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Editorial

RANK/RANKL pathway in cancer: Biological activity beyond bone?

Roughly 15 years have gone by since the identification of the main characters involved in the interplay of the RANK–RANKL system. This gave rise to the race for elucidating their biological role [1]. In those years, in vivo silencing experiments for both Receptor Activator of Nuclear Factor κ B (RANK) and its ligand RANKL showed that RANK, and RANKL negative mutant mice exhibit severe osteopetrosis due to a complete lack in osteoclast activation.

RANKL is produced mainly by the osteoblastic lineage and immune cells, and is able to link to the osteoclast surface receptor, RANK, stimulating bone resorption through osteoclastogenesis and multinucleated mature osteoclasts activation [2]. As the years progressed, it has become clearer that targeting the RANK–RANKL interaction could be of great relevance in the treatment of diseases characterised by increased osteoclast activation, including osteoporosis and bone metastases. This led, within a few years, to preclinical and clinical development of denosumab, a fully humanised monoclonal IgG2 antibody targeting RANKL.

Denosumab has been shown to reduce vertebral, hip, and non-vertebral fracture risk in postmenopausal women, and to be effective in delaying skeletal-related events in bone metastatic solid cancers and multiple myeloma [3]. In addition to this effort in first elucidating and then targeting the RANK–RANKL axis in the metastatic setting, considerable data were collected. This led to a more complex and comprehensive view concerning the abrogation of this pathway in cancer.

It has been demonstrated that RANKL plays a role in mammary tumourigenesis. It acts as a major paracrine effector for the mitogenic action of progesterone in mouse mammary epithelium as well modulating oestrogen-dependent expansion and regenerative potential of mammary stem cells [4,5]. Moreover RANK overexpressing transgenic mice showed a higher incidence of breast cancer after multiparity or medroxyprogesterone acetate treatment, whereas RANK silenced trangenic mice [4] or mice treated with recombinant osteoprotegerin, [5] had decreased incidence and delayed onset of tumourigenesis in these systems.

Murine model experiments even demonstrated that RANKL is able to act as chemo- attractant and as pro-migratory factor in RANK-expressing breast and prostate cancer cell lines. Moreover, RANKL inhibition has been demonstrated to reduce bone lesions and tumour burden in vivo in melanoma model of bone metastasis [6].

All this evidence is, at least in part, confirmed by exploration in human samples where RANK expression level in the primary tumour correlates with the occurrence of bone metastases, and RANK-expressing cancer could be found in up to 80% of bone metastases originated from solid tumour [7,8]. However, a direct pro-metastastic mechanism may not be responsible for the supposed pro-tumoural role of RANK–RANKL pathway.

Currently the best described model is known as the “bone vicious circle”. In this model, RANK enables cancer cells to migrate to bone to where RANKL is abundantly expressed by osteoblasts. Some tumour cells may directly express RANKL, whereas others further enhance RANKL expression by cell-to-cell contact of tumour cells with osteoblastic cells. This leads cancer cells to enter the vicious circle where they stimulate osteoclasts that express RANK. Bone degradation by osteoclasts creates further space for expansive tumour growth within the bone microenvironment and releases a variety of growth factors and cytokines stored in the bone matrix, including parathyroid hormone-related peptide (PTHrP), IL-6, and transforming growth factor (TGF)-β, that boost even further the proliferation of cancer cells [9].

Recently, it has been shown that dendridic cells (DCs) also express RANK, and therefore can be stimulated by RANKL. RANKL-stimulated DCs respond by up-regulation of co-stimulatory molecules CD86, CD205 and cytokines such as TNF-α, IL-6, and IL-10 leading to $CD4^+25^+$ T-regs ($Foxp3^+$) expansion and subsequent local and systemic immunosuppression [10]. Intriguingly, in the in-vivo breast cancer $Her2^+$ model, RANK expression on tumour cells has clearly been shown to enhance metastatic spread and this phenomenon was strictly dependent on the presence of FoxP3+ T-reg cells most important function, according to this model, appeared to be as the major source of RANKL in cancer microenvironment [11]. Furthermore, DCs from bone marrow with metastatic prostate cancer selectively and highly express RANK, while blockade of RANK/RANKL signalling pathway disables the effects of DC-mediated T-reg-cell expansion and, consequently, delays tumour growth in murine models [12]. Thus, according to the data in the literature, it is possible to hypothesise the existence of an unknown “immune vicious circle” where RANKL produced by cancer cells or mesenchymal cells (in response to factor secreted by cancer cells) could stimulate DCs leading, in turn, to $FoxP3^+$ T-reg cell expansion that in turn produces further RANKL. The final effect of this positive feedback loop could be to enhance tumour invasiveness through an increase of RANK activation in cancer cells as well immune evasion properties through the increase in T-reg activity and the well-known, adverse effects of $FoxP3^+$ lymphocytes in cancer [13].

Recently, denosumab demonstrated a significant delay in the onset of detectable bone lesions in men with castration resistant prostate cancer compared to placebo [14]. This could indicate some kind of efficacy of the compound as anticancer agent and

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consequently an indirect confirmation of the role of RANKL in cancer progression. Currently, we are still far from fully understanding what really happens when disrupting the RANK/RANKL axis in the "real world" and at the same time we do not know which patients could benefit from this approach over and above the effects of denosumab as an anti-resorptive agent. Given the decrease in tumourigenesis observed in mice treated with RANKL blocking agents, we can speculate that denosumab may have a role in breast cancer prevention as well as in reducing tumour relapse after surgery. Finally, it has been demonstrated that tumour-infiltrating FoxP3+ lymphocytes and RANK expression by tumour cells are factors which limit the efficacy of neoadjuvant treatments in breast cancer [15,16]. These results suggest that the use of denosumab in this particular setting might provide additional benefits to the patients. Elucidating these mechanisms more clearly will be an exciting challenge for translational research in bone oncology and could provide the opportunity to exploit RANK inhibition strategies both within and beyond the bone.

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Francesco Pantano
Department of Medical Oncology, Campus Bio-Medico University of Rome, Rome, Italy

INSERM, Research Unit U1033, University of Lyon-1, Faculty of Medicine Laennec, rue Guillaume Paradin, 69372 Lyon cedex 08, France

Philippe Clézardin
INSERM, Research Unit U1033, University of Lyon-1, Faculty of Medicine Laennec, rue Guillaume Paradin, 69372 Lyon cedex 08, France

Daniele Santini*
Department of Medical Oncology, Campus Bio-Medico University of Rome, Rome, Italy
E-mail address: d.santini@unicampus.it

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* Corresponding author. Tel.: +39 6225411311; fax: +39 6225411933.