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Review

Initiation of genetic instability and tumour formation: a review and hypothesis of a nongenotoxic mechanism

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Abstract. Genetic instability in tumours results in cell-to-cell variability of genome which parallels the cell-to-cell variability of microscopic morphology and of behaviour (tumour cell heterogeneity) of these lesions. Genetic instability is therefore strongly supported as the fundamental process by which normal tissue cells become neoplastic.

The commonest current suggestion for the mechanism of initiation of carcinogenesis is a 'direct hit' mutation of a 'cancer critical' gene in a somatic cell by carcinogenic agents. However, this mechanism does not account for the activity of carcinogens which are not mutagens, and does not explain why many mutagens are not carcinogens.

This paper proposes a nonmutational (nongenotoxic) mechanism of initiation of genetic instability in previously normal cells as follows:

1) During S phase of local tissue stem cells, carcinogen binds to and disables the proofreading enzyme for a new DNA strand.
2) While it is disabled, the proofreading enzyme fails to correct illicit changes in the nucleotide sequence(s) for one or more genes for proofreading fidelity or repair of DNA in the new strand of DNA, which passes to one daughter cell.
3) When this daughter cell is a continuing stem cell, the resulting cell line remains immortal, and retains its prior differentiation commitment to produce daughter cells of a particular type. However, the acquired genetic instability in this cell line causes secondary mutations which lead to uncontrolled growth, and the heterogeneous morphologic and behavioural features of a tumour resembling the parent cell type.

Key words: Genetic instability; tumour; carcinogenesis; nongenotoxic; stem cells; mutation; DNA polymerase; DNA proofreading.

Introduction

Tumours are in vivo cell lines characterised by uncontrolled proliferation and immortality for as long as the surrounding tissues support them. In malignant cases, cells invade underlying tissues and metastasise to distant organs of the body. Tumours are common among cell types which maintain themselves by mitosis of local tissue stem cells (e. g. the epithelium of the colon), and virtually never occur in cell types which do not undergo mitosis (e. g. neurons in adults). Tumour cells usually retain variable resemblance to their cell type of origin, but show complex morphological abnormalities which frequently vary from cell to cell (pleomorphism) and microscopic focus to focus in the same lesion [1–3]. The degrees of these morphological abnormalities do not perfectly correlate with behaviour among tumours of the same type, and show even less correlation among tumours of differing cell types [1–3]. Among carcinogenic agents (physical, chemical, viral), there is imperfect correlation of carcinogenic potency with mutagenicity. Indeed, some carcinogens are not mutagenic, and many mutagens are not carcinogenic [4, 5].

Consideration of the mechanism(s) of carcinogenesis has involved two separate issues. The first issue is the identity of the particular cell type, cell process, cell structure, metabolic process or other aspect of cell function which is the primary target of carcinogenic agents. The second issue is how carcinogenic agents initiate the abnormality of

the proposed 'target' structure or function of the susceptible cell.

In recent years, tumours have been recognised to be genetically unstable [6, 7] (i.e. their genomes vary from cell to cell in the same tumour), and this abnormality has been documented early in the formation of neoplasms [7–9]. Genetic instability can explain the complex morphological and behavioural abnormalities of tumours [2, 3]. Although this strongly supports the notion of genetic stability as the fundamental process which is deranged in neoplasia, its precise relationship to the 'initial step' by which carcinogens tumour formation is unclear.

This paper reviews theories of carcinogenesis, first in relation to cell- and tissue targets of carcinogens, and second in relation to the initial step of tumour formation. The nature of stem cells, the interactions of carcinogens with proteins, and the enzymatic (i.e. protein) basis of the mechanisms for prevention of genetic instability in normal cells are emphasised. A new hypothesis is suggested, by which the initial step of carcinogenesis is the induction of genetic instability by a direct and nonmutational inhibition of enzymes which proofread newly synthesised DNA during mitosis of local stem cells.

Nongenetic tissue processes, and cell process targets in carcinogenesis

Tissue processes

In the middle of the 19th century, most theories of carcinogenesis involved abnormalities of hyperplastic responses of tissues to agents such as chronic irritation or parasites [10]. In the 1870s, Cohnheim proposed that cancers arose from left over embryonic cells in adult tissues [10–12]. Von Hansemann, in the 1890s suggested that tumours arose by a process of reverse differentiation (anaplasia) of local proliferative cells to embryonic ones (fig. 1, also reviewed [2]).

In this period of time, initial steps of carcinogenesis were little discussed, as tumours were considered to arise by a gradual local change of cell and tissue behaviour, and adequate biochemical methods did not exist to support further studies.

Theories of neoplasia as a disorder of some local tissue process remain current. Abnormal hyperplasia has long been recognised as a frequent preliminary morphologic change of human tumours, for example of the endometrium [13], and in experimental lesions [14]. Abnormal wound healing was proposed as the basic process of cancer by Haddow [15], and later workers suggested that local hormones controlling cell proliferation, for example 'chalones' [16], may mediate these abnormal responses.

Abnormal differentiation as the fundamental process of carcinogenesis has been extensively investigated. Pierce [17] studied differentiation of teratoma cells, and proposed that abnormal cytoplasmic molecules controlling expression of genes for differentiation were most important for cancer development. Additional theories of abnormal differentiation as the basis of cancer have been provided by Fiala [18], Trosko and Chu [19], Braun [20] and Muller [21].

Local tissue stem cells as the target of initiation of carcinogenesis (fig. 1)

Local tissue stem cells are the defined as cells, which, after embryonic differentiation, persist in adult tissues and are committed to producing specialised cells of one type only by repeated asymmetric mitoses [22–24]. The mitoses are asymmetric because one daughter cell proceeds to specialise according to the cell type, while the other daughter cell retains the features of the local stem cell line (nonspecialised, but able to produce cells which

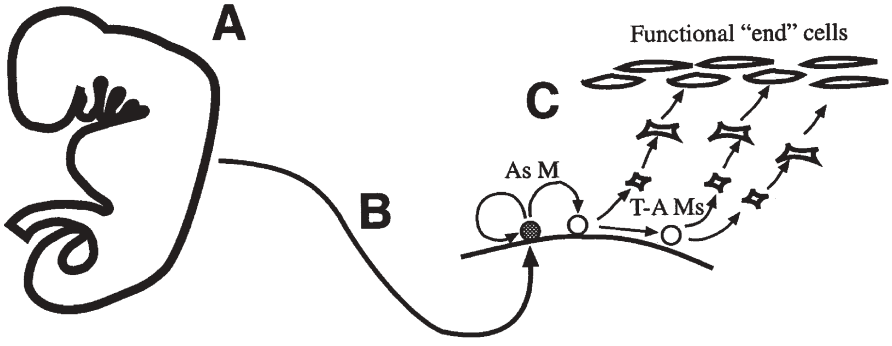

Figure 1. Relationship between embryonic development, local tissue stem cells, local specialisation of cells and some theories of carcinogenesis. (*A*) In embryonic development, lineage-specific cell lines appear which lead to local tissue stem cells (cross-hatched circle) in postembryonic life. Additionally (not shown), embryonic cells show controlled invasion of mesenchyme (to form internal organs) and entry of cells into the bloodstream (embryonic haemopoietic stem cells). (*B*) Von Hansemann suggested that in cancer, the lineage commitment of local stem cells is reversed, so that these cells show the behaviour of embryonic cells (above). (*C*) In postembryonic life, local stem cells (cross-hatched circle) undergo asymmetric mitosis (As M). Subsequently transit-amplifying mitoses (T-AMs) produce specialised functional end cells. Numerous theories have suggested that cancer represents a disorder of this process (see text).

can specialise to the mature cells of the particular type). This asymmetry of mitosis may be controlled by environmental or intrinsic mechanisms [24], and accounts for the immortality of the cell line for as long as the individual lives (one daughter cell is the continuing stem cell), and the ability to produce to specialised cells (the commitment to specialisation of the other daughter cell). Stem cells therefore have the characteristics for neoplastic accumulation, excepting for the uncontrolled proliferation rate. Because tumours are commonest among tissues which include stem cells, and rare among tissues which do not, stem cells have been widely believed to be the target cell of carcinogens for many years [18, 25, 26]. Nevertheless, the reasons for the particular role or susceptibility of stem cells to tumour formation has not been clarified.

Other subcellular structures and metabolic processes

The advent of electron microscopy (late 1940s) raised the possibility that a diagnostic abnormality of a cell structure might be revealed as the basis of cancer. However, no cancer-specific morphological abnormality of any subcellular structure was demonstrated [1, 27]. Nevertheless, functional derangements of cell structures have been proposed as the primary target in carcinogenesis, and include the cell membrane [28], the glycocalyx [29] and intercellular gap junctions [30, 31].

Derangements of several target metabolic pathways have been proposed as primary to carcinogenesis. Warburg, in the 1930s [32, 33], proposed that carcinogenesis was related to excessive anaerobic glycolysis. Later, particular roles of abnormalities of other metabolic pathways, including those of protein metabolism [18] and lipid metabolism [34], were proposed. More recently, free radicals [35, 36], eicosanoids and signalling mechanisms [37], nitric oxide [38] protein kinase C [39] and Id proteins [40] are among many such metabolic concepts which have been suggested to have prime roles in carcinogenesis.

Chromosomes, genes and gene regulators as targets in tumour formation

After the recognition of chromosomes as the structures which carry hereditary information during cell division, abnormalities of chromosomes were proposed to be the basis of neoplasia as early as 1904 [2, 10]. However, not all tumours exhibit chromosomal abnormalities, so that the notion was not widely accepted [41].

In the 1950s, the first gene-regulatory proteins were discovered [42], and subsequently suggested as the basis of cancer. Sherbert [43] and Foulds [44] argued that cancer primarily represented a generalised problem of 'differential utilisation of the genome', rather than a mutation of one gene alone.

Genes for control of cell proliferation

Studies of cells in culture, and particularly of the phenomenon of transformation of growth characteristics [45], led to the discovery of specific endogenous soluble peptides which regulate cell growth and function through interaction with highly specific membrane receptors [46]. Since their discovery, more than 40 of these growth factors have been described, of which some are produced by diverse cell types [46, 47]. Sporn and co-workers [48] suggested that carcinogenesis might involve overexpression of growth factors, with autocrine stimulation of mitosis.

Oncogenes were originally described as genes found in viruses (viral oncogenes), which, when transfected into cultured mammalian cells, caused increased growth and other changes [24, 49, 50]. Many of these genes were later found to be mutant variants of normal mammalian genes [50, 51]. The normal endogenous mammalian genes are called protooncogenes, and their mutant counterparts are known as cellular oncogenes. Many protooncogenes were later shown to have considerable homology with the genes of growth factors or cell signalling enzymes such as kinases [24, 51]. The commonest sequence of events seems to be that viruses infecting human cells are capable of taking some host proliferation genes into their genome, and then effectively transfecting other cells with these genes, either with the original sequence, or as a mutated version [52].

Tumour suppressor genes (anti-oncogenes) are genes for control of cell proliferation [24, 53, 54] which have the opposite effect to growth factor genes. The products of tumour suppressor genes inhibit mitosis in normal tissues. Tumour formation usually requires that both copies of the gene are inactive. Because the tumour is most common among individuals who are homozygous for the mutation, the term 'recessive oncogenesis' has been used [55]. The first tumour suppressor gene was identified in studies of susceptibility to retinoblastoma [56]. Most tumour suppressor genes appear to be active in only a few tissues. However, the p53 gene [57, 58] is present in many tissues. Individuals who inherit only one functional copy of the p53 gene are predisposed to multiple independent tumours in a variety of tissues in early adulthood (Li-Fraumeni syndrome). Mutations in p53 are found in many tumour types throughout adult life, and so may contribute to the formation of a wide variety of tumours [57, 58].

Lodish and co-authors [49] classify genes for inherited susceptibility to tumours according to the function of their protein products:

- Intracellular proteins, such as the p16 cyclin-kinase inhibitor, that regulate or inhibit progression through a specific stage of the cell cycle
- Receptors for secreted hormones (e.g. tumour-derived growth factor β) that function to inhibit cell proliferation

– Checkpoint-control proteins that arrest the cell cycle if DNA is damaged or chromosomes are abnormal
– Proteins that promote apoptosis
– Enzymes that participate in DNA repair.

Multiple gene mutations for regulators of cell-growth control

One attempt to explain tumour formation and its morphological abnormalities by multiple mutations of oncogenes and tumour suppressor genes has been the work of Vogelstein [59, 60], who investigated adenomas and carcinomas of the colon. He proposed that mutations of particular tumour suppressor genes are responsible for each individual step in a morphological series of events for the conversion of a colonic epithelial cell through hyperplasia, low grade adenoma, medium grade adenoma, high-grade adenoma and finally carcinoma.

DNA methylation and cancer

Abnormal DNA methylation in tumour cells has been proposed as a primary disorder of tumour cells [61]. Methylation generally is a mechanism of gene repression or 'silencing' [62, 63]. Abnormal methylation was suggested as the starting mechanism of generalised epigenetic abnormalities of tumours [63–65]. More recent work has shown, however, that the distribution of methyl groups is variable in tumours, ('too much and too little' [66]) so that a definite primary role of abnormal methylation of DNA in the initiation of cancer is not proven.

Other theories of carcinogenesis involving target gene regulators

Transcriptional abnormalities [67] and translational abnormalities [68] of gene regulatory processes, and various cell cycle abnormalities [69, 70] have been proposed as possible mechanisms of carcinogenesis. Mutations of genes for genetic stability are discussed below.

Microarray technology and gene-regulatory theories of carcinogenesis

Microarray technology allows large numbers of genes and their transcript RNAs to be assessed in tissues of tumours. At the present time, it is established that most tumours are characterised by expression and mutational abnormalities of multiple gene regulators. However, no cancer-specific pattern of abnormal expression has been identified [24, 71] and which particular gene among these may be the initial step of carcinogenesis is unclear.

Initiation of carcinogenesis and nongenotoxic effects of carcinogens

Studies in the 1940s of possible synergistic effects of two or more carcinogens by Berenblum and later Mottram [72, 73] showed that in some cases, tumours only appeared when one chemical (an initiator) was applied before another. The second chemical (the promoter) was only able to produce a tumour in skin which had been pretreated with the first, and tissue treated only with the initiator did not develop tumours. It was recognised, however, that some chemicals (complete carcinogens) could have both effects.

Nevertheless, the results led to the popular two-stage concept of carcinogenesis with initiation and promotion being necessary phases of tumour formation [1, 72, 73]. At the time, the mechanism of each of these processes was unclear, but later it was proposed that initiation represented a primary mutation of some particular cancer critical gene [74–77], and promotion was probably related to epigenetic phenomena [78].

Armitage and Doll [79, 80] added tumour progression to the initiation/promotion model as a third stage of neoplasia. Progression later came to be considered to be caused by mutations [81–83] and by mutations arising from genetic instability in particular ([84], and see below). Applying concepts of recessive oncogenesis (see above), Knudson [85–87] has proposed only two mutations are required for carcinogenesis generally.

Nonmutational mechanisms of action of chemical carcinogens

Since the 1930s, chemical carcinogens have been recognised to react with various cellular structures, especially proteins [72, 73, 88]. Haddow, in the 1940s, proposed a hypothesis of initiation of carcinogenesis involving cross-linking of proteins, but the target protein was not specified [72, 73]. Other theories proposed that carcinogens bind to and inactivate proteins controlling cell growth, and it was suggested that avidity of protein-binding correlates with carcinogenicity [88, 89]. Binding of chemical carcinogens to proteins is well established in the literature, and estimations of protein adducts are currently used as a method of monitoring exposures to carcinogenic agents [90–93].

Carcinogens as toxins and stimulators of proliferation

It has long been recognised that almost all carcinogens, if applied to tissues in sufficient quantities, may produce cell damage in some form. However, studies of the relationships between the two phenomena have shown no consistent correlation between carcinogenic potency and either toxic potency [94] or proliferation/hyperplasia-inducing potency of chemical carcinogens [73, 88].

Structural analysis of carcinogens has shown no obvious chemical correlate of carcinogenic activity. However, among polycyclic hydrocarbons an electron-dense area (the 'K region') was proposed by Pullman as having significance for carcinogenic activity [72, 73, 88].

The 'direct gene-hit' mechanism of initiation of carcinogenesis

Theories of mechanism of action of chemical carcinogens currently largely involve chemical reactions of electrophilic derivatives with DNA, forming adducts [4, 5, 88, 89, 95–98]. These carcinogens show selective binding to nucleotides, with some mutations being more common (e.g. GC → TA) than others [98]. However, carcinogens are not known to target any specific genes.

With respect to the effects of adducts bound to DNA on the subsequent rate of DNA synthesis, many carcinogens appear to have no effect, or are associated with cellular changes (hyperplasia) in which DNA synthesis increases. However, 'bulky' adducts often block the progress of DNA polymerase along the template DNA [99, 100].

Physical agents such as ionising radiation [101] and ultraviolet light [102] are thought to produce a variety of effects, including direct effects on DNA and induction of DNA-binding intermediaries from endogenous substances, which cause the mutations of the cancer-critical genes.

Objections to simple gene-hit theories of the initiation of cancer

Nonmutagenic carcinogens and noncarcinogenic mutagens [4, 5, 103, 104]

During most of the 20th century, carcinogens which do not produce mutations in experimental systems were thought to probably be acting by an unspecified promoter effect [105, 106]. Studies of herbicides and other agents, however, and showed that non-mutagenic agents can be complete carcinogens [107]. The terms genotoxic and nongenotoxic carcinogens have been used respectively for mutagenic and nonmutagenic carcinogens [106], and this usage is now widespread.

Little has been suggested of how nonmutagenic carcinogens might act in recent times, and whether or not a relationship exists between nongenotoxic carcinogens and epigenetic theories of carcinogenesis [76, 77, 108, 109] has been little discussed. Similarly, there has been little discussion of how some mutagens are carcinogens and others are not [78, 110].

Mutation is an instantaneous change, but the induction of neoplasia takes months, with only gradual onset of morphological change

This point was made at length by Willis [41], (see [2]), Berenblum [72, 73] and Iversen [111]. This argument retains its validity even for theories of the initiation of carcinogenesis involving direct hits on genes of gene regulators. This is because any mutation acting on morphology and behaviour of the cell (thus excluding genetic-instability mutations, which act indirectly) in carcinogen-treated tissues should have its effects within the lifetime of the affected cell.

Genetic instability: discovery and general mechanisms

Fidelity of replication of the nucleotide sequences of DNA during the S phase of mitosis and of accurate repair of nucleotide sequences of DNA which is damaged in the life of the cell are essential for survival of the organism during development, and the species during reproduction. When the relevant mechanisms fail in a cell line, cell-to-cell variability of genomes (genetic instability) develops among daughter cells. Genetic instability was first recognised in studies of bacteria in the 1950s and 1960s showing that some mutant strains of *Escherichia coli* were more liable to further mutation than others [112, 113].

Since that time, many mechanisms for preserving genetic stability in dividing cells have been elucidated. In eukaryotes, the most important mechanism for replicative fidelity during cell division appears to be an accurate geometric fit of nucleotide to the active site (the fork) of the active site of the DNA polymerase molecule ('insertion fidelity', [114]). The preciseness of the activation sites involve the zinc-finger structure of peptide tertiary structure [24]. Additional major mechanisms of ensuring faithful replication of DNA include proofreading by exonucleases, which shortly after synthesis, check the correctness of the sequence of the newly synthesised chain against the template chain and make corrections.

A second aspect of preservation of cell DNA is the repair of damage, such as spontaneous depurination and deamination [24, 115], which occurs to the nucleotide sequence during interphase. Breaks in single strands, and in both strands of interphase DNA, can be repaired [114], and are so effective that fewer than one per thousand changes caused by damage go uncorrected [24, 114].

The functional sites of all the DNA-proofreading and -repair enzymes depend on highly specific conformations of peptide chains, so that specific configurations of hydrogen bonds and perhaps other weak intermolecular forces, can combine to provide for accurate recognition of appropriate nucleotides for addition to, or replacement in, the DNA chain [114].

Currently, many mechanisms of genetic instability in eukaryotes are recognised, and include mutations of genes of DNA helicase, of chromosome stabilisation, of chromosome segregation during mitosis, of the mitotic spindle checkpoint, of telomerases and of nucleotide excision repair [see 2 and 114–116].

Genetic instability in tumours: identification and significance

Although chromosomal (karyotypic) instability was recognised in tumours during the 19th century [116], this was not widely associated with possible genetic instability of tumours until the 1970s. Ono in 1971 [117], and Nowell in 1976 [118] suggested that chromosomal instability might be a mechanism by which more aggressive tumours arise in previously less aggressive lesions. Cairns in 1975 [119] suggested that mutations in cancer cells analogous to the process of natural selection might play a role in tumour formation ab initio.

However, accumulation of direct evidence of genetic instability playing a role in tumour formation began only with the discovery in the 1960s, by Cleaver [120] that the mutant gene responsible for the increased rates of skin cancer among individuals with xeroderma pigmentosa is for an enzyme associated with repair of DNA. Subsequently, several additional mutations of genes for repair of DNA were found to be associated with susceptibility to hereditary tumours, such as carcinoma of the breast and ovary (BRCA-2 gene) and nonpolyposis colorectal cancer [24]. Cancers occur in multiple organs in one individual in Werner's syndrome (due to a particular mutation for accessory exonuclease and DNA helicase gene), and Bloom's syndrome (due to a mutation for accessory DNA helicase for replication) [24].

Genetic instability has become recognised as a major feature of cancer cells, because large numbers (up to 11,000 genomic events) have been documented per cell [121]. Further, genetic instability has been recognised as occurring early in cancer, as adjacent morphologically normal cells have been shown to be genetically unstable in adjacent and at-risk tissues [7, 8].

Several authors have addressed the significance of genetic instability for carcinogenesis. Riley [122, 123] suggested that initiation of carcinogenesis might be an enhancement of the mutation rate, and that a mutation might induce a metabolic lesion which causes the subsequent increase in mutation rate. Loeb [124–126] has suggested that first step of cancer may be a mutation of genes for DNA polymerase. However, how the first mutation of this gene is acquired was unclear. Schmutte and Fishel [127] suggested that genetic instability was a first step of carcinogenesis, but offered no specific mechanism by which this might be induced.

Proposed nonmutational mechanism of induction of genetic instability, which in turn causes tumour formation (figs. 1, 2)

To summarise the foregoing, a theory of carcinogenesis must accommodate the following: (i) tumours can be induced by mutagenic and non-mutagenic substances; (ii) tumours occur almost exclusively in cell types which are dependent on continuing proliferation of local stem cells; (iii) tumours manifest genetic instability early in their course and (iv) tumours morphologically obey to a greater or lesser degree the specialisation precommitments of their cell of origin.

The proposal of this paper is that the critical event occurs during S phase of mitosis of a stem cell. It is suggested that a carcinogen, or its derivative or secondary product, attaches to the functional site of a DNA replicative-fidelity enzyme (i.e. to a protein), rather than the DNA itself, and causes inaccurate proofreading of a long segment of DNA. This is because there are approximately 100 initiation sites per chromosome, and one DNA polymerase/proofreading molecule for each initiation site. Thus, each DNA polymerase molecule with proofreading enzyme is responsible for approximately 1% of the sequence of a chromosome. Among the DNA inaccurately proofread is the gene for a fidelity or repair mechanism, which is rendered mutant so that its enzyme product will be faulty.

At the end of this mitosis, one daughter cell is unaffected, but the other carries the mutant gene. If the daughter cell with the mutant gene is the continuing stem cell, then genetic instability is established in a proliferating cell line. (If the cell with the mutant gene is in a 'transit-amplifying' cell line [22, 23], the mutation would die out.) Since genes are encoded on both strands of the DNA double helix (24), any particular mutant gene may have a 50% chance of passing to the continuing stem cell.

Formation of tumours after the incorporation of the mutant gene into the stem cell line may not be of great significance if the stem cell divides only occasionally. Secondary mutations would accumulate only slowly. However, if mutations of mitosis-suppressing (mainly tumour suppressor genes – see above) occur either at the time of the first carcinogen-induced loss of accurate proofreading, or at subsequent divisions of the genetically unstable stem cell, tumour cell accumulation would occur more rapidly, with secondary mutations accumulating at a correspondingly faster rate. Mutations of morphology-related genes could occur before mutations of growth-control genes, and thus cause in situ morphological abnormalities as discussed by the author previously [2, 3]. Mutations of specialisation genes would inhibit complete specialisation among some cells. The whole mass of resulting cells would then be likely to include a mixture of proliferating, self-replicating cells, and partially differentiating cells. Invasion and metastasis could occur by de-repression of genes for motility and invasiveness as are characteristically active in embryonic life, and among some particular adult cell types such as leukocytes.

If a carcinogen is both a proofreading enzyme inhibitor and a mitotic stimulant, it becomes a complete carcinogen

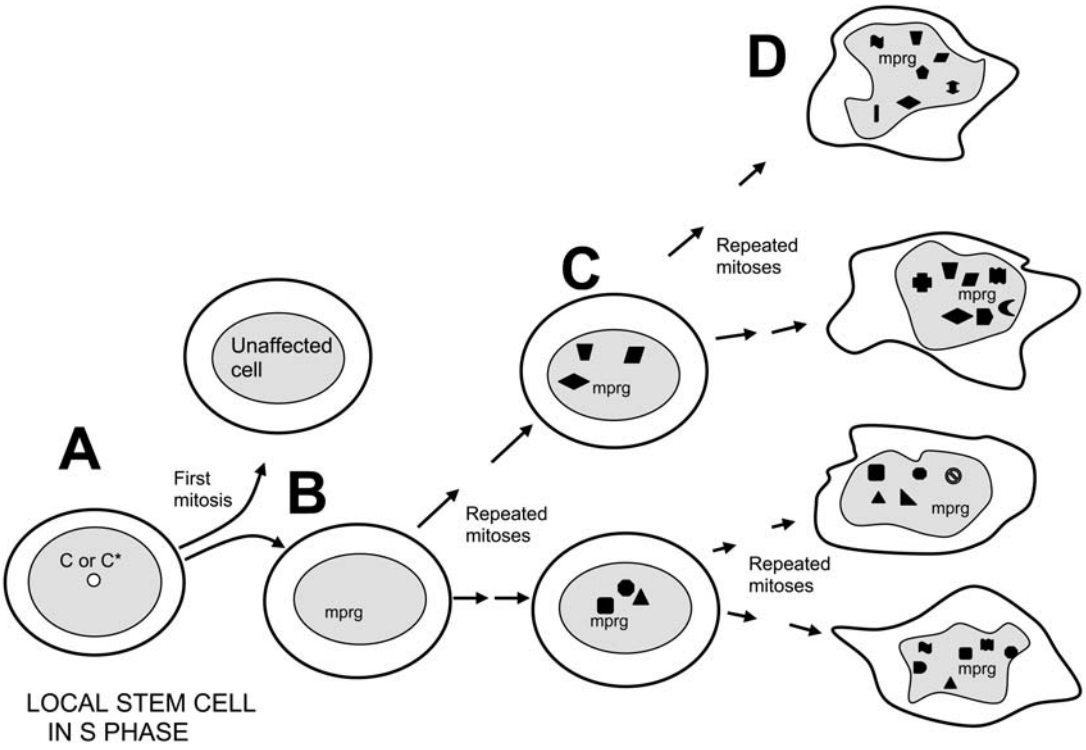

Figure 2. Hypothesis for a nongenotoxic mechanism of induction of genetic instability and neoplasia. (*A*) Local stem cell in S phase with a proofreading site affected by carcinogen inaccurately replicates a gene for replicative fidelity or repair of DNA. (*B*) Mutated gene for replicative fidelity or repair passes to daughter stem cell, causing mutation-based genetic instability. (*C*, *D*) Repeated mitoses result in secondary mutations which cause the stem cell to give rise to a population of cells which accumulate and show variable morphological and behavioural abnormalities. C, carcinogen; C*, activated carcinogen; O, DNA proofreading site disabled by carcinogen while transcribing a proofreading or repair gene; mprg, mutant proofreading or repair gene; other block shapes, secondary mutations.

as recognised in the literature [107], and correspondingly more potent.

It is likely that transit-amplifying mitoses are also affected by applications of carcinogens. However, all daughter cells of these mitoses are eventually lost to the surface. It is possible that during the phase of synthesis of poorly proofread DNA, a mutation occurs for reverse tissue specialisation, so that one of the TA cells reverts to being a stem cell (this is discussed by Sell [26], and the author [2, 3]). Such a sequence is supported by the accumulating evidence of plasticity of behaviour of stem cells [128]. However, because the mechanisms of asymmetric division of stem cells are not understood, it is not (yet) possible to postulate how such a TA cell might be reverted to a stem cell.

Plausibility of the proposed scheme in terms of mitoses of stem cells

Because stem cells are immortal (nonsenescent) and proliferative, only an increase in their mitotic rate is required for cell accumulation to be achieved. Inhibition of any tumour suppressor gene by mutation, or de-repression of any growth factor gene, would probably have this effect.

Numbers of mitoses available for the action of carcinogens

Partial functional derangement of the active site of a fidelity-of-replication site is likely to be a rare event in tissues. However, large numbers of mitoses occur in the lifetime of individuals. Alberts and co-workers [24] suggest that total individual human lifetime mitoses may number 10^{16}, so that stem cell mitoses (as distinct from amplifying mitoses [23, 24]) may well number 10^{12}–10^{13}. The proposal that genetic instability can be induced in a stem cell but not become manifest for long periods because of the requirement for secondary mutations of genes for growth control to occur is consistent with the greatly increasing incidence of tumours with increasing age in the human population [24].

If the functions and fates of transit-amplifying cells are plastic (see [128]), then they may more easily revert to stem cells under the influence of carcinogens than previously thought. Such a phenomenon would mean that the mechanism proposed is even more plausible, as mitoses of

transit-amplifying cells as well as mitoses of stem cells would be available as targets of carcinogens.

Numbers and spatial accessibility of DNA proofreading enzyme molecules during S phase

The human genome consists of 46 chromosomes, and during S phase, approximately 100 replication sites are probably present per chromosome. Therefore, approximately 4600 molecules are active in S phase at any one time. The DNA and associated enzymes are in the cytoplasm during S phase, so that a carcinogen entering the cell may well encounter the proofreading mechanism almost immediately and not be neutralised by other cytoplasmic proteins.

Phenomena of experimental carcinogenesis explained by the hypothesis

Nonmutagenic carcinogens and noncarcinogenic mutagens

The classic experimental model of initiation and promotion of tumours (Berenblum) would be explained if the initiators act by interfering with fidelity enzymes (a toxic effect) and the promoters act as mutagens or mitosis-provoking factors or both.

Nonmutagenic carcinogens [4, 5] (and see above) may act because the initiating mechanism of neoplasia is not mutational, but toxic. However, because the next steps (enhanced proliferation, loss of senescence and so on) require mutation, a carcinogen is more potent if also mutagenic.

Mutagens which are not carcinogens [4, 5] may lack the chemical characteristics to nonlethally derange the DNA fidelity enzyme sites. This is consistent with an observation that many carcinogenic mutagens (such as polycyclic hydrocarbons) are bulky [129], while some noncarcinogenic mutagens (such as trivalent chromium [130]) are not. Alternatively, the carcinogenicity of nickel [131, 132] may relate in some way to the zinc-finger structure of the DNA proofreading site.

Time delays in development of tumours by carcinogens

The events involved in the hypothesis require time, as secondary mutations having the required morphologic and behavioural manifestations require time to develop. The first event is the conferring of potential for genetic instability in the stem cell line. Appearance of the tumour usually will require several months for sufficient mitoses to occur and genetic instability to increase the number of mutations present, so that abnormal morphology or behaviour become manifest.

Mechanism of action of physical and viral agents

Ionising radiations induce tissue damage by well-recognised free-radical mechanisms. The simplest mechanism by which these might affect the enzymic site of the proofreading mechanism is by direct hit on the enzyme during mitosis of a local stem cell. Similarly, ultraviolet light may act directly on the proofreading enzymes rather than by some secondary mechanism involving DNA (see above). Viral infections of cells might release viral proteins, or abnormal endogenous substances which interact with the binding site, and have the same effect.

Nonchemical promoters of experimental carcinogenesis

Any substance or insult which provokes mitosis in the target cell type (such as partial hepatectomy for the induction of hepatocellular carcinomas in experimental animals [133]) hastens the process of neoplasia by providing more mitoses and fidelity-checking sites for the carcinogen to target and for the mutational second steps to occur.

Hormonal initiators and promoters of carcinogenesis

In some experimental models using in-bred strains of experimental animals, hormonal prestimulation is essential for the appearance of tumours, and these sometimes appear without application of any other known carcinogen [88, 134]. The likeliest explanation of these phenomena is that the inbreeding process has already selected animals with abnormal proofreading mechanisms in the stem cells of some their tissues. The hormone then provides the stimulus for cell division and expression of genetic instability and tumour formation, within the short (compared with human) life span of the experimental animal.

Commonly in tumours of hormone-sensitive organs of the body such as the breast or prostate, hormone-antagonist drugs have some clinical benefit [135]. This may be due to elimination of hormone-dependent component of mitotic activity. However, once growth of this population of cells ceases, populations with nonhormone dependent mitotic activity continue to proliferate, so that the therapeutic effect is temporary.

Testing the hypothesis

In any tissue exposed to carcinogen but before tumour appears, the number of cells in which the essential initiating event is taking place would be low. Investigations attempting to demonstrate the key requirement of the hypothesis, namely binding of (labelled) carcinogen to the DNA proofreading site in tissues would take this into account. The number of cells with the lesion would be increased in cultured cell lines which have a high turnover rate and are in a synchronised mitotic cycle [136]. This may therefore be a suitable experimental preparation. To

show that the proofreading site was partially disabled, the relevant enzyme (exonuclease or other) might then be used in assays using homonucleotide sequences in the template DNA strand, as described by Tran and co-workers [137].

Conclusions

This paper addresses the issue of the initiation of genetic instability as the first step in tumour formation by both mutagenic and non-mutagenic carcinogens, especially so that the tumours morphologically resemble their cell of origin. A hypothesis which accommodates these phenomena is proposed based on the literature of several previously distinct aspects of cancer research. It is based on the well-established concepts of interactions of carcinogen with proteins and the prime importance of the stem cells of tumour-susceptible tissues. It is suggested that carcinogens act primarily on the DNA proofreading enzymes (proteins) during mitosis of stem cells. With the inherent properties of stem cells (immortality and prior specialisation-commitment) already conferred, a genetically unstable stem cell line is created which is susceptible to loss of genes controlling proliferation, morphology, specialisation and motile functions.

Cancer is therefore seen as a disorder of genetic stability created in tissue stem cells by carcinogens acting on DNA proofreading mechanisms, and manifest by the biologic effects of diverse secondary mutations of genetic instability rather than any specific effect of mutation(s) of any particular gene(s).

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