Subcutaneous Autografting Leydig Stem Cells: An Approach to Increase Serum Testosterone

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Testis performs two important functions

- Spermatogenesis and Fertility
  - Germ cells
- Production of testosterone
  - Leydig cells
Origin of Stem cell ➔ Maturation of Leydig Cell

SLC ➔ platelet-derived growth factor receptor (PDGFRα+)
ALC ➔ 3β-HSD, LHR

Chen, Zirkin et al. 2010
Testosterone Therapy – Side effects

- Infertility
- Polycythemia
- Elevated estrogen
- Atherosclerosis
- ? MI/ CVA / PE
Strategies to increase serum T without affecting hypothalamic-pituitary axis

• Clomiphene citrate
  – Unlikely to be beneficial in men with elevated LH and testis failure

• Human chorionic gonadotropin
  – dependent on good intratesticular function
Strategies to increase serum T without affecting hypothalamic-pituitary axis

- Whole testis tissue autograft under skin – Makala et al. 2015
- Leydig stem cell transplant – Zang et al. 2017

Major hurdle for clinical translatability
Sertoli cells and Peritubular myoid cells are critical for Leydig stem cell survival and function

Rebourcet et al 2014
Sertoli cells and Peritubular myoid cells are critical for Leydig stem cell survival and function.

Purified Leydig stem cells

Leydig stem cells + peritubular myoid cells + Sertoli cells

Not viable

Viable

Rebourset et al 2014
Subcutaneous Autografting Leydig Stem Cells: Two Novel Aspects

Subcutaneous autograft of Leydig stem cells

Autograft of Combination of Leydig Stem cells + Peritubular myoid cells + Sertoli cells

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**In Vitro Validation of Leydig Stem Cells from adult WT mice**

Stem cells are expanded in culture at 14 days

Stem cells can be differentiated into mature Leydig cells with LH

![Images showing cell culture and differentiation](image)

**PDGFRα (+)**

**Negative control**

**Relative expression changes upon DIM**

- **PDGFRA**
  - Day 2: Control, DIM
  - Day 7: Control, DIM

- **3BHSD**
  - Day 2: Control, DIM
  - Day 7: Control, DIM

**DAPI**

**3BHSD**

**DAPI/3BHSD**

**DIM Day 14**

**Control**

**DIM**

**Negative control**
Quantification of cells in culture – combination of Leydig stem cells with Sertoli cells
Leydig stem cells were isolated from testes and autografted into castrate mice.
Leydig Stem cell were autografted subcutaneously using Matrigel.

1. Serum was harvested
   Levels were checked
   a) Testosterone
   b) LH
   c) FSH

2. Skin grafts were obtained
   1. Expression were checked
      a) 3BHSD
      b) LHR
   2. H&E Staining was performed to validate presence of alien cell population in graft

After 4 weeks, Animals were sacrificed
Structural Analysis of Subcutaneous Autograft Demonstrated Mature Leydig cells

H&E staining showed the presence of alien cells in the autograft skin

Leydig stem cell injection

Recovery of graft at 1 month
Immunofluorescence of Subcutaneous Autograft Confirmed Mature Leydig cells
Immunostaining of Autograft Confirmed Presence of Peritubular Myoid cells and Sertoli cells and Lack of germ cells

α-SMA – Peritubular myoid cells

SOX9 – Sertoli cells

PLZF – Germ cells
Leydig Stem cell Autograft Can Increase Serum Testosterone in 4 weeks

Testosterone production is detectable after Stem cell autograft

Leutinizing Hormone (LH) production is not affected by autograft
Leydig Stem cell Autograft Can Preserve FSH and LH production
Leydig stem cells from Human Testis

**UNDIFFERENTIATED HUMAN STEM LEYDIG CELLS**

Testicular stem cells can proliferate exponentially

3β-HSD expression in differentiated human stem leydig cells

Differentiated human stem leydig cells (HSLC)
Limitations

❖ Increase in serum testosterone level is small – dosage and duration of autograft needs to be determined

❖ Grafts performed in castrate animals to detect changes in testosterone – however high levels of LH may have stimulated Leydig stem cell differentiation

❖ Levels of LH and FSH remained unaltered by autograft in castrate animals.
Summary

❖ Leydig Stem cells were harvested, expanded and differentiated in in-vitro conditions – both mice and humans.

❖ Subcutaneous autograft (Leydig stem + Sertoli + Peritubular myoid cells) were able to maintain a viable population of adult Leydig cells and increase serum testosterone at one month.

❖ Levels of LH and FSH remained unaltered by autograft in castrate animals.
Future directions

- Human Leydig stem cell → graft in immunocompromised NOD-SCID mice → check T, LH, FSH levels
- Long-term effect of autograft on T, LH and FSH levels (~3 months)
- IND (Clinical trial)

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