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Determining Cellular Fate: Pre- and Postnatal Methylation Effects on Gene Expression

The idea of "Designer Babies" has long been a hot topic in both genetics and bioethics; meanwhile, the much less invasive possibility of "Designer Genes" has been largely untouched. "Designing" in the latter sense could theoretically be accomplished through the already naturally occurring process of DNA methylation. While methylation is only one of several epigenetic mechanisms, it is of interest due to its reliable heritability component (as opposed to the less reliable and more specific heritability of chromatin remodeling). DNA methylation refers to the addition of a methyl group to the 5 prime position of a cytosine nucleotide at a CpG (where cytosine occurs next to a guanine) site. Examples of the epigenetic effects of methylation are well documented; however, the frontier of selectively using methylation to essentially engineer qualities within humans is only theoretical at this point. In this, looking into the prenatal and postnatal effects of DNA methylation is the key to understanding if it is possible to artificially select gene expression for a desired phenotype.

I. Natural Methylation Patterns

DNA methylation is a crucial factor in overall animal development, as well as in genomic imprinting and X chromosome silencing. While 60% to 90% of all CpGs are methylated in mammals, the methylation that is the most pertinent to this topic is that which occurs near promoter regions. Methylation of CpG sites near promoter regions can, but not always will, play a role in gene expression (Phillips). The more methylation—

relative to the strength of the promoter— that occurs in these areas, the greater the chance that low or no transcription will occur (Phillips). This repression is greater if the promoter itself is methylated, but distantly methylated sequences can also contribute to repression —especially if the methylation occurs in high frequencies (Bird). However, this specific quality allows for potentially lethal effects. CpG islands (CpGIs), which are dense regions of typically unmethylated CpGs found embedded in the promoters of approximately 60% of all genes, can become inappropriately methylated (in terms of hypermethylation and hypomethylation compared to normal tissue) and thus silenced, leading to a large number of human malignancies (Bird). Most commonly, this is seen in the hypermethylation of the CpGIS of anti-oncogenes, thereby causing the repression of tumor suppressors and the possibility of cancer development (Bird). At the same time, developmentally programmed CpGI methylation (that is not seen in the germ line but is expressed in somatic cells) is involved in genomic imprinting, X chromosome inactivation, and cell differentiation (Phillips), revealing the complexity of methylation patterns.

II. Possible Mechanisms for Methylation to Determine Cell Fate

Currently, the exact role of methylation in gene expression is unknown. Many scientists argue that chromatin-remodeling proteins play a larger role in gene expression than methylation does both because gene silencing can occur before methylation and because histone modification complexes appear to be connected to DNA methylation at certain DNA sequences (Phillips). It has been shown that some genes are silenced before methylation upon them occurs, leading researchers to believe that the methylation occurs simply as a means to “lock” or permanently silence the gene (Bird). Others believe that

the chromatin remodeling proteins serve to allow methyltransferases access to the chromatin because a loss of a remodeling protein leads to only partial, rather than total, methylation loss (Bird).

One thing that has been definitively shown is that shortly after fertilization, the methylation pattern of the gametes disappears; once implantation occurs, a wave of de novo methylation establishes the pattern that causes the majority of CpG methylation and is carried out by the de novo methyltransferases DNMT3A and DNMT3B (Bird). This, along with the peaked expression rates of the methyltransferases in embryonic cells, lends support to the idea that there is a critical period in which methylation programming occurs. If this critical period can be established and then manipulated, gene expression can be altered and desirable phenotypic changes can be made based on the pre-existing genome. Therefore, tapping into the critical period may be a plausible method to determine cell fate.

Another route to changing cell fate may be to work antagonistically toward the methylation patterns already in effect through artificial demethylation. This is based on the finding that, in certain germ cells, the silencing of imprinted genes is reversed post-methylation. This suggests a naturally occurring, pre-existing demethylation mechanism that effectively causes "epigenetic reprogramming," which may be mediated by the removal of amino groups by deamination, causing DNA mismatch repair that abandons the methyl groups (Phillips).

III. Prenatal Methylation: Experimental Results

Experiments aimed to affect prenatal methylation patterns show promise for the possibility of manipulating methylation during the hypothesized "critical period" of

prenatal development in order to produce a specific phenotype. Researches have determined that "the earlier that epigenetic signals are trans- mitted, the more significant the potential changes are in the fetus," directly implying a time-sensitive period of methylation in which genes are turned on or off (Moalem). Following this idea, many experiments have been directed towards fetal development and have yielded results that indicate that utilizing prenatal methylation may be the best way to manipulate phenotypes.

One of the earliest and most cited examples of genetic suppression through DNA methylation is from a study done at Duke University wherein which a combination of prenatal vitamins (that contained methyl donors) given to pregnant mice inhibited the expression of a gene—termed the agouti gene—that caused obesity and yellow fur color (Moalem). This resulted in the pregnant mice producing offspring that lacked obesity and had brown fur simply because the methylation patterns of the mice babies were altered during prenatal development. Similarly, it was observed in a separate experiment that one of the prenatal vitamins, choline, improved mouse memory by methylating a gene that limits cell division in the memory center of the brain (Moalem). Collectively, these experiments add validity to the idea that methylation can be altered in a favorable manner (especially considering the decreased rates of diabetes and cancer seen in the mice with the agouti gene switched off (Moalem)) and lend support to the mechanism of prenatal alterations as a means to determine cell fate.

Several other studies following the agouti study further emphasize the power of methylation during the critical period by demonstrating how even simple changes in the fetal environment can add methyl markers. Experiments showing the effect on a mother's

diet during pregnancy are the main source for this idea. Moreover, they support the hypothesized “critical period” for gene switches. One study showed that a low protein diet during the first four days of pregnancy in rats led to offspring that were prone to high blood pressure because the genes regulating metabolism were adjusted for a low protein environment (Moalem). Similar metabolism adjustments were exhibited in experiments where sheep were underfed during pregnancy and consequently had babies with thickened arteries (Moalem). In humans, this trend is also seen in that pregnant mothers that eat nutrient-poor food while pregnant have children that have metabolism adapted—through methyl markers—for a nutrient-poor living state, leading to obesity as they encounter the reality of a nutrient-rich world (Moalem). Stemming from these conclusions, a phenotype can theoretically be morphed to gain favorable traits, such as a more efficient metabolism, by making small changes to the fetal environment during the critical period.

IV. Postnatal Methylation: Trends and Experimental Results

Unlike prenatal methylation, trends seen in postnatal methylation support the possible use of demethylation to express a desired phenotype. As mentioned beforehand, intolerable hypermethylation can lead to oncogenesis. Extensive data has been found that supports the idea that this hypermethylation is largely due to environmental factors. Across the spectrum, people who smoke show hypermethylation around genes that, if functional, would fight both lung and prostate cancer (Moalem). On an even deeper level, methylation of a single intronic CpG site is found to be associated with hypermethylation of that gene, whose functional loss leads to prostate carcinogenesis (Zhang). Similarly, betel nut chewing has long been associated with oral cancer; interestingly enough, it has

recently been found that this is due to the fact that the betel nut causes hypermethylation of three oral cancer-fighting genes (Moalem). As seen throughout these examples, methylation is something that can be changed in the postnatal lifespan and is not fully determined by genes or prenatal methylation patterns. In this, it is clear that finding a way to demethylate these genes could be a way to change phenotypic expression.

Demethylation within a laboratory setting has been proven to be possible; however, clinical demethylation still lies on the horizon. If this process can be determined, demethylation would be a viable method to change already expressed phenotypes into chosen phenotypes. Successful implementation of this method would be most desirable in terms of reversing disease progression. It also could be useful in stopping disease reoccurrence in cases such as a gene that, when hypermethylated, is related to breast cancer relapse (Moalem).

While the aforementioned examples are contingent upon fixing methylation patterns that are not desirable, certain research shows that demethylation patterns may be naturally edited within an organism's lifetime. For example, Michael Meaney's team noted that rat pups that were given more attention after birth (in terms of getting licked more) exhibited a loss of methyl markers that would have prevented development of part of their brains, causing them to be more relaxed and able to handle stressful situations better than their siblings that were given less attention (Moalem). This idea is also supported by the accumulation of methyl groups on already methylated sites, as seen in disease progression.

V. Current Research

For a long time, the execution of the above discussion was purely theoretical; recently, however, researchers have been implementing techniques to engineer both the artificial methylation and demethylation that the two angles of "designing" genes would need.

Many researchers have attempted artificial methylation as a way to directly turn off genes that are functional and thus contribute to the final phenotype. Examples of the effect of simple measures on methylation patterns during prenatal development are clear in their ability to change phenotypic expression. Based on this idea, if scientists could target methylation of specific genes, they could directly and selectively choose genes to be turned off. At this point, no clear method of artificial methylation has been established. Nonetheless, there is evidence of viable methods on the rise. In my eyes, the most promising method at this point is the use of zinc fingers to direct methyltransferase to specific DNA binding sites that are of interest for methylation. This technique was executed by Brian Chaikind at the National Institutes of Health, where he found that using zinc fingers to flank the desired binding sites localized the methyltranferase—that was broken into fragments in an attempt to stop methylation at undesired regions—to the binding sites and greatly increased methylation at the target site relative to other attempts at artificial methylation (Chaikind). In fact, the results showed 50% to 60% methylation at the target site and nearly undetectable methylation at non-target sites (1.4% +/- 2.4%) (Chaikind). This experiment therefore shows great progress towards developing effective artificial methylation and ultimately, towards designing the genome.

In support of the demethylation approach, certain research has shown that demethylation could be a possible clinical tactic for disease recovery and phenotypic

manipulation. Many reports cite the use of enzymes that work specifically to demethylate. For example, the study on methylation at a single site in prostate cancer uses a low dose of 5-aza-2'-deoxycytidine (5-aza-dC) to reactivate gene expression—referred to as the "limited demethylation approach" (Zhang). However, the inability to direct the demethylation outside of a laboratory setting limits clinical use, despite the functionality of the enzymes.

VI. Conclusion

The main limiting factor of purposefully targeting methylation or demethylation exists in the fact that the scientific community is still unsure of how exactly methylation works. DNA methylation is highly nuanced and largely unknown in its operational mechanism. Some data shows that methylation may have channels through paternal lineages (Moalem). Other data shows that environmental factors affecting the offspring even before conception (Moalem). Contemporary research even shows that methylation may ultimately be secondary to histone methylation in terms of targeting gene silencing (Newell-Price). These points, despite not being proven, are divergent from previous hypotheses and signal the need to define the mechanism of methylation. Once the means by which DNA methylation functions is defined, new frontiers can be explored and the possibilities are endless. This is applicable and pertinent to everyone, considering that it would allow people a measure of control over their own genes and health—saving them from misfortunate genotypes.

Despite the limitations of methylation in terms of genetic engineering, current knowledge of DNA methylation still allows for great strides within the medical field and beyond. Mainly, it is useful in terms of tracking methylation to see disease progression

status. Many health centers are investing in technologies to track cancer growth through methylation concentration. For instance, in India, Reliance Life Sciences is working on a way to use the degree of methylation at known tumor suppressor genes to see how far an individual is from developing oral cancer (Moalem). In Berlin, a real-time PCR assay for DNA methylation has been shown to be successful in tracking even some of the lowest concentrations of methylated DNA and may be on its way to widespread use (Cottrell).

The possibilities for the realm of methylation are endless. DNA methylation studies have already sparked a new field of epigenetics for RNA methylation, which may be linked to energy homeostasis. Additionally, the patterns of methylation have helped informed our understanding of transposons in that transposable elements in hybrids tend to be highly methylated and that demethylation of transposons can potentially stimulate expression (Bird). All of these findings signal that, as a whole, DNA methylation needs to be explored further for it can provide answers as well as introduce powerful tools in the still budding science of epigenetics.

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