



Energy metabolism in bone tumors: Fuel selection and adaptation

Edith Bonnelye

► To cite this version:

Edith Bonnelye. Energy metabolism in bone tumors: Fuel selection and adaptation. Bone Cancer Bone Sarcomas and Bone Metastases - From Bench to Bedside, 2021. hal-03698238

HAL Id: hal-03698238

<https://hal.science/hal-03698238>

Submitted on 17 Jun 2022

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Energy metabolism in bone tumors : Fuel selection and adaptation

Edith Bonnelye

CNRS ERL 6001/INSERM U1232, Institut de Cancérologie de l'Ouest ; Bd Jacques Monod -
44805 Saint-Herblain-France

edith.bonnelye@inserm.fr

Abstract

It has long been recognized that cancer cells exhibit altered patterns of metabolism that allow them to meet the biosynthetic demands of rapid proliferation and overcome metabolic stress imposed by the microenvironment. In this chapter, we aim to recapitulate knowledge on energetic metabolism and reprogramming in osteo-oncology. Diagnosis and staging of bone metastases using ¹⁸Fluorine labeled Glucose, choline and folate and emission tomography and computerized tomography (PET/CT) reflect tumor cells energetic metabolism. Metabolites and associated gene regulators involved in bone metastases and/or osteosarcoma progression are also described including, glucose, lactate, amino-acid, cholesterol, fatty acids and derived mitochondrial activity ROS. Moreover several master regulators of energy metabolism in normal physiology, metabolic syndromes and cancer (PPAR γ , ERR α , PGC1 α , CREB, MYC, RAS, EWS-FLI, TMPRSS2-ERG and mTOR) are identified in bone metastases and primary bone tumor. Interestingly, factors that were first detected in bone physiology (RANKL/OPG/RANK, Runx2, OPN, OCN, POSTN and SOST) have been recently identified as regulators of energetic metabolism. Finally, in clinic, treatments targeting metabolism (everolimus, statin,...) have an impact on bone metastases regression, suggesting that targeting energetic metabolism may constitute a new therapeutic pathway in osteo-oncology.

Research Highlights:

- High diversity of metabolic fuels to feed bone tumor.
- Strong energetic metabolism genes program allow tumor cells to survive and progress in bone microenvironment.
- Osteomimicry may contribute to bone tumor adaptation to their metabolic environment.

Key words: osteo-oncology, metabolites, energetic metabolism and reprogramming signaling, osteomimicry

I. Introduction

Bone tumors include primary bone tumor like osteosarcoma (OS) and bone metastases, which are frequent complications of many cancers but are especially common from breast and prostate cancer. Bone tumors can lead to occurrence of metastases (lung for OS), skeletal-related events, including fractures and spinal cord compression, bone pain and disability, contributing to morbidity and mortality in patients with advanced cancer. Understanding tumor cells survival and progression mechanisms when anchored in the bone is essential for the safe and the identification of new biomarkers or therapeutics in the clinic. Transcriptional reprogramming of energetic metabolism is a hallmark of cancer and is today the best proof of the incredible flexibility of tumor cells allowing them to survive, escape and adapt to their new environment. Therefore, identifying metabolic signatures (glucose-lactate (Warburg), Fatty Acid, Amino-Acid, oxidative stress, OXPHOS...) should contribute to early bone lesions detection as well as the characterization of tumor cells reprogramming metabolic status allowing a better therapeutic choice throughout the disease.

II. Imaging of cellular energy metabolism in Osseous Metastatic Disease.

Since bone metastases remain the main cause of morbidity in patients with metastatic breast and castration-resistant prostate cancer (CRPC), early and accurate detection of bone lesions as well as follow up of the disease during and after therapy, is becoming crucial (Fig.1). In this regard, the use of radiotracers revealing energetic metabolism has shown the capabilities in detection bone metastases. First glycolytic metabolism has been documented by molecular imaging by using the glucose analog ^{18}F -fluorodeoxyglucose ^{18}F -FDG-PET for bone metastases detection derived from breast (BCa) and prostate cancer (PCa) [1][2]. GLUT1 is overexpressed in BCa and PCa and is responsible of the ^{18}F -fluoro-2-deoxy-D-glucose uptake

[3]. Also the hyperpolarized (HP)¹³C-pyruvate MRI had been shown to provide real-time assessment of the glycolytic pathways in PCa bone metastases combined with conversion of pyruvate to lactate and increased expression of lactate deshydrogenase isoform A (LDHA) [4]. Beside aerobic glycolysis, cancer cells in bone can use additional sources of energy. Indeed, an abnormal regulation of the phospholipid metabolism in cancer cells metastatic to bone can be visualized by using the ¹⁸Fluorine-18-choline- and ¹¹C-choline PET/CT scanning of PCa bone metastases [5]. Interestingly, bone metastases type seem to be associated to more specific metabolism since osteolytic lesions show more metabolic activity than the osteoblastic type and are better reveal with the ¹⁸F-FDG. Recently, more specific radiotracers reflecting tumor cells energetic metabolism had been identified like the folate hydrolase Prostate Specific Monoclonal Antibody (PSMA) in PCa cancer [6]. PSMA participates to the folate-mediated one-carbon metabolism essential for purine and thymidine monophosphate (dTMP) biosynthesis, mitochondrial protein translation, and methionine regeneration, which give tumor cells expressing it a proliferative advantage [7]. PSMA can be coupled with the ¹⁸Fluorine or the gallium-68 for PET to visualize bone metastases but ¹⁸F-PSMA seems to outperform the ⁶⁸Ga-PSMA [6][8][9]. A new PET compounds the anti-3-¹⁸F-FACBC or ¹⁸F-fluciclovine (a synthetic analog of the amino acid L-Leucine) that was tested for the detection of PCa relapse was also shown to detect bone metastases [10]. Leucine is known to be an essential amino-acid that is obtained by the diet and used in protein biosynthesis by activating mTOR signaling [11]. Moreover, Leucine is involved in insulin resistance, type 2 diabetes mellitus (T2DM) and dietary restriction of Leucine improved glucose and pyruvate tolerance, increased adipose mass and decreased lean mass in mice [11][12][13]. The impact of Leucine on glucose up-take, mitochondrial biogenesis and fatty-acid oxydation suggests that PCa cells that metastases to bone detected by the ¹⁸F-fluciclovine are capable to metabolize Leucine and to use this amino-acid for their global metabolism and their progression. Interestingly, ¹⁸F-fluciclovine provided

a statistically significant better performance in terms of bone lesion detection rate as compared with ^{11}C -choline [14].

III. Metabolites Contribution to the fertile soil for bone lesions development.

Bone metastases arising from the primary tumor cells must adapt their new metabolic environment to fuel their biology through different phases allowing anchorage, dormancy and/or progression stages. Several studies have characterized metabolites that are used or produced by tumor cells in bone (Fig.2, Table.1).

A. Glucose and Lactate

Similar to many tumor cells and as mentioned previously, bone metastases used glucose and glycolysis to progress [1][2][15]. Indeed, glycolytic enzyme such as phosphoglycerate kinase 1 (PGK1) secreted by PCa cells can regulate bone formation at the metastatic site by increasing osteoblastic activity, osteomimicry and decreasing osteoclastic function [16]. Moreover, glucose is used for ATP generation through lactate production and *via* the pentose phosphate pathway (PPP) for nucleotide synthesis that is essential for cell proliferation. Accordingly, comparison of 28 paired primary metastatic breast cancers revealed differential expression patterns of PPP proteins and the expression of the 6-phosphogluconolactonase (6PGL) in bone metastases was associated with shorter overall survival [17]. Similarly, MDA-MB-231 cancer cells are described to release a large amount of lactate [18]. In fact, Warburg *et al* observed increased venous lactate levels in tumor-bearing limbs of rats compared to controls [19]. It has been reported that the release of lactate at the bone site by glycolytic BCa cells is mediated by the monocarboxylate transporters (MCT4), and that this protein is more expressed in metastases to bone compared to other sites like brain, lung or liver [18][20]. Interestingly, MCT4 and MCT1 overexpression is also observed in osteosarcoma (OS) and is linked to poor prognosis, tumor growth, metastatic process and chemotherapy response [21][22]. Moreover,

in the context of the bone, increase acidosis due to lactate released by tumor cells may strongly stimulate bone metastases progression, by increasing not only tumor cells aggressiveness, but also by modulating bone cell functions and inducing cancer-related bone pain, showing a major impact that may have the energetic metabolism of metastatic cells on the global bone physiology [23][24] (Fig.2). More precisely, low pH can directly contribute to the mineralized matrix destruction since a large majority of the matrix is alkaline mineral (hydroxyapatite). Moreover, lactate uptake by osteoclasts has been described through MCT1, a transporter highly expressed by the bone resorbing cells, stimulating osteoclasts resorption activity and deciphering the lactate as a metabolic fuel for the oxidative metabolism of osteoclasts [18]. Osteoblasts can also perceive tumor metabolic acidosis that inhibits most of their bone formation functions by altering alkaline phosphatase and extracellular matrix proteins expression, inhibiting mineral deposition [25]. On the other side, low pH induces pro-osteoclastogenic factors *i.e* receptor activator of nuclear factor kappa-B ligand (RANKL), TNF α , parathyroid hormone- protein PTH/PTHrP receptors, IL-6, IL-8 and the chemokine (C-C motif) ligand 5 (CCL5) over-expression in osteoblasts, conducting the bone forming cells toward a pro-tumoral phenotype by activating osteoclasts resorption and lead to more bone destruction [26]. With the addition of osteoclasts and osteoblasts, mesenchymal stem cells (MSC) may be sensitive to low pH. Indeed acidosis can induce MSC stemness maintenance and stimulate TGF- β , IL-8 secretion [27] [28]. MSC under low pH conditions, may also contribute to tumor cells aggressiveness, tumor immune escape and bone resorption in OS. Co-culture with MSC incubated with short-term acidosis can stimulate OS cells clonogenicity and invasiveness through NF-kB signaling (RelA, RelB) and downregulated cytokines secretion (CSF2/GM-CSF, CSF3/G-CSF, CCL5, CXCL5, CXCL1, IL6 and IL8) in MSC [29]. Finally, extracellular lactate, have been shown to affect myeloid lineages, by decreasing monocytes motility (contrasted with the stimulation of tumor cells migration), reprogramming

macrophages into a tumor-supporting M2 phenotype and by inhibiting differentiation of monocytes into dendritic cells [30] [31] [32]. Similarly, high lactate levels alter lymphoid lineage, by inhibiting CD8⁺T cells function through depleting extracellular glucose stores (T cell anergy), impairing IFN γ -producing T cells and NK cells and NK generation contributing to the phenomenon of tumor immune escape [33] [34] [35]. Beside its participation into the bone metastases vicious circle, the lactate contribution to cancer-related bone pain is also strongly documented [23]. The low pH induced by extracellular lactate can stimulate acid-sensing receptors highly expressed by sensing neurons (nociceptors) that innervate the bone marrow and the periosteum including transient receptor potential vanilloid (TRPV1), acid-sensing ion channels (ASIC1-3), and metabotropic proton-sensing G protein-coupled receptors (GPCR) thereby creating the formation of neuromas at the origin of the oncologic pain observed in many patients with bone metastases [36] [37] [38]. Interestingly, TRPV1, ASIC and GPCR receptors are also expressed in osteoclasts and osteoblasts [39]. Moreover, the GPCR receptor OGR1, a dual membrane receptor for both protons (extracellular pH) and lysolipids, expression level can be stimulated by RANKL treatment in osteoclasts and can mediate RANKL expression in osteoblasts in response to lactate acidosis [40][41]. Finally, low pH in the bone marrow microenvironment stimulate the release of nociceptive and inflammatory mediators by bone cells such as the nerve growth factor (NGF), the brain-derived neurotrophic factor (BDNF) causing axonal chemoattraction and IL6, IL8 and IL1 promoting tumor-associated hyperalgesia [42] [43].

B. Amino-acid and nucleotides

In parallel to lactate, several studies have identified other metabolites linked to bone metastases energetic metabolism. One of them compared bone / lung metastatic cells to liver metastatic cells, associated lung and bone metastases to an increased glutamine uptake and glutamine derived TCA (Tricarboxylic Acid) cycle while oxidative glutamine was reduced in

liver metastases. Very recently metastatic OS was also described to require glutamine up-take and glutaminase-1 (GLS1) activity for proliferation and metastatic progression. In OS, glutaminase inhibition induces a decrease in glycolysis and TCA cycle, decrease of fatty acid and pyrimidine and Uracil metabolism driving beta-alanine and energy metabolism towards ketogenesis [44].

Genome-wide gene expression profiling of the highly bone metastatic variant MDA-MB-231(SA) cell line as compared to the parental MDA-MB-231 cells also revealed the up-regulation of the phosphoglycerate dehydrogenase (PHGDH), phosphoserine aminotransferase 1 (PSAT1), and phosphoserine phosphatase (PSPH), three genes involved in the L-serine biosynthesis pathway, in the MDA-MB-231(SA) [45]. Interestingly, L-serine is known to be essential for the osteoclasts differentiation, and therefore may contribute to the osteolytic bone metastasis. In bone metastases derived from PCa, tissue obtained from patients with high-risk tumors with or without diagnosed bone metastases were profiled showing increased amino-acids synthesis and metabolism (glutamic acid, asparagine, phenylalanine, threonine, tyrosine, adenosine). Reinforcing data obtained in bone metastases derived from BCa and ¹⁸F-fluciclovine imagery in PCa, serine and leucine were also found increased in bone metastases [10] [45]. In parallel, by Raman Spectroscopy, proteins and amino acid (methionine, phenylalanine, glutamic acid, alanine, glycine, tryptophane) were the second component that was identified increasing with bone tropism and highly present in the MDA-MB-231 osteotropic subclone B02 cells compared to their parental cell line. In contrast, cytochrome C, DNA and nucleotide were inversely related to capacity for tumor cells to metastasize to bone [46].

C. Cholesterol and Fatty acid

Incidence of skeletal metastases from PCa strongly increases with age and obesity, two conditions associated with bone marrow progressive infiltration by fat cells [47][48]. By using

diet-induced obesity models *in vivo*, Herroon *et al* demonstrate the stimulatory effects of marrow fat on growth and progression of skeletal prostate tumors [49]. They demonstrate that lipids from marrow adipocytes induces expression of the Fatty acid binding protein 4 (FABP4), a lipid chaperone, interleukin IL-1 β , and the inducible Heme oxygease 1 (HMOX-1) in prostate skeletal tumors from obese mice and in bone metastases samples from PCa patients [49]. Increased metabolism of fatty acids (Arachidonic acid, Stearic acid, Linoleic acid) were also revealed in bone metastases *versus* normal bone and *versus* prostate cancer tissues [50]. They also noted the high level of cholesterol in PCa bone metastases associated with intense immunohistochemical staining of enzymes for cholesterol influx (low density lipoprotein receptor (LDL-R), scavenger receptor class B type1 (SR-B1)), deciphering the cholesterol as a metabolic fuel for tumor cells. *De novo* synthesis of cholesterol in tumor cells is also suggested through high expression of the 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase in PCa bone metastatic cells, an enzyme that catalyzed the reduction of HMG-CoA into mevalonate, a cholesterol precursor [50]. Reinforcing this data, the expression of fatty acid synthase (FASN) is observed in 90% of bone metastases in CRPC and is coexpressed with the constitutively active AR variant V7 (AR-V7) in 39% of bone metastases [51]. Selective FASN inhibitor antagonizes CRPC growth through inhibition of glucose incorporation into lipids (oleic and palmitoleic acids and membrane lipid composition), accumulation of polyunsaturated FA (docosapentaenoid acid, docosahexaenoic acid, eicosapentaenoic acid and arachidonate) and increased carnitine metabolism resulting in ER stress and the inhibition of protein synthesis [51]. In BCa, Lysophosphatidic acid (LPA) producing enzyme Autotaxin (ATX) promotes the progression of osteolytic bone metastases in animal models and is expressed in 25% of bone metastases [52][53]. Moreover mature osteoclast secretome determined by metabolomics profiling using LC-MS show increased secretion of Arachidonic Acid (AA) and a decrease of lysophosphatidylcholines (LPC) from osteoclasts. This shift in

lipid osteoclastokines stimulates BCa cell lines growth, migration, survival and intravenous administration of osteoclast-secreted lipids exacerbate syngeneic Py8119 cell bone metastases *in vivo* [54]. They also stimulate prometastatic genes, osteopontin (OPN), cyclooxygenase 2 (COX2), integrin- β 3, RANKL, PTHrP and interleukin-1 β expression in BCa cell [54]. Contradictory, BCa Raman Spectroscopy shows that lipids (triacylglycerols, cholesterol, fatty acid, membrane lipids) were inversely proportional to bone metastatic ability [46].

D. Reactive oxygen species (ROS)

Lung and bone metastases also demonstrated an increase in mitochondrial activity and reactive oxygen species (ROS) production [55]. These metabolic differences were due to a decrease in lung and bone metastases of hypoxia-inducible factor 1alpha (HIF-1alpha) conducting to the decrease on the gene encoding pyruvate dehydrogenase kinase (PDK1) and the inactivation of pyruvate dehydrogenase (PDH) which blocks the conversion of pyruvate to acetyl-CoA and suppress the TCA cycle [55]. In contrast, the antioxidant proteins Peroxiredoxin 2 (PRDX2) that is highly expressed in the lungs metastatic BCa cells was shown to have different impact in lung and bone microenvironment. Indeed, high levels of PRDX2 in lungs metastatic breast cancer model contributed to decreased ROS production and stimulation of metastatic progression while in bone metastases model PRDX2 induced an inhibition of osteolytic progression [56]. Because of the high-energy demands of osteoclasts, bone-resorbing cells contain a large number of mitochondria that are the major source of ROS. Interestingly osteoclasts used ROS as secondary signaling molecules during NF-kB ligand (RANKL)-induced differentiation and activation and stimulate bone destruction [20] [57]. In contrast, deacetylation of FoxOs by Sirt1 prevents FoxO association with β -catenin and potentiates Wnt signaling, leading to decreased ROS and stimulation of osteoblasts proliferation [58][59]. Concomitantly, by coupling Raman Spectroscopy and multivariate Curve Resolution (MCR)

analysis Marro *et al* show that lipid, nucleotide and cytochrome C level was higher in B02/PRXD2 cells compared to B02 while amino acid level was lower [46].

IV. Energetic reprogramming genes in osteo-oncology.

A. Transcription factors and cofactors

Peroxisome proliferator-activated receptor γ (PPAR γ) is a master regulator of energy metabolism involved in the adipocytic lineage, thermogenesis and mitobiogenesis [60]. During aging, increased activity of Cebp β /PPAR γ 2, or the decreased expression of the TGF- β /BMP, Wnt/ β -catenin, and IGF-I signaling pathways induced MSCs commitment changes, creating an unbalanced proadipocytic and anti-osteoblastic MSC allocation [61]. These changes lead to reduced osteoblast differentiation and increased adipocytes formation conducting to a new bone microenvironment metabolic status in favor of lipids enrichment. The master genes of this network are Cebp β and PPAR γ , two critical transcription factors that initiate a cascade of other factors that enhance differentiation of adipocytes. Endogenous ligands, including the 15-deoxy-delta12, 14-prostaglandin J2 (15d-PGJ2) that derived from arachidonic acid through cyclooxygenase, and the oxidized lipid (Linoleic acid through lipo-oxygenase), 9-hydroxyoctadecadienoic acid (9-HODE) and 13-HODE activate PPAR γ promote the progression of adipogenesis [48][60][61]. In bone metastases, 15d-PGJ2 inhibited viability, migration, invasion, and PTHrP production in MDA-MB-231. Conditioned medium from MDA-MB-231 treated with 15d-PGJ2 also decreased the RANKL/osteoprotegerin (OPG) ratio in osteoblasts. Osteolytic lesions progression induced by MDA-MB-231 cells was also limited by subcutaneously injected 15d-PGJ2 concomitantly with serum PTHrP levels [20]. Interestingly, FABP4 and PPAR γ pathways interaction was described in skeletal prostate tumors, revealing a pro-tumoral function of PPAR γ in PCa cell aggressiveness in bone [49]. In another study, Yen *et al* show that 15d-PGJ₂ is also a potential regulator of OS through the

PPAR γ -dependent pathway by promoting apoptosis through ROS-mediated JNK activation, downregulation of p-Akt and protein kinase A (PKA)-PLK1 pathways [62]. Beside ligands, coactivators PPAR γ coactivator 1 α (PGC-1 α), can also stimulate PPAR γ activity [48][60][61].

PGC1 α is largely known as a master co-regulator of multiple energetic metabolic pathways through nuclear transcriptional metabolic program [63] [64] [65] [66]. In bone metastases, increased PGC1 α suppressed PCa bone lesion development and integrative metabolomics and transcriptomics revealed that PGC1 α induced a catabolic state, characterized by a reverse Warburg effect, high ATP production and limited production of fatty acid and lactate [67]. This metabolic switch was dependent of PGC1 α interaction with the nuclear receptor Estrogen Related Receptor alpha (ERR α): high PGC1 α level (PGC1 α^{high} /ERR α) conducting to PCa tumor suppression and low level (PGC1 α^{low} /ERR α) to PCa progression [67] [68].

Similar to PPAR γ , ERR α is a nuclear receptor strongly involved in transcriptional metabolic program (adipogenesis, lipogenesis, thermogenesis, neoglucogenesis). In cancer, ERR α is involved in rapid responses to metabolic stress, drug resistance and energetic reprogramming (Glycolysis, pyruvate, OXPHOS, fatty acid oxydation, glutamine, lactate and One-carbon metabolism) [68][69][70][71][72][73]. In bone, ERR α is involved in osteoblasts formation and osteoclasts differentiation and polarization [74][75][76][77]. This receptor is also considered with MYC, as a main regulator of osteoclasts metabolic status by being at the origin of a metabolic switch to an oxidative state required for their differentiation through RANKL pathway [78]. Moreover cholesterol is suspected to activate ERR α activity in osteoclasts and inhibiting ERR α in osteoblasts culture induce a switch into the adipocytic lineage suggesting that low level of ERR α may contribute to lipids enrichment of the bone microenvironment [79][80]. In osteo-oncology, ERR α was found to play a dual function since this receptor acts as an inhibitor and a pro-tumor factor in BCa and PCa-derived bone metastases progression respectively. To understand that functional duality, ERR α was

associated in BCa derived bone metastases with limitation of osteoclasts differentiation and with CD8⁺ T lymphocytes recruitment. Concomitantly increased OPG, CCL17 and CCL20 expression level and inhibition of TGFβ3 signaling was found [81][82]. Conversely, activation of PCa derived bone metastases was due to an increase of bone remodeling (osteoblasts and osteoclasts) and increased level of VEGF, Wnt5a and TGFβ1 expression in tumor cells, and to an active metastatic niche *via* the up-regulation of Periostin in cancer-associated stromal cells [83].

Beside PPARγ and ERRα, PGC1α can also interact and regulate transcriptional activity of other nuclear factors such as nuclear respiratory factor 1-2 (Nrf1-2), Forkhead box O3 (FoxO3a), and cyclic-AMP (cAMP) response element-binding protein (CREB) [63][64]. Nrf1-2, and FoxO3a are master regulators for mitobiogenesis, antioxidant defense and rapid responses to metabolic stress [84][85] and CREB is acting as a metabolic sensor, involved in the adaptation to nutrient restriction and in glycolysis (lactate production), and detoxification of ROS by catalase [86][87][88]. In osteo-oncology, MDA-MB-231 BCa cells exhibited higher CREB expression than non-metastatic MCF-7 cells and CREB expression was increased by several soluble factors, such as IL-1, IGF-1, and TGF-β, factors linked to bone metastases progression [89]. More precisely, IL1β was described to stimulate Wnt signaling *via* NFκB and CREB, promoting BCa cancer stem cells colony formation in bone marrow and bone metastases progression [90]. Reinforcing these data, osteolytic lesions induced by MDA-MB-231 cell were inhibited by a dominant-negative form (K-CREB) [89]. CREB-AP1 was also implicated into treatment response in OS and in the regulation of PTHrP, OPG and Bone Sialoprotein (BSP) in BCa which are closely involved in cancer metastasis and bone destruction [89][91][92]. Moreover, sterol regulatory element-binding protein 2 (SREBP-2) was similarly under the transcriptional control of CREB in BCa inducing matrix metalloproteinases (MMPs) expression, key matrix degradative enzymes involved in bone metastases progression [93].

Altogether, if all these studies on master regulators of energy metabolism (linked to the co-activator PGC1 α) show their involvement in bone lesions progression, their metabolic function in osteo-oncology remains elusive, even though increased expression of ERR α in 4T1 model was associated in the primary tumor to a glycolytic phenotype with increased Glut1, PGC1 α , PGC1 β , Cytc, Ldha and Sod2 mRNA level and decreased of Mcad, Pdk1, Ldhb and Mct1 [94].

Myc and Ras, two other transcription factors, are also both linked to osteo-oncology and implicated in energetic metabolism: glycolysis, glutaminolysis, and mitochondrial biogenesis, rendering cells (tumor cells, osteoclasts) glucose and glutamine dependent for c-Myc and increases glycolytic flux into lactate, de novo nucleic acid synthesis and increases NADH shuttling and oxygen consumption for Ras signaling [95][96][97][98]. MYC oncogene has been implicated in the pathogenesis of human OS and upon MYC inactivation, osteosarcoma exhibited a slight reduction in uptake of ¹⁸FDG, even-though these tumors continue to accumulate ¹⁸FDG at higher levels compared to normal bone [99]. Moreover, OS cells, which harbor mutations driving c-Myc over-expression, induced OS osteoblastic differentiation and ectopic bone formation [100]. Also, in PCa, c-Myc could participate to osteolytic lesions by being up-regulated by estrogen receptor (ER) β 2 that can regulate stimulators of bone lesion progression Runx2, Twist1 and Dickkopf homolog (DKK1) [101][102]. For the oncogen Ras, clinical studies demonstrate that H-Ras codon 61.2 mutation may have some predictive roles for bone metastases in prostate carcinoma [103]. Moreover, even though if it is rarer, bone is the most common site of initial for metastatic follicular thyroid carcinoma (FTC) where Ras mutation was the most commonly identified (55%) [104]. Oncogenic Ras promotes the metastasis of DU145 to multiple organs, including bone and activation of one of the effector pathways downstream of Ras, RalGEF, or WNT signaling and phosphorylated Runx2 (S294 and S312 in human Runx2) led to bone metastases [105][106][107]. Moreover, a positive correlation was noted between OPG production level and K-Ras mutation status in pancreatic

cancer suggesting a link in bone metastases progression [108]. Ras up-regulation and activation was also observed in lung adenocarcinoma that metastasized to bone through the stimulation of Cut and Homeobox DNA-binding domains (ONECUT2) [109]. Moreover, Ras signaling can be regulated by Myc signaling (Myc-associated zinc-finger prot (MAZ)) in bone metastases from PCa [110]. Similarly, Plexin-B1, a semaphorin (SEM4D) receptor that was found to be mutated in 89% of PCa bone metastases, stimulate cell motility and invasion by increasing ratio of active/inactive R-Ras [111]. All together these data describe the expression of well-known energetic metabolism regulators (ERR α and CREB, oncogen Myc and Ras) in bone metastases, suggesting that they can also function as such in osteo-oncology.

B. Genetic fusion EWS-FLI and TMPRSS2-ERG

Fusion between erythroblast transformation-specific (ETS) family transcriptional factor Fli with the Ewing sarcoma breakpoint region 1 (EWS) in PCa events had been linked to metabolic changes and aggressiveness. First, EWS/FLI fusion that is driving the Ewing sarcoma (ES) is a master regulator of metabolism reprogramming in ES. EWS/FLI function results in decrease glycolysis (decrease expression of hexokinase-1), extra cellular acidification rate and low mitochondrial membrane potential suggesting decrease oxidative metabolism, and biosynthesis [112]. This decrease of glycolytic intermediates is linked to increased expression of phosphoglycerate dehydrogenase (PHGDH), phosphoserine aminotransferase (PSAT), phosphoserine phosphatase (PSPH), and serine hydromethyltransferase (SHMT1 and SHMT2) expression, enzymes that participate to the commitment of the glycolytic intermediate 3-phosphoglycerate to serine and glycine synthesis contributing to biosynthetic pathways (precursors for protein, lipid and nucleic acid synthesis) and to 1-carbon metabolism (methyl donor recycling and epigenetic states) [112].

Transmembrane serine protease 2 (TMPRSS2) with another ETS transcription factor ETS-related gene (ERG) fusion also influence the metabolome with a switch of glycolysis to FA

metabolism in PCa. Decreased levels of sugars (glucose, mannose, maltose), may be due to rapidly consuming glucose and elevated levels of lipids metabolism (phosphocholine, phosphoethanolamine, choline-associated metabolites, cerebronic acid, 2-hydroxybehenic and tricosanoic acid (C23 :0)), as observed in TMPRSS2-ERG+ cells and patients [113][114]. Decrease of sphingolipid pathway and increased of acylcarnitines confirm altered FA metabolism in ERG-positive PCa samples [115]. The observed increased of lipids metabolism is in accordance with previous data showing that obesity is associated with poorer prognosis primarily with ERG-positive tumors [116]. Increase lipids metabolism was linked to increase expression level of acetyl-CoA carboxylase alpha (ACACA), FA synthase (FASN), carnitine palmitoyltransferase 1A (CPT1) and long-chain acyl-CoA synthetase 3 (ASCL3) and decrease citrate with low level of aconitase 2 (ACO2). Decrease of carnitine palmitoyltransferase 2 (CPT2), peroxysomal protein enoyl-CoA ligase -1 (EHHADH) and long-chain-fatty-acid-CoA ligase 1 (ACSL1) was also described [115]. Concerning glucose metabolism, high ERG was associated with increased expression of pyruvate kinase (PKM2), 6-phosphogluconolactonase (PGLS), transketolase (TKT) and ribokinase (RBKS) suggesting that ERG may redirect carbohydrate intermediates to pentose phosphate pathway [113]. Decreased levels of spermine, putrescine, gluconic acid, and citrate and increase of tryptophane, tyrosine, isoleucine, aspartate and metabolites belonging to the purine catabolism (Inosine, Xanthine, Adenine and Uric acid) level were also described [113][114][115]. Interestingly, TMPRSS2/ERG has been recently described as a pro-bone metastases factor from PCa by enhancing osteoblastic phenotype through the direct regulation of endothelin-1, Collagen Type I Alpha 1 Chain and Alkaline Phosphatase expression [117]. Now, TMPRSS2/ERG function as a regulator of energy metabolism in osteo-oncology needs to be addressed in the future.

C. mTOR: glycolysis and autophagy

mTOR is known to sense alteration in nutrients (amino acids and glucose), oxygen and stress and is mainly involved in anabolic processes such as proteins and lipids synthesis, stimulates glycolysis and inhibits autophagy conducting to tumor cell proliferation and progression [118]. In bone, mTOR may interact with PPAR γ signaling, Wnt/ β -catenin signaling and the RANK/RANKL thus participating to bone homeostasis [119]. In bone metastases, mTOR is involved early at the BCa cells anchorage stage in the establishment of the bone osteogenic niche, since adherens junctions between osteoblasts (N-cadherin) and tumor cells (E-cadherin) activates mTOR, conducting stimulation of eIF4, protein synthesis and tumor cells proliferation and survival [120][121]. Under ATP availability, metastatic dissemination to bone can be stimulated by the inactivation of the nutrient-sensing AMPK through the tumor necrosis factor receptor-associated protein-1 (TRAP-1), preventing autophagy, stimulating migration and overcoming metabolic stress [122]. Concomitantly to the inhibition of the AMPK activation, the Akt/mTOR/S6K1 was found hyperactivated in a TNBC metastasis to bone model derived from MDA-MB-231 cells [123]. In PCa, inhibiting mTOR may also alter bone metastasis progression by limiting bone colonization and osteolytic lesions induced by PC3 cells *in vivo* and by counteracting osteoclastogenesis and stimulation of osteoblast activity [124]. Beside bone metastases, genetic screen and common insertion site (CIS) analysis of primary tumors and metastatic osteosarcoma identified osteosarcoma-associated genes enriched in the PI3K-AKT-mTOR: RAF1, AKT2, YWHAE, GAB1, GRB2, GSK3B, EIF4E, PTEN, MCL1, SEMA4d, NF1, SEMA6d and ZFP217 [125]. Among them, five genes are known to be implicated into energetic metabolism; AKT2 (insulin-stimulated glucose uptake and glycolysis), glycogen synthase kinase-3 β (GSK3B)(mitochondrial function and hepatic energetic balance), eIF4E (mediating the effects cellular energetics on mRNA translation and protein synthesis), PTEN (autophagy, mitophagy) and MCL1 (mitochondria, response to glucose, apoptosis) [126] [127] [128] [129] [130]. Also, up-regulation of eukaryotic translation

elongation factor 1 delta (EEF1D) and of Glycoprotein non-metastatic melanoma protein B (GPNMB) promotes OS (MG63 and U2OS cells) proliferation and metastasis by facilitating Akt-mTOR signaling pathways [131][132]. P2X7, an ATP-gated ion channel is also highly expressed in OS and stimulate mTOR/HIF1 α /VEGF signaling promoting growth and metastasis of Human HOS/MNNG [133].

Cancer progression is also attributable to a dysfunction of autophagy, a dynamic catabolic process that has dual roles in promoting cell survival as well as cell death [134]. Rab5a, a small-GTPase which regulates vesicle traffic for autophagosome formation, was associated to high grade dysplasia in the mammary gland and detected in bone metastases together with Beclin-1 [135]. The HGF/Met/HIF1 α axis was also associated with autophagy failure and prevention of bone metastases [135]. In OS, Tumor-suppressing STF cDNA 3 (TSSC3) is positively correlated with ATG5 expression and can promote autophagy *via* inactivating the Src-mediated PI3K/Akt/mTOR pathway inhibiting proliferation, migration and invasion of OS cells [136]. However, this link between mTOR and autophagy in OS was also documented by showing that the combination of inhibitors of mTOR and inhibitors of autophagy could enhance OS MG63 cells apoptosis [137]. In OS, autophagy is strongly described as a tumor survival and cytoprotective mechanism to provide tumor cells energy under stress conditions and also decrease drug sensitivity [138]. Indeed, studies linked to HSP90 and its family member HSP90AA1 described autophagy in OS as a cytoprotective process and a process involved into drugs resistance [139][140][141]. Indeed, inhibition of autophagy by 3-methyladenine (3-MA) treatment decreases proliferation rate and promotes apoptosis, reversing the autophagy-induced-cytoprotection due to low level of HSP90 (geldanamycin) or high level of HSP90AA1 in OS cells [139]. They further show that HSP90AA1 induces autophagy by impairing PI3K/AKT/mTOR pathway [141]. Beside mTOR, ROS/JNK is also a key regulator of autophagy in OS. Ma *et al.* had reported that cinobufagin can trigger apoptosis and autophagy

through the activation of the ROS/JNK/P38 axis [142]. The transcription factor Wilms tumor suppressor 1 (WT1) is also associated with autophagy in OS, through the AKT/JNK pathway and the expression of arrest-specific 1 (GAS1) [143]. Investigation on the role of Wnt/ β -catenin signaling in the induction of autophagy in OS show that activating the Wnt/ β -catenin pathway could increase the sensitivity of beclin 1-overexpressed OS cells to gemcitabine-induced cell death [144]. Several MicroRNAs (miRNA) are also associated with the regulation of autophagy and play an important role in the resistance or sensitivity to chemotherapy. miR-199a-5p that directly target beclin-1, inhibits cisplatin-mediated autophagy and reinforces proliferation in OS inhibition [145]. miR-140-5p is also observed in OS treated with chemotherapy, and overexpression of miR-30a enhanced Dox-stimulated apoptosis and inhibited autophagy [146][147].

D. Osteoblastic and osteoclastic factors: osteomimicry and energetic metabolism

Factors that were first detected in bone physiology have been recently linked to metabolic syndromes. RANKL and OPG represent osteoblast-derived paracrine cytokines that are essential for osteoclasts functions and are commonly detected in breast and prostate cancer and highly expressed in bone metastases [148]. Recently, epidemiological studies have linked OPG to insulin resistance in pre-diabetes and type 2 diabetes in postmenopausal women and in obese adolescents [149][150][151] [152]. Elevated serum OPG was also found in diabetic (Type 2) nephropathy and cardiovascular risk (Type 1) [153][154][155]. Other findings imply that OPG exert a common defensive effect on the pathophysiologic alterations responsible for nonalcoholic fatty liver disease [156]. Furthermore, RANKL is proposed as predictor of incident type 2 diabetes mellitus and epigenetic regulation (methylation) of RANKL gene have influences on obesity and adipokine levels [157]. Moreover hepatocyte-specific RANK knockout mice prevent the insulin resistance induced in mutated mice after 4weeks of high-fat

diet [158] and overexpress RANKL (HuRANKL-Tg+) in mice develop sarcopenia while inhibition of RANKL (Denosumab) and OPG improved muscle insulin sensitivity, glucose uptake, and decrease myostatin [159], providing evidence that the RANKL/RANK/OPG axis may be implicated in energetic metabolism of tumor cells in bone. Interestingly, $ERR\alpha$, that play a central role at the interface of cancer and metabolism, is a direct regulator of OPG and RANK in BCa cells and can inhibit bone metastases progression suggesting that OPG and RANK regulation may, beside impacting osteoclast (OPG), also participate into $ERR\alpha$ energetic reprogramming function in tumor cells that metastasize to bone [81][94][160]. Other factors identified in bone cells, like periostin a secreted cell adhesion protein and sclerostin, a cysteine-knot-containing protein mainly released by osteocytes had also been linked to metabolic syndromes and bone metastases. More precisely, Periostin can promote liver steatosis and hyperglyceridemia and is increased in plasma and associated with glucose and lipids of subjects with obesity and Type 2 Diabetes [161][162]. In contrast, besides an increase in bone volume, *Sost*^{-/-} mice exhibit a reduction in adipose tissue accumulation in association with increased insulin sensitivity [163]. High level of Slerostin was also found in children and adolescents with Type 1 Diabetes and is described as a biomarker for early Atherosclerosis in obesity [164] [165]. Similar to OPG/RANKL axis, Periostin and Slerostin are markers of osteolytic lesions derived from BCa suggesting that, both factors may impact bone metastases energetic metabolism [166][167][168][169]. In line with bone markers, Runx2 is abnormally expressed in myeloma and in highly metastatic breast and prostate cancer cells and is associated to bone metastases progression [107][170][171][172]. Interestingly, Runx2 is also associated to catabolic process and can enhance autophagy by stimulating trafficking of LC3B vesicles in metastatic breast cancer cells [173]. Runx2 can also alter glucose metabolism in breast cancer cells. It is associated with expression of glycolytic genes; increased glucose uptake and Runx2 knockdown can increase sensitivity to metformin [174]. Conversely, glucose *via* the activation

of PI3K/Akt pathway stimulates Runx2 but also OPN and osteocalcin (OCN) in osteoblasts [175]. Interestingly, OPN is involved in atherogenesis and hyperglycemia and is also highly expressed in invasive BCa and PCa cells. It is acting as pro-bone metastatic factor and is considered as a biomarker in OS [176][177]. If OPN is implicated in primary tumor proliferation, angiogenesis, invasion and anchorage-independent expansion of tumor cells, it was also described as a modulator of glucose metabolism [178][179]. Osteopontin-a, but not by osteopontin-c could increase glucose level, stimulates lipid signaling through STAT-3 and the stimulation of sn-glycero-3-phosphocholine but down regulates the levels of lactate and taurine in BCa [179]. Osteopontin-a stimulation of cellular glucose levels, may supply the energy source for osteopontin-c mediated anti- anoikis suggesting that OPN may support the survival of circulating tumor cells. Beside the PI3K/Akt pathway, glucose can also upregulate OPN *via* FoxO1 activation that is closely associated with insulin resistance and diabetes [180]. OCN is one of the main osteogenic hormone linked to energy metabolism. OCN is secreted by osteoblast and through resorption is released into active undercarboxylated active OCN in the blood to act as a hormone that can regulate energy metabolism *via* enhancement of glucose uptake and increase mitochondria biogenesis in muscle, increase insulin production in pancreas, increase insulin sensitivity and decrease lipid accumulation in the liver and stimulate adiponectin expression in adipose tissue [181]. Recently, clinical and preclinical studies allow to identify circulating OCN-positive cells (cOC) in BCa bone metastases. Progressive disease group present higher baseline cOC and a decrease in bone metastases free survival. In murine, cOC increased at early time points when micrometastases were detectable by histology but not observed by bioluminescence imaging and its expression correlated bone metastases tumor burden [182].

V. Treatments targeting tumor cells energetic metabolism in bone.

Reinforcing pre-clinical data, PI3k/mTOR inhibitor rapamycin was found to inhibit osteolytic lesions progression by counteracting osteoclasts and stimulating osteoblasts in breast and prostate derived bone metastases [124][183][184]. Interestingly, rapamycin can also stimulate OPG secretion by host stromal cells inhibiting osteolytic lesions [185]. Finally results from the BOLERO-2 study suggest beneficial effects of the combination therapy exemestane with everolimus (inhibitor of mTOR) by lowering disease progression in the bone and suppress increased bone turnover and resorption observed in exemestane alone [186][187][188]. Everolimus was also shown to decrease mTOR in OS [189]. Denosumab, a fully human monoclonal antibody directed against RANKL that inhibits osteoclasts differentiation, decrease bone metastases progression from BCa and PCa patients and has been shown to improve muscle strength, insulin sensitivity and glucose uptake in mice suggesting that it may also regulate tumor cell energetic metabolism [190][191][192]. Interestingly, the very commonly drugs the aminobisphosphonates (NBP), used for treatment of metastatic bone disease, prevent prenyl protein transferase of the mevalonate pathway, a major metabolic pathway that plays a key role in sterol isoprenoids, such as cholesterol and androgen synthesis [193][194]. Other mevalonate pathway inhibitors, statins (Atorvastatin, Fluvastatin, Simvastatin), a class of lipid-lowering drugs that target the mevalonate pathway rate-limiting enzyme dominant, the 3-hydroxyl-3-methylglutaryl-Coenzyme A reductase (HMG-CoAR), have also been described in bone metastatic treatment and recently in the prevention of osteonecrosis of the jaw [195][196][197]. Accordingly, targeting of SREBP-2 with Fatostatin, which specifically inhibits SCAP (SREBP cleavage-activating protein) and prevents SREBP activation, attenuated breast cancer-induced osteolysis *in vivo* [91]. Moreover, the combination of Simvastatin and Metformin (activator of AMPK activity) synergistically reduces CRPC cell viability and metastatic properties by stimulating AMPKa activity and decreasing HMG-CoAR, reducing total cellular cholesterol and its synthesis in PCa cells [198]. Interestingly, concerning statins actions, ERR α activation

by cholesterol was shown to mediate Simvastatin and zoledronate effects on osteoclasts, muscle and macrophages suggesting that statins and NBP may mediate their effects on bone metastases through the nuclear receptor [79]. Beside metastases, Simvastatin is described to induce apoptosis of OS cell lines through the regulation of the RhoA-AMPK/p38MAPK signaling and to exert anticancer effect via the geranylgeranylation and c-Jun activation [199]. Lovastatin, NBP and metformin also improve OS cell sensitivity to doxorubicin and cisplatin by increasing their cytotoxicity [200]. In line with lipid metabolism regulation, NADPH oxidase activation is involved in LPA stimulating osteolysis through at least three signaling including, LPA, RANKL and TGF β and NADPH oxidase inhibitors Phycocyanin (anti-oxidant: ROS inhibitor) and statins can decrease osteolytic bone metastases driven by LPA generation [201]. Finally, selective FASN inhibitor antagonizes CRPC growth through metabolic reprogramming [51] and epidemiology has linked omega-3 intake to increase bone density and decrease osteolysis in patients treated with aromatase inhibitor therapy [202].

Conclusions and perspectives

Multiple mechanisms contribute to primary bone tumor and bone metastases development and among them metabolic flexibility, enabling tumor cells to adapt fluctuations in nutrients is becoming a key step in survival of metastatic cells in bone suggesting that targeting energetic metabolism may constitute new therapeutic pathway into osteo-oncology. Several genes implicated in osteomimicry are described these days in metabolic syndromes and involved in metabolites metabolism (glucose, lipids) suggesting their implication, not only on bone micro-environment alteration, *i.e* osteoclasts, osteoblasts, CAF or immune cells, but also in energetic fuel used, re-programming and adaptation for survival and progression in bone lesions. On the other side, master genes and co-regulators of multiple energetic metabolic pathways identified

in metabolic diseases, should also constitute new research avenues to decipher new mechanisms of action of tumor cells in bone anchorage, survival, dormancy and progression. In conclusion, identifying metabolic signatures could therefore constitute, not only new therapeutic paths but also a new means of monitoring tumors metabolic status contributing to a better-personalized medicine.

References

1. Peterson L, O'Sullivan J, Wu Q, Novakova-Jiresova A, Jenkins I, Lee J, et al. Prospective study of serial 18F-FDG PET and 18F-fluoride (18F-NaF) PET to predict time to skeletal related events, time-to-progression, and survival in patients with bone-dominant metastatic breast cancer. *J Nucl Med*. 2018;1823–30.
2. Pietrzak A, Czepczynski R, Wierchoslawska E, Cholewinski W. Detection of the Prostate Cancer Bone Metastases: Is It Feasible to Compare 18F-fluorocholine PET/CT, 18F-fluorodeoxyglucose PET/CT and 99mTc-methyl Diphosphonate Bone Scintigraphy? *Urol J*. 2018 Sep;242–7.
3. Gonzalez-Menendez P, Hevia D, Alonso-Arias R, Alvarez-Artme A, Rodriguez-Garcia A, Kinet S, et al. GLUT1 Protects Prostate Cancer Cells From Glucose Deprivation-Induced Oxidative Stress. *Redox Biol*. 2018 Jul 17;112–27.
4. Chen H, Aggarwal R, Bok R, Ohliger A, Zhu Z, Lee P, et al. Hyperpolarized 13 C-pyruvate MRI Detects Real-Time Metabolic Flux in Prostate Cancer Metastases to Bone and Liver: A Clinical Feasibility Study . *Prostate Cancer Prostatic Dis*. 2020 Jun; 269–76.
5. Scalzi P, Baiocco C, Genovese S, Trevisan A, Sirotova Z, Poti C. . Evaluation of bone metastases by 18F-choline PET/CT in a patient with castration-resistant prostate cancer treated with radium-223. *Urologia*. 2017;61–4.

6. Pianou N, Stavrou P, Vlontzou E, Rondogianni P, Exarhos D, Datseris I. More advantages in detecting bone and soft tissue metastases from prostate cancer using 17F-PSMA PET/CT. *Hell J Nucl Med*. 2019 Apr;6–9.
7. Zheng Y, Cantley L. Toward a Better Understanding of Folate Metabolism in Health and Disease. *J Exp Med*. 2019 Feb 4;253–66.
8. Pyka T, Okamoto S, Dahlbender M, Tauber R, Retz M, Heck M, et al. Comparison of Bone Scintigraphy and 68 Ga-PSMA PET for Skeletal Staging in Prostate Cancer. *Eur J Nucl Med Mol Imaging*. 2016;2114–21.
9. Sachpekidis C, Baumer P, Kopka K, Hadaschik B, Hohenfellner M, Kopp-Schneider A, et al. 68 Ga-PSMA PET/CT in the Evaluation of Bone Metastases in Prostate Cancer. *Eur J Nucl Med Mol Imaging*. 2018 Jun;904–12.
10. Amorim B, Prabhu V, Marco S, Gervais D, Palmer W, Heidari P, et al. Performance of 18 F-fluciclovine PET/MR in the Evaluation of Osseous Metastases From Castration-Resistant Prostate Cancer. *Eur J Nucl Med Mol Imaging*. 2020 Jan;105–14.
11. Lynch C, Adams S. Branched-chain amino acids in metabolic signalling and insulin resistance. *Nature Reviews Endocrinology*. 2014;723–36.
12. Fontana L, Cummings N, Arriola Apelo S, Neuman J, Kasza I, Schmidt B, et al. Decreased Consumption of Branched-Chain Amino Acids Improves Metabolic Health. *Cell Reports*. 2016 Jul;520–30.
13. Duan Y, Fengna L, Yinghui L, Tang Y, Kong X, Feng Z, et al. The Role of Leucine and Its Metabolites in Protein and Energy Metabolism. *Amino Acids*. 2016 Jan;41–51.
14. Nanni C, Schiavina R, Brunocilla E, Boschi S, Borghesi M, Zanoni L, et al. 18F-Fluciclovine PET/CT for the Detection of Prostate Cancer Relapse: A Comparison to 11C-Choline PET/CT. *Clin Nucl Med*. 2015 Aug;386–91.
15. Hanahan D, Weinberg R. Hallmarks of cancer: the next generation. 2011 Mar 4;646–74.
16. Jung Y, Shiozawa Y, Wang J, Wang J, Wang Z, Pedersen E, et al. Expression of PGK1 by prostate cancer cells induces bone formation. *Mol Cancer Res*. 2009 Oct;1595–604.
17. Cha Y, Jung W, Koo J. Differential Site-Based Expression of Pentose Phosphate Pathway-Related Proteins among Breast Cancer Metastases. *Dis Markers*. 2017 Feb 2;
18. Lemma S, Di Pompo P, Porporato P, Sboarina M, Russell S, Gillies R, et al. MDA-MB-231 breast cancer cells fuel osteoclast metabolism and activity: A new rationale for the pathogenesis of osteolytic bone metastases. *Biochim Biophys Acta Mol Basis Dis*. 2017 Dec;3254–64.
19. Warburg O, Wind F, Negelein E. The metabolism of tumors in the body. *J Gen Physiol*. 1927 Mar 7;519–30.
20. Kim H, Jung W, Koo J. Site-specific metabolic phenotypes in metastatic breast cancer. *J Transl Med*. 2014 Dec 14;354.
21. Zhao Z, Wu M, Zou C, Tang Q, Lu J, Liu D, et al. Downregulation of MCT1 inhibits tumor growth, metastasis and enhances chemotherapeutic efficacy in osteosarcom through regulation of the NF- κ B pathway. *Cancer Lett*. 2014 Jan 1;150–8.
22. Liu Y, Sun X, Huo C, Sun C, Zhu J. Monocarboxylate transporter 4 (MCT4) overexpression is correlated with poor prognosis of osteosarcoma. *Med Sci Monit*. 2019 Jun 9;4278–84.
23. Yoneda T, Hiasa M, Nagata Y, Okui T, White F. Acidic microenvironment and bone pain in cancer-colonized bone. *Bonekey Rep*. 2015 May 6;690.
24. Avnet S, Di Pompo G, Lemma S, Baldini N. Cause and effect of microenvironmental acidosis on bone metastases. *Cancer Metastasis Rev*. 2019 Jun;133–47.
25. Arnett T. Acidosis, hypoxia and bone. *Arch Biochem Biophys*. 2010 Nov 1;103–9.

26. Frick K, Bushinsky D. Metabolic acidosis stimulates RANKL RNA expression in bone through a cyclo-oxygenase-dependent mechanism. *J Bone Miner Res.* 2003 Jul; 1317–25.
27. Massa A, Perut F, Chano T, Woloszyk A, Mitsiadis T, Arvet S, et al. The effect of extracellular acidosis on the behaviour of mesenchymal stem cells in vitro. *Eur Cell Mater.* 2017 Apr;252–67.
28. Bischoff D, Zhu J, Makhijani N, Yamaguchi D. Acidic pH stimulates the production of the angiogenic CXC chemokine, CXCL8 (interleukin-8), in human adult mesenchymal stem cells via the extracellular signal-regulated kinase, p38 mitogen-activated protein kinase, and NF-kappaB pathways. *J Cell Biochem. J Cell Biochem.*;1378–92.
29. Avnet S, Di Pompo G, Chano T, Errani C, Ibrahim-Hashim A, Gillies R, et al. Cancer-associated mesenchymal stroma fosters the stemness of osteosarcoma cells in response to intratumoral acidosis via NF-κB activation. *Int J Cancer.* 2017 Mar 15;1331–45.
30. Goetze K, Walenta S, Ksiazkiewicz M, Kunz-Schughart L, Mueller-Klieser W. Lactate enhances motility of tumor cells and inhibits monocyte migration and cytokine release. *Int J Oncol.* 2011 Aug;453–63.
31. Colegio O, Chu N, Szabo A, Chu T, Rhebergen A, Jairam V, et al. Functional polarization of tumour-associated macrophages by tumour-derived lactic acid. *Nature.* 2014 Sep 25; 559–63.
32. Gottfried E, Kunz-Schughart L, Ebner S, Mueller-Klieser W, Hoves S, Andreessen R, et al. Tumor-derived lactic acid modulates dendritic cell activation and antigen expression. *Blood.* 2006 Mar 1;2013–21.
33. Brand A, Singer K, Koehl G, Kolitzus M, Schoenhammer G, Thiel A, et al. LDHA-Associated Lactic Acid Production Blunts Tumor Immunosurveillance by T and NK Cells. *Cell Metab.* 2016 Nov 8;657–71.
34. Fischer K, Hoffmann P, Voelkl S, Meidenbauer N, Ammer J, Edinger M, et al. Inhibitory effect of tumor cell-derived lactic acid on human T cells.
35. Calcinotto A, Filipazzi P, Gironi M, Iero M, De Milito A, Ricupito A, et al. Modulation of microenvironment acidity reverses anergy in human and murine tumor-infiltrating T lymphocytes. *Cancer Res.* 2012 Jun 1;2746–56.
36. Hiasa M, Okui T, Allette Y, Ripsch M, Sun-Wada G, Wakabayashi H, et al. Bone Pain Induced by Multiple Myeloma Is Reduced by Targeting V-ATPase and ASIC3. *Cancer Res.* 2017 Mar 15;1283–95.
37. Mach D, Rogers S, Sabino M, Luger N, Schwei M, Pomonis J, et al. Origins of skeletal pain: sensory and sympathetic innervation of the mouse femur. *Neuroscience.* 2002; 155–66.
38. Wakabayashi H, Wakisaka S, Hiraga T, Hata K, Nishimura R, Tominaga M, et al. Decreased sensory nerve excitation and bone pain associated with mouse Lewis lung cancer in TRPV1-deficient mice. *J Bone Miner Metab.* 2018 May;274–85.
39. Ludwig M, Vanek M, Guerini D, Gasser J, Jones C, Junker U, et al. Proton-sensing G-protein-coupled receptors. *Nature.* 2003 Sep 4;93–8.
40. Krishtal O. The ASICs: signaling molecules? Modulators? *Trends Neurosci.* 2003 Sep; 477–83.
41. Yang M, Mailhot G, Birnbaum M, MacKay C, Mason-Savas A, Odgren P. Expression of and role for ovarian cancer G-protein-coupled receptor 1 (OGR1) during osteoclastogenesis. *J Biol Chem.* 2006 Aug 18;23598–605.
42. Di Pompo G, Lemma S, Cinti L, Rucci N, Ponzetti M, Errani C, et al. Intratumoral acidosis fosters cancer-induced bone pain through the activation of the mesenchymal tumor-associated stroma in bone metastasis from breast carcinoma. *Oncotarget.* 2017

- Apr 13; 54478–96.
43. Li Y, Jia Y, Cui K, Li N, Zheng Z, Wang Y, et al. Essential role of TRPC channels in the guidance of nerve growth cones by brain-derived neurotrophic factor. *Nature*. 2005 Apr 14; 894–8.
 44. Ren L, Ruiz-Rodado V, Dowdy T, Huang S, Issaq S, Beck J, et al. Glutaminase-1 (GLS1) inhibition limits metastatic progression in osteosarcoma. *Cancer Metab*. 2020 Mar 5; 8:4
 45. Pollari S, Käkönen S, Edgren H, Wolf M, Kohonen P, Sara H, et al. Enhanced serine production by bone metastatic breast cancer cells stimulates osteoclastogenesis. *Breast Cancer Res Treat*. 2011 Jan; 421–30.
 46. Marro M, Nieva C, De Juan A, Sierra A. Unravelling the Metabolic Progression of Breast Cancer Cells to Bone Metastasis by Coupling Raman Spectroscopy and a Novel Use of Mcr-Als Algorithm. *Anal Chem*. 2018 May 1;5594–602.
 47. Duque G. Bone and Fat Connection in Aging Bone. *Curr Opin Rheumatol*. 2008 Jul; 429–34.
 48. Rosen CJ, Ackert-Bicknell C, Rodriguez J, Pino A. Marrow Fat and the Bone Microenvironment: Developmental, Functional, and Pathological Implications. *Crit Rev Eukaryot Gene Expr*. 2009; 109–24.
 49. Herroon M, Rajagurubandara E, Rudy D, Chalasani A, Hardaway A, Podgorski I. Macrophage cathepsin K promotes prostate tumor progression in bone. *Oncogene*. 2013; 32(12):1580–93.
 50. Thysell E, Surowiec I, Hörnberg E, Crnalic S, Widmark A, Johansson A, et al. Metabolomic characterization of human prostate cancer bone metastases reveals increased levels of cholesterol. *PLoS One*. 2010 Dec 3;
 51. Zadra G, Ribeiro C, Chetta P, Ho Y, Cacciatore S, Gao X, et al. Inhibition of De Novo Lipogenesis Targets Androgen Receptor Signaling in Castration-Resistant Prostate Cancer. *Proc Natl Acad Sci U S A*. 2019 Jan 8;631–40.
 52. Peyruchaud O, Leblanc R, David M. Pleiotropic Activity of Lysophosphatidic Acid in Bone Metastasis. *Biochim Biophys Acta*. 2013 Jan;99–104.
 53. Shim S, Shin E, Lee C, Koo J. The Expressions of Autotaxin-Lysophosphatidate Signaling-Related Proteins in Metastatic Breast Cancer. *Int J Clin Exp Pathol*. 2019 Aug 1; 2920–30.
 54. krzeszinski J, Schwaid A, Cheng W, Jin Z, Gallegos Z, Saghatelian A, et al. Lipid Osteoclastokines Regulate Breast Cancer Bone Metastasis. *Endocrinology*. 2017 Mar 1; 477–89.
 55. Dupuy F, Tabariès S, Andrzejewski S, Dong Z, Blagih J, Annis M, et al. PDK1-Dependent Metabolic Reprogramming Dictates Metastatic Potential in Breast Cancer. *Cell Metab*. 2015 Oct 6;577–89.
 56. Stresing V, Baltziskueta E, Rubio N, Blanco J, Arriba M, Valls J, et al. Peroxiredoxin 2 specifically regulates the oxidative and metabolic stress response of human metastatic breast cancer cells in lungs. *Oncogene*. 2013 Feb 7;724–35.
 57. Callaway D, Jiang JX. Reactive Oxygen Species and Oxidative Stress in Osteoclastogenesis, Skeletal Aging and Bone Diseases. *J Bone Miner Metab*. 2015 Jul; 359–70.
 58. Lyer S, Han L, Bartell SM, Kim H, Gubrij I, de Cabo R, et al. Sirtuin1 (Sirt1) Promotes Cortical Bone Formation by Preventing β -Catenin Sequestration by FoxO Transcription Factors in Osteoblast Progenitors. *J Biol Chem*. 2014 Aug 29;24069–78.
 59. Almeida M, Porter R. Sirtuins and FoxOs in Osteoporosis and Osteoarthritis. *Bone*. 2019 Apr; 284–92.
 60. Rosen E, Spiegelman BM. PPARgamma: A Nuclear Regulator of Metabolism,

- Differentiation, and Cell Growth. *J Biol Chem*. 2001 Oct 12;37731–4.
61. Moerman E, Teng K, Lipschitz D, Lecka-Czernik B. Aging Activates Adipogenic and Suppresses Osteogenic Programs in Mesenchymal Marrow Stroma/Stem Cells: The Role of PPAR- γ 2 Transcription Factor and TGF- β /BMP Signaling Pathways. *Aging Cell*. 2004 Dec;379–89.
62. Yen C, Hsiao C, Chen W, Wen Y, Lin Y, Chang T, et al. Cytotoxic Effects of 15d-PGJ2 Against Osteosarcoma Through ROS-mediated AKT and Cell Cycle Inhibition. *Oncotarget*. 2014 Feb 15;716–25.
63. Handschin C, Spiegelman BM. Peroxisome Proliferator-Activated Receptor Gamma Coactivator 1 Coactivators, Energy Homeostasis, and Metabolism. *Endocr Rev*. 2006 Dec; 728–35.
64. Tan Z, Luo X, Xiao L, Tang M, Bode A, Dong Z, et al. The Role of PGC1 α in Cancer Metabolism and Its Therapeutic Implications. *Mol Cancer Ther*. 2016 May;774–82.
65. Scarpulla R. Metabolic Control of Mitochondrial Biogenesis Through the PGC-1 Family Regulatory Network. *Biochim Biophys Acta*. 2011 Jul;1269–78.
66. Bost F, Kaminski L. The Metabolic Modulator PGC-1 α in Cancer. *Am J Cancer Res*. 2019 Feb 1;198–211.
67. Torrano V, Valcarcel-Jimenez L, Cortazar A, Liu X, Urosevic J, Castillo-Martin M, et al. The Metabolic Co-Regulator PGC1 α Suppresses Prostate Cancer Metastasis. *Nat Cell Biol*. 2016 Jun;645–56.
68. Audet-Walsh E, Giguère V. The Multiple Universes of Estrogen-Related Receptor α and γ in Metabolic Control and Related Diseases. *Acta Pharmacol Sin*. 2014 Dec 15;
69. Deblois G, St-Pierre J, Giguere V. The PGC-1/ERR Signaling Axis in Cancer. *Oncogene*. 2013 Jul 25;3483–90.
70. Tam I, Giguère G. The journey of the estrogen-related receptors in the cancer realm. *J Steroid Biochem Mol Biol*. 2016 Mar;13–9.
71. Audet-Walsh E, Papadopoli D, Gravel S, Yee T, Bridon G, Caron M, et al. 3 The PGC-1 α /ERR α Axis Represses One-Carbon Metabolism and Promotes Sensitivity to Anti-folate Therapy in Breast Cancer. *Cell Rep*. 2016 Feb 2;920–31.
72. Park S, Safi R, Liu X, Baldi R, Liu W, Liu J, et al. Inhibition of ERR α Prevents Mitochondrial Pyruvate Uptake Exposing NADPH-Generating Pathways as Targetable Vulnerabilities in Breast Cancer. *Cell Rep*. 2019 Jun 18;3587–601.
73. Park S, Chang C, Safi R, Liu X, Baldi R, Jasper J, et al. ERR α -Regulated Lactate Metabolism Contributes to Resistance to Targeted Therapies in Breast Cancer. 2016 Apr 12;323–35.
74. Bonnelye E, Merdad L, Kung V, Aubin JE. The orphan nuclear estrogen receptor-related receptor alpha (ERR α) is expressed throughout osteoblast differentiation and regulates bone formation in vitro. *J Cell Biol*. 2001 May 28;153(5):971–84.
75. Wei W, Wang X, Yang M, Smith L, Dechow P, Sonoda J, et al. PGC1 β mediates PPAR γ activation of osteoclastogenesis and rosiglitazone-induced bone loss. *Cell Metab*. 2010 Jun 9;:503–16.
76. Bonnelye E, Saltel F, Chabadel A, Zirngibl R, Aubin J, Jurdic P. Involvement of the orphan nuclear estrogen receptor-related receptor alpha in osteoclast adhesion and transmigration. *J Mol Endocrinol*. 2010 Dec;365–77.
77. Bonnelye E, Aubin JE. An energetic orphan in an endocrine tissue: a revised perspective of the function of estrogen receptor-related receptor alpha in bone and cartilage. *J Bone Min Res*. 2013;28(2):225–33.
78. Bae S, Lee M, Mun S, Giannopoulou E, Yong-Gonzalez V, Cross J, et al. MYC-dependent Oxidative Metabolism Regulates Osteoclastogenesis via Nuclear Receptor ERR α . *J Clin Invest*. 2017 Jun 30;2555–68.

79. Wei W, Schwaid A, Wang X, Wang X, Chen S, Chu Q, et al. Ligand Activation of ERR α by Cholesterol Mediates Statin and Bisphosphonate Effects. *Cell Metab.* 2016 Mar 8; 23(3):479–91.
80. Bonnelye E, Kung V, Laplace C, Galson DL, Aubin JE. Estrogen receptor-related receptor alpha impinges on the estrogen axis in bone: potential function in osteoporosis. *Endocrinology.* 2002 Sep;143(9):3658–70.
81. Fradet A, Sorel H, Bouazza L, Goehrig D, Depalle B, Bellahcene A, et al. Dual function of ERRalpha in breast cancer and bone metastasis formation: implication of VEGF and osteoprotegerin. *Cancer Res.* 2011 Sep 1;72:5728–38.
82. Bouchet M, Lainé A, Boyault C, Proponnet-Guerault M, Meugnier E, Bouazza L, et al. 2 ERR α expression in bone metastases leads to an exacerbated anti-tumor immune response. *Cancer Res.* 2020 May 4;
83. Fradet A, Bouchet M, Delliaux C, Gervais M, Kan C, Benetollo C, et al. Estrogen related receptor alpha in castration-resistant prostate cancer cells promotes tumor progression in bone. *Oncotarget.* 2016 Nov 22;77071–86.
84. Olmos Y, Valle I, Borniquel S, Tierrez A, Soria E, Lamas S, et al. Mutual Dependence of Foxo3a and PGC-1alpha in the Induction of Oxidative Stress Genes. *J Biol Chem.* 2009 May 22; 284:14476–84.
85. Spiegelman BM. Transcriptional Control of Mitochondrial Energy Metabolism Through the PGC1 Coactivators. *Novartis Found Symp.* 2007;282:60–3.
86. Altarejos J, Montminy M. CREB and the CRTC co-activators: sensors for hormonal and metabolic signals. *Nat Rev Mol Cell Biol.* 2011 Mar;12:141–51.
87. Fusco S, Ripoli C, Podda M, Ranieri S, Leone L, Toietta G, et al. A role for neuronal cAMP responsive-element binding (CREB)-1 in brain responses to calorie restriction. *Proc Natl Acad Sci U S A.* 2012 Jan 10;109(2).
88. Steven A, Leisz S, Wickenhauser C, Schulz K, Mougiakakos D, Kiessling R, et al. Linking CREB function with altered metabolism in murine fibroblast-based model cell lines. *Oncotarget.* 2017 Oct 27;97439–63.
89. Son J, Lee J, Kim H, Ha H, Lee Z. cAMP-response-element-binding Protein Positively Regulates Breast Cancer Metastasis and Subsequent Bone Destruction. *Biochem Biophys Res Commun.* 2010 Jul 23;399:13–14.
90. Eyre R, Alf  rez D, Santiago-Gomez A, Spence K, McConnell J, Hart C, et al. Microenvironmental IL1 β Promotes Breast Cancer Metastatic Colonisation in the Bone via Activation of Wnt Signalling. *Nat Comm.* 2019 Nov 1;10:5016.
91. Jie Z, Xie Z, Zhao X, Sun X, Yu H, Pan X, et al. Glabridin Inhibits Osteosarcoma Migration and Invasion via Blocking the p38- And JNK-mediated CREB-AP1 Complexes Formation. *J Cell Physiol.* 2019 Apr;161:71–82.
92. Detry C, Lamour V, Castronovo V, Bellahcene A. CREB-1 and AP-1 Transcription Factors JunD and Fra-2 Regulate Bone Sialoprotein Gene Expression in Human Breast Cancer Cells. *Bone.* 2008 Feb;43:422–31.
93. Jie Z, Xie Z, Xu W, Zhao X, Jin G, Sun X, et al. SREBP-2 Aggravates Breast Cancer Associated Osteolysis by Promoting Osteoclastogenesis and Breast Cancer Metastasis. *Biochim Biophys Acta Mol Basis Dis.* 2019 Jan;1695:115–25.
94. Vargas G, Bouchet M, Bouazza L, reboul P, Boyault C, Gervais M, et al. ERR α promotes breast cancer cell dissemination to bone by increasing RANK expression in primary breast tumors. 2019 Feb;950–64.
95. Dang C. Therapeutic targeting of Myc-reprogrammed cancer cell metabolism. *Cold Spring Harb Symp Quant Biol.* 2011; 369–74.
96. Indo Y, Takeshita S, Ishii KA, Hoshii T, Aburatani H, Hirao A, et al. Metabolic Regulation of Osteoclast Differentiation and Function. *J Bone Miner Res.* 2013 Nov;

- 2392–9.
97. Suely Kazue Nagahashi M, Sueli Mieko Oba S. Metabolism and Brain Cancer. *Clinics (Sao Paulo)*. 2011;(Suppl 1):33–43.
98. Chesney J, Telang S. Regulation of Glycolytic and Mitochondrial Metabolism by Ras. *Curr Pharm Biotechnol*. 2013;251–60.
99. Arvanitis C, Bendapudi P, Tseng J, Gambhir S, Felsner D. (18)F and (18)FDG PET Imaging of Osteosarcoma to Non-Invasively Monitor in Situ Changes in Cellular Proliferation and Bone Differentiation Upon MYC Inactivation. *Cancer Biol Ther*. 2008 Dec; 1947–51.
100. Mori T, Sato Y, Miyamoto K, Kobayashi T, Shimizu T, Kanagawa H, et al. TNF α Promotes Osteosarcoma Progression by Maintaining Tumor Cells in an Undifferentiated State. *Oncogene*. 2014 Aug 14;4236–41.
101. Dey P, Jonsson P, Hartman J, Williams C, Strom A, Gustafsson JA. Estrogen Receptors beta1 and beta2 Have Opposing Roles in Regulating Proliferation and Bone Metastasis Genes in the Prostate Cancer Cell Line PC3. *Mol Endocrinol*. 2012 Oct 1;
102. Croset M, Goehrig D, Frackowiak A, Bonnelye E, Ansieau S, Puisieux A, et al. TWIST1 expression in breast cancer cells facilitates bone metastasis formation. *J Bone Miner Res*. 2014 Aug;1886–99.
103. Anwar K, Nakakuki K, Shiraishi T, Naiki H, Yatani R, Inuzuka M. Presence of Ras Oncogene Mutations and Human Papillomavirus DNA in Human Prostate Carcinomas. *Cancer Res*. 1992 Nov 1;5991–6.
104. Cracolici V, Kadri S, Ritterhouse L, Segal J, Wanjari P, Cipriani N. Clinicopathologic and Molecular Features of Metastatic Follicular Thyroid Carcinoma in Patients Presenting With a Thyroid Nodule Versus a Distant Metastasis. *Am J Surg Pathol*. 2019 Apr; 514–22.
105. Yin J, Pollock C, Tracy K, Chock M, Martin P, Oberst M, et al. Activation of the ralgef/ral pathway promotes prostate cancer metastasis to bone. *Mol Cell Biol*. 2007 Nov; 7538–50.
106. Chen W, Liu S-Y, Chang Y-S, Yin J, Yeh H-L, Mouhieddine T, et al. MicroRNA-34a Regulates WNT/TCF7 Signaling and Inhibits Bone Metastasis in Ras-activated Prostate Cancer. *Oncotarget*. 2015 Jan 1;441–57.
107. Ge C, Zhao G, Li Y, Zhao X, Pannone G, Bufo P, et al. Role of Runx2 phosphorylation in prostate cancer and association with metastatic disease. *Oncogene*. 2016 Jan 21;366–76.
108. Kanzaki H, Ohtaki A, Merchant F, Greene M, Murali R. Mutations in K-Ras Linked to Levels of Osteoprotegerin and Sensitivity to TRAIL-induced Cell Death in Pancreatic Ductal Adenocarcinoma Cells. *Exp Mol Pathol*. 2013 Apr;372–9.
109. Ma Q, Wu K, Li H, Li H, Zhu Y, Hu G, et al. ONECUT2 Overexpression Promotes RAS-driven Lung Adenocarcinoma Progression. *Sci Rep*. 2019 Dec 27;20021.
110. Yang Q, Lang C, Wu Z, Dai Y, He S, Guo W, et al. MAZ Promotes Prostate Cancer Bone Metastasis Through Transcriptionally Activating the KRas-dependent RalGEFs Pathway. *J Exp Clin Cancer Res*. 2019 Sep 5;391.
111. Wong O.-W, Nitkunan T, Oinuma I, Zhou C, Blanc V, Brown RS, et al. Plexin-B1 Mutations in Prostate Cancer. *Proc Natl Acad Sci U S A*. 2007 Nov 27;19040–5.
112. Tanner J, Bensard C, Wei P, Krah N, Schell J, Gardiner J, et al. 1 EWS/FLI is a Master Regulator of Metabolic Reprogramming in Ewing Sarcoma. *Mol Cancer Res*. 2017 Nov; 1517–30.
113. Hansen A, Sandsmark E, Rye M, Wright A, Bertilsson H, Richardsen E, et al. Presence of TMPRSS2-ERG Is Associated With Alterations of the Metabolic Profile in Human Prostate Cancer. *Oncotarget*. 2016 Jul 5;42071–85.

114. Meller S, Meyer H-A, Bethan B, Dietrich D, Maldonado S, Lein M, et al. Integration of Tissue Metabolomics, Transcriptomics and Immunohistochemistry Reveals ERG-And Gleason Score-Specific Metabolomic Alterations in Prostate Cancer. *Oncotarget*. 2016 Jan 12;1421–38.
115. Dudka I, Thysell E, Lundquist K, Antti H, Iglesias-Gato D, Flores-Morales A, et al. Comprehensive Metabolomics Analysis of Prostate Cancer Tissue in Relation to Tumor Aggressiveness and TMPRSS2-ERG Fusion Status. *BMC Cancer*. 2020 May 18;437.
116. Pettersson A, Lis R, Meisner A, Flavín R, Stack E, Fiorentino M, et al. Modification of the association between obesity and lethal prostate cancer by TMPRSS2:ERG. *J Natl Cancer Inst*. 2013 Dec 18;1881–90.
117. Delliaux C, Tian T, Bouchet M, Fradet A, Vanpouille N, Flourens A, et al. TMPRSS2:ERG gene fusion expression regulates bone markers and enhances the osteoblastic phenotype of prostate cancer bone metastases. *Cancer Lett*. 2018 Sep 7;32–43.
118. Saxton R, Sabatini D. mTOR Signaling in Growth, Metabolism, and Disease. *Cell*. Mar 9;960–76.
119. Irelli A, Sirufo M, Scipioni T, De Pietro F, Pancotti A, Ginaldi L, et al. mTOR Links Tumor Immunity and Bone Metabolism: What Are the Clinical Implications? *Int J Mol Sci*. 2019 Nov 21;5841.
120. Wang Y, Klijn J, Zhang Y, Sieuwerts A, Look M, Yang F, et al. Gene-expression profiles to predict distant metastasis of lymph-node-negative primary breast cancer. *Lancet*. 2005 Feb 19;671–9.
121. Bertoldo F, Silvestris F, Ibrahim T, Cognetti F, Generali D, Ripamonti C, et al. Targeting bone metastatic cancer: Role of the mTOR pathway. *Biochim Biophys Acta*. 2014 Apr;248–54.
122. Caino M, Chae Y, Vaira V, Ferrero S, Nosotti M, Martin N, et al. Metabolic stress regulates cytoskeletal dynamics and metastasis of cancer cells. *J Clin Invest*. 2013 Jul;2907–20.
123. Khotskaya Y, Goverdhan A, Shen J, Ponz-Sarvise M, Chang S, Hsu M, et al. S6K1 promotes invasiveness of breast cancer cells in a model of metastasis of triple-negative breast cancer. *Am J Transl Res*. 2014 Jul 18;361–76.
124. Mancini A, Colapietro A, Pompili S, Del Fattore A, Delle Monache S, Biordi L, et al. Dual PI3 K/mTOR inhibition reduces prostate cancer bone engraftment altering tumor-induced bone remodeling. *Tumour Biol*. 2018 Apr;1010428318771773.
125. Moriarity B, Otto G, Rahrmann E, Rathe S, Wolf N, Weg M, et al. A Sleeping Beauty forward genetic screen identifies new genes and pathways driving osteosarcoma development and metastasis. *Nat Genet*. 2015 Jun;615–24.
126. Saxena S, Mathur A, Kakkar P. Critical Role of Mitochondrial Dysfunction and Impaired Mitophagy in Diabetic Nephropathy. *J Cell Physiol*. 2019 Nov;19223–36.
127. Ramirez-Peinado S, Alcazar-Limones F, Lagares-Tena L, El Mjiyad N, Caro-Maldonado A, Tirado O, et al. 2-deoxyglucose Induces Noxa-dependent Apoptosis in Alveolar Rhabdomyosarcoma . *Cancer Res*. 2011 Nov 1;6796–806.
128. Penniman C, Suarez Beltran P, Bhardwaj G, Junck T, Jena J, Poro K, et al. Loss of FoxOs in Muscle Reveals Sex-Based Differences in Insulin Sensitivity but Mitigates Diet-Induced Obesity. *Mol Metab*. 2019 Dec;203–220.
129. Varela A, Simoes A, Teodora J, Duarte F, Gomes A, Palmeira C, et al. Indirubin-3'-oxime Prevents Hepatic I/R Damage by Inhibiting GSK-3 β and Mitochondrial Permeability Transition. *Mitochondrion*. 2010 Aug;456–63.
130. Topisirovic I, Sonenberg N. mRNA Translation and Energy Metabolism in Cancer:

- The Role of the MAPK and mTORC1 Pathways. *Cold Spring Harb Symp Quant Biol.* 2011;355–67.
131. Jin R, Jin Y, Tang Y, Yang H, Zhou X, Lei Z. GPNMB Silencing Suppresses the Proliferation and Metastasis of Osteosarcoma Cells by Blocking the PI3K/Akt/mTOR Signaling Pathway. *Oncol Rep.* 2018 Jun;3034–40.
 132. Cheng D-D, Li S-J, Zhu B, Zhou S-M, Yang Q-C. EEF1D Overexpression Promotes Osteosarcoma Cell Proliferation by Facilitating Akt-mTOR and Akt-bad Signaling. *J Exp Clin Cancer Res.* 2018 Mar 6;50.
 133. Zhang Y, Cheng H, Li W, Wu H, Yang Y. Highly-expressed P2X7 Receptor Promotes Growth and Metastasis of Human HOS/MNNG Osteosarcoma Cells via PI3K/Akt/GSK3 β / β -catenin and mTOR/HIF1 α /VEGF Signaling. *Int J Cancer.* 2019 Aug 15;1068–82.
 134. Kundu M, Thompson C. Autophagy: basic principles and relevance to disease. *Ann Rev Pathol Mech Dis.* 2008;427–55.
 135. Maroni P, Bendinelli P, Resnati M, Matteucci E, Milan E, Desiderio MA. The Autophagic Process Occurs in Human Bone Metastasis and Implicates Molecular Mechanisms Differently Affected by Rab5a in the Early and Late Stages. *Int J Mol Sci.* 2016 Mar 25;443.
 136. Zhao G, Gao Z, Zhang Q, Tang X, Yf L, Zhang Z, et al. TSSC3 Promotes Autophagy via Inactivating the Src-mediated PI3K/Akt/mTOR Pathway to Suppress Tumorigenesis and Metastasis in Osteosarcoma, and Predicts a Favorable Prognosis. *J Exp Clin Cancer Res.* 2018 Aug 9;188.
 137. Horie R, Nakamura O, Yamagami Y, Mori M, Nishimura H, Fukuoka N, et al. Apoptosis and antitumor effects induced by the combination of an mTORinhibitor and an autophagy inhibitor in human osteosarcoma MG63 cells. *Int J Oncol.* 2016 Jan;37–44.
 138. Cuervo A. Autophagy: in sickness and in health. *Trends Cell Biol.* 2004;70–7.
 139. Mori G, D'Amelio P, Faccio R, Brunetti G. Bone-immune cell crosstalk: bone diseases. *J Immunol Res.* 2015;
 140. Gao J, Aksoy B, Dogrusoz U, Dresdner G, Gross B, Sumer S, et al. Integrative Analysis of Complex Cancer Genomics and Clinical Profiles Using the cBioPortal. *Sci Signal.* 2013 Apr 2;p11.
 141. Xiao X, Wang W, Li Y, Yang D, Xiaokang L, Shen C, et al. HSP90AA1-mediated Autophagy Promotes Drug Resistance in Osteosarcoma. *J Exp Clin Cancer Res.* 2018 Aug 28;201.
 142. Ma K, Zhang C, Huang M, Li W-Y, Hu G-Q. Cinobufagin induces autophagy-mediated cell death in human osteosarcoma U2OS cells through the ROS/JNK/p38 signaling pathway. *Oncol Rep.* 2016 Jul;90–8.
 143. Mo H, He J, Yuan Z, Mo L, Wu Z, Lin X, et al. WT1 is involved in the Akt-JNK pathway dependent autophagy through directly regulating Gas1 expression in human osteosarcoma cells. *Biochem Biophys Res Commun.* 2016 Sep 9;74–80.
 144. Tao H, Chen F, Liu H, Hu Y, Wang Y, Li H. Wnt/ β -catenin Signaling Pathway Activation Reverses Gemcitabine Resistance by Attenuating Beclin1-mediated Autophagy in the MG63 Human Osteosarcoma Cell Line. *Mol Med Rep.* 2017 Aug;1701–6.
 145. Li Y, Jiang W, Hu Y, Da Z, Zeng C, Tu M, et al. MicroRNA-199a-5p inhibits cisplatin-induced drug resistance via inhibition of autophagy in osteosarcoma cells. *Oncol Lett.* 2016 Nov;4203–8.
 146. Wei R, Cao G, Deng Z, Su J, Cai L. miR-140-5p Attenuates Chemotherapeutic Drug-Induced Cell Death by Regulating Autophagy Through Inositol 1,4,5-trisphosphate

- Kinase 2 (IP3k2) in Human Osteosarcoma Cells. *Biosci Rep* . 2016 Oct 14;e00392.
147. Xu R, Liu S, Chen H, Lao L. MicroRNA-30a Downregulation Contributes to Chemoresistance of Osteosarcoma Cells Through Activating Beclin-1-mediated Autophagy. *Oncol Rep* . 2016 Mar;1757–63.
 148. Renema N, Navet B, Heymann M, Lezot F, Heymann D. RANK-RANKL signalling in cancer. *Biosci Rep*. 2016 Aug 5;pii: e00366.
 149. Mashavi M, Menaged, Shargorodsky M. Circulating osteoprotegerin in postmenopausal osteoporotic women: marker of impaired glucose regulation or impaired bone metabolism. *Menopause*. 2017 Nov. 24(11);
 150. Duan P, Yang M, Wei M, Liu J, Tu P. Serum Osteoprotegerin Is a Potential Biomarker of Insulin Resistance in Chinese Postmenopausal Women with Prediabetes and Type 2 Diabetes. *Int J Endocrinol*. 2017;8724869.
 151. Suliburska J, Bogdanski P, Gajewska E, Kalmus G, Sobieska M, Samborski W. The association of insulin resistance with serum osteoprotegerin in obese adolescents. *J Physiol Biochem*. 2013 Dec;847–53.
 152. Kotanidou E, Kotanidis C, Giza S, Serbis A, Tsinopoulou V, Karalazou P, et al. Osteoprotegerin increases parallel to insulin resistance in obese adolescents. *Endocr Res*. 2019 May;9–15.
 153. Candido R. The osteoprotegerin/tumor necrosis factor related apoptosis-inducing ligand axis in the kidney. *Curr Opin Nephrol Hypertens*. 2014 Jan;69–74.
 154. Chang Y, Lin K, He S, Hsieh M, Hsiao J, Shin S. Serum osteoprotegerin and tumor necrosis factor related apoptosis inducing-ligand (TRAIL) are elevated in type 2 diabetic patients with albuminuria and serum osteoprotegerin is independently associated with the severity of diabetic nephropathy. *Metabolism*. 2011 Aug;1064–9.
 155. Fekih O, Triki H, Triki S, Neffati F, Chouchane S, Guediche M, et al. Osteoprotegerin as a marker of cardiovascular risk in children and adolescents with type 1 diabetes. *Pediatr Diabetes*. 2017 May;230–6.
 156. Reid P, Holen I. Pathophysiological roles of osteoprotegerin (OPG). *Eur J Cell Biol*. 2009 Jan;1–17.
 157. Kalkan R, Becer E. RANK/RANKL/OPG Pathway Is an Important for the Epigenetic Regulation of Obesity . *Mol Biol Rep*. 2019 Oct;5425–32.
 158. Kiechl S, Wittmann J, Giaccari A, Knoflach M, Willeit P, Bozec A, et al. Blockade of receptor activator of nuclear factor- κ B (RANKL) signaling improves hepatic insulin resistance and prevents development of diabetes mellitus. *Nat Med*. 2013 Mar;358–63.
 159. Bonnet N, Bourgoin L, Biver E, Douni E, Ferrari S. RANKL Inhibition Improves Muscle Strength and Insulin Sensitivity and Restores Bone Mass. *J Clin Invest*. 2019 May 23;3214–23.
 160. Thewes V, Simon R, Schroeter P, Schlotter M, Anzeneder T, Büttner R, et al. Reprogramming of the ERR and ER Target Gene Landscape Triggers Tamoxifen Resistance in Breast Cancer. *Cancer Res*. 2015 Feb 15;720–31.
 161. Lu Y LX Jiao Y, Xiong X, Wang E, Wang X, Zhang Z, Zhang H, Pan L, Guan Y, Cai D, Ning G, Li X. Periostin promotes liver steatosis and hypertriglyceridemia through downregulation of PPAR α . *J Clin Invest*. 2014;124(8):3501–13.
 162. Luo Y, Qu H, Wang H, Wei H, Wu J, Duan Y, et al. Plasma Periostin Levels Are Increased in Chinese Subjects with Obesity and Type 2 Diabetes and Are Positively Correlated with Glucose and Lipid Parameters. *Mediators Inflamm*. 2016;6423637.
 163. Kim S, Frey J, Li Z, Kushwaha P, Zoch M, Tomlinson R, et al. Sclerostin influences body composition by regulating catabolic and anabolic metabolism in adipocytes. *Proc Natl Acad Sci U S A*. 2017 Dec 26; E11238–47.
 164. Faienza M, Ventura A, Devecchio M, Fusillo A, Piacente L, Aceto G, et al. High

- Sclerostin and Dickkopf-1 (DKK-1) Serum Levels in Children and Adolescents With Type 1 Diabetes Mellitus. *J Clin Endocrinol Metab.* 2017 Apr 1;1174–81.
165. Popovic D, Mitrovic M, Tomic-Naglic D, Icin T, Bajkin I, Vukovic B, et al. The Wnt/ β -catenin Signalling Pathway Inhibitor Sclerostin is a Biomarker for Early Atherosclerosis in Obesity. *Curr Neurovasc Res.* 2017;200–6.
 166. Sasaki H, Yu C, Dai M, Tam C, Loda M, Auclair D, et al. Elevated serum periostin levels in patients with bone metastases from breast but not lung cancer. *Breast Cancer Res Treat.* 2003 Feb;245–52.
 167. Contié S, Voorzanger-Rousselot N, Litvin J, Clézardin P, Garnero P. Increased expression and serum levels of the stromal cell-secreted protein periostin in breast cancer bone metastases. *Int J Cancer.* 2011 Jan 15;352–60.
 168. Gineyts E, Bonnet N, Bertholon C, Millet M, Pagnon-Minot A, Borel O, et al. The C-Terminal Intact Forms of Periostin (iPTN) Are Surrogate Markers for Osteolytic Lesions in Experimental Breast Cancer Bone Metastasis. *Calcif Tissue Int.* 2018 Nov;567–80.
 169. Gkatzamanidou M, Dimopoulos M, Kastritis E, Christoulas D, Mouloupoulos L, Terpos E. Sclerostin: a possible target for the management of cancer-induced bone disease. *Expert Opin Ther Targets.* 2012 Aug;761–9.
 170. Trotter T, Li M, Pan Q, Peker D, Rowan P, Li J, et al. Myeloma cell-derived Runx2 promotes myeloma progression in bone. *Blood.* 2015 Jun 4;3598–608.
 171. Taipaleenmaki H, Browne G, Akech, Zustin J, van Wijnen AJ, Stein JL, et al. Targeting of Runx2 by miR-135 and miR-203 Impairs Progression of Breast Cancer and Metastatic Bone Disease. *Cancer Res.* 2015 Apr 1;1433–44.
 172. Colden M, Dar A, Saini S, Dahiya P, Shahryari V, Yamamura S, et al. MicroRNA-466 inhibits tumor growth and bone metastasis in prostate cancer by direct regulation of osteogenic transcription factor RUNX2. *Cell Death Dis.* 2017 Jan 26;e2572.
 173. Tandon M, Othman A, Ashok V, Stein GS, Pratap J. The role of Runx2 in facilitating autophagy in metastatic breast cancer cells. *J Cell Physiol.* 2018 Jan;559–71.
 174. Tandon M, Chen Z, Othman A, Pratap J. Role of Runx2 in IGF-1R β /Akt- and AMPK/Erk-dependent growth, survival and sensitivity towards metformin in breast cancer bone metastasis. *Oncogene.* 2016 Sep 8;4730–40.
 175. Liu Z, Jiang H, Dong K, Liu S, Zhou W, Zhang J, et al. Different Concentrations of Glucose Regulate Proliferation and Osteogenic Differentiation of Osteoblasts via the PI3 kinase/Akt Pathway. *Implant Dent.* 2015 Feb;83–91.
 176. Han X, Wang W, He J, Jiang L, Li X. Osteopontin as a Biomarker for Osteosarcoma Therapy and Prognosis. *Oncol Lett.* 2019 Mar;2592–8.
 177. Pang X, Gong K, Zhang X, Wu S, Cui Y, Qian B-Z. Osteopontin as a Multifaceted Driver of Bone Metastasis and Drug Resistance. *Pharmacol Res.* 2019 Jun;235–44.
 178. Zhao H, Chen Q, Alam A, Cui J, Suen K, Soo A, et al. The Role of Osteopontin in the Progression of Solid Organ Tumour. *Cell Death Dis.* 2018 Mar 2;356.
 179. Shi Z, Mirza M, Wang B, Kennedy M, Weber G. Osteopontin-a Alters Glucose Homeostasis in Anchorage-Independent Breast Cancer Cells. *Cancer Lett.* 2014 Mar 1;47–53.
 180. Zhang Q, Wang C, Tang Y, Zhu Q, Li Y, Chen H, et al. High Glucose Upregulates Osteopontin Expression by FoxO1 Activation in Macrophages. *J Endocrinol.* 2019 Aug;51–64.
 181. Karsenty G, Ferron M. The contribution of bone to whole-organism physiology. *Nature.* 2012;314–320.
 182. Lee K, Lee K, Kim T, Hutomo F, Sun H, Cheon G, et al. 1 Circulating Osteocalcin-Positive Cells as a Novel Diagnostic Biomarker for Bone Metastasis in Breast Cancer

- Patients. *J Bone Miner Res*. 2020 May 7;
183. Hussein O, Tiedemann K, Murshed M, Komarova S. Rapamycin inhibits osteolysis and improves survival in a model of experimental bone metastases. *Cancer Lett*. 2012 Jan 28;176–84.
 184. Abdelaziz D, Stone L, Komarova SV. Osteolysis and pain due to experimental bone metastases are improved by treatment with rapamycin. *Breast Cancer Res Treat*. 2014 Jan;227–37.
 185. Hartwich J, Orr W, Ng C, Spence Y, McLaughlin J, Furman W, et al. Rapamycin increases neuroblastoma xenograft and host stromal derived osteoprotegerin inhibiting osteolytic bone disease in a bone metastasis model. *J Pediatr Surg*. 2013 Jan;47–55.
 186. Baselga J, Campone M, Piccart M, Burris H, Rugo H, Sahmoud T, et al. Everolimus in postmenopausal hormone-receptor-positive advanced breast cancer. *N Engl J Med*. 2012 Feb 9;520–9.
 187. Gnant M, Baselga J, Rugo H, Noguchi S, Burris H, Piccart M, et al. Effect of everolimus on bone marker levels and progressive disease in bone in BOLERO-2. *J Natl Cancer Inst*. 2013 May 1;654–63.
 188. Hortobagyi GN. Everolimus plus exemestane for the treatment of advanced breast cancer: a review of subanalyses from BOLERO-2. *Neoplasia*. 2015 Mar;279–88.
 189. Pignochino Y, Dell’Aglia C, Basirico M, Capozzi F, Soster M, Marchio S. The combination of sorafenib and Everolimus abrogates mTORC1 and mTORC2 upregulation in osteosarcoma preclinical models. *Clin Cancer Res*. 2013;2117–2131.
 190. Fizazi M, Carducci M, Smith R, Damião J, Brown L, Karsh L, et al. Denosumab Versus zoledronic acid for treatment of bone metastases in men with castration-resistant prostate cancer: a randomised, double-blind study. *Lancet*. 2011 Mar 5;813–22.
 191. Sousa S, Clézardin P. Bone-Targeted Therapies in Cancer-Induced Bone Disease. *Calcif Tissue Int*. 2018 Feb. 102AD;
 192. Smith M, Coleman R, Klotz L, Pittman K, Milecki P, Ng S, et al. Denosumab for the Prevention of Skeletal Complications in Metastatic Castration-Resistant Prostate Cancer: Comparison of Skeletal-Related Events and Symptomatic Skeletal Events. *Ann Oncol*. 2015 Feb;368–74.
 193. Luckman S, Hughes D, Coxon FP, Graham R, Russel G, Rogers M. Nitrogen-containing Bisphosphonates Inhibit the Mevalonate Pathway and Prevent Post-Translational Prenylation of GTP-binding Proteins, Including Ras. *J Bone Miner Res*. 1998 Apr;581–9.
 194. Coleman R, Rathbone E, Brown J. Management of cancer treatment-induced bone loss. *Nat Rev Rheumatol*. 2013 Jun;365–74.
 195. Yang Z, Su Z, DeWitt J, Xie L, Chen Y, Li X, et al. Fluvastatin Prevents Lung Adenocarcinoma Bone Metastasis by Triggering Autophagy. *EBioMedicine*. 2017 May;49–59.
 196. Manoukian G, Tannir N, Jonasch E, Qiao W, Haygood T, Tu S-M. Pilot Trial of Bone-Targeted Therapy Combining Zoledronate With Fluvastatin or Atorvastatin for Patients With Metastatic Renal Cell Carcinoma. *Clin Genitourin Cancer*. 2011 Dec;81–8.
 197. Adachi N, Ayukawa Y, Yasunami N, Furuhashi A, Imai M, Sanda K, et al. Preventive Effect of Fluvastatin on the Development of Medication-Related Osteonecrosis of the Jaw. *Sci Rep*. 2020 Mar 27;5620.
 198. Babcook M, Shukla S, Fu P, Vasquez E, Puchowicz M, Molter J, et al. Synergistic Simvastatin and Metformin Combination Chemotherapy for Osseous Metastatic Castration-Resistant Prostate Cancer. *Mol Cancer Ther*. 2014 Oct;2288–302.
 199. Kany S, Woschek M, Kneip N, Sturm R, Kalbitz M, Hanschen M, et al. Simvastatin

- Exerts Anticancer Effects in Osteosarcoma Cell Lines via Geranylgeranylation and c-Jun Activation. *Int J Oncol* . 2018 Apr;1285–94.
200. Nilsson S, Huelsenbeck J, Fritz G. Mevalonate Pathway Inhibitors Affect Anticancer Drug-Induced Cell Death and DNA Damage Response of Human Sarcoma Cells. *Cancer Lett*. 2011 May 1;60–9.
 201. McCarty M, DiNicolantonio J. Suppression of NADPH Oxidase Activity May Slow the Expansion of Osteolytic Bone Metastases. *Healthcare (Basel)*. 2016 Aug 25;60.
 202. Hutchins-Weise H, Picho K, Watkins B, Li Y, Tannanbum S, Claffey K, et al. High-dose eicosapentaenoic acid and docosahexaenoic acid supplementation reduces bone resorption in postmenopausal breast cancer survivors on aromatase inhibitors: A pilot study. *Nutr Cancer*. 2014;66–76.

Legends

Fig.1: Imaging energetic metabolism of bone metastases.

Fig.2: Metabolites and molecular signalings involved in energy metabolism in bone tumors.

Table.1: Genes involved in energetic program of tumor bone lesions.

BM: Bone metastases, OS: Osteosarcoma, ES: Ewing Sarcoma

Table.1

Gene/miR name	Metabolic function	Cancer type	References
ATX	LPA synthesis	BM (BCa)	[52, 53]
ASIC1-3 OGR1, TRPV1	pH sensing (protons) (Lactate induce low pH)	BM (BCa) Osteoblasts	[36-38, 40, 41]
BDNF	Induced by low pH	BM (BCa)	[42, 43]
Beclin-1	Autophagy	BM (BCa)	[135]
CREB	Metabolic sensor Glycolysis-ROS detoxification	BM (BCa) / OS	[89, 92]
EEF1D	Protein metabolism mTOR signaling	OS	[131]
ERR α	OXPPOS Warburg effect Glutamine, Folate, Lactate, FA oxidation	BM (BCa and PCa)	[81-83]
EWS/FLI	Metabolism reprogramming Decrease glycolysis	Ewing Sarcoma	[112]
FABP4	Lipid transport	BM (PCa)	[49]
FASN	Fatty Acid synthesis	BM (PCa)	[51]
GLUT1	Glycolysis	BM (BCa and PCa)	[2]
GLS1	Glutamine uptake	OS	[44]
GPNMBr	mTOR signaling	OS	[132]
HMG-CoA	Cholesterol (synthesis)	BM (PCa)	[50]
HSP90 HSP90AA1	Autophagy mTOR signaling ROS/JNK/P38	OS	[139-142]
LDHA	Lactate metabolism	BM (BCa and PCa)	[4]

LDL-R	Cholesterol influx	BM (PCa)	[50]
MCT-1	Lactate uptake Lactate fuel	OS Osteoclasts	[21, 22]
MCT-4	Lactate release	BM (BCa), OS	[18, 20-22]
miR-199a-5p miR-140-5p miR-30a	Autophagy	OS	[145-147]
MYC	Glycolysis Glutaminolysis Mitochondria biogenesis	OS BM (PCa)	[99-101]
NGF	Induced by low pH	BM (BCa)	[42, 43]
OCN	Glucose metabolism Insulin Lipids Mitochondria biogenesis	BM (BCa)	[181, 182]
P2X7	ATP-gated ion channel mTOR signaling	OS	[133]
PDK1	Pyruvate metabolism	BM (BCa)	[55]
PGC1 α	Multiple energetic metabolic pathways	BM (PCa)	[67]
PGK1	Glycolysis	BM (PCa)	[16]
6PGL	PPP	BM (BCa)	[17]
PHGDH	L-serine biosynthesis Link with EWS/FLI	BM (Bca) ES	[45, 112]
PPAR γ	Lipids metabolism	BM (BCa)	[20, 49]
PRDX2	Ros metabolism	BM (BCa)	[56]

PSAT1	L-serine biosynthesis Link with EWS/FLI	BM (BCa) ES	[45, 112]
PSPH	L-serine biosynthesis Link with EWS/FLI	BM (BCa) ES	[45, 112]
6PGL	PPP	BM (BCa)	[17]
Rab5a	Autophagy	BM (BCa)	[135]
RAS	Glycolysis, Nucleotide Acid synthesis, OXPHOS	BM (PCa)	[103, 106, 107]
SR-B1	Cholesterol influx	BM (PCa)	[50]
mTOR/S6K	Metabolic sensor	BM (BCa, PCa)	[120, 122-124, 137]
TSSC3	ATG5 correlation mTOR signaling Autophagy	OS	[178]
WNT/ β -catenin	Autophagy (Beclin-1)	OS	[144]
WT1	Autophagy, AKT/JNK GAS1	OS	[143]

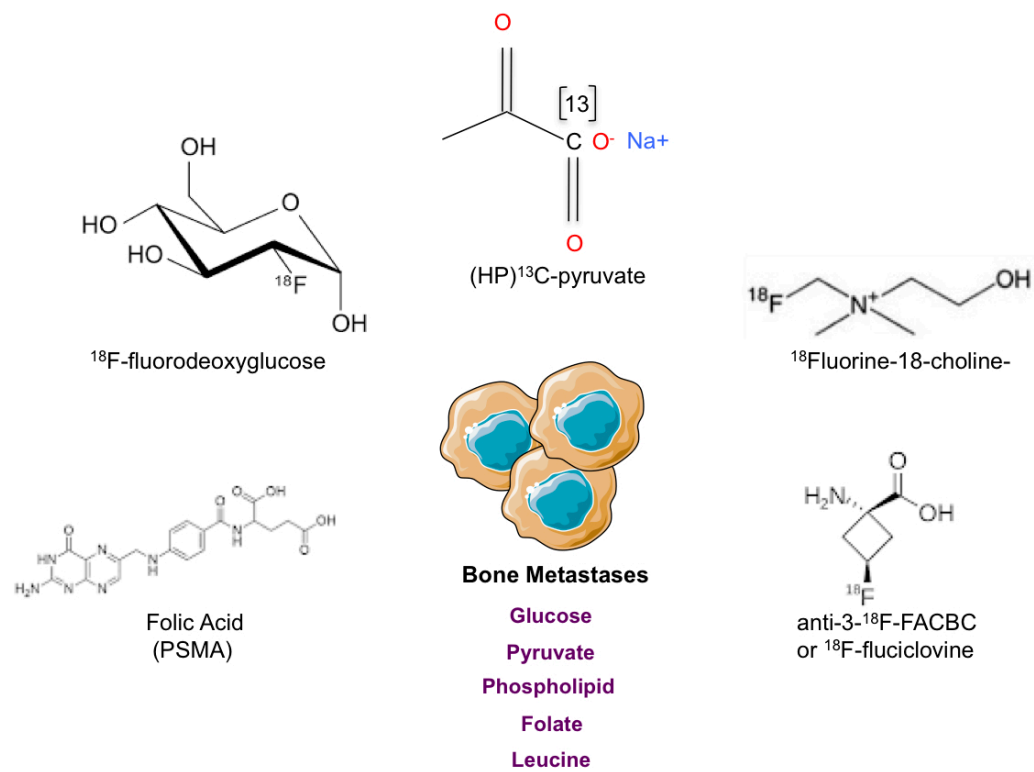


Figure.1:

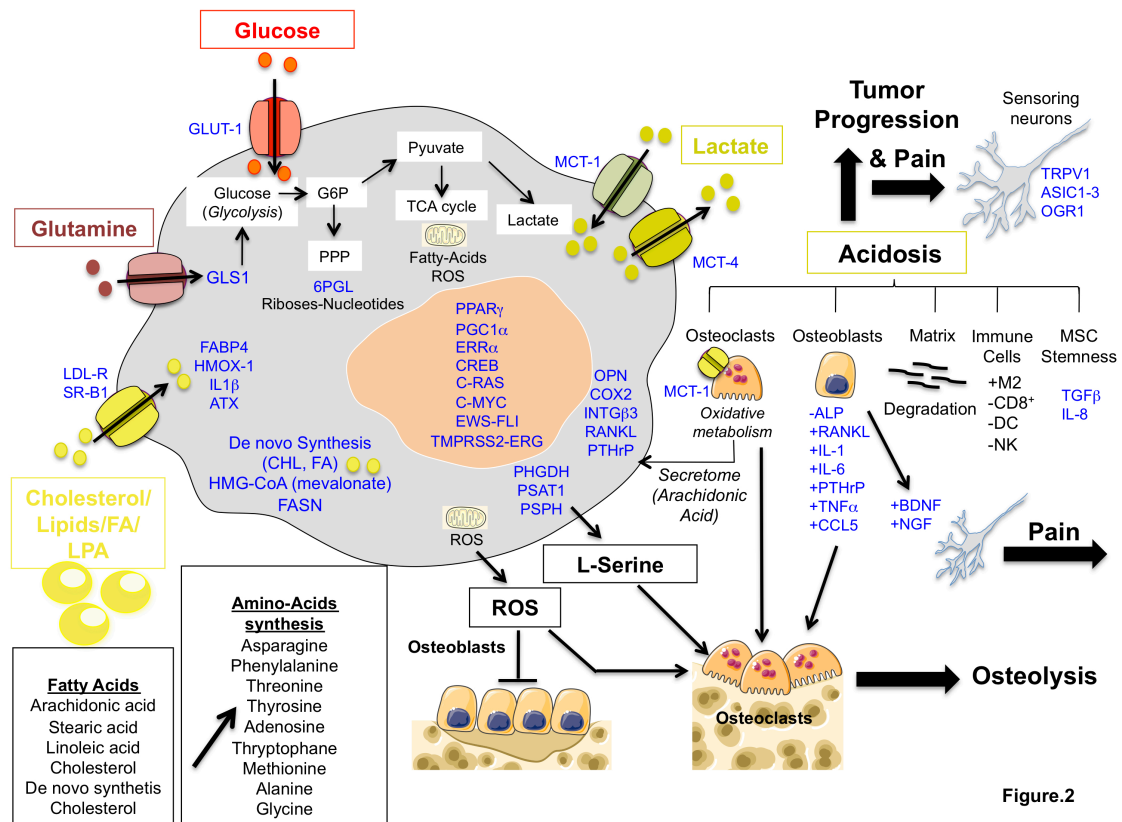


Figure.2