

## REACTIVATION OF DIHYDROOROTATE DEHYDROGENASE BY RESPIRATION RESTORES TUMOR GROWTH OF MITOCHONDRIAL DNA-DEPLETED CANCER CELLS

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Cancer cells without mitochondrial DNA (mtDNA) need to reconstitute oxidative phosphorylation (OXPHOS) by acquisition of host mitochondria to form tumors (1,2), but the reasons why functional respiration is crucial for tumorigenesis remain unclear. Using time-resolved analysis of the initial stages of tumor formation by mtDNA-devoid cells and genetic manipulations of OXPHOS components, we now show that pyrimidine biosynthesis, supported by the respiration-linked dihydroorotate dehydrogenase (DHODH), is strictly required to overcome cell cycle arrest, while mitochondrial ATP generation is dispensable for tumorigenesis. Primed DHODH is present in mtDNA-devoid cells and becomes fully active by complex III/IV respiration after mitochondrial transfer, or by the introduction of an alternative oxidase. Conversely, DHODH deletion interferes with tumor formation even in cells with functional OXPHOS, whereas disruption of mitochondrial ATP synthase has little or no effect. Collectively, our results show that pyrimidine biosynthesis via DHODH is an essential pathway that links respiration to tumorigenesis.

1. Tan, A. S., Baty, J. W., Dong, L. F., Bezawork-Geleta, A., Endaya, B., Goodwin, J., Bajzikova, M., Kovarova, J., Peterka, M., Yan, B., *et al.* (2015). Mitochondrial genome acquisition restores respiratory function and tumorigenic potential of cancer cells without mitochondrial DNA. *Cell Metab* *21*, 81-94.
2. Dong, L. F., Kovarova, J., Bajzikova, M., Bezawork-Geleta, A., Svec, D., Endaya, B., Sachaphibulkij, K., Coelho, A. R., Sebkova, N., Ruzickova, A., *et al.* (2017). Horizontal transfer of whole mitochondria restores tumorigenic potential in mitochondrial DNA-deficient cancer cells. *Elife* *6*.