2008-04-29

Gene expression in nuclear microenvironments for biological control and cancer

Gary S. Stein
University of Massachusetts Medical School

[Cancer Biology & Therapy 6:11, 1817-1824; November 2007]; ©2007 Landes Bioscience

Profiles and Legacies

Gene Expression in Nuclear Microenvironments for Biological Control and Cancer

Gary S. Stein

Correspondence to: Gary S. Stein; University of Massachusetts Medical School; Department of Cell Biology; UMASS Memorial Cancer Center; Worcester, Massachusetts USA; Email: Gary.Stein@umassmed.edu

Original manuscript submitted: 11/14/07
Manuscript accepted: 11/14/07

Previously published as a *Cancer Biology & Therapy* E-publication:
http://www.landesbioscience.com/journals/cbt/article/5294

KEY WORDS

cell proliferation, chromatin, gene expression, nuclear organization, differentiation

ACKNOWLEDGEMENTS

The author is appreciative of the editorial assistance of Elizabeth Bronstein in the preparation of this manuscript. She is a valued member of our research team.

The central theme of my research has consistently been to investigate mechanisms that control proliferation and differentiation with emphasis on regulation that is compromised in cancer. Starting as a graduate student, I have been committed to exploring cell cycle and growth control and tissue-specific transcription. And, I have been fortunate, throughout my career, to have colleagues who nurtured an architectural perspective of gene expression providing a novel dimension to the problem from both fundamental biological and clinical perspectives.

It was a unique opportunity to contribute to the initial characterization of transcriptional regulation that mediates control of the cell cycle. The studies from our laboratory in the early 1970s provided insight into the molecular mechanisms regulating gene expression during the cell cycle at the G_1/S phase transition in normal and tumor cells. To mechanistically examine linkages between proliferation and differentiation we developed a foundation for addressing bone tissue-specific gene expression. Our research group has established aberrations that accompany the onset and progression of skeletal disease and changes in gene expression that are associated with breast and prostate tumor metastasis to bone. A major contribution from our laboratory has been to mechanistically define functional relationships between the subnuclear organization of nucleic acids and regulatory proteins. During the past several years our research group has focused on combinatorial organization and assembly of regulatory machinery for gene expression in nuclear microenvironments for epigenetic control of cell fate and lineage commitment in biological control and cancer.

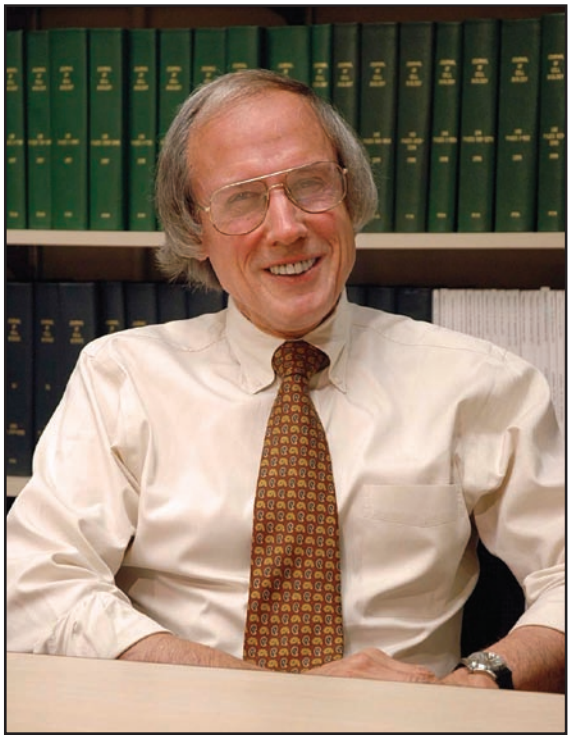
Progress in science does not occur in a vacuum. For me, the longstanding partnerships with Janet Stein, Jane Lian, Andre van Wijnen and Martin Montecino have been both effective and exceptionally meaningful. These are gifted scientists with skill sets and perspectives that have provided a broad-based platform to confront the challenges of growth control and tissue-specific gene expression that is compromised in cancer. Janet is an outstanding nucleic acid biochemist. Jane is a highly talented bone biologist, Andre is an innovative molecular biologist and Martin is an insightful chromatin biochemist. It is gratifying to look back at decades of collaboration and describe "our," rather than my initiatives and contributions.

I never lose sight of the mentors and collaborators who have been truly instrumental in development of strategies and experimental approaches. Sheldon Penman, since the late 1960s, has been the driving force behind pursuit of cell structure-gene expression relationships. Arthur Pardee, Renato Baserga and my thesis advisor Howard Rothstein guided our investigations into the regulatory mechanisms that are operative in cell cycle control; not simply as components of pathways but within the context of physiologically integrated networks and regulatory machinery that is dynamically organized and assembled. Art Pardee had the vision to dissect the components of combinatorial control and recognized the importance of multi-dimensional signaling mechanisms.

A collaboration with Carlo Croce over many years has provided a cancer genetic perspective. Sidney Weinhouse taught me the importance of relating biochemical and molecular mechanisms to cancer as a disease. His guidance solidified my commitment to tumor biology and pathology. A rewarding partnership and friendship with our Cancer Center Director, Dario Altieri, to build a Cancer Center with disease-based programs where tumor biology and pathology are pursued in a seamless manner, has been professionally and personally rewarding.

GARY S. STEIN, PH.D.

Gary S. Stein, PhD, is the Gerald L. Haidak and Zelda S. Haidak Professor of Cell Biology at the University of Massachusetts Medical School (UMMS), Chair of the Department of Cell Biology, Deputy Director for the UMASS Memorial Cancer Center, and a driving force behind establishing the UMMS Stem Cell Program. The central theme of Gary Stein's research has been to discover mechanisms controlling proliferation and differentiation. He pioneered characterization of transcriptional regulation that mediates cell cycle control. His studies have provided considerable insight into the molecular mechanisms governing gene expression during the cell cycle that are compromised in cancer. Gary Stein has had major and lasting impact in skeletal biology, where he established the foundation for addressing bone tissue specific gene expression, and provided valuable insight into aberrations that accompany the onset and progression of skeletal disease and tumor metastasis to bone. He has been instrumental in defining functional interrelationships between the localization of gene regulatory machinery in nuclear microenvironments and has made seminal contributions to mechanisms that direct transcription factors to intranuclear sites that support combinatorial organization, integration and assembly of machinery for activation or suppression of genes within the context of biological control and cancer.



CHARACTERIZATION OF TRANSCRIPTIONAL REGULATION THAT MEDIATES CELL CYCLE CONTROL

In the mid to late 1960s as a graduate student at the University of Vermont, working with Howard Rothstein, I initiated studying cell cycle control using early cleavage divisions of the zebrafish Brachydanio rerio and wound healing in lens epithelial cells as models. At that time, recombinant DNA technology had not been developed and the field of eukaryotic gene expression was in its infancy. Although interpretation of cell cycle regulatory mechanisms was dependent upon results from metabolic labeling and inhibitor studies, we made a series of observations that directed our focus to the G_1/S phase cell cycle transition.[1-4] With the demonstration by Hewson Swift and Ted Borun that histone protein synthesis is restricted to the S phase of the cell cycle, we developed control of histone gene expression as a paradigm for defining transcriptional regulatory mechanisms that are operative at the onset of S phase. During the past forty years we have made a series of contributions to understanding cell cycle and growth control that have impacted on fundamental regulatory mechanisms that support proliferation and directly relate to aberrant gene expression in cancer. By the combined application of in vivo and in vitro experimental approaches that include biochemical, molecular, cellular and in vivo genetic analysis, we have been utilizing the histone gene promoter to identify and characterize the requirements for transcriptional competency at the G_1/S phase transition point in response to factors that combinatorially regulate cell cycle progression and mechanisms that couple histone gene expression with DNA replication.[5-9]

We were one of the first laboratories to establish that transcriptional control is a key regulatory mechanism mediating the G_1/S phase cell cycle transition. We demonstrated that transcription factors modulate control of gene expression in a cell cycle dependent manner.[2] Subsequently, we cloned the first human cell cycle regulatory genes and used them to probe for proliferation-dependent signaling pathways operative at the onset of S phase.[10,11] These studies uncovered principal regulatory mechanisms that temporarily and functionally couple expression of multiple histone genes with DNA replication. We made the initial observation that remodeling of chromatin structure and nucleosome organization supports modifications in transcription for cell cycle progression.[12] This pursuit of nuclear organization as a component of transcriptional control provided us with an orientation that continues to guide our experimental strategies.

In collaboration with Lewis Kleinsmith our laboratory provided the initial observation that phosphorylation of transcription factors is a key component of cell cycle-related gene expression.[3] We identified a series of cell cycle regulatory elements and cognate transcription factor complexes that are responsible for upregulation of gene expression at the G_1/S phase transition. These studies have defined a novel cell cycle checkpoint at the initiation of S phase that is E2F independent as well as temporally, biochemically and functionally distinct from the R point late in G_1 when genes encoding enzymes for deoxynucleotide metabolism are upregulated (e.g. thymidine kinase, dihydrofolate reductase). We have characterized "R-point–S phase" signaling mechanisms.[13,14] Our research group has shown that regulatory factor complexes that mediate transcriptional control at the G_1/S phase transition are stringently cell cycle regulated in normal cells and constitutive in transformed and tumor cells where growth control has been abrogated.[15,16] The experimental approaches we employed included cell free systems, intact cell studies, gene expression profiling at the G_1/S phase transition and the first transgenic animal models for transcriptional control of cell cycle regulated genes.[6] An early lesson we learned is the necessity to pursue multidisciplinary approaches to address the challenges of combinatorial complexity that is operative in biological control.

GENE EXPRESSION CONTROLLING SKELETAL PROLIFERATION AND DIFFERENTIATION AND BONE CANCER

For many years our laboratory, to a large extent due to the insight and dedication of Jane Lian, has been actively engaged in defining molecular, cellular, biochemical, genetic and physiological mechanisms that regulate skeletal development and bone remodeling in vitro and in vivo. We have focused on skeletal pathology and tumors that originate in bone or preferentially interact with the bone microenvironment. Metastatic breast and prostate tumors are the two bone-seeking tumors that we have been examining.

Our laboratory was fortunate to be in a position to provide concepts and experimental approaches that have paved the way for resolving complexities of regulatory mechanisms that control osteoblast proliferation and differentiation including the identification of steroid hormone responsive promoter elements and bone tissue-specific transcription factors and coregulatory complexes.[15,17-20] A breakthrough for us to understand bone cell biology and pathology was the identification of distinct developmental stages (i.e. proliferation, extracellular matrix maturation, extracellular matrix mineralization and apoptosis) that reflect establishment and maintenance of the osteoblast phenotype. Our research group laid the foundation for addressing gene regulatory mechanisms that are operative during development of the osteoblast phenotype by characterizing the promoters of cell growth and bone specific genes as blueprints for responsiveness to physiological regulatory signals. We established osteoblast phenotype development as a widely utilized approach for studying signaling mechanisms operative during osteoblast differentiation, as well as for examining selective responsiveness to physiological and pharmacological mediators at specific stages of bone cell maturation. Our research team was the first to characterize perturbations in osteoblast growth and differentiation in vivo in rodent models of bone metabolic disease (osteoporosis and osteopetrosis) and cancer (osteosarcoma).[16] The sequential stages and developmental transitions of osteoblast maturation have proved to be a paradigm for many investigators to define regulatory parameters of bone biology and pathology.

To bridge the gap between regulatory mechanisms and clinical applications, our laboratory has developed a novel, transplantation-based approach for targeting gene therapy to bone using tissue-specific promoters. The combined insights obtained from characterizing bone-specific regulatory mechanisms in culture, in transgenic mice and in rodent knockout models, as well as in bone marrow-derived osteoblast precursor cells have permitted development of novel approaches for targeting gene therapy to bone using tissue-specific promoters.[21]

Building on our earlier observations that chromatin structure and nucleosome organization are dynamically modified to accommodate expression of genes during the cell cycle we demonstrated that chromatin remodeling of skeletal genes supports bone tissue-specific and steroid hormone-responsive transcription. With Martin Montecino, initially as a graduate student in our laboratory and now as a Professor at the University of Concepcion in Chile, we pioneered understanding of interrelationships between nuclear architecture and control of skeletal gene expression supporting bone cell differentiation. We have functionally linked remodeling of the chromatin structure and nucleosome organization of skeletal gene promoters with competency for interactions with physiologic mediators of transcription.[18,22]

NUCLEAR MICROENVIRONMENTS

Our pursuit of mechanisms that control proliferation and differentiation have been guided by the requirement to understand localization of regulatory complexes within the nucleus where the combinatorial components of gene expression are organized, assembled and integrated. Our appreciation for the role of nuclear organization in control of gene expression, particularly in relation to biological control and cancer has evolved during the past several years. There is recognition that extensive informational content is encoded in epigenetic signatures that go beyond DNA sequences. DNA methylation and histone modifications are providing signatures for epigenetic control of proliferation, differentiation, transformation and tumor progression as components of mechanisms that sustain aberrant gene expression.

We have been addressing regulatory parameters of transcription that are related to localization of nucleic acids and regulatory proteins within the cell nucleus. Relationships between nuclear structure and gene expression have been recognized for some time. Nucleoli are focal sites where the regulatory machinery for ribosomal gene expression resides. Functional compartmentalization of the cell nucleus is reflected by intranuclear sites that are dedicated to replication, repair, cell survival, and RNA processing. The changes in nuclear morphology that occur during hematopoietic cell differentiation are striking and reflect modifications in the organization of nucleic acids and regulatory proteins that support biological control. Transformation and tumor progression are frequently associated with altered nuclear organization. However, the challenge is to mechanistically understand the localization of regulatory complexes within the cell nucleus using criteria that are specific and quantitative. Our objective has been to develop regulatory parameters of nuclear organization into targets for tumor diagnosis and therapy.

Our group is actively engaged in exploring the intranuclear organization of regulatory domains with emphasis on modifications in cancer cells. Our strategy has been directed to the AML/Runx transcription factors that support tissue-specific gene expression (AML1 supports hematopoiesis, Runx2/AML3 supports osteogenesis and Runx3/AML2 supports gastrointestinal cell differentiation) and context-dependent tumor suppression. We have focused on two classes of nuclear microenvironments that mediate organization, assembly and integration of regulatory cues. First, we established that the Runx transcription factors bind to multiple sites of target gene promoters where they are strategically placed to scaffold coregulatory proteins for epigenetic control and serve as endpoints for key signaling pathways. Here, the regulatory signal is the Runx DNA binding domain. Second, we are pursuing a novel dimension to genetic and epigenetic control by intranuclear trafficking.[23,24] An initial component of a mechanism for localization of regulatory complexes within the nucleus came from our identification of a unique 31 amino acid intranuclear trafficking sequence which functions autonomously. Specificity of the Runx intranuclear trafficking signal is supported by a unique sequence and a unique crystal structure. To quantitatively define a signature for positioning of Runx regulatory proteins within the nucleus we developed a strategy that combines high resolution quantitative image processing with point mutations in Runx proteins that are determinants for temporal, spatial and functional parameters of control. Using emerging capabilities of high resolution imaging, we have quantitatively constructed

signatures for colocalization of Runx regulatory proteins within the nucleus that reflect the transformed phenotype.[24-26] We have demonstrated that the T(8;21) chromosomal translocation in AML leukemia disrupts the AML locus and results in aberrant intranuclear trafficking of AML transcription factors that compromise fidelity of tissue-specific gene expression. Our research group has further linked intranuclear trafficking of transcription factors with activity of tissue-specific genes using in vitro and in vivo genetic approaches. We have demonstrated that cancer cells exhibit altered subnuclear distribution of transcription factors supporting linkage of compromised intranuclear trafficking with tumorigenesis. We showed that replacement of the chromosome 21 encoded intranuclear trafficking signal in the AML transcription factors as a consequence of chromosomal translocation that occurs frequently in AML leukemia, redirects a major hematopoietic regulatory protein to sites within the nucleus for transcriptional suppression rather than activation. These findings provide evidence that subnuclear localization of regulatory proteins is linked with formation of osteolytic lesions by metastatic breast cancer cells and the leukemic phenotype.

Recent efforts in our laboratory have been directed to the extent that microenvironments with transcriptional regulatory machinery contribute to epigenetic control. We have been focusing on Runx/AML transcription factors and leukemia-related Runx/AML translocation-fusion proteins in parental and post mitotic progeny cells to investigate mechanistic parameters of cell fate and lineage commitment. We have observed that the wild type and translocation/fusion Runx/AML transcription factors are retained at chromosomal loci during mitosis providing a novel dimension to epigenetic retention of phenotypic gene expression for biological control. An analogous mechanism appears to be operative that sustains the transformed and tumor phenotypes of cancer cells and similar mechanisms epigenetically support cell fate, lineage commitment and coordinate control of proliferation, cell growth and tissue specific gene expression in a broad biological context.[27,28]

COLLABORATION IS KEY TO CURING CANCER

Collective insight into regulatory parameters that govern biological control of proliferation and differentiation and perturbations that are associated with cancer underscore the importance of combinatorial mechanisms. Evidence is accruing for temporal and spatial dimensions to control of gene expression, replication and repair with a requirement for architectural organization, assembly, integration and localization of regulatory machinery. Structure-function interrelationships are beginning to define regulatory networks that mediate physiological control and aberrations that are linked to the onset and progression of tumorigenesis.

It is now apparent that the informational content of macromolecules and macromolecular complexes goes beyond DNA sequences. There is emerging evidence that epigenetic codes and signatures for components of biological control include histone subtypes, post translational histone modifications, DNA methylation, the subnuclear localization of regulatory complexes and mitotic occupancy of regulatory factors for proliferation, cell growth and phenotypic target gene loci.

The challenge we face is to configure the data from high throughput screens in a manner that maximizes insight into mechanisms and therapeutic targets. The scope of the strategies is rapidly growing. The volume and complexities of information that is readily obtainable is extensive and expanding. Traditional boundaries between disciplines need not be an obstacle. Team approaches where partnerships between investigators with perspectives and skill sets that combine the power of genomic, proteomic, cellular, biochemical and molecular approaches provide the platform for novel insight into biological control and innovative options for cancer diagnosis and treatment. From a personal perspective, collaboration has been the most rewarding component of my career. I am confident that advances in understanding cancer biology and pathology through collaboration are more effective and meaningful than any cohort of individual contributions.

References

1. Stein GS, Matthews DE. Nonhistone chromosomal protein synthesis: utilization of preexisting and newly transcribed messenger RNA's. Science 1973; 181:71-3.
2. Stein G, Park W, Thrall C, Mans R, Stein J. Regulation of cell cycle stage-specific transcription of histone genes from chromatin by non-histone chromosomal proteins. Nature 1975; 257:764-7.
3. Thompson JA, Stein JL, Kleinsmith LJ, Stein GS. Activation of histone gene transcription by nonhistone chromosomal phosphoproteins. Science 1976; 194:428-31.
4. Stein GS, Spelsberg TC, Kleinsmith LJ. Nonhistone chromosomal proteins and gene regulation. Science 1974; 183:817-24.
5. Pauli U, Chrysogelos S, Stein G, Stein J, Nick H. Protein-DNA interactions in vivo upstream of a cell cycle-regulated human H4 histone gene. Science 1987; 236:1308-11.
6. van Wijnen AJ, Choi TK, Owen TA, Wright KL, Lian JB, Jaenisch R, Stein JL, Stein GS. Involvement of the cell cycle-regulated nuclear factor HiNF-D in cell growth control of a human H4 histone gene during hepatic development in transgenic mice. Proc Natl Acad Sci USA 1991; 88:2573-7.
7. Ramsey-Ewing A, Van Wijnen AJ, Stein GS, Stein JL. Delineation of a human histone H4 cell cycle element in vivo: the master switch for H4 gene transcription. Proc Natl Acad Sci USA 1994; 91:4475-9.
8. van der Meijden CM, Lapointe DS, Luong MX, Peric-Hupkes D, Cho B, Stein JL, van Wijnen AJ, Stein GS. Gene profiling of cell cycle progression through S-phase reveals sequential expression of genes required for DNA replication and nucleosome assembly. Cancer Res 2002; 62:3233-43.
9. Marashi F, Baumbach L, Rickles R, Sierra F, Stein JL, Stein GS. Histone proteins in HeLa S3 cells are synthesized in a cell cycle stage specific manner. Science 1982; 215:683-5.
10. Lichtler AC, Sierra F, Clark S, Wells JR, Stein JL, Stein GS. Multiple H4 histone mRNAs of HeLa cells are encoded in different genes. Nature 1982; 298:195-8.
11. Carozzi N, Marashi F, Plumb M, Zimmerman S, Zimmerman A, Coles LS, Wells JR, Stein G, Stein J. Clustering of human H1 and core histone genes. Science 1984; 224:1115-7.
12. Chrysogelos S, Riley DE, Stein G, Stein J. A human histone H4 gene exhibits cell cycle-dependent changes in chromatin structure that correlate with its expression. Proc Natl Acad Sci USA 1985; 82:7535-9.
13. Vaughan PS, Aziz F, van Wijnen AJ, Wu S, Harada H, Taniguchi T, Soprano KJ, Stein JL, Stein GS. Activation of a cell-cycle-regulated histone gene by the oncogenic transcription factor IRF-2. Nature 1995; 377:362-5.
14. Miele A, Braastad CD, Holmes WF, Mitra P, Medina R, Xie R, Zaidi SK, Ye X, Wei Y, Harper JW, van Wijnen AJ, Stein JL, Stein GS. HiNF-P directly links the cyclin E/CDK2/p220NPAT pathway to histone H4 gene regulation at the G_1/S phase cell cycle transition. Mol Cell Biol 2005; 25:6140-53.
15. Holthuis J, Owen TA, van Wijnen AJ, Wright KL, Ramsey-Ewing A, Kennedy MB, Carter R, Cosenza SC, Soprano KJ, Lian JB, et al. Tumor cells exhibit deregulation of the cell cycle histone gene promoter factor HiNF-D. Science 1990; 247:1454-7.
16. van den Ent FM, van Wijnen AJ, Last TJ, Bortell R, Stein JL, Lian JB, Stein GS. Concerted control of multiple histone promoter factors during cell density inhibition of proliferation in osteosarcoma cells: reciprocal regulation of cell cycle-controlled and bone-related genes. Cancer Res 1993; 53:2399-409.
17. Owen TA, Holthuis J, Markose E, van Wijnen AJ, Wolfe SA, Grimes SR, Lian JB, Stein GS. Modifications of protein-DNA interactions in the proximal promoter of a cell-growth-regulated histone gene during onset and progression of osteoblast differentiation. Proc Natl Acad Sci USA 1990; 87:5129-33.
18. Breen EC, van Wijnen AJ, Lian JB, Stein GS, Stein JL. In vivo occupancy of the vitamin D responsive element in the osteocalcin gene supports vitamin D-dependent transcriptional upregulation in intact cells. Proc Natl Acad Sci USA 1994; 91:12902-6.
19. Kundu M, Javed A, Jeon JP, Horner A, Shum L, Eckhaus M, Muenke M, Lian JB, Yang Y, Nuckolls GH, Stein GS, Liu PP. Cbfbeta interacts with Runx2 and has a critical role in bone development. Nat Genet 2002; 32:639-44.
20. Miller J, Horner A, Stacy T, Lowrey C, Lian JB, Stein G, Nuckolls GH, Speck NA. The core-binding factor beta subunit is required for bone formation and hematopoietic maturation. Nat Genet 2002; 32:645-9.

21. Hou Z, Nguyen Q, Frenkel B, Nilsson SK, Milne M, van Wijnen AJ, Stein JL, Quesenberry P, Lian JB, Stein GS. Osteoblast-specific gene expression after transplantation of marrow cells: implications for skeletal gene therapy. Proc Natl Acad Sci USA 1999; 96:7294-9
22. Montecino M, Lian J, Stein G, Stein J. Changes in chromatin structure support constitutive and developmentally regulated transcription of the bone-specific osteocalcin gene in osteoblastic cells. Biochemistry 1996; 35:5093-102.
23. Zeng C, van Wijnen AJ, Stein JL, Meyers S, Sun W, Shopland L, Lawrence JB, Penman S, Lian JB, Stein GS, Hiebert SW. Identification of a nuclear matrix targeting signal in the leukemia and bone-related AML/CBFα transcription factors. Proc Natl Acad Sci USA 1997; 94:6746-51.
24. Zaidi SK, Young DW, Javed A, Pratap J, Montecino M, van Wijnen A, Lian JB, Stein JL, Stein GS. Nuclear microenvironments in biological control and cancer. Nat Rev Cancer 2007; 7:454-63.
25. Javed A, Barnes GL, Pratap J, Antkowiak T, Gerstenfeld LC, van Wijnen AJ, Stein JL, Lian JB, Stein GS. Impaired intranuclear trafficking of Runx2 (AML3/CBFA1) transcription factors in breast cancer cells inhibits osteolysis in vivo. Proc Natl Acad Sci USA 2005; 102:1454-9.
26. Vradii D, Zaidi SK, Lian JB, van Wijnen AJ, Stein JL, Stein GS. A point mutation in AML1 disrupts subnuclear targeting, prevents myeloid differentiation, and results in a transformation-like phenotype. Proc Natl Acad Sci USA 2005; 102:7174-9.
27. Zaidi SK, Young DW, Pockwinse SM, Javed A, Lian JB, Stein JL, van Wijnen AJ, Stein GS. Mitotic partitioning and selective reorganization of tissue specific transcription factors in progeny cells. Proc Natl Acad Sci USA 2003; 100:14852-7.
28. Young DW, Hassan MQ, Pratap J, Galindo M, Zaidi SK, Lee SH, Yang X, Xie R, Javed A, Underwood JM, Furcinitti P, Imbalzano AN, Penman S, Nickerson JA, Montecino MA, Lian JB, Stein JL, van Wijnen AJ, Stein GS. Mitotic occupancy and lineage-specific transcriptional control of rRNA genes by Runx2, Nature 2007; 445:442-6.