

Spontaneous mutation rate in retinoblastoma

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Abstract

A novel approach was used to estimate the in vivo mutation rate of the retinoblastoma gene. A mathematical formula can be used to calculate the probability of neoplasia induced by one or more mutations in a population of dividing cells. This formula can then be applied to epidemiological data on hereditary and sporadic retinoblastoma. The analysis yields an estimate of the in vivo mutation rate of 8×10^{-8} /gene/cell division (range 5.5×10^{-8} to 1.3×10^{-7}). The estimated non-replication association in vivo mutation rate is 2.4×10^{-8} /gene/year (range 0 to 6.8×10^{-8}). The formula is an improvement on previous attempts to produce a model of the process of mutation during cell generation. It can be applied to neoplastic disease in both children and adults.

When cells divide there is a finite chance of genetic damage even in the absence of environmental carcinogens. This spontaneous mutation rate is of the order of 5×10^{-8} to 5×10^{-6} /gene/cell generation. As there are about 7×10^{15} cell divisions in a lifetime¹ spontaneous mutation could make an important contribution to the development of cancer in man.² This idea has been explored by Den Otter *et al.*^{3,4} They attempted to calculate the incidence of so-called endogenous malignancy produced by spontaneous mutation in a population of dividing cells. Unfortunately, the mathematical formula they used was incorrect. More recently an improved mathematical formula was published and applied specifically to the incidence of acute lymphoblastic leukaemia (ALL).⁵ The result of that analysis is consistent with the concept that most cases of childhood ALL arise as a result of spontaneous mutation.

Unfortunately, there are no completely reliable methods for measuring the rate of in vivo mutation. Instead, it is inferred in a number of indirect ways. The incidence of sporadic genetic disease in the population gives an estimate of the mutation rate/locus/generation of between 1×10^{-6} and 1×10^{-4} ,³ but this is influenced by selection and parental age, and needs to be corrected because there are many cell generations in one human generation. The number of lymphocytes in peripheral blood with a mutation of the X linked hypoxanthine phosphoribosyl transferase (HRPT) locus can be determined by selective in vitro culture techniques.⁶ The measured mean mutant

incidence in these assays is about 5×10^{-6} ,^{7,8} but selection may occur in vivo, there are several variables in the in vitro assay, and each lymphocyte is the product of a large number of sequential cell divisions from the zygote. The rate of cell mutation can be measured in vitro using log phase cultures.⁹⁻¹¹ These experiments yield estimates of the mutation rate/gene/cell generation in normal cells of 5×10^{-8} to 5×10^{-6} depending on cell type, the culture conditions, and the indicator gene.

A mathematical formula

This formula has been published elsewhere.⁵ If a single cell divides for x generations, without cell loss, there will be 2^x cells in the final generation. If the mutation rate per gene per cell generation is m then the probability that any single cell in the final generation has undergone a specific mutation is mx . The probability that the cell has undergone n independent specific mutations is $(mx)^n$. If n mutations are required for malignancy the number of malignant clones arising as a result of the last round of cell divisions is:

$$[(mx)^n - [m(x-1)]^n]2^x \quad 1$$

The total number of malignant clones (N) is derived by summing over all x generations—that is,

$$N = \sum_{x=1}^{x=x} [(mx)^n - [m(x-1)]^n]2^x \quad 2$$

If there is cell loss in the process then less than 2^x cells will be produced in the final generation and,

$$N = \sum_{x=b}^{x=x} [(mx)^n - [m(x-1)]^n]2^{x-b} \quad 3$$

where 2^{x-b} is the number of cells in the final generation.

It is worth noting that the number of mutant cells in the x th generation has a skewed distribution. The mean of the distribution is specified by mx , but the median of the distribution is less than mx .

Epidemiological and kinetic data

There are about 7×10^{15} cell divisions in a human lifetime.¹ As all cells are derived ultimately from a single cell this means there must be at least 53 generations to produce the fully differentiated cells of the body. In fact, some degree of cell loss is inevitable in this process, and it has been estimated that there are

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55 to 60 cell generations in a lifetime,³ the upper limit of 60 having been deduced from other data.¹² In each human eye there are between 11×10^7 and 13×10^7 rods, about 7×10^6 cones, and 1×10^6 ganglion cells.¹³ These are fully differentiated cells, presumably produced after 55 to 60 cell generations from the zygote. Their cellular precursors are the pool from which retinoblastomas arise.

The incidence of retinoblastoma is 5×10^{-5} /livebirth.^{14,15} This shows little geographic variation, which in itself is evidence in favour of the idea that the tumour arises as a consequence of spontaneous mutation. Of these cases, 35–45% result from a germinal mutation and 55–65% from two somatic mutations.^{14,15} All cases resulting from two somatic mutations are unilateral. Of those who inherit one defective gene, 1–10% are unaffected, 25–40% have unilateral disease, and 65–70% have bilateral disease. The new mutation rate per locus per human generation is 5×10^{-6} .

The mutation rate

In hereditary retinoblastoma the second mutation is a random event and the number of malignant clones in each eye will follow a Poisson distribution. Thus it is possible to infer from the observed distribution of unilateral, bilateral, and no tumour cases (table) that the mean number of tumours in each eye (u) is between 1.7 and 1.8. Thus from equation 3:

$$2m 2^{x-b} = u \quad - 4$$

The incidence of retinoblastoma is 5×10^{-5} /livebirth and about 55–65% of these cases are due to somatic mutations. Thus the expected number of tumours per eye (h) due to somatic mutation (two mutations) is about 1.5×10^{-5} .

From equation 3:

$$\sum_{x=1}^{x=x} [(mx)^2 - [m(x-1)]^2] 2^{x-b} = h - 5$$

Combining equations 4 and 5 and simplifying:

$$m = \frac{2h}{(4x - 6) u}$$

If $x = 55$, $u = 1.75$, and $h = 1.5 \times 10^{-5}$ then $m = 8 \times 10^{-8}$ and $2^{x-b} = 1.09 \times 10^7$.

If x is in the range 50 to 60, u from 1.6 to 1.9, and h between 1.25×10^{-5} and 1.95×10^{-5} then m will be between 5.5×10^{-8} and 1.23×10^{-7} , while 2^{x-b} will be between 6.5×10^6 and 1.7×10^7 .

If $m = 8 \times 10^{-8}$ and $x = 55$ then the mean mutant number of cells per locus per human

generation due to mutations occurring at mitosis would be 4.4×10^{-6} . This is close to the observed rate of 5×10^{-6} . If the difference is due to mutations occurring between cell divisions then the non-replication associated mutation rate, assuming that a human generation is 25 years, is 2.4×10^{-8} /gene/year (range 0 to 6.8×10^{-8} ; if x , u , and h lie in the ranges given above).

Discussion

Mutations in oncogenes or anti-oncogenes induced spontaneously or by environmental mutagens are central to the process of carcinogenesis.¹⁶⁻¹⁸ It is therefore important to determine the mutation rate in vivo. The incidence of sporadic genetic disease varies between 1×10^{-6} and 1×10^{-4} with a mean of 2×10^{-5} .³ If there are 55 cell generations per human generation then the mutation rate per cell generation would be of the order of 4×10^{-7} ($2 \times 10^{-5} \div 55$) if it could be assumed that genetic damage only occurred at mitosis. But this is not the case because the incidence of genetic disease rises with parental age. A second problem is that many mutations are deleterious so that selection occurs and the mutant frequency might underestimate the mutation rate. The frequency of mutant cells produced in vivo can be measured using selective in vitro culture techniques.⁶⁻⁸ For instance, the incidence of six thioguanine resistant lymphocytes, a consequence of in vivo mutation at the X linked HRPT gene, in peripheral blood is $1.3 - 9.3 \times 10^{-6}$.⁸ If one allows for 55 cell generations in a human generation this gives a crude mutation rate/gene/cell generation of 1×10^{-7} ($5 \times 10^{-6} \div 55$), but there are several problems with this approach. These include possible in vivo selection, a lack of information about the ratio of replication associated and non-replication associated mutations, and problems with the in vitro assay. The last includes the fact that the cloning efficiency of lymphocytes in culture is variable, and there is the possibility of both cooperation and inhibition between mutant and non-mutant cells in tissue culture media. The final problem relates to the highly skewed nature of the mutant frequency distribution curve. The mean mutant frequency after 55 generations is 55 m , but the median is much less. Thus the measured mean from a relatively small sample of cases will be below the true mean.

The cell mutation rate can be measured in vitro using cell culture techniques. These use the procedure initially described by Luria and Delbruck for measuring the mutation rate in bacteria.¹⁹ Cells are grown in log phase culture. The number of cells produced is counted and the incidence of mutants is determined using selective culture media as described above. It is then possible to calculate the mutation rate per unit time, where the unit of time is equal to the length of cell cycle in log phase culture. The method does not allow the replication associated mutations and those occurring in other phases of the cell cycle to be measured

Observed distribution of unilateral, bilateral, and no tumour cases of retinoblastoma in patients who have inherited one defective gene, and that expected for varying value of u

Observed	Expected ($u =$ mean number of tumours per eye)				
	$u = 1.6$	$u = 1.7$	$u = 1.8$	$u = 1.9$	
No tumour	1-10%	4%	3.3%	2.7%	2.2%
Unilateral disease	25-40%	32.3%	29.9%	27.6%	25.5%
Bilateral disease	65-70%	63.7%	66.8%	69.7%	72.3%

separately. The second problem is that conditions in log phase culture do not mirror those in vivo in which there are variable intervals between mitoses. The technique has been used with fibroblasts and lymphocytes using the indicator genes HPRT and ouabain resistance.^{9-11 20} The mutation rate has been variously determined between 5×10^{-8} and 5×10^{-6} .

We used a novel approach to calculate the mutation rate in vivo. The method uses a formula for calculating the probability of neoplasia occurring as a consequence of mutation during cell proliferation. This is combined with the extensive epidemiological data on retinoblastoma to produce simultaneous equations which can be solved to yield an estimate of the chance of mutation of the retinoblastoma gene during a single mitosis. The estimate is 8×10^{-8} with a range of 5.5×10^{-8} to 1.23×10^{-7} . The non-replication associated mutation rate was estimated at 2.4×10^{-8} per gene per annum with range of up to 6.8×10^{-8} .

This approach is only possible because more is known about the molecular biology of retinoblastoma than of any other human tumour.²¹ It is generally accepted that deletion of both retinoblastoma genes by mutation is a necessary and sufficient condition for neoplasia. This idea was first proposed by Knudson.¹⁴ More recently the genes have been located on chromosome 13 and the protein product of the gene has been identified.²¹

A problem with this approach is that no allowance can be made for the fact that the first mutation in sporadic cases could confer either a selective advantage or a selective disadvantage on the cell. If the mutation gave the cell a proliferative advantage then the calculated mutation rate of 8×10^{-8} would be an overestimate; if the mutation was disadvantageous then the value of 8×10^{-8} would be an underestimate.

An interesting outcome of the analysis is that the estimated value of 2^{x-b} , which is 1.09×10^7 with a range of 6.5×10^6 to 1.7×10^7 , is much less than the number of rods and cones in a single eye which is of the order of 12×10^7 .¹³ There are two possible explana-

tions for this finding. The first is that only some 10% of the retinal precursor cells are at risk of neoplasia, implying that differentiation beyond a certain point will prevent retinoblastoma developing even if the two crucial mutations occur. Therefore, some mechanism during differentiation must inactivate the oncogenes that the retinoblastoma genes control. The second possibility is that all the retinal precursor cells are at risk of neoplasia, but 90% of the malignant clones that arise are deleted by a cancer surveillance system.

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