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COMMENTARY

Mitochondrial DNA Mutations and Cancer

Lessons from the Parathyroid

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The article by Müller-Höcker et al¹ in this issue of *The American Journal of Pathology* explores mitochondrial DNA (mtDNA) mutations of benign hyperplastic nodules of the hyperfunctional parathyroid gland. The study compares mtDNA mutations in cells differing by the extent of mitochondrial proliferation (ie, clear chief cells, pre-oxyphil, and oxyphil cells). Mitochondrial proliferation in these cells is thought to be a compensatory effect caused by a defect in respiratory chain. The transition from clear chief to oxyphil cells is an example of the oncocyctic transformation, which is also observed in other tissues.

Müller-Höcker et al¹ compare mtDNA mutations in normal cells with mutations in cells defective in cytochrome c oxidase (COX). COX, a key enzyme of the respiratory chain, is partially encoded by the mtDNA. Of particular importance is their discovery of substantial load of COX-inactivating mtDNA mutations and their conclusion that oncocyctic transformation in the parathyroid gland can be caused by COX mutations. This conclusion extends an existing view that oncocyctic transformation is caused almost exclusively by mutations in another respiratory chain enzyme partially encoded by mtDNA [ie, NADH dehydrogenase (ND)]. Although appearing too specialized, the impact of the highlighted study is quite broad as oncocyctic cells give rise to oncocyctic tumors, which are a rare example of tumors where involvement of mtDNA mutations is certain. This study may thus provide one of the missing tiles in the complicated puzzle depicting the role of mtDNA mutations in carcinogenesis.

mtDNA Mutations in Tumors: Drivers or Drifters, Enemies or Friends?

The question whether mutations in mtDNA may be involved in carcinogenesis has a long history of controversy. Perhaps

the most influential discovery was that tumors tend to contain mtDNA mutations that are not found in the surrounding normal tissue.² Because there are hundreds of mtDNA molecules in each cell, mutant mtDNA may be present at various fractions of the total mtDNA (a condition called heteroplasmy) up to 100% (homoplasmy). Strikingly, mtDNA mutations were not just present in tumors, but they constituted a high percentage (>50%) heteroplasmy or even homoplasmy. Thus mitochondrial genomes carrying these mutations somehow had managed to out-compete their wild-type counterparts within tumor cells or tumor precursors, but not in normal tissue. Initially, this observation was perceived as evidence of dramatic positive selection of mutant mitochondrial genomes in tumors, implying that mutations were essential for carcinogenesis. However, later it was shown that somatic mtDNA mutations may reach homoplasmy in tumors by a purely random process similar to fixation of mutations in a population by genetic drift.³ Similar processes also operate in normal cells.^{4,5} Thus the mere presence, high incidence, or high fraction mtDNA mutations in tumors by itself cannot be used as evidence of their involvement in carcinogenesis.

The conclusion that mtDNA mutations can be irrelevant to the tumor even if it contains them at high concentration does not mean that mtDNA mutations cannot play important roles in carcinogenesis. Indeed, the number of studies supporting involvement of mtDNA mutations in cancer has

Accepted for publication September 11, 2014.

Supported by the Ellison Medical Foundation award (K.K.) and Novartis grant no. 14B065 (K.P.).

Disclosures: K.P. receives funding from Novartis.

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increased in the recent years. It appears, however, that mtDNA mutations can both facilitate and impede carcinogenesis. Initially, positive effects of mtDNA mutations on carcinogenesis were discovered, such as down-regulation of apoptosis by mutations in ATP synthase⁶ or stimulation of metastatic potential by increased reactive oxygen species levels associated with mutations in ND genes.^{7,8} On the other side, it has been argued that functional mitochondrial respiratory chain is indispensable for tumor proliferation.⁹ Furthermore, inactivating ND mutations appear to enhance carcinogenesis at low fractions, but they suppress it once mutations approach homoplasmy.^{10,11} This likely occurs via destabilization of hypoxia-inducible factor 1- α , which is considered one of the master regulators of the metabolic adaptation needed for malignancy.¹¹

Comparison of the Distributions of mtDNA Mutations Can Reveal their Roles in Carcinogenesis

As argued above, high incidence or high fraction of mtDNA mutations in tumors do not prove that mutations are involved in carcinogenesis. Fortunately, more detailed analysis, such as comparison of mutation frequency distributions by type, position, etc., can help. Indeed, the nonsynonymous to synonymous mutation ratios, or relative frequencies of mutations in one gene versus another, can be changed by selective pressures only. For example, Khajdakov et al¹² revealed that the proportion of nonsynonymous somatic mtDNA mutations in normal tissues was higher than in tumors. The most natural explanation of this observation is that nonsynonymous mtDNA mutations in tumors are under negative selective pressure, that they impede carcinogenesis. This is an interpretation consistent with other studies.^{10,11,13}

Alternately, instead of fewer nonsynonymous mutations in tumors, there may be more of them in normal tissues in comparison to the distribution of *de novo* generated somatic mutations. Once generated, nascent mutations are subject to positive or negative selective pressures, which change the nonsynonymous to synonymous ratio. From this perspective, higher frequency of nonsynonymous mutations in normal tissues may result either from negative selection in tumors, or from positive selection in normal tissues, or both. Note that positive selection of detrimental mtDNA mutations with age is a well-known phenomenon.¹⁴ Additional experiments are needed to determine which of the two scenarios is correct, and to establish the distribution of nascent mutations, which is technically difficult.¹⁵ Whatever the direction of this effect, the difference in nonsynonymous to synonymous ratio of mtDNA mutations between tumors and normal tissue strongly endorses their involvement in carcinogenesis.

Similar to the difference in the proportion of nonsynonymous mutations, a significant excess of mtDNA mutations in one gene compared to another, in tumors but not in normal cells, would strongly support their involvement in carcinogenesis.

Such unequal distributions of mtDNA mutations are indeed observed in tumors, most notably in the oncocyctic tumors.

Dissimilar Mutational Distributions in Oncocyctic Tumors versus Benign Lesions

Oncocyctic tumors form in various tissues, including the parathyroid gland. Oncocyctic tumors originate from precursor cells, which have already undergone oncocyctic transformation. mtDNA of many oncocyctic tumors have been sequenced and tumor-specific mtDNA mutations determined.¹⁶ Very notably, the resulting distribution is heavily dominated by mutations in ND genes and just as notably depleted of COX mutations. Specifically, among 67 non-synonymous mutations,¹⁶ only 2 (3%) are located in COX genes, whereas 59 (88%) are located in ND genes. The COX-ND difference is highly significant: $P < 0.0001$, Fisher's exact test, odds ratio approximately 10. This estimate considers that COX and ND genes comprise about 20% and 50% of the protein coding part of the mitochondrial genome, respectively.

Such a dramatic disproportion between ND and COX genes implies that mtDNA mutations are important for pathogenesis of oncocyctic tumors, but it is not clear yet at what stage they are involved. One possibility is that ND mutations lead to the oncocyctic phenotype, whereas COX mutations do not. Then ND mutations will appear enriched, and/or COX mutations will appear depleted because in this case only oncocyctic tissue was included in analysis. A second, more interesting possibility is that ND mutations promote tumor progression, whereas COX mutations do not promote or even impede it. This would also result in ND enrichment and COX depletion, because we look at tumors only. As Pereira et al¹⁶ advocated, it would be important to look at mutations in nontumor oncocyctic cells to resolve this dilemma, but such data are not yet available.

Müller-Höcker et al¹ report, unlike Pereira et al,¹⁶ that mtDNA mutations in COX genes are rather common, at least in their set of benign oncocyctic hyperplastic nodules. Moreover, COX mutations (and also other non-ND point mutations) clearly correlate with the progression of the oncocyctic phenotype (clear chief cell—pre-oxyphil cell—oxyphil cell). This suggests that oncocyctic phenotype can be caused by other mutations than ND, including COX mutations. If so, the predominance of ND mutations and depletion of COX mutations in oncocyctic tumors reported by Pereira et al¹⁶ should not be related to the oncocyctic phenotype per se, but rather to tumor progression. This is consistent with recent work reporting ND mutations in tumor progression.^{17,18} Additionally, the observed severe depletion of COX mutations opens a possibility that COX mutations may be actually selected against in oncocyctic tumors (ie, potentially impede tumor progression).

If the above inferences are correct, then the difference between the data collected by Pereira et al¹⁶ and Müller-Höcker et al¹ could rest in the samples; the samples used by Müller-Höcker et al¹ were more benign than tumorous samples used

by Pereira et al.¹⁶ Indeed, the areas analyzed by Müller-Höcker et al¹ are quite small (approximately 1 mm across) and are explicitly classified as benign, though rigorous comparison of pathology is not possible. These inferences will remain highly speculative unless more data on mutations in oncocyctic tumors and even more so on nontumor oncocyctic cells are available for analysis.

In conclusion, the study by Müller-Höcker et al¹ provide a different view on the distribution and role of mtDNA mutations in oncocyctic tumors. These data suggest that ND mutations, which are overrepresented in oncocyctic tumors, may be essential for tumor progression rather than merely for induction of the oncocyctic phenotype. Similarly, COX mutations, which are greatly underrepresented, may impede oncocyctic tumor progression.

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