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Ageing And Cancer As Diseases Of Epigenesis

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Cancer and ageing are often said to be diseases of development. During the past fifty years, the genetic components of cancer and ageing have been intensely investigated since development, itself, was seen to be an epiphenomenon of the genome. However, as we have learned more about the expression of the genome, we find that differences in expression can be as important as differences in alleles. It is easier to inactivate a gene by methylation than by mutation, and given that appropriate methylation is essential for normal development, one can immediately see that diseases would result as a consequence of inappropriate epigenetic methylation. While first proposed by Boris Vanyushin in 1973, recent studies have confirmed that inappropriate methylation not only causes diseases, and it also may be the critical factor in ageing and cancers.

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1. Ageing as an epigenetic disease

There is now substantial evidence that many components of the normal ageing syndrome are due to the accumulation of errors in DNA methylation (*see* Fraga and Esteller 2007). Some of the first evidence for the roles of epigenetic methylation in ageing and diseases came from studies of identical twins. Human monozygous ("Identical") twins account for 1/250 live births. They arise from the same zygote and therefore have the same DNA. However, in many characteristics, there is a relatively high rate of discordance. That is, there are numerous instances where identical twins develop different diseases. These include some conditions that are seen early such as juvenile diabetes and autism, as well as those conditions that are seen later in life, such as ulcerative colitis and various cancers. There is no correlation between the age of onset and the concordance between twins (Petronis 2006).

The cause of this high discordance is not known, but recent evidence suggests that differences in DNA methylation may be involved. One possibility is that DNA methylation patterns can differ between twins, even though their DNA is identical. If a gene becomes methylated when it should not be, it will be turned off, just as if the DNA had been mutated. One loses function either way, and methylation is a much easier way to lose function. Similarly, anomalies arise if a gene becomes unmethylated and then becomes active in the wrong cells.

This appears to be the case for a pair of identical twin girls, where one twin had a severe anomy—a duplicated portion of the spine in the posterior portion of her body. Her phenotype reminded clinicians of a similar phenotype in mice, wherein the *Axin1* gene has been mutated. Axin is an inhibitor of the Wnt pathway in development, and in mice, mutations of the *Axin1* gene cause duplications of the caudal axis—extra spines (and bifurcated tails). Moreover, in mice, methylation of the *Axin1* promoter will repress the gene and prevent its from functioning, also giving an abnormal tail phenotype. Blood samples showed that although both twin girls had the same allele for AXIN1, there was significantly more methylation at this locus in the affected twin than in the unaffected twin or in the twins' parents (Oates *et al*. 2006). The regions around this gene showed no significant differences in methylation.

Most "identical" twins, however, start off life with very few differences in appearance or behaviours, but accumulate these differences with age. Experience counts, and both random events and lifestyles may be reflected in phenotypes. Mario Fraga and colleagues in Manel Estelle's laboratory in Madrid found that twin pairs were nearly indistinguishable

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in methylation patterns when young, but older monozygous twins exhibited very different patterns of methylation. This affected their gene expression patterns, such that older twin had different patterns of DNA expression, while younger twin pairs had very similar expression patterns. Monozygotic twin pairs start off with identical amounts of methylated DNA, Histone H4 acetylation, and Histone H3 acetylation (three epigenetic markers). However as the twins get older, methylation increases in both twins, but to different extents. Similarly, acetylation differences also increase.

Not only does the amount of methylation change as twins age, but so does the pattern of methylation. This can be shown by looking at specific genetic sequences where one cuts with enzymes that are sensitive to methyl groups on the cytosines. Thus, there are some enzymes that will cleave DNA at a sequence containing a C residue, but will not cut that sequence if the C is methylated. As twins age, the there is an increase in the discrepancies between the twin's DNA. Therefore, methylation differences may be critical in causing twins to phenotypically diverge as they grow older and to be discordant for different diseases, as well.

The idea that random epigenetic drift inactivates important genes without any particular environmental cue gives rise to an entirely new hypothesis of ageing. Instead of randomly accumulated mutations – which might be due to specific mutagens – we are at the mercy of chance accumulations of errors made by the DNA methylating and demethylating enzymes. Indeed, our DNA methylating enzymes, unlike the DNA polymerases, are prone to errors. DNA methyltransferases are not the most fastidious of enzymes. At each round of DNA replication, they must methylate the appropriate Cs and leave the other Cs unmethylated, and this is not always done properly.

This hypothesis of random epigenetic drift may have profound affects on physiology. For instance, the methylation of the promoter region of the estrogen receptors are known to increase with age (Issa *et al*. 1994). There is a linear relationship between the methylation of a promoter region in estrogen receptor gene with increased age. The methylation of the promoters of the genes for the alpha and beta estrogen receptors increases with age, resulting in the inactivation of this gene activation in the smooth muscles of the circulatory system. Moreover, methylation of the estrogen receptor genes is even more prominent in the atherosclerotic plaques that occlude the blood vessels. The atherosclerotic plaques showed more methylation of the estrogen receptor genes than did the tissue around it (Post *et al*. 1999; Kim *et al*. 2007) Thus, DNA methylation associated inactivation of the estrogen receptor genes in vascular tissue may play a role in atherogenesis and ageing of the vascular system. This potentially reversible defect may provide a new target for intervention in heart disease.

So we now have a new hypothesis for ageing. There appears to be random epigenetic drift that is not determined by the type of allele or any specific environmental factor. Random epigenetic drift may cause the ageing phenotype as different genes randomly get repressed or ectopically activated. Mistakes in the DNA methylation process accumulate with age, and may be responsible for the deterioration of our physiology and anatomy. If this is so, some genes may be more important than others. The estrogen receptors, for instance, are critical in vascular, skeletal, and muscular health.

2. Cancer

But there are some genes that are even more important to be properly regulated. These are the genes that prevent cancers. There are two types of genes involved in cancer production. The first are oncogenes. These are the genes that promote tumour formation and metastasis (spread of cancer cells throughout the body). They are the genes that promote cell division, reduce cell adhesion, and prevent cell death. The second set of genes are the tumour suppressor genes. These genes usually put breaks on cell division and increase the adhesion between cells. These genes also can cause apoptosis (cell death) of rapidly dividing cells.

These oncogenes and tumour suppressor genes have to be very finely regulated. In the stem cells of the skin, gut, and blood, for instance, there has to be a very carefully controlled rate of cell division. In the epithelial cells that make up your kidney and lungs, cell division is much rarer. Thus, one might get cancers if faulty methylation either inappropriately methylated the tumour suppressor genes or inappropriately demethylated the oncogenes.

In the breast, estrogen receptors can act as oncogenes for estrogen-dependent breast cancer. Moreover, in breast tissue, there is an age-related increase in the methylation of the promoter of the *RASSF1A* gene, whose product is a tumour suppressor. This methylation has been correlated with the risk of breast cancer (Euhus *et al*. 2008). In the colon, however, estrogen stops the proliferation of cells, and the estrogen receptors function as tumour-suppressor genes. Issa and colleagues (1994) showed that in addition to the age-associated methylation of the estrogen receptors, there was a much higher level of DNA methylation in the estrogen receptor genes in colon cancers. Even the smallest colon cancers had nearly 100% methylation of the estrogen receptor promotor. Moreover, microarrays (which detect differences in mRNA populations) identified numerous genes whose methylation patterns changed dramatically in colon cancer cells (Schuebel *et al*. 2007).

In addition to the age-related onset of tumours. DNA methylation can also explain the etiology (cause) of several lifestyle-related tumours. For instance, tobacco smoke is

known to cause cancer, but it is not a very powerful mutagen. Russo and colleagues (2005) and Liu and colleagues (2006) found that lung cancers caused by cigarette smoke had several tumour suppressor genes heavily methylated. These included the genes encoding cell adhesion proteins, apoptosis accelerators, and mitosis inhibitors. In addition, Liu and colleagues (2007) showed that the synuclein-gamma gene, a gene that is not normally expressed in lung tissues, is activated by cigarette smoke. This gene appears to be demethylated, and its activation promotes the spread of the tumour to other parts of the body.

The mechanism by which the synuclein-gamma gene is demethylated gives us an important clue as to how tumours can occur. The current model of tumour formation is a gene-centered model wherein a particular cell accumulates over a dozen new mutations. This seems probabilistically unlikely. But in the cancer model presented here, DNA methylation, not DNA mutation, causes the deficiency of proteins. The cause for the demethylation of synuclei-gamma appears to be the downregulation of a gene that encodes the methyltransferase enzyme DNMT3B, which usually adds methyl groups to DNA.

3. The reciprocity of epigenetic and genetic causation in cancer

The epigenetic causation of cancer does not exclude a genetic cause. Indeed, several studies indicate that these mechanisms augment one another. Numerous mutations occur in each cancer cell, and recent evidence suggests that as many as fourteen significant tumour-promoting mutations are found in each cancer cell (Sjöblom *et al*. 2006). Feinberg and colleagues (2006) have proposed that the first stage of tumour development is an epigenetic alteration of the DNA in a progenitor cell such that it will enable mutations to accumulate in other portions of the genome, and Jacinco and Esteller (2007) have shown that the large number of mutations that accumulate in cancer cells has an epigenetic cause. DNA has several means of protecting itself from mutations. One is the editing subunits on DNA polymerase which get rid of mismatched bases and insert the correct ones. Another mechanism is the set of DNA repair enzymes. These enzymes repair DNA that has been damaged by light or by cellular compounds that are products of metabolism. In cancer cells, the genes encoding these DNA repair enzymes appear to be susceptible to inactivation by methylation. Once these DNA repair enzymes have been downregulated, the number of mutations increases. Therefore, ageing and cancer may be linked in having the common denominator of aberrant DNA methylation. If metabolically or structurally important genes (such as the estrogen receptors) become heavily methylated, they do not produce enough receptor proteins, and our body functions more poorly. If tumour suppressor genes or the genes encoding DNA repair enzymes are heavily methylated, tumours can arise.

In this model of cancer development, genetic changes are not nearly as critical as the epigenetic changes. This provides an organizational framework for a great deal of data that have accumulated indicating that many cancers have normal genomes (reviewed in Sonnenschein and Soto 1999; Gilbert, 2006). Some cancer cells can actually revert back to being normal cells when put into appropriate environments or when given chemicals that cause them to differentiate (Stewart and Mintz 1981; Altucci and Gronemeyer 2001; Bissell *et al*. 2002). So these cancer cells have been thought to be epigenetically rather than genetically altered.

However, even as one finds that epigenetic mechanisms may cause mutations, one also finds that mutations can be the cause of epigenetic silencing. For instance, as many as 5% of all the genes in tumour cells might be hypermethylated (Schuebel *et al*. 2007). One of the most important oncogenes is the Ras small G-protein. This oncogene is known for its ability to initaiate signalling transduction cascades that phosphorylate certain transcription factors that promote tumourigenesis. However, Ras can also act by a second, epigenetic, cascade (Gazin *et al*. 2007). Through a pathway composed of at least 28 proteins (including the DNMT1 methyltransferase), activated RAS is able to silence the *Fas* gene that enables the rapidly dividing cells to commit apoptosis. The active Ras protein thereby recruits DNMT1 to the promoter of the *fas* gene, where it hypermethylates the promoter and stops its tumour-suppressor function.

Tumours can be generated by a combination of genetic and epigenetic means. Changes in DNA methylation can activate oncogenes and repress tumour-suppressor genes, thereby initiating tumour formation. Conversely, oncogenes can cause the methylation of tumour suppressor genes, which also aids in tumourigenesis. Moreover, the tissue environment of the cell may be critical in regulating these processes. This may be especially true in the stem cell niche, where ageing might allow the selection of oncogenic mutations (*see* Marusyk and DeGregori 2008). The complexities of tumours, including their multiple somatic mutations and there resistance to agents that induce apoptotic cell death, may best be explained by a combination of genetic and epigenetic factors more than just on the basis of mutations. Moreover, knowledge of the epigenetic causes of cancer can provide the basis for new methods of cancer therapy.

4. Conclusion

Ageing and cancer have epigenetic components wherein genes can be inappropriately activated or repressed by demethylation and methylation. Physiologically important genes, when repressed, can lead to the changes seen in ageing,

and areas of marked suppression have been associated with atherosclerotic plaques and other phenomena characteristic of the ageing syndrome. When epigenetic processes activate oncogenes or inactivate tumour suppressor genes, cancer can arise. The large number of mutations in tumour cells might emerge from previous epigenetic events that prevent DNA repair functions from taking place. These new insights can lead us to better preventive measures in public health and better treatments through drug design.

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