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Reprogramming of equine fibroblasts into induced pluripotent stem cells (iPSCs)

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Stem cells and their potential as treatments (1)

- Stem cells are non-specialised cells that have the ability to produce different specialised cell types, depending where they are located in the body. There are two main types of stem cells, embryonic and adult.
- Embryonic stem cells (ESCs) can differentiate into all tissue types (pluripotent) whereas adult stem cells can only differentiate into cell types particular of each tissue. For example, skin stem cells only produce skin cells.
- Stem cells provide enormous potential for tissue regeneration.

Stem cells and their potential as treatments (2)

- If stem cells are to be used for treatment it is essential that the patient's body does not reject them. One way to do this is to harvest the cells from the patient's own tissues. This is not possible in the case of ESCs, whereas adult stem cells can be harvested from patients in only limited amounts.

- A solution to this would be to produce stem cells in a test tube. This can be done by nuclear reprogramming of adult patient cells to generate induced pluripotent stem cells (iPSCs) - the ones that can differentiate into all tissue types.

Why generate equine iPSCs?

- Equine iPSCs may offer distinct advantages over other types of stem cells for regenerative medicine:

  1. Generated from a simple skin sample
  2. Patient-specific
  3. Large scale culture possible
  4. Can be used to derive a wide variety of differentiated cells

  HOWEVER, improved methods to produce clinically safe cells in a controlled manner are still needed

- An added benefit is that these are a powerful tool for research that avoids the use of live animals

How we did it

Equine iPSC derivation

Skin biopsy

Fibroblasts

Foal Fibroblasts

Oct4

Sox2

c-Myc

Klf4

Viruses containing four different genes linked to pluripotency were inserted into cultures of skin cells collected from a foal

Cells reprogrammed by the four pluripotency genes were multiplied and tested

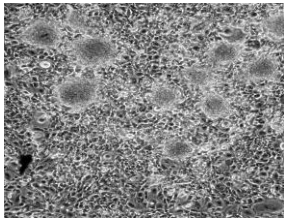
Analyses of pluripotency genes

Test capacity for in vitro (test tube) differentiation

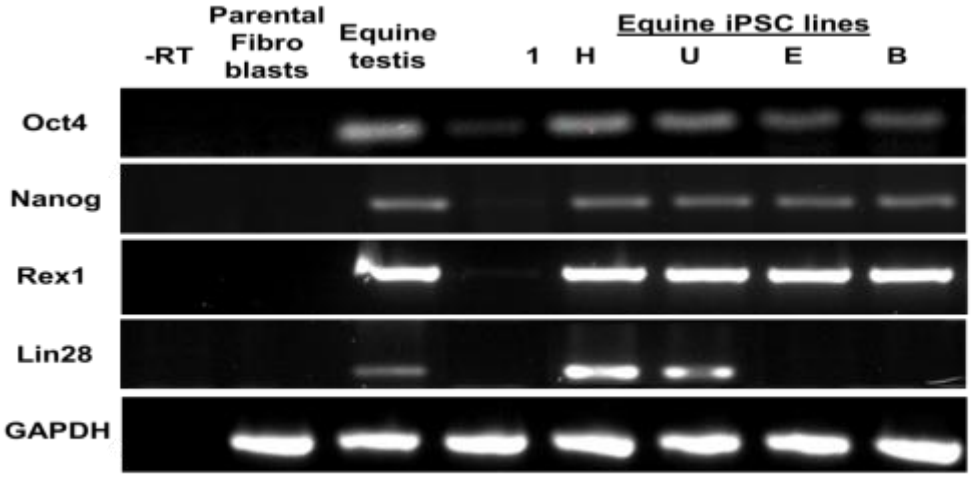
Test capacity for differentiation in vivo (living tissue) mice

Results: we confirmed the cells were the right type

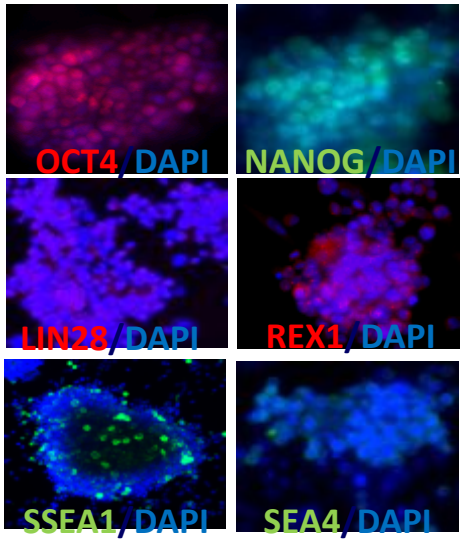
Equine iPSC lines were successfully generated in vitro



Polymerase chain reaction confirmed the presence of different genes naturally associated with pluripotency in equine iPSCs



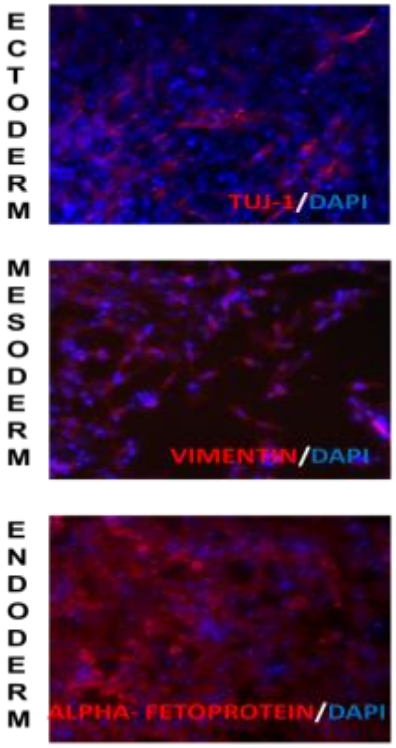
Immunochemical analyses revealed the presence of different markers associated with pluripotency (OCT4, NANOG, LIN28, REX1, SSEA1 and SSEA4)



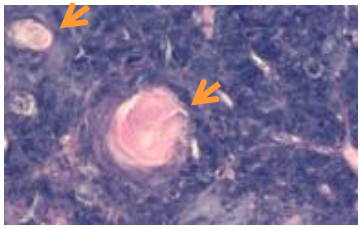
Results: the cells did what we wanted

Equine iPSCs were able to differentiate into different body cell types both in laboratory and living tissue.

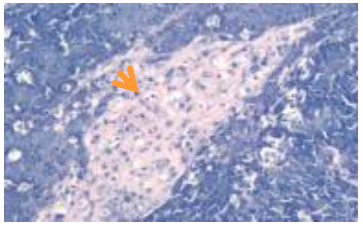
In vitro



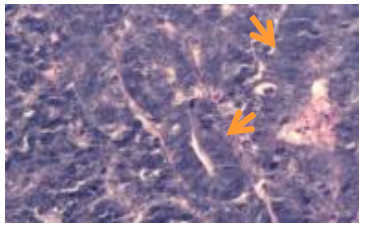
In vivo



Keratinised epithelia



Bone



Glandular epithelia

Conclusions and potential relevance to the Thoroughbred

- Equine cell lines with the ability to differentiate into many cell types both in test tubes and in live animals were generated for the first time.

- These novel iPSCs can be generated from virtually any individual, can be readily expanded in long-term culture and can develop into a variety of potential tissues, thus have unprecedented potential for regenerative stem cell therapy.

- Furthermore, these cells could provide excellent tools to study diseases that are relevant to the Thoroughbred, avoiding the use of experiments on live horses.

Scientist's Summary

- Stem cells are showing promise as a new treatment for many conditions, in particular tendon injuries.
- A stem cell has the ability to transform itself into many different tissue types, depending where it is located in the body.
- If they are to be used for treatment it is essential that the patient's body does not reject them. One way to do this is to harvest the cells from the patient's own tissues. Currently this is done from a bone marrow sample. However, pluripotent stem cells generated in a test tube might be a better source.

Scientist's Summary

- In this project we generated induced pluripotent stem cells (iPSCs) by manipulating the genes within adult cells.
- iPSCs can both self-renew indefinitely and differentiate into virtually all body tissues. These properties, together with the fact that iPSCs can be generated from any given individual, confer these cells with unprecedented biomedical potential.
- In this study, we successfully generated iPSCs from equine skin cells.

Scientist's Summary

- These findings will pave the way for iPSC technology to become a powerful research and clinical tool in equine biomedicine.

- Future studies should develop protocols for efficient cell differentiation into clinically relevant cell types, through which equine iPSCs will facilitate the development of new cell therapies for horses.

- Laboratory models could be developed to study equine diseases and for toxicological screening.