Chemotaxis and Osteogenic Differentiation of Mesenchymal Stem Cells are Induced by Non-Resorbing Osteoclasts

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Introduction:

Osteoclast activity has traditionally been regarded as being restricted to bone resorption. On the other hand, evidence suggests that non-resorbing osteoclasts might also influence osteoblast activity. This assumption is based on observations in patients with osteoporosis, especially with autosomal dominant osteopetrosis Type II, as well as studies with osteopetrotic animal models. Recently, it was demonstrated by an in vitro study that conditioned media collected from non-resorbing osteoclasts induced formation of bone-like nodules in a murine preosteoblastic cell line. The authors suggested, therefore, that osteoclasts secrete an anabolic signal, which is not derived from their resorptive activity. Further studies were aimed at investigating the hypothesis of the anabolic function of non-resorbing osteoclasts by investigating their capability to recruit mesenchymal stem cells (MSC) and to provoke their differentiation towards the osteogenic lineage. Conditioned media collected from non-resorbing human osteoclasts were tested for their ability to induce the expression of a spectrum of osteogenic marker genes together with chemotactic activity in human bone marrow derived MSC. Furthermore, the involvement of PDGF as a signaling factor was investigated.

Materials and Methods:

Culture of osteoclasts and collection of supernatants:
The isolation of human cells was approved by the Ethical Committee of the University of Ulm, Germany. Mononuclear cells were isolated from the peripheral blood of two healthy donors (PBMNC) using Ficoll 1077 density gradient centrifugation. 5x10^6 cells/cm^2 were seeded in 48-well tissue culture plates in 250 µl/well α-medium supplemented with 1% L-Glutamine, 10% FCS, 20-50 ng/ml rhRANKL and 10-25 ng/ml rh-CSF and were incubated at 37°C, saturation humidity and 8.5% CO2 with medium change twice a week. After 21 days the cells were fixed with 4% buffered formaldehyde and stained for tartrate-resistant acid phosphatase (TRAP). Osteoclasts were identified as TRAP-positive multinucleated cells containing at least 3 nuclei. Conditioned media (CM) of osteoclast cultures were obtained from 13 independent experiments with cells from two donors, pooling the media collected during each medium change, and stored at -70°C. Media were added to MSC cultures at a concentration of 50% in MSC-expansion medium to investigate their capability to induce osteogenic differentiation. As a control, MSC-expansion medium containing 50% osteoclast medium not conditioned by osteoclast cultures (non-CM), was used. The migratory response of MSC to CM was investigated by a chemotaxis assay in a Boyden chamber.

Culture of MSC:
MSC were isolated from bone marrow aspirates by density gradient centrifugation. For testing the effect of the CM, 1x10^6 MSC/cm^2 (passage 1 or 2) were seeded onto tissue culture plates in MSC-expansion medium (DMEM supplemented with 10% FCS). After 24h the medium was replaced by MSC-expansion medium containing 50% CM or 50% non-CM (negative control), respectively. To compare both treatments with successfully differentiated cells (positive control), parallel cultures were treated for 22 days with osteogenic differentiation medium (OD medium) consisting of MSC-expansion medium with 0.1 µM dexamethasone, 10 mM β-glycerophosphate and 0.2 mM ascorbate-2-phosphate. The expression of the osteogenic differentiation marker genes bone sialoprotein (BSP), alkaline phosphatase (AP), osteopontin (OP), and transcription factor Runx2 were measured quantitatively by real-time RT-PCR. Calcium deposition was examined by von Kossa staining and AP was detected with an AP staining kit. PDGF-BB and BMP-2 Immunoassay:
BMP-2 and PDGF-BB concentrations in the conditioned media were assessed in duplicate using the Quantikine BMP-2 and PDGF-BB immunoassay (R&D Inc. Minneapolis, USA).

Statistical analysis:
mRNA expression was investigated in MSC treated with conditioned media derived from 7 osteoclast cultures. A nonparametric Wilcoxon signed rank test was performed to evaluate differences between cells treated with conditioned and non-CM. The results of the chemotaxis assay for each tested CM were expressed as the mean of quadruplicate determination a standard deviation. Statistical significance was determined by Student’s t-test. Level of significance: p<0.05.

Results:

Effect of conditioned media on osteogenic differentiation:
With PBMNC from one donor, formation of TRAP-positive multinuclear osteoclasts was successful, resulting in typical inhomogeneous cultures containing numerous osteoclasts as well as mononuclear cells. The cultures obtained from the second donor consisted of only mononuclear cells without any osteoclast formation. All CM of cultures with osteoclast formation (n=9) induced osteogenic differentiation of MSC. Significant up-regulation of Runx2, BSP, and AP compared to the control cultures treated with non-CM was observed. The up-regulation of the osteogenic markers was in the same range as in MSC stimulated with the differentiation medium containing osteogenic supplements (positive control). This was confirmed by cytochemical detection of calcified matrix by von Kossa as well as by AP staining. MSC that were exposed to conditioned media derived from cell cultures without osteoclast formation (n=4) as well as MSC cultured in the presence of non-CM (negative control) did not show any such positive staining. Expression of osteogenic marker genes was also not up-regulated.

Effect of conditioned media on MSC migration:
The conditioned media, which stimulated MSC differentiation, also induced increased migration activity. Mean chemotactic index (CI) of 4.9±1.4 was significantly higher compared to mean CI of 2.1±0.2 measured in cultures stimulated with CM derived from cultures without osteoclast formation.

Functional involvement of PDGF:
The PDGF-BB concentrations in medium with 50% CM from osteoclast cultures ranged between 16 and 101 pg/ml. In media derived from cultures without osteoclast formation the PDGF-BB concentration was significantly lower (2 pg/ml). After pre-incubation of MSC with an inactivating antibody against the PDGF receptor β, a significant decrease of the migratory cell response to CM secreted from osteoclast forming cells was measured. Interestingly, with CM from cultures without osteoclast formation no major influence on cell migration was detected. BMP-2 could not be detected in the various CM media in measurable quantities by ELISA.

Discussion:
The present study demonstrated that supernatants collected from non-resorbing osteoclasts can induce osteogenic differentiation of human MSC and confirmed the hypothesis that osteoclasts might release anabolic signals, which are not linked to their resorption activity. Furthermore, it was shown for the first time that non-resorbing osteoclasts could recruit MSC. While effects on MSC migration might be mainly due to PDGF-BB, the factors inducing osteogenic differentiation remain to be elucidated.

References: