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Category: ORAL

(NOMINATED FOR THE BASIC SCIENCE AWARD FOR THE BEST ORAL PRESENTATION)

Title:

Assessment of cellular heterogeneity in the level of mitochondrial DNA heteroplasmy in mouse embryonic stem cells

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Abstract Text:

Study question

Is there any heterogeneity in the level of mitochondrial DNA (mtDNA)-heteroplasmy among individual colonies (intercolony) and at the single cell level (intracolony) in mouse embryonic stem cells (ESCs), both in pluripotent and differentiated states?, and is there any correlation between ESCs and cells from pre-implantation embryos, in terms of mtDNA-heteroplasmy?

Summary answer

ESCs exhibit variability in the level of mtDNA-heteroplasmy between individual colonies and also at the single cell level, both in differentiated and undifferentiated states. Intracolony heterogeneity is more divergent than intercolony heterogeneity. mtDNA-heteroplasmy in ESCs is closer to trophectoderm (TE) than to second polar body (2PB) of the founder embryo.

What is known already?

MtDNA-heteroplasmy is a condition when more than one type of mtDNA co-exists within a cell. Intercellular variation of heteroplasmic load is frequently seen in individuals with mtDNA mutations. However, it is unresolved how these differences between somatic cells arise and how heteroplasmic mtDNA segregates between daughter cells. To our knowledge, this is the first report showing heterogeneity in mtDNA-heteroplasmy level between and within ESC colonies before and after differentiation and their correlation with parent embryos.

Study design, size and duration
Intercolony heterogeneity was analyzed in ESCs at different passages until 40th passage (n=137) and also in differentiated cells after retinoic acid (RA) stimulated differentiation (n=42). Heterogeneity in 100 single cells from both ESCs and embryoid bodies was investigated. MtDNA-heteroplasmy levels were compared between ESCs and their corresponding 2PB and TE.

Participants/materials, setting, methods
In-vivo fertilized zygotes were recovered from heteroplasmic BALB/cOlaHsd mice. ESCs were derived from blastocysts previously subjected to 2PB biopsy and TE biopsy. The coefficient of variation (CV) in the level of mtDNA heteroplasmy was determined in ESC colonies and in single cells before and after differentiation. $P<0.05$ was considered significant.

Main results and the role of chance
Heteroplasmic ESCs were derived from five embryos previously subjected to second PB and TE biopsy. The CV in pluripotent ESC colonies was 3.7%, 11.6%, 12.5%, 14.7% and 7.4% in lines 1 through 5, respectively. After RA mediated differentiation, the CV in these lines was 2.8%, 21.9%, 12.0%, 0.6% and 35.7% respectively. At the single cell level, bigger divergence was observed, before (L1=16.0%, L2=25.2%, L3=27.3%, L4=12.5%, L5=14.6%) and after differentiation (L1=38.0%, L2=12.4%, L3=26.9%, L4=39.1%, L5=36.4%) compared to the colonies. In line 3, level of mtDNA heteroplasmy in undifferentiated and differentiated single cells was significantly lower compared to that in the colonies. The level of mtDNA heteroplasmy in ESCs was closer to the TE (r=0.6) than to the second PBs (r=0.3) of the founder embryo.

Limitations, reason for caution
These results in a neutral polymorphic mouse model should be extrapolated to mtDNA mutation disorders in humans only with caution.

Wider implications of the findings
Our results support a large degree of heterogeneity at the single cell level in ESCs in terms of mtDNA heteroplasmy. Unbalanced mtDNA segregation in pluripotent cell populations during subsequent cell divisions may lead to alterations in the level of mtDNA heteroplasmy in future somatic tissues. Our research may serve to gain insight into the functional regulation of mtDNA heteroplasmy in humans with mitochondrial disorders.

Study funding/competing interest(s)

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Trial registration number

Not applicable.