Molecular Determinants of Cancer-Induced Bone Pain

Subjects: Oncology | Primary Health Care

Contributor: Marco Ponzetti

Cancer-related pain is arguably the most common consequences of this disease, significantly reducing quality of life and affecting the ability to complete everyday tasks and live a normal life. Among these, cancer-induced bone pain (CIBP) is one of the most prevalent, presenting as movement-related, constant or most commonly, in combination. Bone metastases can then induce CIBP in several ways, many of which are still under investigation. Indeed, bone is a richly innervated tissue, and sensitive neurons can be found in both the periosteum and the bone marrow.

bone pain | osteoclasts | osteoblasts | cancer induced bone pain | bone oncology | bone metastases | vicious cycle

1. Acidity

Osteoclasts, and in particular conditions also osteocytes, use acidity to solubilise the mineralised fraction of the bone matrix, allowing ionic calcium and phosphates to be released into the bloodstream [1]. The acidification process must be tightly controlled to avoid protons leaking towards the bone marrow and having ineffective bone resorption. Physiologically, osteoclasts create a tightly sealed area using podosomes, limiting proton efflux to a discreet area between the osteoclast itself and the bone matrix, called the resorption lacuna [2]. When local bone resorption is complete, osteoclasts release the seal and move to a close area or undergo apoptosis. In normal conditions this process involves little or no proton leakage, and the relatively small number of osteoclasts guarantees that the pH of the bone marrow is not altered by bone remodelling. Cancer-induced osteoclastogenesis creates an acidity excess in the bone marrow by increasing absolute osteoclast number. Additionally, tumour cells also contribute to the acidification of the microenvironment through the Warburg effect, especially considering that bone is not a normoxic tissue, but a hypoxic one (5% pO2) [3], which stimulates cancer cells to switch towards a glycolytic metabolism, and therefore produce lactate and protons. Acidity is therefore an important (and often overlooked) result of the vicious cycle especially on the standpoint of bone pain.

Although the role of acidity in nociception seems clear in fields such as gastroenterology, the molecular mechanisms underlying acidity-induced bone pain are not as clear to date. Recently, studies on mouse and rat models of cancer-induced bone pain (CIBP) revealed that there are two main families of receptors that are activated by acidity: transient receptor potential channel-vanilloid (TRPVs) subfamily members and acid-sensing ion channels (ASICs) [4][5][6][7][8][9]. TRPVs and ASICs are described as proton-activated, cationic current-generating

receptors, and are present in periosteal as well as bone marrow nociceptive terminals. The main molecular players belonging to this family seem to be TPRV1 and ASIC3. Although similar in function, they have different features as far as pH-sensitivity and sensitisation/desensitisation go.

TRPV1 is expressed by calcitonin gene related peptide-positive (CGRP+) C-type neural sensory terminals. It is activated by pH < 6, and sensitised to other stimuli, such as capsaicin, heat and inflammatory mediators between pH 6 and 7 [10][11]. To confirm the role of TRPV1 in CIBP, recent preclinical studies showed that treating mouse models of CIBP with either an antagonist of TRPV1 or administering another TRPV1 antagonist along with morphine, caused reduction (or further reduction in the case of co-administration) of CIBP [4][12].

At variance with TRPV1, ASIC3 is activated by milder acidosis (pH 6.7–7.3), and different models of pain showed that its activation is sufficient to induce nociceptive behaviours [13][14]. Although its role in CIBP seems clear, researchers are still to demonstrate that inhibiting its signalling can reduce CIBP in solid tumours, although a study is present where inhibiting ASIC3 is able to reduce CIBP in multiple myeloma [15]. However, an inhibitor for this channel has been developed and has been used to reduce osteoarthritic pain, and even its progression [16].

The different pH sensitivity of these two receptors convinced investigators that they are used to discriminate between mild and severe extracellular acidification of the bone marrow [17].

2. Neurotrophins

Neurotrophins include a small family of molecules: nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF) and neurotrophin-3 (NT-3). Neurotrophins have a coreceptor—p75—that is common to all of them, and a specific receptor that is tropomyosin-related kinase (Trk)-A for NGF, TrkB for BDNF and TrkC for NT-3 [18]. These are present in both the CNS and the CGRP+ sensory terminals in the bone marrow. Usually, NGF is considered as the most potent pain inducer and blocking its binding to TrkA using a monoclonal antibody (mAb911, Rinat/Pfizer) is able to strongly reduce CIBP in mouse models [19][20][21]. Tanezumab, a humanised version of mAb911, is currently in a Phase 3 clinical trial in association with opioids in a randomised, double-blind, placebo-controlled, multicenter, parallel-group study (Clinical trial NCT02609828) and is probably the drug that currently holds the most promise for pain management in bone metastatic patients, in addition to anti-resorptive drugs (read below).

BDNF could also have a role in sensitising central neurons to pain response [22], therefore general Trk inhibitors are also being developed [23]. Intriguingly, metastatic breast and prostate cancer cells express high levels of NGF and BDNF [24][25], which not only can stimulate nociceptors directly, but may also induce macrophages to secrete TNF-α, IL-6, IL-1β and prostaglandin (PG)E2 [26]. These inflammatory mediators can further activate pain responses, fuel the vicious cycle, sensitise nociceptors and activate other molecular mediators of pain, such as acidity [27][28][29]. A further link between NGF and acidity is that this neurotrophin can sensitise TRPV1 to protons and increase its expression, causing acidity-directed allodynia and hyperalgesia, which further worsens CIBP [10].

3. Inflammatory Cytokines and Chemokines

As mentioned above, ILs can increase osteoclastogenesis and tumour survival, but also induce bone pain. As a matter of fact, IL-1β, arising from cancer-related inflammation, increases macrophage expression of cyclooxygenase (COX)-2, eventually leading to an increased production of prostaglandins, which bind prostanoid receptors on sensory terminals, resulting in CIBP [30][31]. Supporting the conclusion that IL-1β is involved in CIBP, an in vivo mouse model of osteosarcoma showed that this interleukin is not only increased in the tumour area, but also in the spinal cord, and inhibiting its receptor reduces mechanical and thermal hyperalgesia [32].

TNF-α is another notable hyperalgesia-inducing substance: it has been suggested that this happens through the sensitisation of TRPV1 channels, linking back to acidity [33]. Monocyte chemoattractant protein (MCP)-1 has also been directly implicated in CIBP and mechanical allodynia and, as demonstrated by Hu and colleagues, intrathecal administration of an MCP-1 neutralising antibody reduces CIBP in a breast cancer model of bone metastasis [34]. Although other cytokines and chemokines have been correlated to bone pain [35] the link with CIBP is most likely indirect and is dependent on osteoclastic bone resorption.

4. Other Microenvironment- and Tumour-Derived Factors

Although the aforementioned factors would be sufficient to explain most of CIBP (if every hypothesis proves correct), there is still a sizeable side of it that still needs to be explained. As a matter of fact, many other molecular mediators emerged in the last few years.

One of the most important examples is arguably ATP. This molecule is present in every single living cell, and it is never found in the extracellular environment, unless there is cell or nerve damage, which may culminate in necrosis. Extracellular ATP can be sensed by the purinergic receptors P2X (ionotropic) and P2Y (metabotropic) [36]. A member of the former family, P2X3, has been studied extensively in CIBP, since it is expressed quite specifically in small diameter nociceptive fibres throughout the bone marrow and periosteum [37]. Interestingly, treating rats with antagonists for this receptor reduces pain-related behaviours in CIBP models, and the same has been shown in mouse models [37][38]. Nowadays, second generation P2X3 receptor antagonists are being developed to overcome some of the limitations encountered by the first-generation antagonists, such as dysgeusia (i.e., alteration of taste) and hypogeusia (i.e., reduction of taste) (Clinical trial NCT03449134). Nevertheless, the P2X3 antagonist Gefapixant has gone through several Phase 2 clinical trials with promising results for chronic endometriosis-related pain (ongoing, NCT03654326) and bladder pain syndrome (completed, NCT01569438), but still not CIBP.

TGF-β1 and IGF-1 are also two strong candidates for the induction of CIBP. They are highly represented in the organic bone matrix [39] and are therefore released in the microenvironment during tumour-induced bone resorption. Intriguingly, they are already notorious vicious cycle-fuelling factors [40]. A recent report showed that TGF-β1 signalling is crucial for CIBP onset and development in a preclinical study on rats [41]. This makes TGF-β1 an even more interesting molecular target for cancer bone metastases, although the ubiquitous nature of this protein makes inhibition a difficult path to walk. Nevertheless, a number of clinical trials have been completed or are ongoing, and many of them show promising results. As for IGF1, it has been shown to sensitise the TRPV1 receptor, promoting acidity-induced nociception in the context of bone metastases [42]. Another important mediator

of cancer induced bone pain is osteocyte-derived Sclerostin (SOST) [43]. Osteocytes, in addition to mediating pain in response to microfractures, which are often cancer-related [5], release SOST as a consequence of multiple myeloma (MM). SOST is a potent inhibitor of the Wnt/β-Catenin pathway, which in this case appears to be a driver of MM-induced bone disease. In fact, genetically ablating SOST or blocking it with an antibody can strongly reduce osteolysis and consequent acidity-mediated pain [43].

Finally, serotonin which seems to be able to increase sensitivity of ASIC3 in the dorsal root ganglion (DRG) of rats intratibially inoculated with cancer cells [44] and to modulate TRPV1 activity as well [45].

References

1. Teti, A.; Zallone, A. Do osteocytes contribute to bone mineral homeostasis? Osteocytic osteolysis revisited. Bone 2009, 44, 11–16.
2. Cappariello, A.; Maurizi, A.; Veeriah, V.; Teti, A. The great beauty of the osteoclast. Arch. Biochem. Biophys. 2014, 558, 70–78.
3. Eliasson, P.; Jonsson, J.I. The hematopoietic stem cell niche: Low in oxygen but a nice place to be. J. Cell. Physiol. 2010, 222, 17–22.
4. Ghilardi, J.R.; Röhrich, H.; Lindsay, T.H.; Sevcik, M.A.; Schwei, M.J.; Kubota, K.; Halvorson, K.G.; Poblete, J.; Chaplan, S.R.; Dubin, A.E.; et al. Selective blockade of the capsaicin receptor TRPV1 attenuates bone cancer pain. J. Neurosci. 2005, 25, 3126–3131.
5. Wakabayashi, H.; Wakisaka, S.; Hiraga, T.; Sakurai, T.; Tominaga, M.; Yoneda, T. Role of acid-sensing TRPV1 in bone pain associated with cancer colonization in bone. J. Bone Miner. Res. 2005, 20 (Suppl. 1), S32.
6. Nagae, M.; Hiraga, T.; Yoneda, T. Acidic microenvironment created by osteoclasts causes bone pain associated with tumor colonization. J. Bone Miner. Metab. 2007, 25, 99–104.
7. Pan, H.L.; Zhang, Y.Q.; Zhao, Z.Q. Involvement of lysophosphatidic acid in bone cancer pain by potentiation of TRPV1 via PKCepsilon pathway in dorsal root ganglion neurons. Mol. Pain 2010, 6, 85.
8. Lautner, M.A.; Ruparel, S.B.; Patil, M.J.; Hargreaves, K.M. In vitro sarcoma cells release a lipophilic substance that activates the pain transduction system via TRPV1. Ann. Surg. Oncol. 2011, 18, 866–871.
9. Qiu, F.; Wei, X.L.; Zhang, S.Z.; Yuan, W.X.; Mi, W.D. Increased expression of acid sensing ion channel 3 within dorsal root ganglia in a rat model of bone cancer pain. Neuroreport 2014, 25, 887–893.

10. Caterina, M.J.; Leffler, A.; Malmberg, A.B.; Martin, W.J.; Trafton, J.; Petersen-Zeitz, K.R.; Koltzenburg, M.; Basbaum, A.I.; Julius, D. Impaired nociception and pain sensation in mice lacking the capsaicin receptor. Science 2000, 288, 306–313.
11. Nakanishi, M.; Hata, K.; Nagayama, T.; Sakurai, T.; Nishisho, T.; Wakabayashi, H.; Hiraga, T.; Ebisu, S.; Yoneda, T. Acid activation of Trpv1 leads to an up-regulation of calcitonin gene-related peptide expression in dorsal root ganglion neurons via the CaMK-CREB cascade: A potential mechanism of inflammatory pain. Mol. Biol. Cell 2010, 21, 2568–2577.
12. Niiyama, Y.; Kawamata, T.; Yamamoto, J.; Furuse, S.; Namiki, A. SB366791, a TRPV1 antagonist, potentiates analgesic effects of systemic morphine in a murine model of bone cancer pain. Br. J. Anaesth. 2009, 102, 251–258.
13. Deval, E.; Gasull, X.; Noël, J.; Salinas, M.; Baron, A.; Diochot, S.; Lingueglia, E. Acid-sensing ion channels (ASICs): Pharmacology and implication in pain. Pharmacol. Ther. 2010, 128, 549–558.
14. Li, W.-G.; Xu, T.-L. ASIC3 channels in multimodal sensory perception. ACS Chem. Neurosci. 2011, 2, 26–37.
15. Hiasa, M.; Okui, T.; Allette, Y.M.; Ripsch, M.S.; Sun-Wada, G.H.; Wakabayashi, H.; Roodman, G.D.; White, F.A.; Yoneda, T. Bone pain induced by multiple myeloma is reduced by targeting V.-ATPase and ASIC3. Cancer Res. 2017, 77, 1283–1295.
16. Izumi, M.; Ikeuchi, M.; Ji, Q.; Tani, T. Local ASIC3 modulates pain and disease progression in a rat model of osteoarthritis. J. Biomed. Sci. 2012, 19, 77.
17. Yoneda, T.; Hiasa, M.; Nagata, Y.; Okui, T.; White, F.A. Acidic microenvironment and bone pain in cancer-colonized bone. Bonekey Rep. 2015, 4, 690.
18. Skaper, S.D. The neurotrophin family of neurotrophic factors: An overview. Methods Mol. Biol. 2012, 846, 1–12.
19. Halvorson, K.G.; Kubota, K.; Sevcik, M.A.; Lindsay, T.H.; Sotillo, J.E.; Ghilardi, J.R.; Rosol, T.J.; Boustany, L.; Shelton, D.L.; Mantyh, P.W. A blocking antibody to nerve growth factor attenuates skeletal pain induced by prostate tumor cells growing in bone. Cancer Res. 2005, 65, 9426–9435.
20. Jimenez-Andrade, J.M.; Ghilardi, J.R.; Castañeda-Corral, G.; Kuskowski, M.A.; Mantyh, P.W. Preventive or late administration of anti-NGF therapy attenuates tumor-induced nerve sprouting, neuroma formation, and cancer pain. Pain 2011, 152, 2564–2574.
21. Buehlmann, D.; Ielacqua, G.D.; Xandry, J.; Rudin, M. Prospective administration of anti-nerve growth factor treatment effectively suppresses functional connectivity alterations after cancer-induced bone pain in mice. Pain 2019, 160, 151–159.
22. Wang, L.N.; Yang, J.P.; Ji, F.H.; Zhan, Y.; Jin, X.H.; Xu, Q.N.; Wang, X.Y.; Zuo, J.L. Brain-derived neurotrophic factor modulates N-methyl-D-aspartate receptor activation in a rat model of cancer-

induced bone pain. J. Neurosci. Res. 2012, 90, 1249–1260.

23. Ghilardi, J.R.; Freeman, K.T.; Jimenez-Andrade, J.M.; Mantyh, W.G.; Bloom, A.P.; Kuskowski, M.A.; Mantyh, P.W. Administration of a tropomyosin receptor kinase inhibitor attenuates sarcoma-induced nerve sprouting, neuroma formation and bone cancer pain. Mol. Pain 2010, 6, 87.
24. Hondermarck, H. Neurotrophins and their receptors in breast cancer. Cytokine Growth Factor Rev. 2012, 23, 357–365.
25. Molloy, N.H.; Read, D.E.; Gorman, A.E. Nerve growth factor in cancer cell death and survival. Cancers 2011, 3, 510–530.
26. Williams, K.S.; Killebrew, D.A.; Clary, G.P.; Seawell, J.A.; Meeker, R.B. Differential regulation of macrophage phenotype by mature and pro-nerve growth factor. J. Neuroimmunol. 2015, 285, 76–93.
27. Zhang, X.C.; Kainz, V.; Burstein, R.; Levy, D. Tumor necrosis factor-alpha induces sensitization of meningeal nociceptors mediated via local COX and P38 map kinase actions. Pain 2011, 152, 140–149.
28. Binshtok, A.M.; Wang, H.; Zimmermann, K.; Amaya, F.; Vardeh, D.; Shi, L.; Brenner, G.J.; Ji, R.R.; Bean, B.P.; Woolf, C.J.; et al. Nociceptors are interleukin-1beta sensors. J. Neurosci. 2008, 28, 14062–14073.
29. Mamet, J.; Baron, A.; Lazdunski, M.; Voilley, N. Proinflammatory mediators, stimulators of sensory neuron excitability via the expression of acid-sensing ion channels. J. Neurosci. 2002, 22, 10662–10670.
30. Barrios-Rodiles, M.; Chadee, K. Novel regulation of cyclooxygenase-2 expression and prostaglandin e2 production by IFN-gamma in human macrophages. J. Immunol. 1998, 161, 2441–2448.
31. Sabino, M.A.; Ghilardi, J.R.; Jongen, J.L.; Keyser, C.P.; Luger, N.M.; Mach, D.B.; Peters, C.M.; Rogers, S.D.; Schwei, M.J.; de Felipe, C.; et al. Simultaneous reduction in cancer pain, bone destruction, and tumor growth by selective inhibition of cyclooxygenase-2. Cancer Res. 2002, 62, 7343–7349.
32. Baamonde, A.; Curto-Reyes, V.; Juarez, L.; Meana, A.; Hidalgo, A.; Menendez, L. Antihyperalgesic effects induced by the IL-1 receptor antagonist anakinra and increased IL-1beta levels in inflamed and osteosarcoma-bearing mice. Life Sci. 2007, 81, 673–682.
33. Constantin, C.E.; Mair, N.; Sailer, C.A.; Andratsch, M.; Xu, Z.Z.; Blumer, M.J.; Scherbakov, N.; Davis, J.B.; Bluethmann, H.; Ji, R.R.; et al. Endogenous tumor necrosis factor alpha (TNFalpha) requires TNF receptor type 2 to generate heat hyperalgesia in a mouse cancer model. J. Neurosci. 2008, 28, 5072–5081.

34. Hu, J.H.; Zheng, X.Y.; Yang, J.P.; Wang, L.N.; Ji, F.H. Involvement of spinal monocyte chemoattractant protein-1 (MCP-1) in cancer-induced bone pain in rats. Neurosci. Lett. 2012, 517, 60–63.
35. Lozano-Ondoua, A.N.; Symons-Liguori, A.M.; Vanderah, T.W. Cancer-induced bone pain: Mechanisms and models. Neurosci. Lett. 2013, 557, 52–59.
36. Giuliani, A.L.; Sarti, A.C.; Di Virgilio, F. Extracellular nucleotides and nucleosides as signalling molecules. Immunol. Lett. 2018.
37. Kaan, T.K.; Yip, P.K.; Patel, S.; Davies, M.; Marchand, F.; Cockayne, D.A.; Nunn, P.A.; Dickenson, A.H.; Ford, A.P.; Zhong, Y.; et al. Systemic blockade of P2X3 and P2X2/3 receptors attenuates bone cancer pain behaviour in rats. Brain 2010, 133, 2549–2564.
38. Hansen, R.R.; Nasser, A.; Falk, S.; Baldvinsson, S.B.; Ohlsson, P.H.; Bahl, J.M.; Jarvis, M.F.; Ding, M.; Heegaard, A.M. Chronic administration of the selective P2X3, P2X2/3 receptor antagonist, A-317491, transiently attenuates cancer-induced bone pain in mice. Eur. J. Pharmacol. 2012, 688, 27–34.
39. Baron, R. Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism; Favus, M.J., Ed.; ASBMR: Washington, DC, USA, 2003; pp. 1–8.
40. Breuksch, I.; Weinert, M.; Brenner, W. The role of extracellular calcium in bone metastasis. J. Bone Oncol. 2016, 5, 143–145.
41. Xu, Q.; Zhang, X.M.; Duan, K.Z.; Gu, X.Y.; Han, M.; Liu, B.L.; Zhao, Z.Q.; Zhang, Y.Q. Peripheral TGF-β1 signaling is a critical event in bone cancer-induced hyperalgesia in rodents. J. Neurosci. 2013, 33, 19099–19111.
42. Li, Y.; Cai, J.; Han, Y.; Xiao, X.; Meng, X.L.; Su, L.; Liu, F.Y.; Xing, G.G.; Wan, Y. Enhanced function of TRPV1 via up-regulation by insulin-like growth factor-1 in a rat model of bone cancer pain. Eur. J. Pain 2014, 18, 774–784.
43. Delgado-Calle, J.; Anderson, J.; Cregor, M.D.; Condon, K.W.; Kuhstoss, S.A.; Plotkin, L.I.; Bellido, T.; Roodman, G.D. Genetic deletion of Sost or pharmacological inhibition of sclerostin prevent multiple myeloma-induced bone disease without affecting tumor growth. Leukemia 2017, 31, 2686–2694.
44. Wang, X.; Li, W.G.; Yu, Y.; Xiao, X.; Cheng, J.; Zeng, W.Z.; Peng, Z.; Xi Zhu, M.; Xu, T.L. Serotonin facilitates peripheral pain sensitivity in a manner that depends on the nonproton ligand sensing domain of ASIC3 channel. J. Neurosci. 2013, 33, 4265–4279.
45. Holzer, P. The pharmacological challenge to tame the transient receptor potential vanilloid-1 (TRPV1) nocisensor. Br. J. Pharmacol. 2008, 155, 1145–1162.