RANKL/RANK Axis

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Contributor: Aristofania Simatou, Panagiotis Sarantis

Receptor activator of nuclear factor -κB (RANK) and the RANK ligand (RANKL) was first reported in the regulation of osteoclast differentiation/activation and bone homeostasis. Additionally, the RANKL/RANK system is a significant mediator of progesterone-driven mammary epithelial cell proliferation, potentially contributing to breast cancer initiation and progression. Moreover , several studies supported a synergistic effect of RANK and epidermal growth factor receptor (EGFR) and described the RANK's involvement in epidermal growth factor receptor 2 (ERBB2) positive carcinogenesis. Consequently, anti-RANKL treatment has been proposed as a new approach to preventing and treating breast cancer and metastases.

Keywords: RANKL/RANK Axis ; Breast Cancer ; Denosumab ; ERBB2 positive carcinogenesis

1. Definition

The nuclear factor-κB (RANK)/RANK ligand (RANKL)/osteoprotegerin (OPG) axis was identified at the end of the 1990s as a multipotent regulator mechanism of bone remodeling[1]. It is composed of three main components: (a) the receptor activator of nuclear factor-kB ligand (RANKL), (b) the receptor activator of nuclear factor-kB (RANK) and (c) the soluble receptor of RANKL, osteoprotegerin (OPG).

2. Specifics

RANKL, a tumor necrosis factor-alpha superfamily cytokine, was initially identified on the surface of T-cells and dendritic cells (DCs)[2]. It is a type II membrane protein encoded by the *TNFS11* gene and encountered in three isoforms due to alternative splicing of the gene[3]. RANKL1 represents the full-length molecule, while in RANKL2, a branch of the intracellular domain is missing. In RANKL3, the N-terminal fraction is deleted. It has been highlighted either as a soluble or membrane form and is the ligand of the membrane receptor RANK. Soluble RANKL (sRANKL) is derived from the membrane-bound form through alternative splicing or the proteolytic cleavage and can potentially circulate in blood[4]. RANK, a member of the tumor necrosis factor receptor (TNFR) superfamily, encoded by the gene *TNFRSF11A*), is a type I transmembrane protein, including four cysteine-rich repeat motifs and two N-glycosylation sites. The binding of these two molecules leads to the recruitment of adaptor molecules such as TNF receptor-associated factors (TRAFs), the adaptor protein TRAF6 and the activation of a plethora of signaling pathways (JNK, AKT/PKB, NF-kb, MAPK/ERK and Src)[5]. Several studies suggest that oxidative stress is a key pathogenic mechanism of osteoporosis. NRF2 partakes in bone metabolism and has a critical role, providing a balance between the plasma antioxidant and oxidant biomarkers. The expression of RANKL decreases the ratio of NRF2/KEAP1, which decreases the expression of NRF2-related enzymes and favors the increase in ROS activity, a downstream molecular signal of RANKL. NRF2 could also affect osteoclastogenesis through the expression of IL-6[6]. In contrast, molecules with antioxidative activity, such as petunidin, which is a B-ring 5′-O-methylated derivative of delphinidin, act as bone anabolic agents[7]. Additionally, the RANKL/RANK axis is regulated by the soluble receptor osteoprotegerin (OPG) (*TNFRSF11B*), which is a soluble glycoprotein encountered as a 60 kD monomer or a 120 kD dimer. The dimerization of OPG increases its affinity to RANKL, and by binding to it, exerts an inhibitory effect on the axis[8].

3. The Role of the RANKL/RANK Signaling Pathway in Normal Mammary Gland Development

Beyond bone homeostasis, recent studies have pointed out the essential role of the RANKL/RANK axis in mammary gland physiology and its role as a mediator in hormone-driven epithelial proliferation through pregnancy. RANK- and RANKL-deficient mice fail to form lobuloalveolar structures during pregnancy[9]. At the same time, RANK overexpression in transgenic mice with mouse mammary tumor virus promoter (MMTV) controlled RANK, induced proliferation at midgestation and disrupted alveolar differentiation in the mammary epithelia[10].

These observations suggest that RANK's lack of overexpression leads to impaired lobuloalveolar development and consequent lactation defects. Considering the critical role of hormones during pregnancy, several studies in mice have shown that progesterone enhanced RANKL expression in cells that are already characterized by high estrogen and progesterone receptors (ER/PR) on the cell surface[11][12][13]. When RANKL expression is specifically induced in these cells, an ordered alveolar development occurs, and the RANKL signaling pathway seems to be responsible for the primary proliferative response of the mouse mammary epithelium to progesterone for the period of mammary lactation morphogenesis[14]. The RANK-Id2-p21 axis represents two main signaling pathways activated by RANK in mammary epithelial cells. IKK-α catalyzes the phosphorylation, ubiquitination and proteasome degradation of IkBα, leading to its dissociation from NF-kB, which then migrates to the nucleus and induces the transcription of cyclin D1. On the other hand, the inhibitor of DNA-binding protein 2 (Id2) translocates into the nucleus and inhibits the expression of p21, a well-known cell cycle inhibitor. Altogether, these molecular mechanisms lead to increased proliferation and the survival of cells[15].

Progesterone treatment does not seem to affect RANKL expression in human breast cancer cells that express progesterone receptors (PR+), indicating that the progesterone-mediated regulation of the mammary epithelium is limited to normal-expressing, and not extended to RANK-expressing, cancer cells. Additionally, RANKL has been shown to exert a paracrine function, mediating ER/PR- cell proliferation and promoting the progesterone-mediated amplification of mouse mammary stem cells[16][17][18].

4. The Role of the RANKL/RANK Signaling Pathway in Breast Cancer

Following the observation of progesterone being a crucial regulator of RANKL expression, which leads to enhancing the proliferation of mammary epithelial and stem cells, several studies highlight the role of the RANKL/RANK axis in the progression of breast tumorigenesis and bone and lung metastases in mice through the same pathway[19][20][21].

Consequently, anti-RANKL treatment has been proposed as a new approach to preventing and treating breast cancer and metastases[22][23][24]. Analytically, the RANK signaling pathway seems to be involved in all stages of breast cancer development, from the expansion of the partition and enhancing the proliferation of epithelial cells to increasing the resistance of tumor cells to DNA thus damaging agents and promoting metastatic potential[9][25]. Several studies identified the role of RANKL in the acceleration of migration and metastasis of cancer cells. The RANKL/RANK axis is a key molecular link between progestins and epithelial carcinogenesis. The inhibition of RANKL may be considered a putative therapeutic approach for breast cancer. These effects seem to be mediated, at least in part, by the IKK-α–NF-κB signaling pathway[21]. In addition, RANKL/RANK appears to induce epithelial to mesenchymal transition (EMT), migration and invasion through the activation of NF-κB and the upregulation of Snail and Twist in breast cancer cells[23].

The essential role of the RANKL/RANK signaling pathway in the early stages of tumorigenesis was shown in a recent study describing the response of a RANK-positive luminal progenitor (LP) subset (present in BRCA1 mutation carriers) to progesterone-induced RANKL as responsible for the transformation of LPs into basal-like breast tumor cells. The same study suggests denosumab as an effective treatment in the prevention of breast tumorigenesis[26].

Furthermore, the same signaling pathway has been identified as a regulator of the development of resistance to targeted therapies and the proliferation and expansion of cancer stem cell populations[27][28], especially in ERB2+ tumors[29].

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