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The roles of DNA methylation in the stages of cancer

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Abstract

Next year will mark 60 years since Dr. Leslie Foulds outlined his hypothesis that cancer is “a dynamic process advancing through stages that are qualitatively different,”¹ leading the way to our view of cancer progression as we know it today². Our understanding of the mechanisms of these stages have been continuously evolving this past half-century and there has always been an active discussion of the roles of both genetic or epigenetic changes in directing this progression. In this review, we will focus on the roles one particular epigenetic mark - DNA methylation - plays in these various “discontinuous” stages of cancer. Understanding these steps not only gives us a better picture of how this fascinating biological process operates, but opens the doors to new prognostic biomarkers and therapies against these malignancies.

Fundamentals of DNA methylation

DNA methylation is the conversion of the DNA base cytosine (typically within a CpG moiety) to 5-methyl cytosine. This reaction is catalyzed by DNA methyltransferases (DNMTs) of which there are three in mammals (DNMT1, DNMT3A, and DNMT3B). In mice, loss of any of these genes leads to early death^{3,4}. In contrast, the genes that catalyze the “demethylating” reaction (actually a conversion of 5-methyl cytosine to 5-hydroxymethyl cytosine) – known as the TET enzymes (TET1/2/3) appear to be capable of substituting for each other, at least during development, while loss of all three results in a failure of embryogenesis⁵.

The DNA methylation status (decided by the balance between methylation and demethylation activities) for a locus can have a myriad of biological effects, but the most widely-recognized is the regulation of gene expression. Methylated DNA is associated with heterochromatin – a more compact form of DNA and histones which is more difficult for the transcriptional machinery to access (assuming methylation occurs in a transcriptional regulatory element like a promoter or enhancer), leading to loss of expression of that gene (Figure 1).

DNA methylation changes have also been associated with other events key to gene expression like mRNA splicing polyadenylation⁶, transcriptional termination, and other events important to gene expression⁷, making DNA methylation an important regulator of gene expression and, consequently, an important regulator of the fate of each cell in the body⁸. DNA methylation is also an important contributor to genomic imprinting, wherein a particular allele can be silenced by this genomic modification, and this deterministic modification to an allele can have profound effects on physiology⁹.

Differences in methylation between normal and cancer cells was first observed more than 30 years ago.¹⁰ These and other early observations suggested that cancer could be regulated at some level by DNA methylation. Over the years, it has become evident that DNA methylation regulates many of the “discontinuous stages” of cancer progression (Figure 2).

DNA methylation in carcinogenesis

The first suggestion that epigenetic events may be involved in the first step of cancer – carcinogenesis - came from observations in oral squamous carcinoma¹¹ under the term “field cancerization”: the hypothesis that cancer is the result of a carcinogenic agent that transform cells in a directional manner. A variety of carcinogenic events have been proposed as field agents, including DNA methylation. Although carcinogenesis is now viewed as a clonal event^{12,13} that begins with somatic mutations that activate proto-oncogenes and inactivate tumor suppressors, DNA methylation has also been implicated in the initiation of cancer. There are essentially two mechanisms whereby DNA methylation can cause cancer: via gene expression modulation and by contributing to somatic mutations. First, hypermethylation of a tumor suppressor promoter region can lead to decreased expression of the tumor suppressive transcript and – conversely - hypomethylation of a proto-oncogene promoter sequence can lead to increased oncogenic activity from that locus, as has been observed in WNT5 in prostate cancer¹⁴. Thus, the gene regulatory activity of DNA methylation can be an important early event in cancer pathophysiology. However, DNA methylation can also lead to somatic mutation in driver genes, thus activating carcinogenesis. De-amidation of 5’ methyl-cytosine results in a C->T transition, thus leading to a G:T adduct, which is inefficiently repaired. If such an event occurs in a driver gene, it could result in cancer, as has been observed (Table 1 – Methylation to somatic mutation subsection).

Further, DNA methylation has been implicated in maintaining genomic stability by inhibiting expression of potentially disruptive retro-transposons¹⁵, and loss of DNA methylation in these regions leads to increased transcriptional activity of transposons¹⁶. This observation also implicates DNA methylation in maintaining genomic stability; loss of genomic stability is another driver of carcinogenesis. Therefore, DNA methylation can play an important role in many aspects of carcinogenesis, but it also regulates many facets of the later stages of cancer pathophysiology, as we describe below.

Therapeutic sensitivity/resistance

Resistance to treatment (radiation, chemotherapeutics, immunotherapy, etc.) is another capability that tumors must obtain before the tumor can kill the individual. Most cancers are subjected to at least one of more therapeutic strategies (as surgery, chemotherapy, radiation or biologic therapies), generally in some combination, although the order of this combination can differ dramatically between tumor types and between individuals within a tumor type. Treatment resistance is, however, a common occurrence and - over the past 10 years - the role of DNA methylation in the efficacy/inefficacy of each of these treatments has been the focus of considerable investigation. DNA methylation differences were observed between radio-sensitive and radio-resistant cell lines^{17,18}. Specific and reproducible changes in DNA methylation have been observed in cells surviving radiation¹⁷. Most importantly, however, inhibition of DNA methylases with 5-aza-cytidine sensitizes a number of radiation resistant cancers to radiation, demonstrating that DNA methylation plays an important role in radio-resistance in the clinic.

DNA methylation has been studied in more detail when it comes to resistance to chemotherapy. First, DNA methylation changes are associated with tumors/cell lines of varying chemosensitivity. Next, experimental inhibition of DNMT1 – with siRNA - increases chemosensitivity in the ovarian cancer cell line (A2780-AD).

One well-known example of how DNA methylation affects chemosensitivity comes from oligodendrogliomas – a rare lower grade glioma, often associated with mutation in isocitrate dehydrogenase 1 (IDH1). A somatic IDH mutation commonly found in these tumors (and a handful of others) results in changes leading to a modified metabolic product from alpha-keto-glutarate to 2-hydroxyglutarate (2-HG), which inhibits the TET enzymes, leading to a buildup of methylation of the promoter for the DNA repair enzyme O⁶-methylguanosine-DNA methyltransferase (MGMT)¹⁹. This methylation results in loss of MGMT protein expression: since MGMT repairs alkylating agent-derived DNA damage – and the alkylating agent temozolamide is the most common chemotherapeutic drug for this tumor type – loss of MGMT expression leads to the accumulation of alkylation-based DNA damage, and a pronounced sensitivity to chemotherapy²⁰. This admittedly complex set of reactions represents another example of not only how DNA methylation regulates the life of the tumor, but the complex interplay between somatic mutation, DNA methylation, and the pathophysiology of cancer.

Observations like those described above have indicated that epigenetics may play an important role in the sensitivity of cancer to chemotherapy^{21,22}. Our laboratory has shown that treatment of colorectal cancer cell lines increased sensitivity to irinotecan, and this is associated with changes in gene expression²³. It is our hope that we can use these observations to develop improved biomarkers, and consequently a more personalized therapeutic regimen based on each individual's DNA methylation profile.

Recurrence

It was fewer than 10 years ago that differential methylation was first associated with increased risk of recurrence: in this study, in non-small-cell lung cancer (NSCLC), only seven loci were evaluated, but the authors were able to demonstrate that methylation within the promoters of these seven genes could predict which stage I NSCLC patients were at an increased risk of recurrence²⁴. DNA methylation events are also associated with an increased risk of recurrence in breast cancer^{25,26}. Since then multiple groups have identified promoter methylation events as predictors of recurrence in a wide variety of tumor types²⁷ (Table 1).

Metastasis

DNA methylation changes may even be important mediators in the transition to metastatic cancer: dramatic and reproducible changes in methylation have been described in powerful longitudinal studies. Such studies have been done in colorectal cancer²⁸, prostate cancer²⁹ and breast cancer³⁰. E-cadherin (CDH1), is broadly recognized as a target of DNA methylation leading to metastasis in breast cancer³¹: notably, DNA methylation of metastasis-suppressing genes has been observed in circulating tumor cells deriving from a variety of tumor types^{32–34} raising the intriguing possibility that DNA methylation may also be a useful prognostic marker for metastasis. Notably, a pan-cancer meta-analysis identifies signature methylation loci associated with metastases in breast and colorectal cancers³⁵ and methylation at specific loci which predict metastatic potential to the liver. Therefore, the potential of methylated DNA as prognostic, diagnostic, and metastatic markers is an area upon which we expand upon next.

DNA methylation-based biomarkers

Validation of methylation-specific DNA loci in cancer has led to the advent of these genetic alterations' usage as biomarkers. As previously identified, the location of these methylation-marks in nucleotide sequences containing repetitive CpG at gene promoters contributes to gene regulation; distinctly, CpG methylator phenotype ('CIMP') was originally used to describe a subset of colorectal cancers with aberrant methylation, typically at CpG islands in tumor suppressor genes (inhibiting gene expression; including *MLH1*). CIMP high in colorectal cancer is often associated with microsatellite instability (MSI) as well as BRAF mutations. CIMP status -high versus low- CpG island methylation is observed in a variety of other cancers and CIMP status³⁶ is used a prognostic and therapeutic indicator, including in MSS CRC. Notably, in colon cancer hypermethylation at gene promotor regions of mismatch repair genes (MMR), like *MLH1*, inhibits DNA damage repair in tandem repeat loci known as microsatellites (receptive DNA sequences of 10–12 nucleotides), leading to microsatellite instability (MSI)³⁷; these cancers are typically more responsive to chemo- and/or radiation therapy.

More recently, MSI colorectal cancers, which account for 12–15% of all CRC, are noted for better therapeutic response to immunotherapy (PD1/PDL-1 inhibitors) compared with MSS CRC³⁸. Other methylation markers used for the detection of CRC includes detection from

non-invasive screens, including stool samples- the sample detection kit –Cologuard®- was approved by the FDA and two genes with aberrant methylation, NDRG4 and BMP3, are used as diagnostic indicators. Though detection results from these kits- relative to diagnosis through annual colonoscopies- have yet to meet these standardized metrics for the detection premalignant polyps⁴⁵. Identifying stage specific markers remains a priority for better treatment results and prolonged health benefits, particularly those markers that distinguish potential for recurrence; *IGFBP-3* methylation status in Stage II CRC primary tumors has been linked with increased likelihood of recurrence⁴⁹. Similarly, methylation status of *APC*, *p16*, *CDH13*, and *RASSF1A* are linked with recurrence of Stage I, non-small cell lung cancer (NSCLC)²⁴. These data are further described in Table 2 and are adapted from Mikeska, T et al⁵⁰.

MGMT hypermethylation, as previously discussed is also an important predictive biomarker for treatment success; patients with hypermethylated MGMT are more likely to respond to temozolamide treatment in brain cancers (characteristically, glioma phenotypes with IDH1 mutation)^{40,51}. IDH1 mutation, observed in some gliomas, acute-myeloid leukemia (AML), cholangiocarcinoma (CC), and some chondrosarcomas is also an important prognostic biomarker, with prolonged survival observed in glioma, AML, and CC and response to therapy^{19,52–54}. IDH1 itself is an important epigenetic regulator, along with ARID1A, which are often found mutated in these cancers, contributing to hypermethylation.

DNA methylation-based therapies

As discussed previously, altering DNA methylation can sensitize cancer cells (*in vitro* and *in vivo*) to other chemotherapeutic therapies; however, DNA methylation inhibition has also been an effective chemotherapeutic when used alone. Myelodysplastic syndrome – a bone marrow failure disorder that often leads to AML – has been successfully treated with the DNA methylation inhibitor azacytidine⁴⁹. While some of this success is likely attributable to intercalation into DNA and RNA – rather than purely from inhibition of DNA methylation – the effectiveness of a DNA methylation inhibitor in the treatment of malignancy provides evidence of the usefulness of DNA methylation modulation in treating cancer.

Summary

DNA methylation is an important driver of many of the distinct stages of cancer (Figure 2). While somatic mutations are clearly the best-characterized and most powerful drivers of cancer, the dynamic nature of DNA methylation provides a wide variety of mechanisms that guide cancer, many of which may be reversible. Further understanding of how DNA methylation regulates the “qualitatively different” stages of cancer can lead us to improved prognostics and – ultimately – treatments.

Future

The roles of DNA methylation in various aspects of cancer have shown convincingly that it can be an important contributor to pathophysiology of the various stages of cancer. However, most studies have only been able to investigate the effect of global DNA methylation

changes – often coupled with global changes in gene expression – to identify methylation loci that are involved in cancer pathophysiology. Since – as outlined here – DNA methylation can be involved in many aspects of the stages of cancer through multiple mechanisms – it will be important to better understand the role of specific methylation events in each of the stages of cancer. This will require new technologies, some of which are becoming available now, including micro-RNA-induced down-regulation of methylation and demethylation enzymes, targeted methylation and demethylation, and targeted mutation at methylation sites to elucidate the precise role of individual DNA methylation events in each stage of cancer, as well as the interactions between genetic and other epigenetic changes. By delving more deeply into mechanisms of DNA methylation in the various stages of cancer, it will be easier to build better treatments for each patient based on their specific tumor, contributing significantly to new precision medicine therapies in the future.

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Figure 1.

DNA methylation regulates chromatin states: Chromatin conformation is regulated by DNA methylation, along with other epigenetic states. DNA hyper-methylation is associated with silenced loci, while hypo-methylated DNA is associated with active chromatin.

Figure 2.

Timeline for a typical cancer, and how DNA methylation affects each stage

Table 1

Specific methylation events and cancer

<i>Promoter Hypermethylation of tumor suppressors</i>	
Tumor suppressor	Tumor type
hMLH1	colorectal cancer
MGMT	glioma
p16INK4a	lung
p15INK4b	MDS, AML
p14ARF	colorectal
DAPK	HNSCC
VHL	CCRC
RASSF1	lung, breast, prostate, glioma neuroblastoma, kidney
RB	
<i>Methylation to somatic mutation</i>	
Gene	Tumor type
TP53	lung, colon, bladder
RB	bladder carcinoma, small-cell carcinoma
<i>Hypomethylation of proto-oncogenes</i>	
Proto-oncogene	Tumor type
BCL-2	B-CLL
WNT5A, CRIP1, S100P	prostate
<i>Methylation of recurrence suppressors</i>	
Gene	Tumor type
SHOX2	NSCLC
TWIST1	breast
<i>Methylation of chemosensitivity loci</i>	
Gene	Tumor type
MGMT	glioma
MLH1	gastric
p16	gastric
<i>Methylation of metastasis suppressors</i>	
Gene	Tumor type
CDH1	breast, gastric
CDH13	colorectal, breast
APC	colorectal

Table 2

Translational biomarkers: differentially methylated genes in cancers adopted for treatment, diagnostic, and prognostic tests

DNA Methylation Marker	Clinical Determinant
<i>Treatment</i>	
<i>MLH1</i>	PD1/PDL1 Immunotherapy Treatment ³⁸
<i>MGMT</i>	Temozolamide Treatment ^{20,39,40}
<i>Diagnostics</i>	
<i>NDRG4, BMP3, and VIM</i>	Stool-based Assay ^{41,42}
<i>SEPT9</i>	Blood-based Assay ⁴³
<i>SHOX2</i>	Bronchial Fluid Assay ⁴⁴
<i>Prognostics</i>	
<i>IGFBP-3</i>	Prognostic Indicator ⁴⁵
<i>APC, p16, CDH13, and RASSF1A</i>	Prognostic/Recurrence Indicator ⁴⁶⁻⁴⁸