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METABOLIC BONE DISORDERS: FROM BENCH TO BEDSIDE

Vanessa Nicolin, Doriano De Iaco, Roberto Valentini

University of Trieste, Clinical Department of Medical, Surgical and Health Science,
Ospedale di Cattinara Strada di Fiume 447, Trieste

Abstract — Bone is a complex tissue that have many function, when bone remodeling becomes perturbed we can assist at many pathologies. The key factor in these pathologies is a perturbation of RANKL/RANK/OPG pathways. We have tune up a 3D culture system that have the advantage to produce osteoclast cells more physiological than the administration of the recombinant RANKL protein. This can be useful when study new drugs to avoid the cross-reaction with the exogenous cytokines.

Index Terms — TRANS2CARE, bone, disorder, osteoclast, co-culture, RANKL

1 BACKGROUND

Bone is a complex tissue that provides mechanical support for muscles and joints, protection for vital organs, a mineral reservoir that is essential for calcium homeostasis, and the environment and niches required for haematopoiesis. The regulation of bone mass is governed by a complex interplay between bone-forming cells termed osteoblasts and bone-resorbing cells termed osteoclasts, and is guided physiologically by a diverse set of hormones, cytokines and growth factors. The balance between these processes changes over time, causing an elevated risk of fractures with age. Osteoclasts may also be activated in the cancer setting, leading to bone pain, fracture, spinal cord compression and other significant morbidities.

2 OBJECTIVES

Bone remodeling becomes perturbed in a variety of pathologic conditions that affect the skeleton, including post-menopausal osteoporosis and rheumatoid arthritis, in which there is local and/or systemic alteration in the levels of hormones or proinflammatory cytokines that are known to stimulate or inhibit bone resorption in vitro and in vivo. Elucidation of the specific roles played by

RANKL/RANK in various types of cells link bone remodeling with regulation of the function of other organ systems in health and disease. Our objective is to study new types of drugs that could interfere with RANKL/RANK/OPG pathway using a cell system closely mimic the in vivo state and generate more physiologically relevant data.

3 APPROCH & METHODS

3.1 General approach

Our 3D Culture System is animal-free developed of higher-fidelity cell culture models that are more predictive of disease states and drug responses. 3D cultures have potential to greatly improve cell-based drug screening and identify toxic and ineffective substances at an earlier stage of the drug discovery pipeline than animal or clinical trials. It is suitable for abroad range of procedures, including multicellular tumor spheroid and bone turnover assays and high-throughput drug screening assays.

3.2 Methods

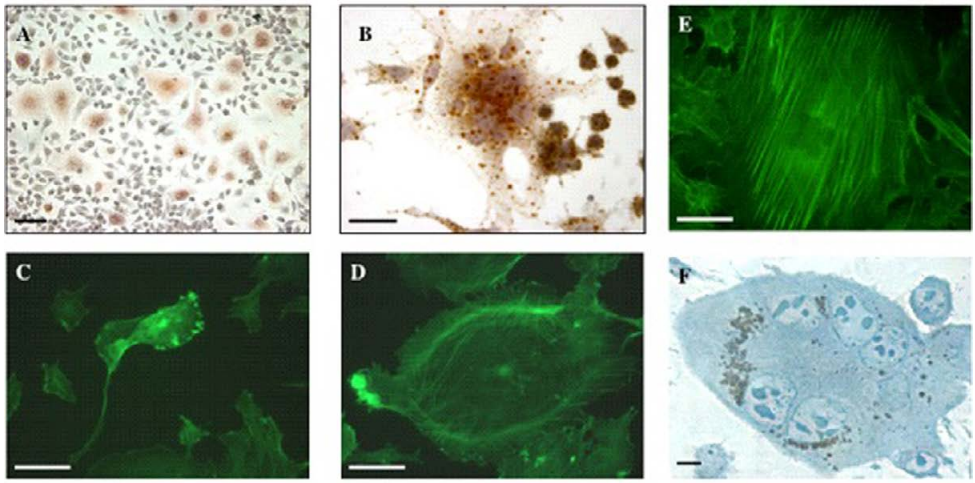
Monocyte/macrophage cell line co-cultured with osteoblast were used as a model system of osteoclastogenesis. Both cell line were cultured in a 24-well plate at a density of 105 cells/mL in RPMI-1640 medium with 2 mM glutamine and modified to a final concentration of 4.5g/L glucose, 1.5 g/L sodium bicarbonate, 10 mM Hepes, 1 mM sodium pyruvate and 10% fetal bovine serum (FBS). Cells were grown for 4 days then washed once in phosphate buffered saline (PBS) pH 7.2 and fixed for 30 min in a solution of 4% paraformaldehyde. After this step osteoclast developed could be observed by TRAP assay, Immoncytochemistry, Transmission electron microscopy (TEM) and scanning electron microscopy (SEM).

4 RESULTS

Many studies of the past decade have focused on the mechanisms by which osteoblasts regulate osteoclast formation. Bone is continuously remodeled by bone formation and resorption processes and the cooperative bone metabolism is tightly regulated to maintain homeostasis. More recently, following commercial availability of a recombinant RANKL protein, another technique of osteoclast generation, negating the need for osteoblasts, has been described and is now routinely used. These new techniques for osteoclast generation allow the study of both osteoclast formation and resorption. Whereas these procedures are highly valuable in that they provide a constant supply of human osteoclasts, their one disadvantage is that they are time consuming, osteoclasts only forming after approx 7–10 days in culture.

We used monocyte/macrophage niche grown as co-culture together osteoblast cells with the aim to propose a more physiological cell culture compared to the system induced by cytokines (Fig.1 A, B). In this study, we have shown monocyte cell line co-cultured with osteoblasts, in absence of exogenous factors, are capable to differentiate into functional osteoclasts capable to resorbe dentin surface (Fig.1 C, D, E, F). In the bone microenvironment, osteoclast formation requires cell-cell interaction of osteoclast precursors cells with osteoblasts and can be achieved by co culturing monocyte precursors cells with osteoblast/stromal cells. The identification of the relationship between these cells (monocyte-osteoblasts-osteoclasts) establishes new approaches for future research into pharmacological control of the same cells in health and diseases.

Fig.1



Coculture of osteoblasts with onocytes after 4 days of differentiation. **A-B**: Rapresentative fields of the TRAP+ cells. Scale bars= 2A 100mm; 2B 50mm.
C: Immunostaining for actin shows a podosome clusters developed at two days of differentiation Bar 50mm;
D: Dynamic actin ring forminig peripheral podosome belt at four days of differentiation Bar 50mm; **E**: Actin stress fibers developed at four days of differentiation during osteoclats migration Bar 50 mm
F: Semithin section of mature osteoclast at 4 days of differentiation Bar:10mm

5 POTENTIAL NEW PRODUCTS & SERVICES

Our 3D cell culture system is useful to test the perturbation inside the bone turnover process. Osteoclast are very difficult to obtain, with traditional methods such as by pharmacologic combination of RANKL and MCSF. Our system guarantee after 4 days obtain functional osteoclasts without the use of exogenous cytokines. The monocyte-osteoblast 3D system is superior because it allows a more physiological environment for experimentation of anti-resorption drugs, stimulating bone turnover in pathologies such as osteoporosis.

6 CURRENT COLLABORATIONS

NIH USA (TRL1-2-3), CNR Rome ITA (TRL1-2-3), Ebri Rita Levi Montalcini Foundation Rome ITA (TRL1-2-3), New York University USA (TRL1-2-3), University of Salerno ITA (TRL2-3), Eli Lilly and Company Indianapolis Indiana USA (TRL3-4), Abiogen Pharma ITA(TRL1-2-3-4).

7 CONTACTS OR COLLABORATIONS NEEDED

We need a collaboration to whom is interested in the R&D of new drugs that require a bone physiological system of cell to cell interactions.

8 COMMUNICATION TOOLS

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Our patents: sintesi e attività biologica di derivati n-alchilcarbazoli come inibitori di STAT3 cod. SA2011000014, categoria ISI-CRUI 06_Pharmacology/Toxicology,
Our Researchgate: Nicolin Vanessa
Our mail contact: Nicolin@units.it
Our facebook: Researcher Work in Progress

9 FUNDS NEEDED (FORECAST)

9.1 For basic research (investigation of biolological mechanisms): 15.000 €

9.2 For applied research (solutions for real-world problems): 15.000 €

9.3 For pilot & demonstrator activities (to develop a prototype) : 30.000 €

10 CONCLUSION

Our 3D culture method have the advantages to be faster, economical and more physiological than previous osteoclastogenic method, also it have many possible application such the drug discovery studies, research on the RANKL/RANK/OPG pathway, studies on bio-mimetic bone interface or morphological studies on osteoclast cells genesis.