Mitochondrial Replacement Therapies: Assessing Global Epigenetic Consequences

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Reprogramming Mitochondrial Disease

3 yo female with transfusion-dependent anemia since birth
- Bone marrow biopsy at 6 mos of age showed ringed sideroblasts
- Developed diabetes, pancreatic insufficiency, lactic acidosis
- Diagnosed with Pearson’s Marrow-Pancreas Syndrome

MITOCHONDRIAL DNA DELETION IN PEARSON’S MARROW/PANCREAS SYNDROME

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Novel 2501 bp deletion
(ND4, ND5, tRNAs: Lcun, Sacg, H)
Reprogramming Pearson syndrome cells

Loss of deleted mitochondria over time in cell culture

Bone marrow fibroblasts

QPCR: 60-70% deleted mtDNA

% heteroplasmy

WT 1 2 4 clone

Normal mitochondria
Mutant mitochondria
Normal phenotype
Disease phenotype

mtDNA proliferation

Random Segregation

Loss of heteroplasmy in cell culture
Cells purged of defective mitochondria: improved growth and blood formation, lack sideroblasts

Induced Pluripotent Stem Cells with a Pathological Mitochondrial DNA Deletion

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Disease-Causing Mitochondrial Heteroplasmy Segregated Within Induced Pluripotent Stem Cell Clones Derived from a Patient with MELAS

Clifford D.L. Foles,⁴ Almudena Martinez-Fernandez,⁴ Ester Perales-Clemente,⁴ Xing Li,⁴ Amber McDonald,⁶ Devin Oglesbee,⁶ Sybil C. Hrstka,⁵ Carmen Perez-Terzic,⁴ Andre Terzic,⁴ Timothy J. Nelson,⁶ Reprogramming captures mtDNA pathology

Cell culture alone can generate healthy patient-matched cells

Future: cell therapies for certain features of mitochondrial disease
Mitochondrial Replacement

Pronuclear transfer in human embryos to prevent transmission of mitochondrial DNA disease

Towards germline gene therapy of inherited mitochondrial diseases

Nuclear genome transfer in human oocytes eliminates mitochondrial DNA variants

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A scenario for assessing the global epigenetic modifications that accompany MRT (human)
Prospects for understanding genetic, epigenetic consequences of MRT, pre-clinically?

- Extensive comparative studies: IVF ES cells vs NT-ESCs, iPSC, pESCs
- iPS cells from patients with mitochondrial dz exist alongside purged, normalized isogenic derivatives
  - Enable experiments to define links between bioenergetic defects, genetic/epigenetic stability, and tissue function
- Single cell analytics can be applied to blastomeres from reconstructed embryos
  - RNA-seq; global methylation analysis; limited locus-specific epigenetic analysis; assessment of mtDNA carryover
- Derivation of ES lines from reconstructed embryos
  - Affords opportunity for gene copy number analysis, whole genome sequencing, wg bisulfite sequencing, extensive epigenetic analysis; functional analysis
- Correlations with organismal level analysis in mice, monkeys
Limitations of analytics

- Single cell analytics limited in their resolution
- Mosaicism among blastomeres
- Potential for skewed analyses from derived ES lines
  - Evolution of genotypes, epigenotypes in culture
the unknown...