Metastatic Bone Disease: Role of Transcription Factors and Future Targets

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Abstract

Progression of cancer from the earliest event of cell transformation through stages of tumor growth and metastasis at a distal site involves many complex biological processes. Underlying the numerous responses of cancer cells to the tumor microenvironment which support their survival, migration and metastasis are transcription factors that regulate the expression of genes reflecting properties of the tumor cell. A number of transcription factors have been identified that play key roles in promoting oncogenesis, tumor growth, metastasis and tissue destruction. Relevant to solid tumors and leukemias, tissue specific transcription factors that are deregulated resulting from mutations, being silenced or aberrantly expressed, have been well characterized. These are the master transcription factors of the Runx family of genes, the focus of this review, with emphasis placed on Runx2 that is abnormally expressed at very high levels in cancer cell lines that are metastatic to bone. Recent evidence has identified a correlation of Runx2 levels in advanced stages of prostate and breast cancer and demonstrated that effective depletion of Runx2 by RNA interference inhibits migration and invasive properties of the cells prevents metastatic bone disease. This striking effect is consistent with the broad spectrum of Runx2 properties in activating many genes in tumor cells that have already been established as indicators of bone metastasis in poor prognosis. Potential strategies to translate these findings for therapeutic applications are discussed.

Keywords

Runx/AML/Cbfa1 factors; Runx2; microRNA; breast and prostate tumors in bone; osteolytic disease; subnuclear targeting; Smads

Introduction

Initiation of the tumor cell phenotype, tumor growth and its metastasis to distal sites progresses through many stages involving responses of the tumor cell to systemic factors

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and the local tissue environment [1]. Each stage of tumor progression involves transcriptional regulation/deregulation with the activation of genes and secreted proteins that drive the proliferative and invasive properties of tumor cells. The earliest event is the epithelialmesenchymal transition mediated by well characterized transcription factors (e.g. HLH Factors, Twist, Snail) and signaling pathways (Notch, Wnt, TGF/BMP, Src) that result in a population of cells designated as tumor-initiating cells [2]. The developing tumor cells acquire properties that are increasingly mesenchymal-like and produce proteins that are components of the bone ECM. For more than a decade, the association of tumor cell expressed sibling proteins, integrins and matrix metalloproteinases have been correlated with bone metastasis. The discovery that the Runx2 transcription factor which controls bone development, is expressed in metastatic breast tumors, was a key finding in understanding the regulation of genes associated with metastatic bone disease. An overwhelming number of Runx2 gene targets are expressed at different stages along the osteoblast lineage; and together with the numerous Runx2 protein-protein interactions with chromatin remodeling factors, mediators of developmental signaling pathways (TGF/BMP, Hox, Wnt, Src, PTHrP) and tumor suppressor proteins, Runx2 has a broad spectrum of phenotype control of a cell. The functional characterization of abnormal and highly expressed levels of Runx2 in metastatic breast and prostate cancer cell lines emphasizes the significance of a master skeletal transcription factor in potentiating tumor cell progression and metastatic bone disease.

1. Runx Transcription Factors and Cancer

The Runx transcription factors (Runx1, Runx2, and Runx3) are essential for organogenesis and regulate phenotypic genes through successive cell divisions determining cell cycle progression or exit in progeny cells. Runx factors not only control lineage commitment and cell proliferation by regulating genes transcribed by RNA Pol II, but also acts as a repressor of RNA Pol I mediated ribosomal RNA (rRNA) synthesis by functional association with ribosomal genes that reside in large discrete foci at nucleolar organizing regions of metaphase chromosomes and in the nucleoli of interphase cells [3]. These Runx chromosomal foci are associated with open chromatin and undergo transition into nucleoli at sites of rRNA synthesis during interphase [3,4]. Enlarged and increased numbers of nucleoli are hallmarks of the tumor cells and the abnormal expression levels of Runx factors in cancer cells and associated with multiple nucleoli implicates Runx factors in deregulated activities of the transformed phenotype [5].

Mutations in Runx genes have been linked to several types of cancer [6]. Runx1 is essential for definitive hematopoiesis; however, numerous translocations with other genes result in a spectrum of leukemias [7]. Runx3 contributes to gut and neural development and functional inactivation of RUNX3 through mutation, epigenetic silencing, or cytoplasmic mislocalization is highly correlated with gastric cancer(s) [8]. Runx2 is a key factor for bone formation [9-11] and is associated with osteosarcoma [12,13]. The oncogenic potential of Runx2 was first indicated from T-cell lymphoma induced by retroviral insertion, synergism between Runx2 and c-Myc [6]. The finding of Runx2 expression in highly metastatic tumor cell lines and aggressive tumor growth in bone further implicated Runx2 activities in promoting tumor properties [14-16]. In normal bone development, Runx2 contributes to regulation of the balance between bone formation and bone resorption through repression and activation of genes that control osteoclast and osteoblast activity. A large number of Runx2 target genes essential for normal bone formation, are also documented to promote tumor growth and invasion [17,18].

2. Runx2 function appears critical at early and late stages of tumor progression

The mechanism(s) by which each of the Runx factors switch their properties from a tumor suppressor role promoting differentiation to normal cell phenotypes to functioning as proteins with oncogenic properties in tumor cells are not clear [6,19-21]. While Runx1 and Runx3 mutations are linked with leukemia and gastric cancer, respectively [6,8] as established by at least a decade of research, Runx2 association with the cancer progression is relatively recent. We find in normal prostate and breast glandular tissue, Runx1 is expressed in the epithelial lining cells analogous to its expression in periosteum and perichondrium [22,23], suggesting a critical function in supporting the phenotype of these cells. It is intriguing to postulate that EMT events may lead to deregulated expression of Runx1 and Runx2 in the resulting tumorigenic cell.

Initial structural alterations characterizing breast cancer include loss of cell polarization and luminal filling of mammary glands [24,25]. In normal mammary epithelial cells Runx2 is expressed at low levels and activates differentiation genes such as β-casein [26]. Ectopic Runx2 expression in normal mammary epithelial cells, induces several key cancer-related genes (Bcl-2, and IL8) and disorganizes acinar architecture to resemble a tumorigenic phenotype in the 3D culture model [16]. Depletion of endogenous Runx2 or expression of a dominant negative or point mutations that prevents fidelity of Runx2 intranuclear localization in highly aggressive metastatic breast cancer cells, reverts cancer cell aggregates into more normal acini-like structures in vitro. The significance of these in vitro studies was confirmed by limited tumor growth in the mammary fat when Runx2 was silenced in the MDA-MB-231 cells [16]. A recent study demonstrated a potential role for Runx2 in early phases of breast cancer by showing a positive association between nuclear Runx2 and estrogen-progesterone receptor gene expression in a small percentage of cells in human tissue samples and thus identifies a biological subtype of breast cancer [27]. These studies suggest that Runx2 not only promotes metastatic properties of cancer cells, but also can initiate tumorigenic properties in normal mammary epithelial cells.

Recent studies suggest possible mechanisms of Runx2 mediated early survival of tumor cells by directly activating survivin expression in prostate cancer cells [28]. Survivin is highly expressed in a range of human tumors and correlates with both accelerated relapse and chemotherapy resistance [29]. These findings indicate that Runx2 negatively affect the apoptotic pathway (by activating Bcl2 and survivin) leading to enhanced survival of tumor cells. Studies have also shown that Runx2 levels increased with breast and prostate tumor growth and are linked to protections of cancer cells against apoptosis by bone morphogenetic protein 7 (BMP7) [30-32]. For example, prostate tumors in the conditional Pten-knockout mouse exhibited higher Runx2 levels during prostate cancer progression, as did BMP7 [30]. The effects of BMP7 are complex being inhibitory to prostate cancer in the normal epithelial cell, but positively correlated with prostate metastasis and tumor growth in bone [30]. The prostate cell line C4-2B which produces osteoblastic lesions express both BMP7 and Runx2 at high levels [33]. This raises interesting possibilities of cross talk between BMP7-Smads and Runx2-Smad transcriptional pathways in prostate cancer progression, as Runx2 has a well established interaction with Smads [34]. Together, these observations support a novel role of Runx2 in regulating survivin expression in malignant epithelial cells and identifying Runx2 as a potential critical factor in BMP7 signaling that prevents prostate cancer cells from undergoing apoptosis.

Evidence for Runx2 involvement in the later metastatic process included the characterization of the bone-related matrix proteins (osteopontin, osteocalcin, bone sialoprotein and other sibling proteins) [35], vascular endothelial growth factor [36], TGFβ1R [37] and matrix metalloproteinases [35,38,39]. Each of these proteins is associated with stages of tumor progression and metastatic events [17]. Importantly, several Runx response elements are

present in these genes identified in bone-seeking metastatic cancer cells. Runx2 is also responsive to integrin signaling [40] and the receptor integrins beta subunits are highly expressed in breast and prostate cancer cells [41,42]. These secreted proteins regulate key cellular processes implicated in cancer progression by increasing the mobility and invasive properties of cancer cells.

A direct functional role for Runx2 in metastatic cell lines was provided by the intratibial model of bone metastasis. In these studies, the highly aggressive MDA-MB-231 cell line was modified with stably integrated mutant Runx2 proteins (dominant negative or a point mutation) or expression of shRNA-Runx2 [15,43,44]. Blocking Runx2 function either by expressing mutant Runx2 proteins or depletion of Runx2 decreases expression of metastatic and osteolytic genes, in vitro invasive properties of MDA-MB-231 breast and PC3 prostate cancer cells and inhibits bone osteolytic properties in vivo[43,44]. These findings suggest that Runx2 function is obligatory for expression of target genes that mediate the osteolytic activity of metastatic breast cancer cells. These events are summarized in Figure 1. Tissue arrays that include biopsy samples of tumors at stages of progression of breast and prostate cancer have detected robust Runx 2 levels at more advanced stages by immunohistochemical studies [27,44].

3. Runx2 Activities Promoting Bone Resorption at the Metastatic Site

Runx2 promotes breast and prostate tumor growth and associated osteolytic lesions in the bone microenvironment, in part through direct transcriptional activation of genes that promote bone degradation, MMP9, MMP13 and other MMPs [39,44]. Understanding of the mechanism by which Runx2 participates in metastatic bone disease is indicated by the studies where Runx2 regulation of key components of the "vicious cycle" of tumor growth and bone resorption was investigated [43]. This cycle involves overproduction of PTHrP by breast cancercells that has a profound effect on tumor cell activities and survival and, when present in the bone microenvironment, results in osteoclastic bone resorption [1,45]. The resorbed bone releases TGFβ-stimulating tumor cell proliferation and consequently increased PTHrP secretion, thus continuing the vicious cycle. Furthermore, PTHrP is regulated by Gli, a Hedgehog signaling factor, and this pathway leads to pathologic consequences in a variety of human tumors [46]. It was recently established thatRunx2 regulates TGFβ-mediated activation of PTHrP through interaction with Hedgehog signaling molecule Gli2 [43]. Runx2 binds to the Indian Hedgehog (IHH) promoter and activates its expression in cancer cells. This regulation further increases PTHrP levels, resulting in operation of the vicious cycle in cancer cells. Runx2 directly contributes to the osteolytic process by regulating the IHH-PTHrP pathway in breast cancer cells that leads to osteoclastogenesis *in vivo*. Importantly, Runx2 was shown to be required for TGFβ-mediated activation of cyclin D1 in breast cancer cells [43]. Thus Runx2 further impacts on the osteolytic vicious cycle as illustrated in Figure 1. Taken together, these studies suggest that metastatic cancer cells, by having higher levels of Runx2, are able to activate components of the vicious cycle and target genes that increase bone loss and promote tumor progression in bone, resulting in the metastatic bone disease.

Prostate cancer cells often metastasize to bone where osteolytic, osteoblastic or mixed lesions are formed [47]. The presence of Runx2 in prostate cancer tissues and bone metastatic cell lines is positively correlated to advanced stages of prostate cancer, in adenocarcinomas and metastatic tumors, as shown by human tissue microarray studies of prostate tumors at stages of cancer progression [44,48]. Negligible Runx2 is found in normal prostate epithelial and nonmetastatic LNCaP prostate cancer cells. Our recent studies in three sublines of prostate cancer cells indicate that Runx2 is highly expressed in subclones of osteolytic PC3 bone metastatic cells. In osteolytic PC3 prostate cancer cells, endogenous elevation of Runx2 levels and diminished p57 protein levels are associated with faster

proliferation in vitro and development of larger tumors in bone [49]. In the intra-tibial metastasis model, high Runx2 levels in PC3 cells are associated with development of large tumors, increased expression of metastasis-related genes (MMP9, MMP13, VEGF, Osteopontin) and secreted bone-resorbing factors (RANKL, PTHrP, IL8) promoting osteolytic disease [44]. These studies further identified the mechanisms of Runx2 function and show that PC3 cells promote osteoclastogenesis and inhibit osteoblast activity.

Of further significance, Runx2 siRNA treatment in PC3 prostate cells or MDA-MB-231 breast cancer decreases cell migration and invasion through Matrigel in vitro, and in vivo shRunx2 stable expression cells blocked the ability of these tumor cell lines to survive in the bone microenvironment ([43,44] and see Fig. 1). The specific mechanisms for this striking effect of Runx2 depletion needs further elaboration, but the observation supports the concept that an osteoblast master transcription factor, having many biological activities, contributes to tumor cell properties in the bone microenvironment.

4. Runx2 Related Molecular Mechanisms in Tumor Cells

Runx family members form co-regulatory complexes with co-activator and co-repressor proteins that include chromatin remodeling factors, pathways and nuclear hormone receptors in both normal and tumor cells. These complexes are organized in unique subnuclear domains to regulate gene transcription. Runx is targeted to the nuclear scaffold and recruits co-regulators to these sites as a mechanism to provide cell and gene type specificity in response to signaling cascades and other regulators of tissue specifications [20]. The known pathways for which there is direct evidence of Runx involvement and which are relevant to the progression of breast and prostate tumor responses and associated metastatic bone disease include TGFβ/BMP [45], Wnt [50-52], PTHrP [45] and Src pathways [53]. Src signaling, which involves non-receptor tyrosine kinases and WW domain proteins, is a key component of osteoclastic resorption and is a strong stimulus of tumor growth. YAP is an intracellular mediator of Src signaling that shuttles to the nucleus, forms a transcriptional complex with Runx2 [54]. Inhibition of Src signaling is highly effective against bone metastatic tumor growth [55-57].

The protein-protein interactions of Runx with BMP and TGFβ/Smads are well documented in regulating normal cell differentiation (Runx1, hematopoietic cells; Runx2, osteoblasts) [58,59]. Loss of the Runx1-Smad interaction in Runx1 translocations in leukemia is a significant contributing factor to loss of the normal differentiation pathway of the hematopoietic cells dependent on TGFβ [60]. Runx3, which has potent antiproliferative and proapoptotic effects through mediating TGFβ/Smad activities in gut epithelial cells, is deregulated in gastric cancer due to silencing of Runx3 or other loss-of-function of Runx3 [8,61]. Runx2-Smad interactions have a critical role in bone formation mediating BMP and TGFβ effects [34,62]. In breast and prostate tumor cells Runx2-Smad complexes form activating target genes that promote tumor growth and osteolytic disease.

Runx2 also responds to canonical Wnt signaling, a pathway particularly enhanced in prostate tumors [50-52]. In normal mesenchymal stem cells and osteoprogenitors, Runx2 expression is activated by canonical Wnt /β-Catenin signaling, while in mature osteoblasts the TCF/LEF transcriptional target of Wnts forms a co-regulatory complex with Runx2 to attenuate genes, such as osteocalcin [63,64]. Several studies show stimulated canonical Wnt signaling mediates osteoblastic lesions produced by PCa 2b and C4-2B cell lines, both of which are associated with Runx2 /Cbfa1. Interestingly, the osteolytic cell line PC3 is characterized by high level of the Wnt inhibitor DDK1, proposed to be the indictor of osteoblastic versus osteolytic lesions induced by prostate cancer cells [65,66], as well as associated with breast cancer cell mediated the osteolytic lesions [67,68]. How Runx2-interfaces with Wnt signaling in breast and prostate tumor cells to mediate the metastatic

bone disease, is not clear. It is likely that both dependent and independent pathways are operative.

Runx2 coregulatory protein interactions include nuclear hormone receptors as another mechanism for regulating tumor cell activities. The androgen receptor binds to Runx2 and abrogates its binding to DNA [69,70]. In the PCa LNCaP cells, Runx2 stimulates androgen receptor (AR) responsive expression of the prostate-specific marker PSA [49]. Runx factors cooperate with an Ets transcription factor to regulate PSA gene expression through 4 RUNX sites in the PSA gene regulatory region [71]. Studies also show that Runx2 is mechanistically linked to TGFβ and androgen responsive pathways that support prostate cancer cell growth [49]. These distinct observations indicate that Runx2 and AR are integrated at several levels to regulate gene expression by DNA binding activity, protein-protein interactions, and through TGFβ signaling. In breast cancer cells, in the presence of estradiol, estrogen receptor alpha interacts with Runx2 and suppresses its transcriptional activity [72]. What is not yet understood is how repression of Runx2 by AR and ER affects tumor cell activities in response to the bone microenvironment.

Mechanisms underlying the upstream global changes in chromatin structure and related gene expression during metastasis progression are poorly understood. Studies in normal osteoblasts suggest that Runx2 regulates differentiation through chromatin remodeling by interacting with homeobox and Hox proteins [73-76] and similar control might exist in cancer cells as these transcription factors are also implicated in cancer progression [77-80]. Alsoexpressed in aggressive metastatic breast cancer cells and tumors is SATB1, a genome organizer that anchors multiple genomic loci and recruits chromatin-remodeling enzymes to regulate chromatin structure and downstream gene expression. The knockdown of SATB1 in MDA-MB-231breast cancer cells altered the expression of >1,000 genes and blocks tumorigenesis by restoring glandular structure-like acinar polarity and inhibits tumor growth and metastasis in vivo [81]. Interestingly, Runx2 is one of the important SATB1 regulated genomic loci shown in breast cancer cells in this study.

The interactions of Runx2 with histone acetyl transferases, as p300 and histone deacetylases (HDACs) is an important component of Runx2 regulation of gene activation and repression of specific target genes at different stages of skeletal development [82]. It is likely that in cancer cells Runx2-HDAC interactions, as well as Runx2 interactions that promote acetylation of histone to increase target gene transcription, alters the landscape of expressed genes in a tumor cells compared to a normal cell. Genetic deletions of different HDACs in the skeleton of mice have shown either anabolic or catabolic effects on bone. HDAC inhibitors (as trichostatin A) promote osteoblast differentiation in vitro [83], reduce osteoclastogenesis in vitro [84] and have beneficial effects in vivo by inhibiting inflammatory arthritis [85]. Studies using HDAC inhibitors clinically (vorinostat) are very effective in reducing tumor volume [86], but their effects on the skeleton are not fully realized.

5. Translational Approaches Based on Unique Properties of Runx2

A unique feature of Runx proteins is a common protein module in the C-terminus, designated the nuclear matrix targeting signal (NMTS). Runx proteins are targeted and reside in subnuclear foci within the nucleus where they recruit co-regulatory proteins and form complexes with co-activator and co-repressor proteins, as well as with nuclear hormone receptors in breast and prostate tumor cells on gene promoters. This is an elegant mechanism for adapting tissue specificity of gene regulation [20]. Each Runx family member is dependent on this module for normal functions. Striking evidence include the loss of this C-terminal module in Runx1 due to translocations with other genes causes leukemias; and deletion of the Runx2 C-terminus prevents bone formation and results in

embryonic lethality equivalent to a null phenotype in mice [11]. Disruption of Runx2 subnuclear targeting with expression of point mutants of nuclear matrix targeting signal (NMTS) or deletion of C-terminal containing NMTS in bone metastatic breast cancer cells showed a significant loss of its transcriptional activity, loss of invasive potential and inhibition in associated bone osteolytic disease in the intratibial mouse model of tumor growth [14,15]. Understanding of subnuclear association of Runx2 in metastatic cancer cells will not only identify novel mechanisms of bone metastatic disease but will also provide opportunity for developing targeted therapies as small molecule inhibition of the subnuclear targeting domain responsible for transcriptional activity.

The inhibition of osteolytic bone disease by inactivation of Runx2 using RNA interference approaches in bone metastatic and prostate breast cancer cells again provides a strong basis for developing Runx2 as a therapeutic target to inhibit tumor metastasis [16,43,44]. Tumor growth was reduced by MDA-MB-231 with shRNARunx2 in the fat pad and in breast and prostate bone tumors with inhibition of osteolytic disease and loss of activation of metastatic genes. These in vivo studies in which Runx2 was completely depleted by an shRNA approach, clearly demonstrate the feasibility of RNA interference for developing specific therapies for bone metastasis.

Another approach to preventing tumor growth by targeting multiple pathways at the same time is through microRNA control. Evidence is accumulating regarding a key role of microRNAs in cancer progression and bone metastasis [87]. Recent study shows that systemic treatment of tumor-bearing mice with miR-10b antagomirs specifically suppresses breast cancer metastasis to lungs but not the primary mammary tumor growth [88] and systemic delivery of synthetic microRNA-16 inhibits the growth of metastatic prostate tumors in bone via downregulation of multiple cell-cycle genes [89]. miRNAs inhibiting breast cancer metastasis were also identified like miR-31, whose expression correlates inversely with metastasis in human breast cancer patients [90]. Recently, miR-200 family and miR-9 have been shown as a suppressor and promoter of metastasis cascade by influencing epithelial-mesenchymal transition respectively [91,92].

We have performed miRNA profiling in metastatic breast cancer cell lines and leukemic cell lines where Runx levels were modulated and identified several potential Runx regulated miRNAs ([93] and our unpublished results). We find that both Runx1 and the t(8;21)-encoded AML1-ETO occupy the miR-24-23-27 locus with Runx1 decreasing and AML-ETO increasing transcription of miR-24. Expression of miR-24 stimulates myeloid cell growth, renders proliferation independent of interleukin-3, and blocks granulocytic differentiation [93]. Thus, a miR-24 inhibitor (antagomir) could have potential in reversing or reducing the AML-ETO phenotype. Several studies have validated miRs targeting Runx2 in bone cells [94,95], but it is not known if these miRs are present in tumor cells. Nonetheless, as a future therapeutic approach for inhibiting bone metastasis, high protein levels of Runx2 in metastatic cancer cells could effectively be reduced by expression of miRs that directly bind to the Runx2 3' UTR to inhibit translation.

Closing remarks

Treating cancer cells, tumor growth and metastasis is a complex process, and combinatorial approaches offer promise where conventional strategies have been ineffective. In summary, mounting evidence supports the role of Runx factors in contributing to oncogenesis by multiple mechanisms that include silencing, mutations or aberrant expression levels of Runx in tumor cells. A direct role of Runx2 in control of tumor metastasis to bone is consistent with a spectrum of genes in metastatic cancer cells that are linked to mobility, invasiveness and angiogenesis. Just as microRNAs (miRNAs) attenuate gene expression to regulate

biological processes and involve the targeting of many genes that affect the responsiveness of tumor cells, Runx factor regulation converges on many target genes and integrates transcriptional control with signaling pathways that promote tumor growth and metastatic bone disease. The potential to capture this opportunity for targeting Runx2 as an approach to treat tumors can amplify 1) therapeutic effectiveness through repression of multiple gene targets, and 2) the different parameters of biological responses used by tumor cells for their survival, growth and metastasis.

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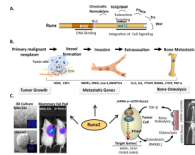
Figure 1. Central role of Runx2 in tumor growth and metastatic bone disease
A: Domain organization of Runx proteins. DBD: DNA binding domain; NLS: nuclear localization signal; NMTS: nuclear matrix targeting signal is responsible for Runx protein sub-nuclear localization. The C-terminal interacts with chromatin remodelers including p300/CBP, HDACs and HATs and mediators of cell signaling pathways like TGFβ/BMP, Src, PTHrP, and Wnt and integrates extracellular responses for Runx function in various cell types.
B: Sequence of metastatic process where primary tumor attracts blood vessels, followed by migration and invasion of tumor cells through the lymphatic or hematogenous circulation and finally colonization of disseminated cells to distal organs such as bone. Among several genes shown to be activated during these events of metastatic process, are Runx target genes.
C: In vivo evidence for Runx2 mediating tumor progression. Left panels: Runx2 knockdown (shRunx2) in bone metastatic MDA-MB-231 breast cancer cells show acini like structure after Runx2 depletion compared to parental cells control which form disorganized structure in 3D culture model. Runx2 knockdown (shRunx2) reduces tumor growth under mammary fat pads as shown by in vivo bioimaging of firefly luciferase labeled MDA-MB-231 cells. Right panel highlights the role of Runx2 in bone osteolytic disease associated with breast cancer metastasis. Breast cancer cells secrete PTHrP in response to TGFβ in the bone microenvironment, which further promotes activation of osteoclasts and causes osteolysis. Expression of shRunx2 or subnuclear targeting deficient mutants (mSTD-Runx2) in metastatic breast cancer cells inhibits osteolysis as shown in intratibial model of tumor growth by abrogating its interaction with co-regulatory proteins.