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REVIEW

The RANK/RANKL/OPG system in tumorigenesis and metastasis of cancer stem cell: potential targets for anticancer therapy

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Abstract: The molecular triad involving receptor activator of nuclear factor kβ (RANK)/RANK ligand (RANKL)/osteoprotegerin cytokine system has been well implicated in several physiological and pathological processes including bone metabolism, mammary gland development, regulation of the immune function, tumorigenesis and metastasis of cancer stem cell, thermoregulation, and vascular calcification. However, this review aimed to summarize several original and up-to-date articles focusing on the role of this signaling system in cancer cell development and metastasis as well as potential therapeutic agents targeting any of the three tumor necrotic factor super family proteins and/or their downstream signaling pathways. The RANK/RANKL axis has direct effects on tumor cell development. The system is well involved in the development of several primary and secondary tumors including breast cancer, prostate cancer, bone tumors, and leukemia. The signaling of this triad system has also been linked to tumor invasiveness in the advanced stage. Bone is by far the most common site of cancer metastasis. Several therapeutic agents targeting this system have been developed. Among them, a monoclonal antibody, denosumab, was clinically approved for the treatment of osteoporosis and cancer-related diseases.

Keywords: cancer, RANK, RANKL, OPG, RANK/RANKL/OPG system, therapeutic, tumor

Introduction

Overview of receptor activator of nuclear factor kβ (RANK)/RANK ligand (RANKL)/osteoprotegerin (OPG) signaling system

The molecular triad involving RANK/RANKL/OPG cytokine system has been observed to influence various physiological and pathological processes throughout the body. These include bone modeling and remodeling, mammary gland development, tumor cell development and migration, and modulation of adaptive immunity (Figure 1).[1–4] The role of this signaling system has been well emphasized in bone where RANKL/RANK signaling mediates osteoclastogenesis and bone resorption via paracrine signaling between osteoblast (RANKL) and osteoclast (RANK) cells. OPG produced by osteoblast and stromal cells acts as a soluble decoy receptor for RANKL and hence prevents osteoclast differentiation and activation by interfering with RANKL–RANK interaction.[1] RANK is also constitutively expressed in mammary epithelial tissues where RANKL works through RANK to provide proliferative and survival signals and thereby promotes the final stages of lactating mammary gland development.[5] It has also been shown to be involved in thermoregulation signaling in females (linked with

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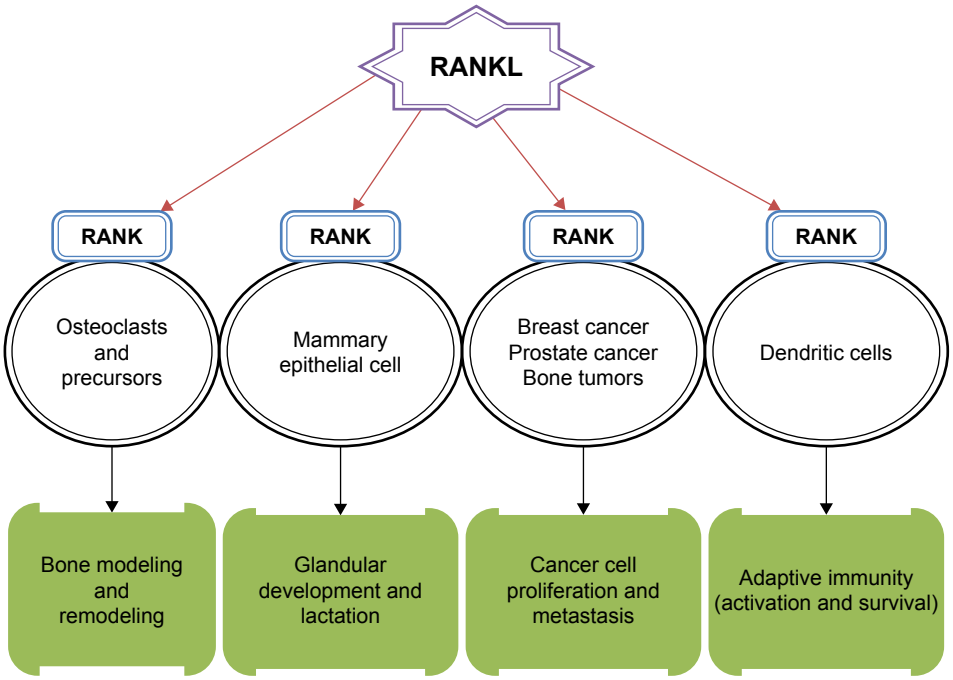

Figure 1 The role of RANK/RANKL signaling system in various physiological and pathophysiological processes.
Abbreviations: RANK, receptor activator of nuclear factor kβ; RANKL, RANK ligand.

ovarian sex hormones) possibly via the COX_2-PGE_2/EP_3R pathway, vascular calcification, and atherosclerosis by cross talking with the renin–angiotensin system.[6–10]

Methods

In this review, both open access scholarly sources and subscription based journals were utilized for the study. Legitimate databases and indexing services including directory of open access journals, PubMed, PubMed Central, Medline, Scopus, and ProQuest were considered for the collection of articles related to the topic of interest. Other supplementary sources such as Google Scholar, WorldCat, and ResearchGate were also used for extensively collecting updated and sufficient information on this topic. For subscription based journals, however, Hinari: World Health Organization for developing countries was used to access such journals (eg, Elsevier journals, subscribed journals in PubMed, Science Direct, etc.). The primary key terms used during data collection were "RANK," "RANKL," "OPG," "RANK/RANKL/OPG system," "cancer," "tumor," and "therapeutic." In each of the databases and web directories, Boolean logic (AND, OR) was routinely applied to connect the key terms. What is more, truncation and alternative terms were also employed to expand the chance of obtaining related articles to the topics of interest. Based on these key terms and following in-depth screening of the relevance of each and every article for the topic, only articles that are highly relevant to this review were included and duplicated articles were excluded from the study in advance. Excluding the background, 73 references were filtered for the body of this review. Coming to the timing distribution of references cited, the majority of references, 52 (71.23%), were published from 2010 to 2017. Data collection was conducted from September 2016 to April 2017.

Overview of RANK/RANKL/OPG system and cancer

Primary tumors will commonly metastasize into bone. Tumors such as breast and prostate cancers typically have a greater chance of inducing secondary cancers within bone.[11] Based on Stephen Paget's seed and soil theory, the bone microenvironment provides a conducive area (fertile soil) in which the seeds (secondary tumors) can easily grow in. RANK and RANKL have been known to be involved in cancer cell migration and development in bone.[12] Secondary tumors within the bone will secrete several growth factors and cytokines which stimulate osteoblast cells. Upon stimulation, osteoblast cells increase the expression and release of RANKL which in turn acts either on the RANK receptor found on the osteoclast cells or soluble decoy receptor (OPG) that circulates in body fluids. RANKL–RANK interaction activates osteoclasts to further release growth factors such as tumor growth factor β and insulin-like growth factor 1 from the bone matrix which in turn stimulate parathyroid

hormone-related peptide production and promote tumor growth. This interaction between tumor cells and the bone microenvironment results in a vicious cycle of bone destruction and tumor growth. Generally, skeletal-related events (SREs) are used to describe a collection of adverse events associated with bone metastases. SREs include pathologic fractures that require surgery or chemotherapy, spinal cord compression, and less frequently malignant hypercalcemia.[11,13,14] RANKL was also observed to be more accurate than conventional markers in the breast cancer subgroup, and was a better predictor of bone progression than N-terminal telopeptides of type I collagen. The study suggested that RANKL could serve as an accurate marker of bone response in metastatic patients. High RANKL levels may identify patients with a shift in bone homeostasis toward bone resorption.[15]

The role of RANK/RANKL/OPG system in solid cancers

Primary bone tumors

Malignant tumors of the bone can be either primary tumors or secondary (metastasized) tumors. Primary tumors include osteosarcoma, multiple myeloma, and giant cell tumor of the bone (Table 1). Osteosarcoma is the most common primary malignant tumor of the bone.[16]

This triad system also plays a significant role in various solid malignancies that are capable of metastasizing to bone. These include breast cancer, prostate cancer, and lung cancer, among others.[22] This triad system is by far strongly implicated in the physiology of mammary gland development and pathogenesis of breast cancer cells.[5,23,24] The role of the RANK/RANKL/OPG triad system goes beyond involvement of breast cancer pathogenesis. The second most common target is prostate cancer. It has also been implicated in the pathogenesis of several and rare malignant tumors such as lung cancer, renal cell carcinoma, hepatocellular carcinoma, and melanoma (Table 2).[22,25]

The regulatory function of RANKL is one of the key factors in progesterone-induced proliferation of the breast. Progesterone has a direct action on progesterone receptor (PR) expressing cells but PR-negative cells are affected indirectly through RANKL-induced paracrine actions leading to the proliferation of mammary epithelial PR-negative cells. RANK induces epithelial to mesenchymal transition and stemness in human mammary epithelial cells and promotes tumorigenesis and metastasis.[26] RANKL stimulation of RANK expressing cells increased multidrug resistance protein 1, breast cancer resistance protein, and lung resistance protein 1 expression and decreased Bim expression through various signaling molecules. These results indicate that the RANK/RANKL system induces chemoresistance through the activation of multiple signal transduction pathways.[27] What is more, expression of RANKL was observed during pregnancy. This evidence suggests that RANKL can be used as a potential breast cancer therapeutic target particularly in young women and pregnancy-associated tumors. RANKL/RANK has also been shown to control *breast cancer 1* gene mutation-driven mammary tumors. Besides, the E3 ubiquitin ligase Cbl-b protein has been shown to improve the prognosis of $RANK^+$ breast cancer patients via inhibiting RANKL-induced cancer cell migration and metastasis (Figure 2).[28–31]

Other solid cancers

RANKL, RANK, and OPG were variably expressed in tumors of the thyroid, including papillary carcinomas, medullary carcinomas, and macrovascular adenomas.[60] Increased

Table 1 The role of RANKL/RANK/OPG system in primary malignant tumors of the bone

Primary bone tumors	RANKL/RANK/OPG system and its role in tumorigenesis	References
Osteosarcoma	– ↑ RANKL/OPG ratio was observed in the serum of patients with osteosarcoma	Grimaud et al[17]
	– In experimental animals, OPG treatment achieved not only the prevention of osteosarcoma-induced osteolysis but also the inhibition of associated tumor development that improved the survival rate in treatment groups	Lamoureux et al[18]
	– RANKL blockade has been shown to prevent and treat aggressive osteosarcomas	Chen et al[19]
Multiple myeloma	Deregulation of the triad system (expression of more RANKL and/or more lysosomal degradation of OPG) enhances osteoclastogenesis	Pearse et al[20]
GCTB	↑ RANKL/OPG ratio was also observed in GCTB The stromal cells within GCTB had increased RANKL/OPG ratio compared to that of non-osteolytic bone tumors	Lewin and Thomas[21]

Note: ↑, increased, high.
Abbreviations: GCTB, giant cell tumor of the bone; OPG, osteoprotegerin; RANK, receptor activator of nuclear factor kβ; RANKL, RANK ligand.

Table 2 The role of RANK/RANKL/OPG system in the development and metastasis of solid cancers

Solid cancers	Methods	Observed molecular mechanisms and effects	References
Breast cancer	*RANK* and *RANKL* gene knockout in mice models (in vivo study)	– Immature lobuloalveolar development (disorganized mammary gland structure) and disabled milk production	Fata et al[5]
		– RANKL-driven hormone (progesterone) dependent proliferation, survival, and expansion of mammary stem cell could each contribute to mammary cancer initiation, progression, and recurrence	Dougall[23]
	Site directed mutagenesis (in mice model) and RANKL blockage	– Gain of function mutation of RANK signaling resulted in ↑ formation of pre-neoplasias and tumors, whereas inhibition of RANKL ↓ tumorigenesis	Gonzalez-Suarez et al[24]
	RANK gene knockout or loss of function mutation in mice model and RANKL treatment	– ↓ and retarded progestin (MPA)-driven breast cancer – RANKL addition ↓ cell death in response to antitumor antibiotics and irradiation	Schramek et al[32]
	In vitro studies (MDA-MB-436/231 breast cancer cells)	– ↑ Expression of RANK, RANKL, and OPG. OPG acts as a decoy receptor for TRAIL and thereby inhibits apoptosis of a range of tumor cells (survival signal for breast cancer cells)	Emery et al[33] Holen et al[34]
	Cancer patients (correlation of Kaplan–Meier survival analysis with serum samples of RANK and OPG)	– ↓ Expression of serum OPG → better overall survival of patients (quantity of life in years)	Santini et al;[22] Owen et al[35]
		– ↑ RANK and ↓ OPG expression → ↑ metastasis of breast cancer to bone	
		– However, contradictory results on OPG were observed • In bone, OPG reduces bone loss (osteolysis) due to breast cancer metastasis; however, in breast cancer, OPG ↓ apoptosis of cancer cell (↑ survival and invasiveness)	Weichhaus et al[36]
	Experimental investigations (breast cancer patients) plus in vitro study (RANKL producing $CD4^+CD25^+$ T-cells)	– High expression of $CD4^+CD25^+FoxP3^+$ Treg cells ↑ aggressiveness of breast cancer phenotype and ↑ RANKL production (major source)	Tan et al[37]
	4T1 and NMuMG cells (ATCC) and MCF 7 cells (lentiviral infection to induce tumorigenesis in normal cells)	– In ER^- and PR^- breast cancer cells → more expression of RANK mRNA levels and generally have poorer prognosis and high degree of invasiveness than controls. Moreover, RANK overexpression has also been demonstrated to induce epithelial to mesenchymal transition and stemness	Palafox et al;[38] Tsubaki et al[39]
	In vitro study on human MDA-MB-231 breast cancer cell lines (MDA-231-RANK cells)	– MDA-MB-231 cells with high RANK expression resulted in greater metastatic growth rate. However, MDA-MB-231 cells with low RANK level primarily remain in situ	Blake et al[40]
		– Administration of RANKL for these cells • ⇑ expression of multiple genes associated with cell invasive behavior (including several matrix metalloproteinases and other genes that can be considered as a bone metastasis gene signature	
	Primary breast cancer samples from the neoadjuvant GeparTrio study (in vitro) plus in vivo experimental study (mice model)	– Pharmacologic inhibition of RANKL attenuates tumor development and metastasis in mice	Pfitzner et al[41]
		– High RANK expression • Higher sensitivity to chemotherapy but a higher risk of relapse and death	
	A nested case–control study	– Higher concentrations of OPG were associated with increased risk of ER^- breast cancer but less likely in ER^+ type	Fortner et al[42]
	Human breast epithelial cells with Brca1 haploinsufficiency cell	– Denosumab has been proposed to interfere with the cross talk between RANKL producing sensor cells and cancer initiating $RANK^+$ responder cells that reside within premalignant tissues of Brca1-mutation carriers	Cuyàs et al[43]

(*Continued*)

Table 2 (*Continued*)

Solid cancers	Methods	Observed molecular mechanisms and effects	References
	Case control study	– Brca1-mutation carriers had lower mean values of free serum OPG, in particular, in Brca1-mutation carriers (P=0.018) compared with controls	Widschwendter et al[44]
	Human case reports	– RANK and RANKL coexpression is associated with poor RFS and OS in patients with TNBC	Reyes et al[45]
	Surgical biopsy sample of prostate cancer patients and in vitro studies (normal human prostate cells, PrEC and human prostate cancer cell lines-LNCaP, DU-145, and PC-3 cells)	– In prostate cancer cells, ↑ RANK, RANKL, and OPG expression was observed indicating more aggressive and advanced stage; however, normal cells have negligible expression of these proteins	Chen et al[46]
Prostate cancer	Comparative study on hormone-insensitive prostate cancer cell lines (PC-3 and DU-145) and hormone-sensitive cell line (LNCaP) under the same conditions	– OPG is considered as a survival factor for prostate cancer cells by blocking TRAIL	Holen et al[47]
	Metastatic cancer cell models	– RANKL → ↑ IKKα (active) and ↓ Maspin (metastasis suppressor protein) leading to disease progression and tumor invasiveness	Luo et al[48]
	ARCaP(E)/ARCaP(M) prostate cancer model and LNCaP clones overexpressing Snail in a stable manner	– RANKL expression upregulates mesenchymal-associated genes (morphogenic conversion from epithelial to mesenchymal type) → more tumor invasiveness (loss of cell to cell adhesion)	Odero-Marah et al[49]
	In murine model of prostate cancer (PC-3) cells, observation of the effect of RANKL inhibitor and/or antimitotic agent (docetaxel)	– Inhibition of RANKL significantly reduced pathologic osteolysis and ⇑ antitumor effect of docetaxel leading to ↓ skeletal tumor burden and prolonged survival	Miller et al[50]
	Case control study (enzyme-linked immunosorbent assay)	– Higher OPG and PSA concentrations have been observed in metastatic bone patients' sera. It seems that elevated levels of serum OPG in patients with prostate cancer reflect the bone metastatic extent and may potentially be used in metastatic patients' follow-ups	Siampanopoulou et al[51]
	Experimental study on murine model (non-castration, castration, and castration + OPG groups)	– The mechanisms of RANK/RANKL signaling are involved in the ADT-induced acceleration of bone metastasis in castration-insensitive prostate cancer	Takayama et al[52]
Lung cancer	Model of lung cancer (human lung cancer [A549] cells)	– RANKL → upregulation ICAM-1 → ↑ tumor migration	Chen et al[53]
	Experimental investigation in human patients and case reports	– Denosumab ameliorated ALK-induced lung cancer • Inhibition of RANK → ↓ PI3K activation → downregulation of Akt/Pkβ (ALK fusion protein downstream signaling)	Curioni-Fontecedro et al[54]
	(NSCLC) bone metastasis models Radiography, longitudinal bioluminescent imaging, and histological analyses	– Recombinant version of OPG (OPG-Fc) ↓ osteolytic lesions and significantly reduce skeletal tumor burden in NSCLC cells	Miller et al[55]
RCC	Histopathologic study of RCC patients with RTQPCR	– In clear cell RCCs, ⇑ RANK mRNA expression ↑ RANK/OPG ratio and low disease-free survival were observed	Mikami et al[56]
HCC	Case report in virus-induced HCC patients	– In hepatitis C virus and hepatitis B virus-induced HCC • RANKL expression → invasiveness and metastasis to bone	Sasaki et al[57]
Melanoma	In vivo study on malignant tumor of melanocytes	– RANKL blockage → ↓ bone metastasis and morbidity	Jones et al[58]
	In vivo study targeting the thymus gland (AIRE)	– RANKL blockage → depletes *AIRE* that expresses MTEC and TSA expression in the thymus gland leading to a window of defective negative selection, rescue melanoma-specific T-cells from thymic deletion and ↑ host survival secondary to tumor challenge	Khan et al[59]

Notes: ↑, increased, unregulated; ↓, decreased, downregulated, attenuated.

Abbreviations: ADT, androgen deprivation therapy; *AIRE*, autoimmune regulator gene; ALK, anaplastic lymphoma kinase; ATCC, American type cell culture; ER, estrogen receptor; HCC, hepatocellular carcinoma; ICAM-1, intercellular adhesion molecule-1; MTEC, medullary thymic epithelial cells; NSCLC, non-small-cell lung cancer; OPG, osteoprotegerin; OS, overall survival; PkB, protein kinase B; PR, progestin receptor; PSA, prostate-specific antigens; RANK, receptor activator of nuclear factor kβ; RANKL, RANK ligand; RCC, renal cell carcinoma; RFS, relapse-free survival; RTQPCR, real-time quantitative polymerase chain reaction; TNBC, triple-negative breast cancer; TRAIL, tumor necrosis factor-related apoptosis inducing ligand; Treg, Regulatory T-cells; TSA, tumor-specific antigens.

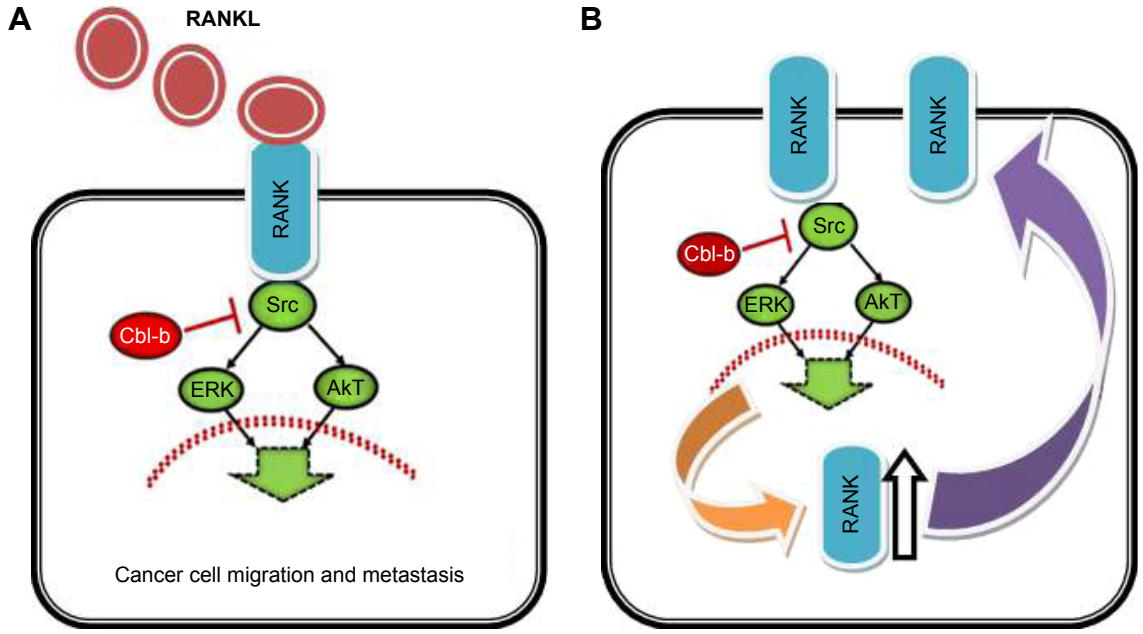

Figure 2 The role of Cbl-b in RANKL-induced breast cancer cell migration and metastasis.
Notes: (**A**) Cbl-b protein inhibited RANKL-induced breast cancer cell migration and metastasis; (**B**) Cbl-b downregulated RANK protein expression by negatively regulating the Src-Akt/ERK pathway.
Abbreviations: ERK, extracellular signal regulated kinase; RANK, receptor activator of nuclear factor kβ; RANKL, RANK ligand.

serum OPG has also been correlated with poor prognosis in gastric carcinoma[61] and bladder carcinoma.[62] RANK overexpression was also considered as a novel esophageal cancer marker.[63]

Nonsolid cancers

Beyond its role in the pathogenesis of several primary and secondary solid malignant tumors, this signaling system has been known to be involved in certain nonsolid cancers such as chronic lymphocytic leukemia and acute myeloid leukemia (AML).

Leukemia

Expression of RANKL was also observed in chronic lymphocytic leukemia and has been known to induce inflammatory cytokines involved in the disease pathogenesis (tumor necrosis factor [TNF], interleukin [IL]-6, and IL-8). A novel Fc-engineered RANK fusion protein was shown to induce natural killer (NK) cell-mediated antitumor immunity against RANKL expressing targets.[12] Besides, RANKL influences the interaction of NK and AML cells by mediating a feedback loop that involves the release of factors by the latter which upregulates RANK on the former. In addition to the immediate inhibitory effects of RANKL-induced factors, RANK is readily available to interact with RANKL expressed by AML cells. This process leads to the activation of a bidirectional signal transduction cascade that causes the delivery of RANK-mediated inhibitory signals to NK cells and perpetuates RANKL reverse signaling process in AML cells.[64]

The adhesion of the freshly isolated lymphoma B cells to bone marrow stromal cells or freshly isolated lymphoma stromal cells inhibited B cell spontaneous apoptosis in culture. This inhibition of apoptosis correlated with decreased cleavage of caspase-3/8 and increased activation of canonical and noncanonical nuclear factor kappa B signaling pathway.[65] Prognostic analysis revealed a higher probability of overall survival in cases with lower RANKL expression (<1.6 and ≥ 1.6, 15.6 vs 12.2 months, $P=0.008$, hazard ratio 0.36, $P=0.008$). The study revealed that RANKL is a promising marker to forecast patients' prognosis in AML.[66]

Potential therapeutic approaches

Considering this molecular triad system as a hot spot in the area of oncology research, several therapeutic agents have been developed over the last decade. These include humanized monoclonal antibodies, herbal medicines, RNA interference technology, and proteolytic enzymes, among others (Table 3).

Table 3 Potential therapeutic agents targeting this signaling system

Therapeutic classes	Examples	Molecular mechanisms and effects	References
Monoclonal antibodies (humanized)	Denosumab	– It binds with RANKL (antigen–antibody interaction) and interrupts RANK–RANKL interaction. It mimics the antagonistic effect of OPG but it does not affect TRAIL and thereby prevents cancer cell survival and migration. It was approved by FDA for treatment	Kostenuik et al;[67] Peddi et al[68]
		– Prolonged treatment with denosumab has sustained activity in GCTB, with a mild toxicity profile	Palmerini et al[69]
		– Systemic therapy inhibits bone resorption by osteoclast-like giant cells	van der Heijden et al[70]
		– Notably, proliferation was markedly reduced in breast biopsies from Brca1-mutation carriers that were treated with denosumab	Nolan et al[71]
Osteoprotegerin-like peptidomimetics	OP3–4	– Selective inhibitors of RANKL without affecting TRAIL	Heath et al[72]
RANK receptor inhibitors	–	– Targets the cytoplasmic motifs of RANK receptor	Kim et al[73]
Peptide RANK antagonists	Novel nonapeptide series	– Targets hinge region of RANK receptor	Téletchéa et al[74]
Proteolytic enzymes	Enteropeptidase	– Cleavage of RANK on NEEDK amino acid sequences	Zhao et al[75]
RANKL targeting peptides	–	– Based on the molecular modeling (3D structure) of OPG	Naidu et al[76]
RNA interference technology	Small hairpin RNAs	– Targets interruption of RANK expression	Ma et al[77]
Organic acid derivatives	Strontium ranelate	– ↑ OPG mRNA expression and secretion	Brennan et al[78]
Anti-inflammatory drugs	Chloroquine	– ↓ RANKL expression – ↑ TRAF3 production	Xiu et al[79]
Herbal medicines	Jolkinolide B	– Root of *Euphorbia fischeriana* (source) ↓ RANKL-induced activation of NF-κB by suppressing RANKL-mediated IκBα degradation	Ma et al[80]
	WEAT	– Inhibited RANKL-induced activation of JNK, NF-κB, and CREB leading to suppression of the induction of c-Fos and NFATc1 (key transcription factors for osteoclast differentiation)	Ha et al[81]
Other agents	Afatinib	– It significantly suppresses RANKL-induced osteoclast formation in BMMs. Consistently, it inhibits the expression of osteoclast marker genes, whereas it upregulates the expression of negative modulator genes. The bone resorbing activity of osteoclasts is also abrogated by afatinib. In addition, it inhibits RANKL-mediated Akt/protein kinase B and c-Jun N-terminal kinase phosphorylation	Ihn et al[82]

Notes: ↑, increased, unregulated; ↓, decreased, downregulated, attenuated.

Abbreviations: BMM, bone marrow macrophages; CREB, cAMP response element-binding protein; FDA, US Food and Drug Administration; GCTB, giant cell tumor of the bone; JNK, Jun N-terminal kinase; NF-κB, nuclear factor kappa B; OPG, osteoprotegerin; RANK, receptor activator of nuclear factor kβ; RANKL, RANK ligand; WEAT, water extract of *Acer tegmentosum*; 3D, three-dimensional; TRAIL, tumor necrosis factor-related apoptosis inducing ligand; NEEDK, aspargine, glutamic acid, glutamic acid, aspartic acid and lysine; TRAF, tumor necrosis factor receptor associated factor.

Conclusion

The role of the RANK/RANKL/OPG system is well emphasized in this review article. Beyond regulation of many biological functions throughout the body, the system has a key role in the pathophysiology of various disorders. This triad system can be considered as a hot spot in the area of experimental oncology. This review revealed that the system is strongly implicated in the development of mammary gland structures and tumorigenesis of lobuloalveolar cells of the breast. It has also been shown to take part in the pathogenesis of several solid and nonsolid cancer types including prostate cancer, lung cancer, renal cell carcinoma, melanoma, and leukemia. What is more, the system is highly associated with tumor invasiveness and metastasis. Considering the role of this triad system in cancer cell development and metastasis, scientists are striving to discover therapeutic agents targeting these TNF-related proteins (RANK, RANKL, and OPG) and their downstream signaling pathways for better treatment of cancer and osteoporosis (bone osteolysis) in the near future. Some of these agents include denosumab, enteropeptidase enzymes, small hairpin RNAs, and Jolkinolide B.

Author contributions

All authors contributed toward conception of the original idea, data analysis, drafting and critically revising the paper and agree to be accountable for all aspects of the work . MS also prepared the final manuscript for publication.

Disclosure

The authors report no conflicts of interest in this work.

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