Murine Periosteum-derived Cells as a Novel Cell Model for Bone Tissue Engineering Research

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Introduction

In recent years, the periosteum has received increasing attention as a possible cell source for tissue engineering1. Human periosteum-derived cells (hPDC) can be easily collected from patients, cultured in vitro and are rich in progenitor cells2. Periosteum from adult mice, however, is difficult to isolate since it is only a few cell layers thick. Given the availability of multiple transgenic mouse models, in vitro and in vivo analysis of murine PDC (mPDC) could provide new insights into the role and therapeutic potential of the periosteum.

In this study we developed a technique to isolate mPDC from long bones of adult mice and compared their characteristics with bone marrow stromal cells (mBMSC). The presence of mesenchymal stem cells (MSC) was determined and the response to hypoxia and an inflammatory mediator, factors that are both present at the early stages of fracture healing, was assessed.

Materials and Methods

Primary mPDC were isolated by collagenase-dispase treatment of the diaphyses of tibiae and femurs of 7-9 week old male C57Bl/6J mice, avoiding digestion of the epiphyses. mBMSC were obtained by flushing the bone marrow.

Trilineage potential was examined by culturing cells in osteogenic, chondrogenic or adipogenic medium. In vivo osteogenic potential of mPDC and hPDC (isolated as previously described3) was evaluated by seeding cells on Collagraft™ and implanting them subcutaneously in nude mice. The presence of MSC in primary mPDC and mBMSC was compared by FACS analysis and by counting colony forming units. Finally, mPDC and mBMSC were cultured in the presence of TNF-α (0, 10 or 100 ng/ml) for 48h or in 0.5 or 21% O₂ for 24h. Effects on gene expression were analyzed by qRT-PCR.

Results

Isolated cells had a fibroblastic morphology in culture. Absence of contaminating cells from muscle, articular cartilage and bone was shown by lack of tissue-specific gene expression, respectively acta1, aggrecan and osteocalcin. Culturing mPDC under appropriate conditions showed differentiation to osteoblasts, chondrocytes and adipocytes. After in vivo implantation, mPDC and hPDC formed a similar amount of bone (9.2±0.9% versus 7.1±0.5%).

Further analyses revealed that mPDC contained a higher percentage of MSC compared to mBMSC (35.7±1.8% versus 13.0±1.1% of CD105+ Sca1+ CD45- cells). This was confirmed by counting colony forming units after seeding cells at low density.

Finally, the response of mPDC to hypoxia and TNF-α was analyzed. Hypoxia increased vegf, sox9, glut1 and ldh-a expression, whereas treatment with TNF-α enhanced bmp2 and mmp9 mRNA levels in a dose-dependent manner. No manifest difference in response was observed between mPDC and mBMSC.

Discussion and Conclusions

The present study shows that mPDC can be isolated and serve as a useful cell model for bone tissue engineering research.

References


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Disclosures

The authors state no conflict of interest.