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Review

Mitochondrial DNA Damage Is Uncommon in Cancer but Can Promote Aggressive Behaviour

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Abstract. *The mitochondrial genome (mtDNA) has been implicated in carcinogenesis. It is more susceptible than nuclear DNA to damage from reactive oxygen species and mutagens, and has a limited DNA repair machinery. Studies of human cancer have shown that a small proportion of tumours carry significant mtDNA mutations but methodological flaws undermine some of these findings. Mutations in mtDNA are often associated with elevated levels of reactive oxygen species and stabilisation of Hypoxia-Inducible Factor-1 (HIF1), but it has not been clearly demonstrated that these relationships are causal. Some mutations in the coding region of mtDNA can confer increased tumourigenicity, motility and metastasis on cells in vitro and in vivo but these mutations are only rarely found in ex vivo samples. Mitochondrial DNA does not play a major role in common types of cancer, but may promote aggressive behaviour in some cases. Shortcomings in mtDNA repair mechanisms could be exploited to promote apoptosis of tumour cells.*

Human mitochondria contain a circular DNA genome of 16,569 base pairs, encoding a number of protein subunits as well as two rRNAs and 22 tRNAs (Figure 1). The proteins form 13 of the 82 subunits in the mitochondrial electron-transport chain and ATP synthase, and the RNAs are used to synthesize these 13 subunits (1). The mitochondrial genome is, therefore, important for oxidative phosphorylation. It contains a regulatory region, the D-loop, which contains initiation sites for transcription, and replication by the mitochondria-specific DNA polymerase-γ. There are several copies of the genome per mitochondrion, and there may be over 100 copies per cell. Their replication is not tied to cell division, in contrast with the nuclear genome. Mitochondrial DNA (mtDNA) is also not associated with histones, but it does associate with various proteins to form nucleoids, each of which contains approximately 5-7 copies of the genome.

In the 1920s, Otto Warburg observed that malignant cells had an altered metabolic phenotype, showing increased glycolysis and lactate production, and reduced oxygen consumption. He hypothesized that this change was responsible for malignant behaviour (2), although more recent evidence suggests that it may be a secondary adaptation to the tumour environment (3). The central role played by mitochondria in carbohydrate metabolism made them early suspects for oncogenesis but it has been difficult to establish whether mitochondrial dysfunction is purely a consequence of damaged nuclear genes and their products, or whether damage to the mitochondrial genome is partly responsible. Whether alterations in mitochondrial metabolism confer a selective advantage on tumours or hinder their proliferation and metastasis has also not been clearly established. This review addresses these questions.

Are Mitochondrial Genomes Damaged in Cancer?

The role of mtDNA in cancer is probably due to acquired mutations, as there are no known cancer syndromes which exclusively follow the maternal line, as mitochondria do. Although the common G10398A polymorphism does increase the risk of spontaneous breast cancer (4-6), this is an exception to the rule that mtDNA polymorphisms are neutral; a large association study found no effect of mtDNA background on colorectal cancer risk, for example (7). On the other hand, a case–control study found germline mutations in cytochrome oxidase subunit I to be associated with prostate cancer (8). Recent advances in sequencing technology have facilitated many studies of mtDNA sequences from cancer specimens and cancer-derived cell lines. These have found increased rates of substitutions, deletions and polynucleotide-tract length changes but interpretation of these data is complicated by heteroplasmy and the high copy number of

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mtDNA. Heteroplasmy is where a cell contains mtDNA copies which have different alleles at a given locus. Heteroplasmy is a continuous variable, in contrast to the binary heterozygous/homozygous dichotomy of nuclear genetics, and a cell may have more than two polymorphisms at a given locus in mtDNA. Low- frequency variants are hard to detect with standard techniques, and too little is known of the regulation of mitochondrial gene expression to predict the effect of low- frequency variants. Most heteroplasmy is probably acquired by somatic mutations but congenital heteroplasmy can occur, which leads to mosaicism, where somatic cells have different genotypes due to random segregation of mitochondria at cell division. This leads to wide variation in the proportion of heteroplasmy in the daughter cells (9). Thus any heteroplasmy found in a cancer clone which differs from that of healthy tissue may be newly-acquired or part of that individual's congenital genetic mosaic. Simple sequencing rarely detects heteroplasmy but high–throughput sequencing can detect rare variants, and some groups have solved the problem by cloning individual mtDNA copies in bacterial artificial chromosomes. Unfortunately *ex vivo* samples often contain a mixture of tumour cells and benign stromal cells, which precludes an accurate assessment of heteroplasmy, although some groups have used laser-capture microdissection to circumvent this by isolating only malignant cells (10). Sequences from healthy somatic tissue can help resolve ambiguity around heteroplasmy but some studies neglect to compare tumour samples with same-patient healthy tissue.

At a population level, mtDNA is more stable than nuclear DNA because it is maternally inherited, thus does not undergo recombination to the extent of nuclear DNA, and mtDNA variants do not segregate independently. This makes it useful in criminal forensics as well as phylogenetics, and there are published datasets of variants grouped by haplotype against which newly-detected variants can be compared (11-13). These have identified highly polymorphic sites, most notably the regulatory region, which contains three highly variable regions (HV1, HV2 & HV3) spanning nucleotides 16,024 to 574. Within HV2 is a polycytosine tract interrupted by a thymine at base 310. Unfortunately many studies of mitochondrial mutations in cancer have failed to consider the spectrum of polymorphisms in the population, and consequently have attributed unwarranted significance to their non-canonical sequences (14).

Polyak *et al*. studied colorectal cancer biopsies, cancer-derived cell lines, and healthy colon tissue from 10 patients, and found 12 somatic mutations distributed among seven out of the 10 cell lines (15). These were homoplasmic in the cell lines but not in the tissues – whether the mixed signal from tissues was from heterogeneity between cells or heteroplasmy within cells was not determined. Hibi *et al*. identified somatic mutations in HV1 and HV2 of mtDNA in seven out of 77 colorectal carcinomas studied (16), while increased mtDNA mutation has been found in other malignancies. Somatic mtDNA mutations have been identified in prostate carcinoma (8, 10, 17), although it is likely that two of the highly mutated samples of Chen *et al*. were contaminated (14) and so the true number of somatic mutations is probably 16 across the D-loops of the 16 prostate specimens they examined. The rate of somatic mutation in coding sequences is much lower, with only three mutations in the cytochrome *c* oxidase 1 (*COI*) genes from 260 prostates (8). In hepatocytic carcinoma, the situation is complicated by pre-existing viral cirrhosis in most cases. The mutation rate in non-cancerous liver tissue from such patients is considerably higher than that of non-cirrhotic patients (18) but even when compared with matched cirrhotic tissue, HCC isolates have further mutations in the D-loop (15 non-polynucleotide length polymorphisms found in 61 specimens) (19) and 13 coding region mutations were found in 44 specimens (20). Squamous carcinoma of the oesophagus is associated with a low rate of D-loop mutation; one article reported on two patients with anomalously high rates (21), but these may be due to sample contamination (14). Disregarding polymorphisms in the length of the poly[C] tract at 303, only four D-loop mutations were found in 75 cases of squamous oesophageal cancer (22, 23). In contrast, squamous carcinomas of the head and neck have a D-loop mutation rate of 24 tumours out of 83. This is associated with frequent non-synonymous coding region mutations (24 out of 83 samples) and tRNA mutations (14 out of 83), compared to the same patients' leukocytes (24). There is also evidence for increased poly-cytosine tract instability in Barrett's oesophagus (25). In myelodysplasia and consequent acute leukaemia, there is mixed evidence for significant mtDNA damage, but unfortunately these studies failed to use same-patient healthy tissues as a control (26, 27). A small minority of glioma isolates contain mtDNA mutations: one study found three out of 15 samples contained somatic mutations, two of which are also common polymorphisms (28); another study found that two out of 17 fully-sequenced glioma samples contained transitions (29), although the second sample shares so many polymorphisms with a European haplo-group U4a that again sample contamination seems likely (14). Eleven complete glioma sequences from Seoane *et al*. all contained a few hitherto undescribed mutations but they omitted same-patient control sequencing, so it is not clear whether these are somatic mutations (30).

Some of these reports have been criticised for failing to properly consider population polymorphisms, and for experimental error and sample mix-up (14). In several cases, the mutations of apparent hypermutator carcinomas correspond closely to a known haplotype (10, 17, 21, 29). Sample contamination is quite likely in most of these cases, although the coincidence could be accounted for if certain

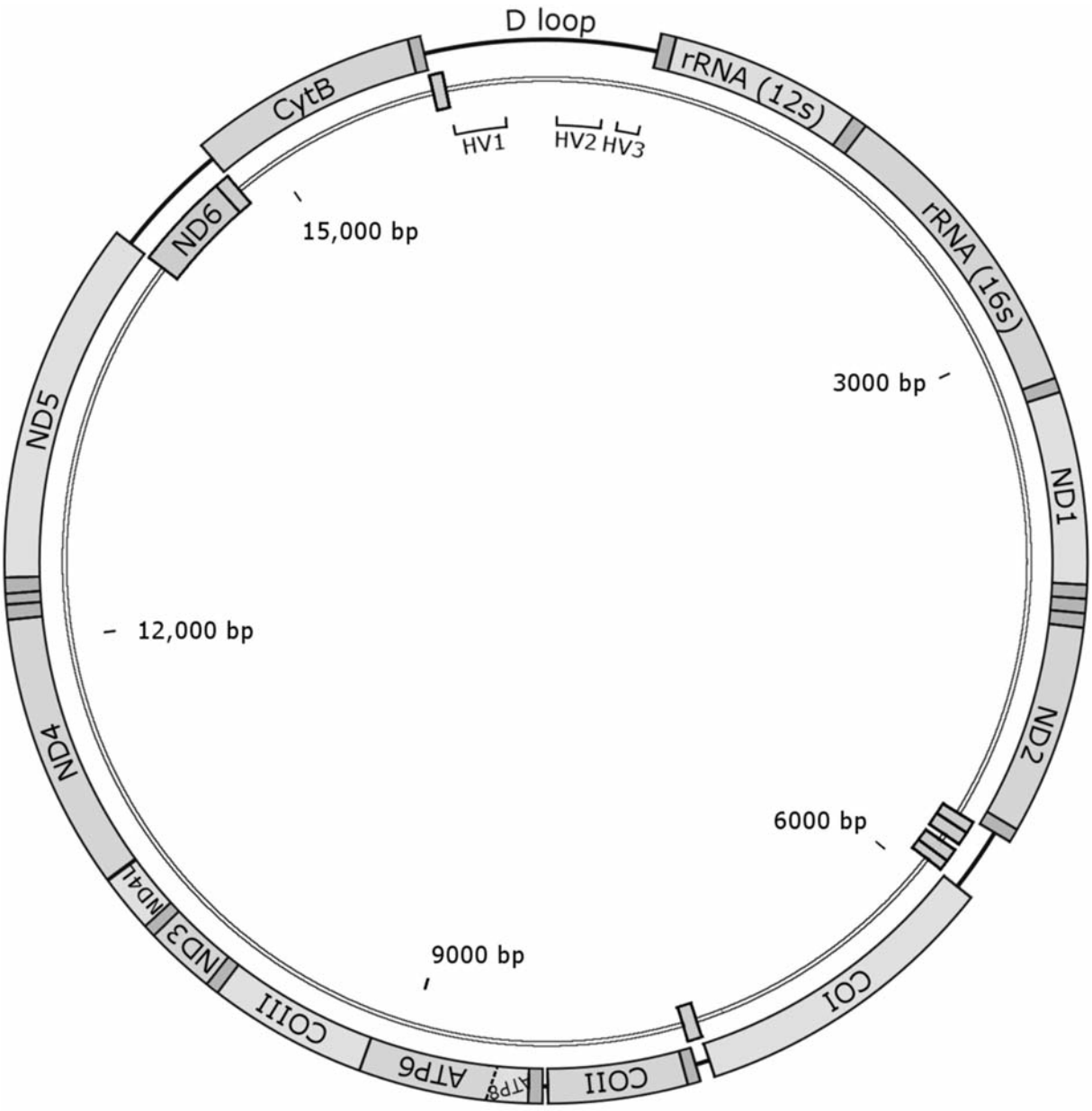

Figure 1. The human mitochondrial genome. Outer ring: Light strand. Inner ring: Heavy strand. ND1-ND6, NADH dehydrogenase subunits 1-6; ND4L, subunit 4L of NADH dehydrogenase; COI, COII and COIII, cytochrome c oxidase subunits 1-3; ATP6 and ATP8, subunits 6 and 8 of ATP synthase; CytB, cytochrome b of cytochrome c oxidoreductase; HV1-HV3, hypervariable regions 1-3. Unlabelled short genes are tRNAs, in clockwise order from the D-loop: Phe, Val, Leu, Ile, Gln, Met, Trp, Ala, Asn, Cys, Tyr, Ser, Asp, Lys, Gly, Arg, His, Ser(2), Leu(2), Glu, Thr, Pro.

mutations were significantly more likely than others, either for steric reasons related to nucleoid structure or because of selective pressures. The discrepancies between the samples and the haplotype must also be attributed to sequencing errors, if contamination did indeed occur. Most sequencing techniques were relatively insensitive to heteroplasmy, which if present in samples, either from the germ line or having arisen in the somatic genome, could explain some discrepancies.

The large number of reports of mtDNA mutations in cancer belies in the infrequency of mutations. In most studies, only a small minority of tumours harboured non-silent mtDNA mutations: five out of 10 colorectal whole-mitochondrial genomes (15), two out of 16 prostate

carcinoma NADH dehydrogenase I (*ND1*) genes (17), three out of 260 prostate *COI* genes (8), 10 out of 44 HCC whole genomes (20), and 24 out of 83 head-and-neck squamous carcinoma whole genomes (24). Although most studies have identified increased rates of mutation in the highly variable D-loop, most of these changes are length polymorphisms of poly-cytosine tracts or other population polymorphisms. Consequently, these mutations are unlikely to be significant in themselves, except insofar as they imply a heightened mutation rate across the genome.

How Are Mitochondrial Genomes Damaged?

In the 1970s, it was reported that UV-induced pyrimidine dimers were not repaired in mtDNA (31), and it was initially believed that there were no DNA repair mechanisms in mitochondria, but rather, damaged mtDNA copies were destroyed and replaced by replication. Subsequently, it has become clear that mitochondria do have base-excision repair mechanisms (32, 33), but do not enjoy the full range of repair mechanisms found in the nucleus. They are also subject to greater mutational stresses: the electron transport chain increases the concentration of DNA-damaging reactive oxygen species (ROS), the mitochondrial membrane potential drives accumulation of lipophilic cations (34), and other lipophilic mutagens damage mtDNA preferentially to nuclear DNA (35, 36).

We have a limited understanding of how damage to mtDNA is mitigated. In addition to having a range of base excision repair proteins and the ability to repair oxidatively damaged bases (32, 33, 37), there is also some evidence for mismatch repair (38), homologous recombination (39), and non-homologous end-joining (40-42). However, with the exception of base excision repair, few DNA repair proteins have been found in mitochondria. Thus increased mutational stresses and limited repair mechanisms are consistent with an increased mutation rate of mtDNA relative to nuclear DNA. This has been experimentally confirmed in model organisms. In *Caenorhabditis elegans*, mtDNA mutation rates are 7-fold higher than for nuclear DNA (43, 44) and they are 10-fold higher in *Drosophila melanogaster* (45). The paucity of repair mechanisms in mitochondria may make mtDNA particularly susceptible to loss of certain nuclear tumour–suppressor genes, such as Xeroderma pigmentosum C (XPC) and Breast Cancer-1 (*BRCA1*). XPC is a genome surveillance protein responsible for some cases of xeroderma pigmentosum, and is involved with nucleotide-excision repair. Knockdown of *XPC in vitro* not only causes increased glycolysis and proliferation, but also accumulation of 7,8-dihydro-8-oxoguanine lesions in nuclear and mtDNA, and characteristic mtDNA deletions (46). It is not clear whether the mtDNA damage is responsible for the transformed phenotype.

Whole mitochondria are removed by autophagy, but whether there are means for disposal of individual genomes is still an open question. The fusion and fission of mitochondria facilitates uneven segregation of mitochondrial contents, and those daughter organelles with reduced membrane potential are targeted for destruction [reviewed in (47)]. However, further details of the regulation of mitochondrial dynamics have proved elusive. The prevailing opinion is that mitochondrial segregation at mitosis is random, and that a controlled number of mtDNA copies is sufficient to account for the expansion to homoplasmy of mutations in various diseases (9), but there is evidence that in some situations, there is preferential propagation of one mtDNA genome over others (15, 48).

Concentrations of ROS are often reported to be high in transformed cells and cancer (24, 25, 49-54) but it is hard to accurately measure such labile species (55), and harder to know whether their presence is causal or merely an epiphenomenon. Experimentally increasing ROS levels cause transformation *in vitro* (56), and attenuating endogenous antioxidant mechanisms increases tumourigenesis *in vivo* (57). Some interventions intended to mitigate ROS have been shown to reduce transformed behaviour and growth *in vitro*, such as addition of antioxidants to media (51, 52, 54), or transfection with ectopic superoxide dismutase or catalase genes (50, 53).

Mitochondrial ROS may also be responsible for age-related accumulation of mtDNA damage. Studies of individual buccal and cardiac cells from young and old individuals have shown increased mtDNA mutations with age which can expand to become the predominant mtDNA species in a cell (58, 59). In cardiomyocytes, these mutations are not randomly distributed but are heavily concentrated in the 16025-16055 region, a highly conserved sequence in the regulatory region of the genome. This supports a functional impact of the changes, which could account for the expansion of these mutants to near-homoplasmy (58). These cells were taken from macroscopically healthy tissue, so the presumed aetiology for mtDNA damage is oxidative stress from aerobic metabolism. The expansion to homoplasmy of these mutations may occur by genetic drift but recombination may also play a role. This occurs rarely in mitochondria: the use of mitochondrial phylogenetics to map human migrations relies on the stability of the haplotype. However, there is evidence for recombination in plants at least (60, 61), and mammalian mitochondrial extracts display homologous recombinatorial activity (39). Mammalian mitochondria also display an absolute requirement for a DNA ligase (62), which could contribute to recombination.

ROS can promote oncogenesis by various means, such as by stabilising HIF1 (63) and attenuating extracellular signal-related kinase/mitogen-activated protein kinase signalling (54). Whether they also do so by damaging mtDNA is uncertain. Experimental exposure to oxidising agents damages mtDNA more than nuclear DNA, with consequences for cell survival (64) and there is some oblique evidence that ROS can damage

mtDNA in spontaneous oncogenesis: mitochondrial transcription factor A (TFAM) is a nuclear-encoded protein which associates with mtDNA, in whose absence mitochondrial function is impaired and ROS production increased. Heterozygous *Tfam* knockout in mice causes a reduction in mtDNA copy number and respiratory chain deficiency, while cardiomyocyte-specific homozygous loss causes progressive cardiomyopathy very like that seen in Kearns-Sayre syndrome (65). Affected cardiomyocytes up-regulate glutathione peroxidase and superoxide dismutase, which may compensate for increased ROS production. In mice heterozygous for adenomatous polyposis coli (*Apc*), heterozygous *Tfam* loss increases small bowel polyp number and ROS production, while overexpressing mitochondrial catalase reduces the number of colonic polyps (53). The effects seen are small but implicate a failure of mtDNA maintenance in ROS-induced tumourigenesis.

Does Mitochondrial DNA Damage Confer a Fitness Advantage to Proliferating Cells?

Investigating the effects of nuclear genetic changes is conventionally undertaken by knocking out endogenous copies and replacing them with engineered versions. In mitochondrial genetics, the high copy number of the genome makes this approach unfeasible. An early solution was to create Rho-negative cells, *i.e.* cells without mtDNA, which was achieved by long–term culture of cells *in vitro* in the presence of ethidium bromide (EtBr). Unfortunately, this non-specific DNA intercalating agent also affects nuclear DNA, so observed changes may be due to nuclear effects or to loss of mtDNA. This treatment caused transformed avian cells to lose their anchorage independence (66), which was restored by fusion with untreated transformed cells to form cybrids with parental mtDNA. However loss of anchorage-independence coincided with greatly reduced anchored growth. Similar experiments using HeLa cells found that cybrids with mitochondria from fibroblasts regained similar tumourigenicity to native HeLa cells (67), and these findings have been replicated in glioblastoma and breast cancer cell lines (68). Rho-negative osteosarcoma cells are also more susceptible to spontaneous and experimental apoptosis (69). These findings suggest that mtDNA is required for optimal growth but there is no evidence that these cells carry any mtDNA mutations which affect growth. On the other hand, some experiments on breast epithelium and breast cancer cell lines have suggested that loss of mtDNA promotes anchorage independence *in vitro* and tumourigenicity *in vivo* (70). Those investigators believe the phenotype is due to signalling caused by the absence of mtDNA, which alters nuclear gene expression, but they did not exclude nuclear mutation or epigenetic changes arising from the mtDNA-depleting treatments (EtBr and ditercalinium).

In mice deficient in the proof-reading domain of DNA polymerase γ, which accumulate mtDNA mutations at 3-8-times the rate of mice with wild-type enzyme, lifespan is halved and apoptosis is increased *in vivo* (71). This supports the theory that ageing is partly due to accumulation of mtDNA damage [reviewed in (72)] but also that diffuse mtDNA mutation can promote apoptosis in tumours. Mice with cardiomyocyte-specific loss of *Tfam* also show increased apoptosis in those cells, with up-regulation of superoxide dismutase-1 and glutathione peroxidase (69). If this is a compensatory response to increased ROS, it appears to fully-counteract the additional oxidative stress, as iron-sulfur complexes in the cardiomyocytes were not compromised. Weinberg *et al*. created mice with activated Kirsten rat sarcoma viral oncogene homologue (Kras) and Cre recombinase-mediated lung-specific knockout of *Tfam* (54). The tumour burden in the lungs was much increased when *Tfam* was knocked-out, which supports a role for *Tfam* in promoting cell survival.

The bulk of evidence thus suggests that mtDNA loss or diffuse mtDNA damage inhibits growth and promotes apoptosis; it certainly does not confer a selective advantage on affected cells. However, these experimental systems do not recreate the state of mtDNA in cancer clones, whose relatively limited mutation burden has arisen by other mechanisms and is subject to selective pressures. This problem was addressed by reciprocal exchange of mitochondria between mouse tumour-derived cell lines (52). Ishikawa *et al*. crossed two isolated cell lines, of high and low metastatic potential, from each of two tumours. Fusion of enucleated cells with Rho-negative cells yielded cybrids whose metastatic potential corresponded with that of the mitochondrial donor cell line but not the nuclear donor line. In contrast, the size of tumours *in vivo* did not correlate with mitochondrial or nuclear donor. Furthermore the transfer of mitochondria from highly metastatic lines to fibroblasts did not confer tumourigenicity. The only mitochondrial gene to be mutated exclusively in the highly metastatic lines was NADH dehydrogenase subunit 6 (*Nd6*) (*Mus musculus* 13885insC, causing a frameshift), and the metastatic phenotype was associated with defects in complexes I and III of the electron transport chain, increased ROS and up-regulation of myeloid cell leukaemia-1 (*Mcl1*), *Hif1α* and vascular endothelial growth factor (*Vegf*). Metastasis was attenuated by siRNA against *Mcl1*, which suggests that the *Nd6* mutation inhibits apoptosis *via* Mcl1. It is worth noting that a highly metastatic colon cancer line was also investigated but its mitochondria did not confer any increase in metastatic potential; unfortunately its mtDNA sequence is not reported.

Contrasting results were obtained from a hypoxia-sensitive glioma cell line containing a missense *ND6* mutation (T14634C), which had no effect on HIF-1 levels but did impair growth and complex I activity, compared to other

hypoxia-tolerant glioma lines. Homology-based models of the mutant ND6 structure suggested an altered conformation (73).

Mutations in other subunits of complex I have also been associated with a transformed phenotype, increased ROS and up-regulation of *HIF1A*. Three *ND2* mutants from head and neck cancer can increase proliferation and anchorage-independent growth *in vitro* when overexpressed in a nuclear vector (24, 50). However, the cells' endogenous copies of *ND2* were still present, which may account for the rather modest increase in proliferation rates. A frameshift mutation in *ND5* also increased growth and *in vivo* tumourigenesis but only when present at only 70% heteroplasmy; cells homoplasmic for the mutation had a lower proliferation rate than wild-type cells (49). The heteroplasmic cells and the ND2 transfectants all displayed increased ROS levels; in addition the *ND2* transfectants had up-regulated *HIF1A* expression. In contrast to these findings, Porcelli *et al*. found frequent mutations in complex I genes in benign oncocytic tumours, causing reduced expression of those subunits, which was associated with reduced HIF-1α levels and no change in ROS labelling (74).

Other loci besides *ND1-6* have also been shown to play a role in malignant behaviour *in vitro* and tumourigenesis: a poly-adenine tract extension (*Mus musculus* 9821insA) in the tRNA-Arg locus confers increased growth, *in vitro* migration, and resistance to apoptosis (51). All of the cell lines studied by Ishikawa *et al*. also had an expansion of one or two extra cytosines at this locus (52). Cybrids with a T8993G in the *ATP6* subunit of ATPase have markedly increased tumour growth *in vivo* and increased ROS production (8).

Many of the mutations identified in studies of coding sequences from tumour isolates are synonymous, and many others are found as polymorphisms in the population. However, it is clear that a minority has the potential to affect phenotype, as they introduce stop codons and frameshifts, or alter tRNA structure, which can cause dramatic inherited disease (75). Some missense and nonsense mtDNA mutations, mostly in subunits of NADH dehydrogenase, have been experimentally shown to cause malignant behaviour. Whether these same mutations confer a fitness advantage in the natural history of carcinomas is less clear. Although some promote 'spontaneous' metastasis (52), other missense *ND6* mutations may actually reduce HIF stabilisation and inhibit pseudo-hypoxia, preventing malignant behaviour (74). The fitness effect of any change depends on the genetic context and local environment. *In vitro* experiments have investigated mtDNA mutations in a variety of cell types, including established *in vitro* cell lines (50, 51), tumour-derived cells (49, 73, 74), fibroblasts and stem cells (3). The incremental effect of a few mtDNA mutations on these cells may be very different from that on clones midway through transformation, and the high selective pressures of a tumour microenvironment are important in determining selective advantage.

A few attempts have been made to correlate mtDNA mutation status with clinical outcome, with mixed results. In gliomas, mtDNA mutation was not associated with prognosis (28), whereas in sporadic breast cancer, mutations in the D-loop correlate with histological grading (76). In HCCs, there have been conflicting reports of associations between grade and mtDNA mutation burden (20, 77). The quantity of mtDNA in breast and thyroid carcinomas did not correlate with grade or metastasis (78), but TFAM expression in colorectal cancer was associated with metastasis and poor prognosis (79). These experiments have no true control group, and so cannot establish causation. Nuclear mutations present in the malignant clone will interact with mtDNA mutations, and altered prognosis may not be due to mtDNA mutations, but rather in spite of them.

What Are The Therapeutic Implications?

Where mtDNA damage does contribute to oncogenesis, clearly a direct reversal of the damage is not a feasible therapeutic option. Any intervention to mitigate the effect of mtDNA damage must therefore act on a downstream signalling cascade or effector protein. Attempts to identify these pathways have met with limited success. Increased ROS production has often been observed, but reliably quantifying ROS is difficult (55), and some groups have observed no change in ROS but an up-regulation in catalase and glutathione peroxidase (69). Most compellingly, attempts to prevent or treat cancer with exogenous antioxidants have unfortunately been unsuccessful (80-83).

The mechanism underlying the Warburg effect is now generally accepted to hinge around stabilisation of HIF1 (63). HIF1 stabilisation can be caused not only by changes to nuclear gene products (84, 85), but also by mitochondrial ROS (86, 87), which may be caused indirectly by various mtDNA mutations. Increased HIF1 levels have been seen in mtDNA mutant cybrids, associated in some cases with altered lactate and pyruvate levels (8, 52). Therapeutics directed against HIF1 have been under investigation for some time [reviewed in (88)], but the important physiological roles of HIF1 limit the specificity of such an approach.

Even if individual mtDNA mutations can be tumourigenic, it is nevertheless the case that widespread mtDNA damage severely limits proliferation and promotes apoptosis (66, 67, 69). For decades mitochondria have been considered to be particularly sensitive to conventional chemotherapy, with their limited DNA repair machinery. Although exploiting this weakness runs the risk of systemic toxicity, if a repair inhibitor could synergize with the increased mutation rate found in malignant cells, it could push malignant clones into apoptosis. Such an approach may be a useful addition to existing therapeutic regimens.

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