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Review article

Role of the RANK/RANKL pathway in breast cancer

Ludwig Kiesel*, Annemarie Kohl

Department of Gynaecology and Obstetrics, University of Münster, Universitätsklinikum, Albert-Schweitzer- Campus 1, 48149 Münster, Germany

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ABSTRACT

The discovery of the OPG/RANK/RANKL pathway two decades ago has initiated novel insights into regulation of bone formation. More recently this pathway has been found to be also relevant in osteoclastic-independent mechanisms, mainly in mammary physiology and breast cancer. RANKL/RANK function is essential for epithelial cell proliferation and cellular survival as well as lobulo-alveolar development. The endogenous OPG functions as a soluble decoy receptor, binding the cytokine RANKL to prevent RANKL from activating its receptor RANK. The regulatory function of RANKL is one of the key factors in progesterone-induced proliferation of the breast. Progesterone has a direct action of progesterone on progesterone-receptor (PR) expressing cells but PR-negative cells are affected indirectly through RANKL-induced paracrine actions leading to proliferation of mammary epithelial PR-negative cells. RANK induces epithelial-mesenchymal transition and stemness in human mammary epithelial cells and promotes tumorigenesis and metastasis. Inhibition of the RANK/RANKL pathway using the monoclonal antibody denosumab can neutralize RANKL and inhibiting its interaction with its receptor RANK. Denosumab is currently used to treat osteoporosis and in prevention of skeletal related events in patients suffering from bone metastases due to solid tumors. As preclinical experiments suggest the RANKL/RANK pathway plays an important role in primary breast cancer development. The interference with the RANK/RANKL system could therefore serve as a potential target for prevention and treatment of breast cancer.

Contents

1. The RANK/RANKL-system: from bone health to breast cancer

The receptor activator of nuclear factor (NF)-κB (RANK), its ligand (RANKL) and the soluble receptor OPG (osteoprotegerin or osteoclastogenesis-inhibitory-factor (OCIF)) as a novel pathway was first discovered in the mid-1990s when a new receptor-ligand system belonging to the TNF-superfamily was found [1]. Since then the role of this pathway has extended from bone health to breast physiology as well as breast cancer, which will be reviewed here.

The discovery of this specific pathway allowed a crucial insight into the normal regulation of bone physiology mainly the regulation and control of bone resorption and remodeling. In this progress the main protagonists are osteoclasts, a specialized cell type of hematopoetic origin, with the ability to resorb inorganic and organic bone matrix. Their antagonists are the osteoblasts with the

* Corresponding author.
E-mail address: l.kiesel@uni-muenster.de (L. Kiesel).

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ability to form new bone. These players are critical for the skeletal mass, structure and strength [1].

The function of osteoprotegerin (OPG) or osteoclastogenesis-inhibitory-factor (OCIF) was first established by functional genomics, using transgenic mice resulting in decreased numbers and activity of osteoclasts [2–5]. These findings demonstrated that OPG suppresses bone resorption thereby increased amount of bone mass. This protein was therefore called "osteoprotegerin" due to its ability to protect bone [1]. However, OPG is an atypical member of the TNF receptor family, since it lacks a transmembrane domain or direct signaling properties [6]. It contains four cysteine-rich TNF receptor homologous domains for binding its specific target RANKL [7]. Knockout-mice lacking either RANK or its ligand RANKL showed an identical phenotype. Due to the absence of osteoclasts and bone resorption these mice developed significant osteopetrosis [8,9].

The cytokine RANKL (receptor activator of nuclear factor κB ligand) formerly referred to as TRANCE (TNF-related activation-induced cytokine) is expressed by bone stromal cells of the osteoblast lineage [10]. It mediates bone resorption [11]. As a member of the TNF-family, numerous cell types including cells of the osteoblast lineage [12,13] or activated T cells [14–16] express at least three different subtypes of RANKL [17,18]. Two of these RANKL-subtypes remain on the cell surface until proteolytically sliced into their soluble forms, showing the capacity to bind their TNF-homology domains. RANKL attaches to its natural receptor RANK [8,19] leading to the fusion of osteoclast precursor cells [20] into cells that show a multinucleated phenotype [21], followed by their differentiation into mature osteoclastic cells [21]. These osteoclasts are then stimulated to attach to the surface [22,23] and activated to resorb bone [5,21]. They also show the capacity to continuously resist cell death by the avoidance of apoptosis [24]. In this process OPG functions as a soluble decoy receptor, binding RANKL in order to prevent RANKL from activating its receptor RANK. It therefore inhibits the catabolic effects of RANKL. Formation, attachment to the bone [22,23], activation [3–5] and survival [24,25] of osteoclasts are effectively stopped. The cortical and cancellous bone volume, density and strength are increased. This precise and balanced interaction between all three participating actors ensures the maintenance of homeostatic bone remodeling. Since OPG directly binds to RANKL the relative balance of the two actors is important for the fragile system to properly function [26,27]. The understanding concerning the interactions of OPG-RANK-RANKL led to the hypothesis that any dysregulation occurring in a disease state including postmenopausal osteoporosis or cancer-induced bone destruction may involve the described pathophysiology [10].

1.1. RANK/RANKL and progesterone signaling in mammary physiology

The RANK/RANKL pathway has also osteoclast-independent effects, e.g., in the normal breast. Mammary tissue consists mainly of two different epithelial cell types. First, luminal epithelial cells, responsible for the production of breast milk. Second, basal, myoepithelial cells, having a contractile function during lactation [28]. Across species, including mouse models and human mammary tissue 30–50% of the cells express estrogen and progesterone receptors [29]. Several distinct steps take place in mammary morphogenesis. First the fetal mammary anlage shows ductal elongation and branching [30]. During puberty the mammary branching continues and the branching of the ductal tree into the mammary fat pad takes place [31]. In case of a pregnancy the developed ductal tree shows significant side branching and the ductal and alveolar epithelium shows expansion and proliferation [32].

During pregnancy several changes occur in mammary biology and structure including mammary gland morphogenesis and formation of a functioning lactating mammary gland [33]. Associated

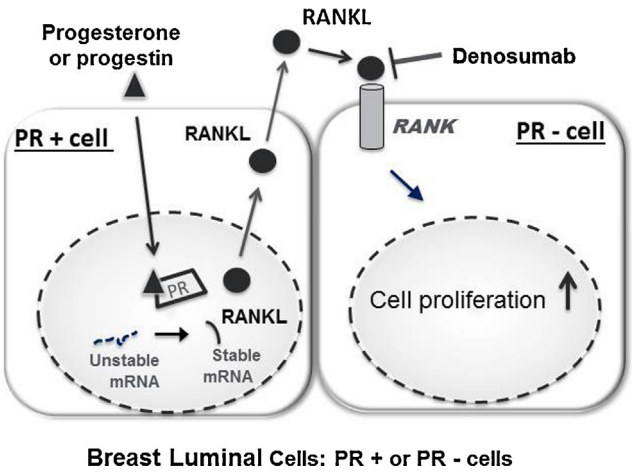

Fig. 1. Mechanisms of signal transduction of RANKL/RANK and progesterone receptor(PR) in PR+ and PR− breast luminal cells (adapted from Ref. [29]).

with these changes in mammary structure the expression of RANK protein shifts. RANK is mainly seen in regions of lobular branching [34]. In the virgin mammary gland of the mouse only low amounts of RANK protein is expressed. Around day 15 of pregnancy a strong up regulation is observed whereas the expression decreases to intermediate levels on the first day of lactation [34]. These results were transferred to human mammary physiology where RANKL expression is also increased by 2.5–3 fold between the 17th and 26th day of the menstrual cycle [35].

RANK or RANKL knockout mice showed distinctive osteoclast-independent effects on mammary morphology similar to those in mice lacking the progesterone receptor [36]. A population of mice exposed to the RANK-/RANKL knockdown manifested severe lactation defects owed to their failure to develop functioning lobulo-alveolar mammary epithelial structures. RANKL shows low expression levels in luminal and basal mammary epithelial cells during the normal female cycle [34]. Under normal conditions, raising lactation hormone levels at mid-gestation pregnancy lead to massive expansion of these breast milk producing cells especially of the ductal branch points [34]. Hence the RANKL/RANK function is essential for epithelial cell proliferation and cellular survival as well as lobulo-alveolar development [33,35]. These changes are induced by a specific up regulation of RANKL expression [35].

The RANK/RANKL-system are regulated by sex hormones [36–38] as well as by prolactin or parathyroid-hormone-related-protein (pTHrP) [39]. One of the clinically important molecular effects involve the interaction of the signaling mechanisms of progesterone and the RANK/RANKL pathway. In this regard progesterone has two distinct cellular actions (Fig. 1). First, there is a direct action of progesterone on progesterone-receptor(PR) expressing cells via cyclin D1-dependent interactions within 24 h. Second, and more importantly PR-negative cells are affected through RANKL-induced paracrine actions leading to proliferation of mammary epithelial PR-negative cells [40].

RANKL and progesterone are co-localized in luminal epithelial cells acting on similar stages of the mammary lactation morphogenesis [39]. Progesterone leads to an up regulation of RANKL in luminal cells expressing progesterone receptors (Fig. 1). In addition RANKL acts also by paracrine effects on cells expressing neither the estrogen- (ER) nor the progesterone receptor in its neighborhood [40–42]. This effect is greater compared to receptor-positive cells in mouse models. In human, however, experiments conducted in human mammary epithelial cells taken either from the primary breast cancer or the contralateral breast showed an more pronounced increase RANKL expression in receptor positive mammary