

Does Prostate-Specific Antigen Contribute to Bone Metastases?

□□ Commentary on Nadiminty et al. p. 1420

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Advanced prostate cancers consistently display common features, i.e., progression to androgen resistance, rising prostate-specific antigen (PSA), and metastases to the skeleton. In this issue of *Clinical Cancer Research*, Nadiminty et al. report a provocative mechanistic link between PSA and bone metastases, in which PSA may alter the pattern of gene expression in bone-forming osteoblastic cells (1).

The gene for prostate-specific antigen is androgen-regulated, however, circulating PSA concentrations are initially reduced when patients are treated with antiandrogen therapy. Patients progress to androgen resistance, however, and usually display rising PSA concentrations. Almost all such patients have tumor metastatic to the skeleton, in which it is a major source of morbidity. Could PSA contribute to bone metastases? PSA is a serine protease and can cleave proteins with known roles in bone metabolism, such as parathyroid hormone-related protein and transforming growth factor β (TGF β ; refs. 2–5). To test the effects of PSA on bone cells, Nadiminty et al. overexpressed PSA in a PSA-negative osteosarcoma cell line, derived from cells in the osteoblast lineage, resulting in dramatic changes in gene expression. When analyzed by cDNA microarrays containing >6,000 genes chosen for cancer specificity, ~500 genes were increased and an equivalent number decreased, with many having potential effects on bone function. The article presents the top 50 genes in the up and down categories.

The genes identified point to three major pathways that control remodeling of the skeleton: (a) the RANK ligand/osteoprotegerin pathway, which regulates osteoclast formation and bone resorption, (b) the Runx2 transcription factor, which controls osteoblast differentiation, and (c) the Wnt signaling pathway, which regulates osteoblasts and bone formation. Metastatic bone lesions in patients with prostate cancer are very heterogeneous. In general, they are characterized by net formation of disorganized, poor quality new bone (6), but at the same time, the patients display very active bone resorption, reflected in the high serum markers of this process.

Prostate cancer bone metastases are generally characterized by activation of both osteoclasts and osteoblasts (Fig. 1), with the latter predominating to give net new bone formation (7). RANK ligand and osteoprotegerin are members of the tumor necrosis factor ligand and receptor superfamilies with opposing actions to stimulate osteoclast formation, activity, and survival (8). These factors are produced by osteoblasts and act

on osteoclasts and their precursors (Fig. 1). The investigators' data suggests that PSA may contribute to the high bone resorption seen in prostate cancer by increasing the ratio of RANK ligand (increased by PSA) to osteoprotegerin (decreased 95 \times by PSA).

Runx2 is a transcription factor essential for osteoblastic differentiation, which is also expressed by breast and prostate cancer cells. The investigators found that it was increased 25 \times by PSA. They also observed that addition to the cells of exogenous, active PSA similarly stimulated Runx2 mRNA. Runx2 is a central transcriptional regulator of the osteoblastic phenotype and also regulates gene expression in cancer cells (9). Thus, active PSA in the bone microenvironment could enhance tumor growth and osteoblastic responses via increases in Runx2 in both tumor cells and bone cells.

The Wnt pathway stimulates osteoblasts and new bone formation (10). Wnt signaling involves a large number of secreted Wnt ligands and a signaling receptor complex on target cells, such as osteoblasts. Additionally, there are a variety of secreted antagonists of Wnt, such as the Dkk proteins and secreted frizzled-related proteins (sFRP). Other researchers have reported that prostate cancer cells stimulate osteoblastic metastases by activation of Wnt signaling (11), but Nadiminty et al. found that PSA increased several Wnt pathway antagonists. Wnt inhibitory factor 1 was the most up-regulated gene (31 \times), whereas sFRP2 was increased 5 \times . Dkk1 inhibits osteoblasts and is increased in multiple myeloma bone disease (12), whereas we have found that it is decreased in endothelin-mediated osteoblastic responses (13), consistent with data that Dkk1 decreases osteoblastic responses to prostate cancer cells (11). The investigators found that β -catenin, the central mediator of canonical Wnt signals to the nucleus, was increased 13 \times . A difficulty in interpreting the significance of these results is the complexity of the osteoblast itself. These cells differentiate from mesenchymal precursors along a multi-step pathway that takes about a month *in vivo*. Later cells in the lineage secrete inhibitors that tonically suppress factors that were active earlier. Osteosarcomas cannot encapsulate all the steps in this complex sequence, therefore, some of the results from the gene arrays may be misleading. The results do point to a role for Wnt signaling in bone metastases, however, that warrants deeper study.

The investigators observed changes in other pathways, including members of the TGF β superfamily: TGF β , its binding protein endoglin, and several BMPs. In early tumorigenesis, TGF β is a tumor suppressor, but advanced cancers lose their growth inhibition and are often stimulated to metastasize by TGF β (14). Prostate cancer cells and bone cells produce TGF β , therefore, the role of the protein in metastases is particularly complex. It may also contribute to androgen independence (15). TGF β requires activation from its precursor form, which can be catalyzed by PSA (4, 5). Thus, PSA may activate TGF β signaling. Bone is an important storage site of TGF β , which is

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Received 1/3/06; accepted 1/3/06.

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© 2006 American Association for Cancer Research.
doi:10.1158/1078-0432.CCR-06-0005

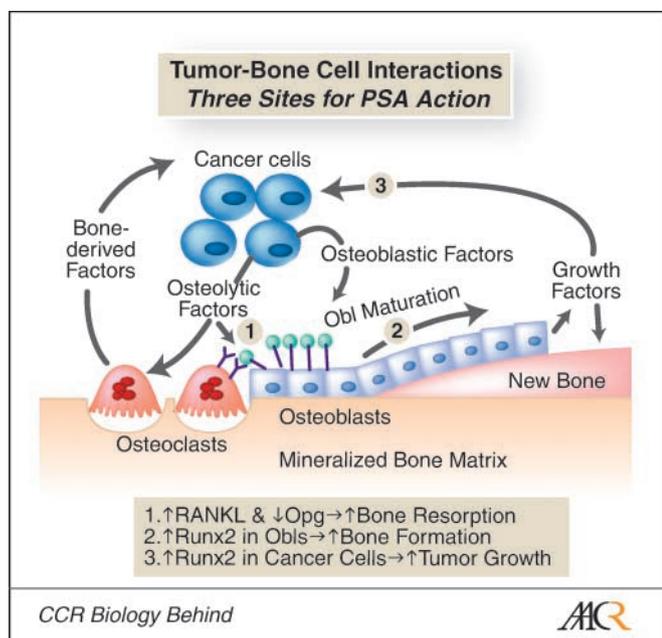


Fig. 1. There are four main players in the bone metastasis microenvironment: tumor cells, bone-forming osteoblasts, bone-destroying osteoclasts, and the mineralized bone matrix itself, which is a major storehouse of immobilized growth factors. PSA protease could alter the activities of various bioactive factors produced by the three cell types or released from the matrix (→), such as TGF β or parathyroid hormone-related protein from tumor cells. PSA could alter the phenotype of the tumor cells and osteoblasts which express it. PSA expression may be altered by factors in the bone microenvironment, such as interleukin-6. Osteoclasts are regulated by the RANK ligand pathway. Osteoblasts express RANK ligand (♣), which binds to the RANK receptor on osteoclasts and precursors (♣), stimulating their differentiation and activation. Osteoblasts also secrete the RANK ligand binding protein, osteoprotegerin (data not shown), which decreases osteoclast formation. PSA may decrease osteoprotegerin.

released during bone resorption (Fig. 1) and could have direct effects on tumor cells (16). A major target of TGF β is connective tissue growth factor, a potent stimulator of tumor cells, bone cells, and angiogenesis. It is released by stromal cells in response to tumor TGF β and is a potent paracrine stimulator of prostate cancer growth (17). It is surprising, therefore, that connective tissue growth factor was decreased 27 \times by PSA. Another counterintuitive result is the observed depression of the endothelin axis by PSA, which decreased endothelin-1 12 \times , whereas increasing the type B clearance receptor 9 \times . Endothelin-1 is an established mediator of osteoblastic metastases (18).

The experiments of Nadiminty et al. focus on mRNAs, which do not necessarily translate into changes in protein. Many genes with possible roles in bone metastases were changed, such as osteopontin, thrombospondin, interleukin-18, and twisted gastrulation homologue-1. These factors need to be validated at the protein level and tested for functional significance in physiologically relevant mouse models. Most studies of bone metastases focus on the final step in the long sequence of events leading from the primary tumor to growth in the bone microenvironment (16, 19). PSA may contribute to some of the intermediate steps, for example, by increasing invasiveness via Runx2 (20). More general provisos are that the experimental system is highly artificial. PSA expression in an osteosarcoma cell line may tell us more about osteosarcomas than about prostate cancer. The experiments rely on a single cell line and a single gene array platform, which may miss important gene

changes. Nonetheless, Nadiminty et al.'s data certainly spotlight more than enough provocative changes in physiologically significant genes.

What might be done next? The effects of PSA overexpression need to be tested *in vivo*. The osteosarcoma cells could be implanted in nude mice and studied for growth and metastasis to sites including bone. It would also be possible to study changes *in vivo* of the identified genes. Does osteoprotegerin decrease or does RANK ligand increase when bone is examined by immunohistochemistry adjacent to tumor cells overexpressing PSA? Is PSA a general regulator of Runx2, and is Runx2 an important mediator of the actions of PSA, at least in prostate cancer cells? These questions could be answered by overexpression and knockdown (with the small interfering RNA) of PSA and Runx2 in prostate cancer cell lines. Mutations interfering with the nuclear import of Runx2 decrease osteolytic bone metastases in animals and invasiveness *in vitro* in a breast cancer model (21).

How does the experimental model relate to the clinical situation? In some ways, Nadiminty et al.'s experimental model is a confusing one because the overexpression of PSA has been targeted into an osteosarcoma. In prostate cancer, the PSA is made by the cancer cells and acts on bone cells in the microenvironment of the metastasis. Prostate cancers frequently express genes associated with osteoblasts, however, such as Runx2 and osteocalcin. Leland Chung has referred to this as an "osteomimetic" phenotype, suggesting that it may contribute to the propensity of prostate cancer to metastasize to bone (22). The data of Nadiminty et al. suggests that PSA expression by prostate cancer cells could activate genes such as Runx2 and osteocalcin, thereby driving the osteomimetic phenotype. This could be tested by altering the expression of PSA by prostate cancer cells and assessing changes in bone-related mRNAs.

What are the molecular mechanisms? How does PSA overexpression change gene expression in the osteosarcoma cells? Activation of the PSA precursor is catalyzed by other proteases (23) and may not occur in osteosarcoma cells. The investigators provide no experimental evidence on this point. Secreted PSA, if active, could cleave other protein factors in the metastatic microenvironment. Alternatively, the PSA precursor could bind to partner molecules, perhaps even translocating into the nucleus, as do other factors active in bone metastases such as parathyroid hormone-related protein, in which they alter gene expression by currently unknown mechanisms. It is even possible that the effects of PSA overexpression are the consequence of the nucleic acid (mRNA or its precursor) and not the protein itself. These possibilities could be tested by either overexpressing proteolytically inactive PSA or coexpressing additional, precursor-activating proteases. In their final figure, the investigators show that added active PSA will increase Runx2 expression, therefore, these more exotic scenarios may not need to be invoked.

If PSA enzymatic activity does directly alter gene expression patterns in the microenvironment of tumors metastatic to bone, then small-molecule inhibitors of the enzyme should dramatically alter the phenotype of prostate bone metastases. Such molecules are being developed. If they decreased or palliated bone metastases in preclinical models, then the clinical development of PSA inhibitors would be indicated (24).

References

1. Nadiminty N, Lou W, Lee SO, et al. Prostate specific antigen modulates genes involved in bone remodeling and induces osteoblast differentiation of human osteosarcoma cell line SaOS-2. *Clin Cancer Res* 2006;12:1420–30.
2. Cramer SD, Chen Z, Peehl DM. Prostate specific antigen cleaves parathyroid hormone-related protein in the PTH-like domain: inactivation of PTHrP-stimulated cAMP accumulation in mouse osteoblasts. *J Urol* 1996;156:526–31.
3. Iwamura M, Hellman J, Cockett AT, Lilja H, Gershagen S. Alteration of the hormonal bioactivity of parathyroid hormone-related protein (PTHrP) as a result of limited proteolysis by prostate-specific antigen. *Urology* 1996;48:317–25.
4. Killian CS, Corral DA, Kawinski E, Constantine RI. Mitogenic response of osteoblast cells to prostate-specific antigen suggests an activation of latent TGF- β and a proteolytic modulation of cell adhesion receptors. *Biochem Biophys Res Commun* 1993;192:940–7.
5. Dallas SL, Zhao S, Cramer SD, Chen Z, Peehl DM, Bonewald LF. Preferential production of latent transforming growth factor β -2 by primary prostatic epithelial cells and its activation by prostate-specific antigen. *J Cell Physiol* 2005;202:361–70.
6. Roudier MP, True LD, Higano CS, et al. Phenotypic heterogeneity of end-stage prostate carcinoma metastatic to bone. *Hum Pathol* 2003;34:646–53.
7. Keller ET, Brown J. Prostate cancer bone metastases promote both osteolytic and osteoblastic activity. *J Cell Biochem* 2004;91:718–29.
8. Roodman GD. Mechanisms of bone metastasis. *N Engl J Med* 2004;350:1655–64.
9. Schroeder TM, Jensen ED, Westendorf JJ. Runx2: a master organizer of gene transcription in developing and maturing osteoblasts. *Birth Defects Res C Embryo Today* 2005;75:213–25.
10. Westendorf JJ, Kahler RA, Schroeder TM. Wnt signaling in osteoblasts and bone diseases. *Gene* 2004;341:19–39.
11. Hall CL, Bafico A, Dai J, Aaronson SA, Keller ET. Prostate cancer cells promote osteoblastic bone metastases through Wnts. *Cancer Res* 2005;65:7554–60.
12. Tian E, Zhan F, Walker R, et al. The role of the Wnt-signaling antagonist DKK1 in the development of osteolytic lesions in multiple myeloma. *N Engl J Med* 2003;349:2483–94.
13. Clines GA, Mohammad KS, Wessner LL, Chirgwin JM, Guise TA. Endothelin-1 stimulates bone formation by regulating osteoblast secretion of the paracrine regulators IL-6, Cyr61, CTGF and Dkk1. *J Bone Miner Res* 2005;20:S249–50.
14. Roberts AB, Wakefield LM. The two faces of transforming growth factor β in carcinogenesis. *Proc Natl Acad Sci U S A* 2003;100:8621–3.
15. Danielpour D. Functions and regulation of transforming growth factor- β (TGF- β) in the prostate. *Eur J Cancer* 2005;41:846–57.
16. Chirgwin JM, Guise TA. Interactions between tumor and bone alter the phenotypes of both. *J Musculoskelet Neuronal Interact* 2003;3:278–81.
17. Yang F, Tuxhorn JA, Ressler SJ, McAlhany SJ, Dang TD, Rowley DR. Stromal expression of connective tissue growth factor promotes angiogenesis and prostate cancer tumorigenesis. *Cancer Res* 2005;65:8887–95.
18. Guise TA, Mohammad KS. Endothelins in bone cancer metastases. *Cancer Treat Res* 2004;118:197–212.
19. Fidler IJ. The pathogenesis of cancer metastasis: the 'seed and soil' hypothesis revisited. *Nat Rev Cancer* 2003;3:453–8.
20. Pratap J, Javed A, Languino LR, et al. The Runx2 osteogenic transcription factor regulates matrix metalloproteinase 9 in bone metastatic cancer cells and controls cell invasion. *Mol Cell Biol* 2005;25:8581–91.
21. Javed A, Barnes GL, Pratap J, et al. Impaired intranuclear trafficking of Runx2 (AML3/CBFA1) transcription factors in breast cancer cells inhibits osteolysis *in vivo*. *Proc Natl Acad Sci U S A* 2005;102:1454–9.
22. Koenenman KS, Yeung F, Chung LW. Osteomimetic properties of prostate cancer cells: a hypothesis supporting the predilection of prostate cancer metastasis and growth in the bone environment. *Prostate* 1999;39:246–61.
23. Borgono CA, Michael IP, Diamandis EP. Human tissue kallikreins: physiologic roles and applications in cancer. *Mol Cancer Res* 2004;2:257–80.
24. Gygi CM, Leibovitch IY, Adlington R, et al. Prostate-specific antigen (PSA)-mediated proliferation, androgenic regulation and inhibitory effects of LY312340 in HOS-TE85 (TE85) human osteosarcoma cells. *Anti-cancer Res* 2002;22:2725–32.