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Adult Stemlike Cells Exclude 'Older' Mitochondria

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Abstract

Asymmetric distribution of damaged cellular constituents may occur during mitosis, resulting in more and less pristine daughter cell pairs. In *Science*, Katajisto et al., (2015) report that mammary stemlike cells (SLCs) unequally apportion older mitochondria to post-division daughter cells, with the daughter containing younger mitochondria maintaining the SLC pool.

Adult stem cells maintain and renew mammalian tissues by asymmetric cell division, in which one daughter retains stem cell characteristics and the other daughter assumes a more differentiated, lineage-specified fate. Accumulating damage to cellular components including lipids, proteins, and nucleic acids is likely to compromise stem cell functions over time and lead to tissue dysfunction with aging (Rossi et al., 2007). The asymmetric partitioning of damaged cell components to one daughter cell versus another is a strategy that favors rejuvenation and longevity in yeast (Aguilaniu et al., 2003; Shcheprova et al., 2008), bacteria (Lindner et al., 2008), and drosophila (Bufalino et al., 2013). Proteins destined for degradation are also asymmetrically distributed between daughter cells in human embryonic stem cell (hESC) and mammalian fibroblast cell lines, resulting in more and less pristine daughter cell pairs (Fuentealba et al., 2008). Now, Katajisto and colleagues report that selective asymmetric partitioning of 'old' mitochondria is required to maintain a stemlike cell (SLC) pool in cultured, immortalized human mammary epithelial cells (hMECs) (Katajisto et al., 2015).

Katajisto used photoactivatable green fluorescent protein (paGFP) (Patterson and Lippincott-Schwartz, 2002) fusion proteins targeted to lysosomes, mitochondria, Golgi, ribosomes, and chromatin with a UV light pulse to differentially tag older fluorescent versus younger non-fluorescent cell components in rounded SLCs and flat, non-stemlike hMECs. Following mitosis, a paGFP-Omp25 fusion protein targeted to the mitochondrial outer membrane showed asymmetric partitioning of the fluorescent signal between daughters of SLCs, but not between daughters of hMECs. paGFP fusion proteins targeted to the other

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four cell components, along with a PKH26 plasma membrane lipophilic dye, showed a symmetric fluorescent distribution in daughter cells from both SLCs and hMECs. To track 'young' versus 'old' labeled mitochondria, mitochondria outer (Omp25) and inner (COX8A) membrane targeted Snap-tag fusion proteins (Keppler et al., 2003) were employed in which red and green linked fluorophores distinguished between newly synthesized and older mitochondrial pools. Following division of SLCs, mainly old, and to a lesser extent young, Snap-tag labels asymmetrically distributed between daughter cells, with each daughter containing the same amount of total mitochondria. Unlabeled mitochondrial proteins synthesized after the Snap-tag labeling reactions balanced the mitochondrial content between cells as they were preferentially apportioned to the daughter cell containing fewer labeled (older) mitochondria-targeted proteins. Analysis of label distribution in SLC mother cells before cell division showed spatial segregation, with proportionally more older labeled mitochondria-targeted proteins localizing near the nucleus, in contrast to young labeled mitochondria-targeted proteins which were dispersed more evenly throughout the cytoplasmic mitochondrial network. This pre-division spatial patterning may have a role in excluding old labeled mitochondria from one of the two daughter cells post-mitosis by an unknown mechanism. Interestingly, differences in mitochondrial membrane potential, $\Delta\Psi m$, were not responsible for the asymmetric segregation of old labeled mitochondria with SLC division.

SLC daughter cells receiving more (designated Pop1) or less (designated Pop2) Snap-tag labeled old mitochondria were FACS-sorted and grown in culture (Figure 1). Pop1 daughters had a flat adherent, non-stemlike hMEC morphology, in contrast to Pop2 daughters, which showed both round and flat cell morphologies, suggestive of SLCs. Mammosphere assays confirmed these morphological impressions, as Pop2 cells generated three-times more mammospheres, a measure of stemness, than Pop1 cells. Blockade of mitochondrial network fission, which is required for PINK1/Parkin-dependent and independent mitochondrial degradation through mitophagy, using the Drp1 fission protein inhibitor mDivi-1, reduced the number of Pop2 cells inheriting mostly young label-targeted mitochondria, and increased the number of Pop1 cells inheriting a mixture of young and old label-targeted mitochondria. A similar result was obtained by impairing the Parkin-dependent mitochondrial quality control system using a siRNA targeting Parkin. Importantly, both perturbations led to less efficient mammosphere formation of the Pop2 cells at the lower, Pop1 rate of production, providing evidence that partitioning of mitochondria containing old versus young-targeted proteins in SLC daughter cells is required for maintaining stemness. The mechanism(s) that link mitochondrial network dynamics and quality control to asymmetric old label-containing mitochondrial apportioning by SLCs, in addition to establishing the perinuclear localization of old labeled mitochondria, requires further investigations.

These intriguing studies selectively linking stemness with mitochondria containing target proteins of different age naturally raise many important follow-up questions. For example, it is unclear whether the asymmetric distribution of old mitochondria to daughter cells occurs in other mammalian adult stem cell types. In flies, proteins damaged during aging are asymmetrically distributed to daughter progeny to maintain intestinal stem cells but this asymmetric distribution of damaged proteins does not occur in germline stem cells or

neuroblasts (Bufalino et al., 2013). Also, as alluded to by the authors, it is unclear whether asymmetric mitochondrial apportionment by contained protein age in immortalized SLCs in vitro will be replicated in mammary stem cells or other stem cell types in vivo. The molecular basis for selective mitochondrial partitioning based on contained protein age compared to age-independent partitioning of other cell structures is not revealed. Perhaps mitochondrial proteins (and lipids?) are more damaged with age than proteins in other organelles or cellular locations due to the redox reactions of the nearby electron transport chain, although the time for this to occur is relatively short for SLCs in the reported assay system. Finally, are mitochondria containing older proteins excluded from one SLC daughter cell simply by their premitotic positioning in the mother SLC or are there additional active or passive processes that cause retention, exclusion, or expulsion of mitochondria based upon protein age during mitosis? Overall, this new study by Katajisto and colleagues shows that the age of protein components associated with a whole organelle, the mitochondrion, is a new factor that potentially affects the maintenance of stemness and adult stem cell pools. Further studies will be required to determine how a cell senses mitochondrial age to trigger effector functions that influence cell fate.

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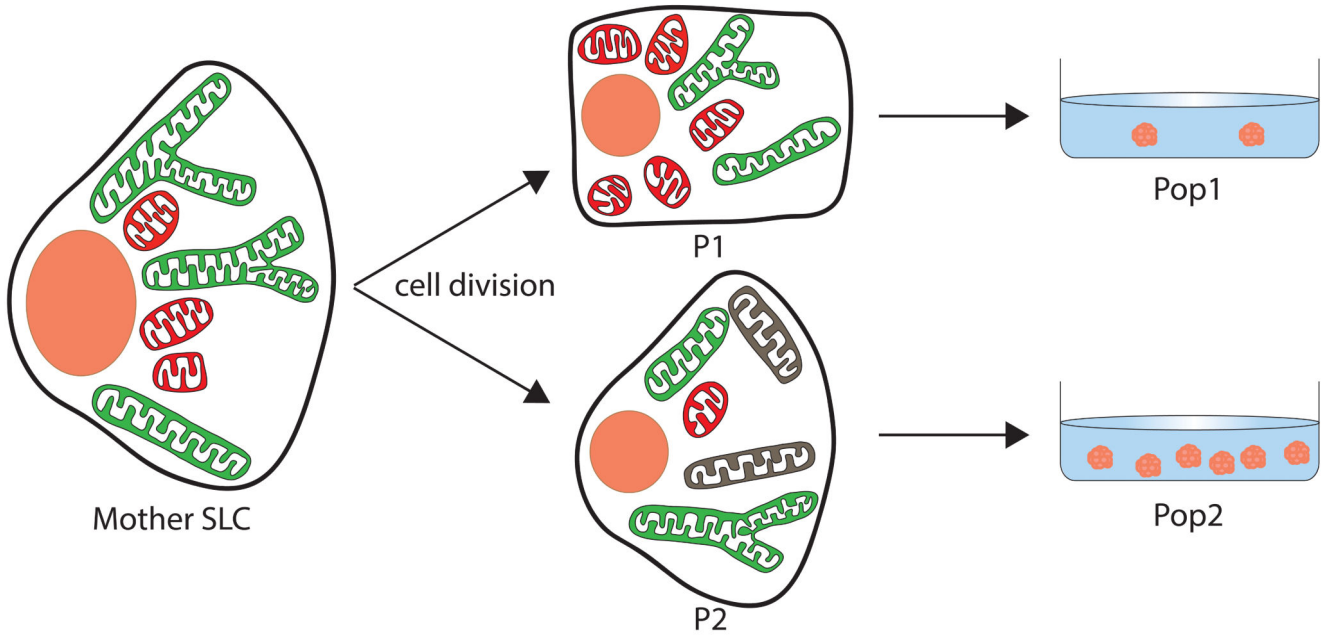

Figure 1. Asymmetric Distribution of Mitochondria Containing Older Versus Younger Membrane Proteins to SLC Daughter Cells Influences 'Stemness'

A mammary SLC (left) expresses genes associated with stemness and shows perinuclear enrichment of labeled older mitochondria-targeted proteins (Omp25 and COX8A; RED), in contrast to labeled mitochondria-targeted younger proteins that are more evenly dispersed throughout the mitochondrial network (GREEN). Cell division generates two daughter cells (P1 and P2) that contain similar total amounts of mitochondria. P1 daughter cells contain proportionally more old red-labeled mitochondria-targeted proteins thanP2 daughter cells that contain proportionally more green-labeled and unlabeled young mitochondria-targeted proteins. A population of P2 daughter cells (Pop2) shows a mixture of rounded SLCs and non-stemlike hMECs and generates three times as many mammospheres, a measure of stemness, than does a population of P1 (Pop1) daughter cells, suggesting that mitochondria-targeted protein age influences the renewal of SLCs. Consistent with this interpretation, disruption of mitochondria partitioning based upon the age of targeted proteins by pre-division treatment with mDivi-1, to alter network dynamics, or siParkin, to inhibit PINK1/Parkin-dependent mitophagy, causes Pop2 cells to form mammospheres at the reduced rate observed for Pop1 cells.