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Hypothesis

How carcinogens (or telomere dysfunction) induce genetic instability: associated-selection model

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Abstract Carcinogens induce carcinogen-specific genetic instability (defects in DNA repair). According to the 'direct-selection' model, defects in DNA repair per se provide an immediate growth advantage. According to the 'associated-selection' model, carcinogens merely select for cells with adaptive mutations. Like any mutations, adaptive mutations occur predominantly in genetically unstable cells. The 'associated-selection' model predicts that carcinogen-driven selection minimizes cytotoxic but maximizes mutagenic effects of carcinogens. A purely mutagenic (neither cytotoxic, nor cytostatic) environment will favor effective DNA repair, whereas any growth-limiting conditions (telomerase deficiency, anticancer drugs) will select for genetically unstable cells. Genetic instability is a postmark of selective pressure rather than a hallmark of cancer per se. Once selected, genetic instability facilitates the development of resistance to any other growth-limiting conditions. As an example, a putative link between prior exposure to carcinogens and the ability to develop a telomerase-independent growth is discussed. © 2001 Published by Elsevier Science B.V. on behalf of the Federation of European Biochemical Societies.

Key words: Carcinogen; Cancer; Selection; Genetic instability; Telomerase

1. Introduction

DNA repair exists to repair DNA. Therefore, one expects that cells that have survived exposure to DNA-damaging carcinogens will effectively repair DNA. It may seem logical that inhibition of DNA repair will increase the cytotoxicity of alkylating agents, which damage DNA. This paradigm is a basis for combining of alkylating drugs (which damage DNA) with novobiocin (which inhibits DNA repair) for the treatment of cancer [1].

On the other hand, a recent publication by Bardelli et al. describes that mutagenic carcinogens, including alkylating agents, select for cells with carcinogen-specific genomic instability. Furthermore, cells with carcinogen-specific genomic instability survive exposure to carcinogens [2]. Although these results are somehow contraintuitive, they have been theoretically predicted [3]. In a highly mutagenic environment, it might be too 'costly' for a cell to repair DNA [4]. According to this view, it is DNA repair that is harmful for cells exposed to a mutagen. Futile cycles of DNA repair is a growth disadvantage in a mutagenic environment [4]. Vice versa defective DNA repair (genomic instability) provides an immediate growth advantage.

However, even if genetic instability has no immediate growth advantage, these phenomena (namely: (i) mutagenic carcinogens select for carcinogen-specific genomic instability, and (ii) carcinogen-specific genomic instability allows cells to survive exposure to carcinogens) can be predicted.

Furthermore, the alternative model explains and predicts a broad range of unrelated events from telomerase-independent growth and cell immortalization to induction of genetic instability in normal cells following treatment of leukemia. But more on this later.

1.1. Carcinogen-specific genetic instability

Different forms of DNA damage require different sensing and repair enzymes. Thus, there are separate types of genetic instability. For example, chromosomal instability (CIN) is manifested by chromosomal rearrangements leading to aneuploidy. Numerous agents including bulky-adduct-forming (BAF) agents cause chromosomal rearrangements. BAF-induced DNA damage is repaired by nucleotide excision. BAF mutagens select for cells with defects in nucleotide excision repair, that in turn precludes the repair of DNA damage caused by BAF [2].

Another type of genomic instability, microsatellite instability (MIN), is characterized by deficiency in nucleotide mismatch repair. Alkylating carcinogens cause G/T mismatches and select cells with a deficiency in nucleotide mismatch repair [2]. In brief, carcinogens favor the breakdown of the specific repair of DNA damage which is inflicted by this carcinogen [3,4].

1.2. Genetic instability

A high mutation rate due to genetic instability is a hallmark of cancer. Genetic instability appears early in carcinogenesis [3,5]. However, elevated mutation rate, per se, cannot explain the rise and expansion of cells with elevated mutation rate [3]. How is genetic instability selected? There are essentially two models that may describe this process. Ironically, they are used interchangeably, as one model, because they share a common element: selection for growth advantage. Genetic instability may provide an immediate growth advantage (direct-selection). Alternatively, genetic instability provides no

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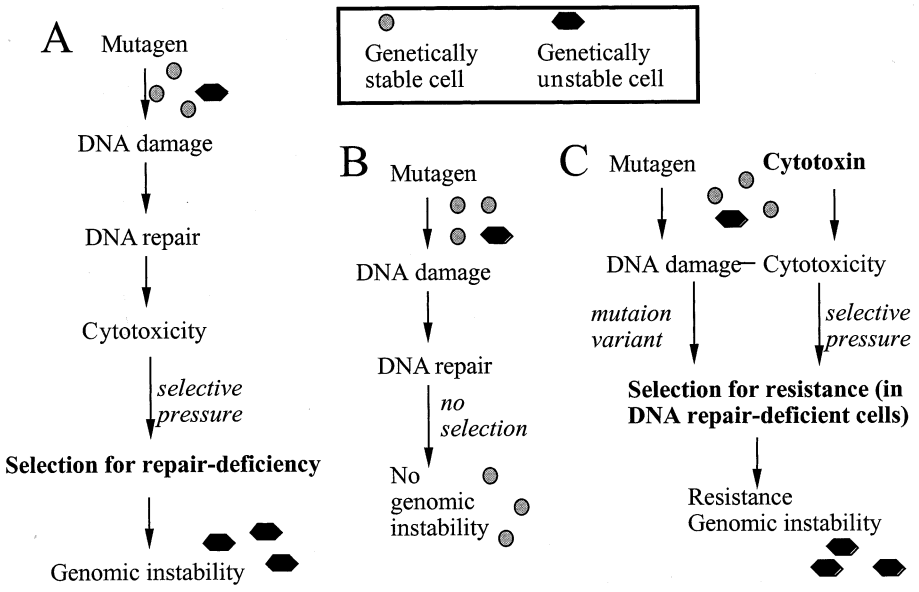

Fig. 1. Two models: selection for genomic instability. A: The direct-selection (mutation-driven) model. DNA repair, rather than DNA damage, causes cytotoxicity. Repair-deficient cells are selected in highly mutagenic environment. B: According to the associated-selection model, pure mutagens (non-cytotoxic agents) do not select for genetic instability. C: Associated-selection (cytotoxicity-driven) model. In genetically stable cells, DNA is repaired, and there will be no pool of mutations to select for resistance. The population of genetically stable cells will be eliminated. In repair-deficient cells, there will be a pool of mutations from which to select for resistance to cytotoxic pressure. The cytotoxicity-driven selection results in both resistance and genomic instability

immediate growth advantage but it is selected in association with growth-promoting mutations.

1.3. Direct-selection model: mutagen-driven selection

In a highly mutagenic environment, DNA repair may limit cell growth [4]. Under highly mutagenic environment, it may be too costly to repair mutations. By employing only one factor (mutagenesis), this concept explains a carcinogen-driven selection. I will refer to this concept as to as 'direct-selection (mutagen-driven)' model (Fig. 1A). Although the appeal of this model is that one single factor (mutagenic environment) both creates a mutation repertoire and selects for genomic instability, the problem is that it must be reconciled with the fact that DNA repair exists, generally speaking, in order to repair DNA.

1.4. Two elements of selection

Selection depends on two essential elements, i.e. availability of genetic variation and selection barrier [5]. Selection pressure is the major driving force in tumorigenesis [6]. Selection barrier eliminates sensitive cells, selecting for adaptive mutations. Adaptive mutations, predominantly arise in cells with a high mutation rate. In other words, genetic instability provides a repertoire of mutants from which cytotoxic pressure selects favorable variants.

In normal conditions, when cells are adapted to their environment, there is no selection pressure. Given that most mutations are deleterious, mutations are repaired and a low mutation rate is sustained (Fig. 1B). A purely mutagenic environment favors effective DNA repair. In theory, a mutagen that is not cytotoxic will not select for genomic instability (Fig. 1B). Under cytotoxic conditions only, a cell should adapt to the environment in order to survive. Then a mutation repertoire is needed. Genetically stable cells cannot overcome a selective barrier [5]. And an exposure to carcinogen is a selective barrier.

1.5. Dissociation of mutagenic and cytotoxic effects

Cytotoxic and mutagenic effects of carcinogens are not necessarily hardwired. In theory, two agents (mutagen and cytotoxin) can replace one carcinogen (Fig. 1C). There are several lines of evidence that mutagenic (DNA-damaging) and cytotoxic effects can be separated. First, many carcinogens (e.g. TPA) are cytotoxic/cytostatic but are not mutagenic [7]. Second, a mutagenic agent by itself is not necessarily cytotoxic. Thus, somatic cells might tolerate the production of large numbers of mutations [7]. Genetically unstable cells (e.g. cancer cells) are highly viable. The cytotoxicity of DNA-damaging (mutagenic) agents in part depends on the activation of apoptotic pathways [8]. In turn, apoptosis can be inhibited without any effects on DNA strand breaks and their repair.

1.6. Association-selection model: cytotoxicity-driven selection

Cytotoxicity-driven selection is the basis for the 'associated-selection' model (Fig. 1C). Why does a carcinogen select for genomic instability? Technically speaking, it does not. A carcinogen selects for mutations that confer resistance to its cytotoxicity, but these mutations happen to occur in genetically unstable cells (Fig. 1C). In other words, 'genetic instability is observed in the final tumor because the instability mutations propels progression and is carried along as a passenger with the clonally selected alterations' [5].

1.7. Direct- versus associated-selection models

According to the associated-selection model, 'when a cell inactivates an instability gene, there is no immediate growth advantage to that cell' [5]. In contrast, the direct-selection model demands that 'if a mutated instability gene is selected

during tumor progression it must be related to the growth advantage of this genetic alteration' [3].

The direct-selection model has emerged to solve 'the paradox that a DNA-damaging and repair-demanding environment potentiates the growth advantage of repair deficiency' [3]. According to the associated-selection model, there is no paradox in the first place. It is a growth-limiting (not 'DNA-damaging and repair-demanding') environment which favors a repair deficiency.

2. Predictions of the associated-selection model

2.1. Carcinogen-specific genetic instability according to the associated-selection model

As discussed, a mutation repertoire is required to select for a carcinogen-resistant phenotype. Under cytotoxic conditions, carcinogen-specific genetic instability merely reflects the need in a mutation repertoire. To acquire a mutation, both DNA damage and failure to repair this damage are needed. Obviously, DNA damage and repair deficiency should match each other. Because carcinogens caused carcinogen-specific damage, it must be complemented by carcinogen-specific repair defects. BAF agents, which induce chromosome breaks, select for the inability to repair these breaks (CIN) [2]. Alkylating agents, which cause nucleotide mismatch, select for a deficiency in nucleotide mismatch repair (MIN) [2].

A cytotoxic mutagen selects for a specific kind of repair deficiency which maximizes the number of mutations. Although the goal is not selection for genomic instability but selection for resistance, it could be only achieved by maximizing a mutation rate. Carcinogen-driven selection minimizes cytotoxic effects and maximizes mutagenic effects of carcinogens (Fig. 1C).

2.2. Cytotoxic/cytostatic conditions favor genetic instability

The associated-selection model predicts that cytotoxic agents, lacking any mutagenic activities, must select for genetic instability. Indeed, hypoxia, low pH, and nutrient deprivation induce genetic instability [9]. As another example, a monomer of plastics (which is not mutagenic) induces cellular

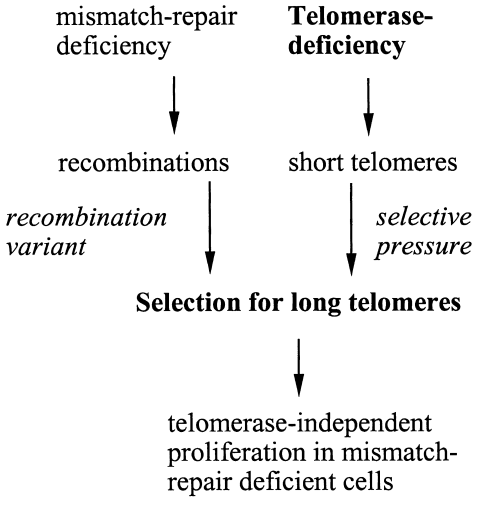

Fig. 2. Genetic instability and telomerase-independent proliferation. As a particular case of the associated-selection model (Fig. 1C), telomerase deficiency and mismatch repair deficiency correspond to cytotoxicity and mutagen, respectively.

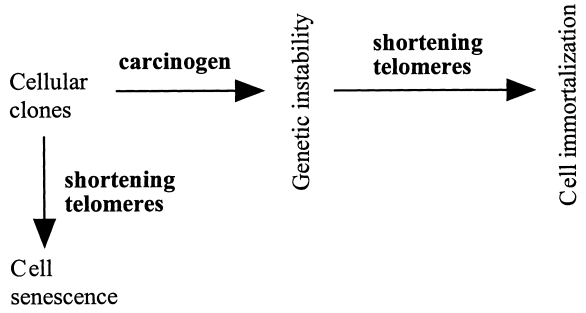

Fig. 3. Carcinogen-selected genetic instability permits immortalization.

transformation and aneuploidy (genetic instability) [10]. A plausible reinterpretation of these phenomena is that hypoxia, deprivation, plastics, etc. select for rather than induce genetic instability. Hypoxia selects for hypoxia resistance causing tumor progression [11]. Genetic instability is an universal phenomenon in cancer because it is 'selected' even by non-mutagenic carcinogens. Due to endogenous sources of mutations [12], carcinogens are not necessarily mutagenic. Furthermore, genetic instability is not restricted to cancer. For example, genetic instability arises in normal T cells from patients who had received chemotherapy for B-lineage acute lymphocytic leukemia [13]. Genomic instability also occurs in ulcerative colitis [14]. Genetic instability is a postmark of selective pressure rather than a hallmark of cancer.

2.3. Defects in mismatch repair promote telomerase-independent proliferation

The title of a recent paper in *Nature* indicates that defects in mismatch repair promote telomerase-independent proliferation in yeast [15]. On the other hand, telomere dysfunctions increase a mutation rate in yeast [16]. This relationship between telomere dysfunction and genetic instability is a reminiscent of the relationship between carcinogens and genetic instability. Carcinogens promote genetic instability and genetic instability promotes cells' survival. Yet, the link between carcinogen-promoted and telomerase-independent growth is not obvious. Unless... we simply consider a telomere shortening as a growth-limiting condition.

2.4. Telomere shortening as a growth-limiting condition

Telomeres (the ends of chromosomes) are essential for chromosomes maintenance. Telomeres shorten with each cell cycle because DNA polymerases do not replicate the very end of telomeres. This shortening limits the number of cell divisions. Telomeres can be maintained (in germline and cancer cells) by telomerase, a reverse transcriptase that 'synthesize' telomeres. But telomeres also can be maintained by an alternative mechanism: by a homologous recombination [17]. Like mutations caused by alkylating agents, a homologous recombination is prevented by mismatch repair machinery.

Telomerase-negative mutant yeast cells undergo telomere shortening over many cell divisions. Survivors maintain their telomeres. Telomere shortening is a growth-limiting condition that selects for longer (recombinant) telomeres. Telomeres recombination occurs in mismatch repair-deficient cells. It is not surprisingly that mismatch repair-deficient cells displayed growth advantage [15]. Technically, defective repair does not

promote proliferation. Defective repair increases the frequency of recombinations, leading to longer telomeres. It is longer telomeres, not genetic instability, which is selected. But longer telomeres occur in cells with mismatch repair deficiency (Fig. 2). Thus, telomerase deficiency selects for mismatch repair deficiency.

2.5. Carcinogen exposure and telomere dysfunction: barriers or promoters

As recently emphasized, it might seem paradoxical that the cytotoxicity caused by telomere erosion promotes cancer initiation [18]. According to the cytotoxicity-driven (associated-selection) model, this is exactly what one should expect. A selective barrier such as telomere dysfunction can either promote or inhibit immortalization and carcinogenesis. Development of cancer in telomerase-deficient mice required inactivation of p53 [19]. Inactivation of p53 leads to genetic instability [20]. Inactivation of p53 converts telomere based crisis from the barrier to tumor development into one that enhances cancer initiation [18]. In theory, a prior exposure to a carcinogen can substitute for p53 inactivation. By selecting for carcinogen-tolerance, carcinogens indirectly select for genetic instability (Fig. 3). In turn, genetic instability may permit telomerase-independent growth (or immortalization) (Fig. 3). Telomere erosions limit growth and cause senescence of cells that were not exposed to carcinogens and retain proficient mismatch repair (Fig. 3). A selective barrier is a hurdle for cells with a low mutation rate but it is a tumor promoter for cells with a high mutation rate.

2.6. Conclusion: combining alternative paradigms

Genetic instability is a well-recognized hallmark of cancer [3,5,7]. The concept of cancer as a mutator phenotype is based on the rarity of mutations in normal cells and the high frequency of mutations in malignant cells [21]. The question is how is genetic instability induced. The concept, which I refer to as the associated-selection model, has been described previously [5], but it was not clearly distinguished from 'coexisting' direct-selection model. In a broader sense, two models which describe selection of genetic instability (direct- and associated-selection) stand between two extreme views. First view is that selection is not necessary for a rise of genetic instability which is, accordingly, a simple accumulation of mutations in genes that repair DNA. Accordingly, carcinogenic environment is essentially mutagenic. For example, raise of genetic instability in chronic ulcerative colitis, a premalignant condition, can be explained by the production of mutagenic radicals. 'The amount of damage may exceed the capacity of the DNA repair machinery, resulting in mutations including in genes that effect genetic instability' [22]. This example however also supports the thesis that cytotoxic (non-mutagenic) environment in ulcerative colitis selects for genetic instability. By dissociating mutagenic and cytotoxic effects of carcinogens, this review envisions the associated-selection model as cytotoxicity-driven selection. The model predicts that a purely mutagenic environment favors DNA repair, whereas cytotoxic environment favors genetic instability. This thesis is best supported by examples of selection for genetic instability by non-mutagenic carcinogens, hypoxia, and anticancer drugs. Genetic instability is a postmark of cytotoxic selective pressure. Although it is a hallmark of many cancers, it is not necessarily restricted to cancer.

On the other extremity, it has been argued that increased mutagenesis is not required to produce the multiple mutations observed in cancer. A raised mutation rate may make evolution (or tumorigenesis) faster, but is not necessary for evolution (or tumorigenesis) to occur [6].

Regardless of whether genetic instability is necessary or unnecessary for tumorigenesis, the associated-selection model predicts an increase in genetic instability during tumor progression. As mentioned a high mutation rate may make tumorigenesis faster [6], and this difference may be crucial for the development of overt cancer in our limited life span. Because the cytotoxicity-driven selection for genomic instability is flexibly associated with carcinogenesis, the time of rise of genetic instability is also flexible: it may precede cancer [14,22], it may appear early in carcinogenesis [23–25], or it may be selected after overt cancerous alterations occur [6,26,27].

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