Trading mtDNA uncovers its role in metastasis

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It has been controversial for many years of whether mtDNA mutations are involved in phenotypes related to cancer due to the difficulty in excluding possible involvement of nuclear DNA mutations in these phenotypes. Although mitochondrial respiratory function is controlled by both nuclear and mitochondrial genomes, the pathogenicity of these mtDNA mutations has been proven by co-transmission of the mutant mtDNAs and mitochondrial respiration defects to mtDNA-less (ρ−) human cells: the resultant trans-mitochondrial cybrids sharing the same nuclear background showed respiration defects, only when they accumulated the mutated mtDNA from the patients. Moreover, we generated trans-mitochondrial mito-mice sharing the same nuclear background, but carrying various proportions of mtDNA with a pathogenic mutation, and provided model systems for studying exactly how mtDNAs with pathogenic mutations are transmitted and distributed in tissues resulting in the pathogenesis of mitochondrial diseases that show various clinical phenotypes. With respect to the involvement of mtDNA in tumor phenotypes, it has been proposed that most chemical carcinogens bind preferentially to mtDNA rather than to nuclear DNA in mammalian cells, and thus, mtDNA should be the major cellular target of chemical carcinogens, and resultant creation of mutations in mtDNAs is responsible for expression of tumor phenotypes.

Although, there has been no direct evidence for creation of mtDNA mutations by chemical carcinogens, and for their contribution to tumor development in mammalian cells, recent studies showed that somatic mtDNA mutations accumulated in human colorectal tumors and in various tumor types rather than in normal cells of the same subjects, probably by the clonal expansion of the mutated mtDNAs along with the repeated division of tumor cells. Many subsequent studies supported preferential accumulation of mutated mtDNAs in tumor cells, suggesting that mutated mtDNAs in tumor cells have acquired replication advantages to be homoplasmic. However, these studies did not address the fundamental question of whether the mutated mtDNAs are involved in tumor development.

Our previous studies directly addressed this issue using trans-mitochondrial cybrids obtained by mtDNA trading between normal and tumor cells, and provided convincing evidence that mutations in nuclear DNA, but not in mtDNA were involved in tumor development in the mouse and in human cultured cells. The possibility that these observations may represent some specific tumor cases can be excluded since there has been no statistical evidence for association of tumor development and pathogenic mtDNA mutations in the patients with mitochondrial diseases expressing respiration defects caused by pathogenic mutations in mtDNA. The possibility that some polymorphic mtDNA mutations that do not induce respiration defects, but somehow contribute to tumor development also can be excluded, because there has been no statistical evidence for the presence of maternal inheritance of tumor development in spite of the strictly maternal inheritance of mammalian mtDNA.

Nonetheless, it was still possible that mtDNA mutations are involved in other processes than oncogenic transformation of normal cells to develop tumors, such as in malignant progression of tumor cells to develop a metastatic potential. Recent studies demonstrated that mitochondrial respiration defects in TCA-cycle enzymes caused by nuclear DNA mutations controls tumor phenotypes as a consequence of induction of a pseudo-hypoxic pathway under normoxia. Thus, some mtDNA mutations also induce the pseudo-hypoxic pathway under normoxia by inducing mitochondrial respiration defects. However, there has been no direct evidence for involvement of mtDNA mutations in malignant progression or in the regulation of the pseudo-hypoxic pathway under normoxia, because of the difficulty in excluding possible contribution of nuclear DNA mutations in these processes.

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Recently, we addressed this issue using trans-mitochondrial cybrids obtained by complete trading of mtDNAs between highly and poorly metastatic mouse lung carcinoma cells (Fig. 1). By this approach, we could provide convincing evidence for the control of malignant progression of tumor cells to develop metastatic potential by mtDNA: All the trans-mitochondrial cybrids with mtDNA from highly metastatic tumor cells expressed high metastatic potential, while those with mtDNA from poorly metastatic tumor cells expressed low metastatic potential, irrespective of whether their nuclear genome was derived from highly or poorly metastatic tumor cells. The findings in our study can be summarized as follows: (1) A missense G13997A mutation in the ND6 gene of mtDNA from highly metastatic lung tumor cells induces a complex I defect, and reversibly controls malignant progression of tumor cells to develop high metastatic potential, while those with mtDNA from poorly metastatic tumor cells expressed low metastatic potential, irrespective of whether their nuclear genome was derived from highly or poorly metastatic tumor cells. The findings in our study can be summarized as follows: (1) A missense G13997A mutation in the ND6 gene of mtDNA from highly metastatic lung tumor cells induces a complex I defect, and reversibly controls malignant progression of tumor cells to develop metastatic potential, but does not control oncogenic transformation of normal cells to develop tumors; (2) The complex I defect simultaneously induces enhanced glycolysis and ROS overproduction, but induction of metastasis is due to ROS overproduction; (3) ROS overproduction induces metastasis not by acceleration of genetic instability as usually proposed, but by reversible upregulation of nuclear-coded genes related to metastasis, such as Mcl-1; (4) ROS scavengers are therapeutically effective in suppressing mtDNA-mediated metastasis.

Thus, our study partly resolves the controversial issue on the relevance or irrelevance of mtDNA mutations in tumor development and/or tumor phenotypes by showing that mutations in mtDNA control development of metastasis in tumor cells. Considering that complex I defects simultaneously induce enhanced glycolysis under normoxia (the Warburg effect) and ROS overproduction, it remains possible that the Warburg effect alone can control metastasis independently from ROS overproduction. More recently, we examined this possibility by generating trans-mitochondrial cybrids with the deletion mutant mtDNA, which can be expected to induce overall respiration defects, and express enhanced glycolysis under...
normoxia, but not express ROS overproduction. The results showed that the Warburg effect alone did not control metastasis.

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