Mitochondrial replacement: Disease prevention and fertility enhancement?

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No conflicts of interest with the topic of the presentation

Outline

Mitochondrial disease

Mitochondrial disease prevention

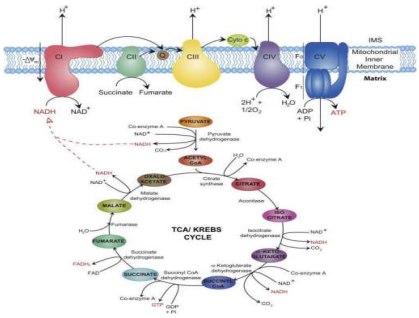
- Preimplantation Genetic Diagnosis
- Mitochondrial replacement Techniques
- Genome Editing

Mitochondrial replacement for fertility enhancement

Pluripotent stem cells as a model for mitochondrial disease

Mitochondrial disease

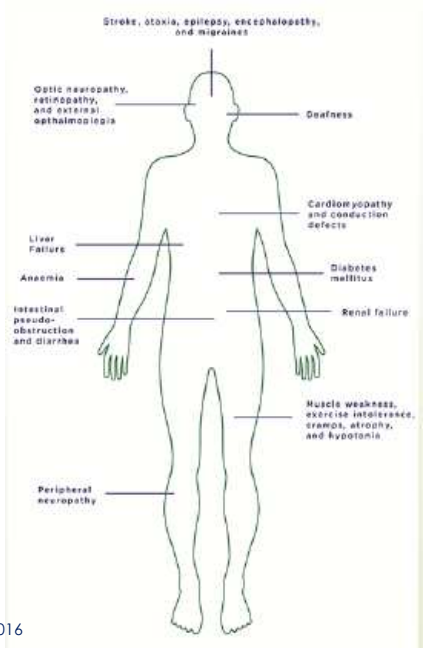
- Clinically heterogeneous group of disorders due to dysfunction of the **mitochondrial respiratory chain** (aerobic metabolism)
- Tissues and organs that are highly **dependent on aerobic metabolism** are preferentially involved in mitochondrial disorders
- Individuals with mitochondrial disorders resulting from mutation of mtDNA may harbor a **mixture of mutant and wild-type mtDNA within each cell (heteroplasmy)**
- The percentage level of mutant mtDNA may vary **among individuals within the same family**, and also **among organs and tissues within the same individual.**
- Single-cell studies and cybrid-cell studies have shown that the proportion of mutant mtDNA must exceed a **critical threshold level** before a cell expresses a biochemical abnormality of the mitochondrial respiratory chain (**the threshold effect**)



Mitochondrial disease

Common clinical features

- Ptosis
- External ophthalmoplegia
- Exercise intolerance
- Cardiomyopathy
- Sensorineural deafness,
- Optic atrophy
- Pigmentary retinopathy
- Diabetes mellitus.
- Encephalopathy
- Seizures
- Dementia
- Migraine
- Stroke-like episodes
- Ataxia
- Spasticity



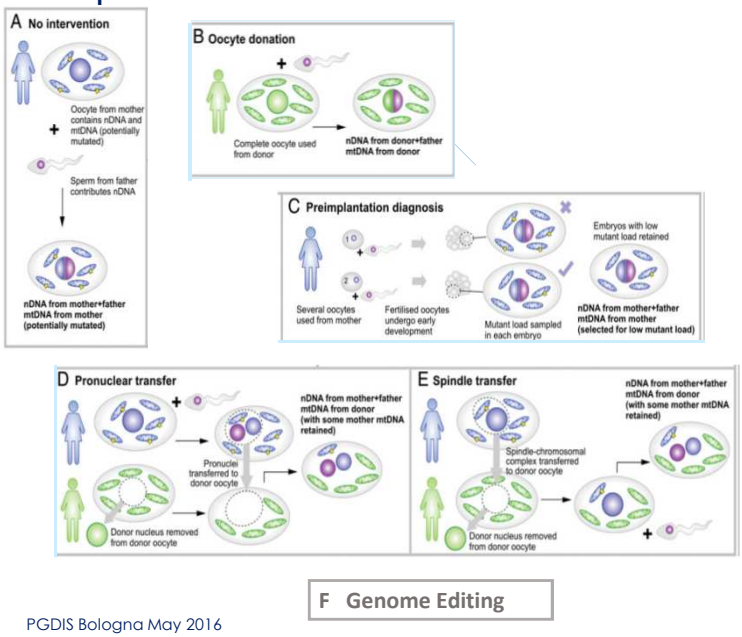
Mitochondrial disease prevention

- A: No intervention
- B: Oocyte donation
- C: Preimplantation genetic diagnosis (PGD)
- D, E: Mitochondrial replacement techniques (MRT):

Spindle Transfer

Pronuclear Transfer

- F: Genome editing (GE)

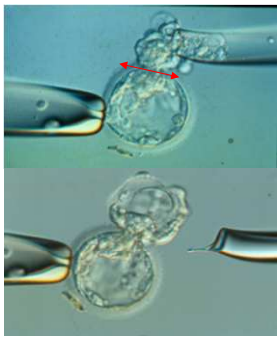


F Genome Editing

PGD

Blastocyst preimplantation genetic diagnosis (PGD) of a mitochondrial DNA disorder

- A PGD case in a 30-year-old carrier of 35% 3243A>G mtDNA mutation load with a daughter affected by mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes (MELAS) syndrome.
- Blastocyst biopsy for PGD of **mutation load and gender.**
- Oocytes and embryos were found to possess a variety of 3243A>G mutation loads from **9% to 90% in oocytes** and **7% to 91% in embryos**.

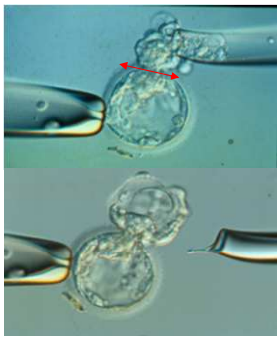


Treff ,2012

PGD

Blastocyst preimplantation genetic diagnosis (PGD) of a mitochondrial DNA disorder

- Highly consistent results were obtained within multiple biopsies of both cleavage- and blastocyst-stage embryos.
- Transfer of **a male embryo, predicted to possess 12% mutation load** by analysis of a trophectoderm biopsy, resulted in the **delivery of a boy** with tissue-specific **mutation loads ranging from undetectable to 15%.**



Treff ,2012

PGD

Mitochondrial disease prevention

- PGD can reduce the risk of transmitting mtDNA disease, it relies on the production of embryos with low levels of mtDNA mutation.
- PGD provides a promising risk reduction strategy for affected families
- PGD is **not useful for homoplasmic women** (100% of mtDNA mutated) or **for heteroplasmic women** who consistently produce **oocytes with high mutation loads**

MRT

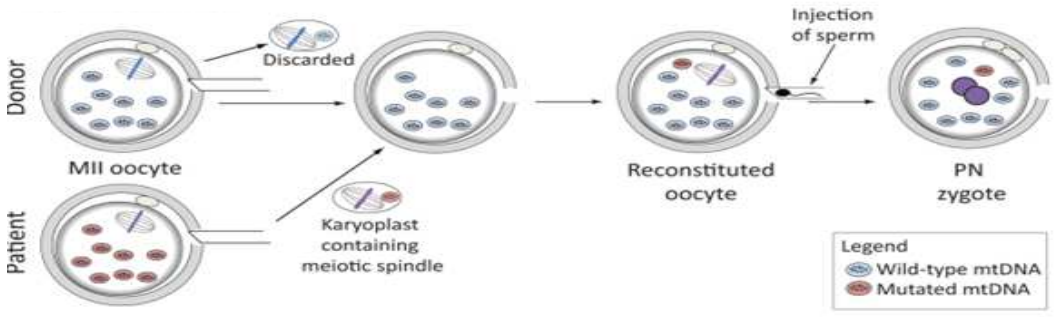
Mitochondrial disease prevention: Mitochondrial replacement techniques (MRT)

Mitochondrial replacement techniques to replace mutated mtDNA with wild-type mtDNA offer women with **high mutation loads** the possibility to have a genetically related child without the risk of transmitting disease.

- Spindle transfer
- Pronuclear transfer

MRT

Spindle Transfer



Richardson, 2014

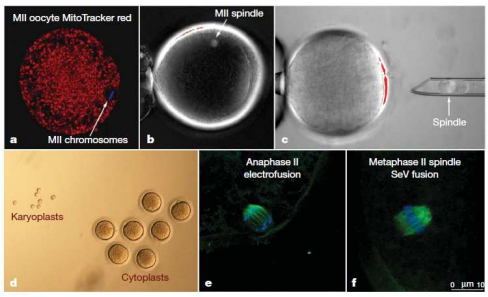
Spindle Transfer

MRT

Mitochondrial gene replacement in primate offspring and embryonic stem cells

- Mitochondrial genome can be efficiently replaced in mature non-human primate oocytes (Macaca mulatta) by spindle-chromosomal complex transfer from one egg to an enucleated, mitochondrial-replete egg.
- The reconstructed oocytes were capable of **supporting normal fertilisation,embryo development and produced healthy offspring.**
- Genetic analysisi confirmed the origin of nuclear DNA from spindle donors and mt DNA from cytoplast donors.

Tachibana ,2009



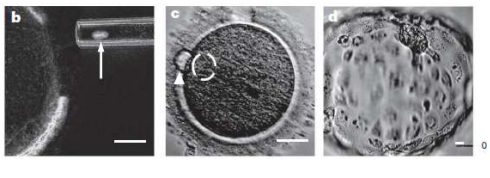
Spindle Transfer

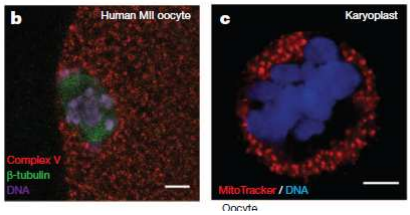
MRT

Nuclear genome transfer in human oocytes eliminates mitochondrial DNA variants

- **Nuclear genome transfer** between unfertilized oocytes of two donors to prevent the transmission of mitochondrial mutations.
- Developmental efficiency to the **blastocyst stage** and **genome integrity** was maintained
- **Mitochondrial DNA** transferred with the nuclear genome was initially detected at levels **below 1%**, decreasing in blastocysts and stem-cell lines to undetectable levels.
- Stem cells and differentiated cells had **normal mitochondrial respiratory chain enzyme activities** and **oxygen consumption** rates.

Paull, 2013





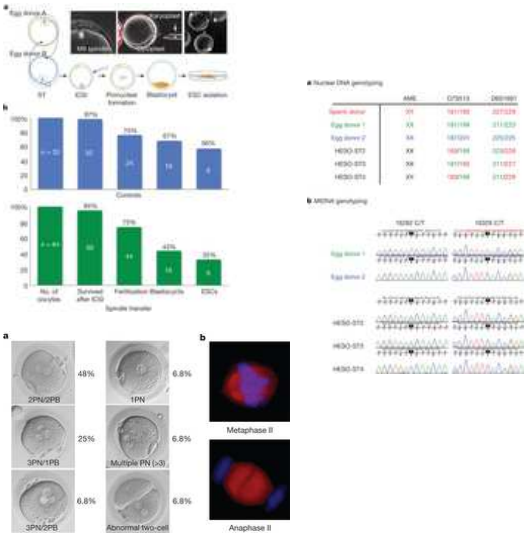
Spindle Transfer

MRT

Towards germline gene therapy of inherited mitochondrial diseases

mtDNA replacement in human oocytes by spindle transfer

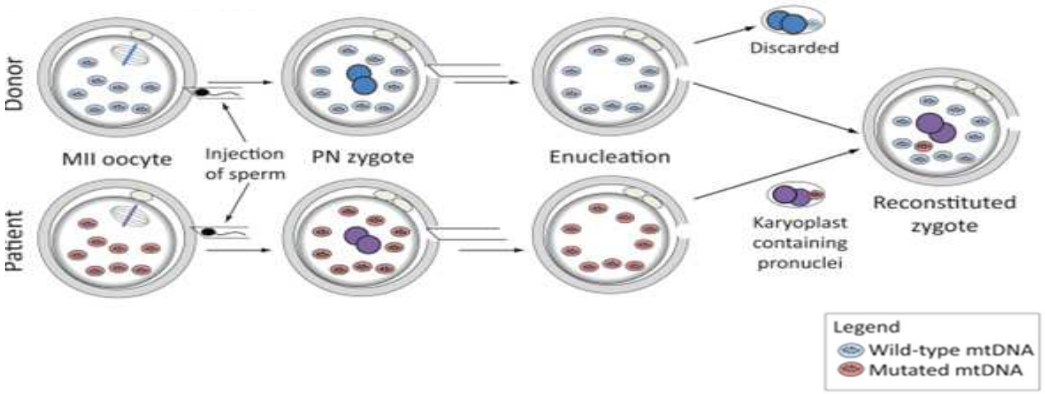
- Of 106 human oocytes donated for research, 65 were subjected to reciprocal ST and 33 served as controls.
- **Fertilization rate** in ST oocytes (73%) was similar to controls (75%);
- A significant portion of **ST zygotes (52%) showed abnormal fertilization** as determined by an irregular number of pronuclei.
- Among normally fertilized ST zygotes, **blastocyst development** (62%) and **embryonic stem cell isolation** (38%) rates were comparable to controls.
- All embryonic stem cell lines derived from ST zygotes had normal euploid karyotypes and **contained exclusively donor mtDNA.**



Tachibana,2013

MRT

Pronuclear Transfer



Richardson, 2014

MRT

Pronuclear Transfer

MOUSE

- Proven to be compatible with development in mice (McGrath and Solter, 1983; Meirelles & Smith, 1997)
- Proven to prevent transmission of an mtDNA deletion in mice (Sato, 2005)

MRT

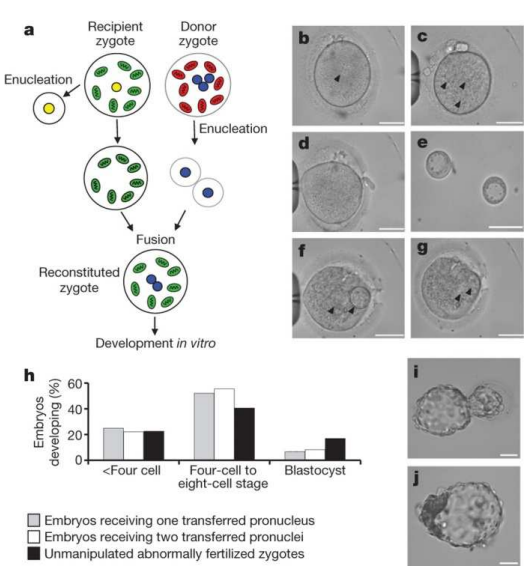
Pronuclear Transfer

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

- Pronuclear transfer is technically feasible in human zygotes (**3PN zygotes**)
- Reconstituted zygotes can develop to the **blastocyst stage** (8% vs 17%)
- How much mtDNA is transferred with the pronuclei? mtDNA **carryover** :

  1st series 7.6%; 2nd series 2%

Craven, 2010



MRT

Safety and efficacy

- Effects of pronuclear transfer on embryo development of <u>normally</u> fertilised eggs
- Can reconstituted embryos develop to **good quality blastocysts** with high efficiency?
- Are these **blastocysts normal**?

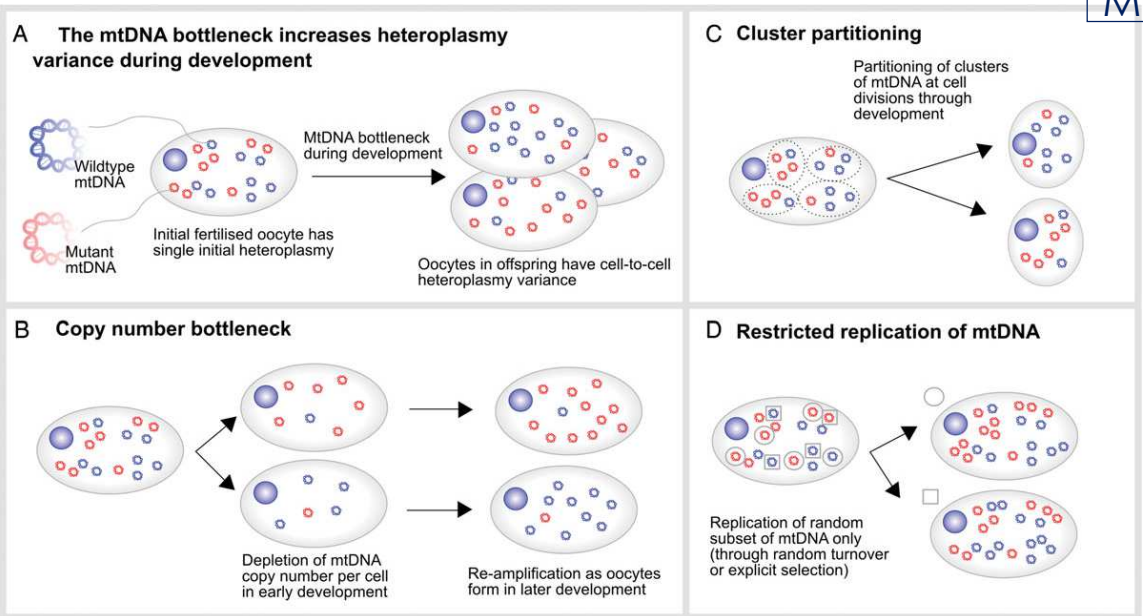
Cell number - Cell death

Gene expression

Chromosomal constitution

mtDNA carryover . **mtDNA bottleneck**

MRT

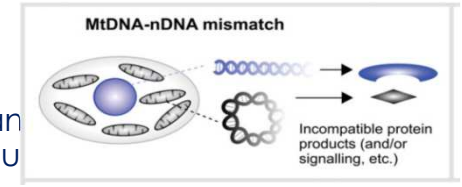


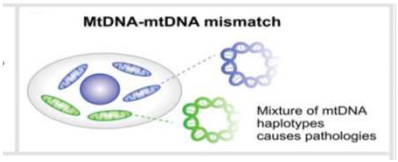
The mitochondrial DNA bottleneck during development

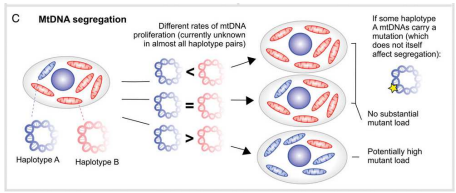
Burgstaller, 2015

MRT

- Supply of **donor oocytes** needed.
- Co-evolution of the mitochondrial an maternal nuclear genomes might resu in adverse effects arising from the creation of a **new combination of mtDNA and nuclear DNA.**
- Evidence of **adverse outcomes following experimentally induced heteroplasmy in mice and Drosophila.**
- **No evidence for incompatibilities** between nuclear and mitochondrial genotypes **in humans**
- Replacement of male embryos would prevent transmission

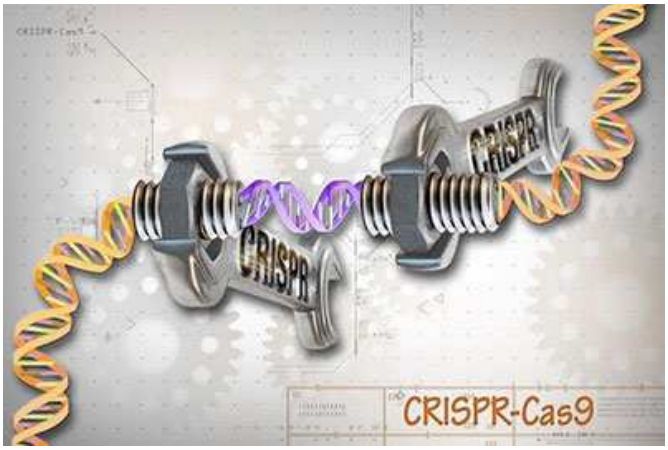






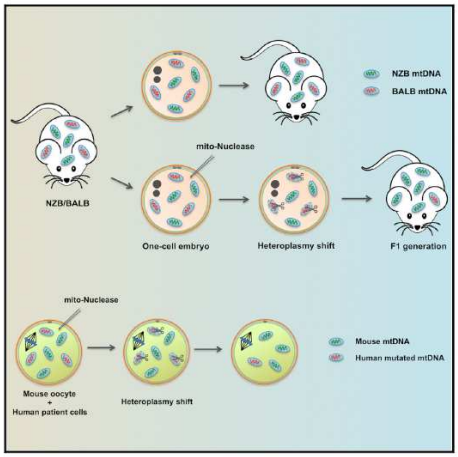
Burgstaler, 2015

Genome editing for mitochondrial disease prevention



GE

Selective Elimination of Mitochondrial Mutations in the Germline by Genome Editing



Reduction of mutated mitochondrial genomes in the germline .
Induction of mtDNA heteroplasmy shift

- Injection of **mito-targeted restriction endonucleases into mouse oocytes and 1-cell embryos**: reduced levels of mutated mtDNA
- Injection **of mito-TALENs against human mutations into artificial human oocytes** (fusion of patient's cells with mouse oocytes using Sendai virus): reduced levels of mutated mtDNA

Reddy, 2015

GE

- Single injection of mRNA into patient oocytes is **technically simpler and less traumatic to the oocyte** compared to mitochondrial replacement techniques
- Transmission of mitochondrial diseases by female carriers directly correlates with the levels of mutated mtDNA present in oocytes.
- Oocytes containing **high levels** of mutated mtDNA that are subjected to **heteroplasmy shift** may result in embryos with low mtDNA copy number that **may fail to implant**.
- **PGD** could be used as a complementary approach to select embryos with **mtDNA copy numbers sufficient for implantation.**

Autologous transplantation into oocytes of mitochondria from oogonial stem cells may enhance embryo development and increase pregnancy rate?

The AUGMENT^{SM} Treatment: Physician Reported Outcomes of the Initial Global Patient Experience

1.-Ovarian biopsy
2.-Isolation of mitochondria from oogonial stem cells
3.- Microinjection of mitochondria + spermatozoon (ICSI)

- Egg precursor cells can be readily isolated from the protective outer lining of the ovarian cortex.
- The AUGMENTSM treatment was initially used in a population of difficult-to-treat patients with a poor prognosis. Each group reported marked improvements in pregnancy rates above the historic IVF success rate for these patients (e.g., 11- and 18-fold increase in ongoing clinical pregnancy rates in the UAE and Canada, respectively).
- Morphogenetic embryo selection and transfer from the AUGMENT treatment group was significantly higher, suggesting that improved embryo quality may have resulted in the improved pregnancy rates observed in these women.

Fakih, 2015

THE USE OF MITOCHONDRIAL TRANSFER TO IMPROVE ART OUTCOME

- Wrong citations (Van Blerkhom, Cohen)
- Wrong control group
- No blinded morphology assessment
- Methodology

Mitoch. Isolation and purification

Number of mtDNA copies needed

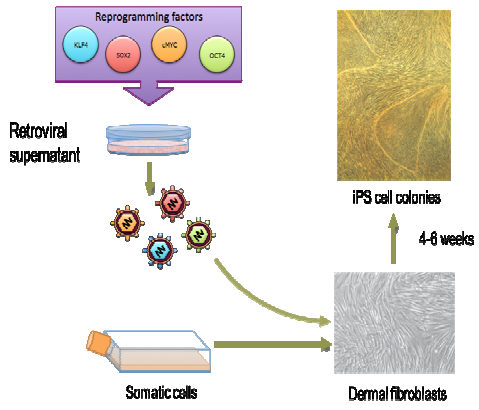
mtDNA heteroplasmy

Epigenetic changes

No animal models

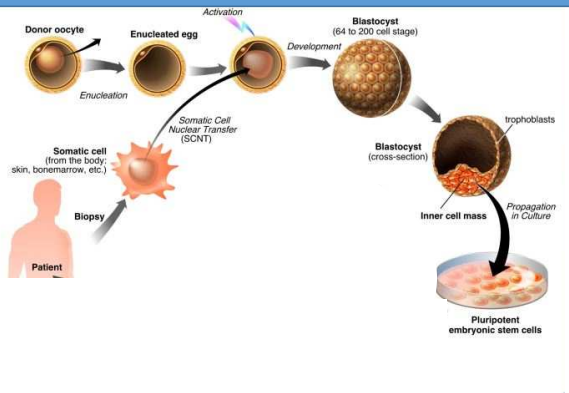
Heindryckx, ESHRE SIG Stem Cells, 2015

Pluripotent stem cells from mtDNA disease patients
- iPS
- SCNT ESC



INDUCED PLURIPOTENT STEM CELLS iPS

SOMATIC CELL NUCLEAR TRANSFER SCNT



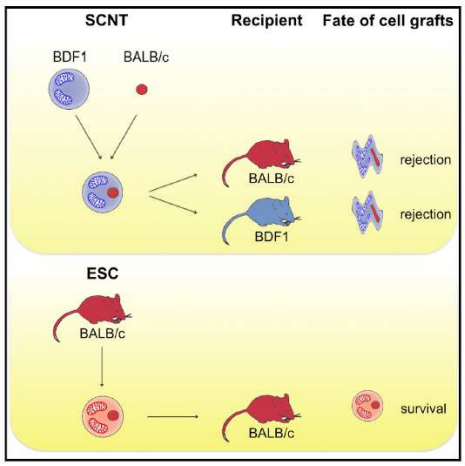
SCNT

SCNT-Derived ESCs with Mismatched Mitochondria Trigger an Immune Response in Allogeneic Hosts

SCNT would allow the creation of patient-matched embryonic stem cells, even in patients with hereditary mitochondrial diseases

Murine transplantation setup

- **Allogeneic mitochondria in NT-ESCs**, which are nucleus-identical to the recipient, may trigger an **adaptive alloimmune response** that impairs the survival of NT-ESC grafts.
- The immune response is adaptive, **directed against mitochondrial content**,and amenable for tolerance induction.
- **Mitochondrial alloantigenicity** should be considered when developing therapeutic SCNT-based strategies.



Deuse, 2015

iPS/SCNT

Metabolic rescue in pluripotent cells from patients with mtDNA disease

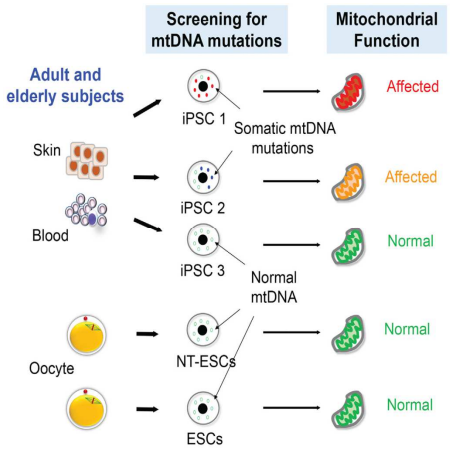
Genetically corrected pluripotent stem cells (PSCs) generated from patients with mtDNA disease.

- Multiple **iPS and SCNT cell lines** obtained from patients with common heteroplasmic mutations including 3243A.G, causing mitochondrial encephalomyopathy and stroke-like episodes (MELAS)5, and 8993T.G and 13513G.A, implicated in Leigh syndrome.
- Generation of multiple **iPS** cell lines allows recovery of clones with **exclusively wild-type mtDNA due to spontaneous segregation of heteroplasmic mtDNA.**
- **SCNT enables correction of homoplasmic mutations** through replacement with donor mtDNA, and generation of PSCs with transcriptional and epigenetic profiles similar to embryoderived embryonic stem cells.
- **Recovery of metabolic function** despite haplotype differences between patient and donor mtDNA suggests that **normal nuclear-to-mitochondrial interactions** are highly conserved within species.

Ma, 2015

iPS

Age-Related Accumulation of Somatic Mitochondrial DNA Mutations in Adult-Derived Human iPSCs



Genetic integrity of iPS?
Accumulation of somatic **mitochondrial genome (mtDNA) mutations** in skin fibroblasts, blood, and iPSCs derived from young and elderly subjects (24–72 years).

- Human iPSC clones derived from **elderly adults** show **accumulation of mtDNA mutations**
- Fewer mtDNA mutations are present in ESCs and iPSCs derived from younger adults
- Accumulated mtDNA mutations **can impact metabolic function in iPSCs**

Kang, 2016

Summary

- Mitochondrial disease prevention can be attempted through PGD, mitochondrial replacement techniques and genome editing.
  - PGD: proven efficiency
  - MRT clinical application about to be initiated in UK. Safety and efficacy?
  - Genome editing. Debate on germline genome editing.
  - Male embryos to be replaced to avoid transmission.
- Controversy over autologous replacement of mitochondria from oogonial stem cells to enhance fertility in poor prognosis women.
- Puripotent stem cells constitute a good model to study mitochondrial function and disease.

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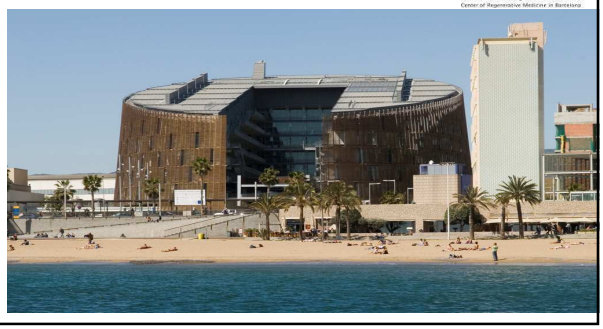

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