Is There a Role for Mitochondrial Genes in Carcinogenesis?1

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Summary

Although defective respiration is not characteristic of all tumors, recent comparative studies on the ultrastructure of normal and tumor cell mitochondria indicate that in malignant cells mitochondria deviate from normal not only in relative abundance but also in the size, form, density, and frequency of appearance of lesions. Normal and abnormal mitochondria may populate the same cell, suggesting that there may be a gradation in respiratory deficiency depending on the proportion of normal to abnormal forms.

Recent advances in mitochondrial genetics suggest that aberrant mitochondria may be formed as a result of the presence of an abnormal mitochondrial genome. In analogy with the petite mutant of certain strains of yeast, animal cells may be transformed by treatment with dyes that alter the structure of their mitochondrial DNA, so that their mitochondria also become deficient in enzymes of the respiratory chain. Whether nutritional or other deficiencies are mutagenic with respect to mitochondrial DNA of animal cells is not known; nor is it known whether mitochondrial mutagenesis is causally involved in carcinogenesis. New knowledge of cytoplasmic genetics and of mitochondrial DNA and membrane structure and dynamics should encourage investigations aimed at examining the possible role of mitochondrial genes in neoplastic transformation.

No one can give thought to the subject of mitochondrial metabolism in relation to cancer without recalling the theory of carcinogenesis proposed about 20 years ago by Otto Warburg (33–35). Although much of what he held to be true is presently given little credence, one of his convictions, namely, that the respiration of tumor tissue is defective, still remains viable, as judged by the number of reports, both pro- and con-Warburg, appearing in the cancer literature.

While mention of Warburg in relation to cancer usually calls to mind his concept of the energy metabolism of tumors, it should be noted that Warburg’s belief that tumor respiration was defective was only 1 of 3 related ideas on carcinogenesis. I have taken the liberty of paraphrasing his main thoughts on this problem in the following sentences. (a) Due to damage to its respiratory apparatus, the tumor cell adapts to the life of an anaerobic organism. (b) The defect in respiration, which is the specific stimulus to the development of cancer, is heritable, since the damaged respiratory apparatus is a part of the mitochondrion, which is itself an autonomous organelle. (c) Dedifferentiation is the result of the replacement of respiration, which depends on the structural integrity of the mitochondrion, by fermentation, the reactions of which are catalyzed by enzymes in solution (state of disorganization).

It does not do Warburg justice that most students of oncology have rejected all of his postulates because of their disagreement with 1 (the 1st). While perhaps based less on fact than on the innate suitability of the concept to his overview of carcinogenesis, his idea of an association of a defective, heritable mitochondrion with cancer may, in due time, turn out to be not entirely farfetched.

In regard to the question of energy metabolism of neoplastic as compared with normal cells, there persists an impression, despite recurrent controversy, of the existence of a genuine difference of respiratory function between the 2 kinds of tissues. In his comprehensive review of the glycolysis and respiration of tumors, Aisenberg summarizes his views, in part, in the statement that “the most striking property of neoplastic energy metabolism remains the high glycolytic rates of slices of tumor tissue,” at the same time recognizing that a high rate of glycolysis is not uniquely restricted to tumor tissue (1).

In a more recent survey of the same field, Wenner (36), while emphasizing that the energy derived from glycolysis by minimal deviation tumors neither predominates nor even comprises an appreciable proportion of the total energy generated by the cell, concluded that vehement glycolysis, aerobic as well as anaerobic, remains 1 of the striking biochemical properties of the cancer cell, particularly in the rapidly growing tumor.

More recently, attention has been directed to properties of tumor cell mitochondria that can be visualized under the electron microscope rather than to their biochemical characteristics. Bernhard, in his review of this field of investigation (3), although underlining the great variability of mitochondria in tumor as compared with normal cells, formed the general impression that cancer cells have fewer mitochondria than do their normal counterparts, their number decreasing with development of the tumor. He also noted numerous swollen mitochondria. While recognizing that mitochondrial swelling might somehow be secondary to intensive growth, Bernhard found the same lesions in cells that were well preserved in all respects and presumably were actively growing at the time of fixation. Summarizing his overall impressions of tumor cell mitochondria, Bernhard was struck by the extraordinary variation in number, size, form, density and frequency of lesions that they pre-

1 Presented at the Conference on Nutrition in the Causation of Cancer, May 19 to 22, 1975, Key Biscayne, Fla. Aided by USPHS Grant CA-03651 from the National Cancer Institute.
Mitochondria and Carcinogenesis

sent. He pointed to the necessity, therefore, of critical morphological control of mitochondrial pellets prepared for biochemical studies. These observations as well as similar observations recorded by others (4, 23, 24) strongly suggest that procedures used to isolate mitochondria from normal tissues, when applied to tumor tissue, may eliminate those of greatest biochemical interest. In any case, when consideration is given to the observed pleomorphism of tumor cells mitochondria and to the lesions that have been noted, there would seem to be little reason to expect uniformity of energy metabolism among all types of neoplasms.

As has already been indicated, I of Warburg's beliefs, namely, that mitochondria are autonomous organelles, deserves greater attention than it has received to date. Warburg derived support for his view that mitochondria are heritable structures from plant genetics, a view that early on recognized the existence of cytoplasmic genes. Among the workers whom Warburg cited for special mention were M. W. Woods and H. G. DuBuy of the National Cancer Institute, who found, in leaves of a variegated species of Nepeta cataria (catnip), mixed cells containing multiple mitochondrial types (37). Most significantly, the mitochondrial phenotypes were transmitted to progeny by non-Mendelian inheritance. Borrowing heavily from Woods and DuBuy, who were themselves convinced of the pertinence of their studies to cancer (11), Warburg proposed that, once a mitochondrion was damaged, it remained so, transmitting its defect to progeny (presumably mitochondria), just as would occur, he asserted, in the case of a damaged nuclear gene. Although there would seem to be reason to allow the speculation that cancer cells, through defects in mitochondrial structure, may have nonfunctioning respiratory chains, the question whether such defects are transmissible is as yet highly conjectural.

My assignment as a participant in this meeting is specifically to consider whether, due to aberrations caused by a change of nutritional conditions, mitochondria can play a role in carcinogenesis. Without at this time speculating on that question, there can be no doubt that mitochondrial function per se is sensitive to the withdrawal from the diet of certain vitamins and trace elements. An extreme example is the effect of a lack of dietary copper. In this condition the loss of activity of cytochrome oxidase is so severe that liver mitochondria of animals killed at the height of the reaction the loss of activity of cytochrome oxidase is so severe. Of special significance was the observation that the petite mutation is spontaneous yet occurs with a frequency that can be orders of magnitude greater than another, the petite mutation is spontaneous yet occurs with a frequency that can be orders of magnitude greater than another. As detected simply by a change of one carbon source for another, the petite mutation is spontaneous yet occurs with a frequency that can be orders of magnitude greater than rates of spontaneous nuclear mutations. Under certain other conditions the rate of mutation can reach 100%. Thus Ephrussi et al. (13) found that acriflavine, an acridine dye, could transform an entire population of cells to the petite form. Of special significance was the observation that the yield of mutant colonies was the same whether the parent cells were of a haploid or diploid strain. This independence of the effect of the dye from gene dosage provided strong evidence from which it was concluded that the mutation was not that of a nuclear but of a cytoplasmic gene. In S. cerevisiae, cytoplasmic genes are contained in the mitochondrion, the genetic message being encoded in the mitochondrial DNA.

The frequency of mitochondrial mutation can be increased even when cells are metabolizing under aerobic conditions. Mass formation of petite mutants of S. cerevisiae
was accomplished by preventing mitochondrial synthesis of ATP by inhibiting respiration with cyanide or antimycin A, while simultaneously blocking uptake of glycolytically formed ATP with an appropriate inhibitor (bongkrekic acid). These results were interpreted to indicate that the constant presence of ATP within mitochondria is essential to normal replication of mitochondrial DNA (30). An alternative interpretation is that interference with energy utilization in mitochondria may be mutagenic for mitochondrial DNA.

In the presence of ethidium bromide, a phenanthridine dye that intercalates between complementary bases in duplex DNA, mitochondrial DNA synthesis is selectively inhibited and preexisting mitochondrial DNA is progressively degraded (15). The petite mutation is thereby enhanced to the extent that mass formation of the respiration-deficient mutant occurs. In a concentration that readily enhances production of mitochondrial mutants, ethidium bromide is without effect on the synthesis or degradation of nuclear DNA. This selective action of the dye has now been shown also to be expressed in animal cells.

When mouse L-cells were exposed to a concentration of ethidium bromide, 1 \( \mu g/ml \), the cell content of cytochrome oxidase and cytochrome \( b \) declined (29). The mitochondria enlarged and were noted to have fewer cristae; those that remained appeared abnormally organized. Both normal and abnormal mitochondria were seen in the same cell, suggesting not only a difference among mitochondria in their sensitivity to the dye but also suggesting that mitochondrial mutation is an effect on individual organelles rather than on the cell as a whole. The latter inference would also be consistent with the observation that the loss of mitochondrial cytochromes is partial and for the fact that the action of ethidium bromide on mouse L-cells is reversible. Thus while damaged mitochondria could not replicate, those that were undamaged would be capable of doing so after removal of the dye. This uneven susceptibility of mitochondria to a mutagen may be an explanation for the survival of animal cells that have been exposed to such agents. Unlike the petite mutant that, when its mitochondria are no longer functional, readily adapts to an anaerobic life, animal cells may survive only when loss of mitochondrial viability is incomplete. In the sense that Warburg intended, there would seem to be no facultative anaerobes among animal cells.

Effects of ethidium bromide on HeLa cells (26), Chang liver cells (16), regenerating liver (10), and the like are similar to those to which attention has already been called, i.e., confirmatory of changes in the concentration of mitochondrial cytochromes following from mutagenic effects of the dye. In all instances, effects of ethidium bromide on mitochondrial but not nuclear DNA were observed.

In the biogenesis of mitochondria, 2 separate and distinct genetic systems are involved in the synthesis and assembly of polypeptide constituents of the membranes and enzyme systems, namely, that of the nucleus (cytoplasmic system) and that of the mitochondrion itself. Which mitochondrial components are encoded in nuclear DNA and which in mitochondrial DNA is presently uncertain. In animal cells, mitochondrial DNA consist of circular, double-stranded light and heavy chains of supercoiled molecules having a molecular weight of 9 to 10 million daltons (19, 28, 32). It has recently been estimated that, in the HeLa cell, genes so far identified on mitochondrial DNA account for about 25% of the potential information contained in the 5-nm-long DNA molecule (2). Of singular interest is the fact that, on animal cell and yeast mitochondrial DNA, genes have been identified that encode for tRNA's that almost without exception are specific for the hydrophobic amino acids (9). It is, therefore, no wonder that all proteins synthesized on mitochondrial ribosomes have so far been found to belong to the class of hydrophobic proteins associated with the inner mitochondrial membrane (6).

In petite mutants that have grossly altered or no mitochondrial DNA, it is found that mitochondrion-like structures are formed that contain an outer membrane, an abnormal inner membrane with poorly developed cristae, Krebs cycle enzymes, and an incomplete respiratory chain (6). These observations are consistent with amino acid incorporation studies (31) that suggest that mitochondrial DNA encodes for the synthesis of hydrophobic polypeptides that are essential for complete assembly of functional inner mitochondrial membranes.

As has been indicated above, the presence in cells of altered mitochondrial DNA results in the synthesis of aberrant forms of mitochondrial structures. What then is the case in malignant cells, in many of which mitochondrial DNA is different from normal? In malignant cells the concentration of mitochondrial DNA is usually several times greater than in normal cells, resembling the concentration found in embryonic cells (20). In mouse ascites cells, in which many damaged mitochondria appear, the mitochondrial DNA has an abnormal topography (21). For a recent review of mitochondrial DNA in malignant cells, see Ref. 24.

Great interest has been shown in a unique uncircular DNA dimer peculiar to mitochondria of human leukemic leukocytes. The frequency of such molecules in cases of chronic myelogenous leukemia was found to be proportionately related to the severity of the disease, while in remission the dimer content declined (8). Dimers of this kind are not, however, limited to human tumors (25) but are found also in the normal human thyroid.

At present at least there is no evidence that the information content of the abnormal dimer molecules of mitochondrial DNA of human leukemic cells is different from that of the normal monomer (24). Thus at present the connection between abnormal mitochondrial DNA of human and animal tumors and abnormal mitochondria is purely a circumstantial one. Mitochondrial damage and abnormalities of mitochondrial DNA are common findings in malignant cells. Mitochondrial mutagenesis, induced by intercalating dyes such as ethidium bromide, produces forms that resemble those seen in tumor cells. Changes in mitochondrial structure and in the concentration of mitochondrial cytochromes correlate with informational content of mitochondrial cytochromes correlate with informational content of mitochondrial DNA, and in vitro abnormal changes in
mitochondrial DNA are accompanied by changes in the morphology and cytochrome content of mitochondria. In the light of the role of mitochondrial genes in directing the synthesis of key components of the mitochondrial network, numerous observations of tumor-associated disturbances of mitochondrial function cannot be ignored. To quote an outstanding authority in the field of cytoplasmic genetics: "In the past only nuclear genes were taken into consideration in planning and evaluation of cancer research studies. With the development of our knowledge about cytoplasmic genetics, it would be useful to reconsider past studies and to design new investigations aimed specifically at examining the possible role of cytoplasmic genes in neoplastic transformation" (27).

References
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