Free Rad. Res. Comms., Vol. 11, Nos. 1-3, pp. 59-63
Reprints available directly from the publisher
Photocopying permitted by license only

IS THE INITIAL EVENT IN CARCINOGENESIS AN ENHANCEMENT OF THE MUTATION RATE?

P.A. RILEY

Dept Chemical Pathology, UCM School of Medicine, Cleveland Street, London W1P 6DB, UK

(Received December 15, 1989; in final form, March 28, 1990)

The age-specific incidence rates of adult cancer indicate that the carcinogenic process is a power function of elapsed time. If the malignant clone arises by a series of mutations, and each mutation is regarded as an independent event with a small instantaneous probability of occurrence, then the slope of the age-specific incidence gives an indication of the number of genes involved. In most cases, the epidemiological data exhibit an age-specific incidence the slope of which is between a fourth and a seventh power of age. Assuming that the mutations involved are deletions and must occur in otherwise viable and proliferative cells, the mutation rate required to generate enough mutant cells to fit the cancer incidence data must be remarkably high in the pre-malignant cell population. The initiation process may thus be an event that results in a raised mutation rate in the affected cell and its progeny. It is proposed that the process of induction involves one or more mutations that induce a metabolic lesion that has the effect of increasing the subsequent mutation rate in the affected clone. A possible mechanism involving free radical generation is suggested and some of the biological implications of the proposal examined.

KEY WORDS: Cancer, carcinogenesis, induction, mutation, pre-malignant, malignant, probability, incidence.

MULTI-STEP CARCINOGENESIS

There is good evidence that cancer is clonal in origin and it is almost universally agreed that the malignant phenotype arises by a series of mutations. From the age-specific incidence of the majority of human cancers it can be deduced that at least four, and possibly as many as seven mutations are required to transform a normal into a malignant cell. This process can be envisaged as a series of transitions between compartments, where the transition between compartments is a function of the mutation rates. A general description[1] of the process of carcinogenesis can be devised based on such a multicompartmental model which is capable of embracing complexities such as the effects of proliferation of, and loss from, compartments. However, such models are somewhat intractable because of the large number of variables. In the treatment outlined below the simplifying assumption is made that the size of the cell population at risk is in a steady state. The time-dependent cumulative probability of a cell having progressed as far as the final (malignant) compartment is calculated on this basis.

THE PROBLEM OF THE MUTATION RATE

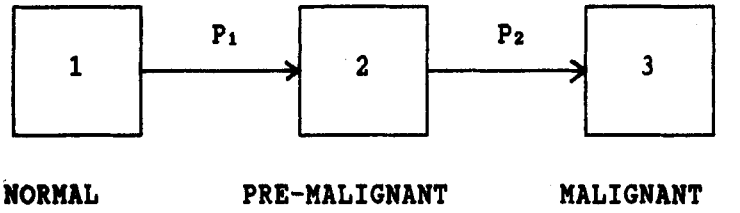
When such calculations are made for multicompartmental models with between 5 and 7 stages it emerges that there is a limited set of mutation rates that will generate

age-dependent cumulative probability curves that are compatible with the known cancer incidence data. In all cases the average mutation rate for each cell at risk is in excess of 10^{-4} mutations per gene per year.

This value is two orders of magnitude higher than the accepted highest estimate of the rate of mutation in normal cells which is 10^{-6} per gene per annum.

TWO STAGE MODEL

To overcome the problem of the high mutation rate it is proposed here that the initial mutation(s) in the series leading to malignant transformation increase the mutation rate for subsequent transfers. This sequence may be envisaged in the form of a 3-compartment model:



The first transfer is subject to the normal mutation frequency ($< 10^{-6}$), but cells having undergone the initial transfer to stage 2 ("pre-malignancy") exhibit a raised mutation rate for the remaining independent steps leading to transference to stage 3 (malignancy).

CUMULATIVE PROBABILITY

Calculations have been made on the basis of this 3-compartment model. The cumulative probability of a cell having reached the malignant state by time t is given by the integral of the products of the individual transfer probabilities for successive periods of time x and $(t\text{-}x)$ respectively, the first and second transfer probabilities being described by:

$$p_1(x) = 1 - \exp(-(\mu_1 x^{g1})$$

$$p_2(t - x) = 1 - \exp(-(\mu_2(t\text{-}x))^{g2})$$

where μ_1, μ_2 and g_1, g_2 are respectively the mutation rates and the number of genes affected for each transfer. Thus, the cumulative probability of becoming malignant by time t is:

$$P_{(t)} = \int_0^t p_1(x)\, p_2(t\text{-}x) \cdot dx$$

Numerical solution of this equation generates curves of the kind shown in Figure 1 which are isomorphic with those obtained from an equivalent steady-state multi-compartmental model (unpublished work).

RESULTS

For the age range 30–80 years the model generates cumulative probabilities of malignant transformation in a single cell in the range 10^{-18} to 10^{-13} as illustrated in Figure ·1. The assumptions generating curve A are: $\mu_1 = 10^{-9}$; $g_1 = 1$; $\mu_2 = 1.5 \times 10^{-4}$, $g_2 = 4$. Curve B illustrates the solution when $\mu_1 = 10^{-6}$; $g_1 = 2$; $\mu_2 = 10^{-2}$; $g_2 = 4$.

Comparison of the model-derived single-cell probabilities with cumulative cancer incidence data calculated by the method of Day[2] from age-specific incidence rates quoted by Burch[3], Mould[4], and Doll and Peto[5], give (with few exceptions) nearly constant ratios which are compatible with the estimated total numbers (N) of cells at risk. Thus, for total male cancers the factor (N) is in the range of 1.6×10^{15} which is the estimated total number of cells at risk in the body. Similar considerations apply to the data for lung cancer ($N = 5 \times 10^{12}$), stomach cancer ($N = 5 \times 10^{13}$), and

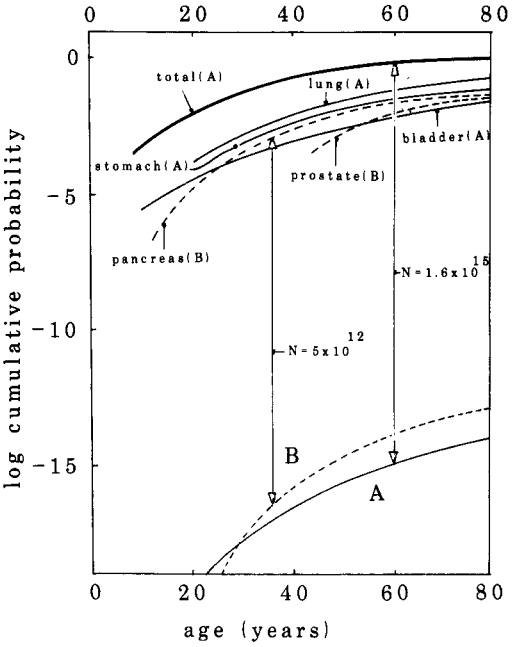


FIGURE 1 *Comparison of calculated cumulative probability and cancer incidence data.* The curves show the logarithm of the cumulative probability (or incidence) at ages between 0 and 80 years (abcissa). Two theoretical curves (A and B) are shown for comparison with selected incidence data for cancer in males. The theoretical curves represent the probability of malignancy arising in a single cell and are derived as described in the text using different assumptions for the number of initiation genes ($g_1 = 1$ for curve A, and $g_1 = 2$ for curve B). In each case the mutation rates involved in the first and second transfers (μ_1 and μ_2 respectively) were chosen to generate curves that fit the incidence data (i.e. for A: $\mu_1 = 10^{-9}$; $\mu_2 = 1.5 \times 10^{-4}$; and for B: $\mu_1 = 10^{-6}$; $\mu_2 = 10^{-3}$). The incidence data correspond to the product of the single cell probability and the number of cells at risk. The number of cells at risk (N) is indicated in two cases by vertical lines e.g. $N = 5 \times 10^{12}$ cells for the pancreas (follows curve B) and $N = 1.6 \times 10^{15}$ cells for all organs affected (approximates to curve A). The corresponding N values for other organs illustrated are given in the text.

bladder cancer ($N = 5 \times 10^{11}$) which reflect curve A. The incidence data for carcinoma of the prostate and carcinoma of the pancreas ($N = 5 \times 10^{12}$) resemble more closely curve B (see Figure 1).

DISCUSSION

It appears that a description of multi-step carcinogenesis in two stages in which the initial stage results in an enhanced mutation rate for subsequent steps is consistent with human cancer incidence data. Moreover, the mutation rate for the first step(s) remains in the accepted normal range.

Mechanisms which give rise to an enhanced mutation rate could include DNA repair deficiencies (c.f. 6) or defective cellular detoxification (e.g. reduced antioxidant defences) increasing the vulnerability of affected cells to external mutagenic agents. The lack of specific vulnerability to extrinsic mutagens, which might be expected from the former (e.g. as in the case of the specific UV-sensitivity of patients with xeroderma pigmentosum), and evidence that the antioxidant and detoxifying capacity of malignant cells is enhanced rather than diminished, tends to favour the alternative hypothesis – that of a metabolic disturbance resulting in the *intrinsic generation of mutagenic species.*

Such a process could involve free radical mechanisms. We are currently investigating the possibility that an increased intrinsic mutation rate results from an initiating event which interferes with cellular electron transport pathways giving rise to increased generation of potentially mutagenic species such as superoxide.

Although the process of induction envisaged is one that is brought about by somatic mutation, it might be anticipated that similar abnormalities could be inherited through the germ-line, possibly as a heterozygous trait. A possible candidate in this category is Bloom's syndrome. This hereditary chromosomal breakage syndrome is associated with a high cancer incidence of early onset (57 malignancies in a total of 130 affected infividuals at a mean age of 24.7 years) and is accompanied by an increased *in vivo* mutation rate.[7,8] Oxygen-derived free radicals seem to be involved, since superoxide dismutase has a protective action.[9,10]

The establishment of a population of cells with a raised intrinsic mutation rate will result rapid divergent cellular evolution with a raised death rate in cells that have acquired deleterious mutations. This implies that cells which have undergone the series of mutations necessary for the expression of the malignant phenotype will comprise a small proportion of the initial premalignant population consisting of cells which have evolved biochemical mechanisms that aid survival. Clearly, this subsumes a wide category of secondary phenotypic characteristics and the implications for early diagnosis and treatment of a metabolically distinguishable pre-malignant sub-population of cells are very wide-ranging.

This evolutionary approach to the development of malignancy outlined above for pre-malignant cell populations is entirely consistent with the widely accepted ideas of tumor progression in established malignancies.[11,12]

Acknowledgements

I thank the Association for International Cancer Research for financial support. I am indebted to Dr S. Gallivan and Professor T.F. Slater for helpful discussions.

References

1. P.A. Riley (1980) A theory of cellular senescence based on Darwinian principles in the light of Linnaeus. *Linn. Society Symposium,* **9**, 111–126.
2. N.E. Day (1982) *Cancer Incidence in Five Continents.* IARC, Lyon. Vol IV pp. 668–670.
3. P.J.R. Burch (1976). *The Biology of Cancer: a new approach,* MTP, Lancaster.
4. R.F. Mould (1983) *Cancer Statistics.* Adam Hilger, Bristol.
5. R. Doll and R. Peto (1981) *The Causes of Cancer,* Oxford University Press, Oxford.
6. P.A. Riley (1982). Is the establishment of a clone exhibiting defective DNA repair the initial stage of carcinogenesis? *Medical Hypotheses,* **9**, 163–168.
7. M. Vijaylaxmi, H.J. Evans, J.H. Ray and J. German (1983) Bloom's syndrome: evidence for an increased mutation frequency *in vivo. Science,* **221** 851–853.
8. R.G. Langlois, W.L. Bigbee, R.H. Jensen and J. German (1980) Evidence for increased *in vivo* mutation and somatic recombination in Bloom's syndrome. *Proceedings at the National Academy of Sciences USA,* **86,** 670–674.
9. I. Emerit and P. Cerutti (1981) Clastogenic activity from Bloom's syndrome fibroblast cultures. *Proceedings at the National Academy of Sciences USA,* **78,** 1868–1872.
10. I. Emerit, P.A. Cerutti, A. Levy and P. Jalbert (1982) Chromosome breakage factor in the plasma of two Bloom's syndrome patients. *Human Genetics,* **61,** 65–67.
11. Foulds L. (1954) The experimental study of tumour progression: a review. *Cancer Research,* **14,** 327–339.
12. Nowell P.C. (1974) in *Chromosomes and Cancer* (Ed. J German). Wiley, New York, 267

Accepted by Prof. H. Sies