Hyaluronan regulated mechanisms that regulate asymmetric gut looping morphogenesis

By Joel Cruz
A long time ago...
Embryonic Development

- Morphogenesis: The creation of shapes and how the organism morphs
- Organogenesis: Specifically involving the morphogenesis of organs and their development
Powerful & Simple

- About 30 feet long
- Inner surface ~ 200m² (tennis court)
- Conserved looping topology
Clinical Application

Normal Rotation  Midgut volvulus
How is this being studied?

- Embryo
- Model Species
Structure of Gut Tube

- Dorsal mesentery suspends gut tube
Looping of Gut Tube

- Looping is an essential step in the development of the gastrointestinal tract
- Starts with 90 degree counterclockwise rotation
How does the gut tube loop?
Cellular Asymmetries
Pitx2
Gpc3/Daam2/Wnt5a, etc...

Davis et al., Dev Cell 2008
Kurpios et al., PNAS 2008
Savin et al., Nature 2011
Welsh et al., Dev Cell 2013
Mahadevan et al., Dev. Cell 2014
Welsh et al., Cell Reports 2015
Hyaluronan (HA) is a strong candidate for driving right side expansion.
Hyaluronan

- The longest polysaccharide sugars
- Ability to absorb and trap water
- Expansion of cell architecture
Coincides with Asymmetries

- HA appears in HH17, but asymmetrically at HH18
- Same time frame when Asymmetries appear
HA depletion results in loss of conserved gut looping
Hyladherins: Tsg6

- HA is a long chain, can have multiple functions
- Hyladherin that covalently bonds to HA
- Allows HA to function
What is the exact role of TSG6 in looping morphogenesis?

- Tsg6 altered to determine its role
- Knock Out (Mutant Mice)
- Knock Down (Morpholino)
TSG6 Knockdown

Knockdown of Tsg6 results in loss of expansion in right DM

R: ctrl MO
R: Tsg6-MO
Loss of looping topology
Loss of looping topology

HH 28

Wild type     Mutant
Loss of looping topology

HH 36

Wild type  Mutant
Conclusion

Knockdown of Tsg6 from the right side affects
   Leftward tilt
   Counterclockwise rotation
   Orientation and positioning of secondary loops
**LEFT**  Pitx2
Gpc3/Daam2/Wnt5a, etc...

**Davis et al., Dev Cell 2008**
**Kurpios et al., PNAS 2008**
**Savin et al., Nature 2011**
**Welsh et al., Dev Cell 2013**
**Mahadevan et al., Dev. Cell 2014**
**Welsh et al., Cell Reports 2015**

**RIGHT**  HA + TSG6

1. Initiates LR
   Asymmetric Morphogenesis the GI tract
2. Establishes correct looping topology
Future directions

1. Characterizing the effect of Tsg6 knockout in gut looping in mice
2. To see whether effects of knockdown could be rescued by overexpression of Tsg6 on the right side,
3. To determine the specific mechanisms by which tsg regulates looping morphogenesis at later stages
Acknowledgements

- Professor Brian Crane, HHMI CHAMPS
- Nev, CHAMPS
- Natasza Kurpios
- Aravind Sivakumar
- Ricky Narvaez
- Adam Oneal
- Rest of Kurpios Lab
- And thank you for your time
Morpholino

- Oligomer molecule
- Modify gene expression
- Knock Down
- Block RNA translation
- Temporary