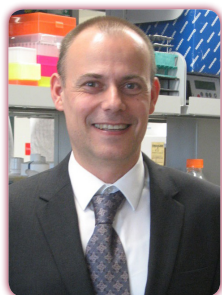


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*Ivo Kalajzic, Brya G. Matthews, Elena Torreggiani, Emilie Roeder,
Igor Matic, and Danka Grcevic*

University of Connecticut Health Center, USA

α Sma expressing perivascular and satellite cells contribute to muscle heterotopic ossification

Heterotopic ossification is formation of bone in atypical locations including muscle. Heterotopic ossification generally occurs as a result of aberrant BMP signaling. However, further studies are required to identify BMP-responsive cells in the muscle and understand how they contribute to heterotopic ossification. Alpha smooth muscle actin (α SMA) is a marker of perivascular cells and mesenchymal progenitors that contribute to bone growth and fracture healing. We aimed to evaluate whether α SMA⁺ cells can contribute to bone formation during heterotopic ossification. To identify and trace cells we used α SMA promoter-driven inducible Cre (α SMACreERT2) combined with a Cre-activated td Tomato reporter Ai9 to generate SMA9 mice. Mature osteoblast/osteocytes were labeled with the Col2.3GFP reporter. To label muscle satellite cells we utilized Pax7CreERT2/Ai9 mice. Pax7Cre/Ai9 labels cells below the basal lamina consistent with a satellite cell phenotype, while SMA9 labels similarly localized cells, in addition to perivascular cells. Pax7Cre/Ai9⁺ satellite cells are CD45/CD31⁻, Sca1⁻ but SM/C2.6⁺. The SMA9⁺ cells are CD45/CD31⁻ and ~60% express satellite cell marker SM/C2.6. 5% express MSC markers Sca1 and PDGFR α and a larger proportion express PDGFR β . During BMP2-induced heterotopic ossification, SMA9⁺ cells comprised 28% chondrocytes and 44% of Col2.3GFP⁺ osteoblasts, while Pax7Cre/Ai9⁺ satellite cells labeled only muscle fibers. Culture of sorted cells indicated that SMA9⁺ and Pax7Cre/Ai9⁺ cells differentiated into myotubes while the negative cells do not. SMA9⁺ cultured cells showed the highest upregulation of osteogenic gene expression after culture in the presence of BMP2. To clarify which subset of SMA9 cells possess osteogenic potential, we subdivided SMA9⁺ cells based on expression of SM/C2.6 and evaluated their ability to differentiate into osteoblasts after transplantation into muscle with BMP2. We observed osteogenic differentiation of SMA9/SM/C2.6⁺ cells suggesting that once the satellite cell population is removed from its niche they can exhibit osteogenic potential. In conclusion, α SMACreERT2 labels a population of mesenchymal progenitor cells in the muscle that contribute to heterotopic ossification induced by BMP2. Our data indicate that the perivascular fraction of cells is responsible for heterotopic ossification; however, muscle satellite cells are capable of osteogenic differentiation after removal from their niche. Further study of these cells may indicate mechanisms by which pathogenic heterotopic ossification occurs.

Biography

Ivo Kalajzic has completed his PhD studies on Identification of stages of mesenchymal lineage differentiation. He is currently Associate Professor at Department of Reconstructive Sciences at University of Connecticut Health Center. His laboratory focuses on studies on regulation of mesenchymal progenitor cell differentiation during bone fracture healing, and the role of notch signaling during repair. Other research projects within his group include the studies on progenitor cells within the muscle, and other tissues, including developing transplantation approaches to treat osteogenesis imperfecta. He has published more than 50 papers with focus on bone biology and orthopedics.

ikalaj@uchc.edu