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Hypothesis

Preoperative epigenetic preparation of patients is a current reality

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Socioeconomic Status Is a Diagnosable Demographic

That Influences Surgical Outcome

And

In Animals, Environmental Stress Modifies the Epigenome To Change Phenotype

And

The Language of DNA is Revealing Methylation or Acetylation, CRISPR-Cas9 with RNA and DNA Surgical Specificity

So

Epigenetics Dwarfs Genetics as a Governor of Phenotype

Thus

Therapeutic Access to the Epigenetic Regulatory System May Permit Personalized Anti-Inflammatory and Anti-Neoplastic Pre-operative Patient Preparation

With disappointing frequency, all surgeons are too often presented with the corpulent, 55-year-old, cigar-chomping banker whose idea of exercise is to walk down a single flight of stairs rather than taking the elevator, and even the 21-year-old single mother of three with no high school diploma who must lock and bolt her door and is constantly short of food stamps. These psychosocial–physiologic demographics of biologic stressors provoke increasingly predictable epigenetic alterations that confer enhanced surgical risk. The purposes of this report are as follows: (1) provide a primer on epigenetics; (2) relate socioeconomic status (SES) to changes in the epigenome; (3) suggest that SES-induced epigenetic alterations can be identified and targeted; and (4) introduce surgeons to the molecular tools for altering the epigenome, which governs the expression of the genome, as an accessible strategy of decreasing surgical risk.

The biology of epigenetics

All 50 trillion cells in the body contain 23 chromosomal pairs that carry about 25,000 identical genes. These genetic recipes dictate which cells become cardiomyocytes as opposed to astrocytes or enterocytes. Chromosomes are constructed from long, noncoding nucleotide segments (introns) interspersed between relatively infrequent protein coding regions (exons). Current estimates suggest that only 5% of human deoxyribonucleic acid (DNA) encodes proteins. Thus, formidably complex eukaryotic cells are built ostensibly with remarkably few instructions. About 60% of the genes that define a cell of a chicken code for the same proteins in human beings. Even more surprising is that as much as 88% of the rat and human genomes code for the same proteins, and the genomes of Eleanor Roosevelt and Madonna are 99% identical. So, some other major control systems must be operative.

Epigenetics describes the strategies cells use to hide some genes and activate others. To squeeze 6-foot–long strings of DNA into a tiny cell's nucleus, the DNA is tightly "wound" into a histone spool called nuclear chromatin. The phenotype of a cell is determined by the activation or suppression of the genes that are wound around its histone spools. Accessibility of this genetic information permits transcription, and this is influenced predominantly by the methylation "capping" of a gene or the acetylation of a lysine on the histone spool (Figure). The addition of an acetyl group to the lysine loosens the histone wrap, making the gene more accessible for transcription. Deacetylation does the reverse.

Throughout the past several decades, it has become clear that these epigenetic instructions are not only heritable but can be influenced and even targeted therapeutically by changes in lifestyle

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and pharmacologic prescriptions. There are five major families of chromatin histones: H1, H2A, H2B, H3, and H4. The letter "K" plus a number designates a specific lysine. Thus, "me3" indicates trimethylation. Therefore, trimethylation of lysine 16 on histone 4 is designated "H4K16me3".

CpG sites are important epigenetic components that influence gene expression. A CpG site is a region of DNA in which a cytosine nucleotide is followed by a guanine nucleotide, separated by only one phosphate group. Cytosines in CpG nucleotides can be methylated to form 5-methylcytosine, which changes the associated gene expression in an epigenetic fashion. There are more than 28 million CpG sites in the human genome. Using arrays and high-throughput sequencing, large fractions of the methylome can now be decoded and potentially targeted therapeutically.[1]

Therefore, we now know the expression of tumor suppressors, DNA repair proteins, and inflammatory cytokine gene targets that we want to promote or inhibit. We are learning to mine the methylome to identify the components that control gene expression. We can identify the numbered lysine that unlocks the histone spool packaging that gene. Furthermore, we have the methylation, phosphorylation, and acetylation enzymes that control the epigenetic activation and suppression of gene transcription. The challenge then becomes whether we can control this process reliably.

SES structurally modifies the genome

Considerable evidence suggests that social adversity triggers biologic pathways. When activated by hormonal changes, free radicals, and cytokines, these pathways can alter the human epigenome and, subsequently, the human phenotype.

In a cohort of 619 infants with nonwhite parental race, lower maternal education, and lower household income correlated with hypomethylation of fetal genes—an alteration linked with low birth weight and a greater propensity for colorectal cancer.[2] Analysis of this cohort also demonstrated that hypermethylation of certain oncogenes was more likely in newborns of never-married mothers. Methylation and cancer risk were less when maternal grandmothers co-resided with infants.[2] This cohort analysis also revealed that increases in prenatal neighborhood disadvantage were associated with greater methylation and a greater cancer risk, indicating a possible additive effect of social disadvantage on methylation changes and subsequent health.[2]

Methylation changes persist into adulthood. The Multi-Ethnic Study of Atherosclerosis study examined life-course associations between DNA methylation of 18 genes related to stress reactivity and inflammation in more than 1,200 non-Hispanic White, Black, and Latino participants older than the age of 55 years.[3] This study reported an association between low childhood SES and DNA methylation of 3 stress-related and 2 inflammation-related genes. Social mobility was correlated with methylation changes in 3 stress-related and 7 inflammation-related genes. Specifically, identifiable methylation patterns have been postulated as the root cause of neoplastic diseases owing to overmethylation of tumor suppressor genes and incessant expression of tumor promoter genes.[4]

SES modifies the epigenome, which influences the response to surgical stress

The process of wound healing involves the activation of more than 300 genes that facilitate mitosis, differentiation, and the specialization of cells. Patients with alcoholism have a greater incidence of non-union of fractures, but the mechanism of this effect is controversial. Sampson et al[5] fed mice an ethanol-based diet for 4 weeks before inducing surgical fracture of the right hind limb. At the time of harvest of the fracture calluses, 35 genes exhibited increased expression and 20 genes exhibited decreased expression owing to alcohol consumption. Patients with evidence of epigenetic depression of these genes via alterations in mRNA expression owing to alcoholism were reported to have a greater risk of non-union after bone fractures, an effect linked to the epigenetic regulation of migration, proliferation, and differentiation with wound healing.[5]

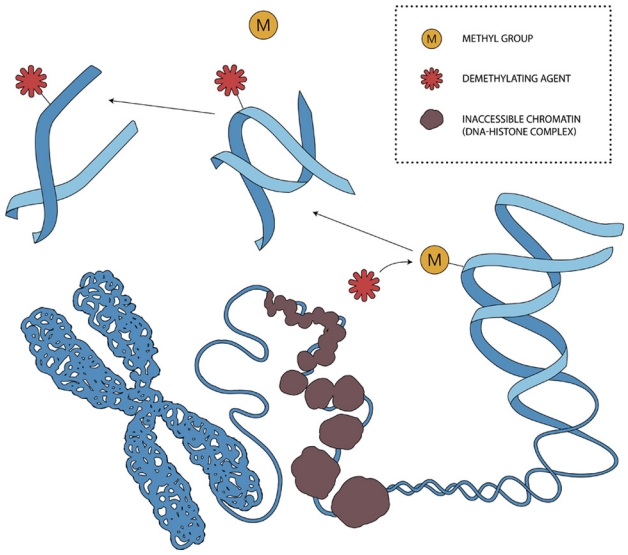

Figure. Let's start with what we think we know. The human genome is composed of 3 billion nucleotide base pairs linked by adenine to thymine and guanine to cytosine rungs in the chromosomal ladder. To fit 23 pairs (46) of these 6-foot–long chromosomes inside a cell's nucleus, many portions of each chromosome are wound tightly into inaccessible balls of nuclear chromatin. The Human Genome Project has identified only about 25,000 protein coding genes, which appear to account for less than 5% of each chromosome. The remaining 95% of each chromosome was labeled the "dark genome" and misinterpreted as "junk DNA." But approximately 100,000 proteins have been identified in human cells. Then, the international Encyclopedia of DNA Elements program (ENCODE) analyzed trillions of permutations of nucleotide sequences within this "junk" DNA and reported that RNA does not simply copy DNA in a mindless, robotic fashion. ENCODE investigators concluded that, although uniquely penetrant genes (such as those which control sickle cell anemia, cystic fibrosis, and eye color) do dictate structural attributes,[4,11] the epigenome, which is environmentally sensitive and responsive but hidden within the zones of "dark" DNA, does the heavy lifting in molding ultimate phenotype.[11] Like musical notes on sheet music, genes can be played softly or loudly, rapidly or slowly—or not at all. They can be played with healthy sonorous chords, or dissonance that causes disease. The epigenome is the air traffic control behind it all. We are at an archaeologic stage in excavating the dark genome. We have only just uncovered the steeples and domes of a complex society and language that has evolved throughout millions of years. Twenty tiny amino-acid fragments of RNA (as micro-RNA, short interfering-RNA, and circular RNA) break off and silence both genes and regulatory locations. Methyl groups appear to constrain gene function. When tumor-suppressor genes are hypermethylated and tumor proliferation genes are hypomethylated, malignant degeneration is likely. It is now feasible to detect both the number and location of methyl molecules on the genome. In addition, acetylation of loci unwinds the histone spool, making previously inaccessible genes operative. Specific genome editing is now feasible,[12] augmenting the therapeutic potential of these RNA interference systems. Cluster Regularly Interspaced Short Palindromic Repeats with CRISPR-associated systems tools, such as CRISPR-Cas9, can cut and splice both DNA and RNA with surgical specificity.

Socioeconomic status influences surgical outcomes and suggests a population with accessible epigenetic targets

Socioeconomic status as estimated by median annual household income is often overlooked during perioperative planning. Bennett et al[6] used a multimodal inference model to analyze data from the National Inpatient Sample of more than 1 million surgical patients older than 16 years of age and reported a

pervasive inverse relationship between patient SES and mortality across 13 complex surgical procedures. Higher SES conferred statistically significant decreases in mortality rates ranging from −2.7% to −13.1% for 10 of 13 operative procedures, including coronary artery bypass grafting, gastrectomy, pancreatectomy, and pulmonary lobectomy. These data support the idea that lower SES increases perioperative mortality and importantly that this difference cannot be explained entirely by other patient or hospital factors. This study concluded that "Patient socioeconomic status is an independent predictor of operative mortality."[6]

The epigenome is therapeutically accessible

Can we learn from oncologists who are modulating the epigenome successfully with histone deacetylase inhibitors in combination with immunotherapy? Both cancer and trauma depress immune responsiveness. When cells become infected or cancerous, they present surface proteins often recognizable by immune system T cells. Once the T-cell attack is initiated, a simultaneous system promotes "checkpoint" molecules to protect healthy cells from activated T cells. CD47 is a checkpoint molecule that sends a "Don't eat me!" signal that interferes with a signal regulatory protein (SIRPα) expressed on macrophages to prevent the destruction of healthy cells.[7] Specific modulation of the desired level of checkpoint inhibition should permit the surgical or oncologic therapist to control the trauma-induced infectious cytokine storm or neoplastic invaders at the optimum level of cure versus morbidity. Epigenetic modifiers of histone acetylation, histone methylation, and DNA methylation targeting the immune checkpoint proteins CTLA4 and PD-L1 are in clinical trials currently for lymphoma.[7]

Epigenetic histone acetylation is driven by histone acetylases and deacetylases. An even more granular epigenetic coding element is DNA base methylation. Drugs that act on the epigenome are histone deacetylase inhibitors and DNA methyltransferase inhibitors. There are now US Food and Drug Administration–approved epigenetic therapies for T-cell cutaneous lymphoma and multiple myeloma.[8] Multiple epigenetic therapies are currently in clinical trials for the treatment of solid organ malignancies. Epigenetically methylated genes associated with the neutrophil accumulation, parakeratosis, and neutrophil chemotaxis characteristic of psoriasis have been identified.[9] These methylated genes (CARD15, KA2N, PTPN22) were identified as inversely methylated relative to gene expression in psoriatic plaques. Chandra et al propose using drugs to control this epigenetically modulated, psoriatic inflammatory response. Immune and inflammatory responses to neoplasia, sepsis, and inflammation may also be controllable via epigenetic therapeutic strategies.

Both in vitro and in vivo studies demonstrate the reversibility of acquired epigenetic programming as a proof of concept. Blumenthal et al[10] studied epigenetic interventions for asthma in mice. In this disease, there is evidence of socioeconomic disparities that can be attributed at least in part to environmental epigenetics. Inhibiting the Bromodomain and Extra-Terminal Domain protein responsible for epigenetically enhanced IL-9 cytokine transcription decreased lung inflammation.[10]

So, a patient's preoperative demographic profile identifies epigenetic targets that are becoming pharmacologically accessible with promising improvements in surgical risk and outcomes.

Conflict of interest

The authors have indicated that they have no conflict of interest regarding the content of this article.

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