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Developing a laboratory model of Setfast in Thoroughbreds by converting skin-derived cells into muscle cells

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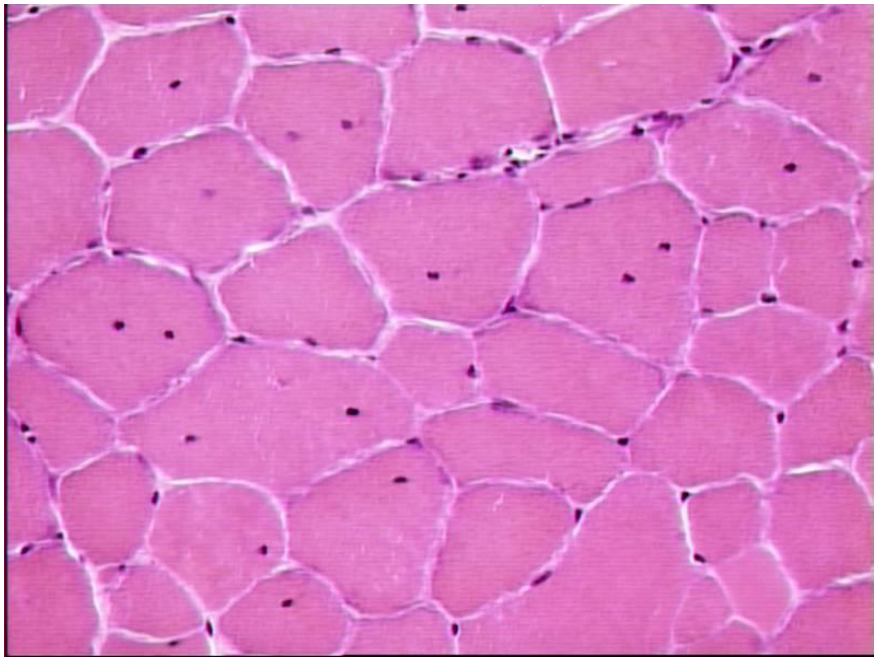
Recurrent Exertional Rhabdomyolysis (Setfast) in Thoroughbreds

- 5-7% Thoroughbreds worldwide affected by RER.
- Results in time lost from training with consequent economic impact.
- Non-specific features in muscle biopsy samples
- It has been proposed that in some horses the condition is due to defective muscle calcium.



Muscle biopsy changes

Recurrent Exertional Rhabdomyolysis (Setfast) in Thoroughbreds

- This painful condition is similar to ‘cramp’ and can be very distressing for the horse. It can lead to the horse having to miss several days of training.

- In racing circles, the disease is more commonly known as ‘setfast’, ‘tying-up’ or azoturia.

What causes Setfast?

- No one knows the exact cause of the condition, but what we do know is that high levels of carbohydrate feed, and irregular, short duration exercise makes its occurrence more likely.

- There is also a strong familial association (i.e. offspring of fillies which suffered from RER are commonly also affected when in training), which hints at a heritable, genetically based defect in muscle metabolism as one possible cause.

What's the best way to study muscle function?

- We know that the muscle cells of many affected horses have an abnormality in the way they handle the calcium molecule.

- To study this previously we had to obtain a piece of muscle from deep within the leg, requiring stitches and some time off, as well as being unpleasant for the horse.

- These researchers asked: Would it be possible to get this muscle sample in a less invasive way?

What's the best way to study muscle function?

- This project was devised to do just that, by taking a tiny pinch of skin, growing the skin cells of the horse in tissue culture in the lab, and then 'persuading' those skin cells to transform into muscle cells by use of some genetic engineering.

- A benefit of the system is that the final muscle cells so produced will share the genetics of the muscle of the horse, and so can be tested for the RER abnormality without the need for a deep muscle biopsy.

Research questions

- Do all Thoroughbreds with Setfast have the same defect?

- What is the underlying cause?

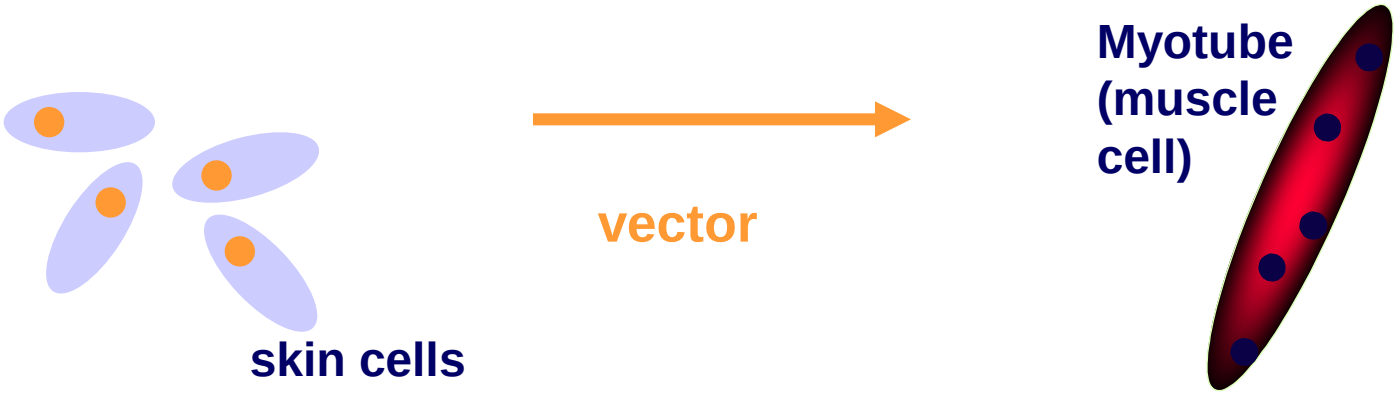
Aims

- Develop an *in vitro laboratory* model of Setfast that:

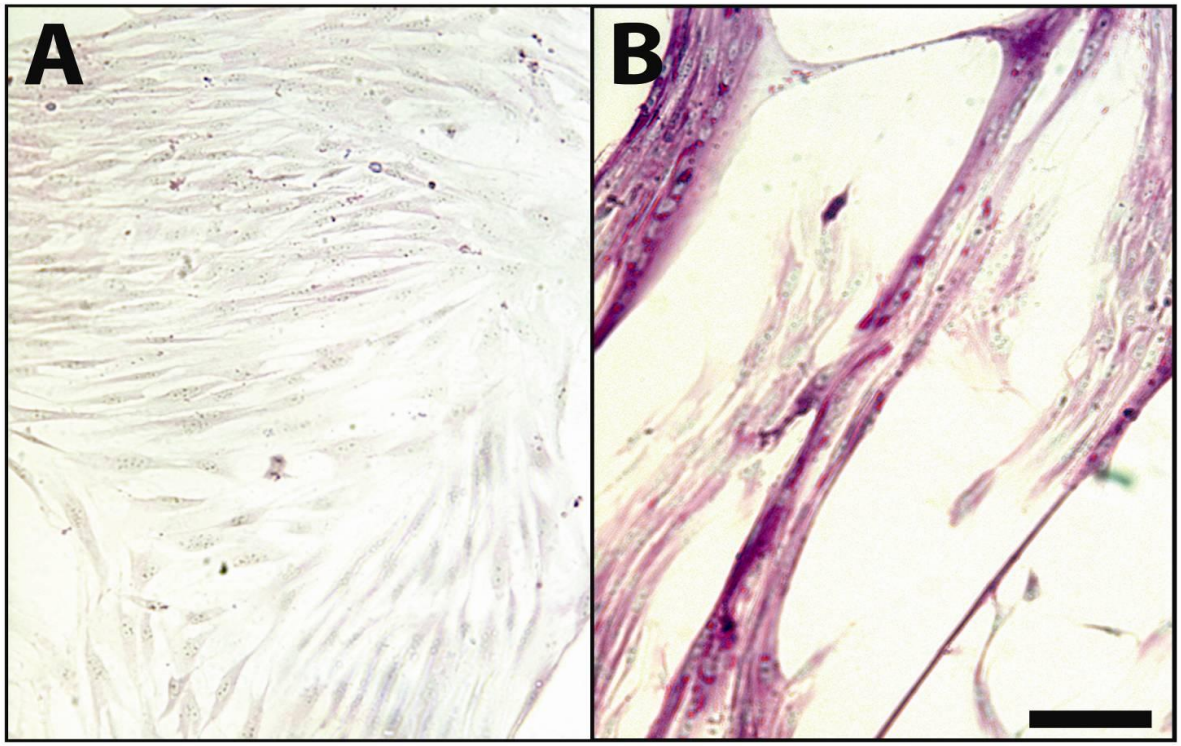
1. Is relatively non-invasive
2. Enables study of the disease mechanism
3. Can be used to evaluate novel treatments

Skin cells to muscle cells

- Culture cells from equine skin samples in the laboratory and convert them to muscle cells using an engineered vector.



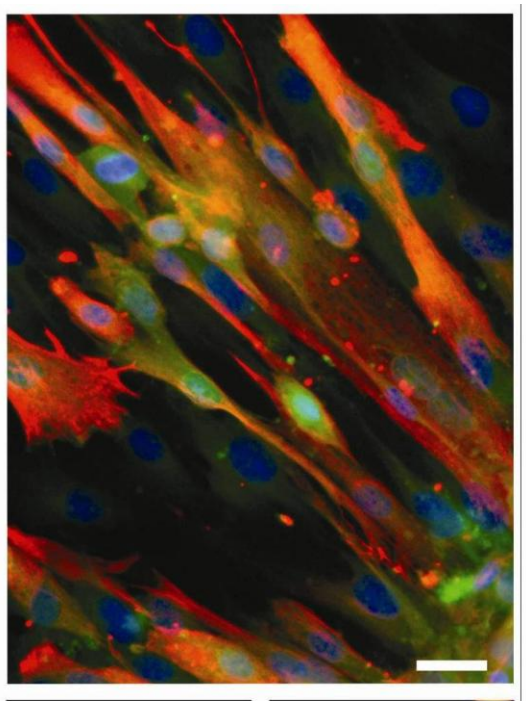
Our vector system converts skin cells (A) to muscle cells (B) in culture



From: Fernandez-Fuente et al. AJVR 2008

Ongoing and future work

- Determine variability between skin-derived muscle cells from different animals and breeds
- Examine responses in setfast-susceptible Thoroughbreds
- Examine responses to pharmacological manipulation (e.g. dantrium)



Muscle cells in culture that have been derived from a horse skin sample

From: Fernandez-Fuente et al. AJVR 2008

Commentary on conclusions

- The project was highly successful, and showed that muscle cells for 'in vitro' (laboratory) testing could reliably be produced from a tiny skin biopsy, easily and painlessly obtained, and requiring no stitches or time off for the horses.

- This will now allow further work on the exact cause of the disease and the efficacy of drugs to treat and control it in the future.

Scientist's summary

- Exertional rhabdomyolysis, or tying up, is a painful syndrome affecting approximately 5-7% of Thoroughbreds.

- Clinical signs vary in severity and include poor performance, muscle cramping and occasionally death. This disorder, aside from important welfare aspects, has a significant economic impact on the UK Thoroughbred racing industry.

- A key basic defect in Thoroughbreds with this disorder is abnormalities in how calcium is handled inside muscle cells. An important way to study this disorder has been to take a muscle biopsy from affected and normal horses but this is invasive and can require rest and pain relief.

Scientist's summary

- A refined technique has now been developed which is reliable and much less invasive to study possible differences in calcium handling between normal and affected horses that is based on collecting a skin biopsy.

- These skin cells are converted to muscle cells in the laboratory, where their properties can be now be studied.

- These findings show both the importance of basic scientific research in the study disease and also now techniques can be used to minimise the adverse effects on horses.

To find out more about HBLB's research go to:

"HBLB's Advances in equine veterinary science and practice"

- http://onlinelibrary.wiley.com/journal/10.1001/%28ISSN%292042-3306/homepage/hblb_virtual_issue.htm