DIFFERENTIAL EXPRESSION OF BONE MATRIX PROTEINS IN ENDOGENOUS UROEPITHELIAL BONE

INDUCTION IN A CANINE MODEL

Yin Xiao1, Wei Shi2, Ben Goss3, Mike Forsythe4, Alistair Campbell5, David Nicoll5, Richard Williams5, Ross Crawford3,4

1Tissue BioRegeneration and Integration, Science Research Centre, Queensland University of Technology, Australia
2Medical Engineering, Queensland University of Technology, Australia
3Prince Charles Hospital, Brisbane, Australia
4Princess Alexandra Hospital, Brisbane Australia

yin.xiao@qut.edu.au

BACKGROUND

Experimental heterotopic bone formation in the canine urinary bladder has been observed for more than seventy years without revealing the origin of the osteoinductive signals. In 1931, Huggins demonstrated bone formation in a fascial transplant to the urinary bladder. Through an elaborate set of experiments, it was found that proliferating canine transitional epithelial cells from the urinary system act as a source of osteoinduction and recipient connective tissue mesenchymal cells in close proximity accept the signal and allow for osteogenetic potential. It was also found that the signal appeared to be released via the basement membrane as this was the side of the cell where bone was formed. This bone formation was unique to urinary tissue including bladder, ureter and renal pelvis, and prostate in the canine model (1). Urist performed a similar series of experiments in guinea pigs as Huggins did in his canine model. After two weeks, mesenchymal cells condensed against the basement membrane of the columnar epithelium and membranous bone with haversian systems and marrow began to form juxtapose the basement membrane. At no time was cartilage formation noted, only direct membranous bone formation. It also demonstrated the expression of BMP’s in the urinary bladder epithelium and provided circumstantial evidence of BMP being the osteoinductive factor in heterotopic bone formation. BMP was expressed in uroepithelial cells at the same time that the mesenchymal cells began to proliferate and condense prior to bone formation. (2)

The aim of this study was to reveal mesenchymal cell differentiation and differential expression of bone matrix proteins during bone formation using a well established animal model of ectopic bone formation in muscle tissues, induced by transitional urinary epithelium.

METHOD

This study was approved by the University of Queensland Animal Ethics Committee. The dogs underwent a midline laparotomy incision followed by mobilization of a right sided myopenitoneal vascularized flap based on a superior epigastric artery pedicle. A sagittal cystotomy is made in the dome of the bladder and the vascularized flap was sutured in place with acryl absorbable, continuous suture. The animals were sacrificed at 6 weeks. The bladder samples were removed and assessed by histology and immunohistochemistry. Sections were incubated with optimal dilution of primary antibody for type I collagen, type III collagen, alkaline phosphatase (ALP), bone morphogenetic protein (BMP)-2 and – 4, osteocalcin (OCN), osteopontin (OPN), bone sialoprotein (BSP).

RESULTS AND DISCUSSION

The mechanism for bone formation induced by the epithelial-mesenchymal cell interactions is not clear. We were able to demonstrate mature lamellar bone formation 6 weeks after transplanting a portion of the abdominal smooth muscle into the bladder wall (Figure 1). The bone formed immediately adjacent to the proliferating transitional uroepithelium, a prerequisite for bone formation in Huggins’ model. There was no comment on the type of bone formation in Huggins’ model and Urist’s guinea pig model did not show any evidence of cartilage formation or enchondral ossification, only membranous bone formation was observed. We report evidence of cartilage formation and therefore enchondral ossification as well as membranous bone formation. Staining for cartilaginous tissue was strongest at the bone smooth muscle interface and the cells hypertrophied and coalesced only to be replaced by bone in the deeper more mature layers of bone. This is very similar histologically to the process of enchondral ossification at the growth plate in the growing skeleton.

CONCLUSION

This study demonstrates transitional epithelium induced differentiation of mesenchymal cells to chondrocytes and osteoblasts in muscle tissue. The sequential expression of bone matrix proteins was related to cell proliferation, differentiation and formation of enchondral and membranous bone. Further information regarding the molecular mechanism of bone formation induced by epithelial-mesenchymal cell interactions will improve understanding of cell differentiation during osteogenesis.

REFERENCES

1) Huggins CB. The formation of bone under the influence of epithelium of the urinary tract. Archives of Surgery, 1931 pp377–408.

Figure 1: Transitional urinary epithelium induced bone formation. New bone was formed six weeks after transplantation of urinary epithelium. This was found directly under transitional epithelium and newly formed bone marrow tissue can also be seen.

In newly formed cancellous bone, bone marrow tissue including osteoblasts, blood vessels, and connective tissue also appears on histological examination. Interestingly, a direct relation between newly formed bone and transitional epithelium was noted. IGF-1 ALP and BMP 2&4 were most strongly expressed in osteoblasts and proliferating mesenchymal cells related to bone formation; whereas, BSP and OCN expressed in mineralizing bone matrix. Type II collagen and VEGF were cellular expressed in chondrocytes differentiated from muscle tissue.