HUMAN FRACTURE HEMATOMA CONTAINS PROGENITOR CELLS WITH MULTI-LINEAGE CAPACITY IN VITRO

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INTRODUCTION:
Fracture site hematoma is known to play an important role in fracture healing. It is clear that, within the hematoma, there is a bone-forming complex, composed of cells and substances which include growth factors. In previous studies, these growth factors have been confirmed to be central regulators of cellular proliferation, differentiation, and matrix synthesis during the fracture healing process. However, a review of the literature shows no reports investigating the cells of fracture hematoma. We hypothesized that the cells could be one of the origins of osteoblasts and chondrocytes in fracture hematoma. In this in vitro study, we investigated whether fracture hematoma cells (HCs) have capacity for multi-lineage mesenchymal differentiation capacity.

METHODS:
Patient characteristics
This study was approved by our institutional ethics committee and informed consent was obtained from all study subjects. Fracture hematoma was obtained from five consecutive patients (age range, 34-66 years; 5 men) during osteosynthesis 2 to 8 days after fracture occurrence. Fracture sites were patella (2), clavicle (1), ulna (1), tibia (1).

Isolation and culture of HCs
Hematoma which formed fibrin-clots, was removed manually before any manipulation or irrigation and placed in sterile polypropylene containers, to avoid contamination during operation. Specimens were then washed with PBS to remove blood, and were minced with scalpels on culture dish into small pieces with original medium, α-MEM containing 10% FBS, α-glutamethione, β-glycerophosphate, and ascorbic acid. Osteogenic differentiation was evaluated by Alizarin Red staining, alkalinephosphatase (ALP) activity. Expression of osteoblast-specific genes, ALP, and osteocalcin (OC), and a housekeeping gene GAPDH (GAP) was also measured by reverse transcription polymerase chain reaction (RT-PCR).

Chondrogenesis
2.5×10⁵ cells were pelleted and cultured for 21 days in chondrogenic medium (Ch+), DMEM-high glucose supplemented with dexamethasone, ascorbate-1-phosphate, sodium pyruvate, ITS+ Premix, TGF-β, and BMP-6. Chondrogenic differentiation was evaluated by Toluidine blue metachromasia. Expression of chondrocyte-specific genes, type-II collagen (ColII) and SOX9, and GAP was also measured by RT-PCR.

Adipogenesis
Cells were plated at 1×10⁵ cells/well in 6-well plates and cultured for 21 days in adipogenic medium (Ad+), DMEM- low glucose supplemented with dexamethasone, isobutyl-methylxanthine, indomethacin, and insulin. Adipogenic differentiation was evaluated by Oil red-O staining. Expression of adipocyte-specific genes, PPARγ and lipoprotein lipase (LPL), and GAP was also measured by RT-PCR.

Flow Cytometry Analysis
1×10⁵ cells at passage 1 were suspended in 20 μg/ml antibody labeled PE, incubated for 30 minutes at 4°C, and cell fluorescence was evaluated by flow cytometry using a FACSaria instrument.

Statistical Analysis
Unpaired t-test was used with a significance level of P < 0.05

RESULTS:
Morphology of HCs
In the primary culture, the adherent cells gave rise to colonies which first became visible around day 5 of culture as cells exhibiting a fibroblast-like spindle shape.

Osteogenesis

Adipogenesis

Chondrogenesis

DISCUSSION:
We successfully demonstrated for the first time that cells derived from fracture hematoma have a multi-lineage mesenchymal differentiation capacity in vitro. Our findings suggest that to accelerate fracture healing, fracture hematoma should not be removed from the fracture site during osteosynthesis. In addition collection and preservation of a part of HCs could provide a useful cell source for tissue engineering in the future treatment of non-union and long-bone defects. Further experiments are needed to examine whether HCs have multi-lineage potential in vivo. This will lead to further progress in the understanding of the composition, role and potential uses of fracture hematoma.