by tumor cells, must be conjugated to the polymeric carriers in such a way that they are released upon accumulation in the tumor. Also, the polymer system itself is not yet optimized, although a twofold increase in accumulation over non-thermally responsive controls is a good starting point. In time, this approach could prove to be a good strategy for targeting cytotoxic drugs to tumors.

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Therapeutic Targets

Osteoprotegerin: a new therapeutic agent for the treatment of bone disease

Physiological bone remodelling depends on an equilibrium between two cellular activities, resorption and apposition. Osteoclasts are the major agents of the resorption process, whereas apposition results mainly from bone-forming osteoblast metabolism. These cellular activities are strongly orchestrated by a large and complicated cytokine network, which has a role in bone development and the maintenance of bone integrity. Osteoclasts and osteoblasts are then interconnected, the first controlling the activation of the second[1,2]. Because osteoclasts are highly specialized elements required for bone resorption, it is not surprising that they are commonly observed at the osteolysis foci, which are associated with systemic bone diseases, for example, hyperparathyroidism and Paget disease. Furthermore, they localize at primary (giant cell tumours, chondrosarcoma) or secondary (bone metastasis) osteolytic tumours and at the membrane around loose joint implants[3]. From these data, the bone resorption can be considered as a consequence of a disturbance in the mechanisms that govern the formation, activation and function of these cells, such as the communication between osteoclasts and osteoblasts.

RANK–RANKL–OPG: a novel signalling pathway

Recent discoveries have elucidated a key signalling pathway between stromal cells and osteoclasts. A novel soluble protein, osteoprotegerin (OPG), which inhibits osteoclastogenesis *in vitro* and *in vivo,* has been cloned[4]. In response to this discovery, an osteoprotegerin-ligand, RANKL (receptor activator of NF-κB-ligand), expressed on the stromal cell membrane, and which binds OPG and stimulates osteoclast differentiation, activation and survival, has also been cloned[5]. Stromal cells also expressed a soluble form of RANKL, explaining that its effects on osteoclasts are maintained in the absence of cell contacts between osteoclasts and stromal cells. Finally, RANK, the third protagonist, is localized at the surface of the osteoclastic lineage and is the appropriate receptor for the OPG-ligand[6]. Among the protagonists of this triad, OPG acts as a decoy receptor (antagonist) and inhibits the binding between RANKL and RANK. Given that, RANK is localized at the surface of the osteoclastic lineage, mature osteoblasts and marrow stromal cells express RANKL, and OPG is ubiquitously produced by a variety of cell types including stromal cells and osteoblasts; therefore, common paracrine pathways could be suggested in the regulation of bone metabolism. Thus, the RANK–RANKL–OPG triad has created a new molecular and cellular dimension for the osteoclastic lineage. If RANK–RANKL–OPG are involved in the physiology of osteoclasts, they are clearly implicated in pathological bone disorders, thus the clinical use of the natural inhibitor OPG can be envisaged.

The role of OPG

Studies have investigated the role of OPG by generating OPG-deficient mice. These $OPG^{-/-}$ mice exhibit a decrease in total bone density, which is characterized by severe bone porosity and a high incidence of fractures similar to post-menopausal osteoporosis[7]; this can be reversed by OPG administration, which inhibits endosteal osteoclasts[8]. The role of RANK–RANKL–OPG has also been investigated in post-menopausal women with osteoporosis. Serum OPG concentrations were increased significantly in post-menopausal women with low bone mass and a high rate of bone turnover[9]. In addition, short-term administration of glucocorticoids significantly suppresses serum OPG, which might participate in the development of glucocorticoid-induced osteoporosis[10]. However, these points were recently discussed by Seck and coworkers who failed to observe the expected changes in the expression of OPG and RANKL in human bone samples at menopause[11].

Gene therapy approach

Despite this controversy, a mouse ovariectomy model of oestrogen deficiency was employed to investigate gene therapy with OPG as a means of preventing osteoporosis[12]. Mice subjected to ovariectomy surgery, followed by immediate adenoviral gene transfer, had significantly higher bone volume with reduced osteoclast numbers. This study demonstrates that a single adenoviral gene transfer can provide sustained delivery of OPG useful in the treatment of osteoporosis. The potential effects of OPG on bone tumours, mainly in hypercalcaemia associated with tumour

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development, have been studied. The data revealed that OPG exhibits hypocalcaemic effects in normal mice and in hypercalcemic nude mice carrying tumours associated with humoral hypercalcaemia of malignancy[13,14]. Similarly, as RANKL is produced by cancer cells[15] and is a key molecule of osteoclast formation for bone metastasis[16], OPG expressed in bone tumours[17] blocks cancer-induced skeletal destruction via its interaction with RANKL (Refs 18–20). Moreover, OPG diminishes advanced bone cancer pain[21]. The balance of bone resorption depends on the local RANKL to OPG ratio, which is enhanced in bone metastases and humoral hypercalcemia of malignancy[22]. The RANK–RANKL–OPG system might also be involved in joint destruction during arthritis pathologies. Indeed, local T-cell activation can lead to RANKL production and subsequent cartilage and bone loss, which can be prevented by OPG treatment[23]. Unexpectedly, the RANK–RANKL–OPG triad is also involved in the vascular calcification process as demonstrated in OPG$^{-/-}$ mice[7,8,24].

Clinical trials

Recently, the results of the first clinical trial with osteoprotegerin (Amgen Company, Thousand Oaks, CA, USA) supported its potential as a therapeutic agent for bone disorders, such as osteoporosis and multiple myeloma. Thus, a randomized, double-blind, placebo-controlled, sequential-dose-escalation study was conducted in post-menopausal women to determine the effect of a single subcutaneous dose of OPG on bone resorption parameters. The results revealed that a single injection of OPG (3 mg kg^{-1}) is well tolerated by the patients and reduces bone turnover for a sustained period[25]. The RANK–RANKL–OPG paradigm has opened new areas in bone research and enables the consideration of novel therapeutic targets in diseases that are characterized by excessive bone resorption.

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