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Review Article

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RANK–RANKL signalling in cancer

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Synopsis

Oncogenic events combined with a favourable environment are the two main factors in the oncological process. The tumour microenvironment is composed of a complex, interconnected network of protagonists, including soluble factors such as cytokines, extracellular matrix components, interacting with fibroblasts, endothelial cells, immune cells and various specific cell types depending on the location of the cancer cells (e.g. pulmonary epithelium, osteoblasts). This diversity defines specific "niches" (e.g. vascular, immune, bone niches) involved in tumour growth and the metastatic process. These actors communicate together by direct intercellular communications and/or in an autocrine/paracrine/endocrine manner involving cytokines and growth factors. Among these glycoproteins, RANKL (receptor activator nuclear factor-κB ligand) and its receptor RANK (receptor activator nuclear factor), members of the TNF and TNFR superfamilies, have stimulated the interest of the scientific community. RANK is frequently expressed by cancer cells in contrast with RANKL which is frequently detected in the tumour microenvironment and together they participate in every step in cancer development. Their activities are markedly regulated by osteoprotegerin (OPG, a soluble decoy receptor) and its ligands, and by LGR4, a membrane receptor able to bind RANKL. The aim of the present review is to provide an overview of the functional implication of the RANK/RANKL system in cancer development, and to underline the most recent clinical studies.

Key words: microenvironment, oncogenesis, RANK, RANKL

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INTRODUCTION

In a physiological context, a healthy tissue microenvironment provides an adapted 3D microarchitecture with essential intercellular signalling, thus ensuring appropriate function. This tissue homoeostasis acts as a barrier to tumour development by inhibiting excessive cell growth and/or migration. Indeed, this fragile equilibrium can be destabilized by any alterations to cell communications, or interaction between cells and extracellular matrix components and consequently can become a fertile environment for cancer cells, promoting their malignant transformation and their proliferation [1]. The conjunction between one or more oncogenic events and this fertile environment can lead to the development of a tumour mass, which is frequently linked to the tumour cells escaping from the immune system [2]. In fact, this description reflects the "seed and soil" theory proposed by Stephan Paget in 1889 to explain preferential metastatic sites depending on tumour subtype [3].

This "soil" or tumour microenvironment is a very complex and dynamic organization, defined by three main "niches" depending on their functional implication: (i) an immune niche involved in local immune tolerance, (ii) a vascular niche associated with tumour cell extravasation/migration and (iii) a metastatic niche (e.g. bone, lung, liver) hosting the metastastic tumour cells [4,5]. The notion of tumour niche was initially described for haematopoietic stem cells, for which the bone microenvironment is composed of complex signalling pathways that carefully regulate stem cell renewal, differentiation and quiescence [6]. The concept of tumour niche was then extended to bone metastases, such as breast or prostate cancers [7–9]. Lu et al. [10] described a model of bone metastasis dormancy in breast cancer where

Abbreviations: EMT, epithelial mesenchymal transition; LRG4, leucine-rich repeat-containing G-protein-coupled receptor 4; OPG, osteoprotegerin; OPGL, osteoprotegerin ligand; RANK, receptor activator of nuclear factor-κB; RANKL, receptor activator nuclear factor-κB ligand; TAM, tumour-associated macrophage; TNF-α, tumour necrosis factor-α; TRAF, TNF-receptor associated factor; TRAIL, TNF related apoptosis inducing ligand; TRANCE, tumour necrosis factor-related activation-induced cytokine.

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VCAM-1, aberrantly expressed, promoted the transition from indolent micrometastasis to proliferating tumour by recruiting and activating *in situ* osteoclastic cells. More recently, Wang et al. [11] analysed the distribution of human prostate cancer cell lines colonizing mouse bones after intracardiac injection of tumour cells and demonstrated that homing of prostate cancer cells was associated with the presence of activated osteoblast lineage cells. These two recent manuscripts are perfect examples of the involvement of the tumour environment in the biology of bone metastases.

The tumour microenvironment thus provides all the factors necessary for cancer cell survival, dormancy, proliferation or/and migration [10] and very often, tumour cells divert this environment in their favour [7–9]. Indeed, this specific microenvironment has recently been involved in the maintenance of cancer cell dormancy [12–14] and may also play a part in drug resistance mechanisms by controlling the balance between cell proliferation and cell death, or by secreting soluble factors that dysregulate the cell cycle checkpoints, the cell death associated signalling pathways, or drug efflux [15,16].

Cell communications in physiological and pathological conditions are promoted by physical contacts involving adhesion molecules and channels, but also by a very high number of soluble mediators called cytokines and growth factors which appear to be the key protagonists in the dialogue established between cancer cells and their microenvironment [16]. These polypeptidic mediators perform their activities in an autocrine, paracrine or juxtacrine manner leading to inflammatory foci and the establishment of a vicious cycle between cancer cells and their local niches [17–19]. These proteins also have endocrine activities and contribute in this way to both the formation of a chemoattractant gradient and the metastatic process.

Considerable diversity in the cytokines and growth factors playing a role in cancer development has been identified in the last four decades. Some of them can be considered to be biological markers for aggressiveness, or to be prognostic factors, whereas others are also regarded as therapeutic targets. Among cytokine families, in the last 15 years, the biology of receptor activator nuclear factor-κB ligand (RANKL) and its receptor RANK has been widely studied in cancer [20–23] and has been identified as a key therapeutic target in numerous cancer entities, as described below. The present review gives a synthesis of RANK/RANKL pathway involvement in the carcinogenesis process. Their direct or indirect activities in oncogenic events will be described, as will their recent therapeutic applications.

RANKL/RANK SYSTEM: DISCOVERY, MOLECULAR AND FUNCTIONAL CHARACTERIZATION

The superfamily of tumour necrosis factor-α (TNFα) is composed of more than 40 members and is associated with a similar number of membrane or soluble receptors. RANKL is one member of the TNF-α superfamily (TNFSF11) and binds to a membrane receptor named receptor activator of nuclear factor-κB (RANK), a member of the TNF receptor superfamily (TNFRSF11A) [20–30]. The interactions between RANKL and RANK lead to specific intracellular signal transduction and are controlled by a decoy receptor called osteoprotegerin (OPG) (TNFRSF11B) [27] (Figure 1).

RANKL

RANKL has alternatively been called tumour necrosis factor-related activation-induced cytokine (TRANCE) [26], osteoprotegerin ligand (OPGL) [27,28] and osteoclastic differentiation factor (ODF) [29,30]. Although RANKL is the name commonly used, the official nomenclature of this cytokine is TNFSF11. RANKL is a homotrimeric type II membrane protein with no signal peptide and existing in three isoforms due to alternative splicing of the same gene [31]. Among these isoforms, the full-length RANKL is called RANKL1, RANKL2 is a shorter form of RANKL1 in which a part of the intra-cytoplasmic domain is missing and RANKL 3 is a soluble form of RANKL, with the N-terminal part of the amino acids deleted [31]. A soluble RANKL can also result from the sheding of membrane-RANKL induced by various enzymes such as the metalloproteinase disintegrin TNF-α converting enzyme (TACE) [32] or ADAM-10, MMP-7, MMP-14 [33,34]. RANKL is expressed by a wide variety of tissues such as the brain, skin, intestine, skeletal muscle, kidney, liver, lung and mammary tissue, but is more highly expressed in bone tissue [35], lymphoid organs and the vascular system [36]. The control of bone remodelling is the predominant function of RANKL. Indeed, RANKL effectively regulates the bone resorption process by stimulating osteoclast differentiation and osteoclast survival [37,38]. Whether RANKL is expressed by osteoblasts, osteocytes, chondrocytes or stromal cells, osteocytes are its main source in adult bone [39,40]. The role of RANKL is not restricted to the bone tissue and RANKL also plays an important role in the immune system, increasing the ability of dendritic cells to stimulate both naive T-cell proliferation and the survival of RANK$^+$ T-cells [25,26,41]. In this context, Wong et al. [27] demonstrated that RANKL is a specific survival factor for dendritic cells. Overall, RANKL is one of the key factors at the crossroad between bones and immunity, a topic called "osteoimmunology" [42].

RANK

RANK, also known as TRANCE receptor [43] and TNFRSF11A, is the signalling receptor for RANKL [25]. RANK belongs to the TNF superfamily receptors and is a type I transmembrane protein. This receptor has a large cytoplasmic domain at its C-terminal domain, a N-terminal extracellular domain with four cystein-rich repeat motifs and two N-glycosylation sites [21]. Its last domain is involved in the interaction with RANKL and the induction of the receptor's trimerization [44,45]. RANK mRNAs have been detected in many tissues such as the thymus, mammary glands, liver and prostate, but more significantly in bone [21,25]. By transducing the cell signalling initiated by RANKL, RANK plays a part in controlling bone remodelling and immunity [46,47].

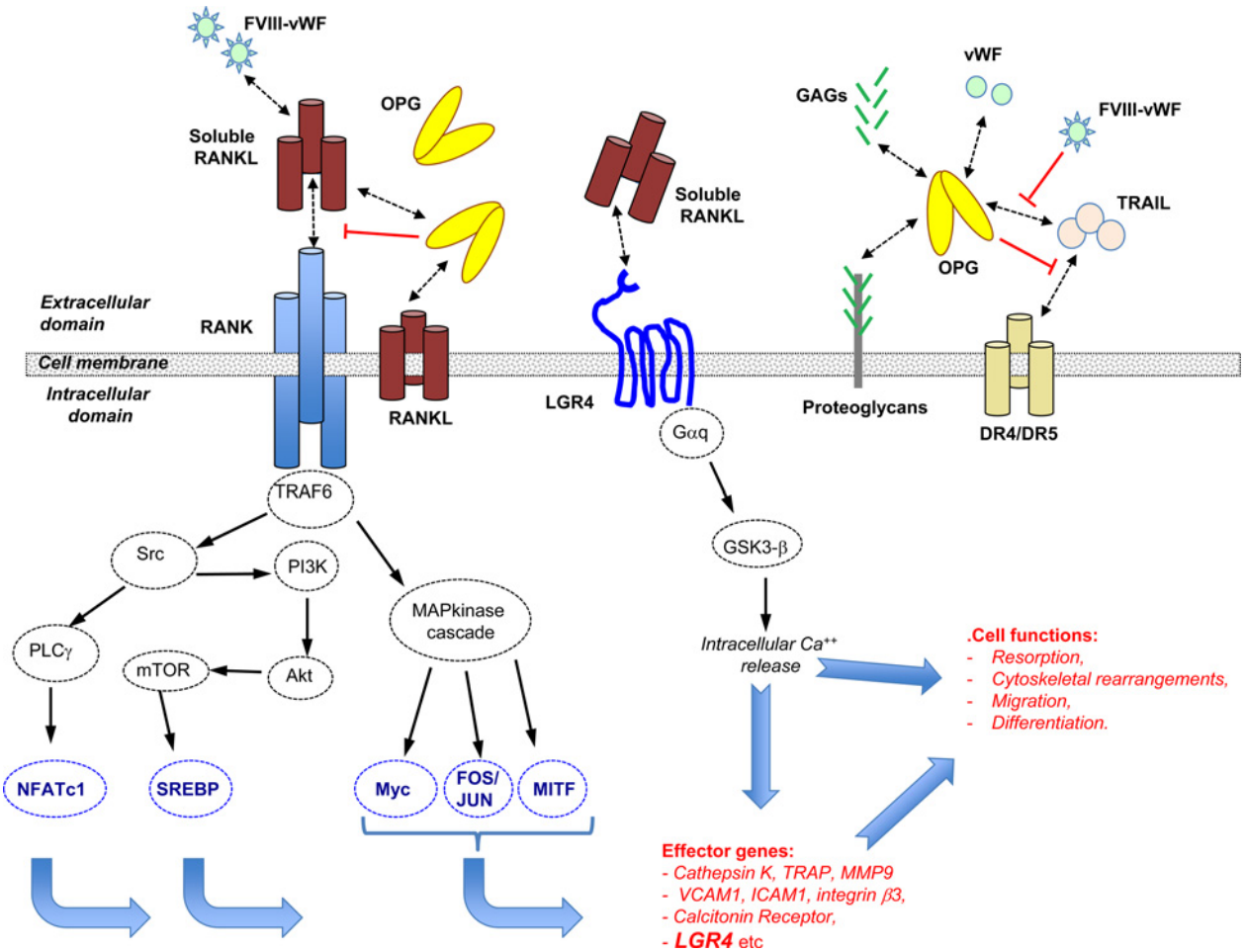

Figure 1 **RANK/RANKL signalling in cancer cells: a very complex molecular network**

RANKL is a trimeric complex produced in a membrane or soluble form. Secreted RANKL can be produced from a specific transcript or by proteolysis of its membrane form. Trimeric RANKL interacts with a trimeric receptor named RANK and triggers a signalling cascade controlling the transcription of numerous effector genes. Additional protagonists intervene to regulate the binding of RANKL to RANK. In this way, OPG acts as a decoy receptor interacting with RANKL, and complex VIII (FVIII-vWF) showed a similar capacity. However, OPG is itself controlled by many ligands, including TRAIL, vWF and glycoaminoglycans (GAGs), and the final inhibitory effect of OPG on RANKL is dependent on its binding to these ligands. Very recently, it has been demonstrated that LGR4 is a new receptor for RANKL which can counterbalance the RANKL activities transmitted by RANK signalling.

Its functional activities have been clearly established by studying the phenotype of RANK knockout mice which exhibit severe osteopetrosis, with a lack of mature osteoclasts, and an absence of lymph node development with impairment in B- and T-cell maturation [48,49]. RANK is then the second key protagonist of "osteoimmunology" [50].

RANK/RANKL AND CANCER

RANK expression identifies cancer cells as RANKL targets

The expression of RANK/RANKL is not restricted to healthy tissues and numerous studies have demonstrated their expression in neoplastic tissues. This wide distribution strengthens the hypothesis of their key role in the oncogenic process (Table 1). Thus, a high percentage of carcinoma cells express RANK mRNA/protein at various levels [51,52]. Indeed, 89% of all the carcinomas assessed exhibit RANK positive immunostaining, and approximately 60% of cases showed more than 50% of positive cancer cells. Interestingly, RANK expression in carcinoma cells is a poor prognostic marker as demonstrated in breast cancer [86,87]. Similarly to prostate cancers, Pfitzner et al. [87] demonstrated that higher RANK expression in the primary breast tumour was associated with higher sensitivity to chemotherapy, but also a higher risk of relapse and death despite this higher sensitivity. RANK expression was also described as being predictive of poor prognosis in bone metastatic patients but not in

Table 1 RANK and RANKL expression in cancers

Cancer subtypes or related organ	RANK expressing tumours (references)	RANKL expressing tumours (references)
Bladder carcinoma	[51]	–
Breast carcinoma	[51–56]	[57–60]
Cervical cancer	[51,61]	[61]
Chondrosarcoma	[62]	[62,63]
Colon and rectal cancers	[51]	–
Endometrial tumours	[51]	–
Oesophageal tumours	[51,64]	–
Giant cell tumours of bone	[65]	[63–66]
Hepatocarcinoma	[51,67]	[67,68]
Lung cancer	[51,69]	[69]
Lymphoma	[51,70]	[71,72]
Melanoma	[73,74]	–
Myeloma	[75]	[75,76]
Neuroblastoma	[51]	[77]
Oral squamous carcinoma	[78]	[78]
Osteosarcoma	[63,79]	[63,79,80]
Prostate carcinoma	[51,73,81,82]	[81,83]
Renal carcinoma	[84]	[84]
Thymic tumours	[51]	–
Thyroid adenocarcinoma	[51,85]	[85]

patients with visceral metastases [88]. Similarly, sarcoma cells also express RANK (18–69 % depending on the series) [79,89,90] and expression is correlated with clinical parameters. Trieb and Windhager [89] described a reverse correlation between RANK expression and the overall survival of patients with osteosarcoma, but not with the response to chemotherapy. These authors observed lower disease-free and overall survival rates in patients presenting RANK positive tumours. Bago-Horvath et al. revealed that RANKL expression was significantly more common in osteosarcoma of the lower extremity than in any other location and did not find any significant correlation between RANKL and disease-free or osteosarcoma-specific survival. However, they did report that RANK expression is a negative prognostic factor regarding disease-free survival, confirming the data obtained by Trieb and Windehager [89]. Interestingly, in 2012, Papanastasiou et al. [91] identified a new isoform of RANK (named RANK-c) generated by alternative splicing and expressed in breast cancer samples. Its expression was reversely correlated with histological grade and RANK-c was able to inhibit cell motility and the migration of breast cancer cells by interfering with RANK signalling.

In several studies [87,90], RANKL expression was not correlated with any clinical outcomes in either carcinoma or sarcoma. However, in one series of 40 patients, Lee et al. [92] showed that RANKL expression was related to poor response to preoperative chemotherapy and a high RANKL level was associated with inferior survival. Recently, Cathomas et al. [93] described an interesting clinical case of an osteosarcoma patient treated with sorafenib and denosumab. RANK and RANKL were expressed by the tumour cells and the authors observed complete metabolic remission for over 18 months strengthening the potential therapeutic value of blocking RANK/RANKL signalling in osteosarcoma [93]. Whereas RANK is expressed by various cancer cell types, its ligand can be produced either by tumour cells or by their environment (Table 1). Consequently, RANKL can then act in a paracrine or autocrine manner on cancer cells. The best example of such paracrine activity is given by the role of RANK/RANKL in the pathogenesis of giant cell tumours in bone. RANK is expressed by giant osteoclasts and the macrophagic component of the tumours, whereas RANKL is produced by stromal cells. Furthermore, exacerbated production of RANKL by stromal cells is directly associated with an increase in osteoclastogenesis and bone destruction [94]. This observation identifies the giant cell tumours in bone as very good candidates for the clinical use of Denosumab [95].

Direct RANK/RANKL signalling in cancer cells: the regulatory activities of OPG and LGR4

RANK, like the other receptors in the TNF receptor superfamily, is characterized by the absence of tyrosine kinase activity and consequently requires adapter proteins named TNF-receptor associated factor (TRAF) in order to transmit cell signalling. The intracellular domain of RANK has two TRAF binding sites able to interact with TRAF-2, -3, -5 and -6 [96,97], but only TRAF6 mutations led to an osteopetrotic phenotype similar to the phenotype of RANK knockout mice, thus underlining the predominant role of TRAF6 in RANK associated signalling among the TRAF family members [96–101]. Consecutively, TRAF6 leads to the activation of Src/PLCγ, PI3K/Akt/mTOR and MAPK (p38, JNK, ERK1/2) cascades which result in the translocation of transcriptional activators including NF-κB, Fos/Jun or MITF and subsequently to the transcription of numerous effector genes involved in bone resorption such as cathepsin K or TRAP, in cell adhesion and motility such as VCAM1 or ICAM1. This explains the various functional impacts that RANKL has on normal and cancer cells (Figure 1).

The first identified regulator of RANKL activities was a soluble protein named OPG [102,103]. OPG is considered to be a ubiquitous protein with predominant expression in bone (osteoblasts, mesenchymal stem cells), immune cells (dendritic cells, T- and B-cells) and vessels (endothelial and vascular smooth muscle cells) [21,104]. OPG acts as a decoy receptor for RANKL, and blocks the RANK–RANKL interaction and RANKL-induced signalling pathways with its N-terminal [11,89]. OPG and RANKL expression are both regulated by inflammatory cytokines released into the microenvironment of cancer cells, and RANKL activities will result from the level of expression and the kinetics of both factors in this microenvironment [21,105]. OPG binds to soluble and membrane RANKL and strongly controls RANKL bioavailability at the cell membrane by facilitating its internalization and reducing its half-life [106]. However, OPG possesses numerous other ligands which

markedly regulate its expression and have an impact on RANKL availability (Figure 1) [104]. In this way, OPG binds to glycosaminoglycans and proteoglycans such as syndecan-1 through its heparin-binding domain with a strong influence on cancer cell development [104,107]. The best illustration of the functional consequence of this interaction in cancer is given by myeloma cells which overexpress syndecan-1 [108]. OPG produced in the bone microenvironment is trapped, internalized and degraded by myeloma cells and the OPG/RANKL balance is then dysregulated in favour of RANKL. The OPG/RANKL imbalance leads to bone resorption, a phenomenon exacerbated by the RANKL production of the myeloma cells. By sequestering OPG, myeloma cells elaborate a microenvironment that facilitates their expansion. Similarly, OPG can be trapped by the proteoglycans and glycosaminoglycans located in the extracellular matrix as shown in osteosarcoma [109]. In addition, OPG binds TRAIL (TNF related apoptosis inducing ligand), a key natural pro-apoptotic and "anti-cancer" factor [110]. By this way, OPG can thus act as an anti-apoptotic and a pro-proliferative factor for cancer cells by blocking TRAIL activity, as shown with prostate carcinoma for instance [111]. Complex VIII (factor VIII-von Willebrand factor) is also able to bind to OPG and increases the complexity of this system by regulating TRAIL-induced cancer cell death [112]. Finally, RANKL expressed by the tumour cells or/and their environment by exerting its action through RANK in an autocrine, endocrine or paracrine manner contributes to establishing the fertile soil needed for tumour cells to be maintained and proliferate. In this picture, OPG and its ligands are notably involved in the bioavailability and biological activities of RANKL.

Very recently, a new RANKL receptor named leucine-rich repeat-containing G-protein-coupled receptor 4 (LRG4) characterized by seven transmembrane regions, has been identified [113]. In this work, Luo et al. [113] revealed that RANKL binds to the extracellular domain of LGR4 and by this way negatively regulates osteoclastogenesis through activation of Gαq/GS3K-β signalling and repression of the NFATc1 pathway (Figure 1). Moreover, *Lgr4* is a transcriptional target of the canonical RANKL–NFATc1, which shows that LGR4 signalling acts as the feedback loop controlling RANKL activities. Interestingly, a mutation in LGR4 encoding gene has been related to an osteoporosis phenotype which can be explained by the new function of LGR4 as a RANKL receptor [114]. Although the involvement of the LRG4–RANKL axis in cancer has not yet been clearly determined, LGR4 nevertheless promotes the proliferation of various tumour cells, including breast, prostate, gastric and hepatic cancer [115]. This proliferation effect was linked to activation of the Wnt/β catenin signalling pathways. LRG4 appears to be a new regulator for prostate development and promotes tumorigenesis [116,117] and the LRG4-Stat3 molecular pathway may control osteosarcoma development [118].

RANKL activities are modulated by the balance between RANKL and their various molecular regulators produced in the microenvironment of cancer cells. RANKL is involved in each stage of tumour development, from the initial oncogenesis process to the establishment of the distant metastases as described below (Figure 2).

The RANK/RANKL axis is involved in the initial phases of tumour development

Initially considered to be a pro-metastatic factor, our vision of RANKL changed when the factor was linked to mammary gland development [119]. RANKL deficiency leads to a defect in the formation of the lobo-alveolar structures required for lactation [120,121]. In addition, RANKL is able to promote the survival and proliferation of epithelial cells simultaneously with the up-regulated expression of RANK during mammary gland development [119–121]. Disturbance in this coordinated mechanism can lead to the formation of pre-neoplasias and subsequently to that of tumour foci, as revealed by Gonzalez-Suarez et al. [122]. These authors established a mouse mammary tumour virus – RANK transgenic mice overexpressing the protein in mammary glands – and reported a high incidence of pre-neoplasia foci (multifocal ductal hyperplasias, multifocal and focally extensive mammary intraepithelial neoplasias), as well as the development of adenocarcinoma lesions in these transgenic mice compared with the wild-type mice. Confirming the involvement of RANKL in the initial oncogenic process, administration of RANK-Fc decreased both mammary tumorigenesis and the development of lung metastases in MMTV-*neu* transgenic mice, a spontaneous mammary tumour model [122]. In a complementary work, this team demonstrated that the RANKL/RANK axis was pro-active in epithelial mesenchymal transition (EMT), promoted cell migration simultaneously with neo-vascularization, and that their expression was significantly associated with metastatic tumours [123]. Overall, their data revealed that RANK/RANKL signalling promotes the initial stage in breast cancer development by inducing stemness and EMT in mammary epithelial cells. A similar process has been confirmed in head and neck squamous carcinoma [124], and in endometrial cancer [125], and RANKL expression has been associated with the EMT and appears to be a new marker for EMT in prostate cancer cells [83].

RANK/RANKL system controls cell motility and consequently contributes to the metastastic process concomitantly with a pro-angiogenic function

Jones et al. [95] provided the first evidence of a chemoattractant activity for RANKL. These authors demonstrated that RANKL produced by osteoblasts and bone marrow stromal cells attracts RANK-expressing cancer cells and induces their migration. This mechanism seems to be relatively universal and was observed in prostate cancer [95,126,127], breast cancer [95], colon cancer [58], melanoma [95], oral squamous carcinomas [128], lung cancer [129], hepatocarcinoma [130], endometrial cancer [131], osteosarcoma [132,133] and renal cancer [134]. RANKL-induced migration is associated with specific signalling cascades, especially the activation of MAP Kinase pathways. The RANKL/RANK axis then regulates cancer cell migration

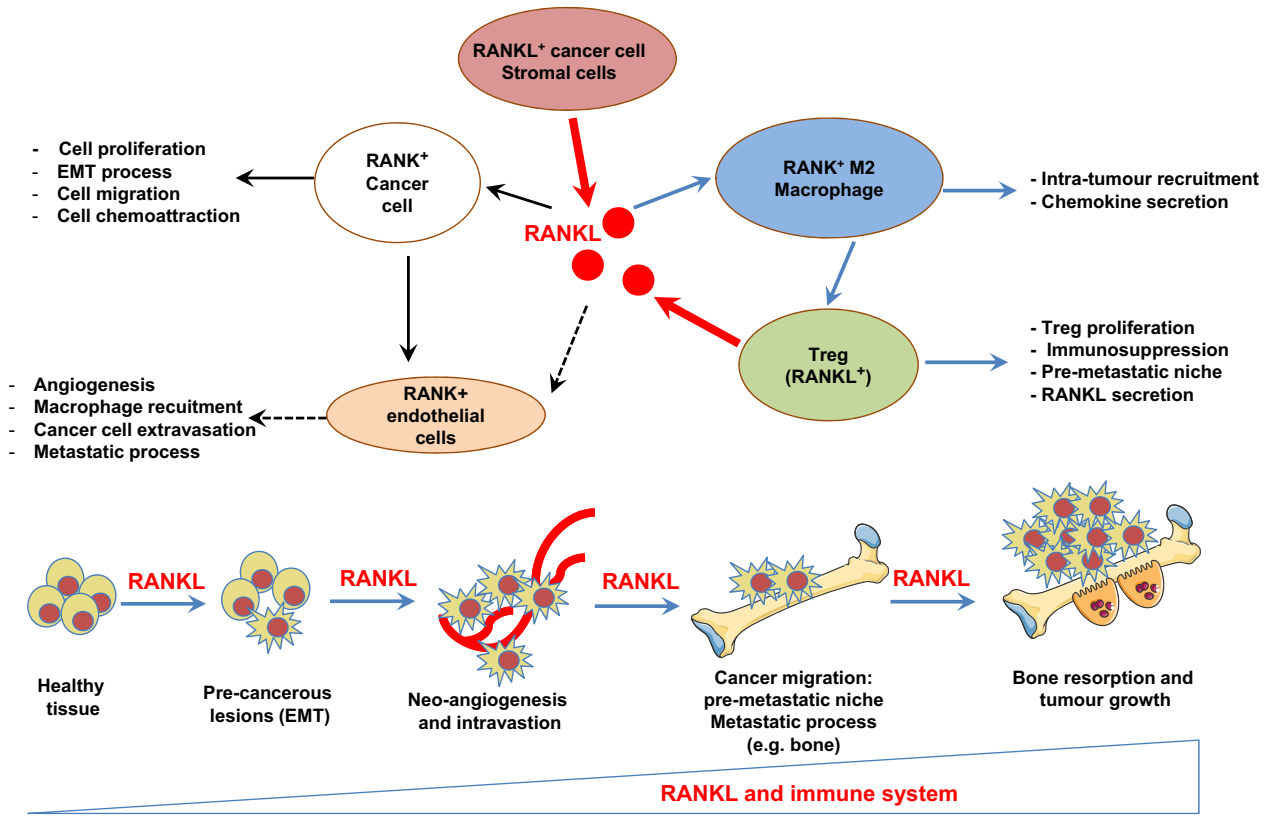

Figure 2 **RANK/RANKL is involved in each stage of cancer development: from pre-cancerous lesions to the establishment of metastases**

Cancer cells are direct targets for RANKL. RANKL initiates the formation of pre-cancerous lesions by facilitating the EMT process and stemness, as well as facilitating tumour growth and the metastatic process by modulating immune and vascular niches. Throughout these processes, RANKL acts as a chemoattractive factor for cancer cells and M2 macrophages. Activated macrophages facilitate both the proliferation of Treg lymphocytes, the main source of RANKL during primary tumour growth, and the initiation of the pre-metastatic niche in bone. RANKL up-regulates the angiogenic process by stimulating the proliferation and survival of endothelial cells and, in parallel, of the metastatic process by promoting the extravasation/intravasation of RANK-expressing cancer cells and their migration to distant organs. The RANKL concentration gradient drives the tumour cells to the metastatic sites.

and RANKL acts as a chemoattractive agent on cells that express one of their receptors.

In addition to its direct effects on cancer cells, RANKL is notably able to modulate the tumour microenvironment, in particular the formation of new blood vessels. Blood vessels are used by cancer cells to deliver large quantities of nutriments and are their main means of migrating so as to invade distant organs. RANK expression was detected in endothelial cells, and by interacting with this receptor, RANKL impacts the angiogenic process by both stimulating angiogenesis through an Src and phospholipase C-dependent mechanism [135,136], and increasing cell survival in a PI3k/Akt-dependent manner [137]. RANKL also induced the proliferation of endothelial cell precursors and the neoformation of vascular tubes [138]. This phenomenon is exacerbated by VEGF, which is frequently secreted by cancer cells and which up-regulates the RANKL response of endothelial cells by an up-regulation of RANK expression and an increase in vascular permeability [139]. These works strengthen the role of RANK/RANKL axis plays in the metastatic process by regulating cancer cell migration and the neoangiogenesis.

Immune cell regulation by RANK/RANKL: setting up fertile soil for cancer cells

RANKL influences the microenvironment of cancer cells by acting on local immunity. The major role of RANKL in the immune system was initially identified in RANKL-knockout mice in which the development of secondary lymphoid organs was impaired, especially the lymph nodes [140,141], but also at the "central" level, where the maturation of the thymic epithelial cells necessary for T-cell development was affected [142,143]. RANKL is also involved in modulating the immune response by inducing T-cell proliferation [25] and dendritic cell survival [26]. T-cells activated as a result of RANKL expression stimulate

Table 2 Main clinical trials based on RANKL targeting in cancers
Source: clinical trial.gov March 2016.

Clinical trial reference	Phase, cancer type	Title	Primary outcome	Patients enrolled	Treatment (references)
NCT01624766	I, Advanced malignancies	A Phase I Trial of anakinra (IL-1 receptor antagonist) or denosumab (anti-RANKL monoclonal antibody) in combination with everolimus (mTOR inhibitor) in patients with advanced malignancies	Maximum tolerated dose	147	Escalating dose of denosumab Starting doses: everolimus 10 mg by mouth daily for a 28 day cycle. Denosumab 120 mg (s.c.) on Day 1 of a 28 day cycle
NCT01419717	III, Advanced cancer	Open-label access protocol of denosumab for subjects with advanced cancer	Subject incidence of treatment-emergent adverse events	129	120 mg denosumab (s.c.) every 4 weeks
NCT01920568	III, Bone metastases from solid tumours	A study comparing denosumab with zoledronic acid in subjects of Asian ancestry with bone metastases from solid tumours	Percent change from baseline in the bone turnover marker (uNTx/uCr)	477	120 mg denosumab (s.c.) injection for a maximum of 13 doses, infusion over $\geq$15 min once every 4 weeks
NCT02470091	II, Osteosarcoma	Phase II study of denosumab, a RANK ligand antibody, for recurrent or refractory osteosarcoma	Disease control rate at months 4 and 12	90	Denosumab (s.c.) on day 1 (days 1, 8 and 15 of course 1 only). Treatment repeats every 4 weeks (28 days) for up to 24 months or 26 courses
NCT00396279	II, Giant cell tumours of bone	An open-label, multicenter, phase 2 safety and efficacy Study of denosumab (AMG 162) in subjects with recurrent or unresectable giant cell tumour of bone	Percentage of patients with tumour response	37	120 mg denosumab (s.c.) once every 4 weeks, with an additional 120 mg doses on Days 8 and 15 of the first month of treatment + daily supplements of at least 500 mg of calcium and 400 IU of vitamin D
NCT00680992	II, Giant cell tumours of bone	An open-label, multicenter, phase 2 study of denosumab in subjects with giant cell tumour of bone	Safety profile of denosumab	530	120 mg denosumab (s.c.) every 4 weeks with a loading dose of 120 mg (s.c.) on study days 8 and 15
NCT01951586	II, Non-small cell lung cancer	A randomized, double-blind, multicenter phase 2 trial of denosumab in combination with chemotherapy as first-line treatment of metastatic non-small cell lung cancer	Relative benefit on overall survival	226	120 mg denosumab (s.c.) every 4 weeks with a loading dose of 120 mg (s.c.) on study days 8 and 15
NCT02129699	III, Non-small cell lung cancer	A randomized, open-label phase III trial evaluating the addition of denosumab to standard first-line anti-cancer treatment in advanced NSCLC	Overall survival	1000	120 mg denosumab (s.c.) every 3–4 weeks + 4/6 cycles of standard (combination of platinum-based agents plus gemcitabine or pemetrexed)

Table 2 Continued

Clinical trial reference	Phase, cancer type	Title	Primary outcome	Patients enrolled	Treatment (references)
NCT00259740	II, Multiple myeloma	An open-label, multicenter phase 2 trial of denosumab in the treatment of relapsed or plateau-phase multiple myeloma	Complete or partial response based on M-protein assessments	96	120 mg denosumab (s.c.) on study days 1, 8, 15 and 29 and every 4 weeks thereafter
NCT01345019	III, Multiple myeloma	A randomized, double-blind, multicenter study of denosumab compared with zoledronic acid in the treatment of bone disease in subjects with newly diagnosed multiple myeloma	Time to the first on-study skeletal related event	1700	120 mg denosumab (s.c.) every 4 weeks
NCT00330759	III, Multiple myeloma	A randomized, double-blind, multicenter study of denosumab compared with zoledronic acid (Zometa) in the treatment of bone metastases in subjects with advanced cancer (excluding breast and prostate cancer) or multiple myeloma	Time to the first on-study skeletal-related event	1779	120 mg denosumab (s.c.) every 4 weeks
NCT02099461	I, Healthy patients, breast	A randomized, stratified, open-label, no-treatment-controlled, parallel group, multicenter phase 1 trial to evaluate the effect of denosumab on cellular proliferation in the human breast	Ratio of post-baseline to baseline Ki-67 index in mammary epithelial cells	82	Healthy volunteers, 60 mg denosumab (s.c.) on day 1 Percutaneous core needle breast biopsies on day 1 (prior to study treatment) and day 28
NCT01545648	II, Early breast cancer	Pilot study to evaluate the impact of denosumab on disseminated tumour cells in patients with early stage breast cancer	Reduction of bone marrow disseminated tumour cells	45	120 mg denosumab (s.c.) every 4 weeks for total of 6 months, then every 12 weeks for two doses, for a total treatment course of one year
NCT01952054	II, Breast cancer	Phase II study of denosumab to define the role of bone related biomarkers in breast cancer bone metastasis	Reduction of circulating tumour cells	35	120 mg denosumab (s.c.) every 4 weeks (+ hormotherapy)
NCT01864798	II, Early breast cancer	A pre-operative window study evaluating denosumab, a RANK ligand (RANKL) inhibitor and its biological effects in young premenopausal women diagnosed with early breast cancer	Geometric mean change in tumour Ki-67 expression	39	Denosumab, from 30 to 120 mg (s.c.) once a every 4 or 12 weeks for 25 weeks

Table 2 Continued

Clinical trial reference	Phase, cancer type	Title	Primary outcome	Patients enrolled	Treatment (references)
NCT00091832	II, Breast cancer with bone metastases	A randomized active-controlled study of AMG 162 in breast cancer subjects with bone metastasis who have not previously been treated with bisphosphonate therapy	Percent change from baseline to week 13 in creatinine-adjusted urinary N-telopeptide	255	Denosumab, from 30 to 180 mg (s.c.) once a every 4 or 12 weeks for 25 weeks
NCT01077154	III, Early breast cancer	A randomized, double-blind, placebo-controlled, multicenter phase 3 study of denosumab as adjuvant treatment for women with early-stage breast cancer at high risk of recurrence (D-CARE)	Bone metastasis-free survival	4509	120 mg denosumab (s.c.) every 4 weeks for 6 months. 120mg (s.c.) every 3 months for the next 4 and a half years Supplementation: oral calcium (at least 500 mg) and vitamin D (at least 400 IU) for 5 years
NCT00321464	III, Breast cancer with bone metastases	A randomized, double-blind, multicenter study of denosumab compared with zoledronic acid (Zometa®) in the treatment of bone metastases in subjects with advanced breast cancer	Time to first on-study skeletal related event	2049	120 mg denosumab (s.c.) every 4 weeks
NCT00089661	III, Non-metastatic breast cancer	A randomized, double-blind, placebo-controlled study to evaluate AMG 162 in the treatment of bone loss in subjects undergoing aromatase inhibitor therapy for non-metastatic breast cancer	Lumbar spine bone mineral density percent change from baseline at month 12	252	Denosumab 60 mg (s.c.) every 6 months, beginning on Study day 1, for a total treatment period of 24 months
NCT02613416	II, Breast biomarkers	Phase II correlative study of denosumab effects on tissue and imaging breast biomarkers (pre- and post-menopausal women diagnosed with stage 0-III breast cancer)	Safety issue	44	120 mg denosumab (s.c.) per month

Table 2 Continued

Clinical trial reference	Phase, cancer type	Title	Primary outcome	Patients enrolled	Treatment (references)
NCT00321620	III, Prostate cancer with bone metastases	A randomized, double-blind, multicenter study of denosumab compared with zoledronic acid (Zometa®) in the treatment of bone metastases in men with hormone-refractory prostate cancer	Time to the first on-study skeletal-related event	1904	120 mg denosumab (s.c.) every 4 weeks Zoledronic acid 4 mg (i.v.)
NCT00286091	III, Non-metastatic prostate cancer	A randomized, double-blind, placebo-controlled, multicenter phase 3 study of denosumab on prolonging bone metastasis-free survival in men with hormone refractory prostate cancer	Bone metastasis-free survival	1435	Denosumab 120 mg (s.c.) every 4 weeks
NCT00089674	III, Non-metastatic prostate cancer	A randomized, double-blind, placebo-controlled study to evaluate AMG 162 in the treatment of bone loss in subjects undergoing androgen-deprivation therapy for non-metastatic prostate cancer	Lumbar spine bone mineral density percent change from baseline at month 24	1468	Denosumab 60 mg (s.c.) at day 1, months 6, 12, 18, 24, 30
NCT01824342	III, Prostate cancer	An open label, single arm, extension study to evaluate the long term safety of denosumab for prolonging bone metastasis-free survival in men with hormone-refractory prostate cancer	Number of patients with treatment-emergent adverse events and deaths	18	Denosumab 120 mg (s.c.) every 4 weeks for up to 3 years

dendritic cells, expressing RANK, to enhance their survival and thereby increase the T-cell memory response [25]. More recently, Khan et al. [144] demonstrated that RANKL blockade can rescue melanoma-specific T-cells from thymic deletion, and increases the anti-tumour immune response as shown in melanoma.

Tumour-associated macrophages (TAMs) accumulate in the tumour microenvironment and, depending on their M2 or M1 phenotype, play a part in tumour growth, angiogenesis and metastasis [145]. RANK is present at the cell membrane of monocytes/macrophages and RANKL acts as a chemoattractant factor for these cells [146]. The M2-macrophages which mainly express RANK is strongly associated with the angiogenic process [147]. RANK/RANKL signalling in the M2-macrophages modulates the production of chemokines, promoting the proliferation of Treg lymphocytes in favour of an immunosuppressive environment [148]. In breast carcinoma, RANKL is mainly produced by Treg lymphocytes ($CD4^+CD25^+$ T-lymphocytes expressing Foxp3). In this context, a vicious cycle is established between TAMs, Treg and tumour cells resulting in tumour growth, the spread of cancer cells and amplification of the metastatic process [149]. In fact, T-lymphocytes appear to be the principal source of RANKL in tumorigenesis. Whether RANKL-producing T-lymphocytes are involved in the initial step of metastatic process or not, T-lymphocytes induce a permissive environment initiating the pre-metastastic niche [150].

RANK/RANKL and bone niche: ongoing clinical trials

When proliferative tumour cells are located in the bone environment (primary bone tumours or bone metastases), they dysregulate the balance between bone apposition and bone resorption in order to create a favourable microenvironment for their growth [151]. In this way, this bone microenvironment becomes a source of therapeutic targets, RANKL being one of them [152]. OPG-Fc was the first generation of drug targeting RANKL to be assessed

in potsmenopausal women [152]. Nevertheless, due to its ability to bind to multiple ligands, and particularly to TRAIL, OPG-Fc based clinical trials have been suspended until the development of a monoclonal antibody targeting RANKL [153]. Denosumab, a fully-humanized antibody targeting RANKL and blocking its binding to RANK, has been developed to bypass this risk [51]. In osteoporotic patients, Denosumab was well-tolerated and a single s.c. dose resulted in a prolonged decrease in bone turnover [154]. The value of blocking RANKL activities has been also demonstrated by the inhibition bone resorption in numerous pre-clinical models of primary bone tumours (Ewing sarcoma [155], osteosarcoma [156,157]), bone metastases (breast [158], prostate [159], non-small cell lung cancer [160]) and in myeloma [161]) and in numerous phase II and III clinical trials (Table 2). In breast and prostate carcinoma patients, bone turnover markers were reduced in a way similar to that in the osteoporosis context and, in addition, delayed the onset of the first skeletal-related event and the risk of multiple SRE [162]. A comparison with bisphosphonate therapy demonstrated the superiority of Denosumab concerning the two previous parameters even if the overall survival rate was similar with both drugs. Additional clinical trials in metastatic diseases are currently in progress and their results will be very informative with regard to the clinical extension of Denosumab in oncology.

CONCLUSIONS

Since their initial discovery in 1997, RANK/RANKL became key actors in first bone remodelling and then more recently in oncology. This molecular axis is clearly involved in all stages of tumorigenesis, including tumour hyperplasia, pre-neoplasia foci formation, cancer cell migration, neo-angiogenesis, immune cell chemoattraction and the establishment of an immunosuppressive environment and initiation of a pre-metastatic niche. In one decade, RANK/RANKL has not only transformed our vision of bone biology but has also strengthened the notion of "seed and soil", conventionally used to explain the metastatic process. Targeting RANK/RANKL signalling has already shown its therapeutic efficacy in osteoporotic patients and its clinical advantages in the management of bone metastases from breast and prostate carcinomas. Current ongoing clinical trials will be crucial for better defining its potential side effects after long term use.

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REFERENCES

1 Bissell, M.J. and Hines, W.C. (2011) Why don't we get more cancer? A proposed role of the microenvironment in restraining cancer progression. Nat. Med. **17**, 320–329 CrossRef PubMed
2 Molon, B., Cali, B. and Viola, A. (2016) T cells and cancer: how metabolism shapes immunity. Front. Immunol. **7**, 20 CrossRef PubMed
3 Paget, S. (1889) The distribution of secondary growths in cancer of the breast. Lancet **133**, 571–573 CrossRef
4 Plaks, V., Kong, N. and Werb, Z. (2015) The cancer stem cell niche: how essential is the niche in regulating stemness of tumor cells? Cell Stem Cell **16**, 225–238 CrossRef PubMed
5 Ordóñez-Morán, P. and Huelsken, J. (2014) Complex metastatic niches: already a target for therapy? Curr. Opin. Cell Biol. **31**, 29–38 CrossRef PubMed
6 Molofsky, A.V., Pardal, R. and Morrison, S.J. (2004) Diverse mechanisms regulate stem cell self-renewal. Curr. Opin. Cell Biol. **16**, 700–707 CrossRef PubMed
7 Wan, L., Pantel, K. and Kang, Y. (2013) Tumor metastasis: moving new biological insights into the clinic. Nat. Med. **19**, 1450–1464 CrossRef PubMed
8 Shiozawa, Y., Berry, J.E., Eber, M.R., Jung, Y., Yumoto, K., Cackowski, F.C., Yoon, H.J., Parsana, P., Mehra, R., Wang, J. et al. (2016), The marrow niche controls the cancer stem cell phenotype of disseminated prostate cancer. Oncotarget, doi: 10.18632/oncotarget.9251
9 Weidle, U.H., Birzele, F., Kollmorgen, G. and Rüger, R. (2016) Molecular mechanisms of bone metastasis. Cancer Genomics Proteomics **13**, 1–12 PubMed
10 Lu, X., Mu, E., Wei, Y., Riethdorf, S., Yang, Q., Yuan, M., Yan, J., Hua, Y., Tiede, B.J., Lu, X. et al. (2011) VCAM-1 promotes osteolytic expansion of indolent bone micrometastasis of breast cancer by engaging $\alpha4\beta1$-positive osteoclast progenitors. Cancer Cell **20**, 701–714 CrossRef PubMed
11 Wang, N., Docherty, F.E., Brown, H.K., Reeves, K.J., Fowles, A.C., Ottewell, P.D., Dear, T.N., Holen, I., Croucher, P.I. and Eaton, C.L. (2014) Prostate cancer cells preferentially home to osteoblast-rich areas in the early stages of bone metastasis: evidence from *in vivo* models. J. Bone Miner. Res. **29**, 2688–2696 CrossRef PubMed
12 Spill, F., Reynolds, D.S., Kamm, R.D. and Zaman, M.H. (2016) Impact of the physical microenvironment on tumor progression and metastasis. Curr. Opin. Biotechnol. **40**, 41–48 CrossRef PubMed
13 Meads, M.B., Hazlehurst, L.A. and Dalton, W.S. (2008) The bone marrow microenvironment as a tumor sanctuary and contributor to drug resistance. Clin. Cancer Res. **14**, 2519–2526 CrossRef PubMed
14 David, E., Blanchard, F., Heymann, M.F., De Pinieux, G., Gouin, F., Rédini, F. and Heymann, D. (2011) The bone niche of chondrosarcoma: a sanctuary for drug resistance, tumour growth and also a source of new therapeutic targets sarcoma. Sarcoma **2011**, 932451 CrossRef PubMed
15 Jones, V.S., Huang, R.Y., Chen, L.P., Chen, Z.S., Fu, L. and Huang, R.P. (2016) Cytokines in cancer drug resistance: cues to new therapeutic strategies. Biochim. Biophys. Acta **1865**, 255–265 PubMed
16 Landskron, G., De la Fuente, M., Thuwajit, P. and Hermoso, M.A. (2014) Chronic inflammation and cytokines in the tumor microenvironment. J. Immunol. Res. **2014**, 149185 CrossRef PubMed
17 Dinarello, C.A. (2006) The paradox of pro-inflammatory cytokines in cancer. Cancer Metastasis Rev. **25**, 307–313 CrossRef PubMed
18 Dranoff, G. (2004) Cytokines in cancer pathogenesis and cancer therapy. Nat. Rev. Cancer **4**, 11–22 CrossRef PubMed

19 Grivennikov, S.I. and Karin, M. (2011) Inflammatory cytokines in cancer: tumour necrosis factor and interleukin 6 take the stage. Ann. Rheum. Dis. **70**, 104–108 CrossRef PubMed

20 Walsh, M.C. and Choi, Y. (2014) Biology of the RANKL–RANK–OPG system in immunity, bone, and beyond. Front. Immunol. **5**, 511 CrossRef PubMed

21 Theoleyre, S., Wittrant, Y., KwanTat, S., Fortun, Y., Redini, F. and Heymann, D. (2004) The molecular triad OPG/RANK/RANKL: involvement in the orchestration of pathophysiological bone remodeling. Cytokine Growth Factor Rev. **15**, 457–475 CrossRef PubMed

22 Wittrant, Y., Théoleyre, S., Chipoy, C., Padrines, M., Blanchard, F., Heymann, D. and Rédini, F. (2004) RANKL/RANK/OPG: new therapeutic targets in bone tumours and associated osteolysis. Biochim. Biophys. Acta **1704**, 49–57 PubMed

23 Yasuda, H. (2013) RANKL, a necessary chance for clinical application to osteoporosis and cancer-related bone diseases. World J. Orthop. **4**, 207–217 CrossRef PubMed

24 Li, J., Yin, Q. and Wu, H. (2013) Structural basis of signal transduction in the TNF receptor superfamily. Adv. Immunol. **119**, 135–153 CrossRef PubMed

25 Anderson, D.M., Maraskovsky, E., Billingsley, W.L., Dougall, W.C., Tometsko, M.E., Roux, E.R., Teepe, M.C., DuBose, R.F., Cosman, D. and Galibert, L. (1997) A homologue of the TNF receptor and its ligand enhance T cell growth and dendritic cell function. Nature **390**, 175–179 CrossRef PubMed

26 Wong, B.R., Josien, R., Lee, S.Y., Sauter, B., Li, H.L., Steinman, R.M. and Choi, Y. (1997) TRANCE (tumor necrosis factor [TNF]-related activation-induced cytokine), a New TNF family member predominantly expressed in T cells, is a dendritic cell specific survival factor. J. Exp. Med. **186**, 2075–2080 CrossRef PubMed

27 Lacey, D.L, Timms, E., Tan, H.L., Kelley, M.J., Dunstan, C.R., Burgess, T., Elliot, R., Colombero, A., Elliott, G., Scully, S. et al. (1998) Osteoprotegerin ligand is a cytokine that regulates osteoclast differentiation and activation. Cell **93**, 165–176 CrossRef PubMed

28 Kong, Y.Y., Boyle, W.J. and Penninger, J.M. (1999) Osteoprotegerin ligand: a common link between osteoclastogenesis, lymph node formation and lymphocyte development. Immunol. Cell Biol. **77**, 188–193 CrossRef PubMed

29 Kodaira, K., Kodaira, K., Mizuno, A., Yasuda, H., Shima, N., Murakami, A., Ueda, M. and Higashio, K. (1999) Cloning and characterization of the gene encoding mouse osteoclast differentiation factor. Gene **230**, 121–127 PubMed

30 Yasuda, H., Shima, N., Nakagawa, N., Mochizuki, S.I., Yano, K., Fujise, N., Sato, Y., Goto, M., Yamaguchi, K., Kuriyama, M. et al. (1998) Osteoclast differentiation factor is a ligand for osteoprotegerin/osteoclastogenesis-inhibitory factor and is identical to TRANCE/RANKL. Proc. Natl. Acad. Sci. U.S.A. **95**, 3597–3602 CrossRef PubMed

31 Ikeda, T., Kasai, M., Utsuyama, M. and Hirokawa, K. (2001) Determination of three isoforms of the Receptor activator of nuclear factor-kappa B ligand and their differential expression in bone and thymus. Endocrinology **142**, 1419–1426 PubMed

32 Lum, L., Wong, B.R., Josien, R., Becherer, J.D., Erdjument-Bromage, H., Schlondorff, J., Temps, P, Choi, Y. and Blodel, C.P (1999) Evidence for a role of a tumor necrosis factor alpha (TNF-alpha)-converting enzyme-like protease in shedding of TRANCE, a TNF family member involved in osteoclastogenesis and dendritic cell survival. J. Biol. Chem. **274**, 13613–13618 CrossRef PubMed

33 Hikita, A., Yana, I., Wakeyama, H., Nakamura, M., Kadono, Y., Oshima, Y., Nakamura, K., Seiki, M. and Tanaka, S. (2006) Negative regulation of osteoclastogenesis by ectodomain shedding of receptor activator of NF-kappaB ligand. J. Biol. Chem. **281**, 36846–36855 CrossRef PubMed

34 Georges, S., Ruiz Velasco, C., Trichet, V., Fortun, Y., Heymann, D. and Padrines, M. (2009) Proteases and bone remodelling. Cytokine Growth Factor Rev. **20**, 29–41 CrossRef PubMed

35 Kartsogiannis, V., Zhou, H., Horwood, N.J., Thomas, R.J., Hards, D.K., Quinn, J.M., Niforas, P., Ng, K.W., Martin, T.J. and Gillespie, M.T. (1999) Localization of RANKL (receptor activator of NF kappa B ligand) mRNA and protein in skeletal and extraskeletal tissues. Bone **25**, 525–534 CrossRef PubMed

36 Collin-Osdoby, P., Rothe, L., Anderson, F., Nelson, M., Maloney, W. and Osdoby, P. (2001) Receptor activator of NF-kappa B and osteoprotegerin expression by human microvascular endothelial cells, regulation by inflammatory cytokines, and role in human osteoclastogenesis. J. Biol. Chem. **276**, 20659–20672 CrossRef PubMed

37 Matsuzaki, K., Udagawa, N., Takahashi, N., Yamaguchi, K., Yasuda, H., Shima, N., Morinaga, T., Toyama, Y., Yabe, Y., Higashio, K. and Suda, T. (1998) Osteoclast differentiation factor (ODF) induces osteoclast-like cell formation in human peripheral blood mononuclear cell cultures. Biochem. Biophys. Res. Commun. **246**, 199–204 CrossRef PubMed

38 Quinn, J.M., Elliott, J., Gillespie, M.T. and Martin, T.J. (1998) A combination of osteoclast differentiation factor and macrophage-colony stimulating factor is sufficient for both human and mouse osteoclast formation *in vitro*. Endocrinology **139**, 4424–4427 CrossRef PubMed

39 Xiong, J., Piemontese, M., Onal, M., Campbell, J., Goellner, J.J., Dusevich, V., Bonewald, L., Manolagas, S.C. and O'Brien, C.A. (2015) Osteocytes not osteoblasts or lining cells are the main source of the RANKL required for osteoclast formation in remodeling bone. PLoS One **10**, e0138189 CrossRef PubMed

40 Hsu, H., Lacey, D.L., Dunstan, C.R., Solovyev, I., Colombero, A., Timms, E., Tan, H.L., Elliott, G., Kelley, M.J., Sarosi, I. et al. (1999) Tumor necrosis factor receptor family member RANK mediates osteoclast differentiation and activation induced by osteoprotegerin ligand. Proc. Natl. Acad. Sci. U.S.A. **96**, 3540–3545 CrossRef PubMed

41 Kong, Y.Y., Feige, U., Sarosi, I., Bolon, B., Tafuri, A., Morony, S., Capprelli, C., Li, J., Elliott, R., McCabe, S. et al. (1999) Activated T cells regulate bone loss and joint destruction in adjuvant arthritis through osteoprotegerin ligand. Nature **402**, 304–309 CrossRef PubMed

42 Takayanagi, H. (2007) Osteoimmunology: shared mechanisms and crosstalk between the immune and bone systems. Nat. Rev. Immunol. **7**, 292–304 CrossRef PubMed

43 Wong, B.R., Josien, R., Lee, S.Y., Vologodskaia, M., Steinman, R.M. and Choi, Y. (1998) The TRAF family of signal transducers mediates NF-kappaB Activation by the TRANCE receptor. J. Biol. Chem. **273**, 28355–28359 CrossRef PubMed

44 Kanazawa, K. and Kudo, A. (2005) Self-assembled RANK induces osteoclastogenesis ligand-independently. J. Bone Miner. Res. **20**, 2053–2060 CrossRef PubMed

45 Télétchéa, S., Stresing, V., Hervouet, S., Baud'huin, M., Heymann, M.F., Bertho, G., Charrier, C., Ando, K. and Heymann, D. (2014) Novel RANK antagonists for the treatment of bone resorptive disease: theoretical predictions and experimental validation. J. Bone Miner. Res. **29**, 1466–1477 CrossRef PubMed

46 Arai, F., Miyamoto, T., Ohneda, O., Inada, T., Sudo, T., Brasel, K., Miyata, T., Anderson, D.M. and Suda, T. (1999) Commitment and differentiation of osteoclast precursor cells by the sequential expression of c-Fms and receptor activator of nuclear factor kappaB (RANK) receptors. J. Exp. Med. **190**, 1741–1754 CrossRef PubMed

47 Nakagawa, N., Kinosaki, M., Yamaguchi, K., Shima, N., Yasuda, H., Yano, K., Morinaga, T. and Higashio, K. (1998) RANK is essential signalling receptor for osteoclast differentiation factor in osteoclastogenesis. Biochem. Biophys. Res. Commun. **253**, 396–400 CrossRef

48 Li, J., Sarosi, I., Yan, X.Q., Morony, S., Capparelli, C., Tan, H.L., McCabe, S., Elliott, R., Scully, S., Van, G. et al. (2000) RANK is the intrinsic hematopoietic cell surface receptor that controls osteoclastogenesis and regulation of bone mass and calcium metabolism. Proc. Natl. Acad. Sci. U.S.A. **97**, 1566–1571 CrossRef PubMed

49 Dougall, W.C., Glaccum, M., Charrier, K., Rohrbach, K., Brasel, K., De Smedt, T., Daro, E., Smith, J., Tometsko, M.E., Maliszewski, C.R. et al. (1999) RANK is essential for osteoclast and lymph node development. Genes Dev. **13**, 2412–2424 CrossRef PubMed

50 Baud'huin, M., Lamoureux, F., Duplomb, L., Rédini, F. and Heymann, D. (2007) RANKL, RANK, osteoprotegerin: key partners of osteoimmunology and vascular diseases. Cell. Mol. Life Sci. **64**, 2334–2350 CrossRef PubMed

51 Santini, D., Perrone, G., Roato, I., Godio, L., Pantano, F., Grasso, D., Russo, A., Vincenzi, B., Fratto, M.E., Sabbatini, R. et al. (2011) Expression pattern of receptor activator of NFkB (RANK) in a series of primary solid tumors and related metastases. J. Cell Physiol. **226**, 780–784 CrossRef PubMed

52 Santini, D., Schiavon, G., Vincenzi, B., Gaeta, L., Pantano, F., Russo, A., Ortega, C., Porta, C., Galluzzo, S., Armento, G. et al. (2011) Receptor activator of NF-kB (RANK) expression in primary tumors associates with bone metastasis occurrence in breast cancer patients. PLoS One **6**, e19234 CrossRef PubMed

53 Bhatia, P., Sanders, M.M. and Hansen, M.F. (2005) Expression of receptor activator of nuclear factor-kappaB is inversely correlated with metastatic phenotype in breast carcinoma. Clin. Cancer Res. **11**, 162–165 PubMed

54 Park, H.S., Lee, A., Chae, B.J., Bae, J.S., Song, B.J. and Jung, S.S. (2014) Expression of receptor activator of nuclear factor kappa-B as a poor prognostic marker in breast cancer. J. Surg. Oncol. **110**, 807–812 CrossRef PubMed

55 Pfitzner, B.M., Branstetter, D., Loibl, S., Denkert, C., Lederer, B., Schmitt, W.D., Dombrowski, F., Werner, M., Rüdiger, T., Dougall, W.C. and von Minckwitz, G. (2014) RANK expression as a prognostic and predictive marker in breast cancer. Breast Cancer Res. Treat. **145**, 307–315 CrossRef PubMed

56 Jones, D.H., Nakashima, T., Sanchez, O.H., Kozieradzki, I., Komarova, S.V., Sarosi, I., Morony, S., Rubin, E., Sarao, R., Hojilla, C.V. et al. (2006) Regulation of cancer cell migration and bone metastasis by RANKL. Nature **440**, 692–696 CrossRef PubMed

57 Owen, S., Ye, L., Sanders, A.J., Mason, M.D. and Jiang, W.G. (2013) Expression profile of receptor activator of nuclear-κB (RANK), RANK ligand (RANKL) and osteoprotegerin (OPG) in breast cancer. Anticancer Res. **33**, 199–206 PubMed

58 Van Poznak, C., Cross, S.S., Saggese, M., Hudis, C., Panageas, K.S., Norton, L., Coleman, R.E. and Holen, I. (2006) Expression of osteoprotegerin (OPG), TNF related apoptosis inducing ligand (TRAIL), and receptor activator of nuclear factor kappaB ligand (RANKL) in human breast tumours. J. Clin. Pathol. **59**, 56–63 CrossRef PubMed

59 Azim, Jr, H.A., Peccatori, F.A., Brohée, S., Branstetter, D., Loi, S., Viale, G., Piccart, M., Dougall, W.C., Pruneri, G. and Sotiriou, C. (2015) RANK-ligand (RANKL) expression in young breast cancer patients and during pregnancy. Breast Cancer Res. **17**, 24 CrossRef PubMed

60 Hu, H., Wang, J., Gupta, A., Shidfar, A., Branstetter, D., Lee, O., Ivancic, D., Sullivan, M., Chatterton, Jr, R.T., Dougall, W.C. and Khan, S.A. (2014) RANKL expression in normal and malignant breast tissue responds to progesterone and is up-regulated during the luteal phase. Breast Cancer Res. Treat. **146**, 515–523 CrossRef PubMed

61 Shang, W.Q., Li, H., Liu, L.B., Chang, K.K., Yu, J.J., Xie, F., Li, M.Q. and Yu, J.J. (2015) RANKL/RANK interaction promotes the growth of cervical cancer cells by strengthening the dialogue between cervical cancer cells and regulation of IL-8 secretion. Oncol. Rep. **34**, 3007–3016 PubMed

62 Hsu, C.J., Lin, T.Y., Kuo, C.C., Tsai, C.H., Lin, M.Z., Hsu, H.C., Fong, Y.C. and Tang, C.H. (2010) Involvement of integrin up-regulation in RANKL/RANK pathway of chondrosarcomas migration. J. Cell Biochem. **111**, 138–147 CrossRef PubMed

63 Grimaud, E., Soubigou, L., Couillaud, S., Coipeau, P., Moreau, A., Passuti, N., Gouin, F., Redini, F. and Heymann, D. (2003) Receptor activator of nuclear factor kappaB ligand (RANKL)/osteoprotegerin (OPG) ratio is increased in severe osteolysis. Am. J. Pathol. **163**, 2021–2031 CrossRef PubMed

64 Yin, J., Wang, L., Tang, W., Wang, X., Lv, L., Shao, A., Shi, Y., Ding, G., Chen, S. and Gu, H. (2014) RANK rs1805034 T>C polymorphism is associated with susceptibility of esophageal cancer in a Chinese population. PLoS One **9**, e101705 CrossRef PubMed

65 Atkins, G.J., Kostakis, P., Vincent, C., Farrugia, A.N., Houchins, J.P., Findlay, D.M., Evdokiou, A. and Zannettino, A.C. (2006) RANK expression as a cell surface marker of human osteoclast precursors in peripheral blood, bone marrow, and giant cell tumors of bone. J. Bone Miner. Res. **21**, 1339–1349 CrossRef PubMed

66 Branstetter, D.G., Nelson, S.D., Manivel, J.C., Blay, J.Y., Chawla, S., Thomas, D.M., Jun, S. and Jacobs, I. (2012) Denosumab induces tumor reduction and bone formation in patients with giant-cell tumor of bone. Clin. Cancer Res. **18**, 4415–4424 CrossRef PubMed

67 Song, F.N., Duan, M., Liu, L.Z., Wang, Z.C., Shi, J.Y., Yang, L.X., Zhou, J., Fan, J., Gao, Q. and Wang, X.Y. (2014) RANKL promotes migration and invasion of hepatocellular carcinoma cells via NF-κB-mediated epithelial–mesenchymal transition. PLoS One **9**, e108507 CrossRef PubMed

68 Sasaki, A., Ishikawa, K., Haraguchi, N., Inoue, H., Ishio, T., Shibata, K., Ohta, M., Kitano, S. and Mori, M. (2007) Receptor activator of nuclear factor-kappaB ligand (RANKL) expression in hepatocellular carcinoma with bone metastasis. Ann. Surg. Oncol. **14**, 1191–1199 CrossRef PubMed

69 Peng, X., Guo, W., Ren, T., Lou, Z., Lu, X., Zhang, S., Lu, Q. and Sun, Y. (2013) Differential expression of the RANKL/RANK/OPG system is associated with bone metastasis in human non-small cell lung cancer. PLoS One **8**, e58361 CrossRef PubMed

70 Fiumara, P., Snell, V., Li, Y., Mukhopadhyay, A., Younes, M., Gillenwater, A.M., Cabanillas, F., Aggarwal, B.B. and Younes, A. (2001) Functional expression of receptor activator of nuclear factor kappaB in Hodgkin disease cell lines. Blood **98**, 2784–2790 CrossRef PubMed

71 Nosaka, K., Miyamoto, T., Sakai, T., Mitsuya, H., Suda, T. and Matsuoka, M. (2002) Mechanism of hypercalcemia in adult T-cell leukemia: overexpression of receptor activator of nuclear factor kappaB ligand on adult T-cell leukemia cells. Blood **99**, 634–640 CrossRef PubMed

72 Barcala, V., Ruybal, P., Garcia Rivello, H., Waldner, C., Ascione, A. and Mongini, C. (2003) RANKL expression in a case of follicular lymphoma. Eur. J. Haematol. **70**, 417–419 CrossRef PubMed

73 Jones, D.H., Nakashima, T., Sanchez, O.H., Kozieradzki, I., Komarova, S.V., Sarosi, I., Morony, S., Rubin, E., Sarao, R., Hojilla, C.V. et al. (2006) Regulation of cancer cell migration and bone metastasis by RANKL. Nature **440**, 692–696 CrossRef PubMed

74 Kupas, V., Weishaupt, C., Siepmann, D., Kaserer, M.L., Eickelmann, M., Metze, D., Luger, T.A., Beissert, S. and Loser, K. (2011) RANK is expressed in metastatic melanoma and highly upregulated on melanoma-initiating cells. J. Invest. Dermatol. **131**, 944–955 CrossRef PubMed

75 Roux, S., Meignin, V., Quillard, J., Meduri, G., Guiochon-Mantel, A., Fermand, J.P., Milgrom, E. and Mariette, X. (2002) RANK (receptor activator of nuclear factor-kappaB) and RANKL expression in multiple myeloma. Br. J. Haematol. **117**, 86–92 CrossRef PubMed

76 Farrugia, A.N., Atkins, G.J., To, L.B., Pan, B., Horvath, N., Kostakis, P., Findlay, D.M., Bardy, P. and Zannettino, A.C. (2003) Receptor activator of nuclear factor-kappaB ligand expression by human myeloma cells mediates osteoclast formation *in vitro* and correlates with bone destruction *in vivo*. Cancer Res. **63**, 5438–5445 PubMed

77 Granchi, D., Amato, I., Battistelli, L., Avnet, S., Capaccioli, S., Papucci, L., Donnini, M., Pellacani, A., Brandi, M.L., Giunti, A. and Baldini, N. (2004) *In vitro* blockade of receptor activator of nuclear factor-kappaB ligand prevents osteoclastogenesis induced by neuroblastoma cells. Int. J. Cancer **111**, 829–838 CrossRef PubMed

78 Chuang, F.H., Hsue, S.S., Wu, C.W. and Chen, Y.K. (2009) Immunohistochemical expression of RANKL, RANK, and OPG in human oral squamous cell carcinoma. J. Oral Pathol. Med. **38**, 753–738 CrossRef PubMed

79 Mori, K., Le Goff, B., Berreur, M., Riet, A., Moreau, A., Blanchard, F., Chevalier, C., Guisle-Marsollier, I., Léger, J., Guicheux, J. et al. (2007) Human osteosarcoma cells express functional receptor activator of nuclear factor-kappa B. J. Pathol. **11**, 555–562 CrossRef

80 Lee, J.A., Jung, J.S., Kim, D.H., Lim, J.S., Kim, M.S., Kong, C.B., Song, W.S., Cho, W.H., Jeon, D.G., Lee, S.Y. and Koh, J.S. (2011) RANKL expression is related to treatment outcome of patients with localized, high-grade osteosarcoma. Pediatr. Blood Cancer **56**, 738–743 CrossRef PubMed

81 Chen, G., Sircar, K., Aprikian, A., Potti, A., Goltzman, D. and Rabbani, S.A. (2006) Expression of RANKL/RANK/OPG in primary and metastatic human prostate cancer as markers of disease stage and functional regulation. Cancer **107**, 289–298 CrossRef PubMed

82 Armstrong, A.P., Miller, R.E., Jones, J.C., Zhang, J., Keller, E.T. and Dougall, W.C. (2008) RANKL acts directly on RANK-expressing prostate tumor cells and mediates migration and expression of tumor metastasis genes. Prostate **68**, 92–104 CrossRef PubMed

83 Odero-Marah, V.A., Wang, R., Chu, G., Zayzafoon, M., Xu, J., Shi, C., Marshall, F.F., Zhau, H.E. and Chung, L.W. (2008) Receptor activator of NF-kappaB ligand (RANKL) expression is associated with epithelial to mesenchymal transition in human prostate cancer cells. Cell Res. **18**, 858–870 CrossRef PubMed

84 Mikami, S., Katsube, K., Oya, M., Ishida, M., Kosaka, T., Mizuno, R., Mochizuki, S., Ikeda, T., Mukai, M. and Okada, Y. (2009) Increased RANKL expression is related to tumour migration and metastasis of renal cell carcinomas. J. Pathol. **218**, 530–539 CrossRef PubMed

85 Heymann, M.F., Riet, A., Le Goff, B., Battaglia, S., Paineau, J. and Heymann, D. (2008) OPG, RANK and RANK ligand expression in thyroid lesions. Regul. Pept. **148**, 46–53 CrossRef PubMed

86 Park, H.S., Lee, A., Chae, B.J., Bae, J.S., Song, B.J. and Jung, S.S. (2014) Expression of receptor activator of nuclear factor kappa-B as a poor prognostic marker in breast cancer. J. Surg. Oncol. **110**, 807–812 CrossRef PubMed

87 Pfitzner, B.M., Branstetter, D., Loibl, S., Denkert, C., Lederer, B., Schmitt, W.D., Dombrowski, F., Werner, M., Rüdiger, T., Dougall, W.C. and von Minckwitz, G. (2014) RANK expression as a prognostic and predictive marker in breast cancer. Breast Cancer Res. Treat. **145**, 307–315 CrossRef PubMed

88 Zhang, L., Teng, Y., Zhang, Y., Liu, J., Xu, L., Qu, J., Hou, K., Yang, X., Liu, Y. and Qu, X. (2012) Receptor activator for nuclear factor κB expression predicts poor prognosis in breast cancer patients with bone metastasis but not in patients with visceral metastasis. J. Clin. Pathol. **65**, 36–40 CrossRef PubMed

89 Trieb, K. and Windhager, R. (2015) Receptor activator of nuclear factor κB expression is a prognostic factor in human osteosarcoma. Oncol. Lett. **10**, 1813–1815 PubMed

90 Bago-Horvath, Z., Schmid, K., Rössler, F., Nagy-Bojarszky, K., Funovics, P. and Sulzbacher, I. (2014) Impact of RANK signalling on survival and chemotherapy response in osteosarcoma. Pathology **46**, 411–415 CrossRef PubMed

91 Papanastasiou, A.D., Sirinian, C. and Kalofonos, H.P. (2012) Identification of novel human receptor activator of nuclear factor-kB isoforms generated through alternative splicing: implications in breast cancer cell survival and migration. Breast Cancer Res. **14**, R112 CrossRef PubMed

92 Lee, J.A., Jung, J.S., Kim, D.H., Lim, J.S., Kim, M.S., Kong, C.B., Song, W.S., Cho, W.H., Jeon, D.G., Lee, S.Y. and Koh, J.S. (2011) RANKL expression is related to treatment outcome of patients with localized, high-grade osteosarcoma. Pediatr. Blood Cancer. **56**, 738–743 CrossRef PubMed

93 Cathomas, R., Rothermundt, C., Bode, B., Fuchs, B., von Moos, R. and Schwitter, M. (2015) RANK ligand blockade with denosumab in combination with sorafenib in chemorefractory osteosarcoma: a possible step forward? Oncology **88**, 257–260 CrossRef PubMed

94 Roux, S., Amazit, L., Meduri, G., Guiochon-Mantel, A., Milgrom, E. and Mariette, X. (2002) RANK (receptor activator of nuclear factor kappa B) and RANK ligand are expressed in giant cell tumors of bone. Am. J. Clin. Pathol. **117**, 210–216 CrossRef PubMed

95 Chawla, S., Henshaw, R., Seeger, L., Choy, E., Blay, J.Y., Ferrari, S., Kroep, J., Grimer, R., Reichardt, P., Rutkowski, P. et al. (2013) Safety and efficacy of denosumab for adults and skeletally mature adolescents with giant cell tumour of bone: interim analysis of an open-label, parallel-group, phase 2 study. Lancet Oncol. **14**, 901–908 CrossRef PubMed

96 Raju, R., Balakrishnan, L., Nanjappa, V., Bhattacharjee, M., Getnet, D., Muthusamy, B., Kurian Thomas, J., Sharma, J., Rahiman, B.A. et al. (2011) A comprehensive manually curated reaction map of RANKL/RANK-signaling pathway. Database (Oxford) **2011**, bar021 PubMed

97 Galibert, L., Tometsko, M.E., Anderson, D.M., Cosman, D. and Dougall, W.C. (1998) The Involvement of multiple tumor necrosis factor receptor (TNFR)-associated factors in the signaling mechanisms of receptor activator of NF-kappaB, a member of the TNFR superfamily. J. Biol. Chem. **273**, 34120–23427 CrossRef PubMed

98 Lomaga, M.A., Yeh, W.C., Sarosi, I., Duncan, G.S., Furlonger, C., Ho, A., Morony, S., Capparelli, C., Van, G., Kaufman, S. et al. (1999) TRAF6 deficiency results in osteopetrosis and defective interleukin-1, CD40, and LPS signaling. Genes. Dev. **13**, 1015–1024 CrossRef PubMed

99 Jin, G., Akiyama, T., Koga, T., Takayanagi, H., Tanaka, S. and Inoue, J.I. (2005) RANK-mediated amplification of TRAF6 signaling leads to NFATc1 induction during osteoclastogenesis. EMBO J. **24**, 790–799 CrossRef PubMed

100 Darnay, B.G., Ni, J., Moore, P.A. and Aggarwal, B.B. (1999) Activation of NF-kappaB by RANK requires tumor necrosis factor receptor-associated factor (TRAF) 6 and NF-kappaB-inducing kinase. Identification of a novel TRAF6 interaction motif. J. Biol. Chem. **274**, 7724–7731 CrossRef PubMed

101 Kim, H.H., Lee, D.E., Shin, J.N., Lee, Y.S., Jeon, Y.M., Chung, C.H., Ni, J., Kwon, B.S. and Lee, Z.H. (1999) Receptor activator of NF-kappaB recruits multiple TRAF family adaptors and activates c-Jun N-terminal kinase. FEBS Lett. **443**, 297–302 CrossRef PubMed

102 Simonet, W.S., Lacey, D.L., Dunstan, C.R., Kelley, M., Chang, M.S., Lüthy, R., Nguyen, H.Q., Wooden, S., Bennett, L., Boone, T. et al. (1997) Osteoprotegerin: a novel secreted protein involved in the regulation of bone density. Cell **89**, 309–319 CrossRef PubMed

103 Yasuda, H., Shima, N., Nakagawa, N., Yamaguchi, K., Kinosaki, M., Mochizuki, S., Tomoyasu, A., Yano, K., Goto, M., Murakami, A. et al. (1998) Osteoclast differentiation factor is a ligand for osteoprotegerin/osteoclastogenesis-inhibitory factor and is identical to TRANCE/RANKL. Proc. Natl. Acad. Sci. U.S.A. **95**, 3597–3602 CrossRef PubMed

104 Baud'huin, M., Duplomb, L., Teletchea, S., Lamoureux, F., Ruiz-Velasco, C., Maillasson, M., Redini, F., Heymann, M.F. and Heymann, D. (2013) Osteoprotegerin: multiple partners for multiples functions. Cytokine Growth Factor Rev. **24**, 401–409 CrossRef PubMed

105 Gonda, T.A., Tu, S. and Wang, T.C. (2009) Chronic inflammation, the tumor microenvironment and carcinogenesis. Cell Cycle **8**, 2005–2013 CrossRef PubMed

106 Kwan Tat, S., Padrines, M., Theoleyre, S., Couillaud-Battaglia, S., Heymann, D., Redini, F. and Fortun, Y. (2006) OPG/membranous–RANKL complex is internalized via the clathrin pathway before a lysosomal and a proteasomal degradation. Bone **39**, 706–715 CrossRef PubMed

107 Théoleyre, S., Kwan Tat, S., Vusio, P., Blanchard, F., Gallagher, J., Ricard-Blum, S., Fortun, Y., Padrines, M., Rédini, F. and Heymann, D. (2006) Characterization of osteoprotegerin binding to glycosaminoglycans by surface plasmon resonance: role in the interactions with receptor activator of nuclear factor kappaB ligand (RANKL) and RANK. Biochem. Biophys. Res. Commun. **347**, 460–467 CrossRef PubMed

108 Standal, T., Seidel, C., Hjertner, Ø., Plesner, T., Sanderson, R.D., Waage, A., Borset, M. and Sundan, A. (2002) Osteoprotegerin is bound, internalized, and degraded by multiple myeloma cells. Blood **100**, 3002–3007 CrossRef PubMed

109 Lamoureux, F., Picarda, G., Garrigue-Antar, L., Baud'huin, M., Trichet, V., Vidal, A., Miot-Noirault, E., Pitard, B., Heymann, D. and Rédini, F. (2009) Glycosaminoglycans as potential regulators of osteoprotegerin therapeutic activity in osteosarcoma. Cancer Res. **69**, 526–536 CrossRef PubMed

110 Emery, J.G., McDonnell, P., Burke, M.B., Deen, K.C., Lyn, S., Silverman, C., Dul, E., Appelbaum, E.R., Eichman, C., DiPrinzio, R. et al. (1998) Osteoprotegerin is a receptor for the cytotoxic ligand TRAIL. J. Biol. Chem. **273**, 14363–14367 CrossRef PubMed

111 Holen, I., Croucher, P.I., Hamdy, F.C. and Eaton, C.L. (2002) Osteoprotegerin (OPG) is a survival factor for human prostate cancer cells. Cancer Res. **62**, 1619–1623 PubMed

112 Baud'huin, M., Duplomb, L., Téletchéa, S., Charrier, C., Maillasson, M., Fouassier, M. and Heymann, D. (2009) Factor VIII-von Willebrand factor complex inhibits osteoclastogenesis and controls cell survival. J. Biol. Chem. **284**, 31704–31713 CrossRef PubMed

113 Luo, J., Yang, Z., Ma, Y., Yue, Z., Lin, H., Qu, G., Huang, J., Dai, W., Li, C., Zheng, C. et al. (2016) LGR4 is a receptor for RANKL and negatively regulates osteoclast differentiation and bone resorption. Nat. Med. **22**, 539–546 CrossRef PubMed

114 Styrkarsdottir, U., Thorleifsson, G., Sulem, P., Gudbjartsson, D.F., Sigurdsson, A., Jonasdottir, A., Jonasdottir, A., Oddsson, A., Helgason, A., Magnusson, O.T. et al. (2013) Nonsense mutation in the LGR4 gene is associated with several human diseases and other traits. Nature **497**, 517–520 CrossRef PubMed

115 Zhu, Y.B., Xu, L., Chen, M., Ma, H.N. and Lou, F. (2013) GPR48 promotes multiple cancer cell proliferation via activation of Wnt signaling. Asian Pac. J. Cancer Prev. **14**, 4775–4578 CrossRef PubMed

116 Liang, F., Yue, J., Wang, J., Zhang, L., Fan, R., Zhang, H. and Zhang, Q. (2015) GPCR48/LGR4 promotes tumorigenesis of prostate cancer via PI3K/Akt signaling pathway. Med. Oncol. **32**, 49 CrossRef PubMed

117 Luo, W., Rodriguez, M., Valdez, J.M., Zhu, X., Tan, K., Li, D., Siwko, S., Xin, L. and Liu, M. (2013) Lgr4 is a key regulator of prostate development and prostate stem cell differentiation. Stem Cells **31**, 2492–2505 CrossRef PubMed

118 Liu, J., Wei, W., Guo, C.A., Han, N., Pan, J.F., Fei, T. and Yan, Z.Q. (2013) Stat3 upregulates leucine-rich repeat-containing g protein-coupled receptor 4 expression in osteosarcoma cells. Biomed. Res. Int. **2013**, 310691 PubMed

119 Fata, J.E., Kong, Y.Y., Li, J., Sasaki, T., Irie-Sasaki, J., Moorehead, R.A., Elliott, R., Scully, S., Voura, E.B., Lacey, D.L. et al. (2000) The osteoclast differentiation factor osteoprotegerin-ligand is essential for mammary gland development. Cell **103**, 41–50 CrossRef PubMed

120 Kim, N.S., Kim, H.J., Koo, B.K., Kwon, M.C., Kim, Y.W., Cho, Y., Yokota, Y., Penninger, J.M. and Kong, Y.Y. (2006) Receptor activator of NF-kappaB ligand regulates the proliferation of mammary epithelial cells via Id2. Mol. Cell Biol. **26**, 1002–1013 CrossRef PubMed

121 Gonzalez-Suarez, E., Branstetter, D., Armstrong, A., Dinh, H., Blumberg, H. and Dougall, W.C. (2007) RANK overexpression in transgenic mice with mouse mammary tumor virus promoter controlled RANK increases proliferation and impairs alveolar differentiation in the mammary epithelia and disrupts lumen formation in cultured epithelial acini. Mol. Cell. Biol. **27**, 1442–1454 CrossRef PubMed

122 Gonzalez-Suarez, E., Jacob, A.P., Jones, J., Miller, R., Roudier-Meyer, M.P., Erwert, R., Pinkas, J., Branstetter, D. and Dougall, W.C. (2010) RANK ligand mediates progestin-induced mammary epithelial proliferation and carcinogenesis. Nature **468**, 103–137 CrossRef PubMed

123 Palafox, M., Ferrer, I., Pellegrini, P., Vila, S., Hernandez-Ortega, S., Urruticoechea, A., Climent, F., Soler, M.T., Muñoz, P., Viñals, F. et al. (2012) RANK induces epithelial–mesenchymal transition and stemness in human mammary epithelial cells and promotes tumorigenesis and metastasis. Cancer Res. **72**, 2879–2888 CrossRef PubMed

124 Yamada, T., Tsuda, M., Takahashi, T., Totsuka, Y., Shindoh, M. and Ohba, Y. (2011) RANKL expression specifically observed *in vivo* promotes epithelial mesenchymal transition and tumor progression. Am. J. Pathol. **178**, 2845–2856 CrossRef PubMed

125 Liu, Y., Wang, J., Ni, T., Wang, L., Wang, Y. and Sun, X. (2016), CCL20 mediates RANK/RANKL-induced epithelial–mesenchymal transition in endometrial cancer cells. Oncotarget, doi: 10.18632/oncotarget.8291

126 Mori, K., Le Goff, B., Charrier, C., Battaglia, S., Heymann, D. and Rédini, F. (2007) DU145 human prostate cancer cells express functional receptor activator of NFkappaB: new insights in the prostate cancer bone metastasis process. Bone **40**, 981–890 CrossRef PubMed

127 Li, X., Liu, Y., Wu, B., Dong, Z., Wang, Y., Lu, J., Shi, P., Bai, W. and Wang, Z. (2014) Potential role of the OPG/RANK/RANKL axis in prostate cancer invasion and bone metastasis. Oncol. Rep. **32**, 2605–2611 PubMed

128 Shin, M., Matsuo, K., Tada, T., Fukushima, H., Furuta, H., Ozeki, S., Kadowaki, T., Yamamoto, K., Okamoto, M. and Jimi, E. (2011) The inhibition of RANKL/RANK signaling by osteoprotegerin suppresses bone invasion by oral squamous cell carcinoma cells. Carcinogenesis **32**, 1634–1640 CrossRef PubMed

129 Chen, L.M., Kuo, C.H., Lai, T.Y., Lin, Y.M., Su, C.C., Hsu, H.H., Tsai, F.J., Tsai, C.H., Huang, C.Y. and Tang, C.H. (2011) RANKL increases migration of human lung cancer cells through intercellular adhesion molecule-1 up-regulation. J. Cell. Biochem. **112**, 933–941 CrossRef PubMed

130 Song, F.N., Duan, M., Liu, L.Z., Wang, Z.C., Shi, J.Y., Yang, L.X., Zhou, J., Fan, J., Gao, Q. and Wang, X.Y. (2014) RANKL promotes migration and invasion of hepatocellular carcinoma cells via NF-κB-mediated epithelial–mesenchymal transition. PLoS One **9**, e108507 CrossRef PubMed

131 Wang, J., Sun, X., Zhang, H., Wang, Y. and Li, Y. (2015) MPA influences tumor cell proliferation, migration, and invasion induced by RANKL through PRB involving the MAPK pathway in endometrial cancer. Oncol. Rep. **33**, 799–809 PubMed

132 Golden, D., Saria, E.A. and Hansen, M.F. (2015) Regulation of osteoblast migration involving receptor activator of nuclear factor-kappa B (RANK) signaling. J. Cell Physiol. **230**, 2951–2960 CrossRef PubMed

133 Beristain, A.G., Narala, S.R., Di Grappa, M.A. and Khokha, R. (2012) Homotypic RANK signaling differentially regulates proliferation, motility and cell survival in osteosarcoma and mammary epithelial cells. J. Cell Sci. **125**, 943–955 CrossRef PubMed

134 Mikami, S., Katsube, K., Oya, M., Ishida, M., Kosaka, T., Mizuno, R., Mochizuki, S., Ikeda, T., Mukai, M. and Okada, Y. (2009) Increased RANKL expression is related to tumour migration and metastasis of renal cell carcinomas. J. Pathol. **218**, 530–539 CrossRef PubMed

135 Min, J.K., Kim, Y.M., Kim, Y.M., Kim, E.C., Gho, Y.S., Kang, I.J., Lee, S.Y., Kong, Y.Y. and Kwon, Y.G. (2003) Vascular endothelial growth factor up-regulates expression of receptor activator of NF-kappa B (RANK) in endothelial cells: concomitant increase of angiogenic responses to RANK ligand. J. Biol. Chem. **278**, 39548–39557 CrossRef PubMed

136 Kim, Y.M., Kim, Y.M., Lee, Y.M., Kim, H.S., Kim, J.D., Choi, Y., Kim, K.W., Lee, S.Y. and Kwon, Y.G. (2002) TNFrelated activation-induced cytokine (TRANCE) induces angiogenesis through the activation of Src and phospholipase C (PLC) in human endothelial cells. J. Biol. Chem. **277**, 6799–6805 CrossRef PubMed

137 Kim, H.H., Shin, H.S., Kwak, H.J., Ahn, K.Y., Kim, J.H., Lee, H.J., Lee, M.S., Lee, Z.H. and Koh, G.Y. (2003) RANKL regulates endothelial cell survival through the phosphatidylinositol 3-kinase/Akt signal transduction pathway. FASEB J. **17**, 2163–2165 PubMed

138 Benslimane-Ahmim, Z., Heymann, D., Dizier, B., Lokajczyk, A., Brion, R., Laurendeau, I., Bièche, I., Smadja, D.M., Galy-Fauroux, I., Colliec-Jouault, S. et al. (2011) Osteoprotegerin, a new actor in vasculogenesis, stimulates endothelial colony-forming cells properties. J. Thromb. Haemost. **9**, 834–843 CrossRef PubMed

139 Min, J.K., Cho, Y.L., Choi, J.H., Kim, Y., Kim, J.H., Yu, Y.S., Rho, J., Mochizuki, N., Kim, Y.M., Oh, G.T. and Kwon, Y.G. (2007) Receptor activator of nuclear factor-kB ligand increases vascular permeability: impaired permeability and angiogenesis in eNOS-deficient mice. Blood **109**, 1496–1502

140 Mueller, C.G. and Hess, E. (2012) Emerging functions of RANKL in lymphoid tissues. Front. Immunol. **3**, 261 CrossRef PubMed

141 Kong, Y.Y., Yoshida, H., Sarosi, I., Tan, H.L., Timms, E., Capparelli, C., Morony, S., Oliveira-dos-Santos, A.J., Van, G., Itie, A. et al. (1999) OPGL is a key regulator of osteoclastogenesis, lymphocyte development and lymph-node organogenesis. Nature **397**, 315–323 CrossRef PubMed

142 Akiyama, T., Shimo, Y., Yanai, H., Qin, J., Ohshima, D., Maruyama, Y., Asaumi, Y., Kitazawa, J., Takayanagi, H., Penninger, J.M. et al. (2008) The tumor necrosis factor family receptors RANK and CD40 cooperatively establish the thymic medullary microenvironment and self-tolerance. Immunity **29**, 423–437 CrossRef PubMed

143 Akiyama, T., Shinzawa, M., Qin, J. and Akiyama, N. (2013) Regulations of gene expression in medullary thymic epithelial cells required for preventing the onset of autoimmune diseases. Front. Immunol. **4**, 249 CrossRef PubMed

144 Khan, I.S., Mouchess, M.L., Zhu, M.L., Conley, B., Fasano, K.J., Hou, Y., Fong, L. and Su, M.A. (2014) Enhancement of an anti-tumor immune response by transient blockade of central T cell tolerance. J. Exp. Med. **211**, 761–768 CrossRef PubMed

145 Cook, J. and Hagemann, T. (2013) Tumour-associated macrophages and cancer. Curr. Opin. Pharmacol. **13**, 595–601 CrossRef PubMed

146 Breuil, V., Schmid-Antomarchi, H., Schmid-Alliana, A., Rezzonico, R., Euller-Ziegler, L. and Rossi, B. (2003) The receptor activator of nuclear factor (NF)-kappaB ligand (RANKL) is a new chemotactic for human monocytes. FASEB J. **17**, 2163–2165 PubMed

147 Kambayashi, Y., Fujimura, T., Furudate, S., Asano, M., Kakizaki, A. and Aiba, S. (2015) The possible interaction between receptor activator of nuclear factor kappa-B ligand expressed by extramammary paget cells and its ligand on dermal macrophages. J. Invest. Dermatol. **135**, 2547–2550 CrossRef PubMed

148 Fujimura, T., Kambayashi, Y., Furudate, S., Asano, M., Kakizaki, A. and Aiba, S. (2015) Receptor activator of NF-[kappa]B ligand promotes the production of CCL17 from RANK+ M2 macrophages. J. Invest. Dermatol. **135**, 2884–2887 CrossRef PubMed

149 Tan, W., Zhang, W., Strasner, A., Grivennikov, S., Cheng, J.Q., Hoffman, R.M. and Karin, M. (2011) Tumour-infiltrating regulatory T cells stimulate mammary cancer metastasis through RANKL–RANK signalling. Nature **470**, 548–553 CrossRef PubMed

150 Monteiro, A.C., Leal, A.C., Gonçalves-Silva, T., Mercadante, A.C., Kestelman, F., Chaves, S.B., Azevedo, R.B., Monteiro, J.P. and Bonomo, A.T. (2013) Cells induce pre-metastatic osteolytic disease and help bone metastases establishment in a mouse model of metastatic breast cancer. PLoS One **8**, e68171 CrossRef PubMed

151 Esposito, M. and Kang, Y. (2014) Targeting tumor-stromal interactions in bone metastasis. Pharmacol. Ther. **141**, 222–233 CrossRef PubMed

152 Bekker, P.J., Holloway, D., Nakanishi, A., Arrighi, M., Leese, P.T. and Dunstan, C.R. (2001) The effect of a single dose of osteoprotegerin in postmenopausal women. J. Bone Miner. Res. **16**, 348–360 CrossRef PubMed

153 Gobin, B., Baud'huin, M., Isidor, B., Heymann, D. and Heymann, M.F. (2012) Monoclonal antibodies targeting RANKL in bone metastasis treatment. In Monoclonal antibodies in oncology (Fatih, M., ed.), pp. 42–53, Uckum, eBook Future Medicine Ltd

154 Bekker, P.J., Holloway, D.L., Rasmussen, A.S., Murphy, R., Martin, S.W., Leese, P.T., Holmes, G.B., Dunstan, C.R. and DePaoli, A.M.A. (2004) single-dose placebo-controlled study of AMG162, a fully human monoclonal antibody to RANKL, in postmenopausal women. J. Bone Miner. Res. **19**, 1059–1066 CrossRef

155 Picarda, G., Matous, E., Amiaud, J., Charrier, C., Lamoureux, F., Heymann, M.F., Tirode, F., Pitard, B., Trichet, V., Heymann, D. and Redini, F. (2013) Osteoprotegerin inhibits bone resorption and prevents tumor development in a xenogenic model of Ewing's sarcoma by inhibiting RANKL. J. Bone Oncol. **2**, 95–104 CrossRef PubMed

156 Lamoureux, F., Richard, P., Wittrant, Y., Battaglia, S., Pilet, P., Trichet, V., Blanchard, F., Gouin, F., Pitard, B., Heymann, D. and Redini, F. (2007) Therapeutic relevance of osteoprotegerin gene therapy in osteosarcoma: blockade of the vicious cycle between tumor cell proliferation and bone resorption. Cancer Res. **67**, 7308–7318 CrossRef PubMed

157 Lamoureux, F., Picarda, G., Rousseau, J., Gourden, C., Battaglia, S., Charrier, C., Pitard, B., Heymann, D. and Rédini, F. (2008) Therapeutic efficacy of soluble receptor activator of nuclear factor-kappa B-Fc delivered by nonviral gene transfer in a mouse model of osteolytic osteosarcoma. Mol. Cancer Ther. **7**, 3389–3398 CrossRef PubMed

158 Zheng, Y., Zhou, H., Fong-Yee, C., Modzelewski, J.R., Seibel, M.J. and Dunstan, C.R. (2008) Bone resorption increases tumour growth in a mouse model of osteosclerotic breast cancer metastasis. Clin. Exp. Metastasis **25**, 559–567 CrossRef PubMed

159 Miller, R.E., Roudier, M., Jones, J., Armstrong, A., Canon, J. and Dougall, W.C. (2008) RANK ligand inhibition plus docetaxel improves survival and reduces tumor burden in a murine model of prostate cancer bone metastasis. Mol. Cancer Ther. **7**, 2160–2169 CrossRef PubMed

160 Miller, R.E., Jones, J.C., Tometsko, M., Blake, M.L. and Dougall, W.C. (2014) RANKL inhibition blocks osteolytic lesions and reduces skeletal tumor burden in models of non-small-cell lung cancer bone metastases. J. Thorac. Oncol. **9**, 345–354 CrossRef PubMed

161 Vanderkerken, K., De Leenheer, E., Shipman, C., Asosingh, K., Willems, A., Van Camp, B. and Croucher, P. (2003) Recombinant osteoprotegerin decreases tumor burden and increases survival in a murine model of multiple myeloma. Cancer Res. **63**, 287–289 PubMed

162 Lipton, A., Fizazi, K., Stopeck, A.T., Henry, D.H., Smith, M.R., Shore, N., Martin, M., Vadhan-Raj, S., Brown, J.E., Richardson, G.E. et al. (2016) Effect of denosumab versus zoledronic acid in preventing skeletal-related events in patients with bone metastases by baseline characteristics. Eur. J. Cancer **53**, 75–83 CrossRef PubMed

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