Drug repurposing for myotubular and centronuclear myopathies

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Drug repurposing

- Drug repurposing is the application of an “old” drug for a completely different and new indication.
- Drug repurposing can also apply to a drug that has no current application but was previously used in clinical trials.
- Key advantages of drug repurposing:
  - Reduced time from discovery to clinical trial (toxicology and dosing studies not needed or only minimally needed).
  - Likely carry less safety risk (as the drug has previous exposure in humans).
  - Reduced cost for development.
- Potential disadvantages:
  - May lack the specificity needed for optimum benefit.
  - May have side effects related to previous indication.
  - May be little industry incentive to partner for development.
- Some examples:
  - Azidothymidine (chemotherapy drug now used for HIV).
  - Sildenafil (angina medicine now marketed as Viagra. Also being studied in Duchenne muscular dystrophy).
- More than 40 new studies per month published in biomedical journals related to drug repurposing.
How does drug repurposing work?

• Targeted development:
  – take a drug with a known mechanism and apply it to a disease where that mechanism should/would be helpful

• Unbiased development:
  – use a library of approved/tested drugs and test them for efficacy in a disease relevant assay
Drug repositioning strategies for MTM/CNM (Dowling lab approach)

• target an underlying pathway
  – PIK3C2B inhibition to achieve “PIP rebalancing”

• target the broad drug repurposing universe
  – large scale drug screen of FDA approved compounds using the zebrafish model system

• serendipity!
Introducing the model systems we use for drug development
Loss of MTM1 in model systems results in accumulation of PI3P

Increase in PI3P expression in mtm1 morphant zebrafish (Dowling 2009, PLoS Genetics)

Increase in PI3P levels in Mtm1 KO mouse muscle

Pierson, 2012 HMG

Buj Bello 2002
In MTM....
Too much PI3P =
Triad defects =
Muscle weakness!

Question:
What happens if we lower PI3P?
- tried this in the MTM mouse
Removing *Pik3c2b* in *Mtm1* KO mice both PREVENTS and REVERSES the phenotype.

Also reverses the muscle histopathology!
PI3K inhibitor wortmannin ameliorates phenotype and improves survival of *Mtm1* KO mice
Summary

• Genetic inhibition of Pik3c2b can both prevent and rescue disease in animal models of MTM
  – Note loss of Pik3c2b alone has no overt effect on mice so the gene/protein is an ideal target for therapy

• Treatment with known pan kinase inhibitors provides some improvement in disease phenotype
  – Inhibitors not specific to PIK3C2B
  – Safety profile of these inhibitors not optimal for daily pediatric use

• Future directions
  – Develop PIK3C2B specific inhibitors
    • trying both drug repositioning as well as creation of new compound
    • collaboration (through E-RARE grant) with Haucke, Bolino, Payrastre, and Laporte
  – Continue search for additional modifiers
    • completing large scale drug screen in zebrafish model
Drug screening on our zebrafish model of XLMTM
Fin degeneration as a screenable phenotype

- Advantages:
  - Easily observed and measured
  - Rescued by mtm1 re-expression (so mutation specific)
  - Improved by pik3c2b knockdown
Day 0: Mutant carrier in-cross

Day 0: Collect embryos (~25% mtm1 mutants)

Day 1: Treat 16 embryos per drug

Day 4: Score severity of fin degeneration
Primary screen results

- Screened 1280 FDA-approved drugs from MicroSource US Drug Library at 10 µM
- 38 initial “hits” (hits = 0/16 fish treated showing fin degeneration)
- 4 “hits” were positive after re-screen and validation testing on larger numbers
SK01 suppresses mtm1 fin degeneration

mtm1^{+/-}

100 µM SK01

mtm1^{-/-}

100 µM SK01

DMSO
Summary 2

- drug screen in zebrafish has yielded 4 novel therapeutic targets
- SK01 has been validated and shows dose-dependent response
  - drugs with similar action to SK01 also suppress the phenotype
  - SK01 also improves swimming and prolongs survival
- now testing SK01 in the mouse model of disease
  - preliminary data shows that it nearly doubles XLMTM mouse survival
- additionally working up other 3 hits
Lastly... a serendipity

Untreated Mtm1 KO survival curve

Tamoxifen “negative controls” have improved survival
Daily tamoxifen improves the MTM mouse phenotype.
Summary 3

- Treatment of XLMTM mice with TAM improves survival
- Treatment of XLMTM mice with TAM reverses central nucleation and triad defects
- Treatment of XLMTM mice with TAM increases muscle strength and motor function
- TAM is thus an ideal "repurposed" drug to bring to the clinic for XLMTM
  - safely used in boys/men and in children
TAM4MTM: tamoxifen therapy for myotubular myopathy

- study jointly funded by CWR/CIHR, Where There’s a Will, there’s a Cure, and the Joshua Frase Foundation
- anticipate enrollment of 12-15 MTM patients at 3 centres (SickKids, NIH, Chicago)
- planning launch in winter 2018/2019
A note on other CNMs

- potential for drugs identified for MTM to work in other forms of CNM
  - this is hopefully likely given that they are not based on increasing MTM1 or restoring MTM1
- great need for new models of other CNMs that are more suitable for drug development
zebrafish model of DNM2
mouse model of RYR1 related myopathy (with features of CNM)

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