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Review

The anti-metastatic micro-environment of the bone: Importance of osteocyte Cx43 hemichannels

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ABSTRACT

Bone metastases of tumor cells are a common and life-threatening feature of a variety of late-stage cancers, including breast cancers. However, until now, much less has been known about the intrinsic anti-metastatic properties of the bones and how these could be exploited to prevent or treat bone metastases. Very recently, native Cx43 hemichannels present in osteocytes have been identified as important anti-metastatic signaling complexes by establishing high local extracellular ATP levels. Moreover, bisphosphonate drugs, applied as adjuvant therapies in the treatment of breast cancer patients and bone diseases, are known to display anti-metastatic properties. Now, it became clear that these compounds exert their effects through osteocyte Cx43 hemichannels, thereby triggering their opening and promoting ATP release in the extracellular micro-environment. Hence, endogenous osteocyte Cx43 hemichannels emerge as important and promising therapeutic targets for the prevention of bone metastases and/or clinical treatment of bone-metastasized breast cancers.

Contents

1. Bone metastasis of breast cancers and clinical use of bisphosphonate drugs

Tumor cells often metastasize to different organs. This is a major life-threatening complication in many patients suffering from cancers, including breast cancer, at a late stage [1]. One of the major target organs for metastasis of cancer cells is the bone tissue. In the bone, osteocytes arise from osteoblasts trapped within the mineralized matrix. Osteocytes dynamically control bone density by impacting the function of bone-forming osteoblasts and bone-resorbing osteoclasts (reviewed in [2]). Different mechanisms for the occurrence of breast cancer metastases in the bone tissue have been proposed, including the production of osteoprotegerin by breast cancer cells and altered Runx2-dependent signaling in breast cancer cells [3,4]. However, much less is known about the intrinsic anti-metastatic properties of the bone micro-environment. In addition to this, the relatively well-tolerated bisphosphonate drugs applied to treat bone loss and bone diseases, including

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cancer treatment-induced bone loss, also reduce the risk of bone metastasis [5,6]. Bisphosphonates are associated with prolonged disease-free survival and with lower breast cancer incidence [7]. A meta-analysis revealed a significant benefit of having adjuvant bisphosphonate treatment in early breast cancer patients who were in post-menopause [8]. Very recently, a European panel has recommended the use of bisphosphonates as an adjuvant in the routine clinical practice for the treatment of early breast cancers, e.g. in post-menopause women [9,10]. Yet, the therapeutic mode of action of bisphosphonates on the bone and the molecular targets participating in its physiological effects remained not fully understood.

2. Cx43 proteins function as gap junctions and hemichannels and are present in osteocytes

A major determinant for the physiological function of osteocytes in bones is the 43-kDa connexin protein (Cx43), a 4-transmembrane-domain protein with intracellular N-terminal, loop and C-terminal domains and two extracellular loops [11]. The Cx43 protein assembles into hexameric channels that can function as "free" hemichannels or "head-to-head"-docked gap junction channels [12,13], establishing intercellular communication and coordination by mediating the passage of low-molecular weight molecules (Mw < 1.5 kDa) [11]. Gap junctions allow the direct exchange of intracellular ions, like K^+, Na^+ and Ca^{2+}, signaling molecules, like IP_3, and molecules with biological functions, including short amino acid peptides and microRNAs. Hemichannels allow the exchange (release or entry) of low Mw molecules with the extracellular environment, establishing local, paracrine signaling networks and micro-environments through ATP, NAD^+, glutamate and prostaglandins [14]. For instance, Cx43 hemichannels can participate in the release of ATP, thereby eliciting purinergic receptor activation and Ca^{2+} signaling in neighboring cells, detectable as intercellular Ca^{2+} waves [15,16]. Physiologically, in osteocytes, Cx43 hemichannels are mechanosensitive and become activated by fluid flow and shear stress [13]. Therapeutically, part of the bone-protective actions of bisphosphonate drugs could be attributed to its ability to trigger Cx43-hemichannel opening, thereby promoting cell survival and suppressing apoptosis in osteoblasts and osteocytes (critically reviewed in [17]). The presence of functional Cx43 hemichannels in osteoblasts and osteocytes is critical for the activation of cell survival signaling pathways, like Src and ERK, in response to bisphosphonate drugs and to protect against *in vivo* bone loss induced by glucocorticoids [18–20]. Very recently, the negative impact of glucocorticoids on osteocytes has been linked to a prominent decrease in Cx43-protein levels [21]. This is due to glucocorticoid-induced inhibition of Akt-mTORC1 signaling, which leads to an upregulation of autophagy [22], a major turn-over pathway for Cx43 gap junctions and hemichannels [22–25]. Indeed, independent studies previously showed that while high doses of glucocorticoids could induce apoptosis in osteocytes, low doses of glucocorticoids induced autophagy in osteocytes [26], which will negatively impact Cx43-protein levels.

3. Cx43 and its role in oncogenesis and cancer hall-marks

3.1. Cx43 in tumor cells

Cx43 expression has been linked to different tumor biological aspects for a variety of cancers, including breast cancers [27,28]. This involves channel-dependent functions by promoting gap junctional and/or hemichannel-mediated intercellular communication and channel-independent functions by sequestering putative gene regulators and by providing Cx43-protein fragments that can translocate to the nucleus and regulate gene transcription [29]. Most insights about the role of Cx43 in the oncogenesis of cancer cells have been obtained on Cx43 channels present in tumor cells, where they impact different "hall-marks of cancer". In different cancer cell types, including glioma and breast cancers, Cx43 expression inhibits cell-cycle progression tumor growth, proliferation and migration, although Cx43 can also enhance cell migration and motility [29–31]. This dichotomous role for Cx43 is also reflected in its altered expression in different stages of tumor formation and spreading. Indeed, Cx43 expression is often lost during the early stages of the formation of primary breast tumors, while Cx43 expression is upregulated during metastasis and secondary tumor formation [28]. As such, Cx43 functions as a tumor suppressor in primary breast tumors, while it can act as either tumor suppressor or tumor promoter in advanced breast cancers at later stages dependent on the context [32].

Moreover, the effects of Cx43 expression on tumor cells might be dependent on its localization. For instance, in prostate LNCAP cancer cells, re-expressed Cx43 occurs at the cell membrane and increases tumor growth and invasion, while in PC-3 prostate cancer cells, re-expressed Cx43 occurs in the cytosol and prevents tumor growth and invasion. Also, in prostate cancer cell lines, Cx43 expression appeared to positively correlate with their increasing metastatic potential [33]. Knocking down Cx43 suppressed the metastatic properties of these cancer cells, while it did not impact their cell growth. Cx43 expression can also impact cell death and survival, processes often dysregulated in cancer cells. Cx43 gap junctions and hemichannels have been implicated in the spreading of cell death and survival factors, e.g. by facilitating the spreading of signaling molecules that become released upon mitochondrial outer membrane permeabilization, an important onset point for apoptosis [34,35].

3.2. Cx43 in target tissues for hosting metastasized tumors

Besides the extensively studied role of Cx43 in the tumor cells, including breast cancers (recently thoroughly reviewed in [28]), Cx43 from host tissues also controls the eventual seeding and growth of the metastases and secondary tumors. Cx43 present in the host tissues promotes cancer cell migration and tissue invasion by forming hetero-cellular gap junctions between Cx43 from the tumor cells and Cx43 from the host tissue [30]. Via these hetero-cellular gap junctions, glioma cells can transfer a specific subset of microRNAs to astrocytes, like miR-5096, which contributed to the pro-invasive effect [36]. The pro-invasive role of Cx43 has recently been observed *in vivo* in the peri-tumoral region between glioma cells and astrocytes [37]. Absence of Cx43 in the tumors or in the astrocytic host environment attenuates invasion in the astrocyte environment. Very recently, further mechanistic insights on the interplay between metastasized tumor cells and astrocytes have been obtained [38]. New Cx43 gap junctions are formed between tumor cells and astrocytes through protocadherin 7, which is expressed in astrocytes and in several cancer cells, including the ones originating from breast and lung tissues. These hetero-cellular Cx43 gap junctions between cancer cells and astrocytes provide an exchange pathway for second messenger cyclic GMP-AMP (cGAMP) signaling molecules from cancer cells towards astrocytes. In astrocytes, cGAMP initiate "stimulator of interferon genes" (STING) signaling, an innate immune response pathway activated upon double-stranded DNA appearance in the cytosol [39]. STING activation results in the production and release of inflammatory cytokines interferon-α and tumor necrosis factor from astrocytes. As a consequence, tumor cells are influenced by these paracrine factors, activating STAT1 and NK-κB pathways, which favor tumor growth and promote chemoresistance [38]. However, besides the pro-metastatic roles of native Cx43, like in astrocytes, Cx43 expression in other host tissues like the lungs can also counteract tumor metastasis and cancer growth in these tissues, as lack of proper Cx43 expression in the host organism facilitated the occurrence of mammary gland tumor metastasis to the lungs [40].

However, until now, the impact of Cx43 channels on tumor metastasis in the bone and their role in the anti-metastatic properties of bisphosphonate drugs were not fully understood. In particular, most studies describing a role for Cx43 in metastasis and invasion reported