

“Evaluating limb regenerating capabilities of osteoprogenitor cells”

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Introduction: Bone fractures are commonly seen in orthopaedics with about 5.6 million occurring each year in the United States.¹ About 5-10% of these fractures fail to properly heal resulting in invasive surgical intervention to treat nonunion, malunion, osteomyelitis, and chronic pain. For a bone fracture to heal, osteoprogenitor cells of the periosteum proliferate and form a cartilaginous callus which then undergoes endochondral ossification to become new bone. Osteoprogenitor cells are integral to this process but sometimes fail to repair the bone resulting in non-union healing. To better understand regenerative failure, we use a comparative model of bone regeneration and non-regeneration.

The mouse, and similarly humans, can regenerate the distal ½ of their digit tip (third phalangeal element or P3). An amputation proximal to this point (P2) results in a cartilaginous callus with no bone growth distal to the amputation plane and dermal scar formation.² It is unknown if regenerative failure in P2 is due to specific factors intrinsic to the P2 osteoprogenitor cells or if environmental factors drive this healing difference.

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 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. We therefore hypothesize that osteoprogenitor cells from regenerative P3 will have a greater ability to proliferate and greater resistance to stress-induced cellular senescence compared to osteoprogenitor cells from a non-regenerative P2.

Methods: Osteoprogenitor cells were isolated from P3 and P2 bones in mice and then cultured in T-25 cell culture flasks. To calculate population doubling time, an initial starting concentration of 100,000 P3 or P2 osteoprogenitor cells are seeded in T-25 flasks. Cells were grown to 80% confluency, and then cells were counted using a hemacytometer. Population doubling was graphed using Population Doublings (PDs) = $\log[(\text{number of cells harvested})/(\text{number of cells seeded})]/\log 2$. To measure levels of senescence in P3 and P2 cell populations with each passage, cells were stained with Senescence-Associated β -Galactosidase (SA- β Gal) and then counted using brightfield microscopy. To measure stress resistance, P3 and P2 cells were placed in 24 well plates with varying concentrations of hydrogen peroxide (0 μ M, 75 μ M, 150 μ M, and 300 μ M hydrogen peroxide dilutions) for 2 hours. H₂O₂ was washed off and cells collected 24 hours later for SA-beta gal senescent staining.

Results/Discussion: 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, though unexpected, shed light on the nuanced 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, contrary to this hypothesis, our observations revealed a contrasting pattern. Specifically, P3 cells displayed an accelerated onset of senescence and a decelerated rate of proliferation compared to P2 cells. This finding suggests that P2 osteoprogenitor cells retain an intrinsic capability for proliferation, at least in vitro, and may be hindered by the in vivo environment. Next, our study will investigate stress resistance. We hypothesize 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. Completion of this project will address if there are intrinsic differences in osteoprogenitor cells in different bones of the body and may help explain why some injuries in bone regenerate better than others.

Significance/Clinical relevance: This study investigates mechanisms differentiating osteoprogenitor cells that lead to tissue regeneration and scar formation after injuries. Greater knowledge of this biological process could improve clinical and surgical treatment strategies for bone fractures.

References:

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