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Review

Mutation, replicative infidelity of DNA and aneuploidy sequentially in the formation of malignant pleomorphic tumors

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Summary. Mutations are thought to be involved in tumor formation because (i) tumor cells transmit their abnormalities to their descendants; and (ii) many carcinogens are mutagens. Aneuploidy is thought to be involved in tumor formation because (i) it is a common phenomenon, especially among malignant neoplasms; (ii) certain particular types of tumors are associated with specific karyotypic changes; and (iii) many immortal tumor cell lines are hyperploid. In recent years, acquired somatic cell replicative infidelity of DNA ("mutator phenotype") has been suggested as a mechanism of tumor formation, because more somatic genomic events occur in malignant tumor cells than could be caused by repeated exogenous mutagenic insults. Previously, theories of the genomic pathogenesis of tumors have involved these mechanisms individually. Here it is suggested that all three mechanisms may play roles in the formation of certain tumor types. For example, a sequence could occur such that first, a mutation affects genomic elements for control of growth, and for replicative fidelity of DNA, leading to "mutator phenotype". Second, when replicative infidelity of DNA results in mutation of genomic elements for mitotic-and-chromosomal stability, aneuploidy develops. Third, an asymmetric mitosis (in the course of the aneuploid stage) could produce occasional cells in which the "bad copy" is lost (or an extra "good copy" is gained) of the original genomic element which had supported replicative fidelity of DNA. These resulting cells would regain fidelity of replication of DNA, and hence could give rise to populations which are relatively genomically stable, hyperploid and immortal.

Key words: Tumors, Hyperploidy, Aneuploidy, Mitosis, chromosomes, DNA replication, Fidelity, Immortality

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Introduction

Abnormalities of chromosomes and mitoses in tumor cells were noted in the late 1880s by Klebs and others (Wolff, 1907). Hansemann (1890-1893) was first to suggest that maldistribution, or other changes of the complements of chromosomes, might be the first step of the conversion of a normal cell to a tumor cell. Hansemann's theory, either in its original form or as modified by Boveri (1914) (see Bignold et al., 2006a, 2006b), has not been popular, because asymmetric mitoses are not necessarily found early in the courses of most tumor types, and experimental tumors can arise without cytogenetic abnormality (Koller, 1957). Thus generally, mitotic and chromosomal abnormalities have been considered as effects, rather than the cause, of some unrelated, uncomprehended fundamental "neoplastic change" of tumor cells (Israel, 1902; Ludford, 1930; Koller, 1949, 1957; Berenblum, 1974).

Mutations have been mentioned as possible causes of tumor formation since the early twentieth century (Murray, 1904; Tyzzer, 1916; Whitman, 1919) and discussed in some detail by Bauer (1928) and Lockhart-Mummery (1934). However, this hypothesis was discounted by some authors such as Rous (1959) because somatic mutations were believed to be rare. Other authors, especially (Willis, 1948) pointed out that single, or even small numbers of mutations cannot explain the complex histopathology of tumors. Yet other authors such as Berenblum (1974) pointed out that mutations with immediate phenotypic effects cannot account for the long latency (delays) which are evident both clinically and experimentally between the application of carcinogen and the appearance of tumor.

In recent years, cytogenetic studies of tumors have revealed that some tumor types are associated to greater or lesser degrees with some particular karyotypic abnormalities. Further, it has been found that the immortal cell lines which can be derived from some tumor types (such as gastric carcinoma and renal cell

carcinoma), are almost all hyperploid (Catalogue of the American Type Culture Collection). As another particular example, the HeLa cell, which has been cultured very extensively and is cytogenetically relatively stable, is approximately tetraploid. These observations form part of the basis of on-going support for aneuploidy as the basis of tumors (Li et al., 2000; Duesberg, 2005; Duesberg et al., 2005; Weaver and Cleveland, 2005; Pathak and Multani, 2006).

Lastly, however, there is the observation that, of the billions of cells in individual tumors, relatively few cells can be induced to give rise to permanent cell lines in culture. Little discussion of how aneuploidy might give rise to only such small numbers of immortal cell lines has been published (Erenpreisa and Wheatley, 2005).

DNA replicative infidelity in tumor cells

In his description of the properties of genes, H J Muller (1922) noted that genes must be reproduced accurately, and that if any errors occur in such replication, the changes are transmitted exactly to daughter cells. Watson and Crick (1953) emphasised that their double helical model of DNA provided a mechanism for faithful replication of DNA sequence. In the 1960s, bacterial strains were identified in which replication of DNA was reproduced less faithfully than in corresponding wild types. For the former, the term "mutator strain" was introduced (Cox and Yanofsky, 1967). Although Speyer (1965) and Nelson and Mason (1972) suggested that replicative infidelity of DNA might occur in tumors, Loeb (Loeb et al., 1974; Loeb and Kunkel, 1982; Loeb, 1996, 2001; Loeb and Loeb, 2000; Beckman and Loeb, 2005) has continuously championed the phenomenon as a type of genetic instability which is relevant to tumor formation.

One significant observation supporting the occurrence of DNA-replicative infidelity in tumors is that the numbers of "genomic events" per cell has been demonstrated to be very high (approximately 10^5 - Stoler et al., 1999). These numbers are not readily explained by recurrent exogenous mutational events, and thus a role for replicative infidelity of DNA as the mechanism of production of large numbers of somatic mutations in tumor cells seems indisputable. Yet another phenomenon supporting a pathogenetic role of impaired replicative fidelity in the formation of tumors (as opposed to being an incidental and additional effect of some unknown neoplastic process), is that mutations of DNA-replicative fidelity-supporting genomic elements are a possible mechanism of latency (delays) between the application of carcinogen and the appearance of tumors *in vivo*. This is because DNA-replicative fidelity does not of itself necessarily produce any immediately-detectable phenotypic change in living tissues, but its effects appear only after further cell divisions/replications of DNA (Bignold, 2003). Nevertheless, a complete theory of tumor pathogenesis is difficult to base entirely on acquired somatic replicative infidelity of DNA. This is because the descendants of any cell line which is unable to replicate its DNA accurately would accumulate all the mutations of their predecessors. Hence, the sub-lines might be expected, sooner or later, to mutate a viability-essential gene and die out. Furthermore, there is no obvious reason why tumors due to DNA-replicative-infidelity alone, should be so commonly hyperploid.

Hypothesis

The suggestion being made here is that the various mechanisms of genomic disturbance (mutation, replicative infidelity of DNA, aneuploidy) may all be involved to greater or lesser degrees in the formation of tumors according to type. Thus benign, non-progressive tumors showing little aneuploidy (e. g. lipomas, fibromas) might be due to simple mutations resulting in excessive growth. On the other hand, for chronic myeloid leukemia, a chromosomal mechanism may initiate the excessive accumulation of these cells. However, for a cyto-biologically complex lesion such as a carcinoma of the colon, a three-step sequence of genomic alteration is postulated. Specifically, the sequence may be as follows: The first step may be mutation of a genomic element which normally support DNA replicative infidelity, so that the resulting cell acquires replicative infidelity of DNA ("mutator phenotype") (Fig.1). The original mutation could occur by any mechanism, including the process involving interference with DNA replication by exogenous carcinogens, as put forward by Loeb since the 1970s (Loeb, 1974; Cheng and Loeb, 1997) and discussed by the present author in terms of experimental carcinogenesis (Bignold, 2003, 2004) and alkylating agents (Bignold, 2006). This mutation could occur in a pre-existing lesion (e.g. an adenoma) which had arisen by a different mechanism. Nevertheless, with respect to the carcinomatous lesion, further mitoses of the cell line with replicative infidelity of DNA would produce more and more cells with ever-accumulating abnormalities. When mutation occurs in genomic elements which are essential for the viability of such cells, these cells are likely die out.

The second suggested step therefore is that, for immortal cell lines to appear, this replicative infidelity of DNA must be counteracted in some way (Fig. 1). It is possible that simple "corrective" mutations could lead to stable, but still abnormal, cell lines. However, another possibility is that supervening aneuploidy provides for cells which regain fidelity of replication. This aneuploid stage could originate because of a mutation of a genomic element for mitotic stability (symmetric distribution of chromosomes at nuclear division). The essence of the aneuploid stage would be a liability to asymmetric mitoses, having the following potential effects (Fig. 1). Broadly the consequences of aneuploidy are likely to be (i) mainly rapid or slow death of the cell line; (ii) "vegetative" state (continuing existence without further

cell division, which is compatible with loss of impaired replicative infidelity genes, because this defect is of no consequence in non-dividing cells); and (iii) immortality (Fig. 1).

In detail: if during an asymmetric mitosis, the mutant genomic elements are distributed to daughter cells according to the degree of asymmetry of the mitosis (Fig. 1[A]), the smaller daughter cell might be non-viable due to loss of cell-essential genomic elements, while the larger cell is likely to remain viable, because its genomic elements are only amplified. Alternatively, in the less common situation of the asymmetric mitosis distributing relatively more of the mutant genomic elements to the larger cell (Fig. 1[B]), that cell would be likely to be non-viable from increased mutation-load. However, the smaller cell of this division is also likely to be non-viable because of loss of cell-viability essential genes, as in the first scenario (above). Yet again, if relatively more mutant genomic elements are distributed to the smaller cell (Fig, 1[C]), and correspondingly fewer to the larger cell, then the smaller cell would be the most likely to die of all the above cells, because it would have both more mutational load and less cell-viability-essential genome. However, the larger cell of this distribution of chromosomes would be more viable than its parent cell, because it would have fewer mutations, and more "good copies" of essential genomic elements. Such "genomically-replenished" cells might

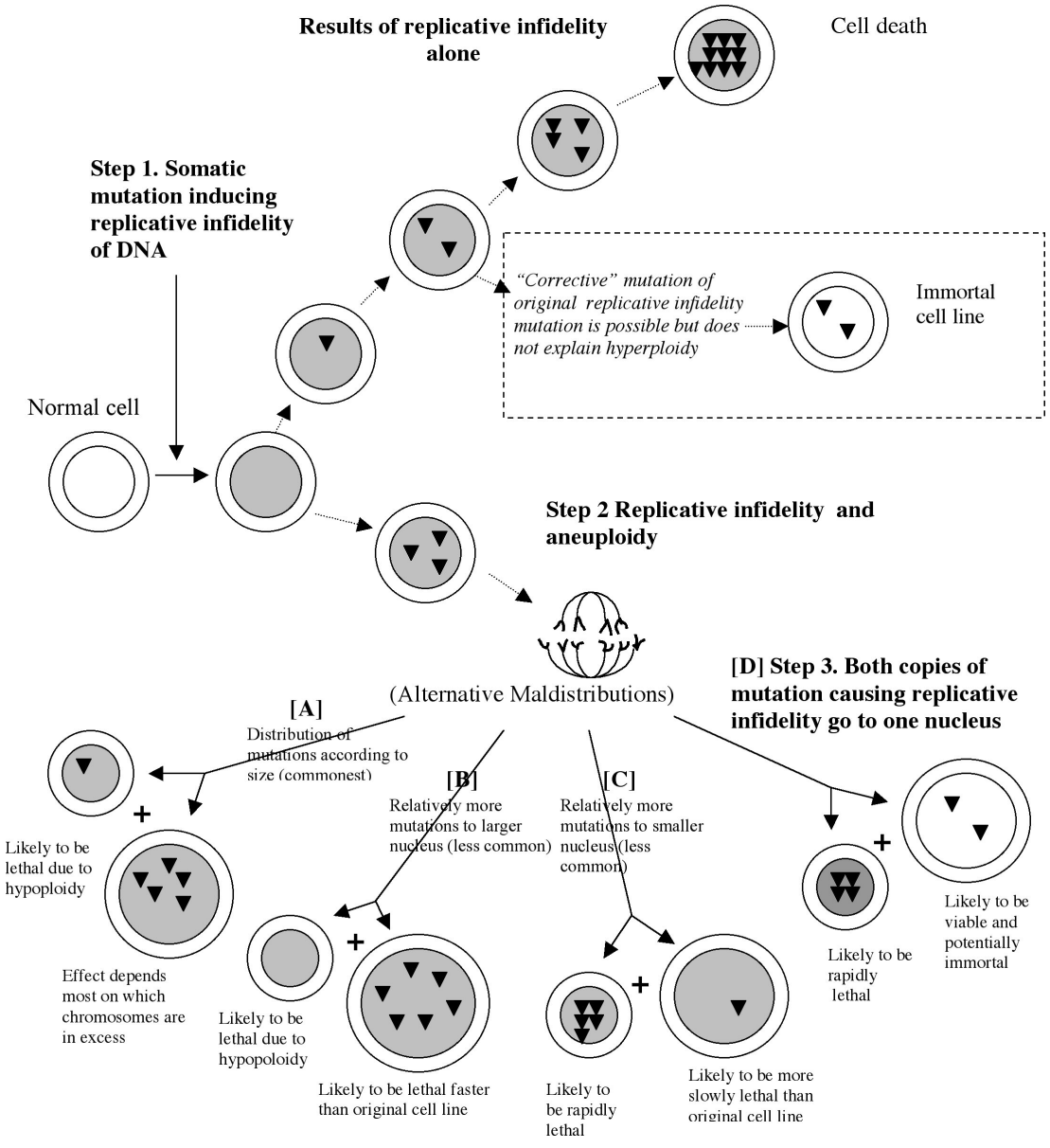

Fig. 1. Diagram of a three-step sequence of genomic alterations which might result in a tumor cell population characterised by large numbers of genomic events (indicating replicative infidelity of DNA); aneuploidy (pleomorphism); and also providing for only a relatively small proportion of "immortal" cell lines. Grey nuclei are those which have acquired replicative infidelity of DNA.
Inverted black triangles are viability-threatening mutations of genomic elements.
Generally, hyperploid cells are more likely to be viable than hypoploid ones because the former have at least one "good copy" of cell-viability-essential genomic elements.
Losses/gains of specific chromosomes may cause other changes, and especially, cells may go into a vegetative state if pro-growth mutations are lost. In these cells, the replicative infidelity mutation becomes irrelevant.
In addition, the effects of any particular chromosomal addition/deletion may be altered by the mutations which occcurred in those chromosomes before the aneuploidy/maldistribution phase began.

give rise to longer-persisting cell lines than others.

The third proposed step (Fig. 1[D]) of the scheme here, is a variant of the last of the possible outcomes of asymmetric cell division (immediately above). If the original mutation which created the DNA-replicative-infidelity trait in the original cell was lost or replaced in this presumably uncommon asymmetric mitosis, the descendants of the cell with both "bad copies" would probably die out relatively quickly, and the cell line deriving from the cell which had shed its "bad copy" would not only be more viable than any other, but also relatively more genetically stable. Such a scheme would result in cells which are particularly rapidly-growing, hyperploid, and immortal, consistent with long-observed tumor cell phenomena. Also, the fact that only a small number of cells in tumors give rise to permanent cell lines in culture, would be provided for.

Discussion

It is stressed that the particular scheme given in detail above is not proposed to be necessarily relevant to all tumor types. In particular, most "benign" tumors and certain "malignant" ones, for example "small-celled" anaplastic carcinomas (mainly of the bronchus) may be due to simple mutational mechanisms. In the case of anaplastic small-celled carcinomas of the bronchus, the immortal cell lines which arise are often euploid, or minimally cytogenetically abnormal (Catalogue of the American Type Culture Collection). Such immortal cell lines might arise by mechanisms such as (i) simple preservation of "stem cell" phenotype, if the original normal cell was a stem cell or (ii) loss of a copy of a senescence gene (Campisi, 2005). However, neither of these mechanisms would explain the regularity of hyperploidy in immortal cells cultured from other tumor types, such as carcinomas of the gastrointestinal tract.

Also unlikely to arise by this mechanism are unusual tumor types, such as carcinoids, which exhibit excess growth and invasion and metastasis, but show little cellular pleomorphism, and rarely undergo any rapid accelerations of cell biological abnormalities (i.e. rarely show "progression"). These tumors may not be significantly genetically unstable at all, and might arise from a single co-mutational "hit" which provides for these features without genetic instability by the mechanism previously suggested (Bignold, 2004, 2005).

According to the general concept (of possibly more than one genome-disturbing mechanism in the pathogenesis of some tumor types) of this paper, no specific genomic element is proposed to be involved in all tumor types, and different ones (protein-encoding sequences, regulatory sequences, transcription factors, histones, micro-RNAs, telomeres etc) might well be involved in different types of tumors, or even in different cases of the same tumor type, or yet again in the same case of a tumor at different phases of its development, or even in different subpopulations of cells within the one tumor. Further, no particular mechanism(s) are specified, of gene activation and inactivation which might underlie excess growth, mitotic and chromosomal instabilities, DNA-replicative-infidelity-type genetic instability, invasiveness, metastatic growth and so on. Particular growth factors or tumor suppressor genes might be involved in the initial excess growth of some tumor types, but mutations of other factors or genes might be caused by DNA-replicative-infidelity-type genetic instability later in the development of the tumor.

Further to this, the concept does not specify any particular mechanism(s) to account the tumorous cell biological phenomena for all tumor types. For example, concerning the acquisition of motility (which is thought to be the basis of invasiveness and metastasis of solid tumors), no emphasis is given to one mechanism over another currently in the literature. Thus, de-repression of motility-suppressing "master-genes" (Frisch, 1997; Carrio et al., 2005), stimulation of latent activities by autocrine motility-enhancing factors (Levine et al., 1995; Silletti and Raz, 1996; Dobashi et al., 2006); or release of inhibited activities by dissolution of desmosomes, other adhesive structures or basement membrane itself (Brinckerhoff et al., 2000; Chrenedk et al., 2001; Chidgey, 2002; Wheelock and Johnson, 2003; Montell, 2005) might occur in different tumor types, or even among different cases of the same tumor type.

Testing the hypothesis

To test this scheme, relevant investigations could be directed at whether or not the replicative fidelity of DNA polymerases can be affected either directly or indirectly by carcinogens, as has been suggested especially by Loeb (reviewed Bignold, 2002, 2003, 2004, 2006). At present, experimental methods have not been established for the easy detection of loci of low-incidence nucleotide sequence changes in the human genome. Thus the implied "hallmark genomic lesion" of a single DNA-replicative-infidelity event would be (say) 0.1% or 1% base changes in 15,000-150,000 base sequences. Complementary DNA-hybridisation techniques are generally only able to detect complete loss of heterozgosity (closer to 100% nucleotide change) in lengths of DNA over $1x10^6$ bases. A possible method of assessment of DNA replicative infidelity was proposed earlier (Bignold, 2004). Nevertheless, in the cells of malignancies, so many secondary genomic events occur either *in vivo*, or *in vitro* during culture of cell lines, that the particular lesion which initiated the tumor may be obscured.

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