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Hypothesis

Do mitochondrial DNA fragments promote cancer and aging?

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Reactive oxygen species are important in carcinogenesis, diseases, and aging, probably through oxidative damage of DNA. Our understanding of this relationship at the molecular level is very sketchy. It has recently been found that in mitochondria oxidative DNA damage is particularly high and may not be repaired efficiently. I propose that oxidatively generated DNA fragments escape from mitochondria and become integrated into the nuclear genome. This may transform cells to a cancerous state. Time-dependent nuclear accumulation of mitochondrial DNA fragments may progressively change the nuclear information content and thereby cause aging. This proposal can be tested experimentally.

Reactive oxygen; Cancer; Aging; DNA; Mitochondria

1. OXYGEN IN CANCER AND AGING

Reactive oxygen species such as the superoxide radical (O_2^-), hydrogen peroxide (H_2O_2), the hydroxyl radical ($OH^\cdot$) and singlet oxygen are produced by normal metabolism, irradiation, or some chemicals including tumor promotors. The very aggressive $OH^\cdot$ is mainly formed by reducing H_2O_2 with O_2^- and heavy metal ions. There is evidence that reactive oxygen plays an important role in multistep carcinogenesis, diseases, and aging [1–7].

Agents which catalytically destroy reactive oxygen species [6,8] and serve as antioxidants [7,9,10] inhibit various steps in neoplastic transformation and defend cells in a manner which may vary among species and tissues [8,11,12]. For example, superoxide dismutase [8], catalase [11], vitamin A analogues [12] and vitamin C [13] suppress cell transformation and, in some cases, inhibit the action of some tumor promotors [8,12,14]. Evidence for the participation of reactive oxygen in carcinogenesis is also found by relating cancer, metabolic rate and life span in various species [15]. Thus, the cumulative cancer risk increases with about the fourth power of age in short-lived species (about 30% of rodents have cancer by the end of their 2–3 year life span) and in long-lived species (about 30% of humans have cancer by the end of their 85 year life span). Cancer has in this context been considered as the price to be paid for longevity.

Aging has been defined as the progressive accumulation of changes with time associated with or responsible for the ever-increasing susceptibility to diseases and death which accompanies advancing age [16]. Several theories of aging have been proposed including that of DNA alterations, lower accuracy of protein synthesis ('error catastrophe'), cross-linking of macromolecules, and failure of the immune system. Yet, our understanding of aging at the molecular level is nil, and aging research is still in a phenomenological phase. The free radical theory of aging [16,17] has received much attention since reactive oxygen species can damage compounds of fundamental biological importance.

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Consistent with it are the increase in life span upon food restriction [18,19] and the high levels of antioxidants in humans which may have facilitated the evolution of man's longevity [20–23]. The important factor responsible for longevity appears to be the basal metabolic rate [24,25], i.e. the quantity of oxygen consumed (on a body weight basis), which is higher in short-lived than in long-lived animals. The free radical theory of aging has recently been criticized by Pryor [26] who suggests that reactive oxygen may act indirectly by causing the age-related increase in diseases, such as diabetes, cataracts, and rheumatism.

The critical targets of reactive oxygen which may be affected during carcinogenesis and aging have not yet been identified with certainty. Because it carries the information between generations of somatic cells, DNA has been given greatest attention.

2. OXIDATIVE DAMAGE TO DNA AND ITS RELATIONSHIP TO CANCER AND AGING

Oxidative damage to DNA could be a major factor in cancer and aging [7,24,25,28]. Damage is caused by reactive oxygen species, lipid hydroperoxides and their radical and aldehydic degradation products [29,30], and oxidation products of aromatic amino acids and purines. Hyperbaric oxygen induces mutations in bacteria [31] and chromosomal aberrations in eukaryotic cells [32,33], probably because the amount of reactive oxygen produced by cellular metabolism in this situation is increased [34]. Indeed, O_2^- and H_2O_2 mutagenize bacteria and induce DNA breaks and chromosomal aberrations [35,36]. Characteristic DNA lesions are single- and double-strand breaks, apurinic and apyrimidinic sites, and products of the 5,6-dihydroxydihydrothymine type. O_2^- induces strand breaks in intracellular DNA [37,38] and liberates nucleobases from nucleotides [39]. Oxygen-dependent DNA damage is also observed with aflatoxin B_1 [40] and phorbol esters [41,42]. The formation of thymine glycol and thymidine glycol in vivo has been used as a possible assay for oxidative DNA damage [28,43]. Very recently, the formation of 8-hydroxydeoxyguanosine in DNA by oxygen radicals was reported [44–49].

Several models relate oxidative DNA damage to aging and cancer [28]. For example, free radicals could react with nuclear DNA and produce somatic mutations including point or frameshift mutations, deletions, and strand breaks. Even without causing a mutagenic event, oxidative DNA damage may lead to cancer and aging, for example by inducing loss of 5-methylcytosine since methylation of cytosine may be important in turning off genes in differentiation [50]. This type of damage would be particularly important for terminally differentiated cells that do not normally undergo DNA replication.

3. MITOCHONDRIA AND OXYGEN

Mitochondria consume about 90% of the cell's oxygen, and the mitochondrial respiratory chain is the source of a continuing flux of oxygen radicals [34,51]. Superoxide production in mitochondria increases with advancing age [52]. In man about 2×10^{20} molecules O_2 are reduced per g of tissue per day. If only 3 parts per 10^8 of this oxygen form $OH^\cdot$ this would be equivalent to an exposure of 1 rad ionizing radiation [27]. Thus, endogenously produced oxygen radicals pose a major threat to aerobically living organisms. This can also be illustrated by another calculation: A rat has a roughly 6-fold higher basal metabolic rate than man. Assuming 1000 mitochondria per cell and a yield of 1% O_2^- from O_2 consumed in mitochondria [34] it follows that about 10^7 O_2^- are formed/mitochondrion per day in a rat weighing 75 g. Despite the presence of antioxidants in mitochondria, a considerable amount of $OH^\cdot$ may therefore be produced. The steady-state concentration of oxygen radicals may be even higher in tumor mitochondria since they have diminished amounts of superoxide dismutase [53,54].

4. MITOCHONDRIAL DNA

Mitochondria possess their own DNA. It is present in multiple copies of double-stranded supercoiled circular molecules which are not covered by histones. During evolution, the size of the mitochondrial genome became progressively smaller. It contains about 16500 base pairs in mammals where it codes for a set of 37 genes specifying 22 tRNAs, 13 mRNAs, and 2 rRNAs. The mitochondrial genome evolves 5–10-times faster

than the nuclear genome of the same organism [55].

There is a general consensus that mitochondria are less efficient in repairing DNA damage and replication errors than the nucleus [56–59]. For example, they lack excision repair and recombinational repair [60,61]. Mitochondria do, however, possess three uracil DNA glycosylases [62–64], two endonuclease activities specific for apurinic/apyrimidinic sites [Tomkinson, A.E. et al. (1988) submitted], endonucleases that act on lesions introduced by high UV doses (Tomkinson, A.E., personal communication), and a DNA ligase [65]. It is reasonable to suppose that these enzymes participate in DNA repair and replication though some of them may also have a role in eliminating damaged DNA molecules.

8-Hydroxydeoxyguanosine is present in DNA isolated from normal rat liver mitochondria at 16-times the level of nuclear DNA [49]. The total oxidative damage of mitochondrial DNA must be very high, since 8-hydroxydeoxyguanosine is just one of several oxidized bases which are formed when $OH^{\cdot}$ reacts with DNA [66]. Besides oxidizing DNA bases, the indiscriminately reacting $OH^{\cdot}$ also causes DNA strand breaks [67]. Indeed, this type of damage is also seen in mitochondrial DNA. For example, adriamycin which damages nuclear DNA in an oxygen-dependent manner, also causes strand breaks in mitochondrial DNA of rat heart in vivo [68. Bleomycin introduces nicks in mitochondrial DNA of mouse fibroblasts [69], and DNA of isolated liver, lung and tumor mitochondria in an oxygen-dependent manner [70]. Also prooxidants like alloxan and *t*-butyl hydroperoxide fragment mitochondrial DNA of rat liver (Richter, C., unpublished). The high steady state of oxidative damage in mitochondrial DNA is most likely due to a copious flux of oxygen radicals, inefficient repair, and the nakedness of mitochondrial DNA.

5. HYPOTHESIS

The large number of mitochondrial DNA copies in most cells and the massive oxidative damage comprising oxidized bases and strand breaks together with the inefficient repair suggest the presence of an appreciable amount of DNA fragments in mitochondria in vivo. Some of these fragments may escape from the organelle and become integrated into the nuclear DNA. The mechanism of DNA transfer from mitochondria to the nucleus and its insertion into the nuclear genome is a matter of speculation. It is important to note, however, that reactive oxygen species destabilize biological membranes [71] and thereby may facilitate the escape of DNA from mitochondria. The subsequent route may be analogous to the insertion of viral or bacterial DNA into nuclear DNA, a common phenomenon in nature.

Integration of mitochondrial DNA fragments into the nuclear genome may transform the cell. This could be due to a variety of events. Genes responsible for the maintenance of normal growth control might become inactivated by insertional mutagenesis. A proto-oncogene might be activated by a promotor insertion mechanism. These events require that the mitochondrial DNA fragments be inserted at particular sites in the nuclear DNA. Alternatively, mitochondrial DNA fragments containing oxidatively damaged sites might be inserted randomly into the nuclear genome. There they may trigger the induction of DNA repair enzymes, some of which are error prone [72–74]. If such repair was sustained for several cell cycles a promutagenic state could be established [5,75,76]. This mechanism (the induction of sustained, error-prone DNA repair) might affect a far greater proportion of the transfected cells than a mechanism requiring the insertion of specific sequences and/or insertion at specific sites. Finally, just random insertion of undamaged DNA fragments might hit an appropriate target, for example a suppressor gene.

The time-dependent accumulation of 'foreign' mitochondrial sequences could also lead to progressive changes in the information content of the nuclear genome and thereby to aging. This may be facilitated by the activities of nuclear repair enzymes because integrated and repaired mitochondrial DNA fragments may no longer be recognized as 'foreign' and therefore be carried over to the next cell generation.

Inter-organellar gene transfer has already been observed. There are reports on mitochondrial DNA sequences being present simultaneously in nuclear and mitochondrial DNA of several species ('promiscuous DNA') (see [77] for a review). These findings strongly suggest that genetic exchange be-

tween mitochondria and nuclei has occurred continuously during evolution. The exciting possibility of transfer of mitochondrial DNA sequences into the nuclear genome during the life-cycle of the fungus, *Podospora anserina*, and its possible role in senescence [78,79] are presently not proven [80].

Alterations in mitochondrial functions, e.g. maintenance of ion homeostasis or ATP supply, have repeatedly been suggested to contribute to cellular transformation since tumor cell mitochondria can differ structurally and functionally from those of normal cells (see [81] for a review), but clear evidence in favour of this suggestion is lacking. There is, however, evidence that some chemical carcinogens primarily attack mitochondria [82–91]. It has also been proposed that mitochondria are the 'molecular clock' in eukaryotes [92] and that mitochondrial genetic damage is one of the fundamental mechanisms underlying aging [93,94].

Most studies in the field of aging are descriptive, and most theories of aging have been difficult to test directly experimentally. The proposal that mitochondrial DNA fragments become incorporated into the nuclear genome and thereby contribute to cancer and aging can be tested with the powerful techniques of molecular biology. Hepatoma cells from hemochromatosis patients may be a good starting material, since iron ions catalyse oxidative mitochondrial DNA damage [49]. Cells in culture with their finite life span, in terms of the numbers of divisions they can undergo, could be used to test the proposal with respect to aging.

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