
REMARKS ON EVOLUTIONARY AND CONCEPTUAL ASPECTS OF THE BASIC MECHANISM OF CARCINOGENESIS

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ABSTRACT. The evolution of metazoans including embryogenesis permits dysdedifferentiation processes that result in a neoplastic phenotype. Since in one study over fifty percent of carcinogens were found not to be mutagenic, other processes must be considered for any basic mechanisms of carcinogenesis. Selecting from potential mechanisms with special reference to epigenetic aspects and drawing from ethionine-induced embryonic gene experiments, it was concluded that direct epigenetic or mutation-perturbed epigenetic mechanism best fit the findings. A basic mechanism derived from this work is as follows. There is a preferential deheterochromatization by carcinogens of embryonic type genes. This is allowed by virtue of a differential type of heterochromatin created during embryogenesis that gives the neoplastic state.

KEY WORDS. Carcinogenesis, evolution, heterocromatin, epigenetic, mutagenesis, DNA-methylation, embryonic genes, ethionine, alpha-fetoprotein, S-adenosyl-L-methionine, dysdedifferentiation.

INTRODUCTION

The cancer cell appears to exist as the result of the evolution of metazoan developmental systems. With the need of embryogenesis (differentiation) comes the reverse potential mechanism of dedifferentiation (or dysdedifferentiation in carcinogenesis). Thus, for example, if embryonic growth factor genes, repressed during the attainment of the adult form, are derepressed, then these embryonic type genes will lead to the expression of an embryonic phenotype—in the case of carcinogenesis, a neoplastic cell.

Biophilosophically, cancer can therefore occur because of metazoan evolution with its accompanying reproductive mechanisms, namely fertilization and the production of embryos.

The basic mechanism of carcinogenesis can be thought of in terms of two major processes, mutagenesis and epigenesis.

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There are also arguments as to the competent cell type. For example, if the competent precursor cell is in a differentiated state and proceeds through a carcinogenic event, the process of dysdedifferentiation (implying an abnormal dedifferentiation) would then occur. However, if the precursor cell is, say, a stem cell, then carcinogenesis would involve dysdifferentiation. In other words, the biology of carcinogenesis would be interpreted, in the latter case, to proceed from a stem cell that is in a mode of differentiating, but becomes perturbed into a cancer cell.

MUTAGENESIS VS. EPIGENESIS

Most carcinogens are thought to be mutagens, however, this is hardly the case. Gold (Gold, *et al.*, 1993) have concluded that among rodent carcinogens, inducing tumors at multiple sites, 19 percent are not mutagenic and, if categorized as positive for inducing tumors at only a single site in one species, 58 percent of the carcinogens tested were not mutagenic.

It seems that there are two possible schemes. First, that mutational changes and epigenetic changes are two separate mechanisms basic to the carcinogenic transformation. Second, that the relevant change needs a transformation of the epigenetic process that then leads to the resultant neoplastic phenotype, even though there has been a separate mutational event (if there is a unified mechanism and all carcinogens are not mutagens). Although this would allow both mutational and epigenetic processes to be involved it is, nevertheless, possibly too restrictive. The following scheme is the most generalized.

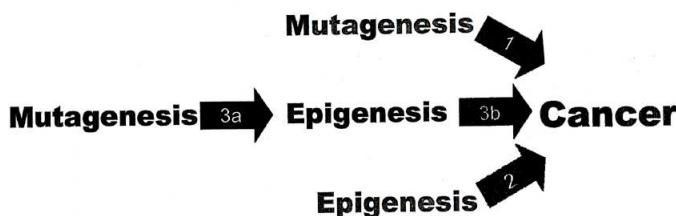


FIGURE 1

Possible pathways of mutagenic and epigenetic events involved in carcinogenesis.

If one uses all possibilities, the scheme would have the following categories: i) mutagenic (1), ii) epigenetic (2), and iii) mutation-perturbed epigenetic (3a, 3b). An epigenetically-induced mutational event (3b, 3a) is disallowed by definition, since epigenetics is defined as being a non-mutational event. However, having said this, one could derive the scenario in which, although an epigenetic event cannot cause a mutation, the mutation could have occurred prior to the epigenetic event as a discrete event. The difference between (3a, 3b) and (3b, 3a) then becomes one of semantics, i.e., how far away temporally can one part of the mechanism be from another required process before it can be considered as only one mechanism.

One could narrow the scheme by suggesting that an epigenetic event needs to be perturbed in order to present with an anomalous situation. Therefore, the final scheme would eliminate number ii) above and leave only i) or iii). If one believes there is only one primary mechanism of carcinogenesis, then it would require the number iii) pathway.

ADDING THE EPIGENETIC PROCESS

A classic example of a mutagenic carcinogen is some alkylating agent that reacts, for example, on N-7 of guanine causing in the bond rearrangement a loss of purine from the deoxyribosyl moiety of a deoxyribonucleotide residue. If this mutational event happens to a critical regulatory gene, such as the p53 tumor suppressor gene, causing the tumor suppressor to be dysfunctional or some mutation in a regulatory portion of the c-myc growth factor gene that disallows its down-regulation, then this would lead or contribute to a progression towards carcinogenesis.

One can add the epigenetic process to this mutational event. As suggested above, a critical mutation might be for a DNA methylase leading to an anomalous epigenetic process. If one administers ethionine, the ethyl derivative of methionine, to rats they develop hepatomas. One of the reactions of ethionine is with ATP to produce S-adenosyl-L-ethionine which inhibits the normal methionine reaction with ATP by ATP:L-methionine S-adenosyl transferase that in turn produces the active methyl groups as S-adenosyl-L-methionine, used by DNA methylases for DNA methylation, an thereby involving epigenetic processes.

One would hypothesize that the inhibition of S-adenosyl-L-methionine synthesis by S-adenosyl-L-ethionine would favor hypomethylation of DNA and, furthermore, such hypomethylations have been, in general, associated with inducing gene activity. If such a hypomethylated DNA of a promoter region of an oncogene occurred, a resultant inappropriately activated growth factor synthesis would be induced.

Ethionine, which is an inhibitor of protein synthesis, surprisingly induces new tRNA methylase activities in adult mouse liver (Hancock, 1968). Fetal liver has high tRNA methylase activity as compared with an adult mouse liver (Hancock, *et al.*, 1967). Therefore the carcinogen-induced activity in adult liver is comparable to that found in the embryonic stages. If one examines a bona fida embryonic type gene activity, namely the gene for alpha-fetoprotein, after only 72 hours of ethionine treatment, this embryonic gene activity could be detected by a radio-immuno-assay and this activity rapidly increased thereafter (Hancock, *et al.*, 1976). Furthermore, this increased gene activity could be down-regulated by the administration of methionine (Forrester and Hancock, 1978) supposedly by increasing the pool concentration of S-adenosyl-L-methionine, allowing normal states of methylation of the alpha-fetoprotein gene to occur.

By theoretical considerations, oncogenes can be considered to be in the set of embryonic type genes, i.e., not being housekeeping or adult state genes. Active embryonic genes, thereby, are those genes active at specific embryonic states. Some embryonic genes would remain in a functional mode in the adult state to be used by certain fast growing tissues such as those involved in erythropoiesis. A recent concept is that such genes are 'leaky' in the adult state for many adult cell types (Ruddon, 1995).

Next is the question of how embryonic genes, which are down-regulated during development, are preferentially up-regulated by carcinogens. This concept is important to those that ascribe to the general principle of the embryonic phenotype as being dominant in cancer cells.

A major process of differential gene control is heterochromatization and deheterochromatization of euchromatin, especially via acetylation, methylation and phosphorylation states of histones. Conceptually, heterochromatin can be placed into two categories—facultative and constitutive.

Constitutive heterochromatin is considered to contain many of the genes that occurred and were more active in the evolutionary background of the species in question. For example, humans have evolved from primitive vertebrates such as the shark, but we have permanently repressed many of these genes while retaining others in an active state, e.g., hox genes involved in segmentation, somite formation, in vertebrates. Interestingly, we have retained genes for gill slit formation only during embryogenesis.

Facultative heterochromatin, on the other hand, is able to be altered in conformation, enabling genes to be turned "on" (accessible to RNA polymerase action, etc.) and "off" via various control processes during the lifetime of the organism.

If there were a differential aspect to the heterochromatic state, e.g., specific embryonic constructs that are formed during the differentiation

period, then such chromatin would give a basis for being preferentially altered by carcinogen-perturbed DNA methylation. This would answer the question of how embryonic genes are uniquely susceptible to carcinogens and thus allow for the overall process of carcinogen-induced embryonic gene activities.

There remains an important consideration. How do promoters become hyopomethylated, if the carcinogen does not perturb S-adenosyl-methionine? Consider a mechanism for dimethylaminoazobenzene. This is not a methylating agent, but if it caused a change in the specific chromatin structure to alter heterochromatin involved in a regulatory segment of an oncogenic promoter region, then it would accomplish a similar result that could be considered an epigenetic change or this, in turn, may cause a secondary epigenetic effect by altering DNA maintenance methylation of a critical DNA region.

SUMMARY

The evolution of epigenetic systems that are used during embryogenesis have allowed for an anomalous process, that of dedifferentiation (or dysdedifferentiation) to occur that results in an abnormal embryonic phenotype termed cancer. In conclusion, the basic mechanism of carcinogenesis, conceptually speaking, can be stated as follows. Carcinogenesis is an induced preferential deheterochromatization of certain embryonic genes crucial to the neoplastic phenotype (oncogenes) by carcinogens, allowing epigenetic hypomethylation of newly exposed promoters to occur. These hypomethylated promoters are maintained in their particular methylation states by DNA maintenance methylases. In other words, these cells would have this new methylation pattern continued, thus stabilizing the neoplastic condition.

These scientific ideas lead to biophilosophical interpretations. If the arrow of evolution has selectively evolved embryonic replicative processes, then these existent genic systems can become perturbed and cause disadvantageous (abnormal) states. Embryogenesis, itself, can be thought of as a non-fission process of replication for complex metazoans. Indeed we find that these developmental embryonic gene states are not only selected by evolutionary processes but are retained—hence, ontogeny recapitulates phylogeny.

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