The role of autophagy during the early neonatal starvation period

Kuma, A *et al.* (2004)Nature 432: 1032-1036

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Autophagy: The Other Programmed Cell Death

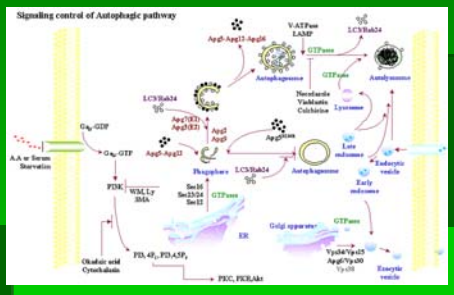
- Degradation of organelles and cellular components by sequestration within an autophagosome and subsequent fusion with a lysosome.
- Adaptive response to starvation.
- Maintains cytoplasmic homeostasis.
  - Degrade long-lived and nonfunctional organelles
- There are 16 known autophagy genes (ATG's) most of which are conserved in higher eukaryotes.

Previous Studies

- Autophagy may play a role in development, pathogenesis, and cell death
  - Dauer formation in *C. elegans*
  - Sporulation in *S. cerevisiae*
  - Larval to pupal development in *D. melanogaster*
  - However, very little genetic studies to support this in higher eukaryotes
- LC3 is the human homolog to yeast Atg8
- Developed transgenic mouse expressing GFP-LC3 in majority of tissues
  - Autophagy induced in response to starvation in young as well as adult mice

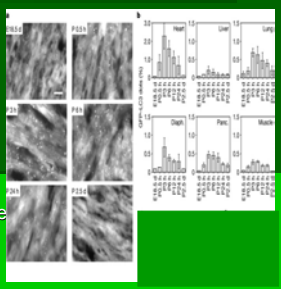
Specific Aim

- Study the physiological role of autophagy during embryonic and perinatal stages using a transgenic mouse model



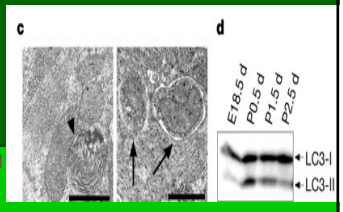
GFP-LC3 Labeled Transgenic Mice

- GFP-LC3 dots, representative of autophagosomes remained at low levels during embryonic stages but increased immediately after birth
- Most up-regulated in high energy organs like heart, lung, and diaphragm



Autophagy is up-regulated at birth

- Autophagic vacuoles seen by EM
- Conjugated LC3, signifying autophagy is transiently up-regulated at birth but returns to basal levels within 24 to 48 hours



Autolysosomes Autophagosomes

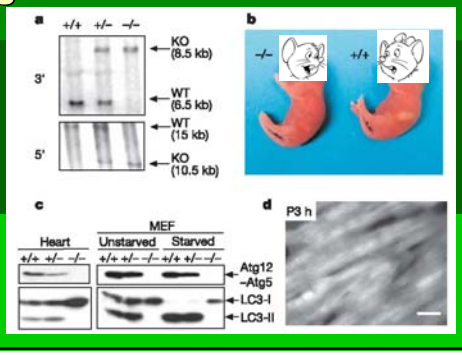
Results

- Autophagy is induced immediately and transiently at birth in tissues that suddenly require a large energy expenditure
  - This may be due to sudden withdrawal from trans-placental nutrition
- Can normal neonates adapt to post-delivery starvation by using autophagy as a means of self-nourishment?

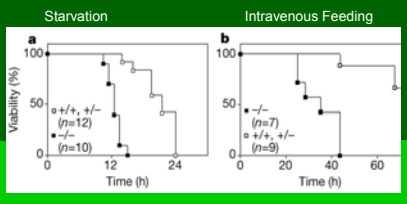
Developing Atg5 -/- Mice

- Atg5 associates with Atg12, a ubiquitin-like protein, a process required for autophagy progression
- Atg5 +/- cells used to make chimeric mice which were backcrossed with wt mice
  - Heterozygous mice were inbred to make homozygous Atg5 knockouts

Can autophagy occur in an Atg5 knockout neonate?



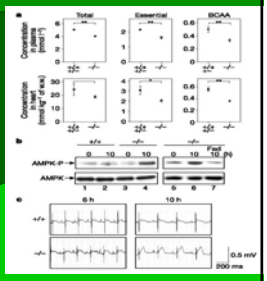
Do Atg5 -/- mutants have a suckling defect?



Results show that there is a nutrient availability defect that causes neonatal death rather than a defect in the mouse's ability to suckle.

Are amino acid concentrations regulated by autophagy?

- Atg5 -/- mutants show severe systemic deficiency in amino acids
- Mutants had low lipid and glucose levels
- Other nutrients were not affected by autophagy
- AMPK, energy sensor is activated in KO but is suppressed by forced feeding
- KO heart has elevated ST segment but no hypoxic damage
  - Possibly respiratory substrates are limited



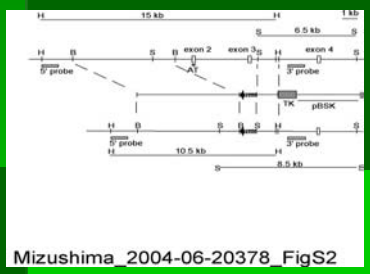
Conclusion

- Autophagy is induced immediately and transiently after birth in response to nutrient deprivation
- Autophagy deficient mice are unable to recycle nutrients so they die within the first two days of life.
- Death can be prolonged by forced milk feeding but is not enough to sustain the mice
- Atg5 mutants are amino acid deprived as well as hypoglycemic and hypolipidemic.

Questions to be Addressed

- Under what other conditions can autophagy contribute to cell viability in a mammalian system?
- How does autophagy regulate plasma amino acid levels?
- What organs/tissues are involved?
- What other organisms use autophagy as an energy accessing source during nutrient deprivation and/or development?

Restriction map to determine presence of the Atg5 allele



S= SpeI

H= HpaI

Mizushima_2004-06-20378_FigS2