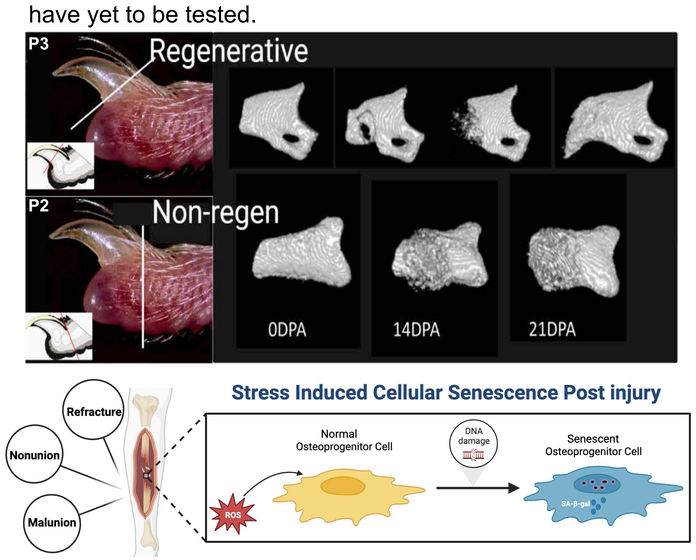
Evaluating limb regenerating capabilities of osteoprogenitor cells

Joshua P. Broussard, BS[1], Sylvia Culpepper, MS[1], Jennifer Simkin, PhD[1]

[1]Dept. Orthopedic Surgery, Louisiana State University Health, School of Medicine – New Orleans, LA, USA

Introduction

- Of the 5.6 million bone fractures that occur yearly in the US, 5-10% of these injuries fail to heal resulting in the need for invasive orthopedic surgical intervention.
- Osteoprogenitor cells are essential in the process of bone healing, but sometimes fail to fully regenerate necessary structures.
- This disparity in healing is exemplified in the mouse model where the distal ½ of the digit tip (P3) can be fully regenerated while an amputation proximal to this point of the phalanx (P2) results in a cartilaginous callus and dermal scar.
- When comparing progenitor cells in regenerative and non-regenerative species, for example in rabbit (regenerative) versus rat (non-regenerative), previous studies suggest regenerative osteoprogenitor cells overcome stress-induced cellular senescence caused by reactive oxygen species (ROS) released upon injury better than non-regenerative counterparts.[3]
- This resistance to stress corresponded to greater proliferative and regenerative abilities. However, two different injuries (regenerative versus non-regenerative) within the same animal have yet to be tested.



Hypothesis

- Regenerative P3 osteoprogenitor cells will have a greater proliferative ability and greater resistance to stress-induced cellular senescence compared to non-regenerative P2 osteoprogenitor cells
- P3 cells will demonstrate heightened stress resistance in comparison to their P2 counterparts. This enhancement in stress resistance could potentially underscore the regenerative cells' ability to evade stress-induced cellular senescence, ultimately facilitating increased proliferative capacity following injury.

Clinical Significance

- This project will help address if there are intrinsic differences in osteoprogenitor cells in different bones of the body and may help explain why some bone injuries regenerate better than others.

Doubling time

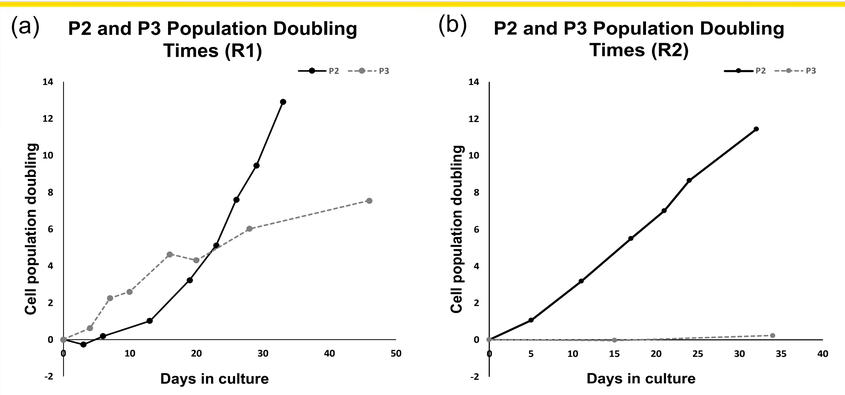


Fig.1 (a,b) P2 (non-regenerative) osteoprogenitor cells exhibit enhanced proliferative ability in vitro compared to P3 (regenerative) osteoprogenitor cells.

Cellular Senescence Assay

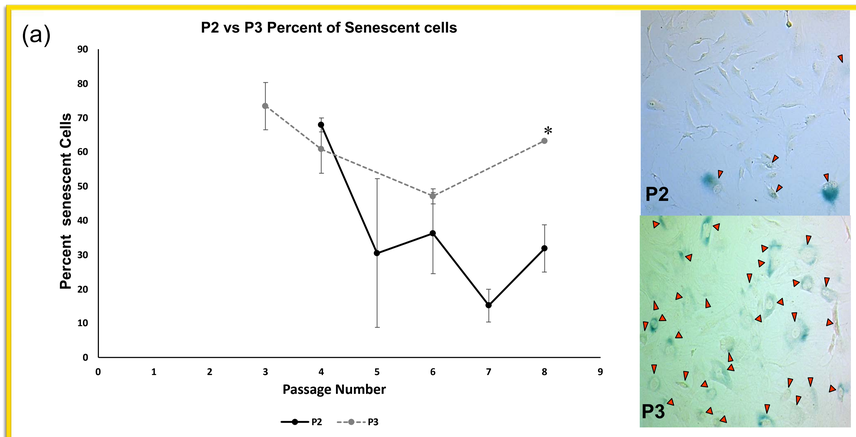


Fig 2 (a). P3 cells show a higher level of senescence in vitro compared to P2 cells (*p<0.05 n=3 per group. Student T-test)

ROS-induced senescence assay

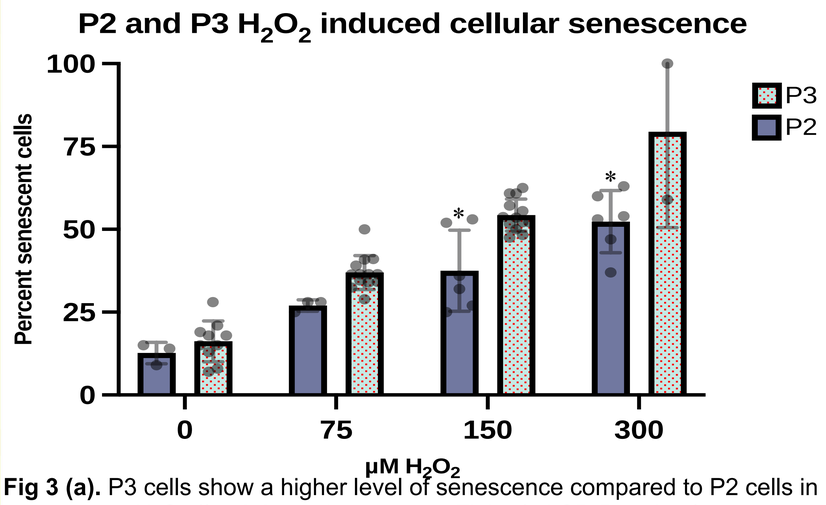
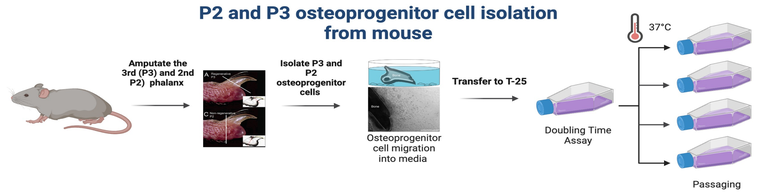


Fig 3 (a). P3 cells show a higher level of senescence compared to P2 cells in response to H_2O_2 (*p<0.05 n=4 per group. Fisher's LSD Post-hoc)

Methods

Doubling Time Assay:

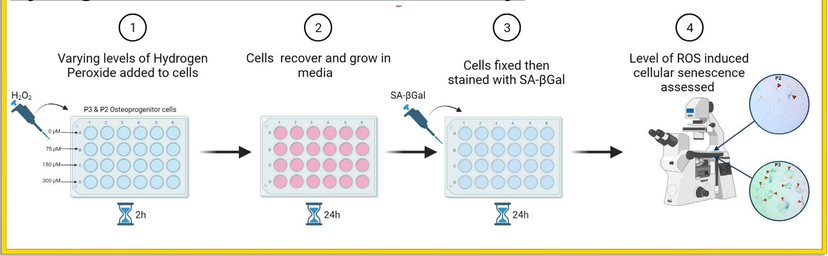


Population doubling was graphed using Population Doublings (PDs) = log [(number of cells harvested)/(number of cells seeded)]/log2.

Senescence Assay:

To measure levels of senescence in P2 and P3 cell populations with each passage, cells were stained with Senescence-Associated β-Galactosidase (SA-βGal), a general marker for cellular senescence, then counted using brightfield microscopy.

Hydrogen Peroxide Stress Resistance Assay:



Discussion and Future Directions

- To determine the intrinsic capabilities of osteoprogenitor cells derived from distinct anatomical regions of the body, we first analyzed the proliferative potential and cellular senescence characteristics of P2 and P3 osteoprogenitor cells in vitro.
- Our findings shed light on the nature of these cells' behavior and provide insights into their regenerative capacities. Initially, we hypothesized that P3 osteoprogenitor cells would exhibit an inherent advantage in terms of proliferative ability and a reduced propensity for cellular senescence when compared to P2 osteoprogenitor cells due to the regenerative ability of the P3 phalangeal element. However, our observations revealed a contrasting pattern.
- **P3 (regenerative) cells displayed an accelerated onset of senescence, a decelerated rate of proliferation,** and **were more susceptible to stress induced cellular senescence** compared to P2 (non-regenerative) cells. This suggests that **P2 osteoprogenitor cells retain an intrinsic capability for proliferation**, at least in vitro, and may be hindered by the in vivo environment.
- In future studies, we will assess the **in vivo** proliferative capabilities of P3 and P2, as well as their resistance to stress-induced cellular senescence. This aims to shed light on the pivotal role played by the wound's microenvironment in shaping the proliferative and stress-resistant capacities of P3 and P2.

References

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