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Special Equipment needs (other than standard computer equipment and powerpoint): NONE

Stemness of Bone Marrow Stromal Cells for Regenerative Properties, **KAITLYN QUESADA[1]*, BRIAN CARTER[1]*, HIROKO OKAWA[2], and ICHIRO NISHIMURA[2]** ([1]UCLA Pre-College Science Education Program, UCLA School of Dentistry, 10833 Le Conte Avenue, Los Angeles, CA 90095, quesadakaitlyn@gmail.com, briancarter0003@gmail.com; [2]UCLA School of Dentistry, 10833 Le Conte Avenue, Los Angeles, CA 90095, hiroko.okawa01@gmail.com, inishimura@dentistry.ucla.edu).

Stem cells are known for having the ability to self-renew and differentiate into various cell types, allowing them to regenerate tissues and replace damaged cells. Previous research was conducted regarding the effect on regeneration and stemness qualities in Bone Marrow Stromal Cells (BMSC) derived from NPAS2 knockout mice. In NPAS2 knockout mice, the NPAS2 gene, a component of the Circadian Clock, is removed. **Objective:** To further investigate stemness and osteogenic differentiation abilities of BMSC in NPAS2 knockout. **Methods**: WST-1 was used to assess proliferation and viability of cells in wild type and NPAS2 K/O mice. RT-PCR was used to find which stem cell markers were expressed in wild type and NPAS2 K/O mice BMSC. ALP staining was used for osteogenic differentiation potential in both wild type and NPAS2 K/O mice BMSC. **Results**: Stem cell markers NANOG and KLF4 were expressed more within NPAS2 K/O mice BMSC than wild type mice BMSC. NPAS2 K/O mice BMSC had a faster proliferation rate and underwent osteogenic differentiation faster than wild type mice BMSC. **Discussion**: After reviewing multiple experiments dealing with the suppression and knockout of NPAS2, reports indicate results that support our data. An article that claims "silencing NPAS2 expression [within tumor tissue] could promote cell proliferation, cell invasion and increase the wound healing ability (Xue X.)" **Conclusion:** We conclude that the lack of NPAS2 is able to keep FBMSC in a more stem-like state and rapidly increases recovery speeds of wounds on mice femur bone tissues.

Stemness of Bone Marrow Stromal Cells for Regenerative Properties

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Introduction

Stem cells are known for having the ability to self-renew and differentiate into various cell types, which allows them to regenerate tissues and replace defective or damaged cells. Previous research was conducted regarding the effect on regeneration and stemness qualities in Bone Marrow Stromal Cells (BMSC) which were derived from NPAS2 knockout mice. A NPAS2 knockout mouse is one which NPAS2, a protein which is a component of the Circadian clock gene, has been removed. **Objective:** To further investigate stemness and osteogenic differentiation abilities from NPAS2 knockout BMSCs. **Methods**: WST-1 was used to stain and view the numbers of cells that had proliferated from Wild type and NPAS2 K/O mice. RT-PCR was used to find out which stem cells markers were expressed in Wild type and NPAS2 K/O mice BMSC. ALP staining was used to check the osteogenic differentiation potential in both wild type and NPAS2 K/O mice BMSC. **Results**: Stem cell markers NANOG and KLF4 were expressed more within NPAS2 K/O mice BMSC than wild type mice BMSC. NPAS2 K/O mice BMSC had a faster proliferation rate and underwent osteogenic differentiation faster than wild type mice BMSC. **Discussion**: After reviewing multiple experiments dealing with the suppression and knockout of NPAS2, reports indicate results that support our data. Such as an article that claims "silencing NPAS2 expression [within tumor tissue] could promote cell proliferation, cell invasion and increase the wound healing ability (Xue X.)" **Conclusion:** We conclude that the lack of NPAS2 is able to keep FBMSC in a more stem-like state and rapidly increases recovery speeds of wounds on mice femur bone tissues.

- Stem cells regenerate to form new cells and tissues; one specific type is Bone Marrow Stem Cells
- NPAS2 is a protein is which is a component of the Circadian clock gene

Hypothesis

NPAS2 knockdown BMSCs have high stemness and osteogenic differentiation ability.

Aims

1. Measure stem cell marker gene expression using RT-PCR
2. Measure cell proliferation using WST-1
3. Observe osteogenic differentiation using ALP staining

Materials & Methods

- Femur Bone Marrow Stromal Cells from B6 mice
- Medium (DMEM+10%FBS+1%Penicillin Medium)
- QIAGEN RNeasy Mini Kit
- Gene Markers for real-time RT-PCR
- WST-1
- ALP staining agent

Results

Figures 1 and 2:

The expression of stemness marker genes

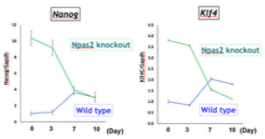


Figure 3:

Cell proliferation of BMSC (WST-1 assay)

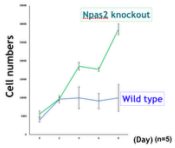
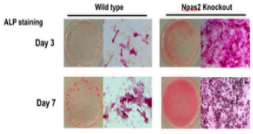


Figure 4:

Osteogenic differentiation of Npas2 Knockout BMSC



Discussion

- By knocking out NPAS2,
  - There was a high expression of Nanog and KLF4
    - More gene markers being expressed correlates directly to higher stemness levels
  - Cells proliferated at a faster rate and produced faster
  - There was more bone formation taking place

Conclusion

To conclude, our hypothesis proved to be true as the cells showed many characteristics of maintaining high stemless levels, such as more proliferation and bone formation, in our results when having NPAS2 knocked down.

Limitations

- Time constraint (six weeks)
- Inability to handle animals

Future Studies

Further test stem cell markers such as STRO-1 which can identify a cell surface antigen expressed by stromal elements in human bone marrow.

References

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