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Review Article

P2X7 Receptor Function in Bone-Related Cancer

Elena Adinolfi, Francesca Amoroso, and Anna Lisa Giuliani

Department of Experimental and Diagnostic Medicine, Section of General Pathology, University of Ferrara, Via Borsari 46, 44121 Ferrara, Italy

Correspondence should be addressed to Elena Adinolfi, elena.adinolfi@unife.it

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Modulation of tumor microenvironment by different mediators is central in determining neoplastic formation and progression. Among these molecules extracellular ATP is emerging as a good candidate in promoting cell growth, neovascularization, tumor-host interactions, and metastatization. This paper summarizes recent findings on expression and function of P2X7 receptor for extracellular ATP in primary and metastatic bone cancers. Search of mRNA expression microchip databases and literature analysis demonstrate a high expression of P2X7 in primary bone tumors as well as in other malignancies such as multiple myeloma, neuroblastoma, breast, and prostate cancer. Evidence that P2X7 triggers NFATc1, PI3K/Akt, ROCK, and VEGF pathways in osteoblasts promoting either primary tumor development or osteoblastic lesions is also reported. Moreover, P2X7 receptor is involved in osteoclast differentiation, RANKL expression, matrix metalloproteases and cathepsin secretion thus promoting bone resorption and osteolytic lesions. Taken together these data point to a pivotal role for the P2X7 receptor in bone cancer biology.

1. Introduction

Primary bone cancers, malignancies that originate directly from bone cells, are quite rare diseases. According to USA National Cancer Institute about 2810 people were diagnosed, and 1500 died of bone and joint cancer in 2011. A similar incidence, one affected individual every 100.000, was reported in the same year by the Italian Association for Cancer Research (AIRC). Primary bone cancers generally originate in long bones of limbs and affect children or young adults accounting for 6% of all new pediatric cancer per year [1]. Among primary bone cancers osteosarcoma is the most frequent [2]. Two different osteosarcoma variants are known: a conventional high-grade form intramedullary located at the metaphysis of long tubular bones, frequent in adolescents, and a rarer low-grade variant arising at the surface of long bones, showing a better prognosis, more frequent among adults than young people. Frequency of low-grade is 20 times lower than high-grade ones [3].

A peculiar primary tumor, causing bone damage without need of spreading from the original site, is multiple myeloma. Multiple myeloma is a clonal B-cell malignancy characterized by an accumulation of mature plasma cells in the bone marrow, leading to bone destruction and failure of normal hematopoiesis [4, 5]. The incidence is 3–9 cases/100.000/year; it is more frequent among elderly with a slight male prevalence. Multiple myeloma remains an incurable disease even with the use of proteasome inhibitor bortezomib, immuno-modulatory drugs (thalidomide or lenalidomide), and high-dose chemotherapy associated to autologous stem cell transplantation, as part of first-line therapy [6]. In multiple myeloma, tumor and stromal cells interact via adhesion molecules and cytokine networks to simultaneously promote tumor cell survival, drug resistance, angiogenesis, and disordered bone metabolism.

Bone metastasis of extra-bone high-grade solid tumors is more frequent than primary bone cancers. The rate of bone metastasis is about 70% in breast, melanoma, lung and prostate cancer and about 15–30% in colon, stomach, bladder, uterus, rectum, thyroid, and kidney carcinomas [7]. Symptoms related to bone metastasis include pain, fractures, spinal cord compression, and hypercalcemia leading to poor quality of life and reduced life expectancy. It is estimated that the appearance of bone metastasis can reduce the five-years

survival rate of breast cancer patients from 98% to 26% and that of prostate cancer patients from 100% to 33% [8].

Both primary and secondary cancers involving the skeleton can cause osteoblastic (sclerotic) or osteolytic lesions, although generally the final histological picture is a mixture of the two. Osteoblastic lesions originate from proliferation of osteoblasts, while osteolytic lesions are generally due to osteoclast activation caused by factors secreted by cancer cells. Common sites of metastasis in the skeleton are the spine, rib cage, limbs and skull. Once settled into the bone, tumor cells release factors that activate matrix resorption leading to bone destruction; this facilitates cancer spread and proliferation [8, 9]. Since bone resorption activity is followed by an increase in bone formation, as it occurs in normal remodeling, these two processes are intimately linked and typically present at sites of bone metastasis. Osteolytic metastasis are common in breast cancer, mainly due to stimulation by tumor cells of osteoclast differentiation and activity [10]. An associated local bone formation usually occurs, presumably in an attempt to activate repair, but this response is often inefficient, thus leading to final bone loss. In multiple myeloma tumor cells in the bone marrow cause exclusively osteolytic lesions, with almost complete absence of bone formation [4]. This seems to be due to suppression of osteoblast activity. On the contrary, prostate cancer metastasis are primarily osteoblastic, with possible presence of osteolytic components [11]. Most of the factors implicated in osteolytic metastatization are also involved in the pathogenesis of osteoporosis to the point that pharmacological treatment of osteolytic metastasis and osteoporosis is similar.

PTHrP (parathormone-related protein) and TGF-β (transforming growth factor beta) are among the most important osteolytic mediators [9]. PTHrP secreted by breast cancer and other tumors, is a well-known potent stimulator of osteoclast activity in bone metastasis. PTHrP acts through the release of RANKL (receptor activator of nuclear factor kB ligand) which binds to RANK receptor on osteoclasts, the system RANK-RANKL being the main pathway for osteoclast differentiation and activation. Factors released by metastatic cells or by the primary tumor activate bone resorption, this in turn triggers release of TGF-β from bone matrix. TGF-β and tumor-derived PTHrP are believed to act in a vicious cycle of local bone destruction in osteolytic metastasis: TGF-β released in active form during osteoclastic resorption of bone matrix stimulates PTHrP production by tumor cells. In turn, PTHrP mediates bone destruction by stimulating osteoclasts [4, 12]. Furthermore, TGF-β released from bone exerts suppressive effects on T lymphocytes and NK cells, thus reducing immune cell response to tumor [13].

Growth of primary bone tumors and development of bone metastasis are complex processes involving bone-tumor cells crosstalk mediated by several different cytokines and growth factors. In this context the study of molecules moulding tumor micro-environment is of great importance for a better understanding of cancer biology and to find new and efficacious therapies. Here we suggest a possible role of extracellular ATP and its receptor P2X7 in the development of both primary bone cancers and skeletal metastasis.

2. Role of ATP and P2X7 Receptors in Cell Growth and Carcinogenesis

In recent years investigation of tumor microenvironment has gained great importance for the understanding of tumor formation and progression [14]. Several molecules are released by tumor cells, acting as promoters of proliferation, allowing for immune system escape, helping cell matrix infiltration, neovascularization and distant site invasion. Interestingly, mediators of inflammatory-immune response can also influence cancer progression [15]. A good candidate molecule for many of these functions is extracellular ATP. This nucleotide has been recently shown to accumulate both at inflammatory sites [16, 17] and into the interstitium of solid tumors of different origin [18]. ATP release by dying tumor cells, following chemotherapy, is also associated to immunogenic cell death [19]. Immunogenic cell death implies that the host immune system is essential for antitumor effect of certain chemotherapeutic agents such as anthracyclines, and that molecules released from dying tumor cells act as danger signals to activate the host immune response. In this context ATP released from neoplastic cells drives recruitment of dendritic cells and T lymphocytes into the tumor site [20, 21]. Effects of ATP are mediated by two families of plasma membrane purinergic receptors: P2Y, metabotropic G coupled receptors, and P2X, ligand gated ion channels [22]. The P2X7 receptor is involved in many of the tumor-promoting and immune-modulatory effects of extracellular ATP. Like other members of the P2X family, P2X7 mediates cation fluxes across the plasma membrane but thanks to its peculiar C terminal tail it also gates a large nonselective pore [23]. Opening of this pore is coupled to the well-known P2X7 cytotoxic activity usually triggered by high (i.e. mM) pharmacological ATP concentrations. On the contrary, basal tonic P2X7 activation mediated by endogenous ATP release causes cell proliferation [24, 25]. Proliferation and other tumor transformation hallmarks seem to be dependent on the channel activity since they are retained also by cells expressing a C-terminal truncated P2X7 splice variant, which lacks pore-forming activity [26]. Several reports suggest an association between P2X7 and cancer [27]. In different cell models P2X7 expression supports organellar calcium increase, NFATc1 activation, matrix infiltration and cell growth [24–26, 28, 29]. There is good evidence that P2X7 receptor can influence energy production by increasing mitochondrial potential and intracellular ATP levels [25], a condition linked to the PI3 K/Akt pathway that positively influences cell proliferation [30]. Moreover, P2X7 activation alters the biochemical composition of tumor by causing release of microvescicles [31, 32] and secretion of cytokines [33], tissue factor [34], matrix metalloproteases (MMPs) [35] and prostaglandins [36]. Furthermore, P2X7 itself can be a conduit for ATP release [37]. Direct participation of P2X7 in tumor progression was demonstrated in a recent *in vivo* study by our laboratory [38]. We showed that P2X7 inhibition by either pharmacological tools or RNA interference caused a dramatic reduction of tumor masses, and vice-versa that P2X7 overexpression accelerated tumor growth. Interestingly P2X7 expressing tumors showed a

thicker vascular network and higher secretion of vascular endothelial growth factor (VEGF). Accordingly, tumor growth was inhibited by administration of the anti-VEGF antibody bevacizumab [38].

3. Extracellular ATP and P2X7 as Modulators of Osteoblasts and Osteoclasts Responses

The role of P2X7 receptor in bone cells has been extensively studied and was recently appraised in several reviews [39–42]. P2X7 receptor is expressed by both osteoblasts [43] and osteoclasts [44, 45] of different species and plays a central role in mediating osteoblast-osteoclasts crosstalk via calcium oscillations [46] and other signaling pathways [39]. One of the main roles attributed to P2X7 receptor in osteoblasts is to promote cell growth and osteodeposition [36] through a series of different pathways including c-fos [47], ERK [48], PI3 K [49], and COX [36]. Moreover, P2X7 likely mediates osteoblast ATP release as shown by the inhibitory effect on nucleotide release of P2X7 blockers [50, 51].

Skeletal disorders, such as osteoporosis and tumor-induced bone resorption, are caused by increased activity of bone-resorbing osteoclasts. The role of P2X7 in osteoclast biology is still poorly understood. It has been suggested that P2X7 participates in cell fusion, a central step in osteoclastogenesis [52, 53], but osteoclasts from P2X7 KO mice are normal in number and size [54, 55]. P2X7 receptor might, however exert its activity on osteoclast fusion indirectly by extracellular adenosine generation [53] or, simply, by increasing survival via RANKL [44] and NF-κB [56] pathways. The effect of P2X7 knock-down on bone phenotype probably depends on the different mice models considered [57]. Whatever is the role of P2X7 in osteoclast fusion and activation, a reduced activity of the receptor has been associated to increased susceptibility to osteoporosis [58]. All the known polymorphisms of human P2X7 have now been studied in different postmenopausal women cohorts [59–61]. These studies revealed an association between different complications of osteoporosis and loss of function of P2X7; a lower incidence of vertebral fractures in women expressing a gain of function receptor polymorphism was also evident [60]. These data suggest that, depending on the P2X7 polymorphism carried, one could be more or less exposed to osteolytic bone cancer complications.

4. P2X7 and Cell Metabolism in Cancer: Warburg Effect and Signaling

Ability to adapt to unfavorable conditions is a key feature of cancer cells, making them more and more aggressive. Tumor-cell survival runs through a reorganization of metabolic pathways to balance energy generation and production of biosynthetic intermediates. Aerobic glycolysis (also known as "Warburg effect") is known to be the preferred metabolic path adopted by cancer cells, in presence of oxygen. Lactate release, as a consequence of glucose degradation, is observed in many solid tumors and leukemias. Detection of increased glucose uptake in tissues is commonly used for diagnosis of cancer by positron emission tomography (PET) [62]. In a recent paper, Grol and colleagues showed that in the osteoblast like MC3T3-E1 cell line, P2X7 activation triggers, via PI3 K, release of lactate and increased glucose metabolism [49]. PI3 K activates the serine threonine kinase Akt, one of the most studied paths involved in tumor progression and aggressiveness [63, 64]. Indeed, activation of PI3 K/Akt pathway has been correlated with many cellular critical events such as proliferation, apoptosis, metabolism, adhesion, cytoskeleton modifications, tumorigenesis, metastatization, and drugs resistance [65, 66]. A direct effect of P2X7 activation on Akt has been shown in several cell lines [30, 67–72]. In some models such as neuroblastoma [70] and non-small-cell lung cancer [72], P2X7 was reported to reduce Akt phosphorylation while in others such as astrocytes [68], neurons [69, 73], and osteoblasts [49], the P2X7-Akt axis promoted proliferation and survival. Another Akt-mediated effect during tumor development is induction of HIF-1α that in turn leads to VEGF production and neovascularization [74, 75]. One might speculate that P2X7-mediated VEGF secretion from tumoral masses [38] could be dependent on PI3 K/Akt also in tumor proliferating osteoblasts; VEGF being a known positive regulator of osteoblastic lesions [76].

5. P2X7 Receptor in Primary Bone Tumors

A search of EMBL-EBI Atlas database (http://www.ebi.ac.uk/) revealed an association between P2X7 overexpression and different malignancies including blood and bone tumors [77]. In particular, P2X7 expression was increased in osteosarcoma, Ewing's sarcoma, chondromyxoid fibroma, and multiple myeloma. Moreover, P2X7 receptor was found to be expressed and active in multiple myeloma cell lines where it mediates MMPs activation [78].

Osteoblasts and osteosarcoma were among the first cell models in which a proliferative activity of P2X7 and ATP was suggested [36]. Although direct *in vivo* proof of an oncogenic role of the receptor in osteosarcoma is missing, several experimental findings point to such an involvement. Osteoblast like (MC3T3-E1) and osteosarcoma cells lines (SaOs-2, HOS) generally show high expression of P2X7 at mRNA, protein, and functional level [43, 79, 80]. P2X7 is expressed in MSC osteoblastic precursors [81] and is highly and constantly detected during osteoblast differentiation [82]. P2X7 activity has been associated to proliferation and osteodeposition [36] as well as to upmodulation of the osteosclerotic factor FosB [83] in osteoblasts [84]. In a recent study, Liu and Chen demonstrated a trophic effect of ATP on HOS cells that was abolished by suramin, a P2 purinoceptors antagonist. Suramin also inhibited ATP-dependent cytosolic calcium increases.The ATP growth promoting effect was likely mediated via both P2X4 and P2X7 [80]. A role for P2X7 receptor in osteoblasts proliferation and osteogenesis was also indicated by Panupinthu et al. who reported reduced cell growth and osteodeposition by calvarial cell cultures from P2X7 KO mice [36]. P2X7 activated pathways in osteoblasts include cyclooxygenase (COX), lysophosphatidic acid, and prostaglandin E2 [36]. A further study by Gavala et

al. showed that P2X7 dependent AP-1/Fos-B activation was responsible for COX-2 expression [84]. Moreover, mechanical stimulation triggers ATP release and P2X7-dependent activation of several kinases, including ERK [48] and PI3 K [49]. It is tempting to speculate that a condition, such as cancer, in which extracellular ATP levels are known to be upregulated [18], might mimic mechanical loading causing and stimulating osteoblast proliferation. Furthermore, mouse osteoblasts and osteoclasts constitutively release ATP into extracellular microenvironment via P2X7-dependent pathway [51].

NFAT is one of the main pathways activated through Ca^{2+} and calcineurin following P2X7 stimulation [29, 85–88]. We have shown that NFATc1 activation is central for P2X7 trophic activity as treatment with the NFATc1 inhibitors cyclosporine and VIVIT obliterates P2X7-dependent cell growth [29]. Moreover, P2X7-positive tumors overexpress NFATc1 [38]. On the other hand NFAT has a central role also in osteoblast biology. Mice expressing in osteoblasts a constitutively nuclear NFATc1 variant, NFATc1(nuc), develop bone masses characterized by osteoblast overgrowth [89]. Accordingly, viable NFATc1-deficient mice have defects in bone formation, in addition to impaired osteoclast development. Calcineurin/NFAT-signaling in Osteoblasts controls the expression of chemoattractants for monocytic osteoclast precursors, thereby coupling bone formation and bone resorption, and regulating bone mass [89]. Elevated levels of NFAT are among factors necessary for *in vitro* invasiveness of mice metastatic osteosarcoma cell lines [90]. Finally, calcineurin/NFAT pathway is implicated in prostate cancer bone metastasis. Prostate tumor cells that engraft in the bone stimulate osteoblasts by secreting growth-promoting factors among which endothelin 1 (ET-1). In osteoblasts ET-1 activates calcineurin, causes nuclear translocation of NFAT and, thus, osteoblasts stimulation [91]. On the other hand, a negative role for NFAT in osteoblasts has been proposed by Choo et al. who demonstrated that constitutively active NFAT inhibits alkaline phosphatase activity and mineralization [92]. These observations might suggest that P2X7 could cause NFAT activation and osteoblast proliferation both in primary and metastatic osteoblastic lesions.

Moreover, Ca^{2+}-NFAT signaling is essential for osteoclast differentiation [93]. Intriguingly, NFAT activation in osteoclasts has also been related to malignant progression of multiple myeloma. Several studies reported osteoblast NFAT reduction associated with decreased osteoclastogenesis, following myeloma treatment [94–97].

P2X7 was shown to induce ATP secretion from both osteoclasts and osteoblasts [51]. Increase in extracellular ATP is followed by extracellular adenosine accumulation viaCD39 and CD73 ectonucleotidases [98]. Adenosine was demonstrated to stimulate proliferation of MC3T3-E1 osteoblastic-like cell line [99]. Furthermore, HCC1 cells release increased amounts of IL-6 and osteoprotegerin following adenosine receptor stimulation [100], likely modulating osteoclastogenesis and bone resorption.

VEGF production was found increased in different experimental tumors expressing P2X7 receptor [38]. The P2X7-VEGF connection in tumors is also supported by the finding that P2X7 activation in rat C6 glioma cells is linked to increased release of proinflammatory factors (MCP-1, IL-8 and VEGF) and to tumor-cell migration [101]. Accordingly, patients with osteosarcoma showed increased VEGF plasma levels, and this was reduced following tumor removal by surgery [102]. Moreover, VEGF secretion is known to be central in malignant progression of multiple myeloma; the first lymphohaemopoietic tumor in which increased angiogenesis was detected, and which greatly benefits of the treatment with VEGF-targeted agents [103].

6. P2X7 Receptor Activity in Bone Metastasizing Cancers

The complications of bone metastasis are thought to be due to the perturbation of the interaction between osteoblasts and osteoclasts. This disruption is thought to be caused by tumor-derived humoral mediators produced by the metastasized cancer cells within the bone marrow. Among factors regulating bone cancer metastasis RANKL plays a central role, as demonstrated by the efficacy of an anti-RANKL antibody in the therapy of such secondary tumors [104]. During physiological bone remodeling RANKL, produced by proliferating osteoblasts, causes activation of osteoclasts ensuring a balance between osteodeposition and bone resorption. However, the presence of cancer cells can alter bone microenvironment causing an increased production of RANKL, favoring osteoclasts activation and osteolysis. Several factors, such as IL-1, IL-6, COX2/prostaglandins, and VEGF, can cause positive shifts in RANKL production [10, 105]. All these mediators are released upon P2X7 stimulation from different cell types, including osteoblasts [36], immune [106, 107], and cancer cells [38, 108, 109]. Moreover, P2X7-mediated ATP secretion [26, 37] from tumor cells [18] could itself upregulate osteoblasts RANKL expression [44]. Extracellular ATP is rapidly degraded to adenosine, which can directly modulate osteoclasts formation through A2 receptors [53]. P2X7-dependent Rho-kinase1 (ROCK) activation has been demonstrated in several cell types [31, 110–112], including osteoblasts [113]. ROCK is known to activate changes in cell morphology, adhesion, and motility, and is associated to P2X7-dependent cell blebbing, a response that might be related to invasive phenotype of P2X7-expressing cells [26, 38, 114]. Interestingly, an increased ROCK signaling has been shown to contribute to breast cancer invasiveness [115] as, if overexpressed, the kinase conferred a bone metastatic phenotype to a human breast cancer cell line in an *in vivo* model. ROCK is also known to mediate activation of the proresorptive factor PTHrP through activation of TGF-β signaling [116].

Several studies reported an association between primary tumors causing bone metastatization and P2X7 overexpression and function (for a recent review see [27]). Upregulation of P2X7 in breast cancer was shown for the first time by Slater et al. who demonstrated an association between receptor expression and tumor invasiveness [117]. Recently, Jelassi et al. also showed an involvement of P2X7 in breast cancer metastasis formation [114]. P2X7 activation in a

highly aggressive breast cancer cell line (MDA-MB-435s) caused increased *in vitro* cell motility and extracellular matrix infiltration [114]. Accordingly, P2X7 inhibition significantly reduced *in vivo* cell migration in a zebra fish embryo metastatic model [114]. As previously reported in macrophages [118], P2X7 receptor activation caused release of a broad range of cathepsins, including cathepsin k, also from breast cancer cells [114]. Another bone-degrading enzyme which is secreted in a P2X7-dependent fashion is MMP9 [35]. Cathepsin k and MPPs release in resorption pits during bone degradation make these enzymes attractive therapeutic targets to block osteolysis [11]. Since cathepsin k inhibitors showed adverse effects during clinical trials [11], pharmacological inhibition of upstream pathways, such as P2X7, could prove useful in therapy.

A peculiar case of tumor causing osteolytic metastasis is neuroblastoma, which is the second most common pediatric malignancy worldwide and is responsible for 15% of childhood cancer deaths [119]. Patients with high-risk metastatic disease (stages III-IV) show a mere 42% survival rate, despite treatment [120]. Few years ago Raffaghello et al. showed that P2X7 receptor is expressed in specimens from neuroblastoma patients and in all neuroblastoma cell lines examined [109]. Interestingly, in these cells P2X7 receptor lacks its well-known cytotoxic activity but rather supports proliferation [109]. Wu et al. have recently reported expression of P2X7 also in murine neuroblastoma cell lines [121]. In these cells P2X7 inhibits differentiation [121], promotes proliferation [70], and microvesicles release [122]. P2X7 implication in neuroblastoma progression is further supported from *in vivo* experiments from our laboratory showing a 2-3 fold reduction in neuroblastoma tumor masses upon P2X7 silencing [38]. Bone involvement is observed in 55–68% of neuroblastoma patients who present metastatic disease at diagnosis [123], particularly at the bony orbit. Bone metastasis is generally associated with osteolytic lesions due to either direct action of cancer cells on osteoclasts or to an indirect involvement of osteoblasts finally causing bone resorption [123]. Although direct evidence of P2X7 involvement in neuroblastoma bone metastatization is still missing all experimental evidence points to such an association [38, 53, 109].

Patients with advanced prostate carcinoma generally develop osteoblastic metastasis due to deregulation of physiological bone remodeling. In these patients prostate cancer cells are adjacent to large numbers of osteoblasts, which are responsible for woven bone deposition [76]. P2X7 expression by prostate cancer cells has been reported long time ago [124, 125]. Furthermore, ATP treatment increases prostate cancer cell invasion [126]. The cross-talk between prostate cancer cells and osteoblasts contributes to metastatic development. If on one hand cancer cells secrete TGFβ, VEGF and other factors promoting osteoblast proliferation and differentiation, on the other osteoblasts can prompt cancer cells to secrete MMP9 and stimulate tumor cell growth [76]. P2X7 activation has been involved in secretion of almost all the above factors either from osteoblasts [44] or from cancer cells [35, 38].

7. Conclusions and Future Perspectives

Over-expression of a potentially cytotoxic receptor such as P2X7 by cancer cells is puzzling. However, the discovery that this receptor has oncogene-like properties may provide a logical explanation for this finding [38, 114]. Due to altered skeletal phenotype of P2X7 KO mice, bone has been one of the tissues in which P2X7 activity has been best characterized. Nevertheless, there is only sporadic evidence linking bone related cancers and P2X7 so far. Here we propose that P2X7 might have a central role in bone cancer development and progression by causing NFATc1, PI3K/Akt, ROCK, VEGF activation, thus driving osteoblast proliferation in primary bone tumors and osteoblastic metastasis. P2X7 might also stimulate bone resorption by causing osteoclast activation [53] as well as secretion of cathepsins and MMPs [35, 114] thus contributing to osteolytic metastasis formation.

The processes of bone resorption and bone formation are tightly coupled and treatments that primarily target the osteoclasts generally exert secondary inhibitory effects on bone formation. The discovery of new pathways occurring in tumorigenic transformation and aggressiveness of bone-related cancers made clinicians pay attention to new targeting drugs: blocking bone lesions acting on osteoblasts/osteoclasts regulation, like bisphosphonates do, is not the only chance anymore. Clinical trials with RANKL blocking antibody (denosumab) produced very encouraging results showing that denosumab was tolerated better than bisphosphonates (zolendronic acid) and even increased patient survival [104]. Inhibitors of mTOR, an Akt downstream effector are in clinical trials for the treatment of multiple bone related cancers [127]. In particular, rapamycin is known to arrest cell growth in osteosarcoma [128, 129] and breast cancer [130].

Since P2X7 receptor is an upstream regulator of all the paths inhibited by the RANKL and mTOR blockers, it is an attractive therapeutic target for bone-related diseases too. Several P2X7 antagonists are currently in phase I and II clinical trials for the treatment of chronic inflammatory diseases, showing so far excellent safety profiles [131]. These drugs are, in principle, available to be used at patient's bed and could be a good therapeutic opportunity for those cancers, such as neuroblastoma and multiple myeloma, which still lack an efficacious cure.

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