Cancer: Evidence Consistent with Epigenetic Carcinogenesis

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Abstract: This brief review outlines the accumulated evidence which favours a mechanism of cancer generation that is dependent on defective vertical transmission of the pattern of epigenetic control of genetic expression. This model is based on the initiating lesion involving the process that copies the epigenetic features when stem cells undergo mitosis.

Keywords: Malignant phenotype, abnormal transmigration, gene silencing, DNA methylation, defective epigenetic copying, initiation, promotion.

MALIGNANCY AND TRANSMIGRATION

The necessary and sufficient cellular properties that confer malignant status are proliferative capacity and the ability to transgress tissue boundaries. In animals such properties are normally present in embryonic cells and are essential for development of the organism. The structure and function of multicellular organisms depends on the generation and cooperative regulation of cell types brought about by differential activation of genes – an epigenetic process regulated by DNA methylation. In particular, the migratory behaviours of the component cells are expressed in a controlled manner and are switched off during the developmental process to yield the morphology of the fully formed organism. This morphological organisation requires the establishment of migratory boundaries that limit the range of movement of different classes of cells [1,2].

Given the requirement for cancer cells to exhibit anomalous migration there are three ways in which malignancy can arise:

1. Failure of epigenetic silencing of migratory genes during embryogenesis and tissue differentiation giving rise to developmental and childhood cancers. In most instances the details of the failure to activate the appropriate gene silencing is not clear but the basis of the phenomenon is demonstrated by the ability of normal embryonic development to normalise the malignant behaviour of teratocarcinoma cells [3].

2. In cell lineages that retain transmigratory capacity, such as leucocytes, fully differentiated white cells are prevented from proliferating outside the confines of their haemopoietic compartments. Failure to silence proliferative controls in cells with normal transmigratory ability may lead to haematological malignancies which differ in some essential respects from those of other cancers.

3. In adult tissues, the acquisition of transmigratory properties by proliferating stem cells will lead to cancer. This route accounts for the majority of human malignancies.

Confining attention to adult cancer, the process of acquisition of abnormal transmigratory property requires an explanation and one possibility is that it is the result of one or more somatic mutations affecting the expression of a set of genes involved in the control of cellular migration, as such resulting in effect in the re-expression of genes active during embryogenesis. However, given the relatively low mutation frequency in normal cells this might be expected to be a rare occurrence whilst there is a great deal of evidence to suggest that cancer cells and their precursors exhibit a high degree of genetic variability.

Diagnostic Features of Cancer Cells

Among the notable diagnostic features of cancer cells is their cytological abnormality which include pleomorphism, large nuclear size, increased nuclear to cytoplasmic ratio, nuclear hyperchromatism and prominent nucleoli, irregular chromatin distribution, karyotype instability, abnormal mitosis, abnormal cytoplasmic structures and lack of normal differentiation [4]. This is associated with chromosome instability (CIN) and a wide range of genetic abnormalities.

Such a scenario might be considered to have arisen by somatic evolution resulting from hypermutability [5]. But a raised mutation rate generating this range of structural and functional abnormalities would be expected to initiate an immune response to the multiple

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abnormal products. Yet a remarkable feature of cancer biology is that the immune system fails to react to the presence of grossly deranged cells [6] and special measures are necessary to elicit a response. On the other hand, if the abnormally expressed gene products were 'self' components it would account for the lack of an intrinsic immunogenic response.

Thus, an alternative explanation to account for the multiple abnormalities of cancer cells is that they arise from a defect in the control of the pattern of gene expression. This is the basis of the theory of epigenetic carcinogenesis [7,8].

DNA Methylation and Epigenetic Control

As adumbrated above, the process of development and differentiation involves the separation of embryonic cells into classes of cells that have different functions and properties. This process comes about by the activation and silencing of sets of genes. The gene silencing is brought about by an epigenetic mechanism that is based on the methylation of specific regions of DNA. The process involves the methylation of cytosine residues within CpG dinucleotides in specific regions of the genome which has profound effects on gene expression as proposed by Holliday & Pugh [9] and Riggs [10] and constitutes the basic mechanism for generating the different gene expression profiles essential for normal development.

In order to retain the established epigenetic pattern generated in each different cell type it is essential that a mechanism exists to ensure that when the cell divides the epigenetic pattern is transmitted to the mitotic progeny. It is the faithful somatic inheritance of this epigenetic maintenance mechanism that may be at risk.

In eukaryotes the basic unit of chromatin is the nucleosome which consists of 1.65turns of DNA wrapped round an octamer of histones that include two copies of the core histones H2A, H2B, H3and H4 [11]. During mitosis the structure of nucleosomes is broken down and the DNA released. After DNA replication has taken place the nucleosomal structure is reassembled. The reconstitution of the chromatin [12] is a complex process but in essence the reassembly of the nucleosomes is guided by the pattern of DNA methylation which is associated with certain histone modifications [13,14].

In order to perpetuate the correct pattern of gene expression the DNA methylation pattern has to be accurately copied to the newly replicated strand of DNA and this is carried out by a methylating enzyme (DNMT1) associated with the replisome which binds to hemi-methylated DNA [15] although there is evidence that some of the methylation process is completed after the nucleosome reassembly has taken place, involving DNMT3a and DNMT3b which are associated with the histone complex.

It is highly likely that, because of the importance of the fidelity of this process, that the accuracy of copying the epigenetic pattern is subject to some proof-reading mechanism and it has been suggested that the p53 - associated apoptosis mechanism performs such a function [16,17] and the high frequency with which p53 has been shown to be inactivated in cancer cells [18] is consistent with this proposal.

Epigenetic Carcinogenesis

Failure of fidelity in copying the DNA methylation pattern would lead to disturbance of the pattern of gene expression and could result in anomalous activation of migratory genes and thus lead to malignant behaviour of affected cells. Therefore, the essence of the theory of Epigenetic Carcinogenesis is that malignancy arises as a result of defective epigenetic copying. Moreover, since new errors will arise each time the affected cell undergoes mitosis the process will lead to the production of clones with diversifying structural and functional abnormalities and associated derangement of the chromatin architecture with resultant widespread chromosome instability CIN) [19]. Hence, this process of defective epigenetic copying provides a rational explanation for the diagnostic features of cancer.

Two-Stage Carcinogenesis

Assuming the origin of the defective epigenetic copying is the result of somatic mutation in an affected cell the process leading to malignancy can be viewed as a two-stage process consisting of two stages: the initiation step which is the result of somatic mutation and the promotion step which results from the defect induced by initiation.

1. The Initiation process of somatic mutation(s) generates the faulty epigenetic copying process. As several processes are involved in copying the methylation pattern there are several targets which include the methylation enzyme DNMT1 which is known to be essential since DNMT1-knockout animals fail to undergo differentiation [20]. In addition there is evidence that the p53-associated scrutiny is of great importance.

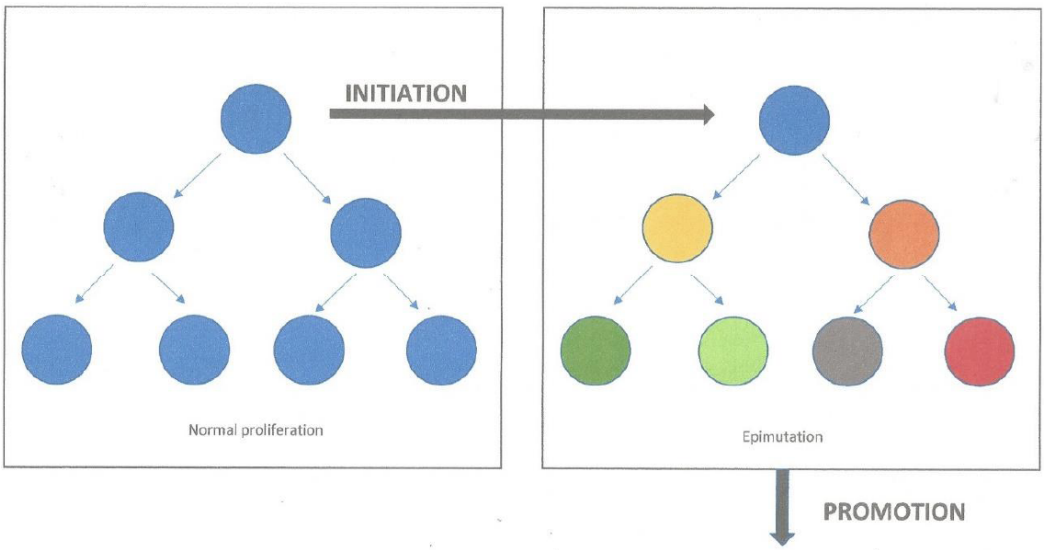

Figure 1: Schematic outline of the two-stage model of Epigenetic Carcinogenesis showing (on the left hand side) normal stem cell mitosis in which the pattern of gene expression (represented by the blue colour) is repeated at each division. The ***initiation*** process, resulting from mutation of the genes involved in the epigenetic copying process, gives rise to a cell from which the division products exhibit progressive gene expression abnormalities. This ***promotion*** step, in which there is progressive variation in gene expression pattern, is indicated by the different colours of the clonal products. The attainment of the ***malignant phenotype*** by expression of transmigratory characteristics is indicated by the red cell.

2. The Promotion stage comes about by the failure of the transfer of the epigenetic pattern in cells that bear the mutation(s) affecting the epigenetic copying. At each mitosis there is the propensity to produce new errors with the resultant genetic variability and clonal selection. The expression in the affected clonal offspring of the transmigratory genes gives rise to the malignant characteristics.

This set of events is summarised in the scheme illustrated in Figure **1**.

Given this scenario it is possible to derive a model of carcinogenesis [21] similar to that of Armstrong & Doll [22] in which the initiation rate for a tissue can be stated as:

$$I(t) = S(\mu t)^g$$

Where *I* is the number of cells having undergone the necessary initiating mutations, μ is the mutation rate, *t* the time and *g* represents the number of genes that need to be mutated to result in a cell displaying defective epigenetic copying, and *S* is the size of the stem cell population.

Turning to the process of progression of an initiated cell towards the malignant state, since the occurrence of epigenetic error in an initiated cell is confined to mitosis the probability of the incidence of malignancy will be a function of stem cell proliferation rate (*R*). Also, the overt malign behaviour of affected cells is dependent on the likelihood of the activation of transmigratory function and thus represents only a proportion of the epigenetically deranged cells (represented by *k*). Therefore, the equation for the cumulative frequency over time of the occurrence of malignant cells is given by:

$$M(t) = \frac{g!}{(g+2)!} SRk\mu^g t^{(g+2)}$$

Which can be utilised for comparison with lifetime risk of cancer for different tissues [23].

Clearly there are many factors in addition to the size and proliferation rate of the stem cells in question which need to be taken into account in the interpretation of this simplified model. This applies in particular to the number of susceptible genes giving rise to faulty epigenetic copying and their mutation rate, as well as the relative probability of the deranged pattern of genetic expression leading to overt malignant behaviour. Nevertheless, making the simplifying assumptions regarding metabolic constancy of these values it suggests that the estimated cancer risk for tissues would be a linear function of the stem cell population size and the mean proliferation rate which is consistent with the data of Tomasetti & Vogelstein [24]. Also, tissues in which mitosis is absent (such as CNS) or relatively rare (such as striated muscle) will not develop cancer, whereas tissues with high proliferation rates (such as epithelia) will have raised incidence of

malignancy as observed [25]. By a similar argument the cancer risk will be modified by factors that affect the stem cell proliferation rate, such as inflammation, hormones and age [26,27].

CONCLUSION

Given this coherent model of the process of carcinogenesis are there any implications for the diagnosis and therapeutic approach to cancer? If the significant initiating lesions affect the epigenetic DNA methylation processes by generating mutant versions of these enzymes then diagnostic tests capable of detecting these materials might be expected to be highly sensitive. Also the possibility that immunological agents specifically targeting cells exhibiting these abnormal components might prove to be highly effective.

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