

Carcinogenesis:

When transmission of epigenetic information goes awry

Genes are blueprints that code for proteins and in turn, proteins drive most activities within our cells. But between genes and their protein products is the layer of epigenetics – genes may be expressed differently depending on the heritable, epigenetic features that switch them on and off. Charting new territories in carcinogenesis, Professor Patrick Riley at University College London explores the idea that cancers arise when inheritance of epigenetic information goes awry.

Although we all started as a single cell with a half-genome from each parent, that single cell quickly divided, new cells were created and became different from each other. This process of cell differentiation enables multicellular organisms to contain hundreds of different cell types, each capable of carrying out specialised functions. It was initially thought that these differences were the result of the loss of parts of genetic material from the fertilised egg. However, transplantation studies revealed that each cell expresses only a proportion of the genes in its total repertoire, and that the answer lies in the way that each cell deploys its genome. Epigenetics is the process by which cells designate some genes for use and others for storage. In essence, epigenetic changes allow cells to regulate their gene expression without permanently changing the genes themselves.

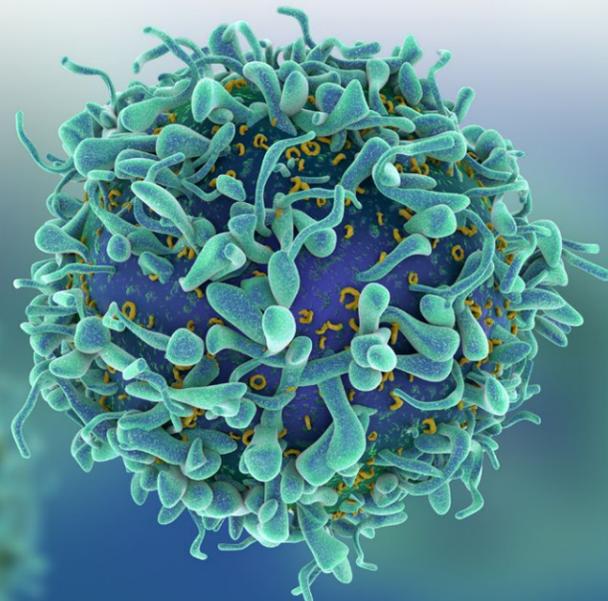
Pushing the boundaries of our knowledge of the fascinating field of epigenetics is Patrick Riley, Emeritus Professor of Cell

Pathology at University College London. Professor Riley suggests that defects in the somatic inheritance of epigenetic information offer an explanation for the development and characteristics of cancer.

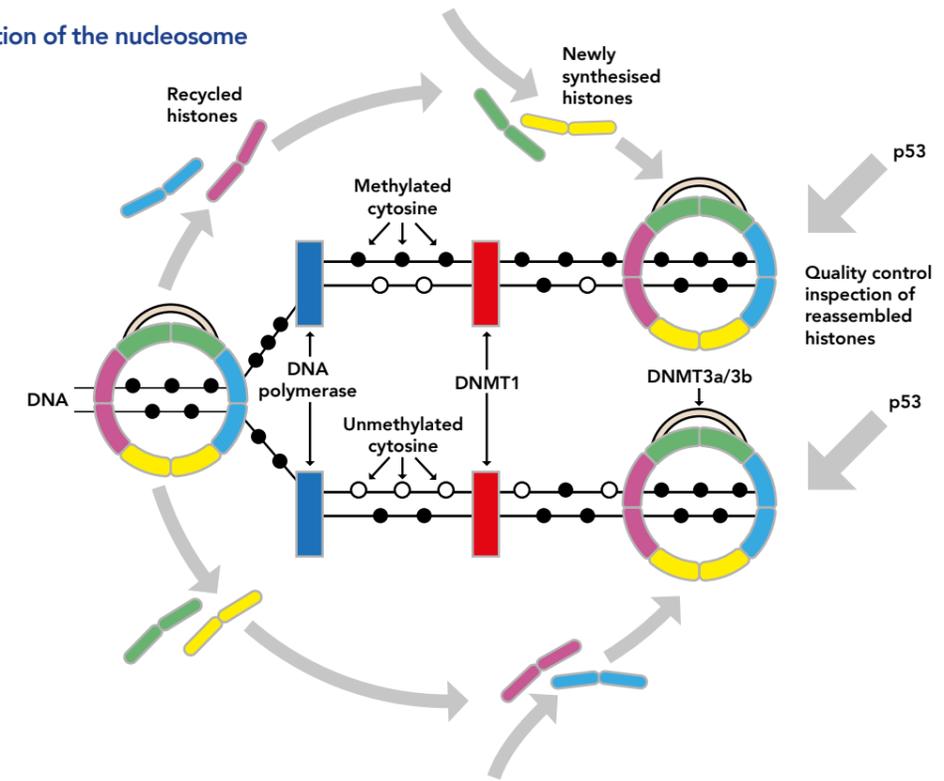
TURNING GENES ON AND OFF

The process of differentiation involves gene silencing and relies on small, reversible changes to the DNA complex that makes up chromosomes; these include DNA methylation and histone modification. Within a cell, segments of DNA are tightly packed around proteins called histones to form nucleosomes. In turn, nucleosomes are strung together to create chromatin, the fibre from which chromosomes are made. This tightly packed coiling provides a way of strictly controlling gene activity (or gene expression).

To activate a gene, the portion of DNA containing the gene must be accessible. Histones control how tightly the DNA is packed, and thus



Duplication of the nucleosome



Highly schematised view of the stages in the duplication of the nucleosome organisation. The double-stranded DNA on the left is shown with the pattern of methylated cytosines (●) associated with a set of histones forming the initial nucleosome structure with associated proteins (in this case DNMT3a and 3b). The histones are removed and the two strands of DNA separated. Each of the single strands of DNA is copied by DNA polymerase, with the newly-synthesised complementary strands containing unmethylated cytosines (○). DNMT1 attaches to the hemi-methylated DNA and methylates sufficient of the unmethylated cytosines to enable the DNA to reassemble with the initial set of histones (some recycled and some newly synthesised as indicated by the arrows). With the proper nucleosome structure reformed, the associated DNMT3 enzyme complex completes any residual cytosine methylation reconstituting the initial nucleosomal pattern and ready for inspection by the p53 system. Possible sources of error are: (1) faulty methylation by DNMT1; (2) faulty reconstitution of nucleosome through faulty synthesis, modification or selection of histones; (3) failure to complete cytosine methylation due to absence of attachment or malfunction of DNMT3a/3b; (4) failure of the quality control system to detect nucleosomal abnormality.

how readable the genetic information is. Loosely packed nucleosomes (as in euchromatin) allow the DNA to be transcribed and the genes expressed. In contrast, tightly packed nucleosomes (as in heterochromatin) prevent access of transcriptional machinery so that genes on these stretches of DNA are silenced. The major way in which genes are silenced involves the attachment of methyl groups to specific cytosine bases of the DNA molecule. This modification to part of the DNA determines the way that DNA associates with histones, consequently regulating which genes are able to be expressed. Epigenetic markers such as methyl groups keep cells on the straight and narrow – ensuring they divide in an orderly manner and remain true to their nature (e.g. that kidney cells remain kidney cells).

PRESERVING THE STATUS QUO
In order to perpetuate the correct pattern of gene expression it is essential that at each cell division (when DNA is replicated), specific methylation patterns are replicated too. That is, DNA methylation has to be accurately copied to the newly made strands of duplicated DNA (i.e. correct transmission

during embryonic development) or failure of expression of previously active genes.

ERROR-PRONE EPIGENETIC INHERITANCE

In a 2014 publication, Prof Riley proposed that carcinogenesis could be a result of a process involving defective transmission of epigenetic information, building on an idea first suggested in 1979 by Holliday¹. This concept is seen as a rational explanation for the main characteristics of cancer including the disturbances of chromosome structure and function, and the variable and abnormal patterns of gene expression resembling hypermutability.

Professor Riley suggests that defects in epigenetic inheritance offer an explanation for carcinogenesis.

of essential epigenetic information). Since the methylation pattern of DNA ultimately determines the chromosomal structure, any defective copying of DNA methylation could lead to altered chromosomal structure, resulting in inappropriate re-expression of previously silenced genes (such as those expressed

¹ Holliday R. (1979) A new theory of carcinogenesis. *Br J Cancer*; 40:513–522.

The crucial characteristic of malignant cells is abnormal migratory behaviour and as Prof Riley explains: "plants, whose cell walls preclude migration, do not get cancer. In animals, cell migration is an important feature of embryonic development but is strictly limited in the adult." He noted that any defective expression of migratory behaviour normally associated with development would be catastrophic, particularly if it were a result of unscheduled expression of normal genes, as this would go unrecognised by the immune system.

Prof Riley also observes that cancer does not occur in non-dividing cells, suggesting that the crucial genetic errors arise only during DNA replication, as would be the case if they resulted from failure of fidelity of epigenetic copying.

CONFIRMATORY OBSERVATIONS
Research by others is consistent with Prof Riley's conjecture. All cancers display abnormal DNA methylation patterns, they show variable and abnormal gene expression, as well as chromosomal anomalies. In addition, it has been shown that the likelihood of malignant change

Professor Riley suggests that an aberrant quality control system is an important factor in malignancy.

occurring in a tissue is related to the number of stem cells and proportional to the rate of stem cell growth and proliferation.

Interestingly, there are differences between the majority of adult cancers and cancers of childhood (developmental malignancies). However, they both are explicable within the concept of an epigenetic origin. Developmental cancers appear to arise from the failure of differentiation. As Prof Riley explains: "childhood cancers arise from a lack of initial DNA methylation necessary to silence certain genes which impose restricted genetic patterns on the tissues of the developing organism. In these cases, the genesis of malignant behaviour appears to rest on the failure to initiate the proper epigenetic pattern rather than in the failure to perpetuate it."



The key to carcinogenesis may lie, not in genetics, but in epigenetics.

POSSIBLE SOURCES OF ERROR-PRONE EPIGENETIC TRANSMISSION

Prof Riley's current research has focused on unpicking the processes that may be responsible for defective epigenetic transmission, in particular the faulty methylation of DNA. The addition of methyl groups to DNA is controlled at several different levels, and is carried out by a family of enzymes called DNA methyltransferases (DNMTs). One particular enzyme, DNMT1, is of crucial importance because it recognises hemi-

developing into tumours, and is the most common mutation detected in over 50% of cancers. Prof Riley puts forward the intriguing idea that the p53 system might be viewed as the 'guardian of the epigenome,' suggesting that one of its functions is to detect differences between the epigenomes of the division products of differentiated cells. Those that are detected as abnormal are earmarked for elimination by apoptosis (controlled cell death). In effect, p53 safeguards the accurate transfer of the epigenetic pattern, so that the differentiated pattern of gene expression and silencing is retained.

THE FUTURE: EPIGENETIC THERAPY?

If the proposed model of carcinogenesis is correct, the initiating lesions consist of mutations affecting one or more components of the epigenetic copying mechanism, and it is probably unrealistic to be able to reverse the defective process; but it may be possible to devise an agent able to sensitively detect abnormal post-division methylation patterns and thus enable the development of uniquely targeted cytotoxic therapy.

However, in view of the apparent importance of the gatekeeper function of p53, there may be scope for preventive treatment by the introduction of extra copies of the gene, especially in high-risk individuals. There is evidence that the comparatively low cancer incidence in elephants is due to multiple copies of the p53 gene.

methyated strands of DNA. During DNA replication in differentiated stem cells, the methylation pattern is maintained by a DNMT1 complex which methylates the newly-synthesised strand of DNA. Another important step involves the reassembly of nucleosomes and the incorporation of the correct histones. These reconstituted structures determine the associated enzyme complexes that are important in the replication of the correct methylation pattern (as indicated in Scheme 1). Clearly there are several steps in this copying process that may be rendered defective by initiating mutations, such as, for example, by a mutation affecting the activity of DNMT1.

QUALITY-CONTROL MECHANISM

A common defect in cancer cells involves inactivated p53. Acting as a 'safety net,' p53 prevents abnormal cells from



Behind the Research

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Research Objectives

Professor Riley, University College London, investigates the effect of epigenetic changes on carcinogenesis, the formation of cancer.

Detail

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Bio

Patrick Riley qualified in 1960 from UCHMS. He joined Claude Rimington's Department of Pathological Chemistry in 1966 and worked on free radical mechanisms in pathology, including lysosomal damage and photosensitisation with Trevor Slater. He was successful in cultivating melanocytes *in vitro* and collaborated with Peter Sutton on culturing cancer cells. He spent much of his academic career studying melanocytes and melanoma, and developed experimental approaches to melanoma therapy involving metabolically targeted pro-drugs activated by tyrosinase.

Collaborators

- Dr Mark Burkitt
- Professor Roger Dean
- Professor Charles Harding
- Professor Ferdy Lejeune
- Mr Brian Morgan
- Professor John Vince



References

- Riley P A. (2014) Failure of fidelity of vertical transmission of epigenetic patterning as the basis for cancer. *Melanoma Research*, 24: 424-427.
- Riley P A. (1985) *Pathological Migration: From Melanin to Malignancy*. University College London.
- Riley PA. (2018) Epimutation and Cancer: Carcinogenesis Viewed as Error-Prone Inheritance of Epigenetic Information. *J. Oncol.* 1-4.
- Riley P A. (1982) Is the establishment of a clone exhibiting defective DNA repair the initial stage of carcinogenesis? *Med. Hypotheses*, 9: 163-168.
- Riley P A. (2017) The Epigenetic Theory of Carcinogenesis: p53 as the Guardian of the Epigenome. *J. Oncol. Canc. Res.*, 1: 1-4.
- Riley P A. (2018) Cancer Incidence in Relation to Species Size: The Peto Paradox in the Light of the Theory of Epigenetic Carcinogenesis. *J. Med. Oncol.*, 1: 11-15.

Personal Response

Pharmaceuticals that reverse epigenetic changes have exciting potential for a variety of cancers. How far are we from making this a realisation?

Unfortunately, although epigenetic engineering has exciting potential in some areas, I do not see a significant future for it in cancer because (given that the proposed model is along the right lines) the fundamental mechanistic error will be repeated each time a cell from an affected clone divides – that is why the phenomenon is so devastating. Advances in genetic insertion might enable cancer to be eradicated by increasing the number of copies of p53 in the human genome, as suggested at the end of the article.

At present the most promising therapeutic advances seem to be in the field of immunology, and an interesting development might be to couple a powerful antigenic molecule to an agent capable of detecting anomalously methylated DNA, thus alerting the immune system to any epigenetically-defective cell.

