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Stem Cell Derived Osteoprogenitors and their Role in Bone Repair Using Morphogenic Activators

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Abstract

Bone constantly cycles through a dynamic process of breakdown and remodeling. Osteoblasts are the specialized mesenchymal stem cells that have a major role in bone formation and the remodeling process whereas their counterpart osteoclasts, handle bone resorption. Embryonic stem cells can be partially differentiated into Progenitor cells, and we worked with #18, a candidate for being an osteoprogenitor that has the potential to respond to morphogenic activators. In the case of bone remodeling, TGF- β 2, BMP-2 and an abundance of CA⁺⁺ have been shown to be potential activators of differentiation into osteoblasts. Eight different trials were conducted with the cells using different combinations of the three morphogenic activators. After inducing the cells with the activators, we performed Immunohistochemistry (IHC) to analyze the expression of osteocalcin, which is the enzyme that binds calcium to mineralize bone. The cells with varying activator combinations showed different physiology with a variance in the cell shape, structure, and spacing. The greatest results were from the combination of TGF- β 2 and BMP-2, which is consistent with #18 operating as an osteoprogenitor. A 3D construct model of #18 seemed to have a similar structure to that of an osteon, possibly indicating the formation of bone. We took slices of the model and performed an IHC staining for Osteocalcin, Prolyl Hydroxylase (5B5), and Collagen I. We saw a strong positive signal for Col I and 5B5, and a slight positive signal for Osteocalcin. This information confirmed that #18 is an osteoprogenitor and is able to assemble bone.

Introduction

Human embryonic stem cells (hESC) are primordial, unspecialized cells that can differentiate into any other cell type. BioTime's business is to direct these hESC into progenitor cell lines with the potential to become differentiated into a variety of different cell types. One of Biotime's cell lines, progenitor # 18, is a Mesenchymal stem cell (MSC) line. Specialized MSC's can be potentially differentiated into muscle, tendons, ligaments, fat, and bone. MSC's are sensitive to the timing of growth factor presentation and differentiation can be specialized toward osteoprogenitors responsible for bone formation and remodeling. We encompassed our research around the question of whether Progenitor #18 cells have the potential to be differentiated into primary bone cells called osteoblasts. Osteoblasts distinguish themselves from other mesenchymal cells through the production of osteocalcin, which is responsible for the calcification of bone. This research could advance efforts in the field of bone repair.

Materials and Methods

Cell Culture

- A P-60 culture of Human embryonic Progenitor line # 18 was used from BioTime.
- The culture was grown in DMEM 10 % FBS medium and treated to a 5ml PBS followed by Trypsin.
- After centrifugation cell line was divided evenly into an 8 well slide each individual well was exposed to a combination of three morphogenic activators: BMP-2 (10 uL), TGF- β 2 (10 uL), and CA⁺⁺ (2.5 uL)
- After 4 days of incubation images were taken for morphology and culture was returned to 37° C incubator

Cell Culture Immunohistochemistry (IHC)

- 7 days after adding morphogenic activators, IHC was conducted.
- Cell were separated, marked by pap-pen and fixed to slide using Acetone methanol solution for 10 minutes at room temperature.
- Cells were stained for 50 minutes with a 1:200 dilution of primary antibodies, Osteocalcin and Collagen I
- A second antibody was used at a 1:200 dilution of goat antimouse IgG 594 Red.
- Cell culture was examined by conventional fluorescent microscope (Axiovert 40 CFL) with images taken from Nikon D600

3D Construct Immunohistochemistry (IHC)

- 3D model, created by the progenitor line, was sectioned by a cryostat
- Slices were taken and placed on glass slides to be observed with a microscope
- Two different slides were divided up and stained for 50 minutes.
- Slide 1 was stained with Osteocalcin
- Slide 2 was stained with Collagen I

Results

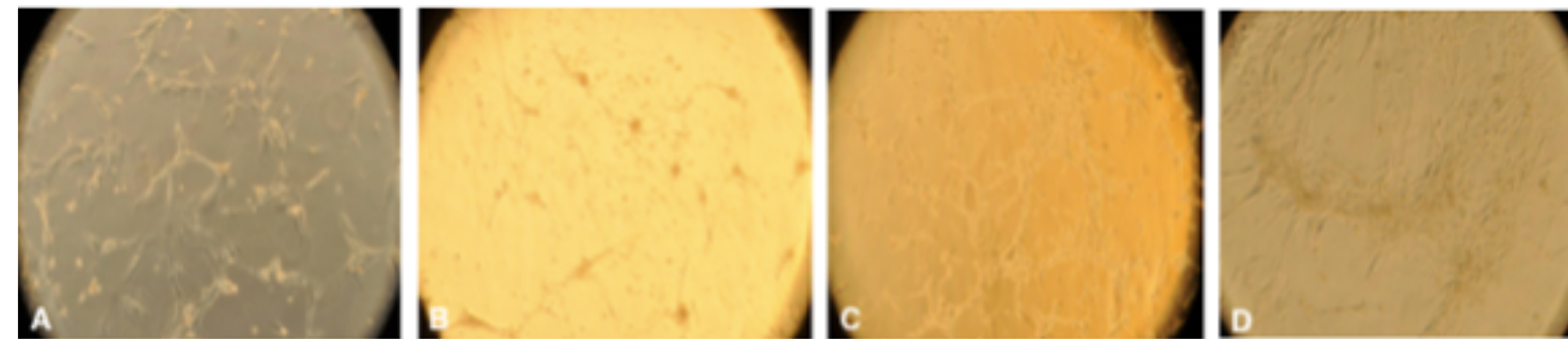


Figure 1: Changing morphology in response to morphogenic activators in #18 (40x)

P60 plate of line #18 in the presence of morphological activators resulted in clear morphological changes. (A) The control line shows the normal morphology of line #18. (B) Cells induced with BMP-2 began to spread out and become confluent. (C) Cells induced with TGF- β 2 began to cluster more after induction. (D) The cells treated with a combination of BMP-2 and TGF- β 2 presented as larger aggregates.

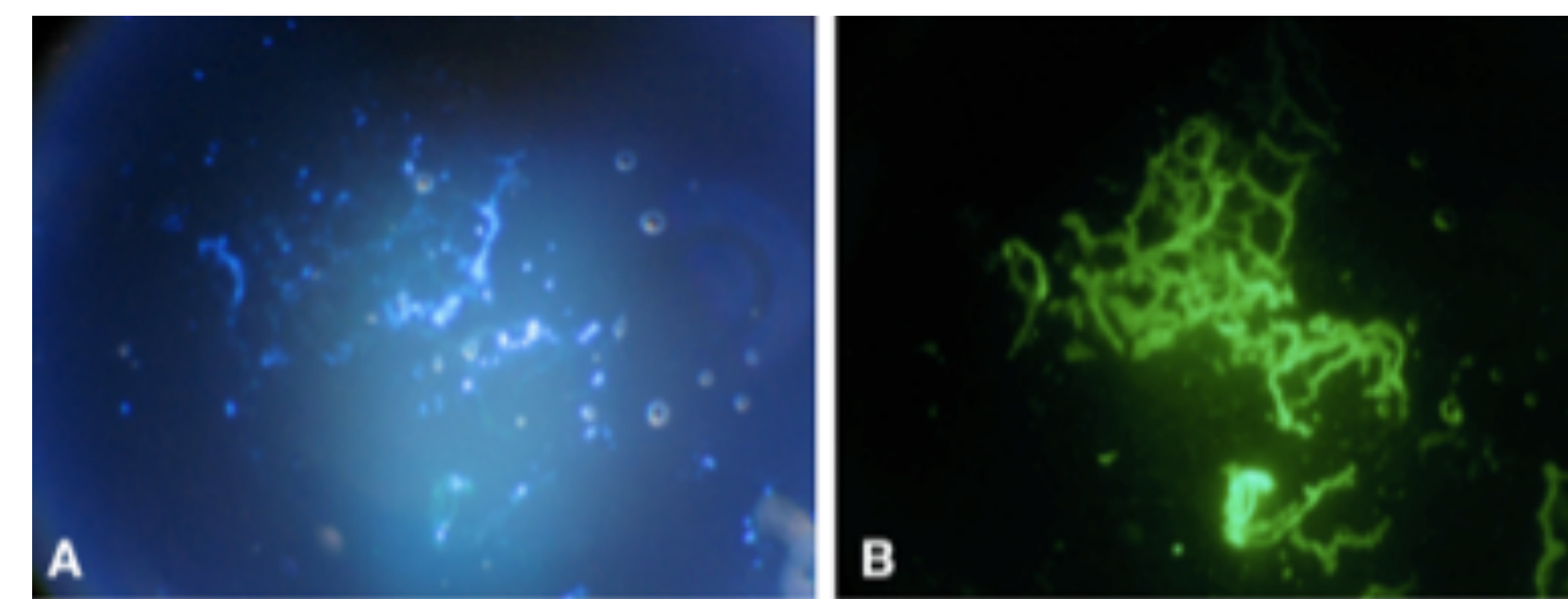


Figure 2: Collagen Expressed in 3D Model (40X)

After taking slices of the 3D model, immunohistochemistry was performed to characterize the expression of Collagen I. (A) The DAPI stain presents expression of Collagen I (B) Collagen I does not completely overlap the nuclei signaling that collagen is made in the vicinity of the cells.

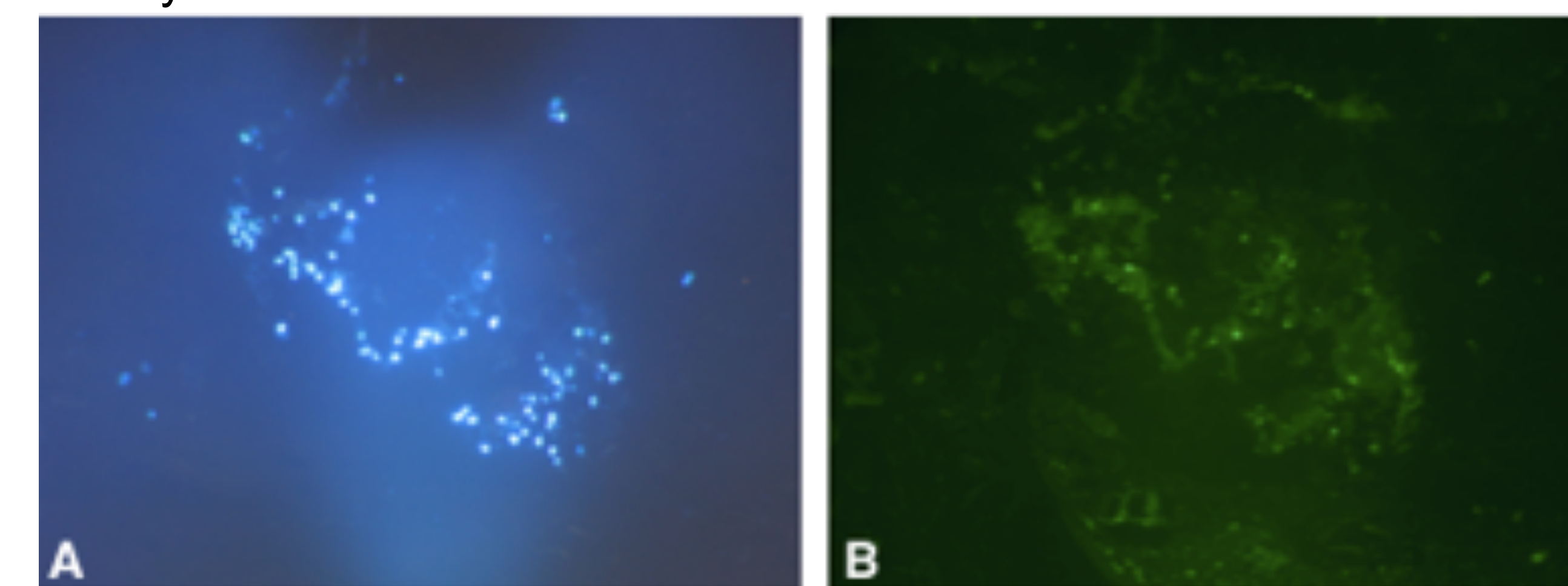


Figure 3: Osteocalcin Expressed in 3D Model (40X)

After taking slices of the 3D model, immunohistochemistry was performed to characterize the expression of Osteocalcin. (A) The DAPI stain expresses a positive expression for Osteocalcin. (B) IHC shows Osteocalcin does not completely overlap with the nuclei and is secreting osteoid beyond the boundaries of the cell, forming bone.

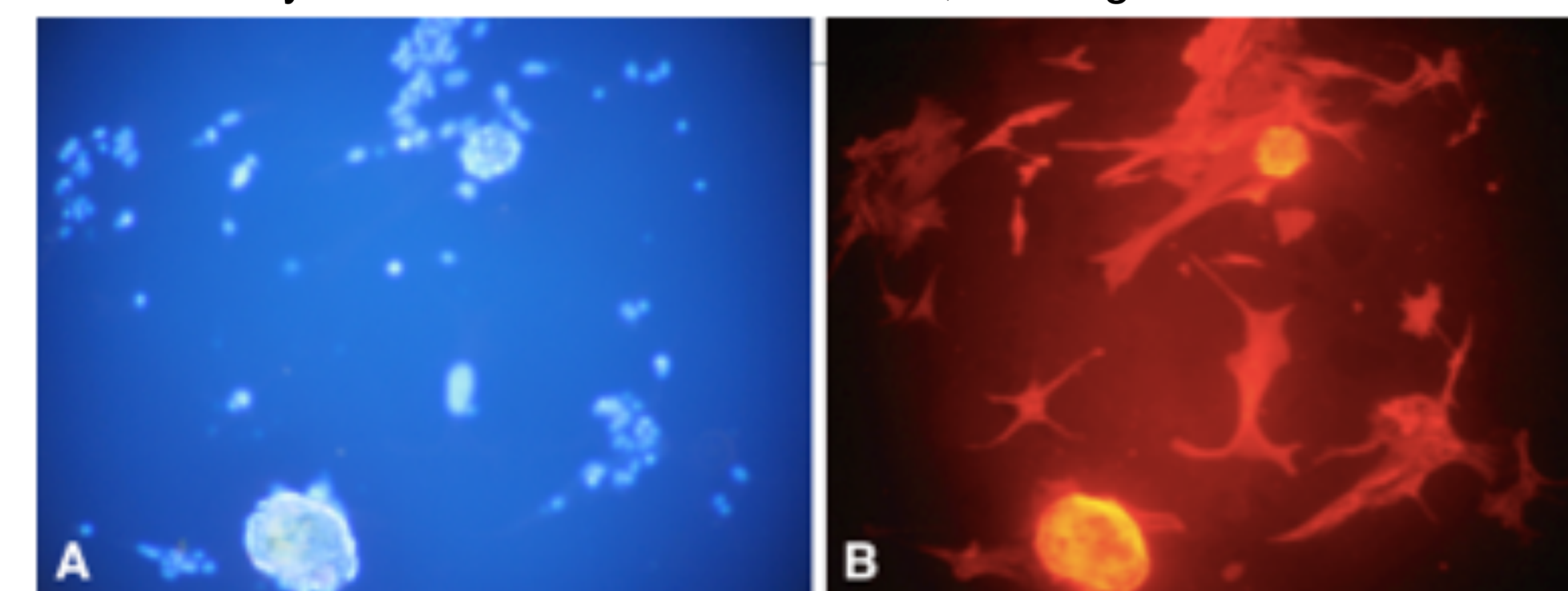


Figure 4: Progenitor cells induced with BMP-2 and TGFB-2 express Osteocalcin

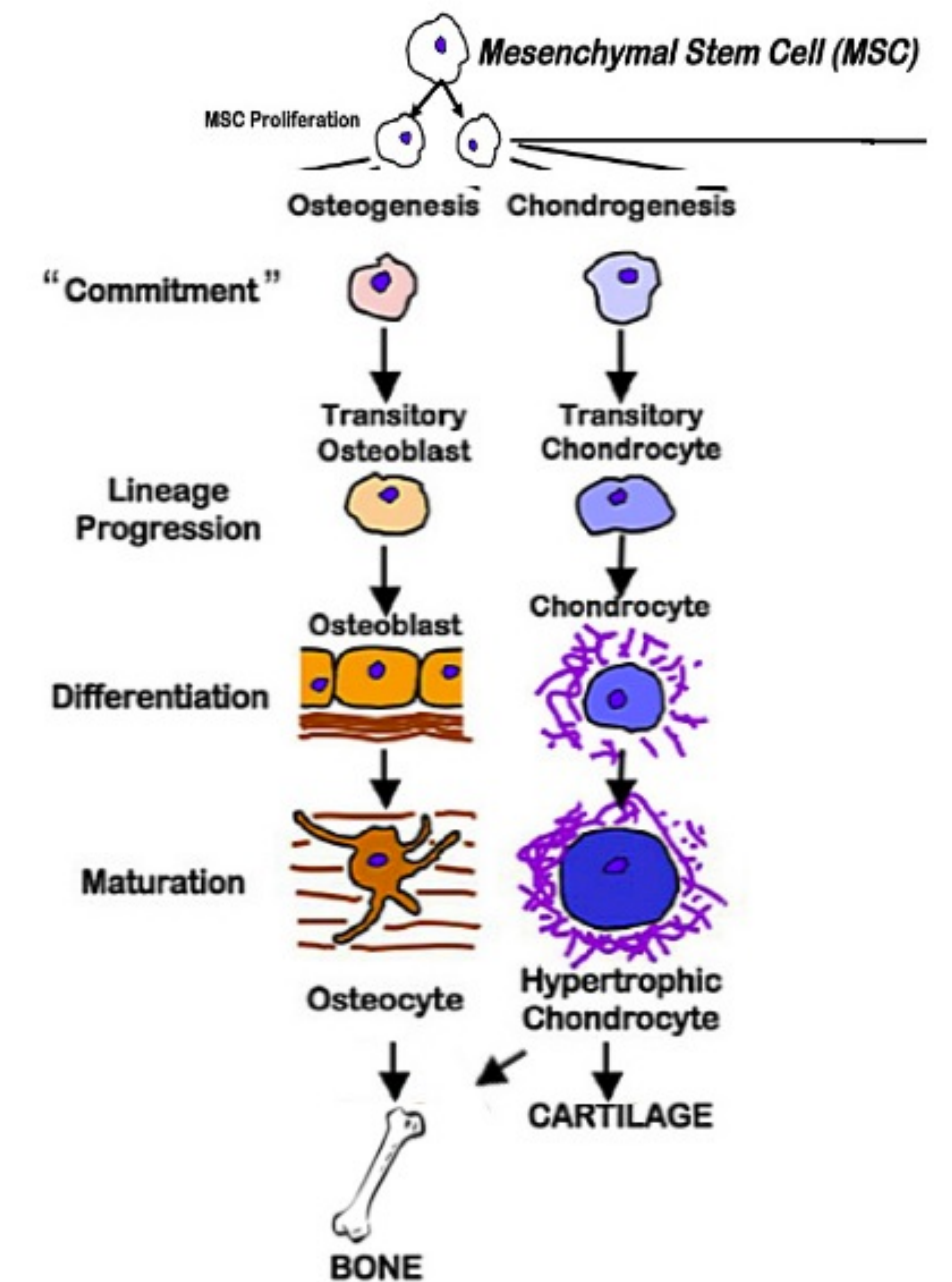
(A) Blue dots show DAPI staining of nuclei therefore the position of living cells. (B) Red staining shows that IHC staining was positive for Osteocalcin.

Acknowledgements

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Figure 5: Embryonic Stem Cell (ESC) Differentiation to Bone Cells

Our collaborator "BioTime inc" took ESCs and derived Mesenchymal Stem Cells (MSC) #18. We tested to see if these cells could be manipulated into becoming bone cells (osteoblasts) by adding BMP-2 and TGFB-2. We saw Collagen 1 and Osteocalcin in IHC which indicates positive bone cell formation.



Conclusions

- Human Embryonic Stem Cells can be partially differentiated into progenitor cell lines. We chose #18 as a candidate for the formation of osteoblasts.
- We tested factors that trigger Osteoblast formation and saw that the combination of TGFB-2, BMP-2 was successful.
- We conclude that cell line #18 is an osteoprogenitor with the potential to assemble bone.

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