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Journal of Bone Oncology

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Research paper

The role of extracellular calcium in bone metastasis



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ARTICLE INFO

Article history:

Received 21 December 2015

Received in revised form

15 June 2016

Accepted 20 June 2016

Available online 7 July 2016

Keywords:

Calcium

Bone metastasis

CaSR

Ion channels

ABSTRACT

This review summarizes the role of extracellular calcium, as found present in the bone tissue, in the process of bone metastasis.

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1. Introduction

The microenvironment of bone tissue is characterized by a unique composition. Due to the constitutive degradation and rebuilding by osteoclasts and osteoblasts it presents a high concentration of growth factors and calcium. Tumor cells recognize extracellular calcium by the calcium-sensing receptor (CaSR) or the P2X-receptor, and mediate calcium influx or efflux through ion channels and transporters. In renal, breast and prostate cancer, calcium has been shown to promote bone metastasis via CaSR. In addition, the ion channels TRPV6 and SK3 have been shown to be relevant factors in the formation of bone metastasis. This would implicate that extracellular calcium plays a role in the formation of bone metastasis via varying routes. This review studies the impact of extracellular calcium, as found present in bone tissue, on bone metastasis.

Abbreviations: AKT, AKT8 virus oncogene cellular homolog; BMP's, bone morphogenetic proteins; cAMP, cyclic adenosine monophosphate; CaSR, calcium-sensing receptor; COPD, chronic obstructive pulmonary disease; ERK, extracellular signal-regulated kinase; ET-1, endothelin-1; FGF, fibroblast growth factor; IGF, insulin-like growth factor; JNK, jun N-terminal kinase; MAPK, mitogen-activated protein kinase; M-CSF, macrophage colony-stimulating factor; PDGF, platelet-derived growth factor; PGE-2, prostaglandin E-2; PKA, protein kinase A; PLC, phospholipase C; PSA, prostate specific antigen; PTEN, phosphatase and tensin homolog deleted on chromosome 10; PTHrP, parathyroid hormone-related protein; RANK, receptor activator of NF- κ B; RANKL, receptor activator of NF- κ B ligand; SK3, small conductance calcium-activated potassium channel 3; TGF β , transforming growth factor beta; TRP, transient receptor potential

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1.1. The shaping of metastasis through bone microenvironment

As early as 1889, Stephen Paget developed the so-called “seed and soil” theory, which postulates a highly significant importance of the microenvironment in the secondary site in organ specific metastasis [1]. This implicates that different organs have a particular characteristic (“soil”) which is preferred by metastasizing tumor cells of specific feature (“seed”). The bone is an organ with a unique microenvironment, differing from all other organs of the body. Due to the circumstance that bone tissue is constitutively degraded by osteoclasts and rebuilt by osteoblasts, numerous growth factors and cytokines are released, including transforming growth factor beta (TGF β), insulin-like growth factors I and II (IGF I and II), fibroblast growth factor (FGF) and platelet-derived growth factors (PDGF), as reviewed by Roodman [2]. Among the high concentration of these bioactive agents, bone is characterized by a high concentration of calcium ions. Whereas in peripheral blood plasma calcium is found present in a concentration range between 2.2 and 2.6 mM, the concentration in bone plasma is around 10 mM, in the extracellular space of bone tissue even around 20 mM [3]. This reflects an ever increasing calcium concentration beginning outwith the bone and progressing to the inner bone tissue. Extracellular calcium affects cells by various different mechanisms: These can be calcium channels as well as calcium binding proteins. The calcium-sensing receptor (CaSR) is an important calcium binding receptor in bone tissue, which seems to be highly significant for the formation of bone metastasis.

1.2. Bone metastasis induced by CaSR

The heterodimeric G-protein coupled CaSR is the most important receptor on the cell surface for detecting extracellular

calcium. In healthy tissue, CaSR is responsible for the physiological regulation of calcium homeostasis in several organs such as kidney, breast, gastrointestinal tract, bones and parathyroid glands, as reviewed by Tennakoon et al. [4]. The binding of calcium on CaSR activates a variety of signaling pathways.

Although CaSR may also have a tumor suppressing capacity in e.g. gastric and colon cancer [5,6], it has been demonstrated to be involved in bone metastasis of several tumor entities, such as renal cell carcinoma, prostate carcinoma and breast cancer. In renal cell carcinoma tissue from patients developing bone metastases during a five year period after nephrectomy, CaSR was expressed distinctly higher than in the tissue of patients without bone metastases. This was independent of the development of metastases in other secondary sites such as the lung, or if the patient remained free of metastases. Primary renal carcinoma cells from patients with bone metastases showed a similar CaSR expression and were highly sensitive to calcium treatment concerning cell proliferation and chemotactical migration directed to enhanced calcium concentration. The CaSR inhibitor NPS 2143 elucidated the role of CaSR on the calcium-dependent effects. Signaling pathways including AKT, phospholipase C γ 1 (PLC γ 1), p38 α , JNK and PTEN have been identified as being responsible for the increased metastatic behavior of CaSR-bearing renal cancer cells after calcium treatment [7].

Saidak et al. also showed a significantly higher migratory capacity in a breast cancer cell line metastasizing into bone (MDA-MB-231) compared to cells with a lower bone-metastasizing behavior (MCF7 and T47D) or cells not metastasizing into bones (BT474). The involvement of CaSR in these effects was proved by using CaSR inhibiting siRNA. The ERK1/2 MAPK and PLC β signaling pathways have been shown to be involved in these effects [8].

Similar to the situation in healthy renal and breast tissue, CaSR is also expressed in prostate tissue. Using microarray analysis, Feng et al. demonstrated a significantly higher CaSR expression in primary prostate cancer tissue from patients with bone metastases compared to those without bone metastases [9]. This reflects the

former results that, in contrast to the non-skeletal metastatic prostate cancer cell line LNCaP, in bone metastatic prostate cancer cell lines PC-3 and C4-2B CaSR was highly expressed and calcium treatment induced an enhanced proliferation. As observed in renal cancer, in prostate cancer the AKT signaling pathway also seems to be involved in CaSR dependent bone metastasis [10].

Chirgwin and Guise defined a “vicious cycle” in bone metastasis, activated when metastasizing tumor cells come in contact with bone tissue. Growth factors such as TGF β , released during bone degradation by osteoclasts, stimulate tumor cells to secrete parathyroid hormone-related protein (PTHrP), prostaglandin E-2 (PGE-2) and macrophage colony-stimulating factor (*m*-CSF), which increase the expression of RANKL (receptor activator of NF- κ B ligand) on immature osteoblasts [11]. This again activates osteoclastogenesis via RANK on osteoclast precursors, leading to enhanced osteolysis, resulting in an enhanced secretion of growth factors and calcium, again activating tumor cells [12]. However, not only growth factors are able to activate a “vicious cycle”: An activation of CaSR by calcium also induces a release of PTHrP by several tumor cells thus facilitating bone metastasis, as reviewed by Saidak et al. [8]. This process is mediated by the ERK1/2 signaling pathway (Fig. 1) [13]. In prostate cancer, which forms mostly osteoblastic metastases a similar “vicious cycle” under involvement of prostate specific antigen (PSA) and endothelin-1 (ET-1) is suggested [14].

In addition to CaSR, calcium ion channels are also relevant for the effect of extracellular calcium on cancer cells, thus influencing bone metastasis. A selection of calcium ion channels bearing impact in bone metastasis is described below.

1.3. The inducement of bone metastasis through calcium-dependent ion channels

Calcium ion channels are capable of changing the intracellular calcium concentration and activating several signaling pathways. The calcium-selective channel TRPV6 [15] has been shown to play

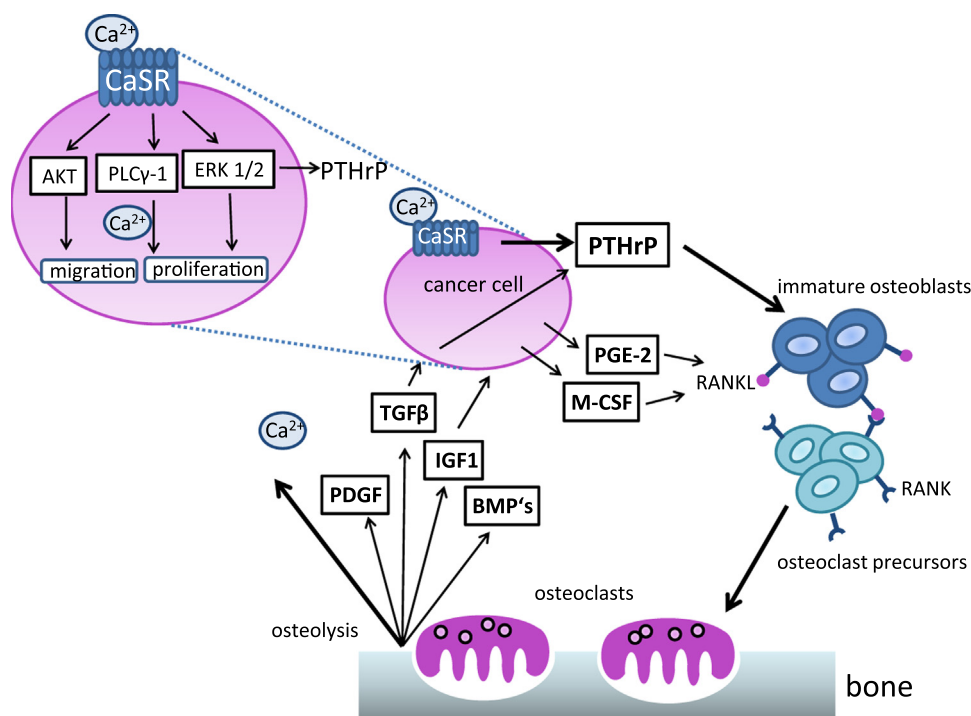


Fig. 1. “Vicious cycle”. Calcium and other growth factors trigger the secretion of PTHrP, PGE-2 and M-CSF in cancer cells. This leads to an increased expression of RANKL on immature osteoblasts, which activates the formation of osteoclasts by precursors. The osteoclasts induce enhanced bone degradation, whereby calcium and growth factors such as PDGF, TGF β , IGF1 and bone morphogenetic proteins (BMP's) are released.

a role in bone metastasis. Prostate carcinoma cells overexpressing TRPV6 show highly aggressive behavior. When prostate PC-3 cells are transfected with the TRPV6 gene and infected into the bone marrow cavity of the tibia of mice, osteoblastic lesions and enhanced formation of bone metastasis occur [16].

Another member of the TRP family, TRPM7, a cation channel with kinase activity, may also play a role in bone metastasis. Neuroblastoma cells transfected with TRPM7 highly accumulate in bone tissue. However, TRPM7 activity seems only to induce cell migration into bone tissue, but does not seem to stimulate cell proliferation. This would suggest that bone metastasis formation reflects a combination of several factors [17].

The SK3 channel, a calcium activated potassium channel, has also been shown to be associated with bone metastasis [18]. In a complex with Orai1, located in lipid rafts of the cell membrane, it controls the calcium influx and so regulates cancer cell migration, leading to the formation of bone metastases. This effect depends on the cAMP-PKA pathway [19].

Furthermore the P2X-receptor has been discussed as contributor for bone metastasis [20]. Extracellular nucleotides can trigger a P2X-mediated influx of calcium, thus leading to increased cell proliferation and survival through the AKT pathway [21].

2. Conclusion

Bone metastasis depends on induction of proliferation and less on tumor cell seeding. The “seed and soil” theory of Paget implicates a balance between the influence of seeded tumor cells and of the microenvironment of the secondary organ. However, in bone tissue the microenvironment seems to be the main influential factor in facilitating bone metastasis. In prostate and breast cancer it is not the number of tumor cells seeded in bone tissue that affects the development of bone metastases but the growth supporting microenvironment of the bone, created by bone turnover [22]. The revival of dormant cancer cells in the bone tissue may also be influenced by changes in the bone microenvironment since in myeloma cells dormancy was shown to be switched off by osteoclasts remodeling [23]. However, an effect of calcium in this process could not be shown. Apart from other bone specific cytokines and growth factors, calcium is one of the key players in the formation of bone metastasis and should be taken into consideration when planning therapeutic strategies for preventing bone metastasis. In other diseases such as chronic obstructive pulmonary disease (COPD) or allergic asthma, calcilytics inhibiting CaSR are discussed as new therapeutics [24,25] and could also be used for preventing bone metastasis. Furthermore, the calcium mimetic Ra-223, blocking CaSR, is an example of a new bone-targeted therapy with potent antitumor effects in first trials [26].

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