cancer.⁷ DNA methylation can have effects not only by methylating promoters of tumor suppressor genes, but also more indirectly by silencing other genes, which code inhibitors for known oncogenes. A subgroup of patients who have high levels of gene promoter methylation at specific loci are said to exhibit a particular CGI methylator phenotype or 'CIMP'⁸. In a paper by Fang et al. published last year, researchers found a group of patients with similar breast tumors that showed a highly characteristic DNA methylation profile with specific hypermethylation at a subset of loci. This group was defined as having a breast CpG island methylator phenotype (B-CIMP) and they discovered that the presence of the B-CIMP in tumors was associated with low metastasis and good odds of survival, while the absence of the B-CIMP came with high metastatic risk and greater chance of death.⁹ Thus, patterns of hypermethylation can tell us not only about the initial causation of cancer but also start to give us clues about the prognosis of cancer types based on epigenomic profile.

Some cancer cells have also been noted to show global DNA hypomethylation where 5 methyl-cytosine is even less frequent than in normal cells⁶. While the causation and mechanisms behind this remain somewhat unclear, profound hypomethylation may lead to instability in the genome, making an individual more prone to genetic changes to the underlying code such as mutations, deletions or translocations.¹⁰ In addition, since methylation usually regulates gene expression within a cell by silencing certain genes

that are intended to stay “switched off”, this hypomethylation could potentially reactivate some genes that are normally silenced.⁶

From various examples that look at the epidemiology of a cancer type related to a single gene, we know that many silencing events function as drivers in carcinogenesis. However, the reason behind these alterations is still somewhat unknown. In a paper entitled ‘Dissecting DNA hypermethylation in cancer’, Marcos Estecio and Jean-Pierre Issa reasoned through a couple possibilities outlined in Figure 2 below.¹¹

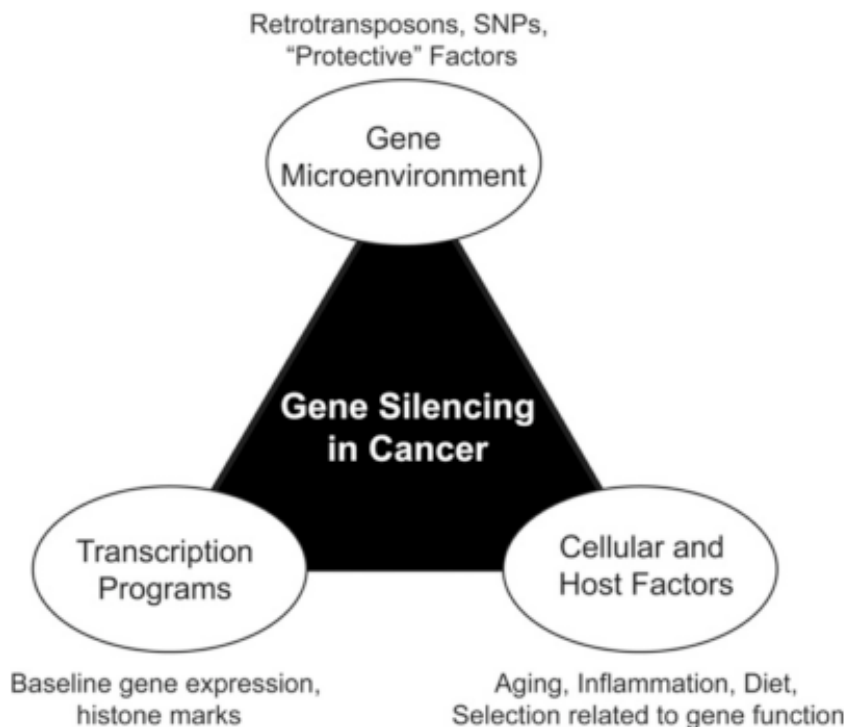


Figure 2. Factors thought to influence de novo DNA methylation in cancer. (Estecio et al).

While there is not scope to go into all the theories presented in this paper, they include individual differences in genetic microenvironment that may increase the chance

of methylation, transcription programs and various other factors related to the host's internal and external environment. One interesting new perspective is how aging acts as a host factor for gene silencing. We are all aware that aging is one of the greatest risk factors for most adult diseases and the classical thinking is that as you get older, you accumulate genetic mutations and are increasingly exposed to potential environmental carcinogens. Well just as you arguably accumulate genetic mutations, you accumulate 5-methylcytosine, which has been shown through both mouse and twin studies.¹²¹³ Aging accounts for a large proportion of epigenetic variation in normal tissues and so can probably also explain some of the variation seen in cancerous cells. Estecio and Issa also mention inflammation as a possible factor in promoting de novo DNA methylation and Richard Stein discusses this idea in more detail in a review article called 'Epigenetics – The Link between Infectious Diseases and Cancer'. According to Stein, around 20% of cancers have been causally linked to human pathogens and epigenetics may lend explanation to why infections are sometimes controlled and other times progress into malignancies.¹⁴ Hepatocellular carcinoma is the third leading cause of cancer death worldwide and risk of developing this disease is about 100 times higher in individuals who are chronic carriers of the hepatitis B virus. In addition, human papillomavirus is one of the most well known infectious agents that leads to cancer and has been implicated particularly in cervical and anal cancers as well as respiratory papillomatosis. Fernandez et al found that individuals infected with these pathogens displayed progressive methylation as acute infection became chronic and eventually developed into premalignant lesions and then full-blown cancer.¹⁵ In a paper published

in 2010, Peterson et al. examined the silencing of *Trefoil Factor 2* in *Helicobacter pylori* positive gastric cancers. *TFF2* increases gastric inflammation and expression of this gene is often lost in neoplasms. Here, they found that DNA methylation of the CGI at the *TFF2* promoter began at the time of *H pylori* infection and increased throughout gastric tumor progression.¹⁶ There are numerous other examples of epigenetics revealing a missing link between infection and cancer development and overall it appears that increased DNA methylation that occurs as a result of an infection makes a region more prone to later malignant transformation.¹²

While the 'how' and 'why' of de novo DNA methylation in cancer development is still a bit of an unknown, we are beginning to see the use of aberrant DNA methylation in various clinical applications. Epigenetic changes may act as a biomarker for diagnosis and prognosis. There is currently no gold standard for what DNA methylation patterns mean and there is great variability for different cancer types and different genes. For instance, increased frequency of methylated genes was found to be correlated with poor prognosis in colorectal cancers and some leukemias but then better prognosis in acute myeloid leukemia.¹¹ We saw earlier that the presence of a CIMP could be very predictive and in their paper 'Cancer Epigenetics' Issa and Taby outline numerous clinically relevant epigenetic biomarkers, many which have very high sensitivity and specificity.⁶ These biomarkers have potential to be used in diagnosis as well as assessment of prognosis and response to therapeutics such as chemotherapy. Further therapeutic potential exists in the possibility of pharmacologically relieving some of the deleterious effects of DNA methylation and chromatin remodeling through drugs

that reverse the process of DNA methylation. The table below taken from the Issa and Taby paper lists some of the epigenetic acting drugs that are FDA approved. These drugs fall under two general classes: DNA methylation inhibitors and HDAC inhibitors. DNA methylation inhibitors act by bonding irreversibly with DNMTs resulting in induced DNA demethylation.⁶ HDAC inhibitors prevent the action of Histone deacetylases, thus block the pathway that causes DNA to condense in heterochromatin causing gene silencing. Antionette Perry outlines a variety of epigenome-targeted therapy agents that could treat prostate cancer, including Valporic acid, a member of the short chain fatty acid class of HDAC inhibitors that was shown to inhibit growth of prostate cancer cells *in vitro* and to reduce tumor xenograft growth in athymic mice.¹⁷ Both DNA methylation inhibitors and HDAC inhibitors seem to be able to reactivate gene expression, however the exact mechanism is still somewhat unclear and a potential problem arises in the fact that these inhibitors are not gene selective so there could be undesired and detrimental side effects elsewhere in the body. A number of clinical trials are currently underway and it seems like that with increased understanding

EPIGENETIC-ACTING DRUG	CLINICAL INDICATION	MAJOR DATA	SUPPORTING LITERATURE
DNA methyltransferase inhibitors			
5-azacytidine (azacitidine)	Symptomatic MDS	16% overall response rate; 66% hematologic improvement/transfusion independence	Kaminskas 2005 ¹³⁷ Fenaux 2009 ¹³⁸
5-aza-2'-deoxycytidine (decitabine)	Intermediate and High-risk MDS	73% objective response rate; 34% complete response rate	Kantarjian 2007 ¹³⁹
Histone deacetylase inhibitors			
Suberoylanilide hydroxamic acid (vorinostat)	Progressive, persistent, or recurrent cutaneous T-cell lymphoma	30% objective response rate	Mann 2007 ¹⁴⁰
Romidepsin (depsipeptide)	Progressive, persistent, or recurrent cutaneous T-cell lymphoma	34% overall response rate; 6% complete response rate	Piekarz 2009 ¹⁴¹

Figure 3. Table taken from Taby and Issa showing the epigenetic acting drugs that have been approved by the US Food and Drug Administration (FDA).

of reversible epigenetic mechanisms involved in specific cancer types, we will be see more and more epigenome targeted therapy.

Epigenetics is rapidly emerging as a field that is important not only to understanding developmental biology but also to give us clues into genetic mechanisms that may lead to cancers and other chronic diseases. DNA methylation is the most extensively studied epigenetic change and in each cell only about five percent of genes are expressed, the rest are methylated and thus silenced or “turned off”. Although for the most part, DNA methylation patterns do not change much from cell to cell, areas that do experience changes profoundly impact cell differentiation and disease pathology. Research over the past decade has shown a consistent link between epigenetic changes and different types of cancer. Both losses and gains of DNA methylation are observed in various cancers and thought to contribute to pathophysiology by inactivating tumor suppressor genes or causing chromosomal instability. While the fact that these epigenetic changes exist is undisputed, the underlying reason has yet to be fully elucidated and we are slowly unraveling the connection between an individuals genetic microenvironment and factors that may make individuals more prone to de novo DNA methylation or other epigenetic changes that lead to cancer. In addition, we are increasingly seeing epigenetics emerge as the link between pathogens that are known to be carcinogenic and their affect on and disease causation and pathophysiology. Patterns of methylation have been recognized in various different cancer types. In some cases, these patterns can be used as biomarkers to aid diagnosis and prognosis and there is potential for new cancer

therapies that target the epigenome. In the wider scientific context, this paper barely scratches the surface of the complexity and depth that currently defines this exciting area of research. But it nonetheless highlights the validity of some of this new research and ways it might be used in the future to treat one of our most significant health concerns.

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