African and European Cybrids Display Unique Basal Transcriptional and Energetic Profiles

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ABSTRACT

Introduction: Human mitochondrial DNA (mtDNA) heritage significantly influences disease sensitivity, but the cellular mechanisms and functional distinctions among mtDNA lineages are not fully understood. MtDNA is strictly maternally inherited and encodes for transcripts responsible for the synthesis of respiratory electron transport chain complexes. Accordingly, changes in mtDNA sequence affect the efficiency of oxidative phosphorylation and influence cellular energetics.

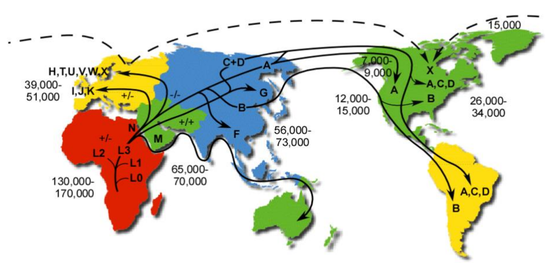
Ancient human migration out of Africa, across continents, and into new environments led to the accumulation of regional, non-pathogenic mtDNA variants that conferred metabolic advantages specific to their environments, forming mtDNA haplotypes. These haplotypes group into phylogenetically distinct haplogroups, which are characterized by unique energetic profiles and have been identified as key modulators of sensitivity to chronic and transmissible diseases.

Hypothesis: We hypothesize that these non-pathogenic mtDNA variants – haplogroups – confer functional differences between trans-mitochondrial (cybrid) cell lines carrying mtDNA from African and European haplogroups.

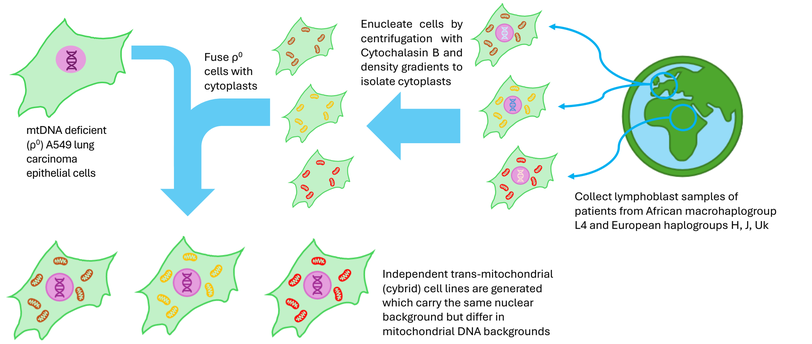
Methods: To test this hypothesis, we generated cybrids using A549 lung carcinoma epithelial cells harboring mtDNA from European haplogroups H, J, and Uk, as well as the African haplogroup L4. We evaluated differences in global transcriptome using RNA-sequencing and cellular energetics using Seahorse Assay between the African and European-derived cybrids.

Results: RNA-sequencing analysis revealed significant differences in gene expression levels of the total transcriptome between the African and European cybrids. Using pathway enrichment analysis, we found basal enrichment of transcripts involved in antioxidant defense, amino acid metabolism, and the integrated stress response in African cybrids relative to European cybrids. Seahorse Assay further revealed differences in cellular energetic profiles, specifically in extracellular acidification rate and non-mitochondrial oxygen consumption rate between the African and European cybrids.

Conclusion: These results show that subtle nucleotide differences in mtDNA have profound effects on the nuclear transcriptome and confer variation in cellular energetics. The cybrid approach in this project could serve as a useful tool to study differential sensitivity to disease driven by energetic defects and ultimately provide greater insight into cellular mechanisms causing such variation.



METHODS



Cybridization

- To generate ρ^0 mtDNA deficient cells, A549 lung carcinoma cells were cultured in 50ng/uL ethidium bromide for 16-18 weeks
- Cytoplasts of lymphoblasts sourced from African patients from the L4 haplogroup and European patients from the H, J, and Uk haplogroups were isolated
- ρ^0 cells were fused with the cytoplasts and select clonal populations of the newly generated trans-mitochondrial cybrids were expanded, generating independent clones of cybrids carrying mtDNA from different haplogroups

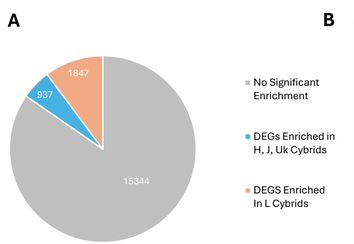
RNA Sequencing Analysis

- Cybrids derived from three African patients carrying the L4 haplogroup mtDNA and from three total European patients carrying H, J, and Uk haplogroup mtDNA were cultured in parallel overnight, and RNA was isolated the following day
- RNA sequencing was performed on all samples, and results were expressed as relative levels of gene enrichment between the African and European haplogroup-derived cybrids
- Enriched gene sets with a false discovery rate<0.25 and normalized p-val<0.05 were identified

Energetic Assays

- The Mitostress Test energetic assay was performed using Seahorse XF Pro in a 96 well format on 10 cybrids: two clones each of cybrids carrying the H, J, Uk, and two independent L4 haplogroup mtDNA
- To determine oxygen consumption rate (OCR) and extracellular acidification rate (ECAR), the following drugs were injected in microwells and O_2 and H^+ concentrations were measured: oligomycin, two FCCP concentrations, and rotenone + antimycin A
- Differences in OCR and ECAR were evaluated between L and H, J, and Uk cybrids

RESULTS



B

Enriched Pathways

Pathway	NES	NOM p-val	Genes	Source
Alanine Aspartate Glutamate Metabolism	-2.14	0	27	GSEA
Amino Acid Metabolism	-2.08	0	79	GSEA
ISR: Target Gene	-1.73	0.01	55	Space
Antioxidant Defenses	-1.72	0.01	25	Space
Stress ISR/UPR/Mitophagy/ROS	-1.7	0.021	149	Space
Stress ISR/UPR	-1.68	0.03	109	Space
Stress ISR/UPR/Mitophagy/ROS/Raas	-1.67	0.006	208	Space
Amino Acid Metabolism	-1.63	0	82	Mitocarta
Glutamate Metabolism	-1.61	0.02	8	Mitocarta
Antioxidant Defense: Glutathione Metabolism	-1.6	0.028	14	Space

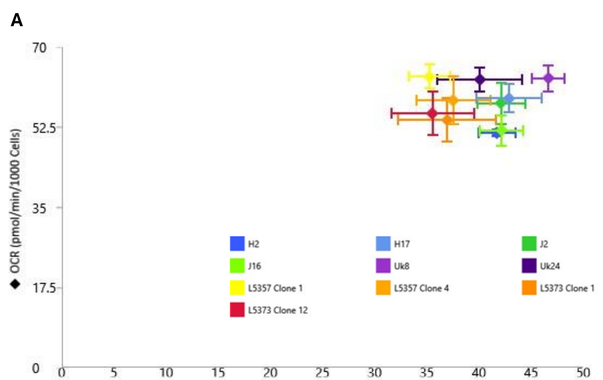
Enrichment in L cybrids vs H, J, Uk cybrids

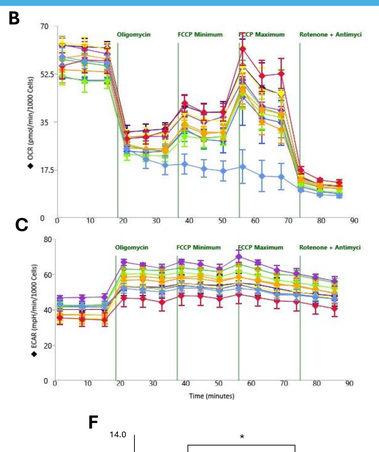
Figure 1. RNA sequencing and pathway analysis reveal basal transcriptional differences between African L and European H, J, Uk cybrids

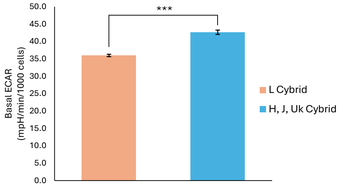
A. Differentially expressed genes (DEGs) enriched in either the African or the European-derived cybrids at a significance of P-adj<0.05 constitute over 15% of the total transcriptome in the African and European-derived cybrids at basal conditions. Notably, the African cybrids display nearly twice as many positive-fold enriched DEGs as the European cybrids.

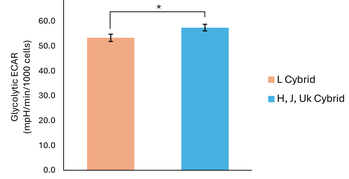
B. Pathway enrichment analysis from the RNA-sequencing analysis data revealed significant enrichment of transcripts involved pathways including amino acid metabolism, the integrated stress response (ISR), and antioxidant defense in the African cybrids relative to the European cybrids at basal conditions.

RESULTS









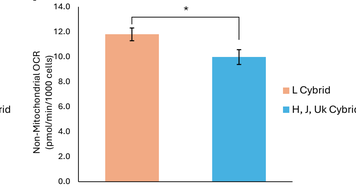


Figure 2. Mitostress Test performed on four L cybrids and six total H, J, Uk cybrids shows differences in energetic profiles between African and European-derived cybrids

A. Energetic profiling using OCR (oxygen consumption rate) and ECAR (extracellular acidification rate) values of individual cybrids reveals grouping of similar cellular energetics within the African-derived cybrids and within the European-derived cybrids
B. OCR of each cybrid during the Mitostress Test plotted against time intervals with the following drug injections: Oligymycin (Complex V/ATP Synthase inhibitor), FCCP (mitochondrial electron transport chain uncoupler), Rotenone & Antimycin A (Complex I & Complex III Inhibitors)
C. ECAR of each cybrid, measured simultaneously during the Mitostress Test, plotted against time intervals with drug injections
D. Basal ECAR was significantly ($P = 1.91x10^{-11}$) elevated in the European-derived cybrids compared to African cybrids
E. Glycolytic ECAR was evaluated as ECAR during Oligomycin treatment, and was significantly ($P = 0.048$) elevated in the European-derived cybrids relative to African-derived cybrids
F. Non-mitochondrial OCR -- the average OCR in total mitochondrial complex inhibition -- was significantly elevated ($P = 0.049$) in the African-derived cybrids compared to European-derived cybrids

CONCLUSIONS

- mtDNA differences between the African and European mtDNA backgrounds induce significant transcriptional differences at basal conditions
- L cybrids display enrichment in transcripts involved AA metabolism, ISR, and antioxidant defense relative to H, J, Uk cybrids
- Energetic profiling of the cybrids indicates the efficacy of cybrids in recapitulating haplogroup differences
- L and H, J, Uk cybrids display differences in extracellular acidification rate and non-mitochondrial oxygen consumption rate

REFERENCES & ACKNOWLEDGMENTS

1) Keane, P. C., Kurzawa, M., et al. (2011). Mitochondrial Dysfunction in Parkinson's Disease. *Parkinson's Disease*, 716871
2) Human mtDNA Migrations. [Image]. (n.d) Retrieved from www.mitomap.org
3) Wallace, D. C. (1995).1994 William Allan Award Address. Mitochondrial DNA variation in human evolution, degenerative disease, and aging. *American Journal of human Genetics, 57(2)*, 201.
4) Wallace, D. C. (2005). A mitochondrial paradigm of metabolic and degnerative diseases, aging, and cancer: A dawn for evolutionary medicine. *Annu.Rev.Genet., 39*, 359-407

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