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PP147

Involvement of the co-receptor RAMP2 in the progression of breast cancer-induced osteolytic lesions

Alfredo Cappariello[1], Nadia Rucci[2], Mattia Capulli[2], Maurizio Muraca[1] & Anna Teti[2]
[1]Children Hospital Bambino Gesù, Rome, Italy; [2]University of L'Aquila, L'Aquila, Italy.

Bone is the primary site of metastasis for breast cancer, which leads mainly to osteolytic lesions, Cancer cells can expand into the bone for their ability to 'dialogue' with resident cells, interfering with the physiological processes of bone turnover. In this study, a large-scale analysis comparing gene expression of biopsies of bone and visceral metastases from human breast cancer patients showed that the receptor (G protein-coupled) activity modifying protein-2 (RAMP2) gene, encoding for a co-receptor calcitonin-receptor-like receptor, was overexpressed 2.7-fold in bone metastases. Gene expression also showed a significant increase of components of the RAMP2-pathway, both receptors (calcitonin-receptor-like receptor, +2.08-fold) and ligands (amylin +1.22-fold). To elucidate the potential role of RAMP2 in osteolytic lesions, we stably transfected the human osteotropic breast cancer cell line MDA-MB-231 with RAMP2 (MDA-RAMP2) and found an increased ability of *in vitro* migration and proliferation, compared to empty vector transfected (MDA-empty) cells. Moreover, osteoclast precursors treated with conditioned medium (CM) from MDA-RAMP2 cells showed a significant increase of osteoclast differentiation (+2.1-fold, $P=0.01$) and function (pit index: +6.1-fold, $P=0.0001$) compared to MDA-empty-CM treated preosteoclasts. Semi-quantitative RT-PCR revealed an increase in RankL/Opg ratio in primary osteoblasts treated with MDA-RAMP2-CM, indicating further pro-osteoclastogenic action of tumour cells mediated by osteoblasts. We also observed that MDA-RAMP2 cells formed oncospheres larger (+2.61-fold, $P=0.04$) but less numerous (−2.87-fold, $P=0.02$) than MDA-empty cells, indicating a reduced stemness in favour of proliferation and differentiation. Finally, *in vivo* experiments of intratibial injection of MDA-RAMP2 cells in Balb-c nu/nu mice showed an increased osteolytic area (+1.6-fold, $P=0.048$) compared to MDA-empty cell injected tibias. In conclusion, our data suggest that RAMP2 plays a role in tumour aggressiveness and promotes the growth of cancer cells in bone through their ability to communicate with the resident cells, thus contributing to the osteotropism of breast cancer cells.

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Direct administration of zoledronate acid improves bone structure in local osteoporotic lesion of ovariectomized rats

Yohei Matsuo, Masafumi Kashii, Tsuyoshi Sugiura, Tokimitsu Morimoto, Hirotsugu Honda, Takashi Kaito, Motoki Iwasaki & Hideki Yoshikawa
Department of Orthopedic Surgery, Osaka University Graduate School of Medicine, 2-2 Yamadaoka, Suita, Osaka, Japan.

Objective
To examine the efficacy and safety of direct administration of zoledronate acid (ZOL) on local osteoporotic lesion of ovariectomized rats.
Methods
Six weeks later after ovariectomy, 16 6-month-old female SD rats were divided into the two groups with no differences of body weight and BMD of the proximal tibia. In the group L, 50 μl ZOL at a dose of 67 μg/kg were locally injected into the bone marrow between the two drilled holes and 50 μl saline was systemically administrated by s.c. injection. In the group S, 50 μl saline was locally injected, and 50 μl ZOL at a dose of 67 μg/kg was systemically administrated. Local osteoporotic lesions induced by ovariectomy (Area 1: cancellous bone area of right proximal tibia between the two holes, Area 2: left side mirror area) were analysed using *in vivo* micro-CT at 2, 4, 6, and 8 weeks later after administration.
Results
In the group L, BMD of the locally injected Area 1 continuously increased until week 8 (+41%), but BMD increased and stayed constant in the group S (+17%). In the group L, BMD of the Area 2 continuously decreased until week 8 (−12%), but BMD maintained at the pre-treated level in the group S. In the group L, BMD and microstructural parameters of the Area 1 were significantly higher than the group S at week 4, 6, 8, and these parameters of the Area 2 were significantly lower than the group S at week 6, and 8.
Conclusions
ZOL is the most potent bisphosphonate that strongly inhibits osteoclast function with high binding affinities for bone. Taking advantage of these characteristics, we showed that direct administration of high-dose ZOL to local osteoporotic lesion have more beneficial effects on local bone structure than the systemic administration, and have no influence on other bone tissue.

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Cytotoxicity of picocyanobacteria strains of the genera *Cyanobium* on osteosarcoma cells

Rosário Martins[2,3], Margarida Costa[3], Mónica Garcia[1], Piedade Barros[2], João Costa-Rodrigues[1], Vitor Vasconcelos[3,4] & Maria Fernandes[1]
[1]Laboratory for Bone Metabolism and Regeneration, Faculdade de Medicina Dentária, Universidade do Porto, Porto, Portugal; [2]CISA – Centro de Investigação em Saúde e Ambiente, Escola Superior de Tecnologia da Saúde do Porto, Instituto Politécnico do Porto, Porto, Portugal; [3]CIIMAR – Centro Interdisciplinar de Investigação Marinha e Ambiental, Universidade do Porto, Porto, Portugal; [4]Faculdade de Ciências, Universidade do Porto, Porto, Portugal.

Marine cyanobacteria have been recognized as an important source of bioactive compounds. The cytotoxicity on cancer cell lines has been extensively explored and several cyanobacteria metabolites are already described as potential anticancer compounds or are considered useful templates for the design of new anticancer drugs. The majority of compounds have been isolated from filamentous or colonial cyanobacteria that growth in high densities along shores. In contrast, picoplanktonic forms have rarely been explored since, for these strains, there is a need for culture for biomass production. From our LEGE cyanobacteria culture collection we selected a panel of seven strains of the picocyanobacteria genera *Cyanobium* in order to explore it's potential as anticancer agents. Strains were cultured under laboratory conditions. Freeze-dried biomass was extracted using methanol and dichloromethane to a crude extract and then fractionated using hexane, ethyl acetate and methanol. The cytotoxicity of crude extracts and fractions was evaluated in the osteosarcoma cell line MG63 by the reduction of the bromide 3-(4,5-dimetil-tiazol-2-il)-2,5-difenil-tetrazolio (MTT) and confirmed by the lactate dehydrogenase (LDH) assay. From the results, four of the seven *Cyanobium* strains were found to induce a significant decrease in cell viability. The highest percentage of inhibition of tumor cells growth was observed within the ethyl acetate, which is therefore, promising in terms of isolation of bioactive compounds.

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PP150

Inhibition of osteoclastogenesis by proton pump inhibitors on co-cultures of human osteoclasts and breast cancer cells

Sara Reis[1,2], Maria Fernandes[1] & João Costa-Rodrigues[1]
[1]Laboratory for Bone Metabolism and Regeneration, Faculdade de Medicina Dentária, Universidade do Porto, Porto, Portugal; [2]Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, Porto, Portugal.

Proton pump inhibitors (PPIs) are a class of drugs particularly used in gastric disorders. They promote a decrease on gastric acid secretion by inhibiting the H^+/K^+ ATPases. Osteoclasts are cells specialized in bone resorption by H^+ translocation to the bone surface. Thus, PPIs might be regarded as potential tools to modulate osteoclast resorption activity, particularly in conditions that are associated with a hyperactivation of osteoclasts, like it happens, in bone osteolytic metastasis. Breast cancer is one of the most frequent tumours that originate bone osteolytic metastasis. In this context, this work intended to characterize the effects of three PPIs on human osteoclastogenesis in co-cultures of human osteoclasts and breast cancer cells.
Osteoclastic precursors were isolated from human peripheral blood and were co-cultured with two different breast cancer cell lines (T47D and SK-BR-3). Cell cultures were treated with a concentration range (10^{-7} to 10^{-3} M) of omeprazole, esomeprazole and lansoprazole. Cell cultures were characterized throughout a 21-day period for total protein content, tartarate-resistant acid phosphatase (TRAP) activity, TRAP+ multinucleated cells and the presence of cells with actin rings and expressing vitronectin and calcitonin receptors.
The presence of breast cancer cells, particularly T47D cells, greatly induced